Horticulture Innovation Australia

Final Report

SPLAT Cuelure based management of Queensland fruit fly (Bactrocera tyroni)

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Summary

The Queensland fruit fly (QFF), *Bactrocera tryoni* (Froggatt) is a significant pest of horticulture and a threat to market access both domestically and internationally.

There are a number of control options available for QFF, including MAT (Male Annihilation Technique), chemical treatments, baits, and post-harvest disinfestation. Existing MAT options are labour intensive and some options can lead to worker exposure to toxic chemicals. Further, few control methods are suitable for organic properties or urban eradication programs due to their inherent human health and environmental risk. The SPLAT (Specialised Pheromone and Lure Application Technology)-MAT option investigated in this study is readily applied, and allows for automated application that is suitable for urban eradication programs and Organic producers. This project provides data on the efficacy of SPLAT MAT for QFF management, the durability of spinosad as a toxicant with SPLAT and provide base data that can be used to supplement an application for registration of the product.

Laboratory bioassays and cage attraction trials were carried out in New South Wales, along with field cage attraction trials and field attraction trials. One field orchard trial was also carried out to assess the effectiveness of SPLAT-CL 5% (Specialised Pheromone Lure Application Technology-Cue Lure) with different insecticides (spinosad, malathion or alphacypermethrin) against nil treatment and an industry standard MAT (Male Annihilation Technique) Wick (Bugs for Bugs). Overall, SPLAT-CL + spinosad compared favourably with current standard techniques for male annihilation (see resultant scientific refereed publication - Reynolds et al, 2016).

Field trials were conducted in three locations in Queensland (Stanthorpe, Gatton and Bundaberg) to assess the efficacy of SPLAT employed as a MAT control of QFF using spinosad as a toxicant. Monitoring traps and sentinel fruit were used to assess the efficacy of the SPLAT and Comparisons were made with a commercial standard treatment of MAT and an untreated control.

The populations of QFF after application of SPLAT MAT was not significantly different to that of the commercial standard MAT. Plots treated with SPLAT MAT or traditional MAT had lower mean populations of QFF and fruit damage than the untreated plots for all sampling times, with trap counts significantly lower on four occasions.

The physical properties of SPLAT performed well in the conditions at Gatton. Although rainfall was minimal during the trial period, SPLAT was visible after 4 weeks when it was due for reapplication. To assess SPLAT's ability to withstand heavy rainfall events commonly experienced in Queensland further work is required.

The results of this study suggest that a novel male annihilation technique (SPLAT-CL plus spinosad) is as effective as industry standard male annihilation controls, and warrants further research to manage QFF populations. This demonstrates that SPLAT MAT + Spinosad option is a suitable alternative to current MAT programs providing the options of automated application in conventional orchards and provides an additional tool for use in organic orchards and urban situations. The attraction of QFF to SPLAT-CL 5% is comparable to SPLAT-CL 20%, however further field trials are required to confirm this result. SPLAT-CL + spinosad may be a reduced-risk alternative for other MAT industry standards,

including wicks-CL + malathion or blocks-CL + malathion for *B. tryoni* and other CL-responding pest fruit flies, such as *Bactrocera cucurbitae* Coquillett, because it contains a reduced-risk insecticide that poses a lower risk to humans and the environment and does not require labour-intensive handling and placement.

Data from this project and associated trials has been passed to a private consultant (Competitive) for consolidation and assessed for submission of a registration application with APVMA. Costs for this submission will be met be ISCA through Organic Crop Protectants.

Keywords

Queensland fruit fly, *Bactrocera tryoni*, specialized pheromone and lure application technology, SPLAT, integrated pest management, Male annihilation technique, MAT

Introduction

Recent and ongoing reviews of registered agricultural pesticides have reduced grower access to pesticides that control a range of pests including Queensland fruit fly *Bactrocera tryoni*. There are a number of new products and strategies that are being developed in the USA and Mediterranean region for other species of fruit fly that may be suitable for use in Australian horticulture. To assess these and other options for the sustainable management of *B. tryoni* a range of research projects are developing the tools, including SIT, improved lures and area wide management strategies. The SITplus research program aims to develop a high quality male-only QFF, which will be rendered sterile using X-Ray and used to control wild QFF populations for the benefit of Australian horticulture. The efficacy of such programs depends upon the effective suppression of wild populations prior to the release of sterile flies. The SPLAT Cuelure based Male Annihilation Technique (MAT) is a potential option for contributing to the desired level of suppression. If successful, the method will also be suitable for urban eradication programs, reducing bi-weekly applications of protein baits with fortnightly application of SPLAT. In urban areas this also reduces the volume of treatment applied from approximately 250 ml to 6ml, greatly reducing the occupational health and safety risks associated with carrying heavy backpacks.

This project aims to assess the efficacy of SPLAT Cue-lure (ISCA Technologies USA) as a management option for control of *B. tryoni* in a diverse range of susceptible crops such as summer fruit, citrus, mangoes, banana, cherry, apples, pears, tomatoes, and avocado. The SPLAT Cue-lure uses Cue-lure to attract male *B. tryoni* and spinosad as a toxicant, attraction distance has been reported up to 400m but the distance appears to be related to humidity, temperature and the landscape. This use of SPLAT with Cuelure is a form of MAT which has been successfully used for fruit fly management in a number of countries but has usually involved less benign toxicants such as maldison. SPLAT is also significantly easier to apply than most MAT systems therefore reducing labour costs. Traditional MAT blocks are Caneite blocks which must be soaked in a lure/toxicant mix allowed to dry and then attached to trees individually, whereas SPLAT can be applied from a tractor or by air if required. The research will include a series of field efficacy trials in four States to provide data from a wide range of crops and environmental conditions. Laboratory trials associated with the research will investigate the weathering rates to assist with developing and optimising the timing of applications in field base management programs.

While this research project does not include trials on *Ceratitis capitata (Mediterranean fruit fly)* experience gained, skills developed and knowledge acquired from this project will assist in development of future research and management programs.

Methods

New South Wales Research

Trial methodology followed discussions and publications of Dr Roger Vargas, USDA, Hawaii, who has expertise with developing SPLAT and registering it for use in the USA against *Bactrocera dorsalis* and other pest fruit flies. The scientific methodology followed for the trials is shown in more detail in the appendices.

Insects

Bactrocera tryoni pupae were obtained from a low stress colony at NSWDPI, University of Newcastle, Ourimbah Campus, North Loop Road, Ourimbah. The fly was reared under standard conditions in a controlled temperature room (i.e., 26±2ºC, 65±10% RH and a light: dark period of 14: 10, with simulated dawn and dusk as the lights ramped up and down at the beginning and end of the light phase), or a similarly maintained colony at Waite Campus in South Australia.

Weathering of Cue Lure treatments

To assess the longevity of SPLAT formulations under field conditions, treatments were evaluated by applying approximately 0.02 g (Contact and feeding toxicity bioassay—see below), 1 g (Cage attraction trial—see below), and 2 g (Field trials – see below) of each SPLAT treatment onto the surface of wooden ice-cream sampler sticks (1.7 by 9.5 cm^2; Stsellsok [A1Packaging] Merrylands, NSW, Australia). The sticks were then hung on a weathering line in direct sunlight in Sydney, Australia. Mean (±SEM) daily air temperature, relative humidity and rainfall were recorded (Appendix 9, Fig 1, 2). The three treatments were tested for each of five aging periods (0, 1, 2, 4, and 8 weeks), with the exception of field-based weathering trials which included an additional weathering treatment, 12 weeks. The results of laboratory-based trials were used to provide a guide to application frequency in field application trials described below.

Contact and Feeding Toxicity Bioassay (Trial 1)

A laboratory contact/feeding bioassay was developed to determine the relative toxicity of the differentially aged formulations indicated above, at 22 ± 2ºC, 60º±% RH, and a photoperiod of 16:8 (L:D) h using laboratory-reared males at the Elizabeth Macarthur Agricultural Institute (EMAI), NSW DPI, Menangle, NSW.

Outdoor Cage Attraction Trial (Trial 2)

An outdoor cage study was established to quantify the toxicity of fresh and the 5 weathered treatments after up to 24 hours exposure in mesh cages (Bugdorm, Taiwan) deployed at EMAI. The SPLAT CL treatment without spinosad was evaluated to determine whether continuous exposure for a maximum period of 24 h is lethal to males.

Field Cage Attraction Trial (Trial 3)

Field cage bioassay studies were conducted to compare the response of *B. tryoni* to weathered (0 1, 2, 4 & 8) treatments including SPLAT 5% CL+Spinosad, SPLAT 20% CL+Spinosad and an industry standard, QFF Wick (Bugs for Bugs) in walk-in field cages, each enclosing a large potted citrus (orange) plant, at EMAI. Each cage received one treatment, suspended in a single Lynfield trap. Temperature and relative humidity were recorded using data loggers (Tinytag). Two hundred male flies aged 8 days, were released into each field cage and daily trap capture recorded and trapped flies removed for 5 consecutive days.

Field Attraction Trials (Trials 4, 5 & 7)

Three field attraction trials were conducted in a mixed fruit (pome, stone, and quince fruit) orchard located at EMAI, which had not been treated with any pesticide in over four years.

A field trial (trial 4; non-weathered) was conducted over two periods; 12 February 2014 – 23 July 2014 and 26 November 2014 – 11 February 2015 to quantify the relative toxicity of the three non-weathered MAT CL treatments associated with a toxicant (spinosad or malathion), i) SPLAT CL 5% + Spinosad, ii) MAT block (Appendix 9, Fig. 4) or iii) MAT wick. Lures were changed every 4 weeks. Traps were checked for fruit flies every 7 days and mortality recorded.

A field trial (trial 5, weathered) was conducted over two periods; 8 October 2014 – 12 November 2014 and 18 February 2015 – 25 March 2015 to quantify the relative toxicity of three weathered (0, 1, 2, 4, 8 or 12 weeks) MAT CL treatments associated with a toxicant (spinosad or malathion), i) SPLAT CL 5% + Spinosad, (Appendix 9, Fig. 5) ii) MAT block or iii) MAT wick. Weathered lures were changed every 7 days, at which time the traps were checked for fruit flies and mortality recorded.

A field trial (trial 7, non-weathered) was conducted from 18 March 2015 – 3 June 2015 to assess the relative attraction of three non-weathered MAT CL treatments without a toxicant, i) SPLAT CL 5% no toxicant, ii) SPLAT CL 20% no toxicant or iii) MAT NSW DPI wick no toxicant. Each trap had a 10mm square Dichlorvos-impregnated strip in the bottom of the trap to kill the flies entering the traps. Lures were changed and traps catches cleared every 7 days, at which time fly identification and mortality counts were recorded.

Wedderburn Orchard Trial (Trial 6)

A field trial in a 2 Ha mixed fruit orchard 'Wedderburn Orchard', Miverna Rd, Wedderburn (GPS Coordinates: 34 08 49.6" S, 150 48 52.8" E) was conducted from December 2014 to February 2015 to assess the effectiveness of SPLAT 5% CL with different insecticides (spinosad, malathion or alphacypermethrin) against nil treatment and an industry standard MAT Wick (Bugs for Bugs). The orchard was divided into 10 plots and 2 replicates (5 plots per replicate). Two cue-lure baited (Bugs for Bugs) Lynfield traps were placed centrally within each plot (Appendix 9, Fig. 6). Whole sentinel organic stone fruit were suspended in mesh bags throughout each treatment plot. Traps were checked weekly and sentinel fruit were collected every 3-7 days, and placed in a controlled environment room (see Insects above), individually over moistened vermiculite to determine the number of pupae (count) and flies (identification, count & sex) that eclosed. Two SPLAT spots were applied to each tree at approximately 1.5 m above the ground as recommended by ISCA after trials conducted with B. dorsalis in Hawaii.

Queensland Field Research

Field trials were conducted to evaluate the efficacy of SPLAT against Queensland fruit fly, *Bactrocera tryoni.*

Field trials were conducted in three locations in Queensland. Trial sites included large scale commercial orchards at Gatton and Pikedale (near Stanthorpe), consisting of non-fruiting stonefruit. The third trial site was located at Gin Gin (near Bundaberg), with trials conducted in blocks of Murcott mandarins shortly after fruit set.

Three treatments were to be evaluated at each location. Treatments included: SPLAT, a commercial standard treatment of MAT deployed at 10 units/ ha, and an untreated control. Each treatment was replicated twice at each location in two separate orchard blocks. Block sizes ranged from 0.46 ha to 9.72 Ha (Figures 1-3).

Male monitoring traps, consisting of BioTraps containing a FT Cuelure wafer (BioTrap Australia PTY LTD), were installed on each trial site. A total of 12 traps (i.e. two per treatment replicate block) were installed at Gatton, Stanthorpe and Bundaberg on the 19th January, 28th January and 4th February 2016 respectively. Traps were serviced regularly with catches of fruit flies sent to the DAF Market Access laboratory for counting and identification to species. Trapping results for Queensland fruit fly include counts for both pest species (*B. tryoni and B. neohumeralis).*

On the day monitoring traps were installed, MAT was applied to orchard blocks at Bundaberg and Stanthorpe. However at Gatton, existing 3 month old MAT devices were not replaced until 2 weeks after traps were installed. In SPLAT and Untreated blocks all existing MAT devices were removed in order to obtain an accurate assessment of SPLAT.

Due to the research permit not being obtained, SPLAT could only be applied to one orchard. Gatton was selected due to the treatment blocks not exceeding the allowable size for use of the generic APVMA small-scale permit for conducting trials with agvet chemicals (PER 7250). Trials at Stanthorpe and Bundaberg were abandoned after 65 days and 85 days without the application of SPLAT. Trials were cancelled to prevent crop losses in untreated mandarin orchards at Bundaberg and at Stanthorpe because of low numbers of fruit flies and leaves dropping in the stonefruit orchards. At Gatton, SPLAT was applied to trees at a rate of 600 spots/ ha, with spots consisting of 1 ml of product. SPLAT was applied to the trunk of the trees at approximately 1.5 m above the ground using 50 ml plunger style syringes. The first application of SPLAT was made on the 29th February 2016 (6 weeks after traps were installed). A second application was applied 4 weeks later on the 29th March 2016.

Four weeks after the first and second application of SPLAT, sentinel fruit were hung in each treatment block to assess the efficacy of the treatments. Ten certified organic gala apples were hung in the canopy of two neighbouring trees, in three locations within each block. A total of 180 apples were used throughout the orchard for this purpose. After 3 days in the field the fruit were removed and transported to the DAF Market Access laboratory. The second installation of fruit was left in the field for 7 days to increase the exposure time for fruit fly oviposition, as results from the first installation found no infestation after 3 days. Fruit were incubated in ventilated plastic containers for at least 14 days at 26°C and 70 RH before they were cut into pieces and assessed for the presence of fruit flies.

Temperature and rainfall observations were obtained from the Bureau of Meteorology (www.bom.gov.au) for the trial period. Climate records are only presented for Gatton as SPLAT was only applied to this site. The closest weather station to the Gatton trial site was approximately 11.1 km away and located at the University of Queensland, Gatton (weather station- 040082).

Data from Gatton only are reported due to SPLAT not being applied to the other sites. The trapping data was analysed using repeated measures residual maximum likelihood (REML). A power model was fitted to account for any correlation between trap clearance dates.

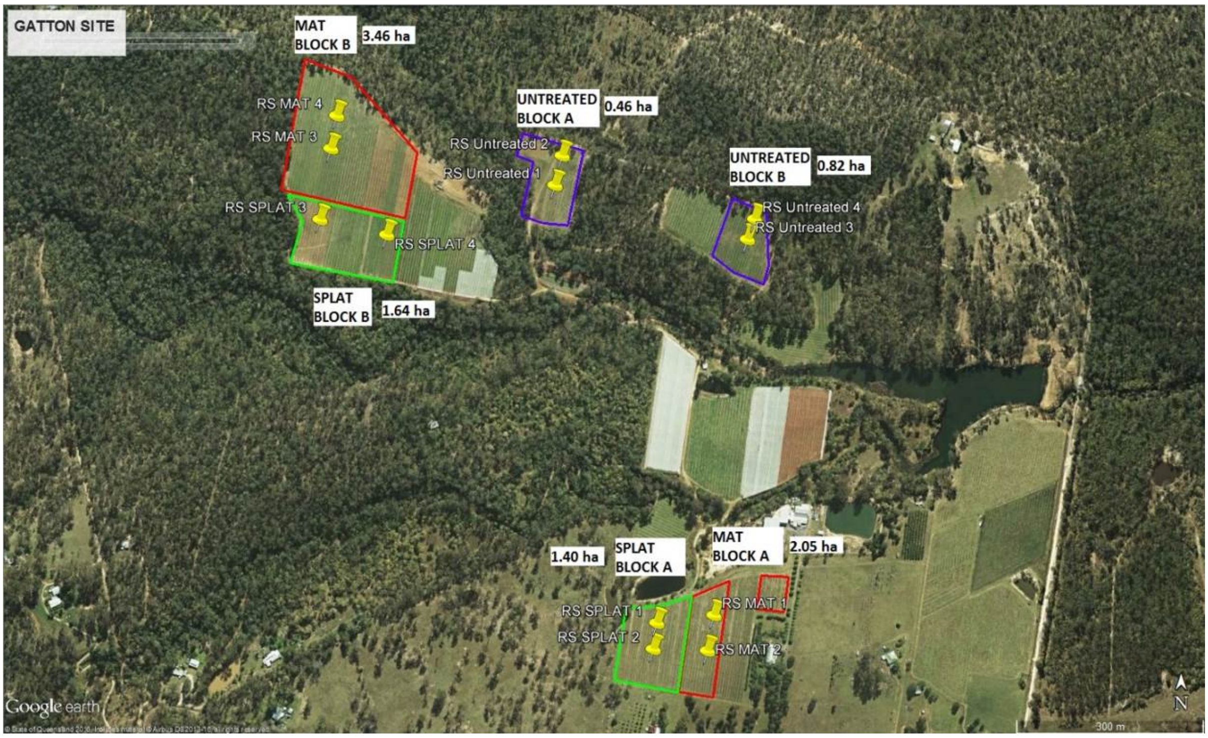

Figure 1: Gatton Field Site

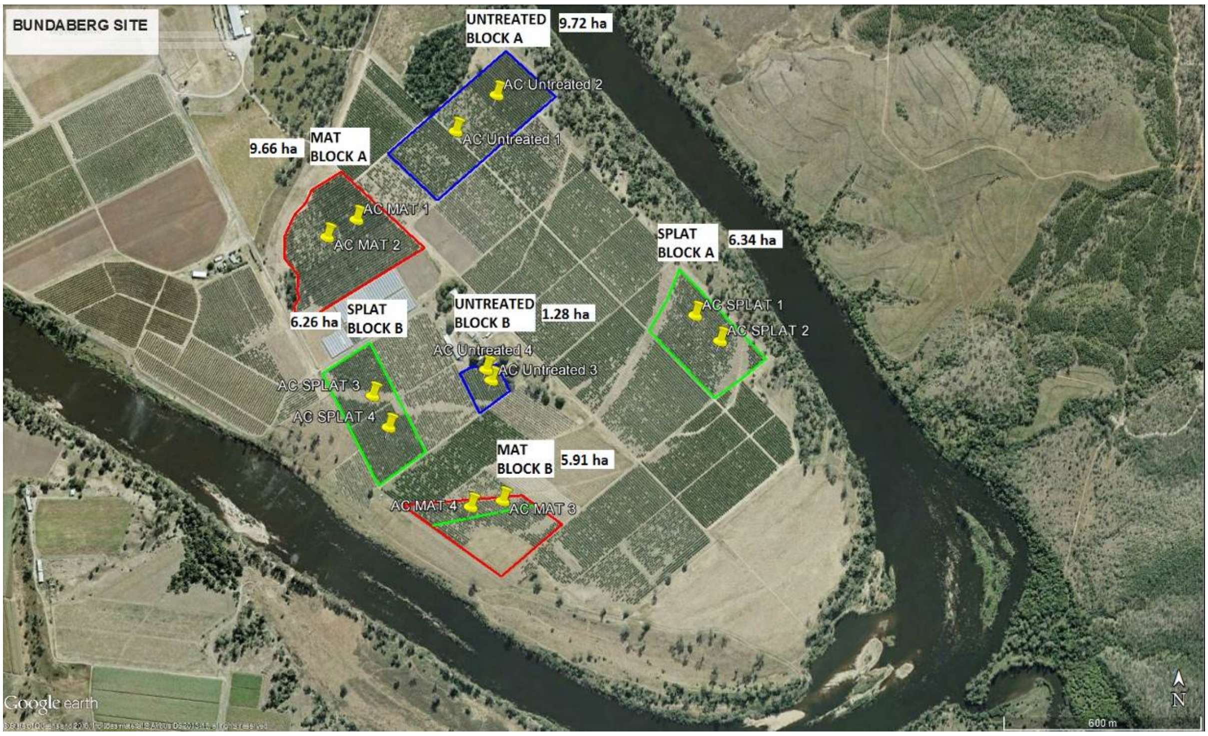

Figure 2: Bundaberg Field site

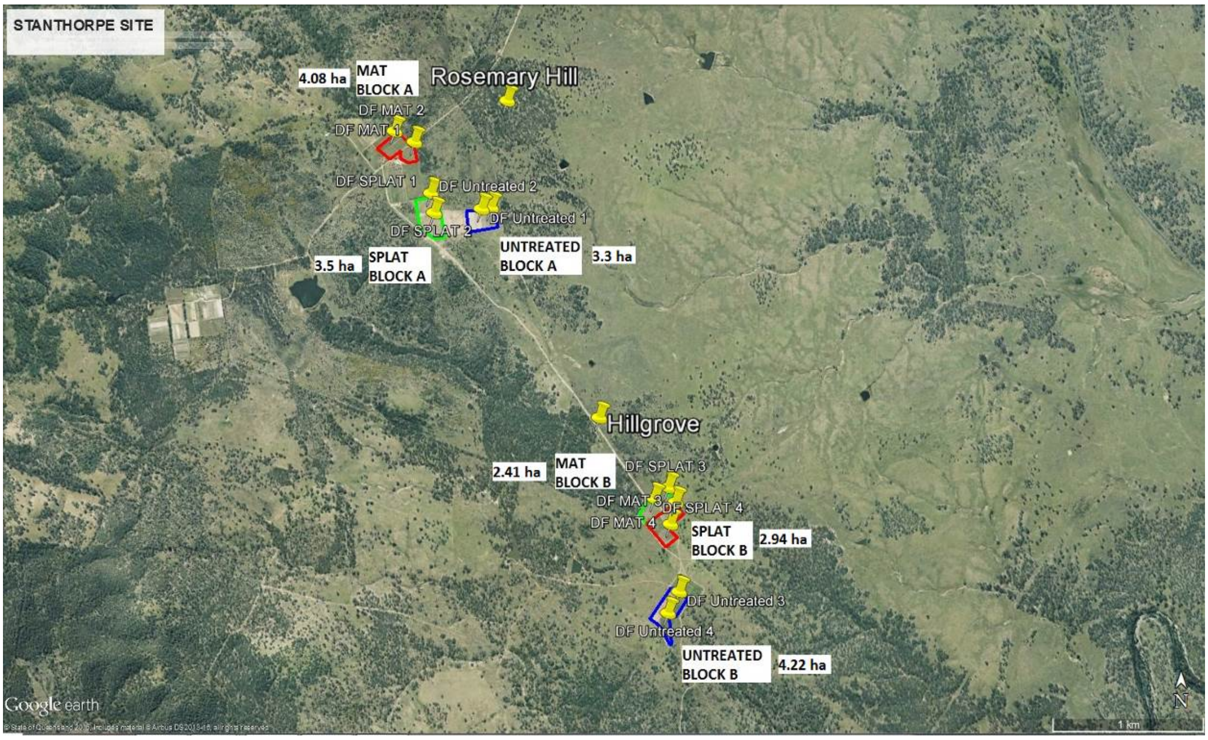

Figure 3: Stanthorpe Field Site

Results and Discussion

New South Wales Research

The results of trials 1-6 are detailed in Appendices 2-7 respectively.

In the contact and feeding bioassay (trial 1; Appendix 2), the lures with MAT cups killed *B. tryoni* within 2 hours of exposure in all weathering periods. Lures with SPLAT CL 5% had no effect on *B. tryoni* on all occasions; however lures with SPLAT-5%CL+Spinosad were effective for up to 2 weeks of weathering. In this feeding and contact bioassay, males were only exposed to each treatment for a maximum of 5 minutes. Given the strong feeding behaviour of male QFF show towards cue-lure, and the fact that males had to be coaxed from feeding on both the SPLAT-5% CL + Spinosad and SPLAT-5% CL, it would be reasonable to assume that under field conditions, males would have access to the toxicant for a longer period.

In the outdoor cage study (trial 2, Appendix 3) the SPLAT- 5%CL+Spinosad performed as well as the MAT cups under all weathering treatments. These results are consistent with our prediction that when males are allowed to feed over a longer period on the lure/toxicant mix, they imbibed enough toxicant to induce mortality across all weathered treatments trialled.

The field cage trial (trial 3; Appendix 4) shows that SPLAT 5% CL + spinosad is equally attractive to male *B. tryoni*, as SPLAT 20% CL + spinosad at all weathering periods tested. After the completion of an orchard trial (trial 7) to determine the attraction of males to three attractants, it was determined (by Peter Crisp) that the SPLAT CL 5%, was defective, and therefore the results of this trial are not reliable, and so not reported. The toxicant added to the SPLAT CL in this case was also used in trials of other insects conducted at Waite and gave results inconsistent with previous replications of these trials. As a result any trials conducted with that batch of toxicant were considered unreliable.

The non-weathered SPLAT small-scale orchard trial (trial 4; Appendix 5) show that SPLAT 5%CL + Spinosad is as effective as or better than MAT Blocks and over twice as effective as MAT Wicks (Wald $\chi^2_{df=2}$ = 15.6; p <0.001). There were several non-target effects of all MATs. *Dacus absonifacies* flies were trapped in very low numbers and could not be analysed (MAT blocks: 7, MAT wicks: 1 and SPLAT CL 5%+ spinosad: 1), however the impact on this species of SPLAT 5%CL is considered very minor. A total of 439 *Dacus aequalis* males were trapped, most in March, however there was no significant difference between SPLAT CL 5% and the current industry standards tested (Wald $\chi^2_{df=2}$ = 1.67; p = 0.44). Therefore, non-target effects are considered minimal.

The weathered field study (trial 5, Appendix 6) trap catches were similar for SPLAT-CL + spinosad and blocks-CL + malathion, and both had higher trap catches than wicks-CL + malathion at all weathering periods, except week 12.

The results of trials 1, 2 and 5 were published in the *Journal of Economic Entomology*, which is shown in Appendix 8.

The trapping results of a small-scale commercial field trial at Wedderburn Orchard (trial 6, Appendix 7a), showed that there was no significant difference between any of the treatments and the control ($F_{df=4,\ 46}$ = 1.39; p =0.252). There could be several reasons for this, including that the treatments in treated plots, 'pulled' flies from the control plots. It should be noted that the MATs (Bugs for Bugs) were also placed at a higher rate than the recommended rate (10 – 20 per Ha, Dan Papacek, pers.

comm. July 2016). Sentinel fruit indicates a reduced number of flies from treatment plots compared with the control plots, however this was not significant (Wald $\chi^2_{df=4}$ = 4.79; p = 0.691) (Appendix 7b). Future trials will need to include greater replication and also be timed with areas and periods when fly populations are at their highest, to maximize the opportunity to observe any statistical differences.

Trial images are shown in Appendix 9.

Queensland Field Research

Trapping results

At Gatton, the cumulative number of fruit flies trapped (combined counts of *B. tryoni and B. neohumeralis*) were higher in Untreated blocks (251 flies total) compared to those treated with SPLAT (150 flies total) and MAT (114 flies total) (Figure 4).

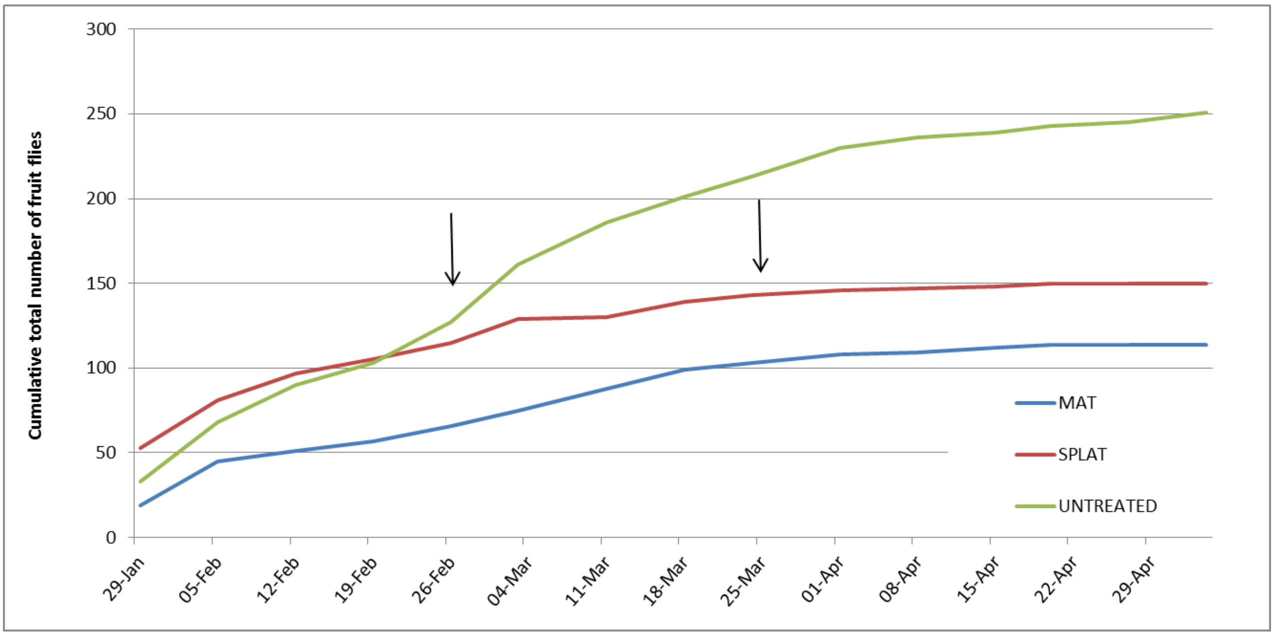

Figure 4: Cumulative total number of trapped flies at Gatton comparing Untreated, SPLAT and MAT blocks (Arrows indicate when SPLAT was applied).

Following the application of SPLAT on the 29/2/16, the mean number of flies trapped was not significantly different to the commercial standard MAT at any of the sampling times. Traps located in SPLAT blocks had fewer flies trapped than the Untreated blocks for all sampling times, with trap counts significantly lower on four occasions. MAT also had significantly lower means than the Untreated on four sampling occasions (Figure 5).

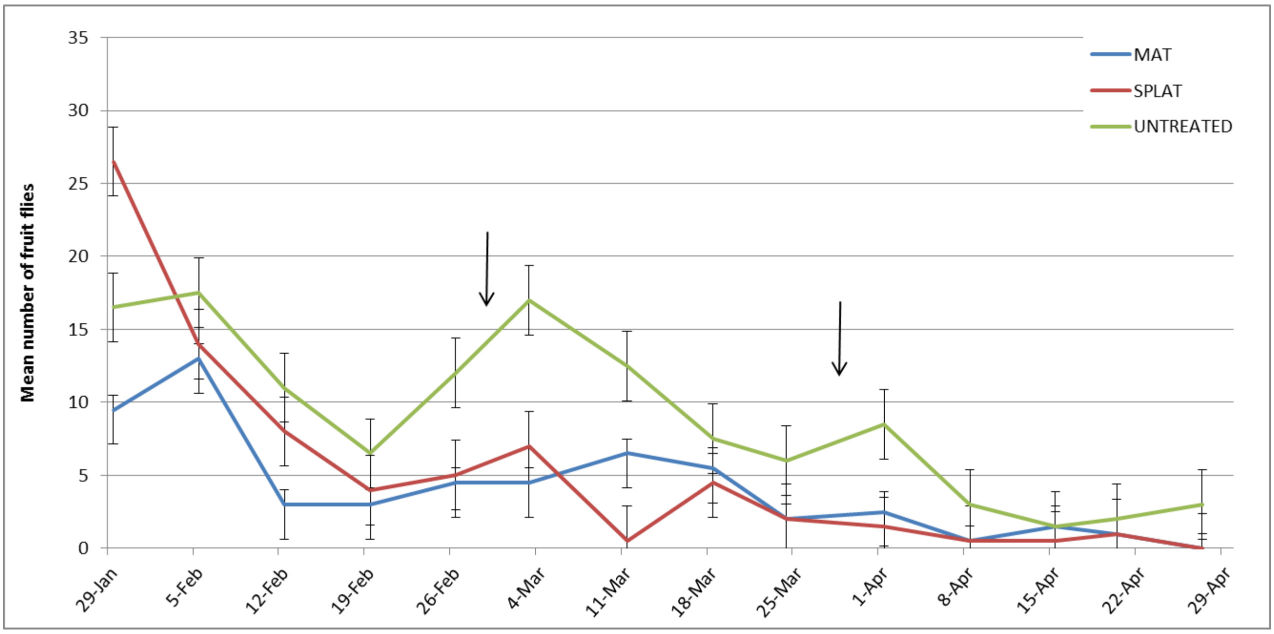

Figure 5: Mean (+/- SE) number of trapped flies per treatment block from Gatton.

(Arrows indicate when SPLAT was applied).

In total, five fruit fly species were trapped over the trial period at Gatton. These included *B. tryoni, B. neohumeralis, B. bryoniae, B. quadrata and Dacus aequalis. Bactrocera tryoni* was the most common fly caught, accounting for 90.58% of the catches (Figure 6).

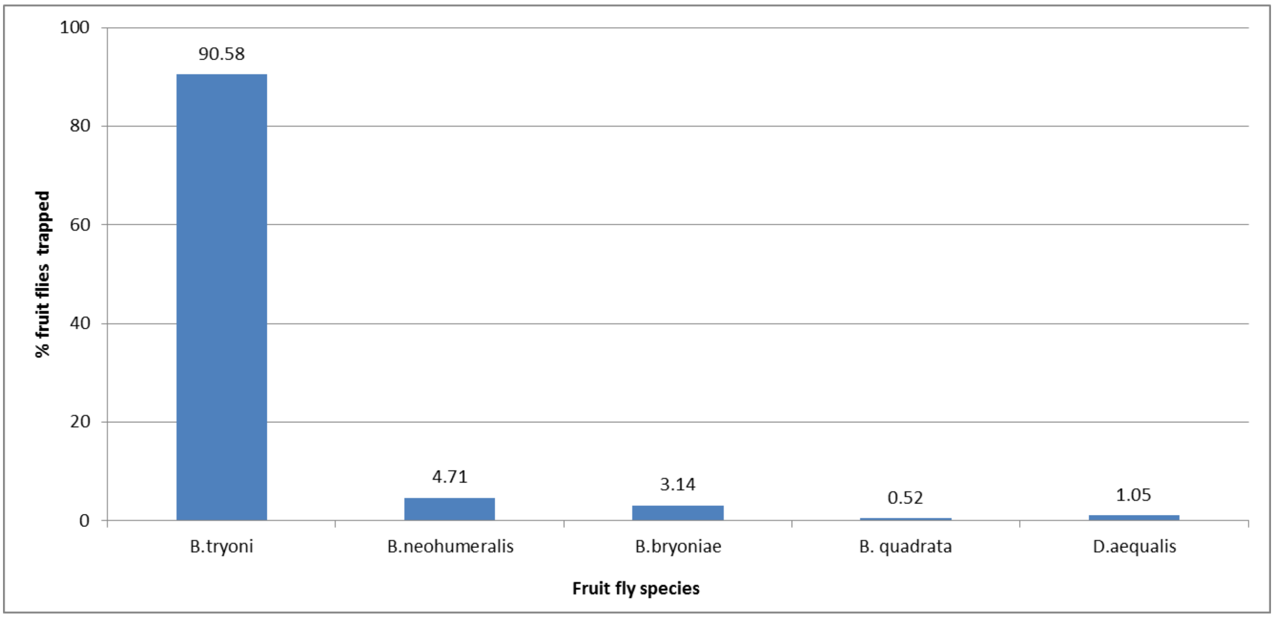

Figure 6: Percentage of fruit flies species trapped at Gatton

Fruit assessments

Sentinel fruit were hung in each treatment block to attract potential females to lay in order to assess the efficacy of each treatment. No fruit flies were reared from the 360 organic gala apples that were hung in the treatment blocks.

Physical properties of SPLAT

Throughout the trial period the physical properties of SPLAT were observed to assess how the product was performing under field conditions in the area. Within 1 week after application, observations found that SPLAT left a greasy residue on the trees that spread over the surface of the bark (Figure 7).

Figure 7: Greasy SPLAT residue on bark 5 days after application

SPLAT appeared to last up to 4 weeks under field conditions at Gatton at which time SPLAT was reapplied. When SPLAT was applied to the fork of the branches in a large spot, this was longer lasting than when it was smeared thinly onto the trunk of the tree (Figure 8). When applied thinly, it dried out and flaked off the tree within several weeks. Figure 9 shows the two SPLAT applications, with one spot four weeks old and the other spot newly applied. Although SPLAT appeared to withstand the weather conditions at Gatton, it should be noted there was very little rainfall after the first application of SPLAT on the 29 February 2016 (Figure 10).

Figure 8: SPLAT applied to the trunk of a tree after 4 weeks

Figure 9: Four week old SPLAT next to newly applied spot.

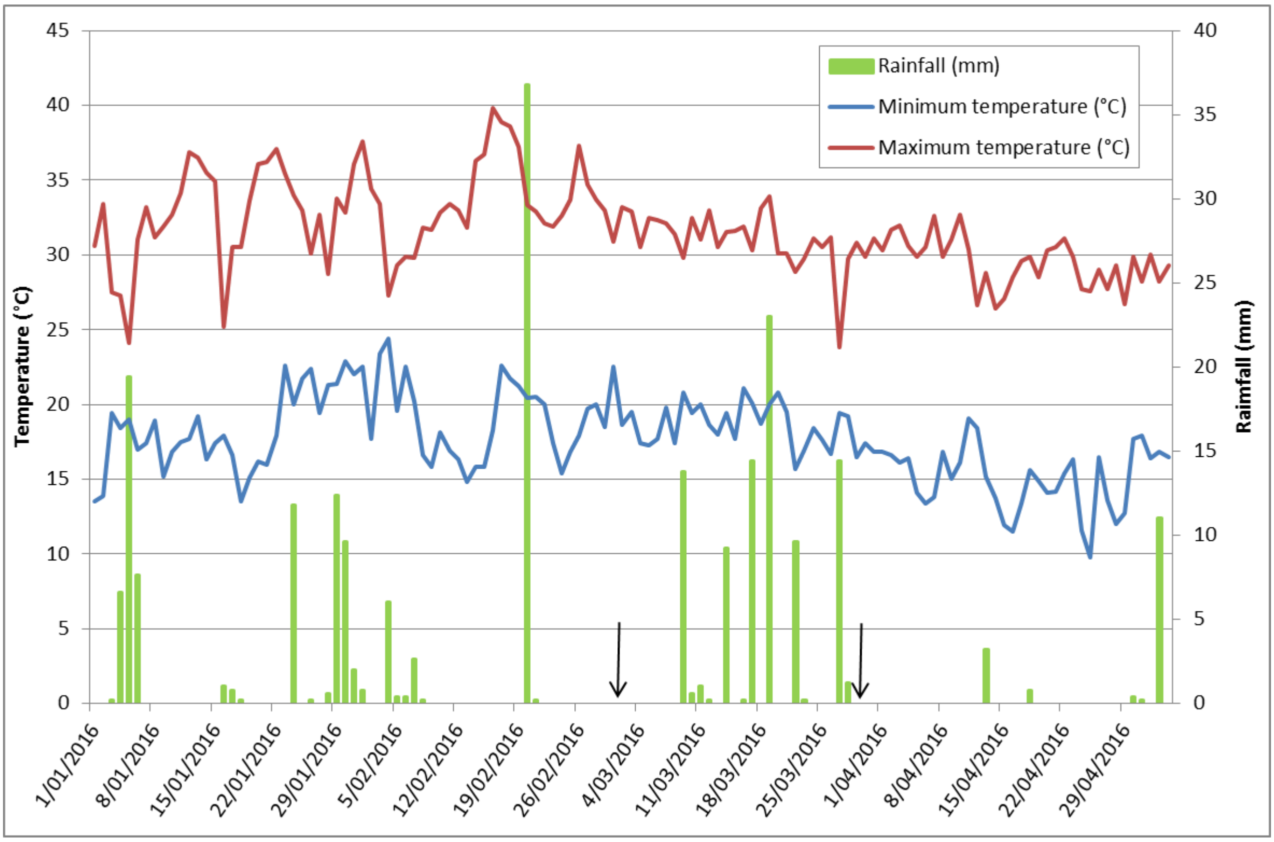

Figure 10: Temperature and Rainfall observations for Gatton during the trial (January-May 2016)

Sentinel fruit failed to find differences between the treatments as no flies emerged from any of the fruit. This assessment method may have been more effective if the trial was conducted during or immediately after harvest, when fruit fly pressure is higher.

The physical properties of SPLAT performed well in the conditions at Gatton although rainfall was minimal during the trial period. SPLAT was visible after 4 weeks when it was due for reapplication. To assess SPLAT's ability to withstand heavy rainfall events commonly experienced in Queensland further work is required.

Field trials conducted in Queensland demonstrate the efficacy of SPLAT MAT treatments were as effective as current MAT technologies and significantly reduce populations of Queensland fruit fly and associated damage compared with untreated control plots. These results are similar to those reported in a number of refereed papers related to the control of Mediterranean fruit fly in Hawaii using SPLAT MAT. Discussions with participating growers found that there is strong interest in using SPLAT for the control of male and female fruit flies. If reapplication intervals for female treatments can be extended from 7-10 days to several weeks, while also being able to resist rainfall, the use of this technology could potentially reduce production costs. The Formulation persisted for 4 weeks in the field in a period of moderate rainfall.

Discussions with participating growers found that there is strong interest in using SPLAT for the control of male and female fruit flies. If reapplication intervals for female treatments can be extended from 7-10 days to several weeks, while also being able to resist rainfall, the use of this technology could potentially reduce production costs.

Outputs

i. Data on the relative efficacy and cost effectiveness of SPLAT CL as a control option for Queensland Fruit Fly in the selected crops. Data sets have been developed in laboratory studies, field cage and field trials that demonstrate the efficacy of SPLAT 5%CL as an alternative to current MAT options. While field data has been collected and results presented in this report, further work in collaboration with commercial partner is required for registration purposes.

ii. Optimised SPLAT CL application and implementation into a systems approach for the control of QFF. This will include programs developed for each crop and re-application intervals determined from weathering and trapping data. This will allow growers to make informed decisions on QFF management on their properties. These decisions will be able to be based on efficacy, workplace safety and economic viability. The data presented from the weathering trials conducted in the laboratory trials and field cages trials and verified by partial and full field trials provides a base for reapplication frequency and spot size. However, the supplier of the toxicant, Spinosad is due to change in late 2016, it is therefore recommended that some of the trials are repeated to show equivalence.

iii. Standard operating procedure (SOP) developed for QFF control using SPLAT CL, which can be integrated into current programs. SOPs will be developed using data generated in the current study, once equivalence between the toxicant currently supplied by Dow Agrichemical and the material from the new supplier has been established.

iv. Research published in peer-reviewed journals

A peer-reviewed publication has been published in the *Journal of Economic Entomology*. A second manuscript is nearing completion. It is expected that at least one more publication will be generated from this work.

v. Presentation

Invited presentation (Dr Olivia Reynolds) on integrated management options, including SPLAT, at two workshops 'Managing Queensland fruit fly in blueberries without Dimethoate', Woolgoolga and Coffs Harbour, 13-14 July 2016.

Outcomes

I. This study shows that SPLAT CL 5% + Spinosad is as effective as other industry standards for the control of *Bactrocera tryoni*. Non-target effects are minimal, and where they occur, are not different to current industry MAT standards.

II. It has been found that SPLAT MAT can suppress populations of QFF to below economic thresholds. Results of field trials have demonstrated that the SPLAT MAT provides at least equivalent control as more traditional MAT applications and is easier to apply and maintain.

III. The technique reduces reliance on organophosphate usage in QFF management programs which will reduce worker exposure and reduce the disruption of beneficial organisms that results from the use of broad-spectrum insecticides, it also provides a tool that can be used in conjunction with sterile insect technique where adults are exposed to Cuelure prior to release.

IV. The increased use of SPLAT CL MAT as part of an Area Wide Management program that includes SIT will provide opportunities to maintain and improve market access for National and International trade through ALPP and PFPP's. The techniques will allow for lower residue on produce and reduce the risks associated with MRL's for some pesticides being lowered by trading partners such as Japan, Indonesia and some European countries.

Recommendations

It has been established that SPLAT is an effective alternative to current industry MATs. The following recommendations are necessary before commercial adoption of this technology.

1. Evaluate combined data sets collected as part of this research for compilation of a registration application to the APVMA for use of SPLAT CL as a control option for Queensland fruit fly in susceptible commercial crops and urban eradication programs. Some data from the field trails in Queensland may benefit from further analysis.

2. Identify any gaps in the data and develop a strategy to gather any information required to achieve registration. This may include equivalence trials if an alternative source of spinosad is sourced or if spinosad is replaced with other toxicants.

3. Assess field efficacy under a wider range of conditions and crops, if required to meet registration requirements for SPLAT.

4. Evaluate the rates at which the toxicant and the lure are breaking down/becoming less toxic/attractive in SPLAT, to allow improvements to the lure.

5. Conduct oviposition studies to understand the ability of females to oviposit after feeding on SPLAT 5% CL + spinosad (& other toxicants).

6. Conduct studies to determine the effectiveness of SPLAT incorporating a female attractant; this is strongly desired by growers. There is currently significant investment in improving male lures and developing a reliable female lure. As these are developed the opportunity to improve the efficacy of SPLAT MAT need to be investigated.

Scientific Refereed Publications

Reynolds, O.L., Osborne, T., Crisp, P. & Barchia, I.M. (2016) Specialized Pheromone and Lure Application Technology as an Alternative Male Annihilation Technique to Manage *Bactrocera tryoni* (Diptera: Tephritidae). *Journal of Economic Entomology*. 109 (3): 1254- 1260.

DOI: http://dx.doi.org/10.1093/jee/tow023 tow023; First published online: 27 March 2016. Appendix 8.

IP/Commercialisation

No IP or commercialisation is associated with the project. However, the is interest in putting a registration data package together for submission to APVMA or development of further research programs if required.

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Cowley, J.M. ; Page , F.D. ; Nimmo , P.R. ; Cowley, D.R. (1990) Comparison of the effectiveness of two traps for *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) and implications for quarantine surveillance systems. Journal of the Australian Entomological Society, Vol.29 (pt.3), pp.171-176

Vargas Roger I. ; Piñero, Jaime C. ; Mau, Ronald F. L. ; Stark, John D. ; Hertlein, Mark ; Mafra-neto, Agenor ; Coler, Reginald ; Getchell, Anna (2009) Attraction and mortality of oriental fruit flies to SPLAT-MAT methyl eugenol with spinosad. *Entomologia Experimentalis et Applicata*, Vol.131(3), pp.286-293

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New South Wales Research

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Queensland Research

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- DAF biometrician- Dr Carole Wright for assistance with statistical analysis and trial design.

Appendices

Appendix 1. Detailed trial methodology

Appendix 2. Mortality (logit(P) ± SE and mean (%)) of male Queensland fruit fly, *Bactrocera tryoni* allowed to feed or contact (1min 30sec – 5 mins) weathered SPLAT- CL + spinosad, SPLAT- CL (no toxicant) or wick-CL + malathion after 2h, 4h, 24h and 48h.

Appendix 3. Mortality (logit (P) ± SE and mean (%)) of male Queensland fruit fly, *Bactrocera tryoni* exposed continuously to weathered SPLAT- CL + spinosad, SPLAT- CL (no toxicant; control), wick-CL + malathion or nil treatment (blank) at 4h, 24h and 48h.

Appendix 4. Mortality (%) of male Queensland fruit fly, *Bactrocera tryoni* exposed to fresh MAT, SPLAT CL 5%+spinosad or SPLAT CL 20%+spinosad after 24h, 48h, 72h, 96h or 120h.

Appendix 5. Lynfield trap (baited with either non-weathered MAT Block, MAT Wick or SPLAT 5% CL + spinosad) captures (logit (P) ± SE and mean (%)), of adult male Queensland fruit fly, *Bactrocera tryoni* and *Dacus aequalis*, each week from 12 February 2014 – 23 July 2014 and 26 November 2014 – 11 February 2015. Lures were changed every 4 weeks.

Appendix 6. Trap captures of wild male Queensland fruit fly, Bactrocera tryoni in Lynfield traps baited with non-weathered and weathered CL treatments at Menangle, New South Wales, Australia in 2015.

Appendix 7a. The mean number of Queensland fruit fly, *Bactrocera tryoni*, caught in cue-lure baited Lynfield traps, placed within plots treated with MAT, SPLAT 5% CL+Spinosad, SPLAT 5% CL+Cypermethrin, SPLAT 5% CL+Malathion or Control (No treatment) each week.

Appendix 7b. The mean number of larvae, pupae and adults obtained from sentinel fruit placed in treatment plots at Wedderburn Orchard, NSW over an 8 week period, 9 December 2014 to 3 February 2015.

Appendix 8. Reynolds, O.L., Osborne, T., Crisp, P. & Barchia, I.M. 2016. Specialized Pheromone and Lure Application Technology as an Alternative Male Annihilation Technique to Manage Bactrocera tryoni (Diptera: Tephritidae). Journal of Economic Entomology DOI: http://dx.doi.org/10.1093/jee/tow023 tow023; First published online: 27 March 2016.

Appendix 9. Trial images.

Appendix 1. Detailed NSW trial methodology

Insects

Bactrocera tryoni pupae were obtained from a low stress colony at Central Coast Primary Industries Centre, NSWDPI, North Loop Road, Ourimbah, reared in a controlled environment room (i.e., 26 ± 2°C, 65 ± 10% relative humidity [RH], and a photoperiod of 14:10 [L:D] h, with a simulated dawn and dusk as the lights ramped up and down at the beginning and end of the light phase). At Elizabeth Macarthur Agricultural Institute (EMAI), Menangle, NSW, Australia, pupae were placed in 50 mm-diameter plastic Petri dishes, under moistened vermiculite (1:4; water:vermiculite) on the base of cubical mesh holding cages (30 cm^3; Bugdorm, Taiwan) under standard conditions in a controlled environment room (i.e., 26 ± 2°C, 65 ± 10% RH, and a photoperiod of 16:8 [L:D] h). Emerged adult flies were provided with sugar (sucrose) cubes as a source of carbohydrate and a 30 mm-diameter plastic Petri dish containing yeast hydrolysate enzymatic (MP Biomedical, Auburn, OH; 60% protein) as a source of protein and water *ad libitum*. Adult male flies were allowed to mate and were tested when aged 7–10 d, at which time they have normally reached sexual maturity and are responsive to CL. Male annihilation, is based upon this concept that the flies will be attracted, feed, and die.

Weathering of Cue-Lure Treatments

Three male annihilation CL treatments were evaluated in a laboratory feeding bioassay and a cage attraction trial:

1) SPLAT-5%CL + spinosad (hereafter referred to as “SPLAT-CL + spinosad”; 2% active ingredient [a.i.]);

2) SPLAT-CL without spinosad (control);

3) wicks with CL + malathion (hereafter referred to as “wicks-CL + malathion”; 57% a.i.; 0.5 ml per wick maldison [malathion], 1.0 ml per wick 4-(p-Acetoxyphenyl) butan-2-one; Bugs for Bugs, Mundubberra, Qld, Australia), an industry standard currently used in Australia.

SPLAT (blank; ISCA Tech.) was mixed with CL (Bugs for Bugs) + spinosad (Success 2 Naturalyte Insect Control; Dow AgroSciences, Frenchs Forest, NSW, Australia), and CL to achieve the required amount for treatments 1 and 2, respectively. The SPLAT-CL treatment without spinosad was evaluated to determine whether continuous exposure to CL for a maximum period of 48 h would be lethal to males.

In a field attraction trial, treatments (1) and (3) above were evaluated with a third treatment, MAT caneite blocks (hereafter referred to as “blocks-CL + malathion”; 50 by 50 by 12 mm^3 impregnated with 2 ml maldison + 2 ml CL per block), a New South Wales (NSW) government standard used across NSW, Australia.

SPLAT CL 20% + Spinosad was used in trials 3 and 7 and was obtained pre-mixed from ISCA Tech. Approximately 0.02 g (Contact and feeding toxicity bio- assay – see below), 1 g (Cage attraction trial – see below), and 2 g (Field trials) of each SPLAT treatment was placed onto the surface of wooden ice-cream sampler sticks (1.7 by 9.5 cm^2; Stsellsok [A1 Packaging] Merrylands, NSW, Australia). For wicks-CL + malathion, 0.02 g was used in the laboratory bioassay, whereas the whole wick was suspended in cages and Lynfield traps for the cage attraction and field attraction trials, respectively. For blocks-CL + malathion, the whole block was suspended in Lynfield traps.

The required amount of each SPLAT treatment was applied to the surface of each stick using a spatula, and weighed using a balance, to two decimal places. A small hole was drilled in the non-treated end of each stick. Wooden sticks, wicks-CL + malathion, and blocks-CL + malathion were hung on a weathering line suspended between Lilly Pilly, *Syzygium smithii* (Poir.), trees in partially shaded locations and exposed to sunlight, wind, and rain at EMAI.

For each weathered trial, three treatments were tested for each of five aging periods (0, 1, 2, 4, and 8 weeks), with the exception of the field trials, which included an additional 12-week weathered treatment. At the prescribed intervals, each stick, wick or block was utilized in the relevant trial (see below).

Trial Protocol

Contact and Feeding Toxicity Bioassay (Trial 1)

A laboratory contact or feeding bioassay was used to determine the relative toxicity of the three differentially aged CL formulations described earlier, under standard conditions, using laboratory-reared F14–15 generation males. The method was modified from that of Vargas et al. (2009). Individual males were introduced into an experimental mesh cage (30 cm3) containing a particular treatment, gently placed onto the test material, and allowed to feed or come in contact with the lure for 1 min 30 s to 5 min. Only those feeding or in contact with the lure for >1 min 30 s were included in the analyses. After feeding or contact, each male was introduced into individual 500-ml plastic cups containing a cube of agar-based adult diet containing sugar and yeast hydrolysate and covered with fly proof mesh. Eight males were exposed in sequence to the same material; therefore, mortality after 2, 4, 24, and 48 h was calculated as a pro- portion (number of males dead/eight males that fed on or contacted a given treatment). For each of the five aging periods, there were five replicates of each of the three MAT CL treatments.

Cage Attraction Trial (Trial 2)

The relative toxicity of the three MAT CL treatments associated with a toxicant (spinosad or malathion) described earlier was quantified in 47.5- by 47.5- by 93-cm mesh cages (Bugdorm, Taiwan) deployed in a large covered open-sided field laboratory with plenty of airflow at the EMAI using laboratory-reared F15 generation male *B. tryoni* as described earlier. A blank treatment (untreated wooden stick) was also used to assess natural mortality. Cages were spaced at least 2 m apart. For each of the five weathered periods for each test period, a single wick or treated or untreated wooden stick was hung inside each of three mesh cages containing four sugar (sucrose) cubes as a source of carbohydrate and a 30-mm-diameter plastic Petri dish containing yeast hydrolysate enzymatic (as described above) and water *ad libitum*. Twenty-five laboratory-reared males were released per cage between 0900 and 1030 hours. After flies were released, an observer recorded the number of dead males at 4, 24, and 48 h after release. Four temporal replicates were carried out for each treatment–age combination. In total, 300 males were tested for each aging period, 100 males for each treatment–age period (i.e., 1,500 males total). The mean (±SE) trial temperature and RH were 23.5 ±0.1^{o} C and 65.0 ± 0.4%, respectively.

Field Cage Attraction Trial (Trial 3)

A field cage study was conducted utilising three walk-in field cages with walls and roof of white shade cloth, and a floor of white propylene, each enclosing two large potted citrus (Seville orange) plants, located at EMAI. Field cages were furnished with water and feeding stations hung from plant branches. Yeast hydrolysate and sugar *ad libitum* was provided as food. In each field cage, one Lynfield trap (Cowley et al., 1990) was suspended from plant branches 1–1.5 m above the ground. Each cage received one treatment, suspended in the single Lynfield trap, comprising either

(i) QFF Wick (Bugs for Bugs Mundubbera Australia) (active ingredients: 5ml cue-lure + 2ml maldison) maldison (Hy-MAL; Crop Care Australasia, QLD, Australia),

(ii) SPLAT CL 5% + Spinosad or

(iii) SPLAT CL 20% + Spinosad.

Lures were weathered for 0,1,2,4,or 8 weeks as described above. When flies were aged 7 to 10 days, 200 male flies were released into each field cage. Traps were checked on a daily basis at 10 am for 5 days after flies were released and mortality recorded. The mean (± SE) trial temperature, RH, and rainfall were 21.5 ± 0.1°C, 64.4 ± 0.3%, and 57.4 ± 0.3 mm, respectively.

Field Attraction Trial (non-weathered) (Trial 4)

A field trial was conducted over two periods; 5 February 2014 – 23 July 2014 and 19 November 2014 – 11 February 2015. The relative toxicity of the three *non-weathered* MAT CL treatments associated with a toxicant (spinosad or malathion), was quantified in a mixed fruit (pome, stone, and quince fruit) orchard located at EMAI, which had not been treated with any pesticide in over four years. Twelve Lynfield traps were spaced 20 m apart with three traps in each of four rows. Traps were suspended from wire, coated in Petroleum Jelly to prevent ants entering the traps, between 1.5 and 2 metres from the ground. Leaves and branches were cleared from around the trap. Each row held one of of the following MAT CL treatments, suspended in the trap in a random design:

SPLAT CL 5% + Spinosad,

ii) MAT block or

iii) MAT wick,

Lures were changed every 4 weeks. Traps were checked for fruit flies every 7 d and mortality recorded. The mean (± SE) trial temperature, RH, and rainfall for period 1 was 15.1 ± 0.1°C, 62.0 ± 0.2%, and 1.4 ± 0.4 mm, respectively and for period 2, was 22.5 ± 0.1°C, 66.5 ± 0.2%, and 3.8 ± 0.9 mm, respectively.

Field Attraction Trial (weathered) (Trial 5)

A field trial was conducted over two periods; 8 October 2014 – 12 November 2014 and 18 February 2015 – 25 March 2015. The relative toxicity of the three *weathered* (0,1,2,4,8 or 12 weeks) MAT CL treatments associated with a toxicant (spinosad or malathion) was quantified in a mixed fruit (pome, stone, and quince fruit) orchard located at EMAI.

SPLAT CL 5% + Spinosad,

ii) MAT block or

iii) MAT wick,

Eighteen Lynfield traps were spaced 20 m apart with three traps in each of six rows. Each row held one of each MAT CL treatments, suspended in the trap in a random design. Weathered lures were changed every 7 days, at which time the traps were checked for fruit flies and mortality recorded. The mean (± SE) trial temperature, RH, and rainfall for period 1 was 18.6±0.1°C, 62.1±0.4%, and 1.8±0.7mm respectively, and for period 2, 21.5±0.1°C, 68.4±0.32%, and 3.9±1.8mm respectively. The mean lure weathering climate (± SE) trial temperature, RH, and rainfall for period 1 was 14.3±0.0°C, 66.4±0.2%, and 2.1±0.7mm, and for period 2 was 22.2±0.0°C, 69.5±0.2%, and 3.3±0.7mm.

Wedderburn Orchard Trial (Trial 6a&b)

A field trial in a 2Ha mixed fruit orchard (comprising mainly peaches and nectarines but also apricots, plums, citrus (oranges and a small number of mandarins, limes & grapefruit), table & wine grapes, figs and apples (Granny Smith)), 'Wedderburn Orchard', Miverna Rd, Wedderburn (GPS Coordinates: 34 08 49.6" S, 150 48 52.8"E) was conducted from 9 December 2014 to 3 February 2015. The orchard was divided into 10 plots and 2 replicates (5 plots per replicate). Two cue-lure baited (Bugs for Bugs) Lynfield traps were placed centrally within each plot. Two plots were randomly treated with one of five treatments, i) MAT, ii) SPLAT 5%CL + spinosad, iii) SPLAT 5%CL + alphacypermethrin, iv) SPLAT 5%CL + malathion or v) Control (no treatment). The MATs used were Bugs for Bugs wicks, spaced evenly 20m apart (plot 1: 15 MATs, 0.25Ha; plot 2: 9 MATs, approx. 0.175Ha). All SPLAT treatment combinations comprised fortnightly sprays of two 'SPLAT's per tree, 2ml per SPLAT; each tree received total 4ml SPLAT placed on the trunk, at 1.5m height, inside the canopy (where possible). Every week, for 8 weeks, 10 organic whole sentinel stone fruit were suspended in mesh bags throughout each plot. White organic nectarines (Australian Certified Organic Producer 11268A) were used every week, with the exception of week 5 when organic yellow peaches (certified organic by Aus Qal Cert AQ610071) were used, as the nectarines were unavailable. Sentinel fruit remained in the orchard for 6 days, for the first 3 weeks, however concerns over larvae hopping prior to fruit collection and vertebrates consuming the fruit, meant that from weeks 4-8, fruit remained in the orchard for 3 days before collection. Sentinel fruit was placed in a controlled environment room (see Insects above) and placed, individually over moistened vermiculite to determine the number of pupae (count) and flies (identification, count & sex) that eclosed. The mean (± SE) trial temperature, RH, and rainfall were 22.4 ± 0.1°C, 52.5 ± 0.3%, and 1.4 ± 0.5 mm, respectively.

Field Attraction Trial (un-weathered) (Trial 7)

A field trial was conducted from 18 March 2015 – 3 June 2015. The relative attractiveness, of three *non-weathered* MAT CL treatments without a toxicant, i) SPLAT CL 5% no toxicant, ii) SPLAT CL 20% no toxicant or iii) MAT NSW DPI wick no toxicant, was quantified in a mixed fruit (pome, stone, and quince fruit) orchard located at EMAI. Nine Lynfield traps were spaced 20 m apart with three traps in each of three rows. Each trap had a 10 mm square Dichlorvos-impregnated strip in the bottom of the trap to kill the flies entering the traps. Traps were suspended from Petroleum Jelly coated wire, to prevent ants entering the traps, between 1.5 and 2 metres from the ground. Leaves and branches were cleared from around the trap. Each row held one of each MAT CL treatments, suspended in the trap in a random design. Lures were changed and traps catches cleared every 7 days. Fly identification and mortality counts were recorded. The mean (± SE) trial temperature, RH, and rainfall were 14.5 ± 0.1°C, 65.6 ± 0.3%, and 26.8 ± 4.8 mm, respectively.

Statistical Analyses ***Trials 1-3***

Data (proportion of QFF mortality) were analysed using a Generalized Linear Mixed Model (GLMM) to compare the effects of SPLAT and other pesticides at different weathering periods. A logit link was used to relate the observe values and the explanatory variables and all parameters were estimated using a residual maximum likelihood (REML) technique. Because zero or 100 percent mortality inflates the weighting factor of logit transformed data a small value (0.25/N) was added to zero proportion and subtracted from 100%. Multiple comparison tests between treatments were made using the Least Significant Difference (LSD) test at 5% level on the logit scale.

Trial 4

Data were then analysed using a generalized linear mixed model with errors assumed to follow a Poisson distribution. Fix effects include treatment and sampling time (weeks) whereas random effects include replicates, interactions between replicates and treatment and sampling time, and traps. All parameters were estimated using a residual maximum likelihood (REML) technique. Individual pair-wise treatment comparisons were tested using a least significant difference (LSD) test at 5% significant level.

Trial 5

Number of insects (male QFF) were analysed using a generalized linear mixed model with errors assumed to follow a Poisson distribution. A logarithmic link function was used to relate the numbers with chemical and weathering duration fixed effects and replicate random effects. A residual maximum likelihood (REML) method was used to estimate all parameters and treatment mean differences were made on log scale at 5% significance level.

Trial 6

The variables, adult male and adult female, were pooled before analysis. Due to large numbers of zero readings from each fruit, insect counts were totaled within each plot. Data were analysed using Generalized linear mixed model with errors assumed to follow a Poisson distribution. Fixed effect factors include treatment and time (weeks) whereas the random terms include the interaction between treatment and time and plots. A logarithmic link function was used to relate the response variable to the random terms and fixed factors. To reduce variance heterogeneity a small value 0.1 was added to the data, though a dispersion factor was included to accommodate any remaining heterogeneity.

Trial 7

Data were analysed using a generalized linear mixed model with errors assumed to follow a Poisson distribution. Fix effects include treatment and sampling time (weeks) whereas random effects include replicates, interactions between replicates and treatment and sampling time. All parameters were estimated using a residual maximum likelihood (REML) technique. Individual pair-wise treatment comparisons were tested using a least significant difference (LSD) test at 5% significant level.

Detailed procedure of generalized linear mixed models is described in Schall (1991).

Schall, R. (1991). Estimation in generalized linear models with random effects. Biometrika 78: 719-727.

Appendix 2. Contact and Feeding Toxicity Bioassay Results (Trial 1)

Mortality (logit(P) ± SE and mean (%)) of male Queensland fruit fly, *Bactrocera tryoni* allowed to feed or contact (1min 30sec – 5 mins) weathered SPLAT-CL + spinosad, SPLAT- CL (no toxicant) or wick-CL + malathion after 2h, 4h, 24h and 48h.

Treatment	Weathering period (weeks)	Logit (P) ± SE and mean (%) mortality							
		2h	2h	4h	4h	24h	24h	48h	48h
SPLAT - CL (no toxicant)	0	-5.65±1.69	0 f	-6.33±2.52	0 e	-6.11±2.63	0 e	-3.85±1.04	2.5 d
	1	-5.65±1.69	0 f	-6.33±2.52	0 e	-6.11±2.63	0 e	-5.86±2.47	0 e
	2	-3.65±0.68	2.5 e	-4.28±1.15	2.5 d	-4.09±1.15	2.5 d	-3.85±1.04	2.5 d
	4	-5.65±1.69	0 f	-6.33±2.52	0 e	-4.09±1.15	2.5 d	-3.85±1.04	2.5 d
	8	-5.65±1.69	0 f	-6.33±2.52	0 e	-4.09±1.15	2.5 d	-3.85±1.04	2.5 d
SPLAT - CL + spinosad	0	1.45±0.37	80 b	2.3±0.77	87.5 b	3.98±1.14	97.5 b	3.86±1.04	97.5 b
	1	0.43±0.34	60 c	1.53±0.71	77.5 b	2.57±0.8	90 b	5.87±2.47	100 a
	2	-0.32±0.34	42.5 c	1.38±0.71	75 b	2.89±0.85	92.5 b	5.87±2.47	100 a
	4	-2.01±0.41	12.5 d	-0.99±0.71	30 c	0.04±0.68	50 c	0.84±0.58	67.5 c
	8	-5.65±1.69	0 f	-2.17±0.78	15 c	-1.06±0.71	30 c	-0.35±0.57	42.5 c
Wick (CL + malathion)	0	5.65±1.69	100 a	5.97±2.5	100 a	5.99±2.62	100 a	5.87±2.47	100 a
	1	5.65±1.69	100 a	5.97±2.5	100 a	5.99±2.62	100 a	5.87±2.47	100 a
	2	5.65±1.69	100 a	5.97±2.5	100 a	5.99±2.62	100 a	5.87±2.47	100 a
	4	5.65±1.69	100 a	5.97±2.5	100 a	5.99±2.62	100 a	5.87±2.47	100 a
	8	5.65±1.69	100 a	5.97±2.5	100 a	5.99±2.62	100 a	5.87±2.47	100 a

Treatment means were compared on the logit scale.
Means within columns followed by the same letter were not significantly different using LSD, $P \leq 0.05$.

Appendix 3. Outdoor Cage Attraction Trial (Trial 2)

Mortality (logit (P) ± SE and mean (%)) of male Queensland fruit fly, *Bactrocera tryoni* exposed continuously to weathered SPLAT- CL + spinosad, SPLAT- CL (no toxicant; control), wick-CL + malathion or nil treatment (blank) at 4h, 24h and 48h.

Treatment	**Weathering period (weeks)**	**Logit (P) ± SE and mean (%) mortality**					
		4h	4h	24h	24h	48h	48h
No treatment (blank)	0	-4.60±1.21	0.00 d	-4.03±0.94	1.00 c	-2.89±0.59	5.00 de
SPLAT- CL (no toxicant; control)	0	-4.60±1.21	0.00 d	-3.06±0.59	4.00 c	-2.51±0.50	7.00 d
	1	-4.60±1.21	0.00 d	-4.60±1.24	0.00 c	-3.06±0.64	4.00 de
	2	-4.60±1.21	0.00 d	-4.03±0.94	1.00 c	-4.03±1.01	1.00 e
	4	-4.57±1.21	0.00 d	-3.64±0.79	2.09 c	-1.69±0.37	15.87 d
	8	-4.60±1.21	0.00 d	-4.60±1.24	0.00 c	-3.06±0.64	4.00 de
SPLAT - CL + spinosad	0	-0.43±0.25	39.42 ab	0.39±0.25	59.58 a	1.85±0.39	87.00 ab
	1	-0.24±0.24	44.00 a	0.62±0.26	65.00 a	2.55±0.51	93.00 a
	2	-0.92±0.28	29.00 abc	0.11±0.25	54.25 ab	1.26±0.33	78.50 bc
	4	-0.77±0.26	31.96 abc	0.37±0.25	59.46 a	1.43±0.34	80.88 abc
	8	-0.87±0.27	29.50 abc	0.00±0.25	50.04 ab	1.18±0.32	76.58 bc
Wick -CL + malathion	0	-1.00±0.27	27.00 bc	0.12±0.25	53.00 ab	1.88±0.39	87.00 ab
	1	-1.44±0.31	19.12 c	-0.35±0.25	41.17 b	0.88±0.29	70.54 c
	2	-0.85±0.26	30.00 abc	0.24±0.25	56.00 ab	1.66±0.36	84.00 abc
	4	-0.82±0.26	30.77 abc	0.30±0.25	57.50 ab	1.28±0.32	78.15 bc
	8	-0.74±0.26	32.38 abc	-0.02±0.25	49.50 ab	1.37±0.33	79.83 bc

Treatment means were compared on the logit scale.
Means within columns followed by the same letter were not significantly different using LSD, $P \leq 0.05$.

Appendix 4. ***Field Cage Attraction Trial (Trial 3)***

Mortality (%) of male Queensland fruit fly, *Bactrocera tryoni* exposed to fresh MAT, SPLAT CL 5%+spinosad or SPLAT CL 20%+spinosad after 24h, 48h, 72h, 96h or 120h.

Treatment	Weathering period (weeks)	Mortality (%) 24h	Mortality (%) 48h	Mortality (%) 72h	Mortality (%) 96h	Mortality (%) 120h
MAT	0	40.5 abc	49.5 ab	55.4 abc	60.5 abc	66.8 abc
MAT	1	18.6 cd	26.1 bc	30.3 cde	33.9 cd	35.9 de
MAT	2	16.2 cd	25.9 bc	35.6 cde	41.5 cd	48.2 cde
MAT	4	9.0 d	17.5 c	24.6 de	31.4 cd	39.4 cde
MAT	8	15.6 cd	23.9 bc	38.1 cde	44.4 cd	51.5 cde
SPLAT CL 5%+spinosad	0	55.3 ab	65.8 a	73.3 ab	74.9 ab	81.5 ab
SPLAT CL 5%+spinosad	1	26.3 bcd	34.0 bc	38.8 cde	42.0 cd	46.1 cde
SPLAT CL 5%+spinosad	2	19.4 cd	30.0 bc	37.4 cde	44.7 cd	52.2 cde
SPLAT CL 5%+spinosad	4	15.1 cd	34.3 bc	46.4 bcd	53.3 bc	63.7 abcd
SPLAT CL 5%+spinosad	8	17.3 cd	28.6 bc	44.0 bcde	50.2 bcd	57.1 bcde
SPLAT CL 20%+spinosad	0	62.9 a	71.2 a	79.3 a	81.9 a	86.2 a
SPLAT CL 20%+spinosad	1	25.6 bcd	31.8 bc	37.4 cde	41.8 cd	46.6 cde
SPLAT CL 20%+spinosad	2	19.0 cd	26.9 bc	35.4 cde	41.2 cd	47.1 cde
SPLAT CL 20%+spinosad	4	12.7 d	25.4 bc	31.3 cde	36.4 cd	45.1 cde
SPLAT CL 20%+spinosad	8	7.4 d	12.5 c	18.4 e	22.5 d	28.8 e

Means within columns followed by the same letter were not significantly different using LSD, $P \leq 0.05$.

Appendix 5. ***Field Attraction Trials (Trial 4)– non-weathered***

Lynfield trap (baited with either non-weathered MAT Block, MAT Wick or SPLAT 5% CL + spinosad) captures (logit (P) ± SE and mean (%)), of adult male Queensland fruit fly, *Bactrocera tryoni* and *Dacus aequalis*, each week from 12 February 2014 – 23 July 2014 and 26 November 2014 – 11 February 2015. Lures were changed every 4 weeks.

		Logit (P) ± SE and mean (backtransformed) trap captures									
Treatment	Male fly species	Week					Week				
		1	2	3	4	Overall	1	2	3	4	Overall
MAT Block	*B. tryoni*	1.105±0.35a	0.988±0.35a	0.669±0.35a	0.972±0.35a	0.934 ±0.20a	3.02	2.69	1.95	2.64	2.54
MAT Wick	*B. tryoni*	0.547±0.35a	0.281±0.35b	0.307±0.35a	0.508±0.35a	0.411±0.20b	1.73	1.32	1.36	1.66	1.51
SPLAT 5% CL	*B. tryoni*	1.172±0.35a	1.337±0.35a	0.992±0.35a	1.160±0.35a	1.165 ±0.20a	3.23	3.81	2.70	3.19	3.21
MAT Block	*D. aequalis*	-1.817±0.56A	-1.479±0.56A	-1.319±0.56A	-1.282±0.56A	-1.475±0.31A	0.16	0.23	0.27	0.28	0.23
MAT Wick	*D. aequalis*	-2.138±0.56A	-2.073±0.56A	-1.489±0.56A	-1.656±0.56A	-1.839±0.31A	0.12	0.13	0.23	0.19	0.16
SPLAT 5% CL	*D. aequalis*	-2.735±0.56A	-1.238±0.56A	-1.364±0.56A	-1.070±0.56A	-1.602±0.31A	0.06	0.29	0.26	0.34	0.20

Treatment means were compared on the logit scale.

Means within columns followed by the same letter were not significantly different using LSD, P=0.05.

Male *B. tryoni*: There was a strong evidence that treatment effects are significantly different (P<0.001), consistently over the 4 sampling times (P=0.97). Time effects were not significant (P=0.51). Male *D. aequalis*: There was no evidence of a significant difference between the three treatments (P=0.437),) or a treatment x sampling time interaction (P=0.652), although sampling time was marginal (P=0.051).

Appendix 6. ***Field Attraction Trials (Trial 5) – weathered***

Trap captures of wild male Queensland fruit fly, *Bactrocera tryoni* in Lynfield traps baited with non-weathered and weathered CL treatments at Menangle, New South Wales, Australia in 2015.

Treatment		**Weathering period (weeks)**					
		0	**1**	**2**	**4**	**8**	**12**
SPLAT – CL + spinosad	Mean (%)	9.10abA	4.47abBC	6.45bAB	6.29bAB	10.76aA	2.32bC
Wick- CL + malathion		5.46bAB	2.32bC	4.63bAB	3.31bBC	2.32bC	8.61aA
Block- CL + malathion		14.40aA	7.12aB	13.57aA	17.21aA	11.59aAB	2.98bC
SPLAT – CL + spinosad	Logit (P)	2.21±0.20	1.50±0.27	1.86±0.23	1.84±0.23	2.38±0.18	0.84±0.37
Wick- CL + malathion		1.70±0.25	0.84±0.37	1.53±0.27	1.20±0.31	0.84±0.37	2.15±0.20
Block- CL + malathion		2.67±0.16	1.96±0.22	2.61±0.16	2.85±0.15	2.45±0.18	1.09±0.33

Means within columns followed by the same lowercase letter and means within rows followed by the same upper case letters were not significantly different using LSD, $P \leq 0.05$.

Appendix 7a. ***Wedderburn Orchard Trial (Trial 6) – trap catches***

The mean number of Queensland fruit fly, *Bactrocera tryoni*, caught in cue-lure baited Lynfield traps, placed within plots treated with MAT, SPLAT 5% CL+Spinosad, SPLAT 5% CL+Cypermethrin, SPLAT 5% CL+Malathion or Control (No treatment) each week.

		Week										
Treatment		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Overall mean**
MAT	Logit (P)	0.528±0.80	0.362±0.80	1.248±0.80	1.702±0.80	2.121±0.80	1.051±0.80	-0.292±0.80	0.795±0.80	1.120±0.80	1.909±0.80	1.054±0.26a
SPLAT 5% CL+Spinosad		-0.015±0.80	0.557±0.80	1.828±0.80	1.856±0.80	2.009±0.80	1.177±0.80	-0.693±0.80	1.248±0.80	1.426±0.80	1.784±0.80	1.118±0.26a
SPLAT 5% CL+Cypermethrin		0.557±0.80	-0.015±0.80	1.857±0.80	2.084±0.80	2.387±0.80	1.327±0.80	0.362±0.80	1.619±0.80	1.476±0.80	1.870±0.80	1.353±0.26a
SPLAT 5% CL+Malathion		1.149±0.80	-0.693±0.80	2.498±0.80	2.425±0.80	2.213±0.80	1.740±0.80	0.307±0.80	2.012±0.80	1.354±0.80	1.875±0.80	1.488±0.26a
Control (No Treatment)		1.010±0.80	0.000±0.80	2.031±0.80	1.422±0.80	1.942±0.80	1.010±0.80	-1.393±0.80	1.508±0.80	1.504±0.80	1.687±0.80	1.072±0.26a
MAT	Mean	1.70	1.44	3.49	5.49	8.34	2.86	0.75	2.21	3.06	6.75	2.87
SPLAT 5% CL+Spinosad		0.99	1.75	6.22	6.40	7.46	3.25	0.50	3.49	4.16	5.95	3.06
SPLAT 5% CL+Cypermethrin		1.75	0.99	6.41	8.04	10.88	3.77	1.44	5.05	4.38	6.49	3.87
SPLAT 5% CL+Malathion		3.16	0.50	12.16	11.30	9.14	5.70	1.36	7.48	3.87	6.52	4.43
Control (No Treatment)		2.75	1.00	7.62	4.15	6.97	2.75	0.25	4.52	4.50	5.40	2.92

Treatment means were compared on the logit scale.
Means within columns followed by the same lowercase letter and means within rows followed by the same upper case letters were not significantly different using LSD, P=0.05.
There was no evidence that treatment effects are significantly different (P=0.252), consistently over the 4 sampling times (P=0.99). Time effects were highly significant (P<0.001).

Appendix 7b. ***Wedderburn Orchard Trial (Trial 6) - sentinel fruit***

The mean number of larvae, pupae and adults obtained from sentinel fruit placed in treatment plots at Wedderburn Orchard, NSW over an 8 week period, 9 December 2014 to 3 February 2015.

	Mean (log transformed)				Mean (back transformed; 10 fruits)			
Treatment	**Larvae (dead)**	**Pupae**	**Pupae (dead)**	**Adult**	**Larvae (dead)**	**Pupae**	**Pupae (dead)**	**Adult**
Control (No treatment)	-2.724±1.64	1.317±1.12	-1.474±1.36	0.514±1.22	0.07a	3.73a	0.23a	1.67a
SPLAT 5%CL + spinosad	-2.417±1.64	0.157±1.12	-2.585±1.36	-0.311±1.22	0.09a	1.17a	0.08a	0.73a
SPLAT 5%CL + alphacypermethrin	-2.259±1.64	0.410±1.12	-0.887±1.36	-1.009±1.22	0.10a	1.51a	0.41a	0.36a
SPLAT 5%CL + malathion	-2.302±1.64	0.814±1.12	-1.363±1.36	-0.472±1.22	0.10a	2.26a	0.26a	0.62a
MAT Wick	-2.252±1.64	-1.392±1.12	-2.311±1.36	-2.170±1.22	0.11a	0.25a	0.10a	0.11a

Means within columns followed by the same lowercase letter and means within rows followed by the same upper case letters were not significantly different using LSD, $P \leq 0.05$.

Appendix 8. Scientific refereed publication

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Research article

Horticultural Entomology

Specialized Pheromone and Lure Application Technology as an Alternative Male Annihilation Technique to Manage *Bactrocera tryoni* (Diptera: Tephritidae)

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Abstract

The results of this study suggest that a novel male annihilation technique (specialized pheromone and lure application technology [SPLAT] incorporating cue-lure [CL] plus spinosad) is as effective as industry standard male annihilation controls, and is worth exploring further to manage *Bactrocera tryoni* (Froggatt) populations. Three lures were evaluated in a contact and feeding bioassay and a cage attractancy trial: 1) SPLAT-CL + spinosad; 2) SPLAT-CL without spinosad; and 3) wick-CL + malathion. In a field attraction trial, lures (1) and (3) were evaluated with a third treatment, caneite blocks-CL + malathion. Lures were weathered for 0, 1, 2, 4, or 8 wk, with an additional weathering treatment of 12 wk included in the field trial. In the contact and feeding bioassay, lures with SPLAT-CL + spinosad were >97% effective at 48h for up to 2wk weathering; however, wicks-CL + malathion killed *B. tryoni* within 2h of exposure under all weathering periods. In the cage attractancy trial, SPLAT-CL + spinosad was as effective as, or performed better than, wicks-CL + malathion under all weathering treatments. The field study trap catches were similar for SPLAT-CL + spinosad and blocks-CL + malathion, and both had higher trap catches than wicks-CL + malathion at all weathering periods, except week 12. Overall, SPLAT-CL + spinosad compared favorably with current standard techniques for male annihilation and warrants further research. SPLAT-CL + spinosad may be a reduced-risk alternative for wicks-CL + malathion or blocks-CL + malathion for *B. tryoni* and other CL-responding fruit flies, such as *Bactrocera cucurbitae* Coquillett, because it contains a reduced-risk insecticide that poses a lower risk to humans and the environment and does not require labor-intensive handling and placement.

Key words: specialized pheromone and lure application technology, male annihilation technique, malathion, spinosad, Queensland fruit fly

Fruit flies (Diptera: Tephritidae) constitute some of the world's most significant pests of horticulture (Sutherst et al. 2000). The Queensland fruit fly, *Bactrocera tryoni* (Froggatt), is the most significant biosecurity threat to national and international market access for horticultural commodities produced in eastern and south-eastern Australia (Plant Health Australia [PHA] 2008). The polyphagous nature of this pest, recorded on >240 plant species from 49 families (White and Elson-Harris 1992, Hancock et al. 2000), its climatic adaptability, and the expansion of its cultivated host range have, among other factors, enabled its spread throughout northern and eastern Australia. Economic losses owing to *B. tryoni* were estimated at AU$28.5 million annually in 2000, rising to an estimated AU$100 million in lost production if the population is uncontrolled (Sutherst et al. 2000).

In the early 1900s, the notion of managing fruit flies by targeting males was proposed (Froggatt 1909). This concept of "male annihilation" is based upon reducing the number of males available for mating in the population, thereby reducing the number of mated females and breaking the life cycle. The male annihilation technique (MAT) is a form of "attract and kill," whereby an attractant "lure" is included in some form of matrix, which also includes a toxicant. Without an attractant or lure, effective management of tephritids is difficult. In the late 1950s, Willison [unpublished data, as cited by Bateman (1966)] found that males of *B. tryoni* were attracted to a

Appendix 9. Images of SPLAT treatment methods

Fig 1. SPLAT treatments weathering in Lilly Pilly trees at EMAI, Menangle, NSW. Photo credit: Olivia Reynolds.

Fig 2. Male Queensland fruit fly attracted and feeding on a SPLAT treatment weathering in a Lilly Pilly tree at EMAI, Menangle, NSW. Photo credit: Olivia Reynolds.

Fig. 3. A Bugs for Bugs wick used throughout several trials conducted at EMAI, Menangle, NSW. Shown here with a male Queensland fruit fly. Photo credit: Olivia Reynolds.

Fig 4. A MAT block (NSW DPI standard) suspended in Lilly Pilly trees, used throughout several trials, located at EMAI, Menangle. Photo credit: Terry Osborne.

Fig 5. Male Queensland fruit flies feeding on SPLAT 5%CL+Spinosad on a Seville Orange branch. Photo credit: Olivia Reynolds.

Fig. 6. Lynfield trap baited with weathered SPLAT 5%CL (on wooden dispenser), located in the EMAI Orchard, Menangle, NSW. Photo credit: Terry Osborne.