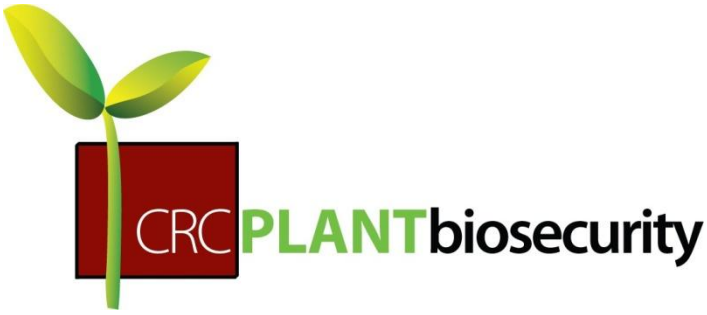

Cooperative Research Centre
for National Plant Biosecurity

Final Report

CRC40136

Insect Eradication (Phase 2)

including Eradication Database Turbo project (CRC40187)

Authors

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1. Executive Summary

Eradication of pest incursions and maintaining area freedom from key pests becomes more important when the mere presence of the pest and the use of pesticides for its control may impact negatively on market access. Eradication will only be feasible if it is economically, environmentally and sociologically sustainable especially if the incursion occurs in urban areas or the surrounding peri-urban zone where farming, hobby farms, recreation, and housing all coalesce.

Eradications in the past have often been characterised by a 'government knows best' approach using broad-spectrum sprays and removal of hosts. The media could be used effectively to promote the program and control dissent. In the internet age of instantaneous and global communication through twitter and social networking this is not a sustainable model. New technologies need to be developed that will effectively eradicate incursions but with few real or perceived side effects.

Compatible and more sustainable technologies such as: mating disruption, the sterile insect technique (SIT), attract & kill, mobile mating disruption, biological insecticides and biological control have the potential to be combined in a robust system that can be modified to deal with different pest incursions. Such systems need to be developed ahead of time and with key stakeholder input/support so that when an incursion occurs the eradication plan can be adopted without delay.

Many exotic Tortricid moths are key pests overseas. The indigenous Australian leaf roller, light brown apple moth (LBAM) (*Epiphyas postvittana*) (Lepidoptera: Tortricidae), was chosen as the model species to develop such an eradication paradigm which we call 'Integrated Insect Eradication'. LBAM has become a pest of a range of orchard (e.g. pome fruit, citrus, stone fruit, etc.) and vineyard crops both in Australia and several overseas countries where it has become established (New Zealand, New Caledonia, England, Ireland, USA States of Hawaii and California. It is a market access threat for exports of Australian fruit commodities such as citrus and is subject to an eradication/containment program in California.

LBAM is an ideal research model for the development of eradication technologies for important threats to Australian and New Zealand Biosecurity such as European grape berry moth (*Lobesia botrana*: this species has recently become established in California and is causing major damage in Chile) and the false codling moth (*Thaumatotibia leucotreta*: a major citrus pest in South African).

Trials were carried out in urban & peri-urban areas in Western Australia and vineyards in South Australia and New Zealand to combine technologies in a holistic approach to pest eradication. Beneficiaries include government and industry jurisdictions involved in pest eradication. There is additional potential for some of the technologies developed to be used for area-wide management of Tortricid pests and as part of a systems approach for market access.

In all trials SPLAT™ (Specialized Pheromone and Lure Application Technology) was highly successful in reducing LBAM populations. In vineyards no mating of virgin female moths in traps was detected until 44 days after SPLAT™ release. Further work to develop automated delivery methods for SPLAT™ and generate registration data is recommended. In an urban environment comparison of cumulative numbers of moths captured in traps from SPLAT™ treated plots and

those in untreated control plots suggested that SPLAT™ achieved greater than 90% trap shutdown for more than 90 days.

In this project we also studied the irradiation biology of LBAM and determined the most effective radiation dose to use in SIT based on flightability, mating competitiveness and inherited sterility. Population modelling provided over-flooding ratios and release frequencies required for the SIT to eradicate localised LBAM populations. However, the resources required to develop and test a functioning SIT program meant that integration of SIT and SPLAT™ was not fully tested although questions about the synergy were raised.

Analysis of eradication programs in Australia showed that almost half of all incursions were detected by agricultural industry members and around 30% were detected by Biosecurity service providers. The science community were a major source of detections of plant pathogens, but detected relatively few arthropods. Of the 124 incursion responses for which the management decision is known, 81% resulted in attempted eradication, 6% resulted in pest management being implemented and 14% resulted in no further action. Of the 59 eradication programmes for which the outcome is known, 72% of those against arthropods and 86% of those against plant pathogens were successful. The higher success rate against pathogens is surprising, but probably reflects the fact that the plant pathogen infestations targeted for eradication were generally much smaller than those of the arthropods targeted for eradication.

2. Aims and objectives

The aim of the project was to investigate the integration of innovative eradication technologies against LBAM as a model for the development of eradication strategies against other exotic moth pests. The main objective was the integration of the sterile insect technique and mating disruption using novel pheromone distribution technologies such as mobile mating disruption and SPLAT™ in urban areas and vineyards.

3. Key findings on Integrated Eradication

1. Data on 131 Australian incursion responses, resulting in 100 eradication programmes against plant pests and plant pathogens, have been compiled in a database accessible at http://b3.net.nz/gerda. Of the 124 incursion responses for which the management decision is known, 100 (81%) resulted in attempted eradication, 7 (6%) resulted in pest management being implemented and 17 (14%) resulted in no further action.

2. In Australia, almost half of all incursions were detected by agricultural industry members and around 30% were detected by Biosecurity service providers (Figure 1). The science community were a major source of detections of plant pathogens, but detected relatively few arthropods. In contrast, 15% of arthropod incursions were reported by members of the general public, but only 3% of plant pathogens. This result contrasts with published data from New Zealand, where half of new organisms were detected by the general public and relatively few by industry. Clearly, passive surveillance is an important component of Biosecurity in both countries, with the agricultural industries providing substantial value for Biosecurity surveillance in Australia.

3. Of the 59 eradication programmes for which the outcome is known, 72% of those against arthropods and 86% of those against plant pathogens were successful.

4. Pharate adult female moths (fully developed moths that have not yet emerged from the pupal case) were more sensitive to radiation than pharate adult male moths.

5. A skewed sex ratio of 2:1 in favour of males in the F_1 generation derived from pharate adults irradiated at 250 Gy suggests that a sterile insect technique (SIT) program would be effective against LBAM providing flightabilty and overflooding ratios reach acceptable levels.

6. Female LBAM irradiated at 300 Gy paired with untreated males demonstrated similar levels of multiple mating (1.7:2 spermataphores/female) as untreated females. Irradiated females had lower fecundity (305 vs. 407 eggs/female) than the untreated females but egg fertility was significantly reduced (0.2%) compared to the untreated moths (64.7%).

7. Wind tunnel and field trials demonstrated that although there was no significant difference in flight behaviour as recorded by an overhead camera coupled with tracking software, there were significant differences between irradiated and untreated males both in successful arrival at lures in the wind tunnel and in recapture rates in traps from field releases of moths in hedgerows and vineyards where flight fitness appeared to decline with increasing radiation dose. This has implications for calculation of overflooding ratios and spatial distribution of release sites for sterile insects. However, the release of semi-sterile male moths, irradiated at a lower dose than fully sterile moths may over come flight fitness issues (key finding 8).

8. None of the 1000 dyed sterile male moths irradiated at 250 Gy released per plot in weeks 6 and 7 after SPLAT^{TM} treatment were recaptured in monitoring traps baited with 3mg LBAM sex pheromone lures, indicating that the treatment was still causing effective trap shutdown.

9. Mating competitiveness testing in field cages indicated that irradiated moths had acceptable competitiveness against wild types.

10. Flightabilty testing in flight tubes indicated that irradiation at 300 Gy reduced flightability of both sexes by about 10%.

11. A population model for LBAM subject to SIT predicted that the probability of eventual population eradication was 0.95 when the ratio of males (irradiated at 300 Gy) to wild type males in monitoring traps exceeds 6:1 but higher overflooding ratios would provide faster eradication at the optimal weekly release interval. The model also suggested that male irradiation at 200 Gy may be optimal because of inherited sterility. Male moths irradiated at 200 Gy are not fully sterile. However, their F1 progeny exhibit full sterility. This means that more sterile moths are produced per irradiated male at a lower radiation dose, as well as having greater flight fitness, than the single sterile moth resulting from a high sterilising radiation dose. Estimates indicated the number of irradiated moths required to eradicate a population would be about 67% lower than the number required when releasing fully sterile moths only, leading to cost savings in an eradication program

12. Mobile mating disruption in which releases of sterile Medflies, treated with topically applied micro-encapsulated LBAM pheromone, at 3000 flies per hectare disrupted moth capture by 95%, 91%, 82% and 85% in delta traps baited with either a virgin female LBAM or synthetic pheromone lures over four consecutive nights. Adequacy of payload, mass application technology, pheromone purity and cost require resolution before the technique advances. This novel tool may

provide a socially acceptable aerial or ground approach for controlling invasive insects with pheromone mating disruption in urban or peri-urban environments.

13. Existing facilities for production of sterile fruit flies may be suitable for production of other insects for SIT but these facilities and the expertise that resides within them are under threat, and the time delay between detection of an incursion and establishment of sufficient insects in culture means that alternative pest management approaches would be required until SIT was ready for use. The SIT is an expensive and research-intensive approach that is only likely to be feasible for use in eradication of an exotic incursion if it is already being used against the pest in another country that is prepared to share information and possibly co-operate with provision of sterile insects. However, knowledge and technology developed in this program may be applicable to new targets.

14. Regulatory barriers against importation of sterile insects need to be overcome if the SIT is to be considered in rapid response options. The SIT is likely to be considered as biological control and therefore the exotic target needs to be gazetted as a target for biocontrol. This is a lengthy process and mechanisms for expediting or pre-empting this need to be developed.

15. Trials in vineyards in Australia and NZ showed SPLAT^{TM} pheromone application at 500-625 dollops ha^{-1} was effective in reducing LBAM numbers to a level where eradication is possible if other complementary treatments are also used.

16. SPLAT^{TM} HD LBAM applied at a rate of around 625 g ai ha^{-1} as approximately 1.0 g dollops appears to provide effective trap suppression (and likely mating disruption) for about 10 weeks and then this effect significantly declines.

17. The 'combination' treatment of the registered rate of the insecticide Prodigy^{TM} followed by the MD treatment with SPLAT^{TM} LBAM HD appears to have provided a very substantial suppression of LBAM adult activity as measured by pheromone trapping

18. Based on the trial results to date and the capacity to mechanize the application of SPLAT^{TM}, the treatment of extensive areas of LBAM host crop and surrounds using this technology is feasible at lesser cost, treatment time and dependence on local landscape features compared to the standard twist-tie technology. These features make SPLAT^{TM} a potentially superior technology compared to existing pheromone products available in Australia for large scale mating disruption of an emergency plant pest incursion.

19. The new four component blend LBAM pheromone identified in NZ offers the potential to increase trapping efficiency and mating disruption compared to the two component blend now used.

20. Virgin female moths placed in traps in SPLAT^{TM} treated and untreated control plots were used to monitor mating disruption in an urban environment. Female moths removed from the traps were dissected to determine presence and number of spermataphores. Mated females were detected in control plots each week but no mating was detected in the organic SPLAT^{TM} treated plots until six weeks after treatment.

21. Plotting cumulative number of moths captured over time provides a means of monitoring the rate of trap shutdown achieved by the SPLAT^{TM} treatment. The rate of moth capture (slope of line) for traps in an urban environment within the SPLAT^{TM} treated plots was consistently lower than in the untreated control plots from immediately after treatment and remained relatively constant thereafter,

whereas the rate of moth capture in the control plots was consistently high until late December when the high summer temperatures probably began to reduce population growth.

22. A plot of % trap shutdown vs. number of days after SPLAT™ application indicated a polynomial relationship of form $y = -0.0009X^2 + 0.0221X + 94.764$ ($r^2 = 0.7165$) where y= % trap shutdown and x= days after application of treatment. The regression suggested greater than 94% trap shutdown for the first 56 days and 90% trap shutdown for a period nearing 90 days after SPLAT™ application.

23. The use of organic SPLAT™ seems well accepted by the community with no complaints received or concerns registered throughout the course of the trial to date.

24. The results obtained using only perimeter treatments indicate that it may be possible to eradicate LBAM by using SPLAT™ treatment alone in an urban area if the area treated is large enough, if multiple treatments are applied and if there is consistent application throughout the treated area. The use of different concurrent SPLAT™ treatments e.g. puffers and SPLAT™ to obtain more effective coverage of an urban area and repeated applications needs to be investigated.

Analysis of Australian incursion and eradication data

We assembled data on Australian state and federal incursion responses against plant pests and plant pathogens. Data were sourced from the published literature, from the Office of the Chief Plant Protection Officer (OCPPO, complete federal records since 2006 and partial records before this), from state departments (notably the Department of Agriculture and Food Western Australia), and media releases. The database is accessible at http://b3.net.nz/gerda, and currently includes information on 131 Australian incursion responses, resulting in 100 eradication programmes. Some of the key findings from these data are summarised below.

In Australia, almost half of all incursions were detected by agricultural industry members, and around 30% were detected by Biosecurity service providers (Figure 1). The science community were a major source of detections of plant pathogens, but detected relatively few arthropods. In contrast, 15% of arthropod incursions were reported by members of the general public, but only 3% of plant pathogens. This result contrasts with published data from New Zealand, where half of new organisms were detected by the general public, and relatively few by industry. Clearly, passive surveillance is an important component of Biosecurity in both countries, with the agricultural industries providing substantial value for Biosecurity surveillance in Australia.

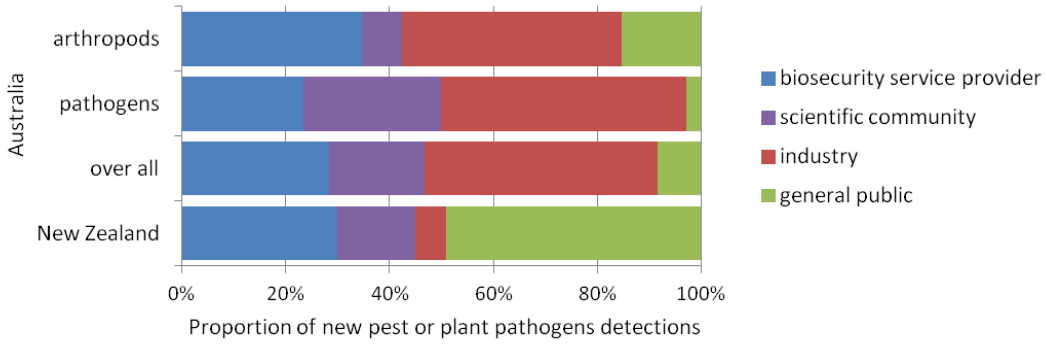


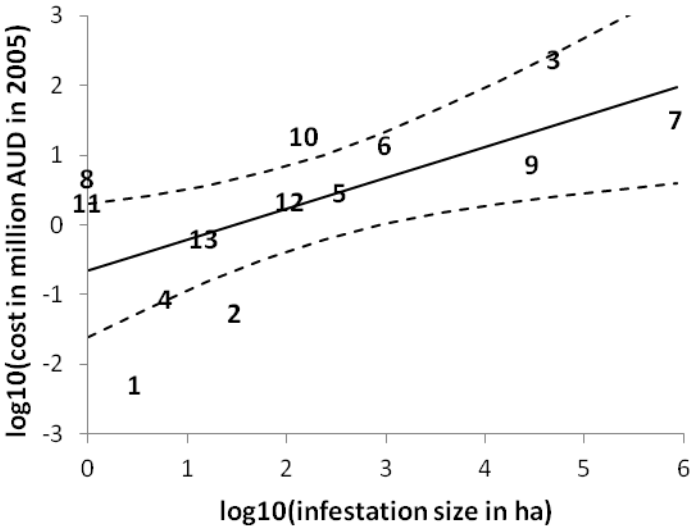
Figure1: Comparison of the sources of new pest and plant pathogen incursion detections in Australia and New Zealand.

Of the 124 incursion responses for which the management decision is known, 100 (81%) resulted in attempted eradication, 7 (6%) resulted in pest management being implemented and 17 (14%) resulted in no further action. In most cases of the latter, the reason given was that the pest or pathogen was already too widespread for eradication to be economically feasible.

Of the 59 eradication programmes for which the outcome is known, 72% of those against arthropods and 86% of those against plant pathogens were successful. The higher success rate against pathogens is surprising, given that these are generally considered to be the more difficult to eradicate, but probably reflects the fact that the plant pathogen infestations targeted for eradication were generally much smaller than those of the arthropods targeted for eradication.

There was a weak (R^2 = 0.44) but significant (p = 0.014) log-log relationship between cost and infestation size for Australian eradications where both data were known (Figure 2). Since this relationship was derived from a range of pest

and pathogen taxa, it may be useful for costing future eradication programmes. Cost is reported for relatively few eradication programmes, but is potentially a very useful datum for Biosecurity managers and decision makers.



1 = tropical fire ant in Kakadu
2 = African big-headed ant in Kakadu
3 = red imported fire ant in Brisbane*
4 = yellow crazy ant in Cairns
5 = yellow crazy ant in Arnhem Land
6 = Argentine ant in Perth
7 = papaya fruit fly in Cairns
8 = Philippines fruit fly in Darwin
9 = Queensland fruit fly in Perth
10 = citrus canker in Emerald
11 = fire blight in Melbourne
12 = chestnut blight in Ovens Valley*
13 = potato cyst nematode in Perth

*eradication still in progress

Figure 2: Relationship between infestation size and cost (standardised to AUD in 2005) in Australian eradications: y = 0.445x – 0.659. Dashed lines show 95% confidence bounds.

Irradiation biology

The radiation biology of two geographically isolated LBAM populations was studied in Perth (W.A) and Lincoln (NZ). Pharate adults fully developed but not emerged from the pupal case and pupae 1-2 days from emergence were exposed to increasing dose up to 300 Gy. Fertility and other life history parameters were measured in emerging adults (parental) and their progeny (F_1-F_3 adults).

Parental fecundity was significantly affected by increasing radiation dose in pharate adults only. For both populations, parental egg fertility declined with increasing radiation. This was most pronounced for the irradiated parental females whose fertility declined at a higher rate than that of irradiated males. At 250 Gy, females from irradiated pupae produced few larvae and no adults at F1. No larvae hatched from 250 Gy-irradiated female pharate adults. At 300 Gy, males still had residual fertility of 2-5%, with pharate adults being the more radio-sensitive.

Radiation-induced deleterious inherited effects in offspring from irradiated males were expressed as increased developmental time in F_1 larvae, a reduction in percent F1 female survival, decreased adult emergence and increased cumulative mortality over subsequent generations. The production of highly sterile F_1 males (≥ 99%) at doses ≥ 250 Gy for both populations opens the prospect of using partially sterile male moths (inherited sterility) versus fully sterile males for release. Greater suppressive potential with partially sterile moths and more competitive moths have been demonstrated in SIT programs with other moths. The skewed sex ratio in the F_1 generation in favour of males at 2:1 (250 Gy) further promotes the release of partially sterile male moths, thus reducing the number of females at the F1 generation that could contribute to survivorship of any wild population.

These results suggest that a SIT program can be applied to control an infestation of LBAM, providing flightabilty and overflooding ratios reach acceptable levels. The challenge is to identify the dose that would provide a balance between insect sterility and field competitiveness. To avoid the risk of releasing potentially fecund and fertile females in the wild when using low doses of radiation, the appropriate dose should be ≥ 250 Gy. For a number of operational reasons, especially difficulties in the separation of pupae from rearing media, irradiation of codling moths (*Cydia pomonella*) and pink bollworm (*Pectinophora gossypiella*) for SIT programs are generally done at the adult stage. However, there are advantages with pupal irradiation in terms of shipment and handling, provided a suitable and effective means of pupal separation from rearing media can be developed. The size difference between the larger female pupae and smaller male of LBAM offers this potential.

To explore the situation if adult irradiation was required for operational reasons adult irradiation trials were carried out in WA with two day old moths irradiated at 250 Gy. Control egg hatch was 85% with fertility of irradiated male parents 6.9 %.and irradiated female parents 2.7%. Some of the larvae from eggs laid by irradiated adult females reached maturity and mated to produce F_2 progeny. Fertility of eggs from pairings of irradiated males and irradiated females was 0.2%. As adult females showed some fertility at 250Gy it was decided to investigate a higher dose. Female moths from 1-4 days old were irradiated at 300 Gy and paired individually with three untreated male moths. Spermataphore dissection was used to confirm mating status. Multiple mating occurred with irradiated (av 1.7 spermataphore/female) and control moths (av 2

spermataphore/female). Average fecundity was 407 eggs/female in the control compared to 350 in irradiated moths. Fertility of irradiated moths was very low (0.2% egg hatch) compared to 64.7% in the control**.** A very small percentage of hatched survived to adulthood (0.03%)**.** The fitness and sterility of these moths was not measured.

Flightabilty of irradiated moths in NZ

Reduced moth fitness from irradiation lowers the effective overflooding ratio of sterile to wild moths. New ways to measure insect quality are being sought to improve field performance of irradiated insects, thus improving the cost effectiveness of the SIT. Trials were carried out in flight chambers, gorse hedgerows and vineyards in NZ to test the fitness of moths irradiated at 100 Gy and 250 Gy compared to untreated moths. Flight success was assessed in a wind tunnel equipped with flight track recording software. Male moths placed in the wind tunnel flew upwind towards septa with pheromone and their success at reaching the septa was measured. Irradiation at 250 Gy reduced arrival success to 49% of untreated controls, during 2-min assays. In general there were no differences in the flight tracks of irradiated and unirradiated males. However, one irradiated moth flew 49 m before reaching the septum <2 m up wind from the release site.

Mark release recapture of males irradiated at 250 Gy indicated reduced male moth recapture in hedgerows (75% of control values of 7.22% of males recaptured) and in vineyards (78% of control values of 10.5% recaptured). Males dispersed similar distances in both habitats and overflooding ratios dropped off rapidly from the release point in both landscapes. Release strategies involving ground releases should consider the effect of limited post release dispersal. Aerial release could solve this problem and warrants investigation.

Field Cage Competitiveness Testing

From September to November 2009 field cage mating competitiveness trials were carried out in Perth in collaboration with Don McInnis of USDA/ARS, Honolulu, Hawaii. The mating ability of sterilized male moths competing with unsterilized wild males for wild females was compared.

We adapted field cage mating competitiveness techniques used to measure competitiveness of irradiated fruit flies. Adult colony moths were irradiated at 300 Gy and released at dusk with unirradiated wild moths into nylon tents. Mating pairs were captured into plastic vials and individual females held separately for oviposition. After eight days females were removed and dissected for presence of spermataphores, an indication of successful mating.

Some cages with male only release were included to investigate if this would result in greater sterility. In other cages mating success was measured indirectly by percent fertility of recaptured females. In these cages moths were released and then recaptured the following morning and held individually for oviposition.

Mating competitiveness is expressed in terms of Relative Sterility Index (RSI) or Fried competitiveness value(C). The RSI is the major index of male sexual competitiveness and measures the proportion of mating achieved by sterile males. A value of 1 indicates all matings were by sterile males, 0.5 half the matings were by sterile males and 0 none of the matings by sterile males. The Fried C value provides an estimate of the overall mating competitiveness of sterile males. It is based on sterility induced in eggs laid by wild females in cage where wild females, wild males, sterile males and/or sterile females are present. Fried C

values of 1 indicate equal competitiveness between sterile and wild males. It is not uncommon to record values greater than 1 and, as results of field cage trials are notoriously variable, many replicates are often required for the true picture to emerge.

Results indicate acceptable competitiveness and that male-only release may improve competitiveness. Estimating competitiveness by egg hatch and not by observing mating pairs appeared to give a more realistic estimation of Fried C values but there was no difference in RSI between the techniques.

Table 1: Competitiveness values for male only and bi-sex release in field cage trials

Treatment	RSI ±SE	Fried C ±SE
Bi-sex	0.4±0.1	0.9±0.4
Male only	0.8±0.1	1.9±1.3
Observed: male only	0.7±0.0	2.4±0.4
Not observed: male only	0.7±0.0	0.7±0.1

Flightability tubes

We have continued testing the suitability of flight tubes to test flightability. This technology is well accepted for fruit fly SIT and offers promise for moths. Pupae were irradiated at 300Gy and placed in 20 cm high tubes and numbers of fliers calculated from moth and unmerged pupae left in the tubes. Flightability of males is much greater than females but irradiation reduced flightability by about 10% for both sexes (Fig.3). It is not known whether low female flightability is an artefact of trial design or if the heavier females are just poor fliers. Certainly in field cage competitiveness tests females have not demonstrated any problems in flying to the top of the field tents.

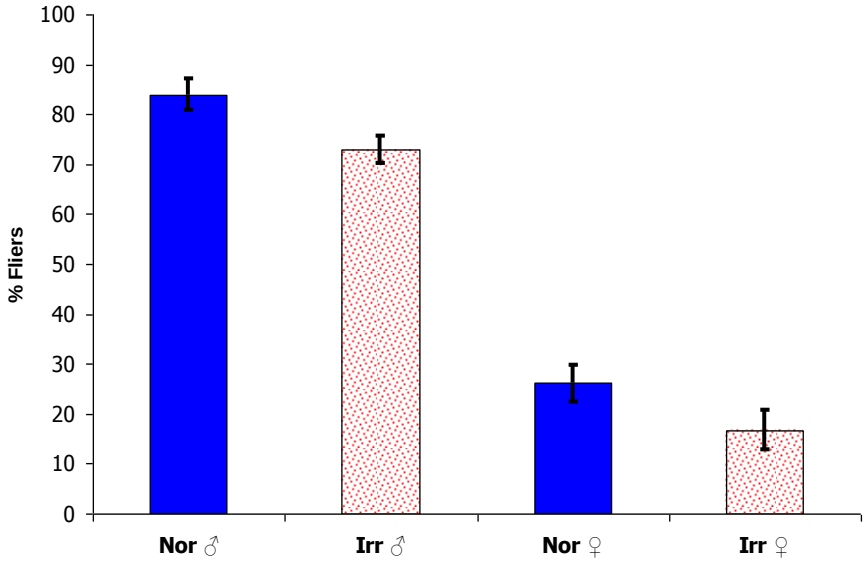


Figure 3: Flightability of unirradiated moths (Nor) and moths emerging from pupae irradiated at 300Gy (Irr).

Modelling of Overflooding ratio

A population model was developed for LBAM subject to the sterile insect technique (SIT). The model was parameterised from the literature and from recent laboratory studies conducted in NZ and WA. Relationships were fitted for several model parameters that vary with irradiation dose, allowing the model to simulate complete sterility at 300 Gy and inherited sterility from lower doses. At 300 Gy, the model suggests that eventual population extinction is 95% probable when the ratio of released to wild males in monitoring traps exceeds 6. Higher overflooding rates would be required to achieve eradication more rapidly. The optimal release interval is approximately weekly. The model showed little advantage in releasing males only compared with releasing both sexes. The critical release rate required to halt population increase declines with decreasing irradiation dose, but at doses of less than 170 Gy there is a risk that irradiated-lineage moths may form a self-sustaining population, making eradication by SIT alone impossible. The model suggests that a dose of around 200 Gy may be optimal because the resulting inherited sterility would reduce by a third the number of factory moths required compared with 300 Gy.

Phenology

Phenology trapping has continued in Perth with traps checked fortnightly. LBAM numbers peak in spring and early summer. Numbers are higher in peri-urban areas with vineyards (Figure 4) than in urban environments (Figure 5). Some vineyards experience consistently high LBAM numbers.

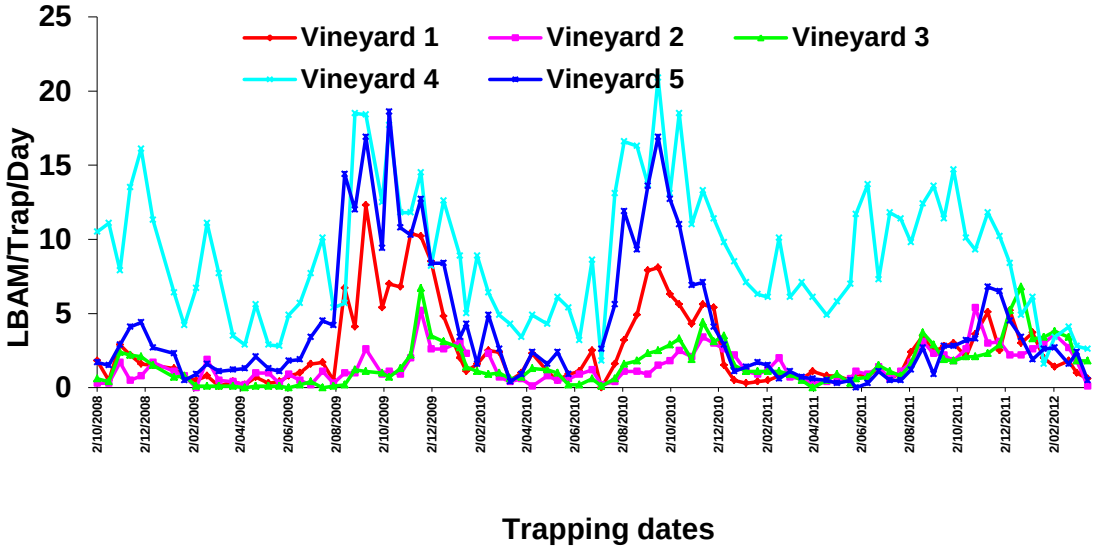


Figure 4: LBAM numbers in traps in individual Swan Valley vineyards

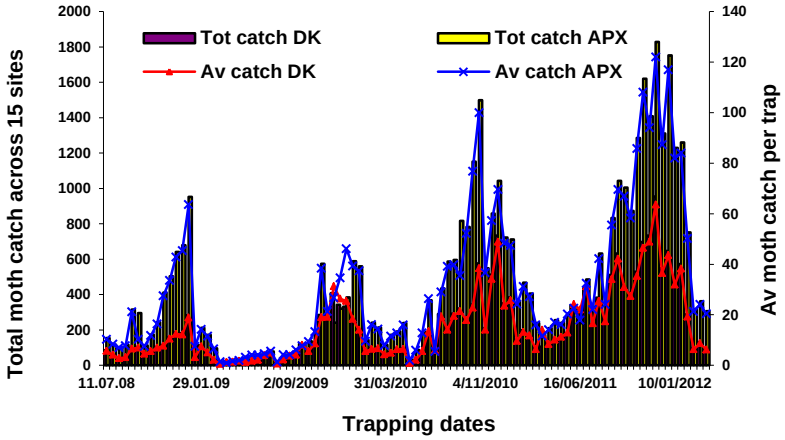


Figure 5: LBAM numbers in traps in Perth urban: Applecross and Dalkeith

Phenology Modelling

Dr Jörg Samietz: Head of Zoology in the plant protection department of Agroscope Changins-Wädenswil, Switzerland visited Plant & Food Research Lincoln from October-December 2012 on sabbatical to write up work on fruit fly phenology modelling. Jörg ran a phenology model for *Epiphyas postvittana* LBAM for Perth climate data. He created four models 2008-09, 2009-10, 2010-11 and 2011-12. Models ran from 1 July to 30 June with winter synchronising the population.

These initial models used daily max - min temperatures from the nearest Bureau of Meteorology site in Perth approximately 10 km from the trial area. The development rate parameters for LBAM were sourced from the literature.

Parameters used for the model were: T_0 low = 7.5°C under which temperature LBAM individuals do not develop, T_0 high = 31.5°C over this temperature LBAM development is assumed to halt and mortality from high temperatures start to occur. Degree days - time taken for development of each stage were: Eggs- 134, Larvae- 346, Pupae- 129 and Eclosion- 30.

It was determined that there were up to six LBAM generations completed per year in Perth. Phenology trapping data of moths from Applecross and Dalkeith were plotted onto the phenology models for each year. In some years the model and trapping were not consistent e.g.2009-2010 (Figure 6, but other years were very similar e.g. 2010-2011 (Figure 7).

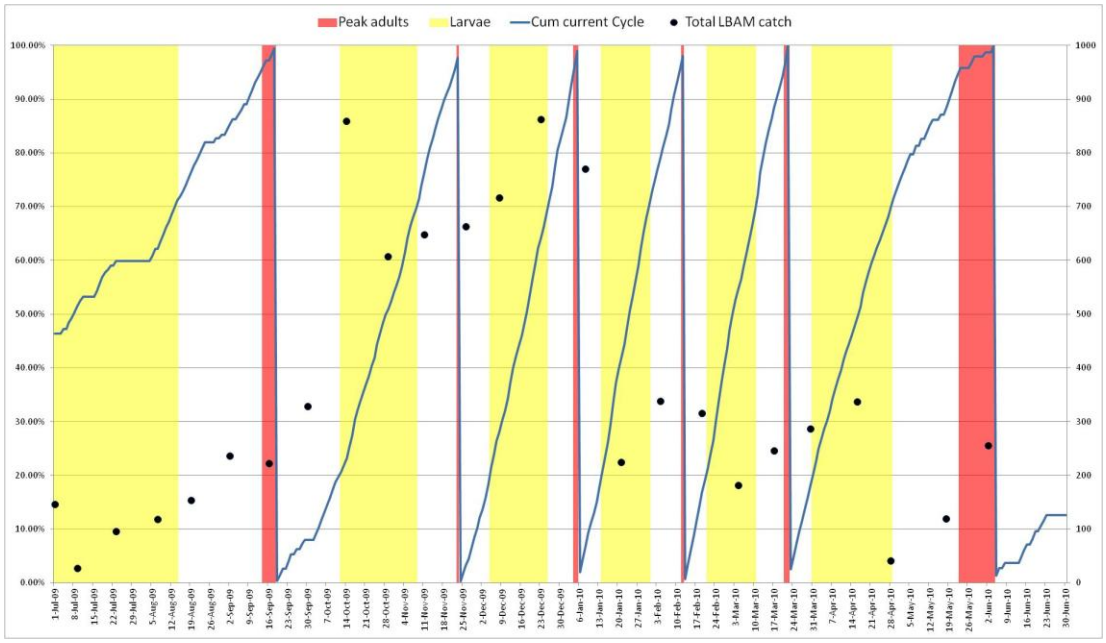


Figure 6: Phenology model and phenology trapping for *Epiphyas postvittana* in Perth Jul 1 2009 - Jun 30 2010.

The red bars indicate the peak timing of the adult stage for each generation. The yellow bars peak larval stage for each generation. The blue line indicates the cumulative development of each generation (0%-egg stage and 100%- adult stage). Overlaid are the trapping data from the male LBAM traps from Perth (black circles●).

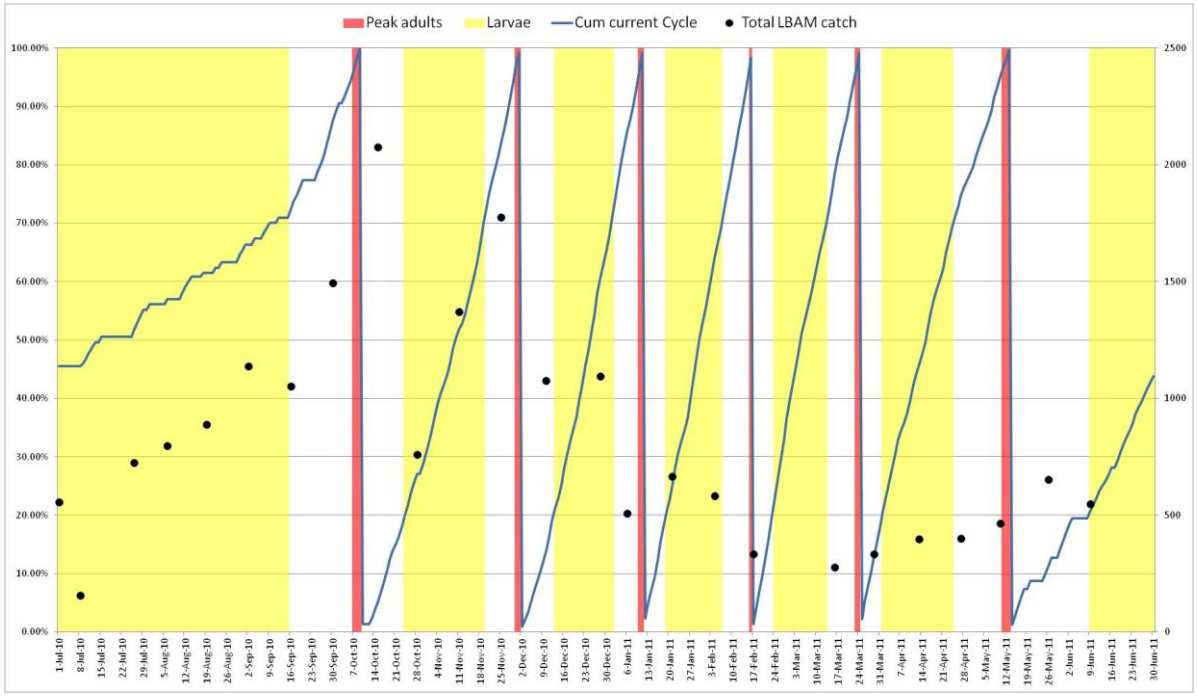


Figure 7: Phenology model and phenology trapping for *Epiphyas postvittana* in Perth 1 July 2010 – 30 June 2011. The red bars indicate the peak timing of the adult stage for each generation. The yellow bars peak larval stage for each

generation. The blue line indicates the cumulative development of each generation (0%-egg stage and 100%- adult stage). Overlaid are the trapping data from the male LBAM traps from Perth (black circles ●).

The model starts with the assumption that at 1 July 50% of the larval development is completed in that average population. After the first two generations had been completed the following four generations peaks were difficult to observe from the phenology trapping data for the male moths. There is likely to be generation overlap, with potentially all stages being present at any time. This asynchrony could be due to variation in the development time of individual moths (from variation within individuals or host plant quality) and the lack of any synchronisation event.

Mobile mating disruption

In 2009 and 2010 Perth trials demonstrated that sterile Mediterranean fruit flies treated with LBAM sex pheromone could disrupt communication in male moths. Medflies topically dosed with moth pheromone (*E*)-11-tetradecenyl acetate showed a no observed effect level (NOEL) of ~10 µg fly^{-1}, with increasing toxicity from 30 to 100 µg fly^{-1}. Greater potency and longevity of attraction and lower mortality were achieved using microencapsulated pheromone. No male moths were captured in traps baited with synthetic lures for one day after release of 1000 pheromone-treated Medflies ha^{-1} in treated 4 ha plots in suburban Perth, Australia. Percentage disruption of delta traps baited with single virgin female moths and also those baited with synthetic pheromone lures on the first four nights after release of about 3000 pheromone-treated Medflies ha^{-1} was 95, 91, 82 and 85%. Disruption of moth catch using pheromone-treated Medflies is a novel development that, with future improvement, might provide a socially acceptable approach for application of the insect mating disruption technique to control invasive insects in urban environments. Adequacy of payload, mass application technology, pheromone purity and cost are issues that require resolution before the technique advances.

SPLAT™ trials in vineyards in SA

Recent advances in the formulation of mating disruption (MD) and lure and kill (L&K) products have resulted in a new range of proprietary products which are well suited to rapid, mechanized, large-scale application from ground or air. As part of Project 40136 three field trials were conducted between 2010-12 to assess the potential of MD and L&K technologies for the suppression and eradication of light brown apple moth (LBAM). In this study the feasibility of developing MD or L&K as a cost-effective tactic to be used in an integrated program with other compatible technologies to eradicate the leaf-roller pest LBAM was investigated in a series of field experiments.

The aim of the first experiment (March-May 2010) was to test the relative efficacy of novel MD and L&K formulations of the LBAM pheromone produced by ISCA Technologies (SPLAT™ HD) against a commercial standard MD treatment (Isomate® LBAM twist ties). The second experiment (November 2010 – February 2011) aimed to determine the optimum dose rate of the product selected as the best performer in the first experiment (the MD product SPLAT™ HD LBAM). The aim of the third experiment (November 2011 – May 2012) was to assess the potential of a dual-tactic program of a selective foliar-applied insecticide

(methoxyfenozide) and SPLAT^{TM} HD LBAM (applied at the chosen 625 g ha^{-1} 'optimal' rate) to eradicate a localized population of LBAM. In addition to these three main experiments, a fourth experiment was conducted in April 2010 to assess the comparative trapping performance of SPLAT^{TM} MD dollops, SPLAT^{TM} L&K dollops, LBAM virgin females and 3 mg LBAM pheromone septa mounted in red delta traps.

The three field trials were conducted in the extensive and largely homogeneous vineyard plantings of Pernod Ricard Australia (Orlando) at Langhorne Creek, South Australia (35^{O} 17' 48'' S, 139^{O} 01' 02''). The site selection was based on a comparative assessment of LBAM activity at Orlando properties in the Barossa Valley and Langhorne Creek using pheromone traps which were checked weekly to fortnightly from August 2009 to January 2010 inclusive, and the relative homogeneity of the two vineyard properties. Given that similar mean numbers of LBAM moths per trap were recorded at both sites, the Langhorne Creek property was chosen over the Barossa site because it was the more homogeneous of the two vineyard landscapes with less trees and topographic undulation.

Pheromone traps were used to measure the activity of LBAM male moths at the experimental sites prior to the placement of the MD or L&K treatments. These trap data were used to help with site and plot selection. Following treatment application the relative differences in the pheromone trap catch of moths in the different treatment plots was used to measure the degree of male disorientation caused by each treatment.

Red delta pheromone traps and red rubber septa loaded with 3 mg of a binary blend of the LBAM pheromone components (95% (*E*)-11-tetradecenyl acetate (*E*11-14ac) and 5% (*E*)-9, (*E*)-11-tetradecadienyl acetate (*E* 9*E*11-14ac) were sourced from Etec Crop Solutions Limited, Auckland, New Zealand. Each trap was fitted with a septum and suspended on cordon wires in the vineyard at approximately 1.0 m above ground level (Figure 8). The traps were fitted with new septa after every 12 weeks of field monitoring. The pheromone traps were checked weekly, cleaned, and the number of LBAM moths recorded.

Figure 8: Red delta pheromone trap in Langhorne Creek vineyard.

In Experiment 1 (7 and 10 weeks pre- and post-treatment respectively) and in the 10-week post-treatment period of Experiment 2 seven pheromone traps were placed in each plot evenly spaced (approximately 16.7 m apart) along a central 100m row transecting the 1.0 ha plot area. During the pre-treatment assessment period in Experiment 2 two pheromone traps were placed 16.7 m distant either side of the centre point of the central row in each plot.

In Experiment 3 nine pheromone traps were placed centrally in each 9.0 ha plot in a 3 x 3 grid pattern. Three traps were placed on each of rows 50, 60 and 70 of the 120-row plots (2.5 m row spacing) and within each of these rows positioned on panels 20, 30 and 40 of the 56-panel rows (each panel 5.4 m length). The traps were assessed and serviced weekly for seven weeks prior to the insecticide treatment application (i.e. 9 September to 3 November 2011), three weeks between the insecticide application and the first of the SPLAT^{TM} applications, 10 weeks between the first and second SPLAT^{TM} applications (i.e. 30 November 2011 to 9 February 2012) and ten weeks following the second SPLAT^{TM} application (i.e 16 February to 26 April 2012).

Virgin female traps were used in Experiments 1 and 3 to measure whether wild male LBAM moths were able to orientate to and mate with the trapped females after the application of the MD treatments.

In Experiment 1 the virgin female traps were red delta pheromone traps (minus the septa and sticky base) which were each fitted with a 70 ml plastic vial (55 mm length and 40 mm diameter with the base and lid replaced with gauze mesh) which was weekly provisioned with a water source (20 ml vial fitted with a dental wick) and three one-day old virgin females. In the pre-treatment period there were two traps per plot, placed 8.4m either side of the mid-point of the sixth row N of the central row of each plot. Post-treatment an additional three virgin female traps were placed at the centre point and 8.4m either side of the mid-point of the sixth row S of the central row of each plot. The virgin female traps were checked twice weekly and females replaced where specimens had died. The traps were run for a total of five weeks pre-treatment and seven weeks post-treatment. The number of males trapped in these cages was recorded weekly.

In Experiment 3 the virgin female traps were cylindrical; approximately 180 mm length and 100 mm diameter, and each constructed from a plastic PET bottle fitted with tapered fly-wire mesh funnel ends with a 7 mm aperture for the males to enter (Figure 9). Each trap was weekly provisioned with a water source (20 ml vial fitted with a dental wick) and a single one-day old virgin female. Four traps were placed centrally in each 9.0 ha plot in a 2 x 2 grid pattern; specifically traps were placed on panels 25 and 35 on each of rows 55 and 65. The traps were run for a total of 16 weeks post-treatment. The caged females were retrieved weekly and dissected to determine their mating status.

Figure 9: A virgin female trap based on a Plant & Food design used in Experiment 3.

Trial Approvals

Because each of the experiments involved the importation and field application of unregistered pest control products (the SPLAT™ MD and L&K formulations), it was necessary to acquire APVMA Research Permit and APVMA Consent to Import approvals for these products for each experiment and we thank APVMA for their prompt attention and approval. The research team wish to acknowledge ISCA Technologies (particularly Agenor Mafra-Neto, Lyndsie Stoltman and Brett Roble) for their technical advice and generous support through free or discounted supply of SPLAT™ product, Pernod Ricard Australia (Orlando) and their site managers Brian Wyatt and Randall Pitt for their generosity and management flexibility in providing access to extensive vineyard planting over the three years of the project and their assistance with the Prodigy™ application, and Dow AgroSciences (Paul Downard in particular) for generously providing a free 15 L sample of Prodigy™ to spray the 54 ha area of the third experiment

Experimental Design

Experiment 1: Relative efficacy of SPLAT™ MD, SPLAT™ L&K and ISOMATE

A five replicate experiment was planned, but because ISCA Technologies shipped a lesser quantity of the two SPLAT™ formulations than had been requested, the experiment had to be reduced to a four replicate design.

A randomized-block design was used to test the following treatments:

1. Untreated control
2. Isomate™ LBAM Plus Pheromone - registered rate of 500 twist dispensers ha^{-1} tied to panel wires. (This treatment equates to 81.6g of *E*-11-teradecen-1–yl acetate and 3.4g of *E*,*E*-9-11-tetradecien-1-yl-acetate being applied ha^{-1}.)
3. SPLAT™ HD LBAM (MD treatment) applied as 1.0 g dollops to 740 panel posts ha^{-1}. (This treatment equates to 70.3g of *E*-11-teradecen-1–yl acetate and 3.7g of *E*,*E*-9-11-tetradecien-1-yl-acetate being applied ha^{-1}.)
4. SPLAT™ HD LBAM plus 5% permethrin (L&K treatment) applied as 1.0 g dollops to 740 panel posts ha^{-1}. (This treatment equates to approximately 66.8g of *E*-11-teradecen-1–yl acetate and 3.5g of *E*,*E*-9-11-tetradecien-1-yl-acetate being applied ha^{-1}.)

Twenty square 1.0 ha plots containing 40 rows (2.5 m row spacing) were marked out in early February 2010 across an approximately 32 ha area of Chardonnay at Pernod Ricard Australia's Langhorne Creek vineyard so that none of the plots had a contiguous face with one another.

The pre-treatment pheromone trap count data for the 20 plots were used to eliminate four 'outlier' plots which had trap counts either substantially higher or lower than the other plots, and to then allocate the 16 selected plots into four replicate blocks. The treatments were then allocated randomly within each block.

The pheromone treatments were applied on 22-23 March 2010 (with the assistance of WA, NZ, VIC and US colleagues) as a single dollop (Figure 10) at a height of ~1.5 m to each panel post (n=740) in each treatment plot. Electric cordless grease guns supplied by ISCA Technologies were used to dispense the SPLAT™ dollops. However these grease guns did not work reliably and resulted in

much 'down time' on maintenance whilst applying the dollops, hence in Experiments 2 and 3 were replaced with plastic syringes.

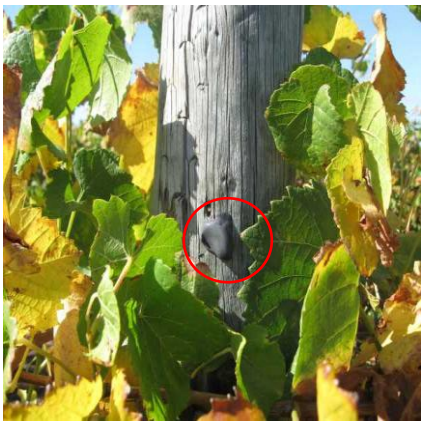


Figure 10: An approximately 1.0 g dollop of SPLAT^{TM} applied to a vineyard panel post.

Experiment 2: Rate comparison with the MD formulation of SPLAT^{TM} HD LBAM

Twenty eight 1.0 ha plots were marked out on 15 September 2010 in blocks of Chardonnay and Cabernet Sauvignon vines at Orlando's, Langhorne Creek. The two pheromone traps in each plot were checked and the catch of LBAM male moths scored weekly for eight weeks, and then the 15 plots for which the cumulative catch was most similar and relatively high were chosen, allocated to each of three replicates (reps 1-3) based on spatial proximity, and within each replicate the five treatments were randomly allocated. Traps were similarly placed in a further 16 1.0 ha plots on 14 November 2010, checked weekly for four weeks, and then 10 plots similarly chosen and allocated to another two replicates (reps 4 and 5) and treatments again randomly allocated within each replicate block.

The randomized-block design was used to test four rate treatments (100, 225, 400 and 625 g ha^{-1}) of SPLAT^{TM} HD LBAM (MD treatment) and an untreated control. The SPLAT^{TM} dollops were applied as ~1.0 g dollops to panel posts at approximately 1.5 m height using 50 ml plastic syringes. The four SPLAT^{TM} rate treatments were applied in the following pattern:

1. 100 dollops ha^{-1} applied in every 4th row to every 2nd post,
2. 225 dollops ha^{-1} applied in every 2nd row to every 2nd post, and an additional 25 random dollops applied across each plot,
3. 400 dollops ha^{-1} applied in every 2nd row to every post, and
4. 625 dollops ha^{-1} applied to 15 posts in every row in a 'treat 3 posts, leave 1 untreated' repeat sequence, and an additional 25 random dollops applied across each plot.

Although there was some variability in the size of dollop applied, the use of the syringes allowed the human applicator to regulate the cumulative amount of SPLAT^{TM} being applied to a plot, and hence the total quantity of SPLAT^{TM} applied corresponded closely to the intended 100, 225, 400 and 625 g treatment doses.

The treatments were applied on 22-23 November 2010 to reps 1-3 and on 13 December 2010 to reps 4-5.

Experiment 3: Localized Eradication of LBAM using a Dual-Tactic (MD plus Insecticide) Approach

Experiment 3 had three treatments in a three replicate, randomized block design:

1. A single spray of the ecdysone receptor agonist Group 18 insecticide Prodigy® (240 g methoxyfenoxide L^{-1}) applied on 7-8 November 2011 (to coincide with LBAM egg hatch and young larval development) at the registered rate of 250 ml product in 1000L water ha^{-1}.
2. The Prodigy® application as per Treatment 1 plus SPLAT™ LBAM HD applied manually using 50 ml plastic syringes in a uniform pattern of 625 x 1.0g dollops ha^{-1} on two occasions 10 weeks apart (28-29 November 2011 and 13-15 February 2012).
3. Untreated control.

The plot size for this experiment was increased from the 1.0 ha size used in Experiments 1 and 2 to a size of 9 ha (300m x 300m), which was considered the minimum size necessary to prevent significant ingress by LBAM moths into the central plot area.

Experiment 4: Comparative Trap Performance

A 4x4 Latin square design with 30 m spacing between each trap was used in April-May 2010 at the Orlando Langhorne Creek vineyard to test the trapping performance of delta traps baited with either:

1. 3mg LBAM septa supplied by NZ Plant&Food Research,

2. 1.0 g dollop of SPLAT™ HD LBAM (MD),

3. 1.0 g dollop of SPLAT™ HD LBAM plus 5.0% permethrin (L&K), and

4. Five virgin female LBAM.

Data Analysis

The trap data for Trials 1-4 were log-transformed to stabilize the variance and analysed by ANOVA using Genstat. Percentage trap shutdown (i.e., suppression of trap catch) was calculated as: 100-((catch in treated traps/catch in control traps)*100).

Results

Experiment 1

This experiment had been designed so that the dosage of active pheromone constituents was reasonably similar between the three pheromone treatments. However, when the weight of SPLAT™ product that had been applied was calculated following application, it was found that rather than the intended dollop size of 1.0 g we had under-dosed and applied a mean weight of 0.84 g per dollop.

As a result of this under-dosing the actual application rate of the primary active ingredient in the SPLAT™ MD and L&K products was respectively about 72.2% and 68.8% of the rate in the standard Isomate™ treatment, and the secondary active ingredient in the SPLAT™ MD and L&K products was respectively about 91.2% and 85.3% of the rate in the standard Isomate™ treatment (Table 2).

Table 2: The proposed versus the actual application rate of the pheromone treatments in Experiment 1.

Treatment	**Proposed application rate (ha^{-1})**	**Actual application rate (ha^{-1})**
Isomate LBAM Plus Pheromone™	500 ties (81.6 g *E*-11[†] & 3.4 g *E*,*E*-9-11[†])	As proposed
SPLAT™ HD LBAM (MD treatment)	740 x 1.0 g dollops (70.3 g *E*-11 & 3.7 g *E*,*E*-9-11)	740 x ~0.84 g dollops (~58.9 g *E*-11 & ~3.1 g *E*,*E*-9-11)
SPLAT™ HD LBAM plus 5.0% permethrin (L&K treatment)	740 x 1.0 g dollops (66.8 g *E*-11 & 3.5 g *E*,*E*-9-11)	740 x ~0.84 g dollops (~56.1 g *E*-11 & ~2.9 g *E*,*E*-9-11)

[†]*E*-11= *E*-11-Tetradecen-1-yl acetate; *E*,*E*-9-11= *E*,*E*-9-11-Tetradecadien-1-yl acetate.

The seasonal activity of LBAM moths varied substantially throughout the nine months that pheromone traps were monitored at the Langhorne Creek vineyard experimental site in 2009-10. The mean number of male LBAM moths trapped per pheromone trap per day in the control plots peaked at 5.0 in mid-November 2009 (the highest density recorded throughout the 3-year study), and then progressively declined during the summer to very low numbers by the beginning of March 2010 (Figure 11). Following the application of the treatments on 22-23 March 2010 the numbers of trapped moths in the control plots steadily increased to reach about 3.0 per trap per week in late April to early May 2010.

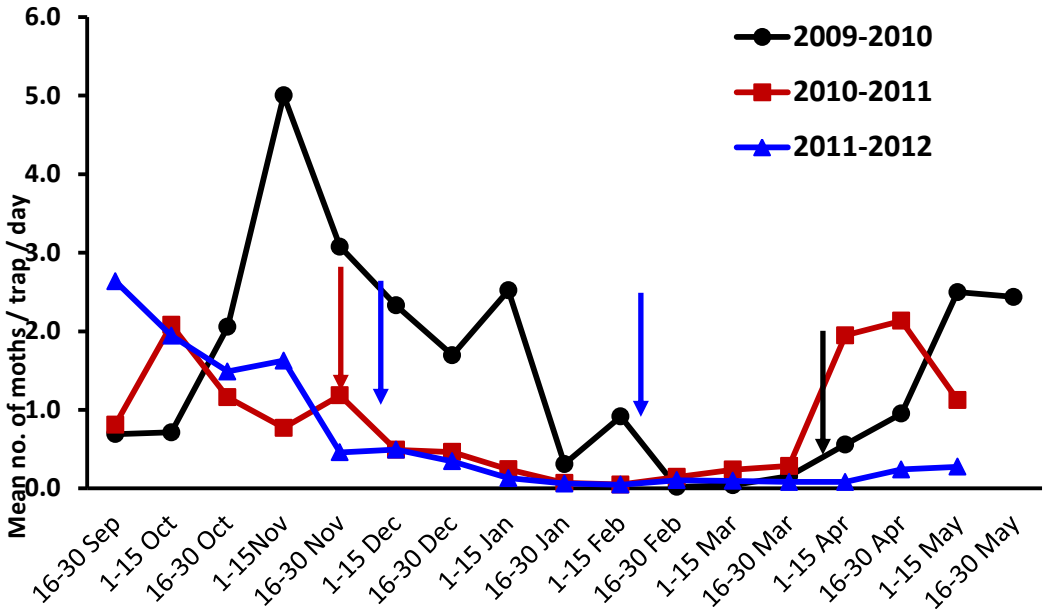


Figure 11: The mean number of male LBAM moths trapped per pheromone trap per day in the untreated plots at the Langhorne Creek vineyard experimental site, September-May in the three years of this study (2009-10, 2010-11 and 2011-12. (Arrows indicate the time of application of the pheromone treatments in each year.)

Following the application of the pheromone treatments on 22-23 March 2010, the relative attractiveness of the virgin female traps compared to the red delta pheromone traps was observed to significantly decline. The reason(s) for this are unclear, but may have been related to the onset of lower ambient air temperatures in April. Despite the decline in the overall numbers of male moths trapped, the virgin female trap catch in the seven weeks following the application of the three pheromone treatments demonstrated a significant trap shutdown as a result of each of the three treatments (Figure 12).

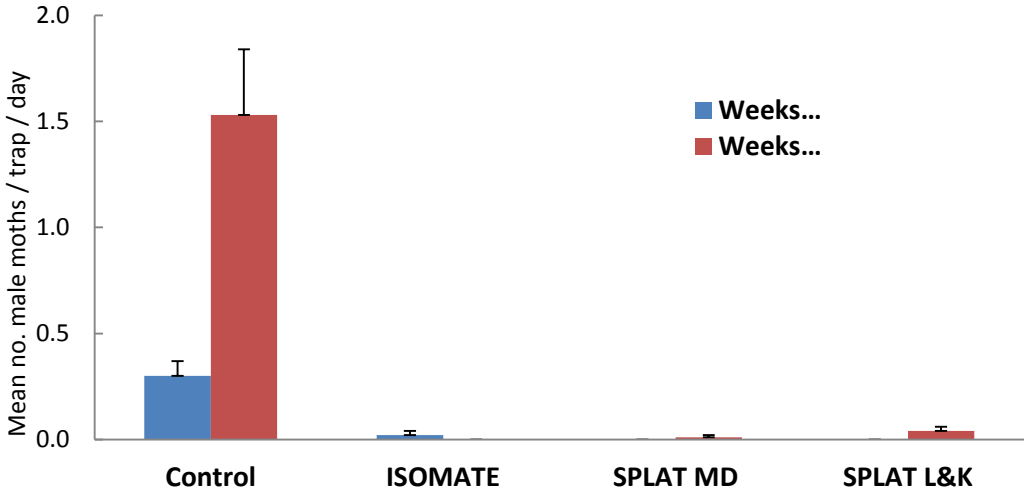


Figure 12: The mean number of male LBAM moths trapped per virgin female trap per day during weeks 1-3 and 4-7 following the application of the pheromone treatments on 22-23 March 2010 (bars indicate sem for each mean value).

Turning to the pheromone trap results, the analysis of the full data-set demonstrated that all three pheromone treatments significantly suppressed the pheromone trap catch (*Fprob*.<0.001) compared to the untreated control, but there was no difference between the three pheromone treatments (Figure 13).

Not unexpectedly, there was a strong trap position effect, with the two pheromone traps that were positioned on the ends of the trapping transect at the plot perimeter in each plot (Traps 1 and 7) trapping significantly higher numbers of moths (*Fprob*.<0.001). This perimeter effect is evident in Figure 13.

With the omission of the control data-set from the analysis, there was still no significant difference between the three pheromone treatments (*Fprob*.=0.277). However, with the omission of the control and the Trap 1 and 7 data, the Isomate™ treatment was shown to provide significantly greater (*Fprob*.=0.045) suppression of the trap catch (mean of 3.6 moths/trap for the 10 week observation time) compared to the SPLAT™ MD (mean of 7.6 moths/trap) and SPLAT™ L&K (mean of 9.1 moths/trap) treatments(Figure 14). However, as previously explained below, there was substantially more pheromone dispensed per ha (approximately 1.42 and 1.13 times the quantity of the *E*-11 and *E*,*E*-9-11 actives respectively) in the Isomate™ treatment plots compared with the SPLAT™ MD and SPLAT™ L&K treatments respectively.

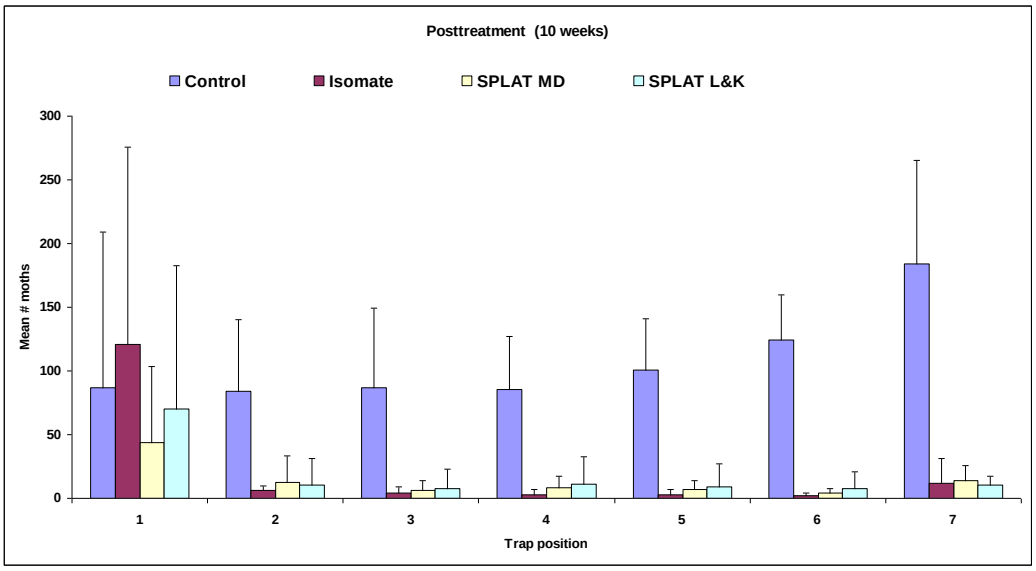


Figure 13: The mean number of moths per trap for trap positions 1-7 for the post-treatment (10 week) trapping period in the control, Isomate^{TM}, SPLAT^{TM} MD and SPLAT^{TM} L&K treated plots (Bars indicate 95% CI for each mean value).

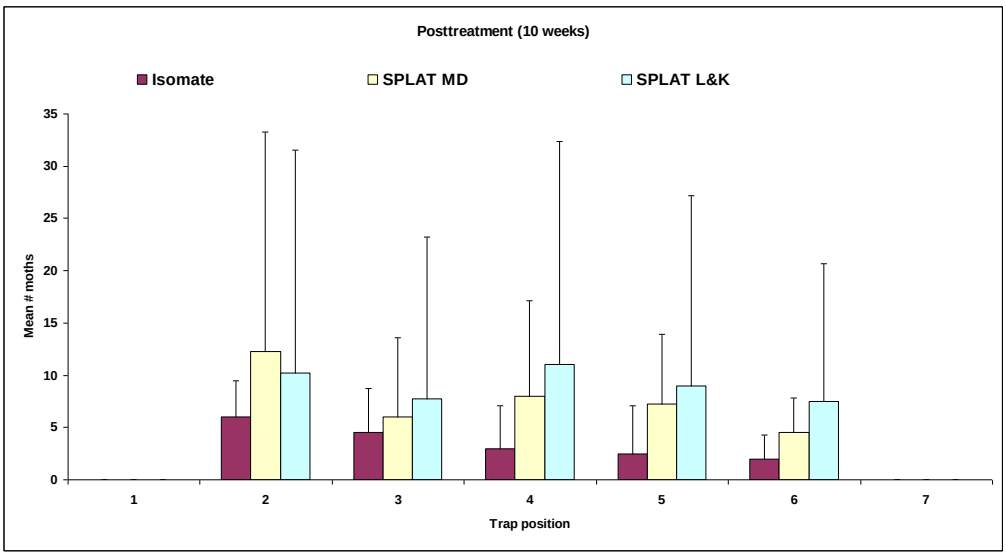


Figure 14: The mean number of moths per trap at trap positions 2-6 for the post-treatment (10 week) trapping period in the Isomate^{TM}, SPLAT^{TM} MD and SPLAT^{TM} L&K treated plots (Bars indicate 95% CI for each mean value).

Analysis of the effect of time on the treatments' suppression of pheromone trap catch revealed that there was no significant difference between the three pheromone treatments for the first three weeks, but for weeks 4-6 and 7-10 the Isomate was significantly better than each of the SPLAT^{TM} treatments (Table 3).

Table 3: Transformed mean numbers of moths per pheromone trap in each of three post-treatment periods.

Weeks post-treatment	Weeks 1-2-3	Weeks 4-5-6	Weeks 7-8-9-10
Isomate™	0.07Aa*	0.26Aa	1.23Ab
SPLAT™ MD	0.04Aa	0.74Bb	1.72Bc
SPLAT™ L&K	0.12Aa	0.75Bb	1.67Bc

*Mean in each row (upper case) and in each column (lower case) followed by different letters are significantly different ($P<0.05$, LSD test), data ln(x) transformed.

Discussion

As previously outlined, the quantity of LBAM pheromone applied in the SPLAT™ MD and L&K treatments was significantly less than the quantity applied in the twist-tie Isomate™ treatment; the rate of the primary active pheromone component was in fact about 30% lower in the SPLAT™ treatments. Despite the lesser pheromone dosing of the SPLAT™ treatments, the results of this experiment demonstrated a similar level of trap shutdown by SPLAT™ HD LBAM, either as a MD or L&K formulation, compared to Isomate in the first three weeks post-treatment. In the subsequent seven week assessment period the Isomate provided significantly greater trap shutdown. However at equivalent initial application rates, a comparable level of trap shutdown would be expected from all three treatment products.

The capacity to mechanize the ground and aerial application of SPLAT™ means that the treatment of extensive areas of LBAM host crop and surrounds using this technology is feasible at potentially lesser cost, treatment time and dependence on local landscape features compared to the standard twist-tie technology. These features make SPLAT™ HD LBAM a potentially superior technology for large scale mating disruption of an emergency plant pest incursion, which could be deployed either alone or in combination with other compatible tactics.

Given the relatively successful and similar performance of both SPLAT™ formulations in this first vineyard field experiment, the project team decided at a June 2010 planning meeting to conduct a dosage rate field experiment with the SPLAT™ HD LBAM MD formulation (rather than the L&K formulation), because the capacity to use a formulation without an insecticidal toxicant would have broader acceptance by both industry and public stakeholders.

Experiment 2

In the 2010-11 season the activity of LBAM moths peaked at a mean of 2.1 LBAM moths trapped per pheromone trap per day in the control plots in mid October 2010, then steadily declined to very low numbers during the summer period of the SPLAT™ experiment, and again increased to a peak of 2.1 moths per trap per day in late April 2011 (Fig. 4).

As described in the Methods, the dates of application of the SPLAT™ treatments differed between replicates 1-3 (22-23 November 2010) and replicates 4-5 (13 December 2010). Further, ten of the trial plots (replicates 1-3 of treatments 1 (100 dollops ha^{-1}) and 2 (225 dollops ha^{-1}), replicate 1 of treatments 3 (400 dollops ha^{-1}) and 4 (625 dollops ha^{-1}) and replicates 1 and 2 of treatment 5 (untreated control) were inadvertently sprayed with insecticide (Avatar™ containing 300 g kg^{-1} of indoxacarb) on December 24 by Pernod Ricard Australia field staff. This spray treatment reduced the moth catch in these plots, and confounded the data set.

With and without the inclusion of the counts for the 10 sprayed plots there was a very well-defined response curve to the rate of SPLAT™ applied as measured by the numbers of male moths trapped in the pheromone trap grid (Figure 15). The level of trap shutdown achieved by the four SPLAT™ rates, measured as the percentage disruption relative to the controls, ranged from 55.6% at the 100 g ai ha^{-1} rate to 93.3% at the 625 g ai ha^{-1} rate (Table 4). These results demonstrate that a substantial disruption of male moth catch can be achieved by the 625 g ai ha^{-1} rate of SPLAT™, and that deployment at this rate has potential as a combination tactic for EPP eradication. The lower rates tested had lesser disruptive effect, but around the 225 to 400 g ai ha^{-1} rate may be a cost-effective pest management tool as an alternative to insecticidal control of LBAM.

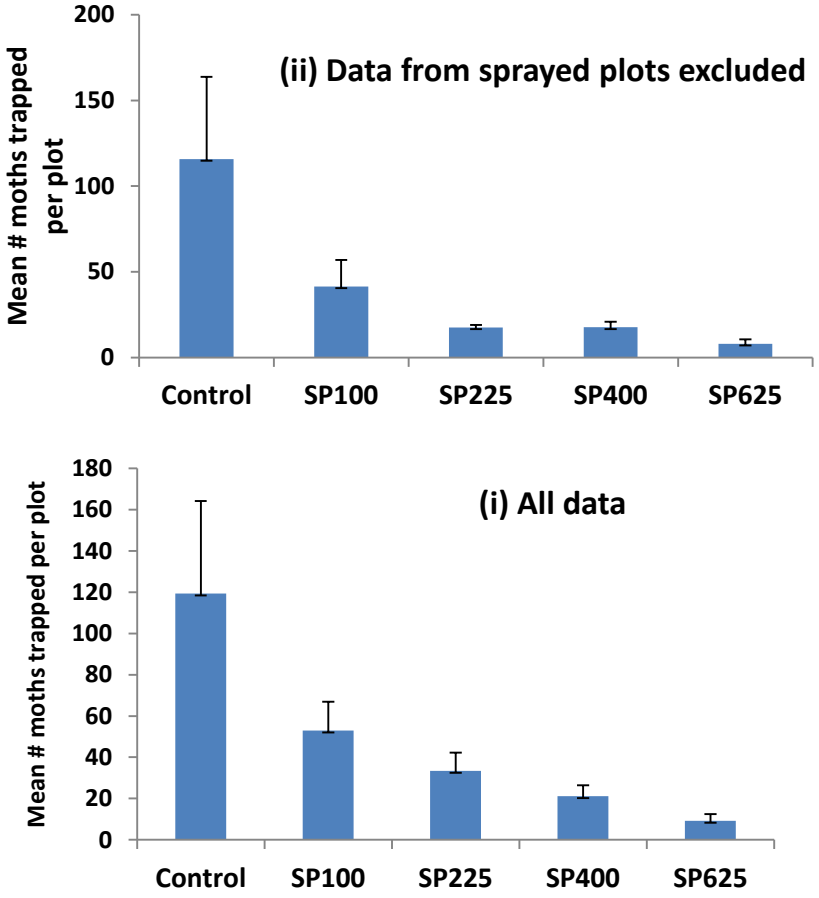


Figure 15: The mean number of moths trapped per plot for the ten weeks following the SPLAT™ treatment application, for (i) all plots and (ii) excluding the

Avatar™-sprayed plots (bars indicate sem for each mean value), 2nd experiment, November 2010 – February 2011.

Table 4: The percentage disruption of LBAM pheromone trap catches for the 10 weeks following the application of four different rate treatments of SPLAT™, 2nd experiment, November 2010 – February 2011.

SPLAT™ treatment (g ai ha^{-1})	% Disruption
100	55.6
225	72.0
400	82.2
625	93.3

Experiment 3

Eradication of a Perennial Crop Pest (Light Brown Apple Moth, LBAM) using Combination Insecticide and Mating Disruption (MD) Technologies

In the 2011-12 season the activity of LBAM moths peaked at a mean of 2.6 LBAM moths trapped per pheromone trap per day in the control plots in mid-late September 2011 and then steadily declined to low numbers during most of the summer to autumn period of the SPLAT™ experiment.

Virgin female trap data

At the time of reporting we had 17 weeks of post-treatment catch data for the virgin female traps. The number of mated females in the traps from the control, Prodigy™ alone and Prodigy™ + SPLAT™ treated plots were 19, 10 and 2 respectively for the full 17 week period. In the first five weeks following the application of each of the SPLAT™ treatments no mated females were found in the traps in the SPLAT™-treated plots (compared to 11 and 3 in the control and Prodigy™ alone plots). Albeit no analysis has been undertaken on these data, the effect of the Prodigy™ alone and Prodigy™ + SPLAT™ treatments on the proportional reduction in the number of mated females broadly corresponds to their observed effect on the reduction of male LBAM trapped in the pheromone traps.

Pheromone trap data

There were no significant pre-treatment differences between the mean numbers of LBAM captured in the pheromone traps positioned in the plots allocated to the control, Prodigy™ alone and Prodigy™ + SPLAT™ treatments.

Consistent with the mode of action of methoxyfenozide, the Prodigy™ spray treatment had no effect on the numbers of LBAM moths trapped in the initial fortnight (10-24 November 2011) following application. However in the 10 week trapping period following the application of the first SPLAT™ treatment (1 December 2011 – 9 February 2012), there was a significant reduction, compared to the control, in the trap catch in the plots treated with Prodigy™ alone (Figure 16). In the subsequent nine week trapping period following the application of the second SPLAT™ treatment (16 February 2012 – 19 April 2012), the residual suppression of the trap catch in the plots treated with Prodigy™ alone was not significant compared to the untreated control.

In both the 10 week trapping period following the application of the first SPLAT™ treatment and the nine week trapping period following the application of the second SPLAT™ treatment there was a significant reduction in the moth catch in the Prodigy™ + SPLAT™ treated plots relative to the untreated plots (Figure 16).

The percentage disruption to the moth catch relative to the controls achieved by the Prodigy™ + SPLAT™ treatment ranged from 73.3% to 100% during the first nine weeks following the 1st and 2nd SPLAT™ applications (Table 5). For 17 of these 18 trapping occasions the percentage disruption was equal to or greater than 93.0%, and for 11 (61.1%) of these trapping occasions there was nil catch of LBAM in the Prodigy™ + SPLAT™ treated plots. In the eighth week following the 2nd SPLAT™ application, when the sudden reduction (73.3%) in disruption level was recorded, two days of extremely high winds occurred. It is possible that the trapping of the eight moths in the Prodigy™ + SPLAT™ treated plots in this eight week was a result of the abnormal wind event. The observed recovery of the disruption level to 93.4% in the following (ninth) week is more consistent with a wind effect than a premature decline in the disruptive effect of the SPLAT™ treatment.

At the time of submitting this report trap monitoring was still underway, and a full analysis of the data has not been conducted. However, the 'combination' treatment of the registered rate of the insecticide Prodigy™ followed by the MD treatment with SPLAT™ LBAM HD has, appears to have provided a very substantial suppression of LBAM adult activity as measured by pheromone trapping. In addition, the female virgin trap data has demonstrated a similar treatment effect in the degree of reduction of male moths captured thus far.

The duration and level of trap suppression (or disruption) observed in this experiment appears similar to that observed in the two earlier experiments. SPLAT™ HD LBAM applied at a rate of around 625 g ai ha^{-1} as approximately 1.0 g dollops appears to provide effective trap suppression (and likely mating disruption) for about 10 weeks and then this effect appears to significantly declines.

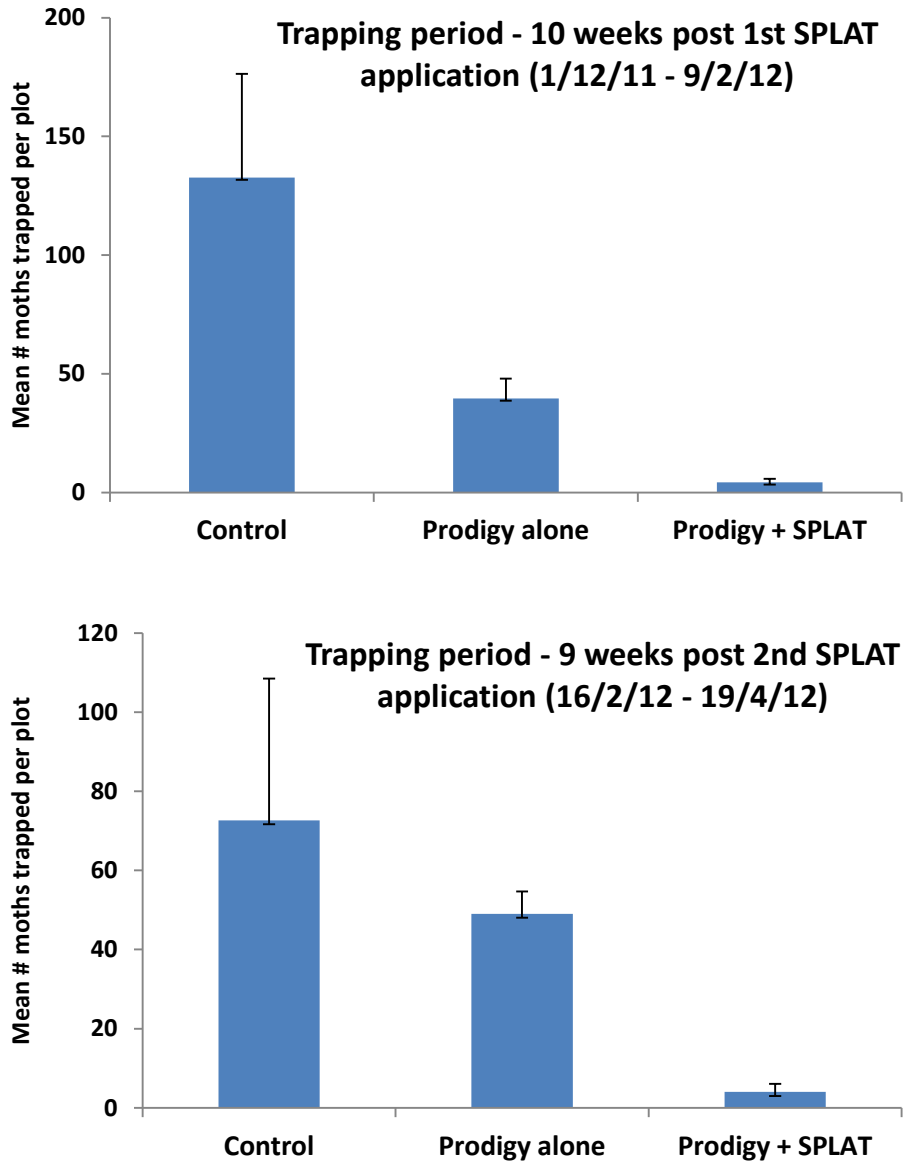


Figure 16: The mean number of LBAM male moths trapped per plot in pheromone traps (placed in the plot centres) in the first ten weeks following the first SPLAT™ application and in the first nine weeks following the second SPLAT™ application (bars indicate sem for each mean value), 3rd experiment, November 2011-May 2012.

Table 5: The percentage disruption of LBAM pheromone trap catches at weekly intervals for the first nine and 10 weeks following the application of the 1st and 2nd SPLAT™ treatments respectively in the Prodigy™ + SPLAT™ treatment, 3rd experiment, November 2011-May 2012.

Weeks post SPLAT™ treatment application	% Disruption	
	1st SPLAT™ application	2nd SPLAT™ application
1	97.4	100
2	98.7	100
3	93.0	100
4	97.7	100
5	100	100
6	100	100
7	100	100
8	100	73.3
9	94.7	93.4
10	-*	61.5

* no moths were trapped in the control plots

Experiment 4

Over the eight week assessment period of this experiment (April-May 2010), which was designed to measure the relative trap performance of four different LBAM attractant sources, a significantly higher (*F. prob.*=0.0074, on ln (x) transformed data) number of male LBAM moths were caught in the traps baited with the 3 mg pheromone septa (mean of 91.3 per trap) compared to those baited with the SPLAT™ MD (mean of 10.0 per trap), SPLAT™ L&K (mean of 16.5 per trap) and virgin females (mean of 13.3 per trap). However there were no significant differences in moth numbers caught in the SPLAT™ MD, SPLAT™ L&K and the virgin female traps. This trial demonstrated a considerable attractant superiority of the 3 mg pheromone septa over the other three tested sources.

The potential of the SPLAT™ LBAM HD formulation (ISCA Technologies, Riverside) of LBAM synthetic sex pheromone for disruption of LBAM mating was demonstrated in three field trials conducted in vineyards at Langhorne Creek, S. Aust. in 2010-12 as part of project 40136. The first trial demonstrated that this product could provide similar levels of trap shutdown as the standard registered LBAM mating disruption twist-tie product(s) when applied at a comparable pheromone dosage per unit area. A second trial demonstrated a strong rate response, and indicated that to achieve the level of pest suppression necessary for eradication a rate of 625g SPLAT™ LBAM HD ha^{-1} or greater would be necessary.

In the third trial, which commenced in November 2011 and will be completed by the end of May 2012, the potential to suppress a field population of LBAM to localized extinction using SPLAT™ LBAM HD applied in combination with a foliar insecticide treatment is being assessed. If competitive attraction is the mechanism responsible for the mating disruption (MD) effect, then the success of MD as a pest management or eradication tactic will be dependent on the pest density, and likely to require deployment in combination with another tactic (e.g. insecticidal control) to ensure the pest population density is sufficiently low for MD to succeed.

Based on the trial results to date and the capacity to mechanize the application of SPLAT™, the treatment of extensive areas of LBAM host crop and surrounds using this technology is feasible at lesser cost, treatment time and dependence on local landscape features compared to the standard twist-tie technology. These features make SPLAT™ LBAM HD a potentially superior technology compared to existing pheromone products available in Australia for large scale mating disruption of an emergency plant pest incursion.

SPLAT™ trials in vineyards in NZ

The zero tolerance of live LBAM larvae in exports significantly raises the requirement for control in the field, although biologically-based pheromone control can be cost-effective to suppress populations. The two component LBAM pheromone has been developed in tools for biotechnical control of the pest, primarily through mating disruption. The new four component pheromone blend, patented by Plant and Food Research New Zealand, has demonstrated greater attraction to LBAM than the standard two component blend. However, the best formulation for control with the four part blend has not been determined.

Two new formulations for mating disruption targeting light brown apple moth (LBAM), *Epiphyas postvittana*, Organic SPLAT™ LBAM, and Hercon® Biotie (biodegradable) were field tested and compared against the standard Isomate® LBAM (500/ha), as a positive control, and an untreated (negative) control at four point source densities. Assessment involved trapping using synthetic lures and caged virgin female LBAM.

A total of 175,776 male LBAM moths were caught in traps baited with both the caged females and lures, from 10 February to 19 May 2011. LBAM catch decreased dramatically after the mating disruption treatments were placed out in the field on 8/9 March 2011.

There was a significant reduction in trap catch with an increasing number of points/ha ($P< 0.001$). The SPLAT™ and Biotie treatment performed equally well ($P = 0.317$) in the trial, as did all the mating disruption technologies (SPLAT™, Biotie and Isomate) at 500 points/ha during the length of this trial ($P = 0.738$).

Biotie dispensers were brittle and impractical for use as supplied, although they were disruptive to the insect. A flexible version (as proposed) would increase their practicality. The vineyard required SPLAT™ to be placed on the tops of posts because they did not want any of the formulation on the fruit or the plants.

The new sex pheromone blend for LBAM was identified from the female sex pheromone gland by El-Sayed et al. (2011). The blend contained two additional compounds to the current two component blend. Five potential blends were compared in a mating disruption trial: the current two-blend pheromone, new four-blend pheromone based on the female gland ratios, one blend containing extra of one of the minor components, one blend containing extra minor component plus an impurity and one blend containing the impurity and none of the expensive diene component. Pheromone blends were mixed in Organic SPLAT™ at 10% by weight and applied to posts in a vineyard at a rate of 500 points per hectare in a small plot (30 x 30 m) with two SPLAT™ dollops per point (183 mg of pheromone per point), and a large plot (80 x 80 m) with one SPLAT dollop per point (91.5 mg of pheromone per point).

The new four-component blend was always ranked first in mating disruption trials, disrupting moth finding behaviour by at least 90%. The percent disruption was similar between trials apart from that of blend 5 (without diene), which performed well in the 183 mg/point trial, ranking second, but was ranked fourth in the 91.5 mg/point trial. The addition of the impurity *Z*11-14Ac, which is present in low purity (thus cost) *E*11-14Ac, reduced disruption success probably because of the male moth's ability to distinguish between sex pheromone lures and disruption pheromone blends. However, the male's ability to distinguish

between the sex pheromone and the mating disruption blend appears to be impaired when *E*9,*E*11-14Ac is absent. A publication is in preparation.

Building on earlier work, this project is currently testing the ability of high purity (expensive) and low purity (33% cheaper) compounds in the current two-component and new four-component formulations for use in mating disruption of LBAM in SPLAT^{TM}. SPLAT^{TM} was applied at a rate of 322 points per hectare (91.5 mg of pheromone per point). Furthermore, as growers have raised concerns about pheromone products being applied to fruit, the pheromone was applied at ~30 cm above ground level, below mechanised fruit harvesting methods.

Mating disruption is being assessed in a vineyard (Feb 24- May 18 2012) by deploying stations with caged virgin females that males can access and leave, but females cannot leave. Seven stations each containing a virgin female moth were placed in a transect running down the central vineyard row within each of 30 one ha plots. All females (n = 210) were left out for a week to mate and then were dissected to determine whether a spermataphore is present (from the male) indicating successful mating. No spermataphore indicates no successful matings have taken place, but the opposite is true if one or more spermataphores are present. As the trial is still underway the data have not been analysed. However, female mating is being disrupted in all treatment plots compared to females in control plots.

Figure 17: Field site in North Canterbury, New Zealand

SPLAT™ trial in an urban environment in Western Australia

The location for the SPLAT™ trial was the leafy up market suburb of Dalkeith close to the Swan River. Before treatments were applied, pre-treatment trapping was undertaken from end July 2011 to end September 2011 to establish a baseline LBAM population within each of the nine treatment plots. Five delta traps baited with the same 3 mg sex pheromone lures were placed within each plot. There was no significant difference in moth populations between plots (Figure 18).

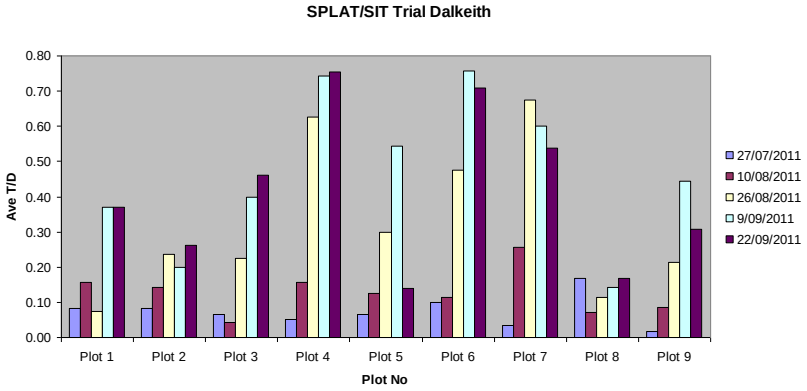


Figure 18: LBAM male population distribution over four weeks in nine plots prior to SPLAT™ application. Each plot had five traps baited with a 3 mg sex pheromone lure.

Organic SPLAT™ HD was applied in late September 2011 manually using 50 ml syringes to deliver dollops of 1 ml on front verge trees (Figure 19), fences, and power poles a least 1 m above ground level. As far as possible a uniform distribution of dollops and a dose rate of 500 mg/ha were achieved in each SPLAT™ -treated plot. Six plots (1 to 6) were treated with SPLAT™. Plots 1, 4 and 6 were earmarked for sterile moth release. The remaining 3 plots (7-9) stood as the Control. Plot size was approximately 2 ha each.

Figure 19: Applying SPLAT™ in Dalkeith

The LBAM population was monitored using nine traps per plot baited with 3 mg LBAM sex pheromone lures, checked at weekly intervals. In addition, from end September date to mid-December 6 LBAM virgin female traps were placed per plot to check the effectiveness of SPLAT™ in reducing mating (Figure 20). Virgin females were put out on Monday and removed on Friday. Each female was then dissected and successful mating with a wild male confirmed by the presence of one or more spermataphore. After mid-December, the use of virgin female moths in traps was discontinued due to high female mortality from the continuous hot temperatures prevailing during this period of the year and predation from ants. Trapping using virgin female moths recommenced from first week of April when weather conditions were more conducive to female survival. A total of 15 traps per treatment containing live virgin females were put out in the control and SPLAT™ plots.

Figure 20: Checking a virgin female trap

Average moth catch in the control and SPLAT™ plots prior to SPLAT™ application was 10 and 18.3 respectively on the week prior to SPLAT™ application. Post-application, this average continued to rise steadily in the control plots to reach a maximum average catch of 129 moths on week 9. Thereafter, the average catch started to drop following the same pattern observed for the past three years in the phenology traps during this period. In SPLAT™ treated plots, the average went down to 0.5 on week 2 post-application and then slowly started to increase to a maximum of 8.83 on week 13 from which it began to decline.

The downward trend in moth catch observed in both the control and SPLAT™ treated plots from the beginning of February, when peak summer conditions started to set in, continued and the average moth catch per trap at end of January was 0.3 and 16.7 in the SPLAT™ and Control plots respectively. The lowest average catch, 0.21 (SPLAT™) and 1.7 (Control), was attained in mid-April. It is interesting to note at the peak of the moth population in November the average catch was 2.2 and 129 respectively.

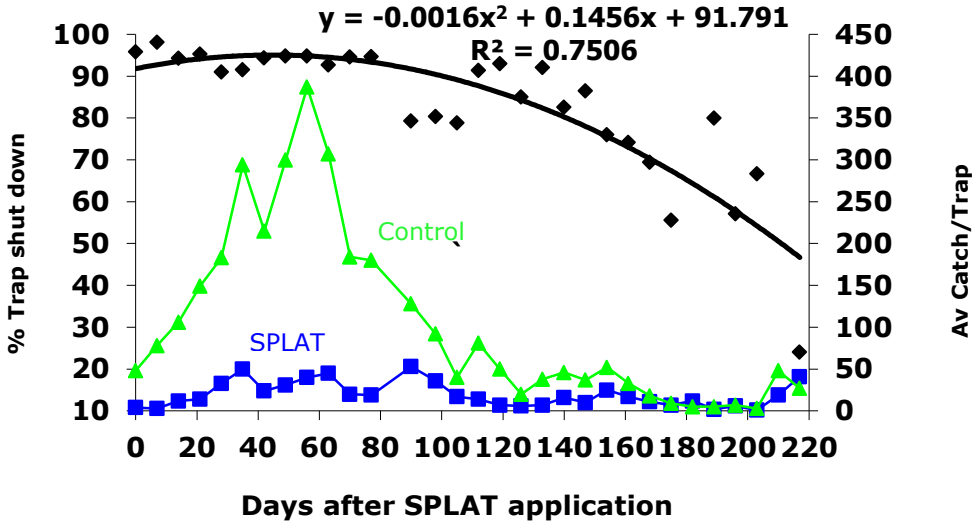


Figure 21: The number of LBAM male moths trapped in pheromone traps and % trap shut down in the first 25 weeks after SPLAT™ application on the 29th -30th September 2011

Average percentage trap shut down in the SPLAT™ plots peaked at 98% on week 2 (Figure 21), remained above 90% until week 12 week before showing a downward trend. The lowest percentage of trap shut down (55.6%) was reached around 175 days after SPLAT™ was applied. A polynomial regression of % shut down against days after application suggests that a 90% trap shut down was achievable for slightly more than 80 days.

Over the 12 weeks after application, only six female moths were found mated out of a total of 360 virgin female put out in all 6 SPLAT™ treated plots (1.6% mating). In the control plots, 66 females were found mated out of total of 180 females used in the control plots (36.7% mating). The first mated females in the SPLAT™ plots only occurred in week 8 demonstrating the efficacy of mating disruption (Figure 22).

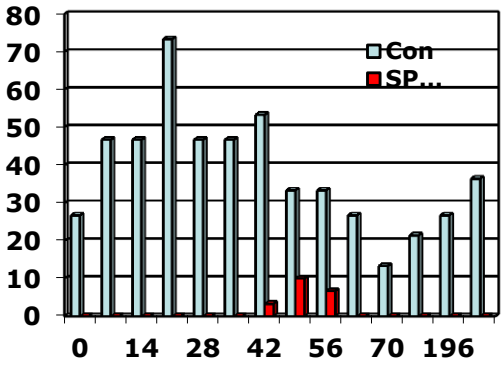


Figure 22: Percentage LBAM female moths in traps found mated in Control plots as compared to SPLAT™ treated plots after SPLAT™ application on the 29th -30th September 2011

After trapping using virgin females recommenced in the first week of April (week 27) female mortality was low. Out of 30 surviving females in the control plots 11

were found mated giving a 36.7% mating success. No females were found to be mated in the SPLAT™ plots.

These results using only perimeter treatments indicate that it may be possible to eradicate LBAM by using SPLAT™ treatment alone in an urban area if the area treated is large enough, multiple treatments are applied, and there is consistent application throughout the treated area. The use of different concurrent pheromone treatments e.g. puffers and SPLAT™ to obtain more effective coverage of an urban area and repeated applications needs to be investigated.

Sterile moth release

For effective sterile insect release in the field large numbers of moths need to be reared, collected, sterilised and released. Economies of scale can only be achieved by rearing large numbers of insects which can be costly. For these trials it was only possible to rear small numbers of moths to test the integration of SPLAT™ and the SIT.

Mr Woods visited mass rearing facilities in the USA and Canada before the rearing and release of sterile LBAM was attempted in Australia. The USDA facility at Moss Landing is a rented factory converted for LBAM mass rearing. Larvae are reared on Pink Bollworm diet trucked over weekly from the Pink Bollworm SIT facility in Arizona. The production target is 100,000 LBAM moths per week but despite the input of considerable resources to the program the maintenance of high levels of LBAM production is still proving a challenge.

The Codling Moth SIT program in Canada was built in 1993 at a cost of $7 million and produces 200 million sterile Codling moths per year at a cost of over 1 million per year. The facility is well designed with air conditioning and boilers in an easily accessed roof area. There is a system to remove moth scales from the building to prevent health concerns. Bulk diet ingredients are combined in large mixers in the roof and dispensed to the floor below.

Moths are released from 4WD motor bikes with a release machine mounted on the front. This comprises of a funnel, conveyor belt and fan mounted on to release the moths. Moths are carried in a small cooler in Petri dishes and are released as the motorbike drives down rows in the field. They aim for at least a 40:1 overflooding ratio of sterile to wild moths.

With fruit flies pupal irradiation is generally used as larvae leave the diet to pupate in vermiculite or sawdust. Pupae are then readily separated from the pupation medium enabling pupal dyeing, irradiation and long distance shipment. The problem in moth SIT is extracting pupae from the diet. Generally larvae will pupate within the diet and removal is difficult and may cause damage to them. In the case of LBAM this is complicated by the presence of webbing made by the larvae before pupation. Therefore moths are allowed to emerge from pupae in the media and are collected before irradiation. This is achieved by moving rearing trays in which moths are ready to emerge into darkened rooms or containers. A UV light attracts the moths to a collection system which, by means of vacuum and cyclonic extraction, collects and holds the moths in a cool room and separates them from the abrasive wing scales. The chilled moths can then be irradiated.

A rearing system based on these studies was built using an initial USDA design (Fig 23). It comprised of light proof metal boxes into which trays of diet were placed. Moths emerging from the diet flew to a fibre optic UV light source at the back of the cage where suction provided by a dust extractor transported them

through a flexible tube to a cyclonic extraction unit which separated the moths out so they fell into a container in a refrigerator where they were held for collection. This system has been used successfully for LBAM in the USA but time constraints and teething difficulties prevented us from using it for the current trials.

Figure 23: Collection system for moths emerging from diet.

Therefore for our trial pupae were removed from the diet medium by hand. This was tedious and time consuming. Moths were separated as pupae into sexes, permitted to emerge, and then irradiated at 250 Gy in a Gammacell 220 irradiator. Only males were irradiated and released and 1000 moths per plot were released in three of the SPLAT™ plots in week 6 and 7. Following the release no dyed moths were recaptured in the monitoring traps, probably because the pheromone disruption effect was still being very effective. To test moth survival and movement, sterile moths were instead released in control plots in weeks 8 to 11. Release of sterile moths resumed in May in both SPLAT™ and control plots, but results are not yet available

Figure 24: Releasing sterile LBAM in Dalkeith

No sterile moths were caught in the traps in the 3 SPLAT™ treated plots for the duration of release (two weeks). In the control plots a very low level of recapture and poor dispersal were observed. Most moths that were captured were within 10 metres of the release point and a few (two moths) at a maximum distance of 35

m. With mating disruption at its lowest since treatment sterile moth release recommenced in May.

Integration of SIT and SPLAT™

This trial has shown SPLAT™ is effective in inhibiting trap catch for a period of months. As it inhibits catch of sterile and well as wild males it is difficult to gauge the dispersal of sterile males in SPLAT™ plots although dispersal in control plots appears to be poor. There are no effective female traps that could be used to catch wild females and measure mating status. It appears that release of sterile males into SPLAT™ treated plots whilst SPLAT™ is still effective will inhibit mating of the sterile males and their effectiveness. Waiting until SPLAT™ becomes ineffective and then releasing is a valid approach especially if SPLAT™ goes from being very effective to not effective over a short period of time and these parameters are known**.**

Implications for stakeholders

The toolbox for eradication is being depleted as older technologies are retired. The use of mating disruption with new application technologies such as SPLAT™ combined with softer insecticides and SIT have to potential to replace some of these lost tools. However, these technologies need continued development to lower their cost and increase their effectiveness and reliability against moth pests. SIT is an expensive and research intensive approach and is only likely to be feasible for use in eradication if it is already being used against the exotic pest in its country of origin, although perceived public acceptance of this tool for eradication is high. Innovative techniques such as mobile mating disruption are a long way from real world usage and due to technical and cost considerations may never be adopted. Integration of pheromones with insecticides provides a solid base for eradication in the orchard and vineyard but there is still a need to develop new techniques to complement mating disruption in urban areas.

4. Recommendations

Transfer of technologies such as SPLAT™ for eradication of pests from other insect orders needs to be investigated. Improvement in eradication efficiency through the development of mechanised application technology and improved formulation is highly likely with further input. Further research is required to develop softer eradication technologies for use in urban areas that can be integrated with pheromones.

5. Abbreviations/glossary

ABBREVIATION	FULL TITLE
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
EPP	Emergency plant pest

6. Plain English website summary

CRC project no:	CRC40136 & CRC40187
Project title:	Insect Eradication(Phase 2) & Eradication Database
Project leader:	Bill Woods, John Kean
Project team:	40136: Max Suckling, Lloyd Stringer, Greg Baker, David Williams, Latif Salehi, Peter Crisp, Ian Lacey, Alven Soopaya, Amandip Kaur, Delyse Campbell 40187: John Kean, Lloyd Stringer, Fiona MacBeth, Ruben Flores Vargas
Research outcomes:	Identification of irradiation dose for SIT, development and testing of mobile mating disruption, development and testing of SPLAT™ in SA, NZ and WA, testing of integrated eradication in the field (SPLAT™ + insecticide) and urban environment (SPLAT™ +SIT), analysis of past eradications in Australia.
Research implications:	New pheromone delivery technologies such as SPLAT™ have the potential to become an important component of integrated eradication strategies in the future.
Research publications:	Kean, J. M., D. M. Suckling, L. D. Stringer and B. Woods. 2011. Modeling the Sterile Insect Technique for Suppression of Light Brown Apple Moth (Lepidoptera: Tortricidae) J. Econ. Entomol. 104(5): 1462-1475 Soopaya, R., L. D. Stringer, B. Woods, A. E. A. Stephens, R. C. Butler, I. Lacey, A. Kaur and D. M. Suckling. 2011. Radiation biology and inherited sterility of Light Brown Apple Moth (Lepidoptera: Tortricidae): Developing a sterile insect release program. J. Economic Entomology 104(6): 1999-2008 Jang E.B., D.O. McInnis, R. Kurashima, B. Woods, and D.M. Suckling.2012. Irradiation of adult light brown apple moth, *Epiphyas postvittana* (Lepidoptera: Tortricidae): egg sterility in parental and F1 generations. J. Economic. Entomology 105(1): 54-61 Suckling D.M., B. Woods, V.J. Mitchell, E.B. Jang, A .Twidle, I. Lacey and A.R. Wallace. 2011. Mobile mating disruption of light brown apple moths using pheromone-treated sterile Mediterranean fruit flies. Pest Management Science 67: 1004-1014 Suckling D. M., L. D. Stringer, V. J. Mitchell, T.E.S. Sullivan, A. M. Barrington, and A. M. El-Sayed. 2011. Comparative fitness of irradiated sterile light brown apple moths (Lepidoptera: Tortricidae) in a wind tunnel, hedgerow and vineyard. J. Economic Entomology. 104: 1301-1308.
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