Horticulture Innovation Australia

Final Report

Farm-wide fruit fly management systems for the east coast of Australia

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Summary

Fruit Fly management in the field has previously relied on relatively cheap effective cover sprays to control Australian pest fruit fly species. These chemicals have now been banned or restricted. Consequently growers require alternative control options for fruit fly.

This project aimed to test a Farm-wide management systems approach to fruit fly control in mangoes based on protein baiting, male annihilation and packhouse grading. A research component aimed to improve aspects within the system.

Application of protein bait sprays to vegetation surrounding a crop is common practice for control of melon fly in Hawaii (Prokopy *et al*, 2003; McQuate & Vargas, 2007) and has recently been evaluated for Queensland fruit fly in strawberry crops (Missenden, 2014). Research evaluated components of a perimeter baiting system for vegetables.

The farm-wide management systems approach based on male annihilation and protein baiting was evaluated at mango farms on the Atherton Tablelands and Bowen. A research component aimed to improve aspects of the system. This included: (1) evaluation of possible non blemishing protein baits; (2) protein bait efficacy; (3) design a bait delivery system suitable for mango; and (4) develop bait station technology and evaluation of male annihilation devices.

A series of small-scale trials was performed to evaluate components of a perimeter baiting system for cucumber fly and Queensland fruit fly in vegetables. In addition, trapping was carried out to assess a cucumber volatile blend (Siderhurst & Jang, 2010) for monitoring and to determine the most effective trap type.

The farm-wide management system was successfully implemented at one of the selected orchards. Fruit fly populations were reduced and damage was limited to four fruits that had previous skin damage. Atherton Tablelands growers did not accept the trunk baiting option. The Bowen grower considered the system feasible and successful. Protein baits evaluated for mango caused unacceptable blemish to skins. A prototype protein bait trunk applicator was designed; however, tree training is required to ensure overhanging fruit do not obstruct the applicator. FT Mallett-CL wafer was shown to be the most effective male lure device. HYM-LURE[TM] protein bait was shown to be the most attractive of the least phytotoxic proteins. The bait station containing 100ml Cera Trap[®] solution and maldison toxicant was the most attractive bait station.

The perimeter baiting vegetable component of the project evaluated several proteins and toxicants for adding to protein baits. Four of the seventeen protein baits were shown to be most attractive to cucumber fly and Queensland fruit fly. Spinetoram, fipronil, clothianidin and abamectin were as effective protein bait toxicant. Forage sorghum and cassava with protein baits applied at above 1.5 m were the best shelter plant/bait height combination for cucumber fly and Queensland fruit fly. Cucumber fly dispersal studies showed that ovipositing within 24 hours of release occurs close to the shelter plant with highest activity between 7–10 am. The cucumber lure captured male and female cucumber flies at several sites and the best trap type was the McPhail trap.

The farm-wide system was successfully implemented at one of the field sites. However, it is recommended that an alternative approach to protein baiting, e.g. protein bait stations be developed for this approach to be more acceptable to mango growers. Other aspects to the system, e.g. male annihilation, could also be improved with more effective devices.

The results of the small scale perimeter baiting studies indicate that this method of control for fruit flies in vegetable crops is possible. However, it is recommended that further research evaluate different models of different models of a perimeter baiting system in large field plots.

Keywords

Fruit fly, *Bactrocera tryoni*, *Bactrocera jarvisi*, *Bactrocera cucumis*, protein baits, cuelure, farm-wide management, perimeter baiting

Introduction

Fruit Fly management in the field has previously relied on relatively cheap effective cover sprays to control all of the important pest fruit fly species endemic to Australia. These chemicals have now been banned or restricted and no alternate chemicals with the same level of efficacy are yet available. Growers are also moving towards systems that guarantee residue free fruit, and consequently the interest in alternative control options for fruit fly, both for field control and market access are becoming more important. The Central Burnett area in Queensland has been trading domestically for many years using a system of bait sprays, male annihilation and pack-house grading to control fruit fly in citrus orchards (Lloyd et al, 2010). It was believed that this system would not be effective in other areas in Queensland, particularly wetter coastal areas where native fruit fly hosts are abundant, and when the host crop is more susceptible to fruit fly attack. Recent work conducted by DAF Queensland in Indonesia has demonstrated that the use of baits and lures can also be very effective in tropical regions and there is renewed interest in demonstrating the same efficacy for cropping systems along the east coast of Australia.

This project aimed to test a farm-wide management systems approach to fruit fly control in mangoes based on protein baiting, male annihilation and packhouse grading. Farm-wide management systems approaches were evaluated on mango orchards on the Atherton Tablelands and Bowen. A research component aimed to improve aspects within the system. The mango skin suffers a phyto-chemical reaction to protein baits. Several options to overcome this issue were investigated as follows: (1) testing of new protein baits that may not cause phytotoxicity; (2) evaluate the efficacy of any promising protein baits against the major pest fruit flies; (3) develop a protein bait spray delivery system designed for mangoes; and (4) develop bait station technology as an alternative to protein baits. Several male annihilation technique (MAT) devices were evaluated for efficacy against Queensland fruit fly, *Bactrocera tryoni* to optimise this control method.

Fruit fly control in vegetable crops has also been based on insecticide cover sprays. The major fruit fly pests of fruiting vegetables are the Cucumber fly, *B. cucumis* and the Queensland fruit fly, *B. tryoni*. Control of *B. Cucumis* is also hampered by the fact that there is currently no commercially available lure to allow pest monitoring. Application of protein bait sprays to vegetation surrounding a crop is common practice for control of melon fly, *B. cucurbitae*, in Hawaii (Prokopy *et al*, 2003; McQuate & Vargas, 2007) and has recently been evaluated for Queensland fruit fly *B. tryoni* in strawberry crops (Missenden, 2014).

A series of small-scale trials was performed to evaluate components of a perimeter baiting system for *B. cucumis* and *B. tryoni* control. In addition, a cucumber volatile blend, recently identified as an effective female-biased attractant for melon fly, *B. cucurbitae* in Hawaii (Siderhurst & Jang, 2010), has shown to have potential for use with *B. cucumis* in Australia (Royer *et al*, 2014). Trapping was carried out to assess this lure for monitoring and to determine the most effective trap type.

Methodology

On-farm evaluation of farm-wide management techniques for control of fruit flies in mango (Appendix 1)

A workshop for potential grower participants was conducted on 1/8/2013. The aims of the workshop were to inform growers of the implementation requirements, the planned outcomes and results from area-wide management in other areas. Four farms were then selected to trial the farm-wide fruit fly management approach to fruit fly control, one at Bowen, two at Dimbulah and one at Mutchilba.

The system included: (a) male lures (MAT cups; www.bugsforbugs.com.au) distributed twice annually (August and November) for cue lure (for *B. tryoni*) at the rate of 10/ha, and once annually for zingerone (for *B. jarvisi*) at the rate of 5/ha; (b) female protein bait application which differed between growers due to varying reluctance to applying protein bait sprays to tree trunks; and (c) monitoring male populations at each trial site with cue lure and zingerone traps at the treatment and control blocks. Fruit were collected at harvest from the Bowen trial site. Dimethoate treatments were applied at the Dimbulah 1 and Mutchilba farms, therefore harvest assessments were not carried out. In 2014/15 farm-wide treatments were only carried out at the Bowen site. Treatments were applied to the original block plus a new 25 ha block separate from the original block.

Comparison of male fruit fly lure technologies (Appendix 2)

The efficacies of four commercial male cue lure technologies were assessed for efficacy as MAT devices to test their efficacy as MAT devices. The replicated trials were conducted in mango orchards near Mareeba, north Queensland. In a separate replicated trial, the effect of weathered lures on efficacy was tested using the Mallett-CL wafer against the standard dental wick.

Research of protein bait phytotoxicity and bait application technologies (Appendix 3)

Two approaches were taken to resolve fruit blemish caused by the baiting treatment; (a) phyto-reactivity of potential proteins was assessed (Northern Territory and Queensland), and (b) bait application technologies that confined the bait to non-fruit areas of the tree were investigated. Research was also conducted to clarify aspects of the solution influencing the phytotoxic response.

Protein phyto-reactivity was assessed in replicated and non-replicated trials during the fruiting seasons of 2013 and 2014. Toxicants were included in treatments as appropriate. Autolysed yeast extract (Fruit Fly Lure™) was included as the control protein in all trials. This protein is commonly used in fruit fly control programs. With the exception of ANAMED™ SPLAT (protein) which was smeared on the skin, all proteins were applied to fruit as a coarse spray. Fruit were scored for phytotoxic blemish 24 hours after treatment application. Bait applicator design was conceptualized prior to the development of mature fruit in 2013. Collaborating farmers advised on fruit distribution on the tree and equipment operational requirements.

Efficacy of least phytotoxic protein baits for attracting *Bactrocera tryoni* and *B. jarvisi* fruit flies (Appendix 4)

Three protein baits that were shown to be the least phytotoxic to mango (cv. R2E2) in phytotoxicity assessments and a commercial protein bait which is very phytotoxic were assessed for efficacy in replicated small cage choice tests. The most efficacious protein bait was then compared to the commercial standard protein bait in replicated large flight cage choice tests. To replicate field situations the protein baits in the large cage trials contained a commercial toxicant. Small and large cage trials were performed with the mango pests *B. tryoni* and *B. jarvisi*.

Comparison of fruit fly protein baits and insecticides (Appendix 5)

Further small scale trials evaluated protein baits for use in perimeter baiting for vegetable crops. Fifteen protein baits were assessed for efficacy in preliminary replicated small cage choice tests against *B. cucumis* and *B. tryoni*. Five of these protein baits were then compared against two previously not tested protein baits in replicated small cage no choice tests.

Development of bait station technologies (Appendix 6)

Prototype protein bait stations based on Cera Trap® solution were assessed as an alternative to phytotoxic protein bait sprays. Initial replicated trials assessed the efficacy of six insecticides presented in a wax matrix bait station. The bait stations were aged and tested at 0, 3, 8 and 12 weeks aging periods in small laboratory cages. The efficacy of the bait stations in the wax matrix and in synthetic membrane at two rates, containing two of the cage tested insecticides and contained in BioTRAP globe traps were evaluated in replicated field trials at two properties on the Atherton Tablelands.

Evaluation of a perimeter baiting system for *Bactrocera cucumis* and *B. tryoni* and trap technologies for the management of *B. cucumis* (Appendix 7)

A series of small-scale trials evaluated components of a perimeter baiting system for management of *B. cucumis* and *B. tryoni*.

1. Five insecticides were assessed as protein bait toxicants in replicated small cage trials. The individual insecticides were mixed with a commercial protein bait.

2. Trials evaluated the attractiveness of plants as roosting sites and hence their potential as perimeter plantings for bait application. Assessments were made of the number of fruit flies feeding on protein bait applied to each of eight plant species.

3. Trials also evaluated the most appropriate height of bait application on the preferred roosting plants, forage sorghum and cassava.

Trials were performed to better understand the behaviour of *B. cucumis* in relation to a host crop plant (zucchini) and a perimeter plant (sorghum or cassava).

1. Trials were performed in a large netted area to determine the dispersal activity of *B. cucumis* from the roosting site to the cucurbit oviposition site. The level of infestation in zucchini fruit placed at varying distances from a sorghum border over a 24 hour period was assessed.

2. Trials were performed in a glasshouse bay to observe the diurnal activity of *B. cucumis* on a host crop plant (zucchini) and a perimeter plant (cassava). Counts of roosting, protein feeding and ovipositing fruit flies were made from dawn to dusk.

Trials also assessed the application of a new cucumber lure as a monitoring tool for *B. cucumis*. Cucumber lure traps were placed in cucurbit crops in the Lockyer Valley, Bundaberg and Bowen. Captured flies were collected weekly. Replicated Trials were also conducted in cucurbits on the Atherton Tablelands to determine the most effective trap type for this lure.

Outputs

On-farm evaluation of farm-wide management techniques for control of fruit flies in mango (Appendix 1)

- *B. tryoni* and *B. jarvisi* monitoring traps at FWM trial sites on the Atherton Tablelands (Dimbulah 1, Dimbulah 2 and Mutchilba) and Bowen showed that at all sites male fruit fly populations were lower in areas treated with male lures compared with those not treated (Figures 4, 5, 7 and 8).

- At the two Dimbulah sites in 2013/14, planned weekly protein bait treatments were not applied consistently by cooperating growers because of time constraints, and intervention by heavy rain which either prevented treatments or washed treatments from treated trunks.

- Similar numbers of female fruit flies in monitoring traps in the perimeter Cera Trap® treated area and the untreated area suggests that the treatment was ineffectual or that trap collections were pre-treatment residents (Figure 6).

- Post-harvest assessment of fruit from treated and untreated areas at Bowen in 2013/14 showed no evidence of eggs or larvae in fruit from the treated area. Six of the 929 fruit from the untreated area, all of reject grade, showed symptoms of oviposition site punctures. In 2014/15, larvae were only found in four skin-damaged fruit from the treated R2E2 treated crop which had only one season of suppression treatments. There were no infested fruit in the Honey gold treated area which received two seasons of treatments. (Table 4).

Comparison of male fruit fly lure technologies (Appendix 2)

- The efficacies of four male cue lure technologies and two dental wick treatments contained within fly trapping devices (BioTRAP Globe trap) were described in mango orchards.

- Of the three commercial lures compared, FT Mallett-CL wafer was shown to be the most effective for attracting male *B. tryoni* (Tables 2 and 3).

- Preliminary information was obtained describing change in FT Mallett-CL wafer lure efficacy with aging when used for male fruit fly annihilation and population monitoring, and the relative efficacies of this lure and the dental wick which is currently used for population monitoring.

- There was no decrease in FT Mallett-CL wafer lure's efficacy at 4 weeks when unprotected in-field (male fruit fly annihilation), but decrease in efficacy was detected at four weeks when enclosed within a trap (male fruit fly population monitoring) (Table 4). The wafer lure consistently attracted more *B. tryoni* than the wick lure over a 91 day monitoring period indicating it's superiority for monitoring populations of these male fruit fly species.

Research of protein bait phytotoxicity and bait application technologies (Appendix 3)

- The phyto-reaction of seven mango varieties to seven proteins of various formulations (paste, powder, and liquid) was assessed (Table 1).

- All proteins caused phytotoxic fruit blemish in assessments in North Queensland (Mareeba–Dimbulah). Selected proteins, some in common with those tested in Queensland, did not cause blemish in the Northern Territory (Table 2).

- Most severe blemish was associated with the paste and gel protein formulations (ANAMED™ SPLAT (protein), DacGel™) (Figure 1). Natflav 500™, HYM-LURE™ and CERABAIT™, at the recommended protein rate for Fruit Fly Lure™ (0.84%), were relatively less phytotoxic to mango (cv. R2E2) than Fruit Fly Lure™ (Table 2).

- The protein component of the protein bait solution (not the toxicant) was shown responsible for causing blemish.

- A prototype applicator was designed that applies bait to the tree trunk (Figure 2), an application point shown by Lloyd *et al* (2005) effective for control of fruit fly in mango. Tree training is needed to ensure low hanging fruit do not obstruct passage of the applicator beneath the tree canopy.

Efficacy of least phytotoxic protein baits for attracting *B. tryoni* and *B. jarvisi* fruit flies (Appendix 4)

- The attractiveness of CERABAIT™, HYM-LURE™ and Natflav 500™ (each at two protein concentrations) and Fruit Fly Lure™ proteins to *B. tryoni* and *B. jarvisi* was described in cage trials (Table 1). CERABAIT™, HYM-LURE™ and Natflav 500™ had been shown in previous research to be less phytotoxic to mango than Fruit Fly Lure™ (Appendix 3, Table 2).

- HYM-LURE™ was found the most attractive protein to both fruit fly species, attraction greater at 0.84% protein concentration compared with 0.42% (Tables 2, 3 and 4).

Comparison of fruit fly protein baits and insecticides (Appendix 5)

- The attractiveness of 17 proteins (Table 1) to female and male *B. cucumis* and *B. tryoni* was described.

- Natflav 500™, Fruit Fly Lure™, Flavex® (Liquid Type FL622) and Flavex® (Powder Type SPA400) were shown the most attractive proteins to both fly species (Figures 1–10).

- The effect of five insecticides (mixed with Fruit Fly Lure™ protein) on female and male *B. cucumis* and *B. tryoni* mortality assessed as alternatives to maldison (Table 2).

- Spinetoram, fipronil, clothianidin and abamectin gave similar *B. cucumis* mortality to maldison after 48 hours exposure, although abamectin was relatively slower to take effect (Table 3, Figure 11). Spinetoram, fipronil and abamectin gave greater *B. tryoni* mortality than maldison after 48 hours exposure (Table 4, Figure 12).

Development of bait station technologies (Appendix 6)

- The effect of six insecticides (Table 1), presented in a wax matrix (bait station), on female and male *B. tryoni* mortality was described.

- Maldison and fipronil caused greatest *B. tryoni* mortality at the first (freshly prepared bait) and last (bait 12 weeks old) assessments (Table 3). None of the insecticides compared lost efficacy over the 12 weeks of weathering (Table 4).

- The efficacies of three bait station designs (wax matrix, synthetic membrane and Cera Trap®) were compared in field trials.

- Cera Trap® caught the highest number of fruit flies of all the bait treatments (Tables 5 and 6). Synthetic membrane (100 ml protein and 5 ml maldison) was the best of the new bait station treatments.

- All bait treatments caught both *B. jarvisi* and *B. tryoni*, and also both sexes of these species (Tables 5–8). All bait treatments caught gravid (egg bearing) females of *B. jarvisi* and *B. tryoni*.

Evaluation of a perimeter baiting system for the management of *B. cucumis* and *B. tryoni* and trap technologies for monitoring *B. cucumis*. (Appendix 7)

- Plant species and feeding height preferences of *B. cucumis* and *B. tryoni* were described in cage trials.

- *B. cucumis* were shown to favour protein applied to sweet corn and forage sorghum (Figure 1), and bait placed at 1 m or 1.5 m height on the plant (Figure 3). *B. tryoni* were shown to favour protein applied to sugar cane and cassava (Figure 2), and bait placed at 2 m height on the plant (Figure 4, Table 1).

- The dispersal capability of *B. cucumis* from a sorghum roosting site into a host crop planting (zucchini) was described. Pupae counts in the host fruit showed that fly dispersal was mainly localised (< 10 m) during 24 hours from release (Figure 5).

- The daylight activity (roosting, protein feeding, and ovipositing) of *B. cucumis* on cassava and zucchini plants was described. Cassava plants, rather than zucchini, were preferred by *B. cucumis* for roosting and protein feeding (Figure 6). Oviposition was highest at 8.30 am, declining thereafter (Figure 7).

- Information describing *B. cucumis* behaviour in the field was obtained by weekly trapping in the Lockyer Valley, Bundaberg and Bowen regions.

- Significant *B. cucumis* catches were only recorded at Lockyer Valley site A (Table 2, Figure 8). Most were caught within the cucurbit crop at a distance of 55 m or greater from the headland during harvest. Outside of harvest, most flies were caught within the crop adjacent to the headland (Figure 9).

- Five trap treatments (trap design and insecticide) for control of *B. cucumis* were compared in two trials in cucurbit field crops (Table 3).

- In both trials, total *B. cucumis* numbers caught in the McPhail trap for the trapping period were higher than the other trap treatments tested (Table 4). This trap caught females and males and *B. cucumis*.

Outcomes

Male *B. tryoni* and *B. jarvisi* reduction in mango was achieved at a farm-wide scale using the commercially available male annihilation technology (male lures) distributed at recommended rates. While the effect of male reduction on male-female population dynamics and the potential risk to fruit quality was not quantified, the result justifies further research investment to improve the efficacy of lure techniques to reduce male fruit fly presence within orchards. The research identified a lure device potentially superior to the lure used in farm-wide management studies (Mallett-CL wafer), and further investment should be made to evaluate its effect on male fruit fly populations in similar studies. Contrary to Tablelands growers, the Bowen grower successfully applied protein baits to tree trunks. The issues (time and overspray risks) raised by the Tablelands growers did not concern the Bowen grower. The farm-wide management system based on male annihilation and protein baiting to tree trunks is feasible.

The research failed to find a bait protein non-toxic to mango fruit or develop workable application equipment to prevent protein contact with fruit. As a result, fruit fly baiting treatment preferences expressed by collaborating farmers during the course of the research has forced a rethink of fruit fly baiting technologies appropriate for mango. Collaborating farmers opinioned that even if protein toxicity was resolved allowing bait application to foliage, they would be disinclined to adopt lure/baiting practices in lieu of cover sprays. They preferred a single application technology (bait station) that controlled the female fly.

Research of several protein bait station concepts was initiated in the project, and while all concepts assessed displayed efficacy for attracting pest fruit flies, the commercially available Cera Trap® was superior in attracting both sexes, and gravid (egg bearing) females of *B. jarvisi* and *B. tryoni*. Opportunities to improve the delivery of the Cera Trap® attractant are currently being investigated.

At the same protein rates, HYM-LURE™ was shown to be less phytotoxic to mango and more attractive to *B. jarvisi* and *B. tryoni* than Fruit Fly Lure™ which is commonly used in fruit fly baiting programs. Its phytotoxic risk disqualifies its use in mango; however its use in other crops that are less reactive than mango warrants research.

The research clarified aspects important for the development of perimeter baiting systems for control of *B. cucumis* and B. *tryoni* in cucurbits; preferred roosting plant species for perimeter planting and bait application, efficacious proteins and insecticides for protein bait treatments, effective protein bait application height, and a lure and trap for monitoring *B. cucumis* populations. These findings need incorporating into a perimeter baiting system and validated under commercial conditions.

Evaluation and Discussion

This project has undertaken a broad range of research of critical aspects of fruit fly control in mango and cucurbits to support growers' interest in alternatives (to in-field cover sprays) that are affective and guarantee chemical free produce and market access. Current knowledge and technologies that can be employed to formulate systems approaches for fruit fly control in these crops based on attractants (lures and baits) is limited. Significantly, mango fruit's sensitivity to protein baits, and the lack of a commercially available male lure for *B. cucumis* have stymied the adoption of such systems.

Farm-wide management studies conducted during the project in mango using commercially available lures and protein baits identified research opportunities which could lead to definition of an effective system for this crop. Male *B. tryoni* and *B. jarvisi* population reduction was demonstrated at all four trial sites treated with male annihilation lures. Protein baiting tree trunks is possible and feasible in conjunction with male annihilation with no fruit damage following two years of treatment. The research gave preliminary information suggesting that a polymer matrix designed lure could be more efficacious than the lure used in the farm-wide management studies and should be researched further as greater male reduction could have a significant impact on fruit fly populations within treated areas.

All protein baits tested on mango in the project caused unacceptable phytotoxic blemish symptoms on fruit. To date, bait phytotoxicity research in mango has largely focused on screening protein products for phyto-reactivity. Protein hydrolysates and yeast autolysates of various formulations (gel, powder, and liquid) have been tested, and all have proven phytotoxic. It seems, therefore, that continued research of this nature has a low chance of finding a non-toxic protein, and if further protein research is contemplated, attention should be given to understanding the factors affecting the phytotoxic reaction. Inconsistent effects shown by protein (some less phytotoxic) and location (NT and Qld) suggest that such research could clarify whether the reaction can be influenced and how.

The idea of applying protein bait to foliage, as is done in other crops, was not favored by mango farmers collaborating in the project because of additional commitment of time, machinery and labor, particularly during harvest. Currently, cover sprays are applied for fruit fly control. For fruit produced under interstate certificate assurance, a minimum of three dimethoate sprays are required starting five weeks from harvest. In contrast, bait is applied during the latter stages of fruit maturation that includes the harvest period, weekly, or more frequently when rainfall reduces efficacy of applied baits. Future investment in baiting techniques should therefore be directed to developing bait station technologies that might require a single application, remain efficacious under rainfall conditions, and pose no risk to fruit quality.

Small-scale laboratory and semi-field trials evaluated protein baits, bait toxicants and bait application to perimeter plantings, as well as behavioural responses of *B. cucumis* and *B. tryoni* in relation to a crop plant and a perimeter plant. These trials have resulted in useful information about the potential efficacy of perimeter baiting in vegetables, such as the most effective baits, toxicants and application technique. Observations of *B. cucumis* and *B. tryoni* behaviour in small-scale trials indicated that in vegetables these species preferentially roost and forage for protein in tall vegetation, such as sorghum and cassava, rather than in a low-growing crop such as zucchini. This suggests that the behaviour of *B. cucumis* and *B. tryoni* in vegetables is similar to that of the melon fly, *B. cucurbitae*, dispersing over a short distance into a crop from a roosting site at the field margin (Nishida & Bess, 1957). Furthermore this suggests that application of protein bait plus toxicant to vegetation on the field margin should theoretically be an effective method of control.

However, the limitations of small-scale trials should be recognized. For instance, trials were of necessity performed using laboratory reared fruit flies. The behavioural activity of wild flies in the field cannot be replicated in small-scale arena trials. Balagawi *et al* (2012) suggested that protein baits were more attractive to fruit flies when applied to the fruiting host plants, however these studies were performed on tree crops. This project has provided initial research on the behavior of pest fruit flies in vegetable crops and further research should concentrate on behavior of fruit flies in the field to validate outcomes of this study.

Trapping of *B. cucumis* flies using the new cucumber lure was highly variable. Very few *B. cucumis* were recovered from the majority of trap sites, in contrast to the findings of Royer *et al* (2014). This may due to the different trap types used in the two studies. The McPhail trap was used in the Royer study and this trap type was shown to be the most efficacious. The trap catches showed a strong female bias. The dry traps although not as efficient as the McPhail trap require less servicing and the captured fruit flies do not spoil. Dry traps could be utilized in situations where regular clearing is not possible. The cucumber lure is thought to have short range attraction and should not be compared to the effectiveness of male lures for other species which attract fruit flies from greater distances. Trap placement may also play a significant role in effectiveness. Due to the roosting behavior of fruit flies in vegetables, the effectiveness of the lure may improve if placed beside shelter plants. Further research is required to evaluate the full potential of this lure.

Recommendations

The following recommendations are made to advance the development of farm-wide management systems for the control of pest fruit flies of mango and vegetables.

- Further trials are required to confirm Mallett-CL wafer use for male fruit fly annihilation in mango (e.g. period of in-field efficacy).

- Cue lure is a relatively weak attractant in comparison to methyl eugenol. Further gains can be made by the development of cuelure analogues that are more volatile. Further investment should be directed towards improving the attraction of this lure.

- Further research of protein phytotoxicity should include studies to understand the factors affecting the phytotoxic reaction.

- Future investment in baiting techniques should therefore be directed to developing 'limited' application technologies that pose no risk to fruit quality.

- Further trials are needed to determine the lowest rate of Cera Trap$^®$ solution needed to achieve optimal attraction from in protein bait stations.

- Research should also be directed towards determining the optimal density of bait stations in relation to the level of fruit fly pressure.

- Field trials need to be conducted to evaluate the efficacy of bait stations in large blocks and that other fauna are not attracted to the protein.

- Trials to generate data to allow registration of spinetoram, fipronil or abamectin as bait toxicants would give growers alternatives to the current limited options.

- Once the protein bait and male lure issues have been resolved it would be recommended that the Farm-wide systems approach be trialed on more properties and over a longer period. Two years is insufficient time to fully test this model. Trials against Indonesian fruit flies showed significant population reductions within the first two years but it wasn't until the third and fourth years that populations were maintained at significantly low levels.

- Large field trials are required to evaluate a perimeter baiting system in vegetable crops. Firstly, the behavior of fruit flies in relation to shelter plants and dispersal into fruit crops should be observed in the field. Secondly, field trials of a model perimeter baiting system should be evaluated.

- Further research on the cucumber lure would determine the best method of utilising it as a monitoring tool.

For mango export markets such as the United States of America that require zero fruit residues of dimethoate and trichlorfon, it is recommended that growers consider implementing male annihilation techniques and protein baiting for pest fruit fly (*B. tryoni* and *B. jarvisi*) control to alleviate the risk of residues from crop cover sprays of these chemicals. These are:

- Male fruit fly annihilation treatments using commercial cure lure lures at the rate of 10/ha for *B. tryoni* and zingerone lures at 5/ha for *B. jarvisi*.

- Trunk sprays containing autolyzed yeast and malathion insecticide to attract and kill female fruit flies.

These technologies should be used in conjunction with: crop monitoring of fruit fly presence using commercial cue lure and zingerone lures and traps for male flies, and Cera Trap® traps for female fruit flies; field hygiene (removal and destruction of ripe and fallen fruit); and pack-house culling of damaged, ripe and fruit fly affected fruit.

Scientific Refereed Publications

No scientific refereed publications were generated.

Intellectual Property/Commercialisation

No commercial IP generated.

References

Balagawi, S., Jackson, K., Hamacek, E.L., Clarke, A.R., 2012. Spatial and temporal foraging patterns of Queensland fruit fly, *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae), for protein and implications for management. *Australian Journal of Entomology* 51, 279-288.

Balagawi, S., Jackson, K., Clarke, A.R., 2014. Resting sites, edge effects and dispersion of a polyphagous *Bactrocera* fruit fly within crops of different architecture. *Journal of Applied Entomology* 138, 510-518.

Clarke, A.R., Powell, K.S., Weldon, C.W., Taylor, P.W., 2011. The ecology of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae): what do we know to assist pest management? *Annals of Applied Biology* 158, 26-55.

Drew, R.A.I., Fay, H.A.C., 1988. Comparison of the roles of ammonia and bacteria in the attraction of *Dacus tryoni* (Frogatt) (Queensland fruit fly) to proteinaceous suspensions. *Journal of Plant Protection in the Tropics* 5, 127-130.

Epsky, N.D., Midgarden, D., Rendon, P., Villatoro, D. and Heath, R.R. 2012. Efficacy of wax matrix bait stations for Mediterranean fruit flies (Diptera: Tephritidae). *Journal of Economic Entomology* 105(2): 471-479.

Hancock, D.L., Hamacek, E.L., Lloyd, A.C., Elson-Harris, M.M., 2000. The distribution and host plants of fruit flies (Diptera: Tephritidae) in Australia. Department of Primary Industries, Queensland, Information Series QI99067.

Heath, R.R., Lavallee, S.G., Schnell, E., Midgarden, D.G. and Epsky, N.D. 2009. Laboratory and field cage studies on female-targeted attract-and-kill bait stations for *Anastrepha suspense* (Diptera: Tephritidae). *Peat Management Science* 65:672-677.

Lloyd, A., Smith, D., Hamacek, E., Neale, C., Kopittke, R.A, Jessup, A., Delima, C.P.F. and Broughton, S. (2003). *Improved protein bait formulations for fruit fly control.* HAL Final Report Project AH00012.

McQuate, G.T., Vargas, R.I., 2007. Assessment of attractiveness of plants as roosting sites for the melon fly, *Bactrocera cucurbitae*, and oriental fruit fly, *Bactrocera dorsalis*, 13 pp. *Journal of Insect Science* 7:57, available online: http://www.insectscience.org/7.57.

Missenden, B., 2014. Alternative fruit fly treatment for interstate access for strawberries: winter window option. Project number BS09022. Horticulture Australia Ltd, Sydney NSW.

Nishda, T., Bess, H.A., 1957. Studies on the ecology and control of the melon fly *Dacus (Strumeta) cucurbitae* Coquillett (Diptera: Tephritidae). *Technical Bulletin, Hawaii Agricultural Experiment Station* No. 34, 44 pp.

Pinero, J,C., Mau, R.F.L., Vargas, R.I. 2010. Comparison of rain-fast bait stations versus foliar bait sprays for control of oriental fruit fly, *Bactrocera dorsalis,* in papaya orchards in Hawaii. *Journal of Insect Science* 10:157.

Prokopy, R.J., Miller, N.W., Piñero, J.C., Barry, J.D., Tran, L.C., Oride, L., Vargas, R.I., 2003. Effectiveness of GF-120 fruit fly bait spray applied to border area plants for control of melon flies (Diptera: Tephritidae). *Journal of Economic Entomology* 96, 1485-1493.

Royer, J.E., De Faveri, S.G., Lowe, G.E., Wright, C.L., 2014. Cucumber volatile blend, a promising female-biased lure for *Bactrocera cucumis* (French 1907) (Diptera: Tephritidae: Dacinae), a pest fruit fly that does not respond to male attractants. *Austral Entomology* 53, 347-352.

Siderhurst, M.S., Jang, E.B., 2010. Cucumber volatile blend attractive to female melon fly, *Bactrocera cucurbitae* (Coquillet). *Journal of Chemical Ecology* 36, 699-708.

Acknowledgements

Appendices

Appendix 1.   On-farm evaluation of farm-wide management techniques for control of fruit flies in mango

Principal Researchers: S De Faveri and S Subramaniam

In 2013/14, four trial sites were selected to trial farm-wide management (FWM) techniques for control of pest fruit flies of mango (*Bactrocera tryoni* and *B. jarvisi*) using male lures and proteins; three were on the Atherton Tablelands (Dimbulah 1, Dimbulah 2 and Mutchilba) and one at Bowen (Table 1). Both the treatment and control (untreated) areas of each trial site were on the same farm except the control area of Dimbulah 1 which was on an adjacent farm managed by the owners of that farm. Male lures (MAT cups; www.bugsforbugs.com.au; Figure 1a) were distributed at the rate of 10/ha for *B. tryoni* and 5/ha for *B. jarvisi*. The MAT cups have a cotton wick dosed with 1 ml of male fruit fly attractant (cue lure for *B. tryoni*, and zingerone for *B. jarvisi*) and 0.5 ml of maldison insecticide. MAT cups were hung within the outer canopy of the tree at approximately 1.8 m height (Figure 1b).

Table 1.   Farm-wide fruit fly management trial sites on the Atherton Tablelands and at Bowen, and dates of male lure and trap distribution, and completion of male fruit fly trapping.

Trial site	Area of treatment block (ha)	Varieties	Male lure and monitoring trap distribution date		Final trap clearance date
			First distribution (*B. tryoni*)	Second distribution (*B. tryoni* & *B. jarvisi*)	(*B. tryoni* & *B. jarvisi*)
2013/14					
Dimbulah 1	24	Honey Gold, R2E2	21/8/2013	14/11/2013	24/2/2014
Dimbulah 2	50	Calypso	10/9/2013	18/11/2013	6/1/2014
Mutchilba	25	Honey Gold, R2E2	28/8/2013	15/11/2013	7/1/2014
Bowen	25	Honey Gold	5/9/2013	8/11/2013	30/1/2014
2014/15					
Bowen	25	Honey Gold & R2E2	29/8/2014	5/11/2014	11/2/2015

(a)   (b)

Figure 1.   (a) male lure (MAT cup), and (b) lure placement in the outer canopy of a mango tree.

Protein application methods planned for trial sites in 2013/14 were dictated by the grower's preferred method of application, and are described in Table 2. At Mutchilba, Cera Trap® traps (Figure 2a) containing a protein based liquid were hung in the perimeter trees of the treatment area on 22 October 2013; one trap placed, on average, every 15 m on the 2.2 km perimeter (140 traps in total). All traps were cleared weekly, and fruit flies identified and counted. At the other trial sites protein trunk treatments (Figure 2b) were preferred by cooperating growers. At Dimbulah 1 and Dimbulah 2, treatments (Fruit Fly Lure™) were applied as a thickened protein solution to every second tree around the perimeter of the treatment area, while at Bowen, treatments (Naturalure®) were applied as a thickened solution to every second tree throughout the treatment area.

In 2014/15, FWM treatments were assessed at Bowen only. Treatments were applied to the area (cv. Honey Gold) used in 2013/14 and also to a separate 25 ha area (cv. R2E2) on the same farm. Following a small cage trial comparing protein attraction it was decided to apply Fruit Fly Lure™ due to improved efficacy over Naturalure®.

Table 2.   Protein baiting methods planned at fruit fly farm-wide management sites on the Atherton Tables and at Bowen.

Trial site	Protein product	Application method
		2013/14
Dimbulah 1	Fruit Fly Lure™ www.bugsforbugs.com.au	Protein bait trunk sprays applied to perimeter trees.
Dimbulah 2	Fruit Fly Lure™	Protein bait trunk sprays applied to perimeter trees.
Mutchilba	Cera Trap® protein solution www.barmac.com.au	Perimeter Cera Trap® traps hung in perimeter trees at 15 m intervals.
Bowen	Naturalure® www.dowagro.com	Protein bait trunk sprays to every second tree of every row four weeks prior to harvest.
		2014/15
Bowen	Fruit Fly Lure™	Protein bait trunk sprays to every second tree of every row four weeks prior to harvest.

In 2013/14 and 2014/15, male fruit fly populations within treatment and control areas were monitored using traps fitted with a MAT cup (www.bugsforbugs.com.au; Figure 3). Four traps containing Cue lure and four containing Zingerone were evenly spaced within these areas. At Mutchilba in 2013/14, Cera Trap® traps were also installed in the treatment and control areas at the time of the perimeter treatment to monitor female fruit fly populations within these areas. MAT cups (treatments and monitoring traps) were replaced every three months (Table 1). Trapping commenced August–September for *B. tryoni* and November for *B. jarvisi*, and continued until completion of harvest (January–February). Trapped flies were collected weekly, and counted and identified to monitor the treatment effects.

<table>
<tr><td>(a)</td><td>(b)</td></tr>
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Figure 2.   (a) Cera Trap® trap, and (b) protein bait on tree trunk.

Figure 3.   Fruit fly monitoring trap.

In 2013/14, post-harvest packing shed assessment of fruit for fruit fly eggs and larval presence was conducted at Bowen only. Fruit of various grades (Table 3) were sampled from packed cartons and reject bins. In addition to visual inspection for eggs and larvae in sampled fruit, a sub-sample of 300 fruit from Bowen was incubated at 27°C and inspected for larval presence in the flesh after 5 days. In 2014/15, fruit samples were collected from the lowest marketable fruit grade (processing grade; class

3). Sampled fruit were graded according to maturity (mature green, or 30–50% yellow), held in rearing containers at 25–27°C for 5 to 7 days, and then assessed for larval presence in the flesh. Fruit with intact and damaged skin were assessed separately. Fruit from the untreated area was not assessed.

Table 3.   Number of fruit of various grades sampled from the treated area (cv. Honey Gold) at Bowen for assessment of fruit fly effects in 2013/14.

Fruit grade	Number of fruit assessed
Premium	609
Class 1	398
Class 2	291
Bulk	370
Bulk incubated fruit	306
Reject	256
Total fruit assessed	2230

Statistical comparison of lure and bait treatment influence on pest fruit fly populations and harvested fruit quality was not possible either within or across farms. Large treated and untreated areas (25–50 ha) were needed to properly assess the efficacy of treatments. Due to limited farm size, treatments could not be replicated within a farm, and there was no scope within farms to select treated and untreated areas of consistent varietal composition and/or size. Male lure treatments were the only treatments common across farms in 2013/14. Data describing indicative response of these treatments (number of male *B. tryoni* and *B. jarvisi* per trap per day) are presented as means accompanied by standard errors as appropriate. Mean flies collected per trap per day (FTD) for the first two collection dates of the treated area (cv R2E2) at Bowen in 2013/14, because of their magnitude, have been omitted from the Figure 7 to limit the scale of the y-axis and improve readability of the figure. These data are presented in the caption of that figure.

Results

2013/14 trials

At the two Dimbulah trial sites, weekly protein trunk treatments were not applied consistently by cooperating growers because of time constraints and intervention by heavy rain which either prevented treatments or washed treatments from treated trunks. Planned weekly protein trunk treatments were applied in the Bowen trial.

At all FWM sites, mean pest fruit fly numbers caught in monitoring traps were consistently lower in the treated areas compared with the control area demonstrating male fruit fly population reduction from the distributed lures (Figures 4 and 5). For the same sampling periods of each site, mean catch numbers of pest fruit fly species at the Atherton Tableland control sites were lower than that at the Bowen control site indicating higher fruit fly pressure at the latter site.
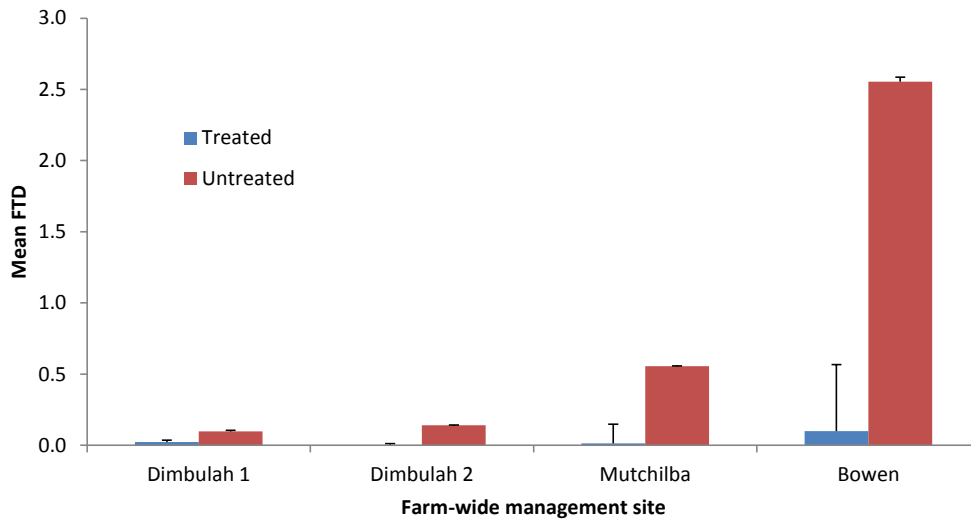
Figure 4.   Mean number of male *B. tryoni* per trap per day (FTD) collected from 10/9/2013 to 6/1/2014 at farm-wide management mango sites. Error bars represent the standard error (+ value) of the mean.
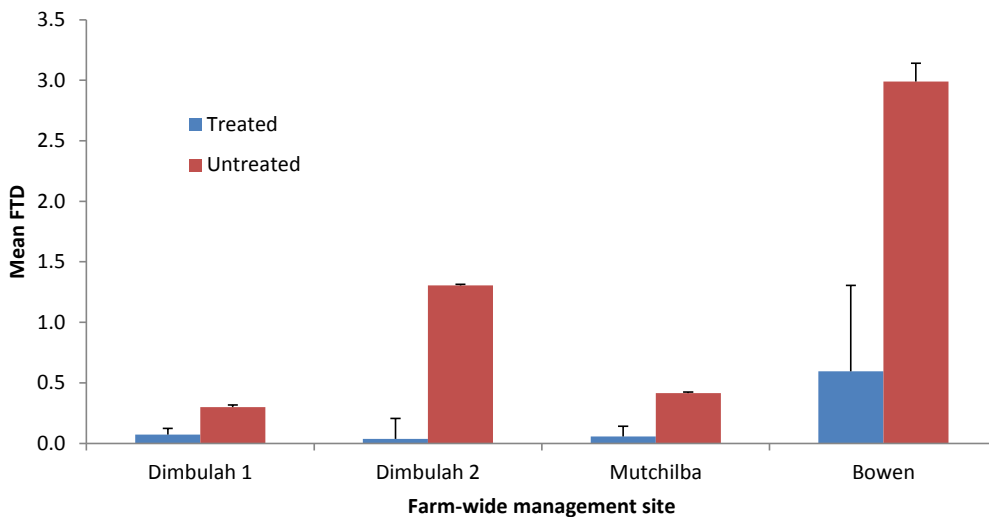

Figure 5.   Mean number of male *B. jarvisi* per trap per day (FTD) collected from 18/11/2013 to 31/12/2013 at farm-wide management mango sites. Error bars represent the standard error (+ value) of the mean.

Over the period that the perimeter treatment was maintained at Mutchilba (22/10/13 to 17/12/13), female *B. tryoni* and *B. jarvisi* were caught in perimeter treatment Cera Trap® traps, and in monitoring Cera Trap® traps within the treated and untreated areas (Figure 6). Female fruit numbers collected from traps at the three trap locations followed a similar trend. Mean FTD for the monitoring traps in the treated and untreated areas were similar and suggests that either the perimeter treatment was ineffectual, or that monitoring trap female fruit fly collections were pre-treatment residents. Eleven female and one male pest fruit flies were captured in traps within the perimeter treatment five weeks after the treatment was applied. Because it was possible that the flies had originated from outside the

treated area, the trial was concluded to allow the grower to begin dimethoate cover sprays.
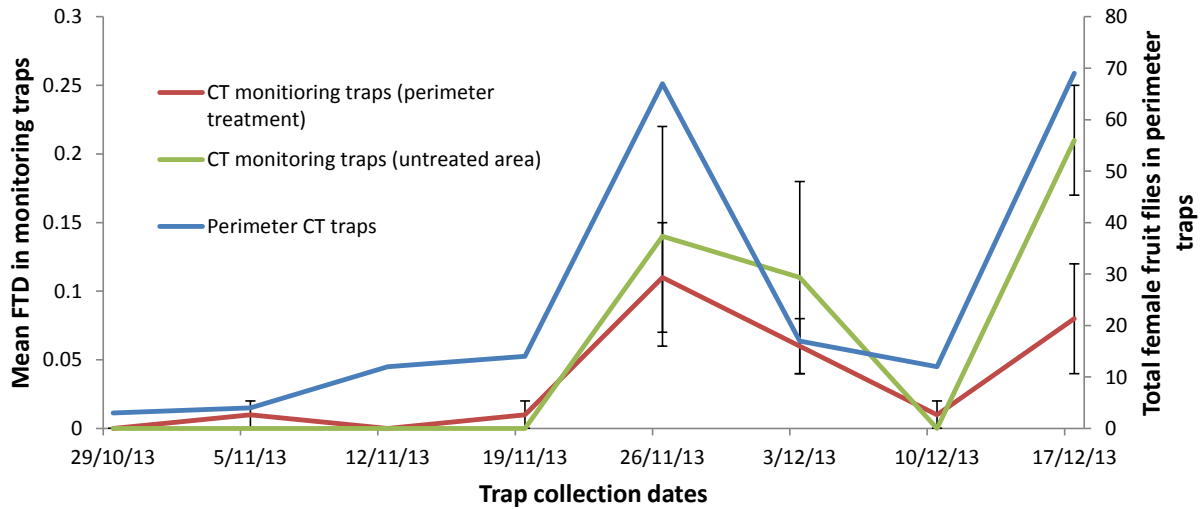

Figure 6.   Mean number of female *B. tryoni* and *B. jarvisi* per trap per day (FTD) in monitoring traps (Cera Trap® traps; CT) in perimeter treated area and untreated area in relation to total number of females of both species collected from perimeter traps surrounding the treated area from 22/10/2013 to 7/1/2014 at Mutchilba. Error bars represent the standard error of the mean.

At Bowen, 2230 fruit from the treated block were assessed for fruit fly eggs and larval presence. The total sampled, that included 256 reject fruit, was inspected for eggs and larvae, and a 300 fruit subsample (bulk grade; 3rd grade green mature, slight skin blemish) were incubated. There were no eggs or larvae found in any fruit grade assessed visually or by incubation. 929 fruit from the untreated area, 875 first grade and 54 reject fruit, were inspected for fruit fly oviposition site punctures (stings). Six of the reject fruit had apparent signs fly stings.

2014/15 trials

Planned weekly protein trunk treatments (Table 1) were applied by the cooperating grower to both treatment areas at Bowen.

On almost all trap clearance occasions, the number of pest fruit flies in traps were lower in the treated Honey Gold and R2E2 areas compared with the untreated area (Figures 7 and 8), again demonstrating fruit fly population reduction from lure and protein treatments. *B. tryoni* and *B. neohumeralis* trap numbers of the R2E2 area were higher than those of the Honey Gold and untreated areas during the first two weeks of monitoring, but declined to lower numbers than the untreated area in subsequent weeks. *B. jarvisi* numbers at the treatment areas were lower than the control area throughout the trial. Trap numbers of both treatment areas increased after the 6th January coinciding with completion of protein baiting and the removal of MAT cups in preparation for tree pruning (hedging).

Figure 7.   Mean number of male *B. tryoni* per trap per day (FTD) collected from traps in mango orchard areas at Bowen untreated and treated with MAT lures and protein baits. FTD for the treated area (cv R2E2) on the 5/9/14 and 12/9/14 were 51.9 (± 21.7) and 111.4 (± 38.3), respectively. Error bars represent the standard error of the mean.

Figure 8.   Mean number of male *B. jarvisi* per trap per day (FTD) collected from traps in mango orchard areas at Bowen untreated and treated with MAT lures and protein baits. Error bars represent the standard error of the mean.

1968 R2E2 and 1997 Honey Gold fruit were assessed for fruit fly eggs and larval presence (Table 4). The proportion of R2E2 fruit with damaged skin was higher than that of Honey Gold (42% *cf* 7%). Larvae were only found in four R2E2 fruit (0.2% of the total fruit sampled); all four had damaged skin, possibly the result of bird feeding. Three of the infested fruit were 30–50% yellow, and one was mature green.

Table 4. Number of fruit of two maturity grades sampled from two farm-wide management treatment areas at Bowen and the number of fruit infested by fruit fly in 2014/15.

Fruit maturity category	Intact skin		Damaged skin		Number pupae
	Total fruit	Number infested	Total fruit	Number infested	
Treated area cv. R2E2					
Mature green	758	0	427	1	3
30-50% yellow	389	0	394	3	7
Total fruit assessed	1147		821		
Treated area cv. Honey Gold					
Mature green	1011	0	62	0	0
30-50% yellow	837	0	87	0	0
Total fruit assessed	1848		149		

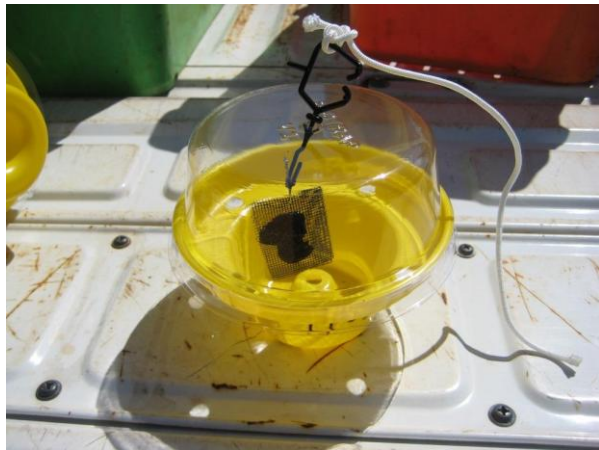## Appendix 2. Comparison of male fruit fly lure technologies

Principal Researcher: D Chambers

The effectiveness of four commercial male fruit fly lure technologies and two dental wick treatments (Table 1) for attracting the pest fruit fly *Bactrocera tryoni* were compared in mango orchards on the Atherton Tablelands in North Queensland; one trial at Mutchilba (cv. Kensington Pride), and the other at Mareeba (mixed varieties). Each lure treatment was enclosed within a BioTRAP Globe trap to trap and quantify fruit fly attraction (Figure 1). Traps were cleared of fruit flies weekly over the periods of monitoring in each trial; 23/9/14 to 16/3/15 at Mutchilba, and 23/12/14 to 3/6/15 at Mareeba. All lures were replaced with fresh ones three months after the start of monitoring.

Table 1.   Male fruit lure treatments compared in mango orchards at Mutchilba and Mareeba.

	Lure treatment		Manufacturer
	Cue lure rate	*Toxicant (active ingredient)*	
SPLAT CL (experimental)[1]		alpha cypermethrin	ISCA Technologies Inc. www.iscatech.com
SPLAT CL (standard)[1]		alpha cypermethrin	ISCA Technologies Inc. www.iscatech.com
MAT cup	1 ml	maldison (0.1 ml)	Bugs for Bugs Pty Ltd. www.bugsforbugs.com.au
FT Mallet-CL wafer	2 ml	maldison (0.5 ml)	BioTRAP Australia Pty Ltd. www.biotrap.com.au
Dental wick (standard)	3 ml	maldison (1.0 ml)	
Dental wick (low dose)	1 ml	maldison (0.5 ml)	

[1] Treatment rate was 5 g SPLAT CL product/lure.

SPLAT CL (experimental and standard)	MAT cup
FT Mallet-CL wafer	Dental wick

Figure 1.   Lure technology treatments (installed within BioTRAP Globe traps) compared in mango orchards at Mutchilba and Mareeba in North Queensland.

Assessment of the effects of aging on male fruit fly lure efficacy

The effect of aging on lure efficacy was studied in a mango orchard near Mareeba (mixed varieties). FT Mallet-CL wafers (2 ml cure lure, 0.5 ml maldison) were aged for two and four weeks prior to comparing with fresh wafer and dental wick (3 ml cue lure, 0.5ml maldison) treatments. Wafers were aged (inside and outside Biotrap Globe traps) within a tree canopy that simulated aging effects within a mango orchard. Following aging, each lure treatment was enclosed within a BioTRAP Globe traps for comparison in the orchard field trial. Fruit flies were collected from traps weekly over the period of monitoring (4/3/15 to 3/6/15).

Lure technology and aging treatments were compared in separate randomised complete block design trials, treatments of both trials replicated five times. Mean fruit fly catch per week for each treatment was calculated from total weekly catches of *B. tryoni* and analysed using a generalised linear mixed model (GLMM). A Poisson distribution was assumed with a log link function. A repeated measures residual maximum likelihood (REML) analysis was also performed to investigate the response over time. A log transformation was required and one was added to all totals to account for the zeroes. It is also

likely that data from the same trap will be correlated, with correlation decreasing over time. To account for this, different correlation models were fitted in the REML analysis and the most appropriate model selected. For all analyses, where a significant (p<0.05) treatment effect was detected, pairwise comparisons were made using the pairwise 95% least significant difference (LSD).

Results

Comparison of male lure technologies

For each respective monitoring period, differences (p<0.001) in mean weekly fruit fly catch amongst lure technologies were found at both Mutchilba and Mareeba (Tables 2 and 3). At Mutchilba, highest mean catch was recorded from traps with FT Mallett-CL wafer. At Mareeba, highest mean catch was recorded with dental wick (standard); however, dental wick (low dose) and FT Mallett-CL wafer performed similarly and better than the remaining treatments. In both trials, mean catch was higher with FT Mallett-CL wafer compared with the other commercial lures SPLAT CL (standard) and MAT cup. Lowest mean catch was recorded from SPLAT CL (experimental).

Table 2.   Mean weekly fly catch of *B. tryoni* over a 174 day monitoring period at Mutchilba as influenced by different lure technologies.

Treatment	Mean weekly fly catch[1]		
SPLAT CL (experimental)	0.29	a	*0.3*
SPLAT CL (standard)	0.86	b	*1.4*
MAT cup	0.86	b	*1.4*
FT Mallett-CL wafer	1.26	c	*2.5*
Dental wick (standard)	0.88	b	*1.4*
Dental wick (low dose)	0.79	b	*1.2*
Average 95% LSD	0.13		

[1]Values in italics are back-transformed means. Means not followed by a common letter differ significantly (p<0.05).

Table 3.   Mean weekly fly catch of *B. tryoni* over a 162 day monitoring period at Mareeba as influenced by different lure technologies.

Treatment	Mean weekly fly catch[1]		
SPLAT CL (experimental)	0.42	a	*0.5*
SPLAT CL (standard)	0.75	b	*1.1*
MAT cup	0.93	c	*1.5*
FT Mallett-CL wafer	1.41	d	*3.1*
Dental wick (standard)	1.67	e	*4.3*
Dental wick (low dose)	1.48	d	*3.4*
Average 95% LSD	0.10		

[1]Values in italics are back-transformed means. Means not followed by a common letter differ significantly (p<0.05).

Influence of aging on lure efficacy

Mean weekly fruit fly catch over the 91 day monitoring period was influenced (p<0.001) by aging (Table 4). At the first trap clearance occasion (day 7 of the monitoring period), mean catch of traps with FT Mallett-CL wafer aged for 4 weeks inside the BioTRAP Globe trap was significantly lower than those with wafer aged for 2 weeks inside the trap. Mean catch with wafers aged 2 and 4 weeks outside the trap was similar at this clearance time.

For the total monitoring period, mean catch of traps with non-aged (fresh) wafers was 2.5 times the number of fruit flies than the standard fresh dental wick that contained 50% higher rate of cue lure attractant. Traps with fresh wafer had a higher (p<0.05) mean catch than those with dental on all trap clearance occasions except clearances on day 70, 84 and 91 of the monitoring period (data not presented).

Table 4.   Mean weekly fly catch of *B. tryoni* at the first trap clearance (day 7) and over the 91 day monitoring period as influenced by different lure aging treatments.

Treatment			Mean weekly fly catch[2]				
Lure type	*Aging time (weeks)*	*Aging method[1]*	At day 7			Total monitoring period	
FT Mallett-CL wafer	0		3.11	c	*22.4*	2.02	c	*6.5*
FT Mallett-CL wafer	2	inside trap	3.37	c	*29.1*	1.72	b	*4.6*
FT Mallett-CL wafer	2	outside trap	2.94	bc	*18.9*	1.92	c	*5.8*
FT Mallett-CL wafer	4	inside trap	2.09	a	*8.1*	1.36	a	*2.9*
FT Mallett-CL wafer	4	outside trap	2.89	bc	*18.1*	1.71	b	*4.5*
Dental wick (standard)	0		2.30	ab	*10.0*	1.24	a	*2.5*
Average 95% LSD			0.01			0.17		

[1]Aged inside or outside a BioTRAP Globe trap. [2]Values in italics are back-transformed means. Means not followed by a common letter differ significantly (p<0.05).

Appendix 3.   Research of protein bait phytotoxicity and bait application technologies

Principal Researchers: P O'Farrell and J Robertson

The response of mango fruit to proteins (Table 1) used for fruit fly baiting was assessed in the Mareeba–Dimbulah region of North Queensland during the flowering seasons of 2013 and 2014. In 2013, proteins were initially evaluated qualitatively in non-replicated trials, and then in a randomized complete block (RCBD) designed comparison of potential proteins (Table 2). In all, the response of seven mango varieties to eight proteins was studied.

Additional research was undertaken as non-replicated trials to initiate understanding of the factors influencing the expression of the blemish symptom; bait solution ingredient (protein or toxicant), bait solution pH, and the phyto-reaction environment.

Three proteins (CERABAIT™, Natflav 500™, and Fruit Fly Lure™) were tested on mature mango (cv. Kensington Pride) as a RCBD trial at Berry Springs, Northern Territory, in September 2013. Bait treatments were applied to fruit as standard (pH 4–4.6) and neutralized (by potassium hydroxide) solutions; however no treatment caused blemish on fruit.

Table 1.   Protein products and mango varieties studied in phytotoxicity trials in 2013 and 2014.

Protein product	Formulation	Protein content	Salt content	Rates tested (per 500 mL)	Bait consistency when applied	Varieties tested
ANAMED™ SPLAT (protein)	paste				paste	A, C, E, F, G.
DacGEL™	powder			6.25 g	gel	A, D.
Fruit Fly Lure™	thick liquid	420 g/L	≤ 1%	10 ml	suspension	A, B, C, D, E, F, G.
Natflav 500™	thick liquid	420 g/L	< 7 ppm (0.0007%)	10 ml	suspension	A, B, C, D, E, F, G.
CERABAIT™	liquid	360 g/L		5, 11.7 ml	liquid	A, B.
Flavex® (Liquid Type FL622)	liquid	140 g/L	17–19%	5 ml	liquid	A, C, E, F, G.
Flavex® (Powder Type SPA400)	powder	420 g/L	4–7%	10 g	liquid	A, C, E, F, G.
HYM-LURE™	liquid	300 g/L	10–12%	2, 5, 10 ml	liquid	A, B, C, E, F, G.

[A]Kensington Pride, [B]R2E2, [C]Honey Gold, [D]Calypso [E]1201, [F]1243, [G]4069.

Toxicants Maldison 500™ (500 g/L maldison; 3.5 mL/L) and HY-MAL™ (1150 g/L maldison; 2.2 mL/L) were included in treatments as appropriate. Autolysed yeast extract (Fruit Fly Lure™) was included as the control protein in all trials. This protein is commonly used in commercial fruit fly control programs. With the exception of ANAMED™ SPLAT (protein) which was smeared on the skin, all proteins were applied to fruit as a coarse spray. Bait solution pH was adjusted with borax or potassium hydroxide (KOH). Fruit were assessed for phytotoxic blemish symptoms 24 hours after treatment application; blemish ratings reflecting the intensity and extent of pink-red discoloration of the skin and ranged from 0 (nil) 1, 2 and 3 (severe).

For the RCBD trial, analysis of variance was used to compare mean phytotoxicity rating for each treatment. Where a significant (p<0.05) treatment effect was found, the 95% least significant difference (LSD) was used to make pairwise comparisons.

Results

Assessment of protein bait phytotoxicity

All proteins assessed in North Queensland, irrespective of formulation, caused phytotoxic fruit blemish. Most severe blemish was associated with baits applied as a paste or gel (ANAMED™ SPLAT (protein), DacGel™) (Figure 1). Natflav 500™, HYM-LURE™ and CERABAIT™, at the same (recommended) protein concentration as for Fruit Fly Lure™ (0.84%), were relatively less (p<0.05) phytotoxic to mango (cv. R2E2) than Fruit Fly Lure™ (Table 2). Bait treatments applied as standard and neutralized solutions in the Northern Territory did not cause fruit blemish.

The protein component of the bait solution (not the toxicant) was shown to be responsible for causing blemish (Figure 2). Bait solution pH had no apparent influence on blemish expression when KOH modified Fruit Fly Lure™ solutions were applied to cv. Calypso (Figure 3). In other assessments, blemish symptoms were slight and obscure, and did not clarify the influence of Natflav 500™ solutions (borax modified) applied to cv. Kensington Pride. Enclosing fruit within plastic bags post treatment with Fruit Fly Lure™ bait solution exasperated blemish symptoms (Figure 4).

Table 2. Phytotoxic response of mature mango fruit (cv. R2E2) to protein treatments at Mareeba, North Queensland.

Treatment		Mean phytotoxic blemish rating	
Protein	*Concentration (%)*		
CERABAIT™	0.36	0.46	a
CERABAIT™	0.84	0.56	a
HYM-LURE™	0.42	0.49	a
HYM-LURE™	0.84	0.66	ab
Natflav 500™	0.84	1.00	b
Fruit Fly Lure™ (control)	0.84	2.15	c
5% LSD		0.43	

Phytotoxic blemish ratings with a letter in common are not significantly (p<0.05).

ANAMED™ SPLAT (protein)
(severe blemish)

DacGEL™
(severe blemish)

HYM-LURE™
(slight blemish, rating 1)

Fruit Fly Lure™
(moderate blemish, rating 2)

Figure 1.   Phytotoxic blemish on mango (cv. Kensington Pride) from four protein products; most severe blemish was associated with baits applied in paste (ANAMED™ SPLAT (protein)) and gel (DacGEL™) consistencies.

Maldison 500™
(no blemish)

Fruit Fly Lure™
(severe blemish)

Fruit Fly Lure™ + Maldison 500™
(slight blemish, rating 1)

Figure 2.   The response of mango (cv. Calypso) to bait ingredients applied alone (Maldison 500™ and Fruit Fly Lure™), or mixed as a prepared bait solution.
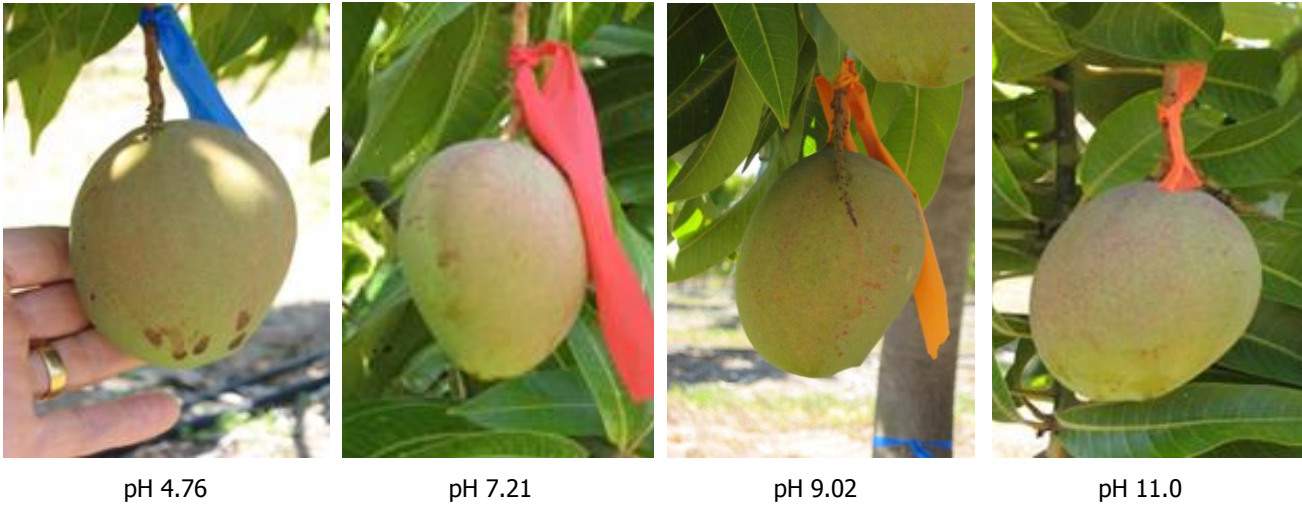
pH 4.76 pH 7.21 pH 9.02 pH 11.0

Figure 3.   Phytotoxic blemish (slight-moderate) on mango (cv. Calypso) from Fruit Fly Lure™ bait treatments applied as standard pH (4.76), and pH adjusted (7.21, 9.02 and 11.0) solutions.

Not bagged. (moderate blemish) Bagged pre-treatment. (slight blemish) Bagged post-treatment. (severe blemish) Bagged pre- and post-treatment. (severe blemish)

Figure 4.   Phytotoxic blemish on mango (cv. Calypso) as influenced by enclosing fruit before and/or after treatment with Fruit Fly Lure™ bait solutions.

Development of bait application equipment

Bait applicator design was conceptualized in consultation with collaborating growers early in the fruiting season of 2013. Several design concepts were considered which took account of tree size and structure, location and density of fruit on the tree, and the use of props to support low hanging fruit (Figure 5). They included application of bait to internal branches from above the canopy, and to non-fruiting areas of the tree (canopy and trunk).

| Low hanging fruit. | Props supporting fruit bearing branches. |

Figure 5.   Potential obstruction to bait application operation equipment operating under the tree
             canopy.

A prototype applicator was initially designed to apply bait to the tree trunk (Figure 6), a placement point previously demonstrated by Lloyd *et al* (2005) as effective for control of fruit fly in mango. Tree training is needed to ensure low hanging fruit do not obstruct passage of the applicator beneath the tree canopy.

A second applicator, a hand lance (Figure 7), capable of applying a measured volume of bait gel to the trunk from an agricultural ATV, was designed and constructed for use in current fruiting season. Various proportions each of Fruit Fly Lure™ protein and Keltrol (Xanthan Gum) thickener were tested to refine the bait viscosity to achieve trunk adherence while maintaining required protein and toxicant rates. The application system was trialed at two of the Farm Wide Management sites with mixed acceptance by cooperating farmers.
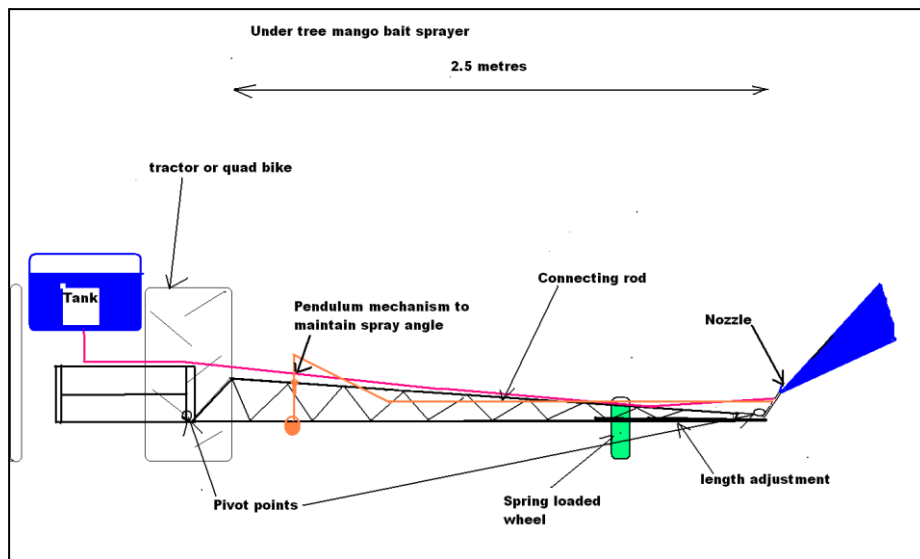
Figure 6.   Prototype applicator designed for applying fruit fly bait to mango tree trunks.

Figure 7.   Hand lance for applying fruit fly bait to mango trunks (figure insert describes a measured amount of bait gel applied to crotch of the tree).

Appendix 4:   Efficacy of least phytotoxic protein baits for attracting _Bactrocera tryoni_ and _B. jarvisi_ fruit flies

Principal Researcher: G Lowe

<u>Protein attraction cage trials</u>

The attractiveness of CERABAIT™, HYM-LURE™ and Natflav 500™ proteins to the fruit flies _Bactrocera tryoni_ and _B. jarvisi_ was assessed by choice experiments in the small laboratory cages and in large glasshouse flight cages (Table 1). These proteins were shown to be less phytotoxic to mango (cv. R2E2) (Appendix 3, Table 2). Fruit Fly Lure™ protein, which has wide commercial use, was included in all trials as a control.

Table 1.   Protein treatments compared in fruit fly attraction cage trials.

Treatment		Treatments compared		
Protein	_Concentration (%)_	_Cage trial 1_	_Cage trial 2_	_Flight cages_
CERABAIT™	0.36	●		
CERABAIT™	0.84	●		
HYM-LURE™	0.42	●	●	
HYM-LURE™	0.84	●	●	●
Natflav 500™	0.42		●	
Natflav 500™	0.84		●	
Fruit Fly Lure™ (control)	0.84	●	●	●

Laboratory cage trials 1 and 2. Treatments were compared in two 60 cm^3 aluminium gauze sided cages in a 12 hour day/night photoperiod room at 27°C temperature (Figure 1). One hundred fruit flies (14–17 days old, of mixed sex and protein deprived) were released into the cages the day before the trial, and given sugar and water.

On the morning of the trial, liquid protein treatments were prepared and placed as 10 x 0.2 ml discrete drops in separate petri dishes positioned randomly on the floor of each cage (Figure 1). The flies were given 30 minutes to acclimatize before recordings of fly response began. The numbers of flies _within_ petri dishes were counted (measure of attraction) every 5 minutes for 30 minutes, and then half-hourly for a period of 4 hours. Six replicates were completed (two per day) for each of _B. tryoni_ and _B. jarvisi_, and protein treatments were reassigned a different position in each replicate to minimize bias within the cages.

B. tryoni and _B. jarvisi_ were analysed separately. Attraction count times were combined into a repeated measures analysis using residual maximum likelihood. Different correlation models were fitted to account for any correlations between the time assessments within each cage. The predicted means on the log scale, the associated standard error and back-transformed means are presented in Tables 2 and 3. Where a significant ($p < 0.05$) treatment effect was found, pairwise comparisons were made using the pairwise 95% least significant difference (LSD).

| Cage | Protein treatment presented within petri dish |

Figure 1.   Gauze sided cage and protein treatment used for laboratory cage trials 1 and 2.

Flight cage trial. HYM-LURE™ (0.84% protein), which was found to be the most attractive to *B. tryoni* and *B. jarvisi* in laboratory cage trials 1 and 2, was compared with Fruit Fly Lure™ at the same protein concentration in two 3 x 1.8 x 1.8 m flight cages (Figure 2). Both treatments contained an insecticide (HY-MAL™; 1150 g/L maldison) at 4.4 ml product/L. The response of *B. tryoni* and *B. jarvisi*, each of two ages (7–10 and 14–17 days old), was assessed in separate trials (four trials in total) during June to August 2014.

Figure 2.   Large flight cage used to compare the efficacy of the proteins HYM-LURE™ and Fruit Fly Lure™ to the fruit flies *Bactrocera tryoni* and *B. jarvisi*.

Five hundred flies (mixed sex and protein deprived) were released into each flight cage at 7 am, two hours before commencement of the trial. The two protein treatments were applied separately to two potted mango trees as a 10 ml spray to the leaves. At 9 am, the trees were placed in opposite corners of the flight cage within catching sheets. Two untreated trees were placed in the remaining corners of

the cage to serve as roosting sites for the flies. At 1 pm, dead fruit flies were collected from the catching sheets, counted and sexed. Four replicates of each trial were completed.

Dead fruit fly counts were analysed by analysis of variance; dead males and females were analysed separately, as well as the overall total count of dead flies of each age group. Where a significant (p<0.05) treatment effect was found, pairwise comparisons were made using the pairwise 95% least significant difference (LSD).

Results

Laboratory cage trial 1

Significant differences (p<0.05) in attraction counts were found for both *B. tryoni* and *B. jarvisi* at various count times which described a trend of greater numbers of flies attracted to HYM-LURE™ 0.84% concentration (data not presented). Across times, HYM-LURE™ (0.84%) attracted greater (p<0.001) numbers of *B. tryoni* and *B. jarvisi* than the other protein treatments (Table 2).

Table 2.	Mean numbers of *B. jarvisi* and *B. tryoni* attracted to different proteins over a 4.5 hour monitoring period in laboratory cage trial 1.

Protein treatment	Mean *B. tryoni* attraction			BT[1]	Mean *B. jarvisi* attraction			BT[1]
CERABAIT™ 0.36%	0.00	a	*0.00*	1.30	0.00	a	*0.00*	1.00
CERABAIT™ 0.84%	0.10	a	*0.68*	1.40	1.51	b	*0.49*	2.51
HYM-LURE™ 0.42%	1.19	a	*0.68*	2.49	3.67	c	*0.49*	4.67
HYM-LURE™ 0.84%	2.88	b	*0.68*	4.18	6.47	d	*0.49*	7.47
Fruit Fly Lure™ (control)	0.05	a	*0.68*	1.34	2.03	b	*0.49*	3.03

Means not followed by a common letter within a column differ significantly p<0.001. Values in italics are standard errors of the transformed means. [1]Back-transformed means.

Laboratory cage trial 2

Significant differences (p<0.05) in attraction counts at various count times were found only for *B. jarvisi*; however for both *B. tryoni* and *B. jarvisi* there was trend of greater numbers of flies attracted to HYM-LURE™ 0.84% concentration (data not presented). Across times, *B. tryoni* attraction to protein treatments was not significantly different, but HYM-LURE™ (0.84%) attracted greater (p<0.001) numbers of *B. jarvisi* than the other protein treatments (Table 3).

Table 3.   Mean numbers of *B. jarvisi* and *B. tryoni* attracted to different proteins over a 4.5 hour monitoring period in laboratory cage trial 2.

Protein treatment	Mean *B. tryoni* attraction			BT[1]	Mean *B. jarvisi* attraction			BT[1]
Natflav 500™ 0.42%	0.84	a	*0.22*	2.31	0.87	a	*0.22*	2.38
Natflav 500™ 0.84%	0.40	a	*0.27*	1.48	1.44	b	*0.17*	4.23
HYM-LURE™ 0.42%	0.97	a	*0.20*	2.63	1.52	b	*0.16*	4.56
HYM-LURE™ 0.84%	1.22	a	*0.18*	3.39	2.49	c	*0.10*	12.07
Fruit Fly Lure™ (control)	0.62	a	*0.24*	1.86	1.48	b	*0.16*	4.38

Means not followed by a common letter within a column differ significantly p<0.001. Values in italics are standard errors of the transformed means. [1]Back-transformed means.

Flight cage trials

Dead female and male *B. jarvisi* and *B. jarvisi* of both age groups (7–10 and 14–17 days old) were retrieved from catching sheets of both protein treatments. Treatments did not influence (p>0.05) the number of dead *B. tryoni* of either sex or age group (Table 4), and only influenced the number of dead 7–10 day old *B. jarvisi* males; the number of dead males was higher (p<0.028) for HYM-LURE™ compared with Fruit Fly Lure™ (Table 5). While not significant, there was a consistent trend of higher fly death associated with HYM-LURE™ compared with Fruit Fly Lure™ for both fly species sex and age.

Table 4.   Mean number of dead *B. tryoni* of two age groups retrieved from catching sheets of protein treatments four hours after release within flight cages.

Protein treatment	Total males and females		Total females		Total males	
7–10 day old fruit flies						
HYM-LURE™	90.5	a	45.5	a	45.0	a
Fruit Fly Lure™ (control)	59.0	a	29.2	a	29.8	a
14–17 day old fruit flies						
HYM-LURE™	63.8	a	36.0	a	27.8	a
Fruit Fly Lure™ (control)	48.0	a	30.2	a	17.8	a

Table 5.   Mean number of dead *B. jarvisi* of two age groups retrieved from catching sheets of protein treatments four hours after release within flight cages.

Protein treatment	Total males and females		Total females		Total males	
7–10 day old fruit flies						
HYM-LURE™	143.2	a	77.2	a	66.0	b
Fruit Fly Lure™ (control)	119.2	a	66.2	a	53.0	a
14–17 day old fruit flies						
HYM-LURE™	129.5	a	70.8	a	58.8	a
Fruit Fly Lure™ (control)	88.8	a	39.2	a	49.5	a

Appendix 5. Comparison of fruit fly protein baits and insecticides

Principal Researcher: L Senior

Comparison of fruit fly protein baits

Preliminary laboratory trials were performed to compare the attraction of cucumber (*Bactrocera cucumis*) and Queensland (*B. tryoni*) fruit flies to 15 proteins (Table 1). Bioassays were performed in four 62 x 68 x 61 cm aluminium gauzed sided cages. Fruit flies were obtained from colonies maintained at the DAF laboratories in Brisbane. *B. cucumis* flies were 10–19 days post-emergence and *B. tryoni* flies were 10–22 days post-emergence, and were protein deprived. Males and females of each species were assessed separately. Four different baits were placed in each cage with 100 flies, and the number of flies per bait counted at intervals over the trial period. An incomplete block design was used to allocate baits to cages, with four replicates per bait.

Table 1.   Protein treatments, dilution rates, and protein comparisons studied.

Protein treatment	Dilution	Preliminary trials	Comparisons with HYM-LURE™	Comparisons with CERABAIT™	Comparisons with Naturalure™
CERABAIT™	10 ml/L			●	
DacGEL™	12.5 g/L	●			
dehydrated yeast autolysate[1]	20 g/L	●			
Flavex® (Liquid Type FL 622)	10 ml/L	●	●	●	●
Flavex® (Liquid Type FL 622)	20 ml/L	●			
Flavex® (Powder Type SPA 400)	10 g/L	●			
Flavex® (Powder Type SPA 400)	20 g/L	●	●	●	●
Fruit fly bait 1314-44C[2]	50:50	●			
Fruit fly bait 1314-44D[2]	50:50	●			
Fruit Fly Lure™	20 ml/L	●	●	●	●
HYM-LURE™	4 ml/L	●	●		
Maurimos	20 g/L	●			
Natflav 500™	60 g/L	●	●	●	●
Naturalure™	154 ml/L				●
Nu-lure®	12.5 ml/L	●			
Prima Fruit Fly Bait	100 ml/L	●			
XXXX Lion Nathan[3]	20 g/L	●			

[1] A dehydrated form of Fruit Fly Lure™. [2] Novel formulations under development by Halcyon Proteins Pty Ltd. [3] A dehydrated beer yeast waste.

The best performing proteins from these trials were Flavex FL 622™ (10 ml/L), Flavex SPA 400™ (20 g/L), Natflav 500™ and Fruit Fly Lure™. These proteins were then compared in individual trials with HYM-LURE™, CERABAIT™ and Naturalure™ using male and female *B. cucumis* flies 8–14 days post-emergence and *B. tryoni* flies 8–17 days post-emergence. For the comparison with HYM-LURE™ only, flies were of mixed sex. Sugar and water were provided for the duration of the trials. Each trial was set up as a randomised complete block design with the five proteins treatments randomised to five positions within a cage. Three cages were run concurrently and repeated twice to give six replicate cages.

Results

Comparison of the top performing proteins with HYM-LURE™

Fruit Fly Lure™ attracted the most cucumber flies up to 120 minutes, after which time all bait was consumed, resulting in a decrease in attraction (Figure 1). HYM-LURE™ attracted the fewest cucumber flies. Conversely, HYM-LURE™ was one of the most attractive baits for Queensland fruit flies (Figure 2).
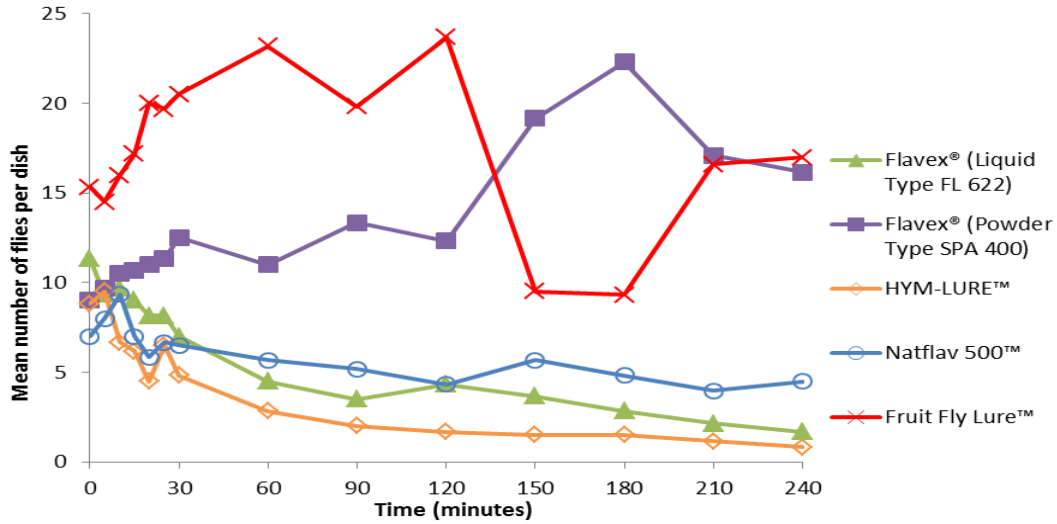

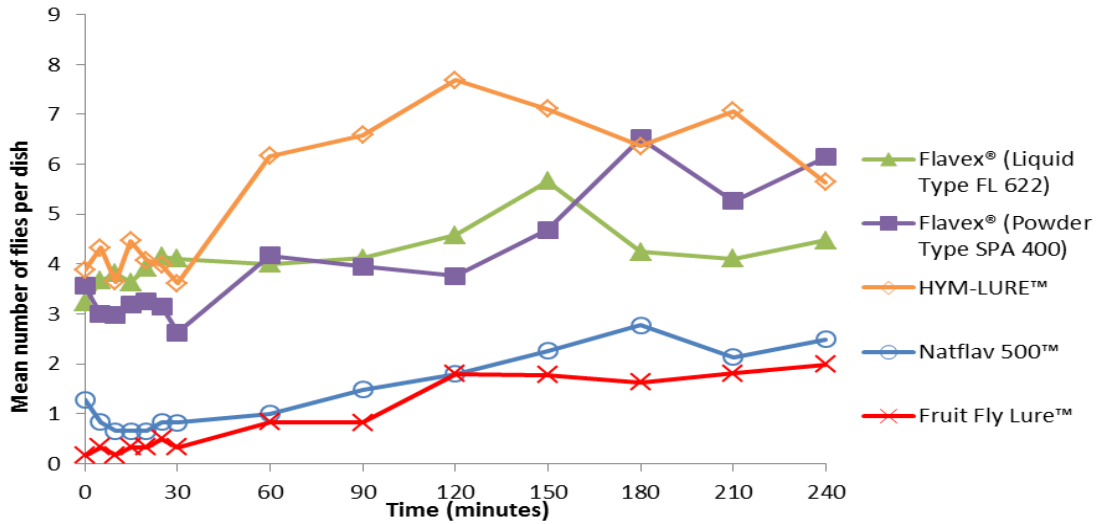Figure 1.   Number of *B. cucumis* (mixed sex) at each of five baits (data points are back-transformed means).

Figure 2.   Number of *B. tryoni* (mixed sex) at each of five baits (data points are back-transformed means).

Comparison of the top performing proteins with CERABAIT™

CERABAIT™ was the least attractive bait for both sexes of both fruit fly species (Figures 3-6).
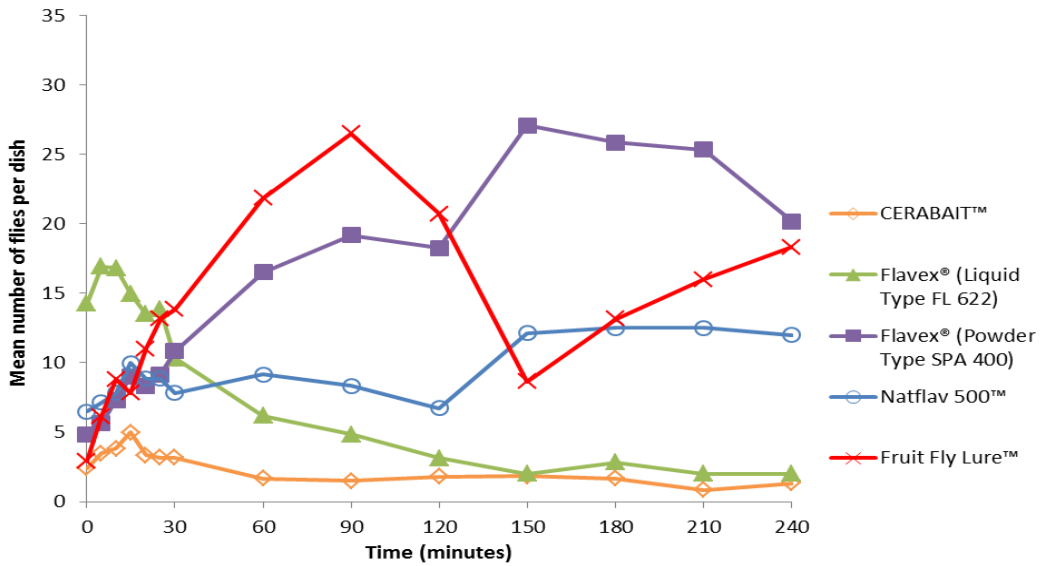

Figure 3.   Number of female *B. cucumis* at each of five baits (data points are back-transformed means).
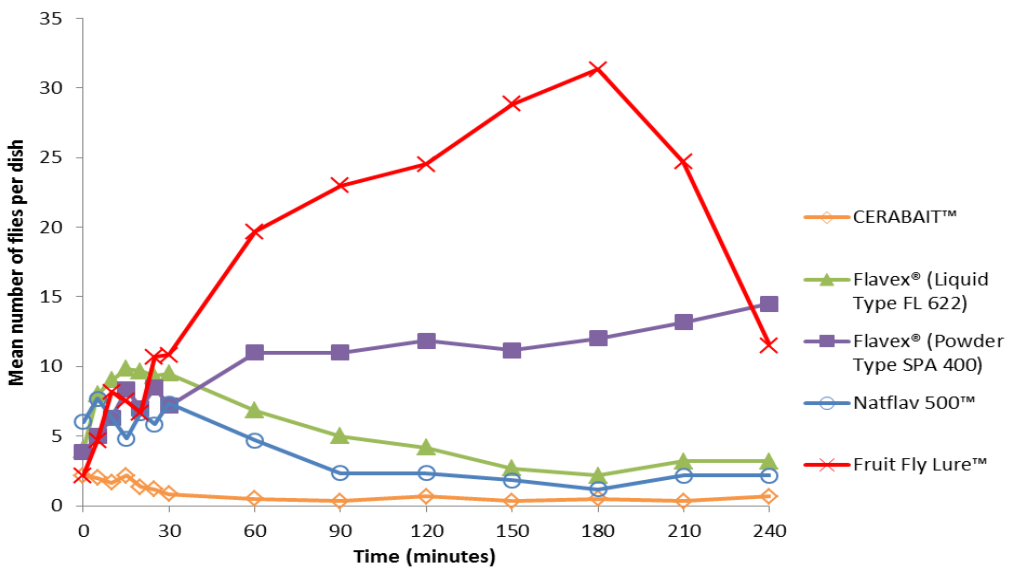

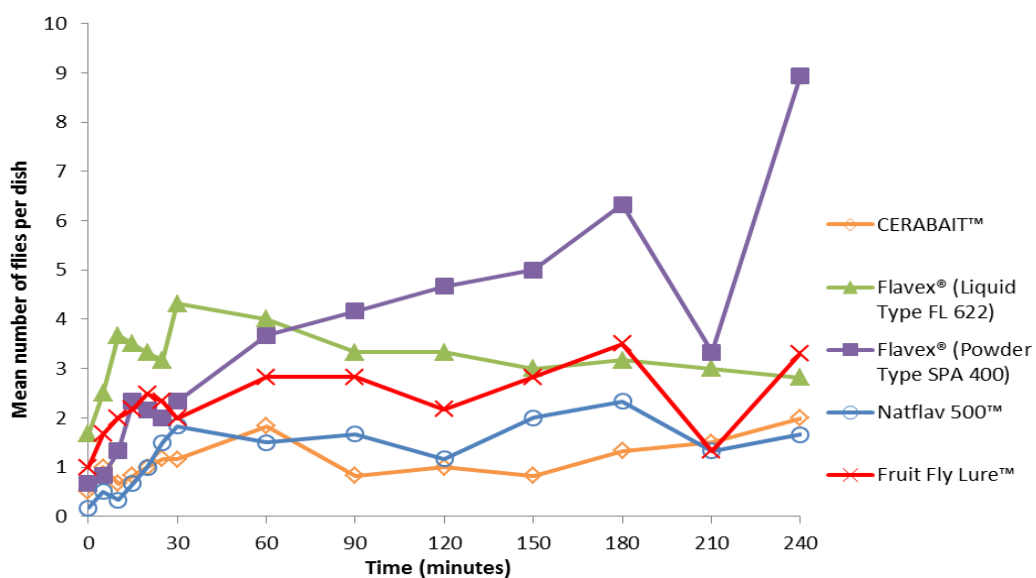Figure 4.   Number of male *B. cucumis* at each of five baits (data points are back-transformed means).

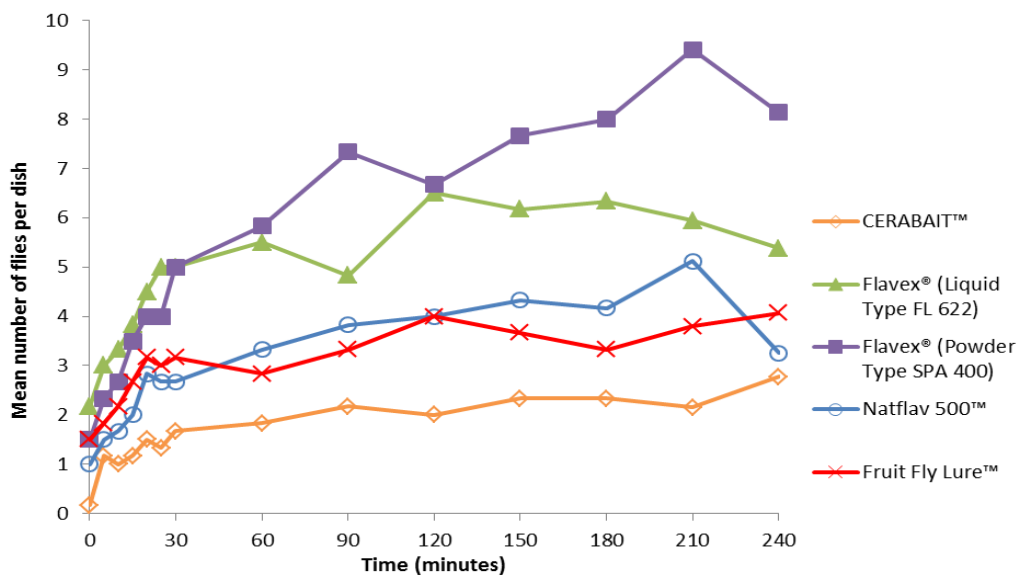Figure 5. Number of female *B. tryoni* at each of five baits (data points are back-transformed means).

Figure 6. Number of male *B. tryoni* at each of five baits (data points are back-transformed means).

Comparison of the top performing proteins with Naturalure™

To ensure a valid comparison between protein baits, spinosad (as Success Naturalyte™) was added to baits at the same rate as Naturalure™ such that all treatments contained 0.24 g spinosad/L. Naturalure™ was consistently the least attractive bait (Figures 7-10).
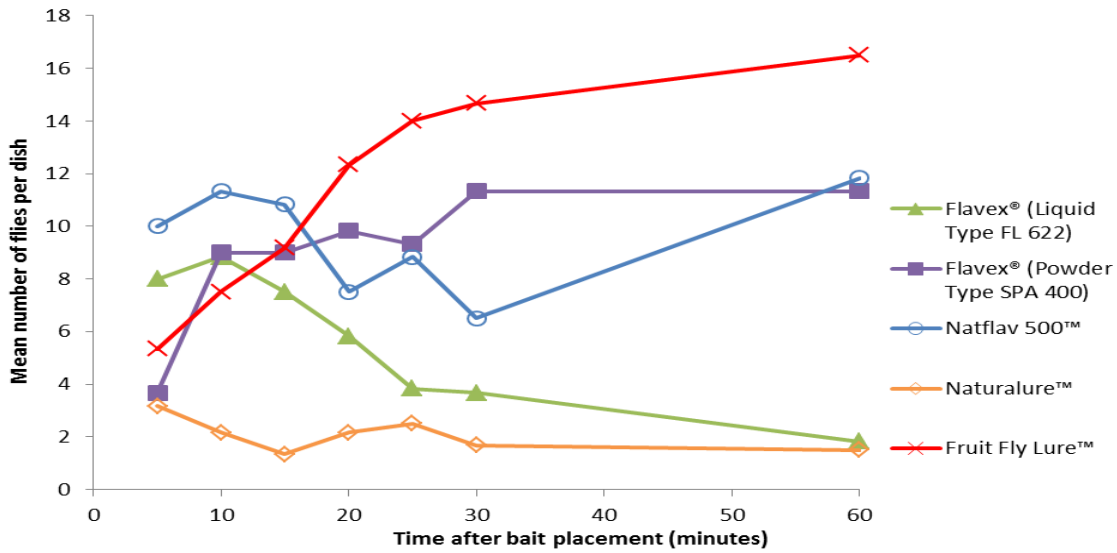

Figure 7.   Number of female *B. cucumis* at each of five baits (data points are back-transformed means).
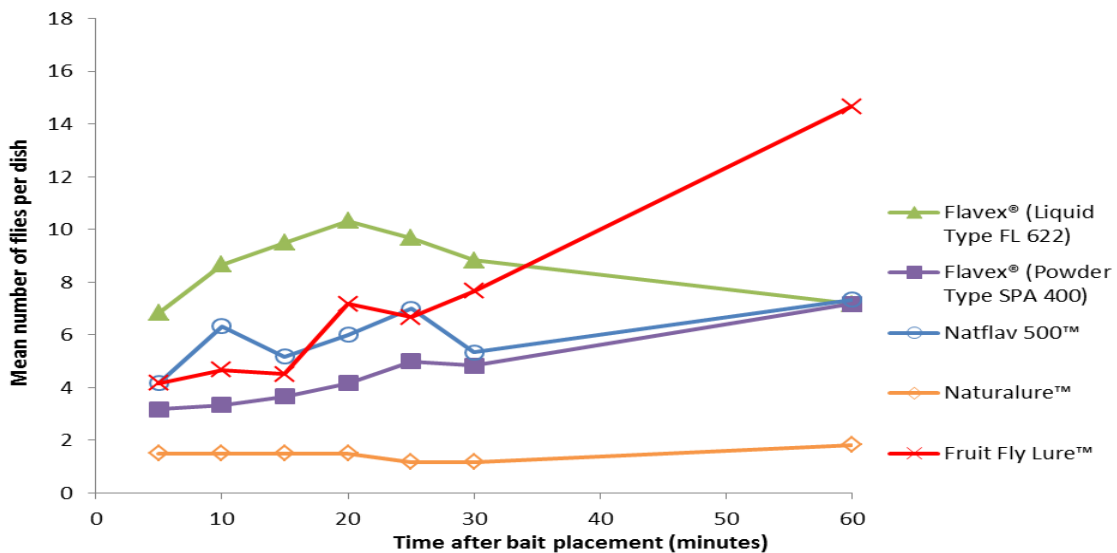

Figure 8.   Number of male *B. cucumis* at each of five baits (data points are back-transformed means).
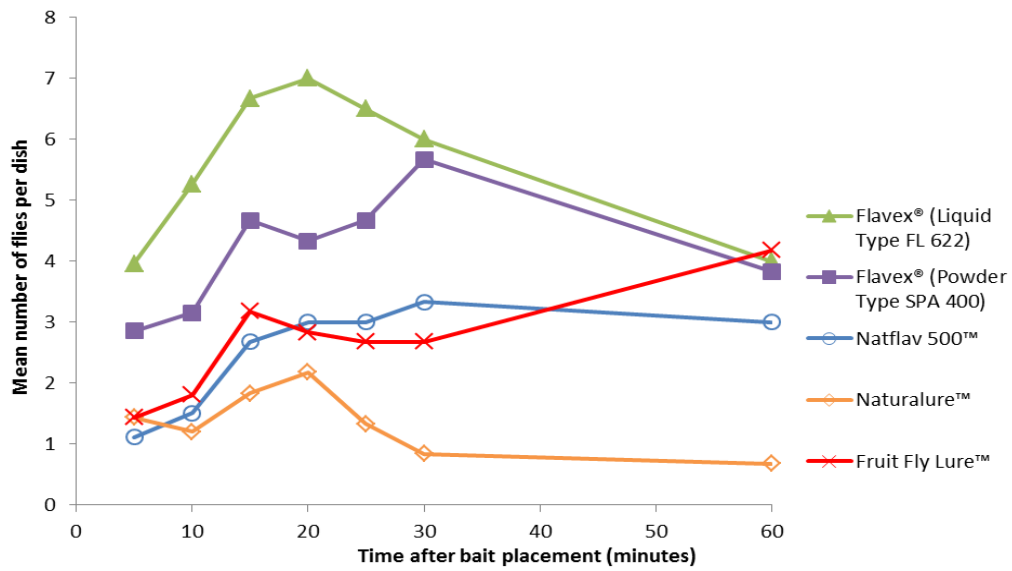
Figure 9.   Number of female *B. tryoni* at each of five baits (data points are back-transformed means).
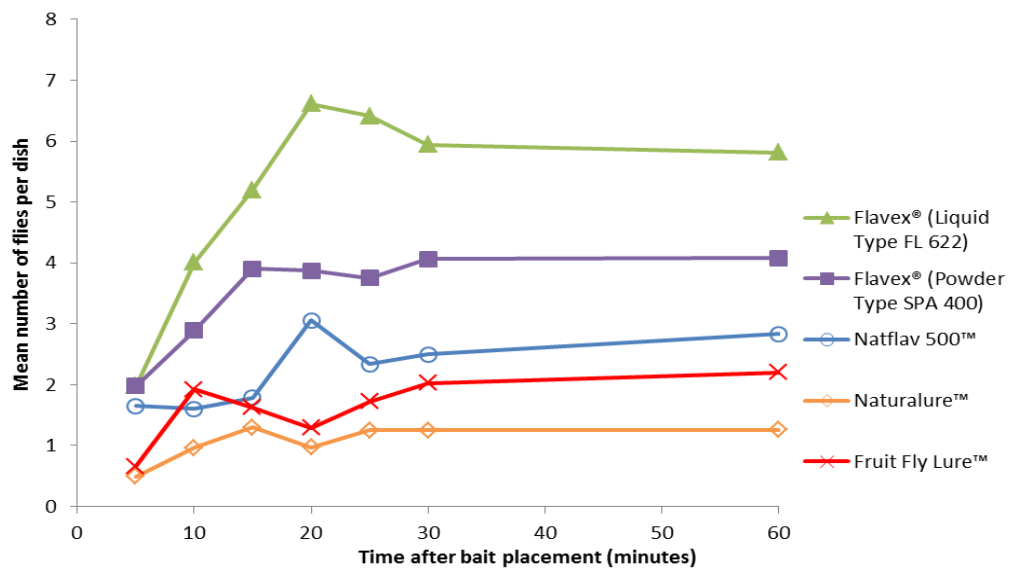

Figure 10.   Number of male *B. tryoni* at each of five baits (data points are back-transformed means).

Comparison of protein bait toxicants

The effect of six insecticides on *B. cucumis*) and *B. tryoni* fruit fly knockdown and mortality were compared (Table 2). Maldison was included as a standard comparison together with a blank bait control (no insecticide) and a no bait control. All insecticide treatments were offered mixed with Fruit Fly Lure™ protein.

Table 2.   Treatments and application rate in bait mix.

Treatment	Insecticide active ingredient concentration	Insecticide rate per litre[1]
Blank bait control		
No bait control		
HY-MAL™ (standard)	1150 g/L maldison	4.35 ml
CroPro STEALTH[©]	18 g/L abamectin	0.25 ml
Success™ Neo	120 g/L spinetoram	0.13 ml
Regent[®] 200SC	200 g/L fipronil	0.23 ml
Fastac[®] Duo	100 g/L α-cypermethrin	4.35 ml
SAMURAI™	500 g/kg clothianidin	0.25 g

[1]All insecticide treatments included Fruit Fly Lure™ protein at 20 ml/L water.

Bioassays were performed in 21 x 21 x 33 cm gauze sided cages. Fruit flies were obtained from the colonies maintained at the DAF laboratories in Brisbane, and were seven days post-emergence and protein deprived. Forty fruit flies (20 male, 20 female) were placed in each cage, provided with sugar and water. Bait (4 ml) was placed into each cage within a Petri dish. Assessments of affected fruit flies (knocked down and dead) were made up to 48 hours post-bait introduction. Assessments of feeding flies were made up to 90 minutes post-bait introduction, in order to determine whether the addition of an insecticide deterred feeding compared with the blank bait control. Four replicates were performed for each treatment, with treatments assigned using a latinised row-column design.

Counts of affected flies and feeding fruit flies at each assessment interval were expressed as a proportion of the total number of flies per cage. In order to analyse the effect of treatment on affected flies over time, assessment intervals were combined in a repeated measures analysis using residual maximum likelihood (REML). Different correlation models were fitted in the REML analysis to account for any correlations between the time assessments within each cage.

Results

There was a significant effect of treatment on the proportion of affected *B. cucumis* ($p < 0.001$). Pairwise comparisons found that knockdown and mortality was significantly higher in all insecticide treatments compared with the controls (Table 3). The quickest response was found in the maldison treatment and the slowest in abamectin reflecting the differences in how quickly these treatments took effect. However, by the end of the trial all insecticides except alpha-cypermethrin had resulted in close to 100% of flies affected (Figure 11).

Table 3.   Results of a repeated measures REML analysis of the combined effect of bait toxicant over time on the proportion of affected *B. cucumis*.

Treatment	Mean number of fruit flies affected	
Blank bait control	0.01	a
No bait control	0.01	a
Maldison (HY-MAL™)	0.72	b
Abamectin (CroPro STEALTH©)	0.29	c
Spinetoram (Success™ Neo)	0.59	d
Fipronil (Regent® 200SC)	0.46	e
α-cypermethrin (Fastac® Duo)	0.55	f
Clothianidin (SAMURAI™)	0.63	g
Average SED	0.02	
Average 95% LSD	0.03	

Means not followed by a common letter differ significantly (p<0.05).
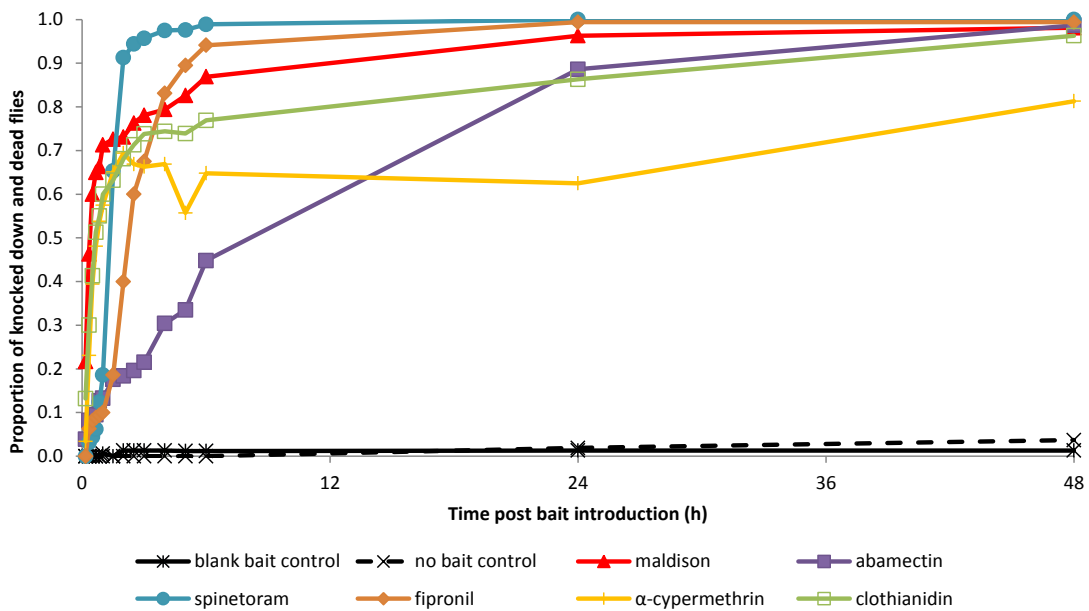

Figure 11.   Proportion of affected (knocked down and dead) *B. cucumis* (data points are back-transformed means).

There was also a significant effect of treatment on the proportion of affected *B. tryoni* (p<0.05); knockdown and mortality was significantly higher in all toxicant treatments compared to the controls (Table 4). The greatest response occurred in maldison and abamectin. By the end of the trial close to 100% of flies were affected in the abamectin, spinetoram and fipronil treatments, whereas only 26% of flies in the alpha-cypermethrin treatment were affected (Figure 12).

Table 4.   Results of a repeated measures REML analysis of the combined effect of bait toxicant over time on the proportion of affected *B. tryoni*.

Treatment	Mean number of fruit flies affected	
Blank bait control	0.01	a
No bait control	0.00	a
Maldison (HY-MAL™)	0.45	d
Abamectin (CroPro STEALTH$^{©}$)	0.38	cd
Spinetoram (Success™ Neo)	0.19	b
Fipronil (Regent$^{®}$ 200SC)	0.34	c
α-cypermethrin (Fastac$^{®}$ Duo)	0.14	b
Clothianidin (SAMURAI™)	0.33	c
Average SED	0.03	
Average 95% LSD	0.07	

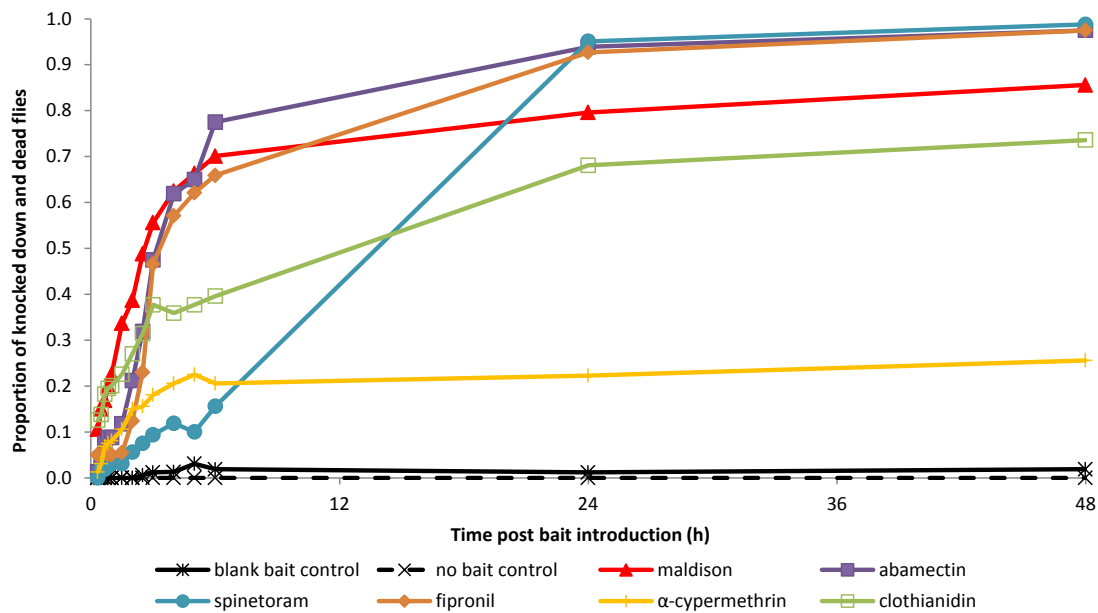Means not followed by a common letter differ significantly (p<0.05).

Figure 12.   Proportion of affected (knocked down and dead) *B. tryoni* (data points are back-transformed means).

Counts of feeding flies indicate that the low efficacy of alpha-cypermethrin may have been due to a repellent effect (Figures 13 and 14).
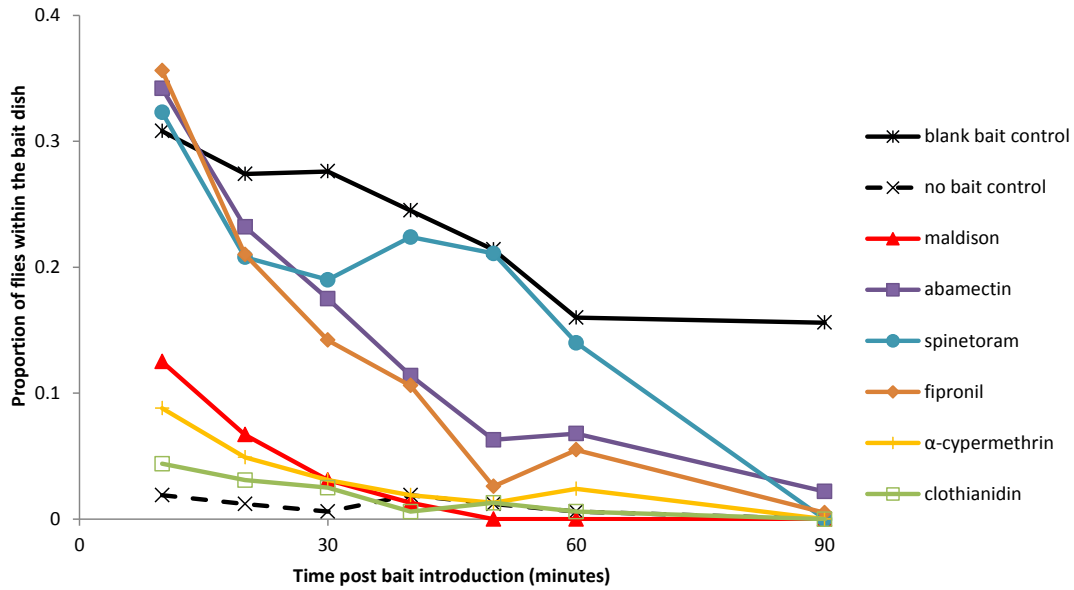

Figure 13.　　Proportion of *B. cucumis* within bait dishes at intervals after bait introduction (data points are back-transformed means).
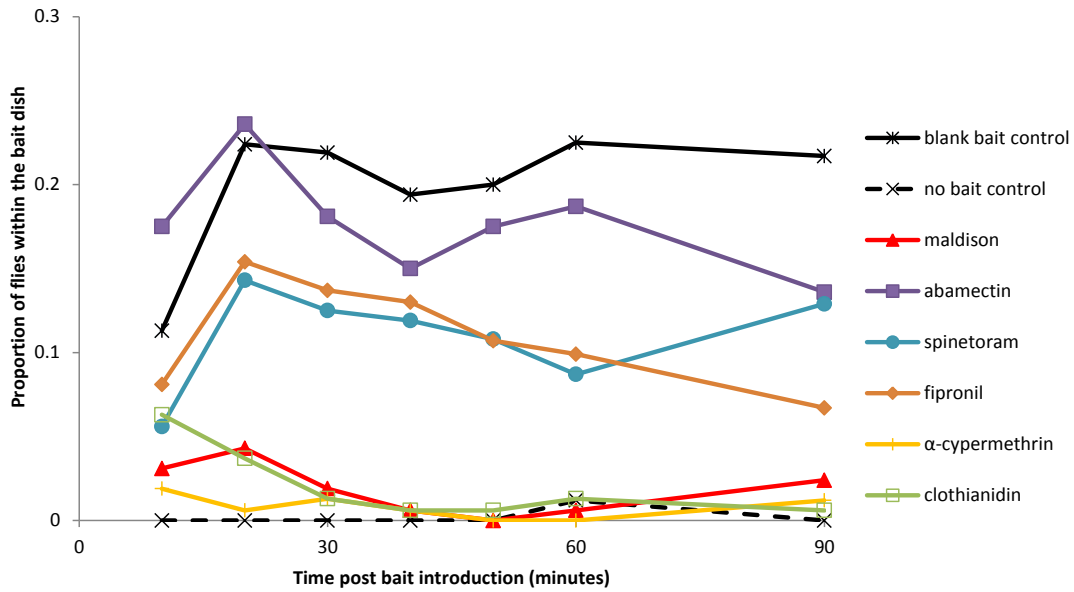

Figure 14.　　Proportion of *B. tryoni* within bait dishes at intervals after bait introduction (data points are back-transformed means).

Appendix 6: Development of bait station technologies

Principal Researchers: S De Faveri and G Lowe

Comparison of insecticides for fruit fly bait stations

The efficacies of six insecticides, presented in a wax matrix (bait station) for fruit fly control, were compared in cage trials at Mareeba July–October 2014. Bait stations (Figure 1) were made by incorporating the treatment rate of insecticide and 100 ml of protein into a wax block (Table 1). They were compared, together with a control (no bait station) and a commercial bait station (Cera Trap®), at four weathering times; when freshly prepared, and after 3, 8 and 12 weeks weathering within a tree canopy that simulated aging effects within a mango orchard.

Table 1.   Insecticides compared in bait station cage trials.

Active ingredient	Rate per bait station (ml)
Abamectin	2
Alpha cypermethrin	5
Clothianidin	2
Fipronil	2
Maldison	5
Spinetoram	2

Treatments were assessed in 60cm x 30cm x 30cm aluminium gauze sided cages inside a poly-roofed nursery igloo. At each weathering assessment time, 100 *B. tryoni* fruit flies (5-10 days old, of mixed sex and protein deprived) were released into each cage between 6.30 and 8.00 am. Cages were then supplied with sugar and water, and wax blocks (insecticide treatments) were hung from the middle of the cage roof.

Counts of apparently dead flies were made on consecutive days; 10 am, 12 pm and 4 pm on the first day, and 10 am, 12 pm and 2 pm on the second. Dead flies were removed following the 4 pm and 2 pm counts. Cages were laid out in a latinised block design to eliminate potential influence of cage position on treatment/fly response. Four replicates (each of 30 hours duration) of each weathering time were completed, two replicates per week over two weeks.

Mortality data (counts, and proportions at 2 pm on day 2) were analysed using generalized linear mixed models. For count data, a Poisson distribution and a log link function was used, and for proportion data, a binomial distribution and logit link function. Where a significant difference was found pairwise comparisons were made using the pairwise 95% least significant differences (LSD).

Comparison of bait stations in field trials

The efficacy of bait station treatments designed to control fruit flies (Table 4, Figure 2), were compared in fruiting mango orchards at Mareeba (cv. Kensington Pride) and Mutchilba (cv. Honey Gold) in North Queensland. Five of the treatments were new bait station concepts (wax matrix and synthetic membrane) containing protein and insecticide at two rates, and each was enclosed within a BioTRAP Globe trap for comparison. The sixth treatment was a commercial fruit fly trap containing liquid protein

(Cera Trap®; Barmac Pty Ltd).

Treatments were arranged as a randomized complete block design; each spaced at 25 m intervals along and across mango rows and hung at 1.5 m height with the canopy of trees. There were five replicates of each treatment. Traps were cleared of flies weekly for the duration of each trial; 12/11/2014 to 18/3/2015 at Mareeba, and 11/11/2014 to 27/1/2015 at Mutchilba. Captured fruit flies were identified to species, counted and sexed. The number of gravid female flies was also recorded.

Count and proportion catch data for *B. jarvisi* and *B. tryoni* were analysed using generalized linear mixed models. For count data, a Poisson distribution and a log link function was used, and for proportion data, a binomial distribution and logit link function. Where a significant difference was found pairwise comparisons were made using the pairwise 95% least significant differences (LSD). The Mutchilba trial was terminated prematurely due to pruning operations. Total fruit fly catches for treatments for the collection period were low (360 compared with 3366 caught at Mareeba), with 85% caught in the final two collections. Only results for total fruit fly catches for the collection period are therefore presented.

Table 2.　　　　Bait station treatments compared in the trials in mango orchards at Mareeba and Mutchilba, North Queensland.

Bait station concept	Treatment code	Protein rate (ml)	Toxicant (active ingredient)	Toxicant rate (ml)
Wax matrix	Wax Mal 100/5	100	Maldison	5
Wax matrix	Wax Mal 50/2.5	50	Maldison	2.5
Wax matrix	Wax Spin 100/2	100	Spinetoram	2
Synthetic membrane	Sin Mal 100/5	100	Maldison	5
Synthetic membrane	Sin Mal 50/2.5	50	Maldison	2.5
Cera Trap®[1]	Cera Trap	600		

[1]BACMAC Pty Ltd (www.barmac.com.au).

Wax matrix	Synthetic membrane	Cera Trap®

Figure 1.　Fruit fly bait station concepts compared in mango orchards at Mareeba and Mutchilba in North Queensland.

Results

Comparison of insecticides for fruit fly bait stations

Across all weathering times, the mean number of dead fruit flies as a proportion of the total number released was significantly higher for the insecticides maldison and fipronil, and lowest for Cera Trap® and the control (p<0.001; Table 3). The mean proportion of dead flies for fipronil increased significantly from the first (fresh) to the last (12 weeks) assessment times, but was not significantly different between assessment times for the other treatments (p=0.031; Table 4).

Table 3.　Mean mortality proportion of flies (*B. tryoni*) across the four weathering periods (0, 3, 8 and 12 weeks) as influenced by insecticide treatments.

Treatment	Mean mortality		Mean (BT) mortality[1]
Abamectin	0.53 c	*0.21*	0.63
Alpha cypermethrin	0.30 c	*0.21*	0.57
Cera Trap®	-1.79 a	*0.25*	0.14
Clothianidin	-0.28 b	*0.21*	0.43
Control (no bait station)	-1.87 a	*0.25*	0.13
Fipronil	2.07 e	*0.32*	0.89
Maldison	2.27 e	*0.31*	0.91
Spinetoram	1.12 d	*0.23*	0.75

Means not followed by a common letter differ significantly (p<0.001). Values in italics are standard errors of the transformed means. [1]Back-transformed means.

Table 4. Mean mortality proportion of flies (*B. tryoni*) at two weathering times, fresh (0 weeks) and at 12 weeks, as influenced by insecticide treatments.

Treatment	Mean mortality Fresh			Mean mortality 12 weeks			Mean (BT) mortality[1] Fresh	12 weeks
Abamectin	0.25	a	*0.41*	0.76	a	*0.43*	0.56	0.68
Alpha cypermethrin	-0.21	a	*0.41*	1.15	a	*0.44*	0.45	0.76
Cera Trap®	-1.98	a	*0.52*	-1.45	a	*0.47*	0.12	0.19
Clothianidin	-0.42	a	*0.41*	0.15	a	*0.42*	0.40	0.54
Control (no bait station)	-1.60	a	*0.51*	-2.03	a	*0.52*	0.12	0.12
Fipronil	0.87	a	*0.43*	3.90	b	*0.97*	0.71	0.98
Maldison	1.80	a	*0.49*	2.67	a	*0.61*	0.86	0.94
Spinetoram	1.76	a	*0.49*	0.87	a	*0.43*	0.85	0.70

Means not followed by a common letter **across** weathering times differ significantly ($p=0.031$). Values in italics are standard errors of the transformed means. [1]Back-transformed means.

Comparison of bait stations in field trials

Weekly fruit fly catches of treatments followed similar trends at Mareeba and Mutchilba for the respective collection periods of each trial (data not presented). At Mareeba, highest catches of both fly species (*B. jarvisi* and *B. tryoni*) were collected over a 35 day period from 13 January to 17 February 2015 (80% of the total of each species caught) (Figure 2).
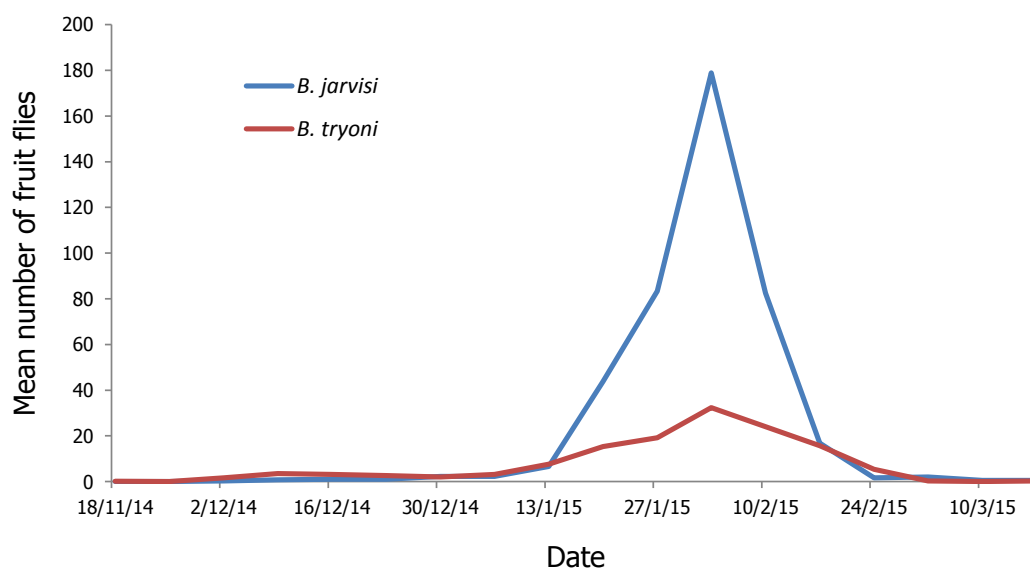

Figure 2. Distribution of weekly fruit fly catches (male and female flies) over the 125 day collection period from 12 November 2014 to 17 March 2015 at Mareeba.

Significantly higher (p<0.001) total catches were collected from Cera Trap® at both trials (Tables 5 and 6). While differences in total catches amongst the wax matrix and synthetic membrane treatments were not consistent at the two trials, catches tended higher for synthetic membrane (100 ml protein and 5 ml maldison) at both locations.

Table 5.   Mean fly catch (*B. jarvisi* and *B. tryoni*) over the 68 day collection period as influenced by bait/toxicant treatments at Mutchilba.

Treatment	Total fly catch (*B. jarvisi* and *B. tryoni*)			BT[1]
Wax Mal 100/5	1.33	ab	*0.47*	3.79
Wax Mal 50/2.5	0.49	a	*0.65*	1.62
Wax Spin 100/2	0.93	ab	*0.54*	2.53
Syn Mal 100/5	2.61	c	*0.32*	13.54
Syn Mal 50/2.5	1.95	bc	*0.38*	7.04
Cera Trap®	3.62	d	*0.279*	37.19

Means not followed by a common letter differ significantly (p<0.001). Values in italics are standard errors of the transformed means. [1]Back-transformed means.

Table 6.   Mean fly catch (*B. jarvisi* and *B. tryoni*) over the 125 day collection period as influenced by bait/toxicant treatments at Mareeba.

Treatment	Total fly catch (*B. jarvisi* and *B. tryoni*)			BT[1]	Proportion *B. jarvisi*			BT[1]
Wax Mal 100/5	1.27	b	*0.27*	58.6	0.78	a	*0.21*	0.69
Wax Mal 50/2.5	1.56	b	*0.23*	78.2	1.07	a	*0.19*	0.74
Wax Spin 100/2	0.00	a	*0.51*	16.4	0.77	a	*0.39*	0.68
Syn Mal 100/5	1.92	b	*0.20*	111.6	1.11	a	*1.16*	0.75
Syn Mal 50/2.5	1.39	b	*0.26*	65.8	1.04	a	*0.21*	0.74
Cera Trap®	3.04	c	*0.11*	313.0	1.26	a	*0.10*	0.78

Means not followed by a common letter within a column differ significantly (p<0.001). Values in italics are standard errors of the transformed means. [1]Back-transformed means.

At Mareeba, both *B. jarvisi* and *B. tryoni* were collected from all treatments (Table 6). *B. jarvisi* were caught in greater numbers (75% of the total caught); however the number, as a proportion of the total *B. jarvisi* and *B. tryoni* caught, was not influenced (p>0.05) by treatments. The proportion of the total number of each fly species caught that were female was also not influenced (p>0.05) by treatments (Tables 7 and 8). Averaged across treatments, 82% of the total *B. jarvisi* caught were females, while only 18% of the total *B. tryoni* caught were female.

Gravid (egg carrying) females of both fly species were caught by all treatments. Differences amongst treatments for the proportion of the total females caught that were gravid were only significant for *B. jarvisi* however, Cera Trap® and synthetic membrane (100 ml protein and 5 ml maldison) caught proportionally higher (p=0.005) numbers than wax matrix and synthetic membrane both with 50 ml protein and 2.5 ml of maldison. Averaged across treatments, 63% of the total numbers of *B. jarvisi* females were gravid, while only 36% of *B. tryoni* females were of this condition.

Table 7.　　Mean fly catch (*B. jarvisi*) over the 125 day collection period as influenced by bait/toxicant treatments at Mareeba.

Treatment	Total *B. jarvisi* catch			Proportion female			Proportion of females gravid			
			BT[1]			BT[1]				BT[1]
Wax Mal 100/5	3.69	ab	*0.29*	40.2	1.54	a	*0.27*	0.82	0.43 ab	*0.17*	0.61
Wax Mal 50/2.5	4.06	bc	*0.24*	58.2	1.53	a	*0.23*	0.82	0.15 a	*0.14*	0.54
Wax Spin 100/2	2.42	a	*0.55*	11.2	1.37	a	*0.46*	0.80	0.61 ab	*0.30*	0.65
Syn Mal 100/5	4.43	c	*0.20*	84.0	1.62	a	*0.20*	0.83	0.82 b	*0.13*	0.70
Syn Mal 50/2.5	3.88	bc	*0.26*	48.6	1.36	a	*0.24*	0.80	0.33 a	*0.15*	0.58
Cera Trap®	5.59	d	*0.11*	267.2	1.65	a	*0.13*	0.84	0.69 b	*0.09*	0.67

Means not followed by a common letter within a column differ significantly (p=0.005). Values in italics are standard errors of the transformed means. [1]Back-transformed means.

Table 8.　　Mean fly catch (*B. tryoni*) over the 125 day collection period as influenced by bait/toxicant treatments at Mareeba.

Treatment	Total *B. tryoni* catch			Proportion female			Proportion of females gravid			
			BT[1]			BT[1]				BT[1]
Wax Mal 100/5	2.91	b	*0.28*	18.4	-1.54	a	*0.27*	0.18	-0.62 a	*0.38*	0.35
Wax Mal 50/2.5	2.98	b	*0.28*	19.6	-1.55	a	*0.22*	0.18	-0.25 a	*0.29*	0.44
Wax Spin 100/2	1.65	a	*0.53*	5.2	-1.36	a	*0.45*	0.20	-0.59 a	*0.64*	0.36
Syn Mal 100/5	3.32	b	*0.23*	27.6	-1.62	a	*0.20*	0.17	-0.51 a	*0.27*	0.38
Syn Mal 50/2.5	2.85	ab	*0.29*	17.2	-1.35	a	*0.23*	0.21	-1.67 a	*0.42*	0.16
Cera Trap®	4.33	c	*0.14*	75.8	-1.65	a	*0.13*	0.16	-0.16 a	*0.15*	0.46

Means not followed by a common letter within a column differ significantly (p<0.001). Values in italics are standard errors of the transformed means. [1]Back-transformed means.

Appendix 7.   Evaluation of a perimeter baiting system and trap technologies for the management of cucumber fly (*Bactrocera cucumis*) and Queensland fruit fly (*Bactrocera tryoni*)

Evaluation of optimal protein baiting techniques in small-scale screening trials

Principal Researcher: L Senior

Comparison of preferred roosting plants for fruit flies

Eight plant species (types) were assessed as preferred roosting sites for the fruit flies *Bactrocera cucumis* and *B. tryoni*. Trials were performed in four 3 x 3 x 2.5 m gauze sided cages within a shade house at DAF Redlands Research Facility (Cleveland, QLD). Two cages were assigned to each fly species, providing individual placements (i.e. corners) for the eight plant types. Each plant type consisted of 2–3 plants per individual placement depending on their size. A clear plastic disc (55 mm diameter) with protein bait applied was fixed to one plant of each plant type. Approximately 300 *B. cucumis* or *B. tryoni* were released into each cage. Flies were mixed sex, 3–7 days post-emergence and protein deprived. Sugar and water were provided. Counts of flies on bait on each plant type were made at 30 minute intervals for two hours. Eight replicates were performed for each fly species. A resolvable incomplete block design was used to allocate plant type to the cages.

Data from each assessment time were combined and subjected to a generalized linear mixed model (GLMM) with a Poisson distribution and log link function. Pairwise comparisons of means were performed using Fisher's protected 95% least significant difference (LSD).

Results

There was a significant effect of plant type on response of *B. cucumis* ($p<0.001$), with higher numbers recorded for sweet corn and forage sorghum (Figure 1). There was no significant effect of time ($p=0.477$), nor any interaction between plant type and time ($p=0.996$).

There was also a significant effect of plant type on response of *B. tryoni* ($p<0.001$), with higher numbers recorded from sugar cane and cassava (Figure 2). There was a significant effect of time ($p<0.001$), with the number of flies on baits decreasing over the two hour trial period. However, there was no interaction between plant type and sampling time ($p=0.991$), indicating that response to plant types was similar across all assessment times.
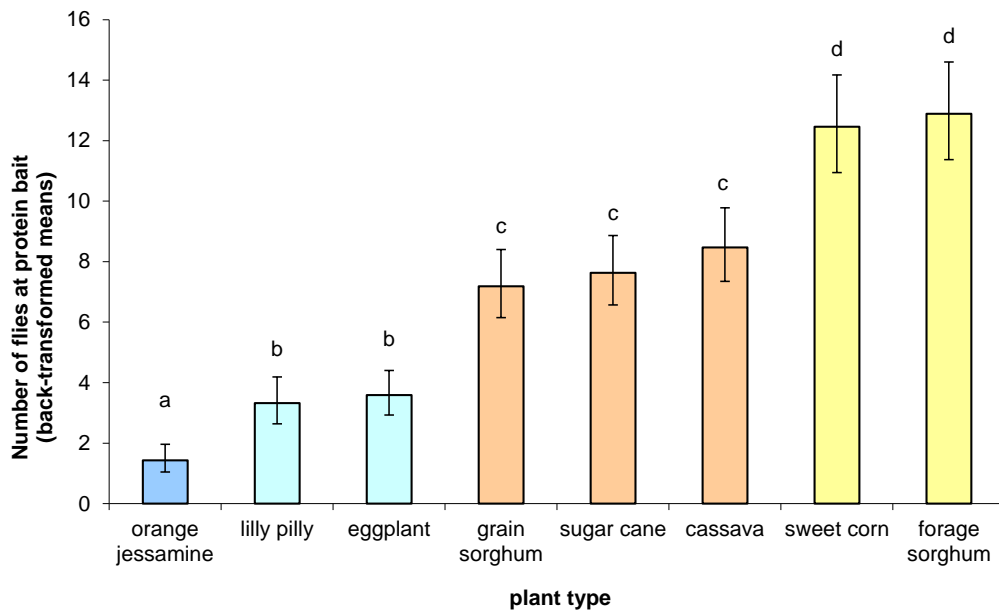
Figure 1.   Mean number of *B. cucumis* on protein baits on each of eight plant types, across all sampling times (back-transformed means ± 1 standard error). Means not followed by a common letter differ significantly (p<0.05).
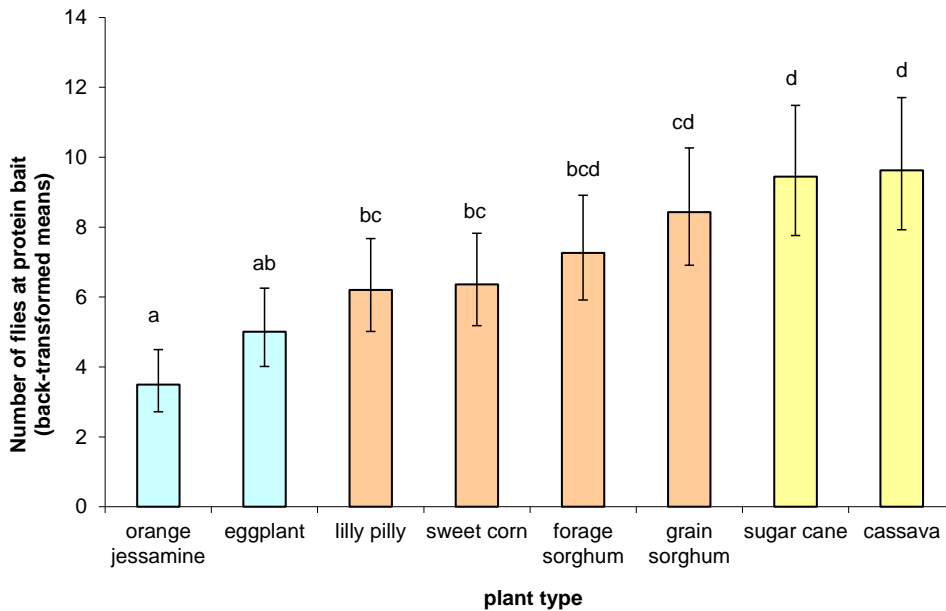

Figure 2.   Mean number of *B. tryoni* on protein baits on each of eight plant types, across all sampling times (back-transformed means ± 1 standard error). Means not followed by a common letter differ significantly (p<0.05).

Optimum height of protein bait application

The optimum height to apply protein bait to forage sorghum and cassava (shown highly attractive to *B. cucumis* and *B. tryoni* respectively), was studied under the same cage conditions as in the previous trial.

The response of each fly species to each plant type was assessed separately.

Three plants of one plant type were placed in a cage, each assigned to a separate corner. A clear plastic disc (55 mm diameter) with protein bait applied was fixed to each plant at 1, 1.5 and 2 m height, giving three baits at each height per cage. Approximately 300 *B. cucumis* or *B. tryoni* (mixed sex, protein deprived, and 4-7 days post emergence) were released into the cage. Sugar and water were provided. Counts of flies per bait were made at 30 minute intervals for two hours. Three replicates were performed for each fruit fly species.

Trials with each plant type and fruit fly species were analysed separately. The mean number of fruit flies at each bait height per cage replicate was calculated. Data from each assessment time were combined and analysed using repeated measures analysis of variance (ANOVA). Pairwise comparisons of means were performed using Fisher's protected 95% LSD. A square root transformation for mean counts was required to improve the assumptions underlying the ANOVA for *B. tryoni* on forage sorghum.

Results

Bactrocera cucumis. There was a significant effect of bait height on cassava (p=0.010), with more fruit flies recorded on baits placed at 1 or 1.5 m compared with 2 m height (Figure 3). There was also a significant effect of time (p=0.002), with mean counts decreasing over time, but no interaction between bait height and time (p=0.240), suggesting a similar pattern of response over time. On forage sorghum, there was no effect of bait height (p=0.341; Figure 3), nor time (p=0.166) and no significant interaction between these two factors (p=0.800).
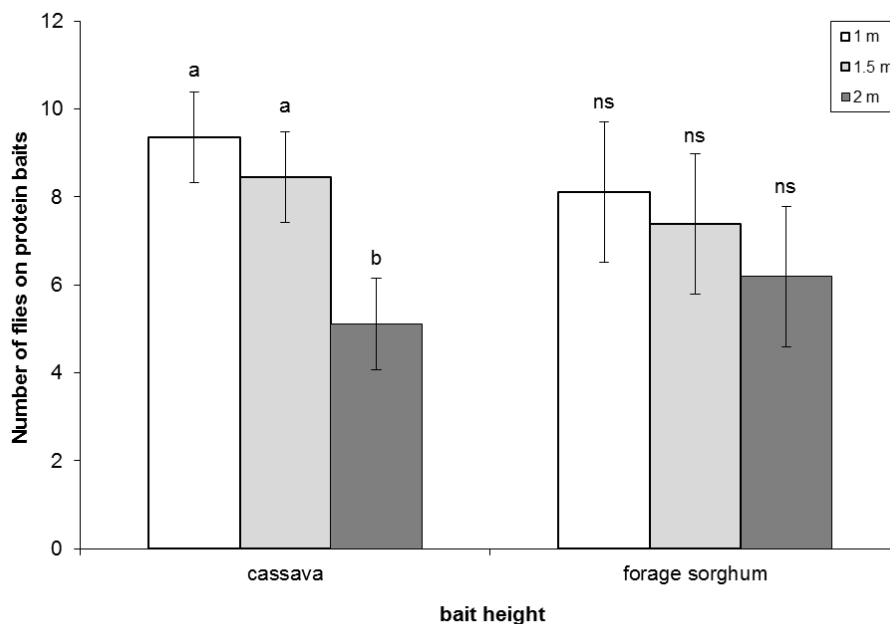

Figure 3. Mean number of *B. cucumis* on protein baits placed at three heights on cassava or forage sorghum, across all sampling times. Error bars represent the 95% least significant difference (LSD). Means within a plant type not followed by a common letter differ significantly (p<0.05). ns = not significant.

Bactrocera tryoni. There was no significant effect of bait height on cassava (p=0.075) (Figure 4). There was a significant effect of time (p<0.001), with mean counts declining over time. There was also a significant interaction between bait height and time (p=0.009). Pairwise comparisons between heights within a time found significantly more flies on 2 m baits compared with 1 and 1.5 m baits at the 30 and 60 minute assessments only (Table 1). On forage sorghum, there was a significant effect of bait height (p=0.015), with more flies on 2 m baits than those at 1 and 1.5 m (Figure 4). There was a significant effect of time (p=0.002), with mean counts declining over time, but no significant interaction between the two factors (p=0.712).
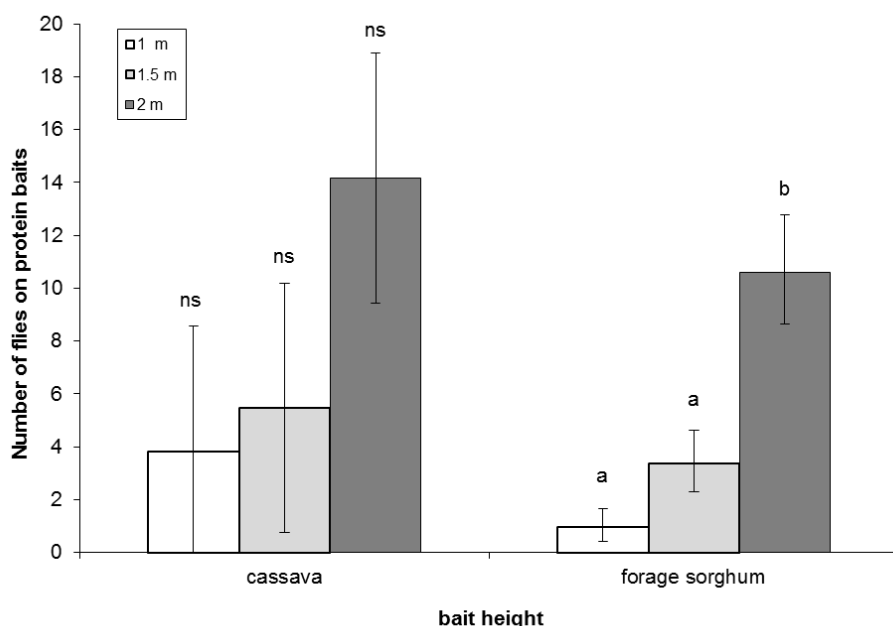

Figure 4.   Mean number of *B. tryoni* on protein baits placed at three heights on cassava or forage sorghum, across all sampling times (forage sorghum means are back-transformed). Error bars represent the 95% least significant difference (LSD). Means not followed by a common letter differ significantly (p<0.05). ns = not significant.

Table 1.   Interaction means resulting from repeated measures ANOVA for *B. tryoni* on protein baits placed at three different heights on cassava.

Bait height (m)	Time after protein bait placement (min)			
	30	60	90	120
1.0	6.6 ab	4.3 ab	2.1 ab	2.3 ab
1.5	8.4 bc	4.9 ab	5.6 ab	3.0 a
2.0	23.9 d	16.7 c	9.6 ab	6.6 ab

Height x Time interaction (comparison between heights): SED = 3.85, 95% LSD = 9.69.

Height x Time interaction (comparison within height): SED = 2.07, 95% LSD = 4.74.

Means not followed by a common letter differ significantly (p<0.05).

Behavioural studies of *Bactrocera cucumis*

Dispersal behaviour

B. cucumis dispersal behaviour into zucchini (host plant) from an adjacent forage sorghum roosting border row was studied in a 57 x 34 m netted area at Maroochy Research Facility (Nambour, QLD). A row of forage sorghum was planted at one end of the netted area, and was 2.3 m in height at the time of the study. Zucchini was planted in rows parallel to the sorghum. Rows 7, 22 and 52 m from the sorghum, were used for the trial.

Four organic zucchini sample fruit, pierced to ensure even oviposition, were placed under each of five plants in each of the three rows of the host plantings. *B. cucumis* (mixed sex, 12–14 days post-emergence, and protein fed) were released into the sorghum. Sample fruit were collected 1, 4, 7 and 24 hours later, and held under controlled conditions (26°C, 70% RH) on drip trays in ventilated containers for a minimum of 11 days to allow eggs to develop to the pupal stage. Counts of pupae were analysed using an ANOVA with a square root transformation. Pairwise comparisons of the interaction means were performed using Fisher's protected 95% LSD.

Results

There was a significant effect of distance from the sorghum roosting site ($p < 0.001$), with more pupae developing in zucchinis nearer the sorghum compared with the furthest row (Figure 5). There was also a significant effect of time following release of flies ($p < 0.001$), with fewer pupae developing in zucchinis left in place for 1 hour compared with those left in place for up to 24 hours (Figure 1). There was a significant interaction between distance and time ($p = 0.047$). At 7 and 22 m, the mean number of pupae was significantly lower in the 1 hour treatment compared with 4, 7 and 24 hours. No significant differences were found between the times for the 52 m row.
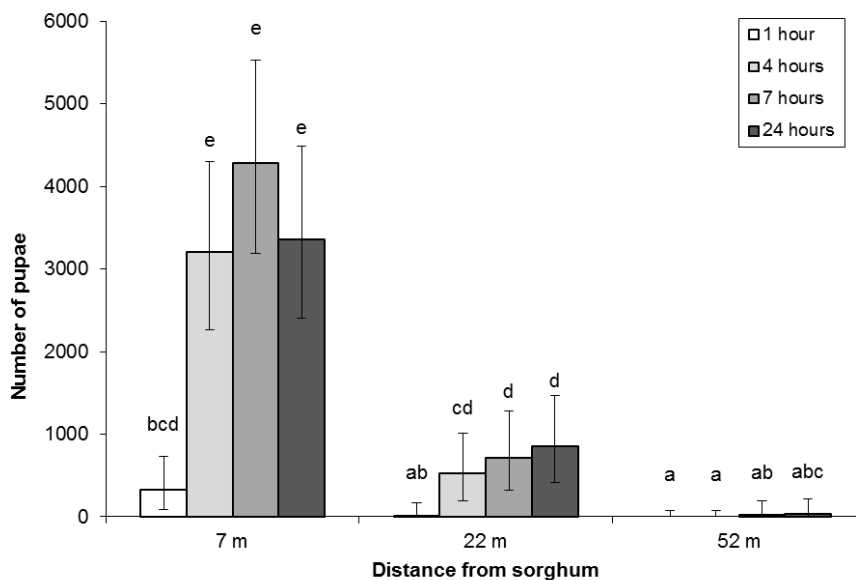

Figure 5.   Mean number of *B. cucumis* pupae in zucchini fruit as influenced by distance from forage sorghum and placement time following fly release (back-transformed means). Error bars represent the 95% least significant difference (LSD). Means not followed by a common letter differ significantly ($p < 0.05$).

Diurnal behaviour

The diurnal behaviour of *B. cucumis* were studied within a 5 x 5 m glasshouse bay at Redlands Research Facility (Cleveland, QLD). Five potted zucchini plants were placed parallel to a row of three potted cassava plants, and a clear plastic disc (55 mm diameter) with protein bait applied was fixed to all plants. One hundred *B. cucumis* (mixed sex, 11-18 days post-emergence and protein fed) were released into the glasshouse bay on the afternoon prior to the start of the trial. Sugar and water were provided.

One organic zucchini fruit was placed under each of the zucchini plants and replaced at intervals, so that five batches of zucchinis were exposed to the flies over the three (diurnal; 6:15 am to 5:30 pm) replicates of the trial. It was not practical to remove and replace fruit flies after each replicate, however additional fruit flies were released. Hourly counts were made of the number of fruit flies on protein baits (assumed to be feeding), on the zucchini fruit (assumed to be ovipositing) and roosting on each plant type. The zucchini fruit were returned to the laboratory after each replicate and incubated under controlled conditions (26°C, 70% RH) for a minimum of 7 days to allow eggs to develop to the pupal stage.

Counts of pupae developing in the zucchini fruit from each time period were analysed using analysis of variance (ANOVA). Counts of feeding, ovipositing and roosting fruit flies were analysed using residual maximum likelihood, with a square root transformation applied to counts of roosting flies.

Results

More *B. cucumis* were recorded roosting on the cassava than any other behaviour characteristic (Figure 2); however, fruit fly numbers roosting on zucchini and cassava suggested only marginal differences between the plant types (p=0.054). There was no significant effect of time of day (p=0.095), and no significant interaction between time and plant type (p=0.279).

While the numbers of protein feeding flies were low throughout the trial (Figure 6), significantly more flies were recorded feeding on the cassava plants compared with the zucchini plants (p=0.006). There was no significant effect of time of day (p=0.416), and no significant interaction between plant type and time (p=0.786).

There was an effect of time of day on the number of ovipositing fruit flies, with the mean number significantly lower (p=0.011) in the first hour (6:30 am) than the next four hours (7:30 am–10:30 am). There was a steady decline in ovipositing fruit flies from 1.30 pm onwards (Figure 6). There was only a marginal effect of time of day on pupae number developing in zucchini fruit (p=0.055). Pupae number peaked at 8:30–10:45 am (coinciding with the peak ovipositing fly number), declining thereafter (Figure 7).
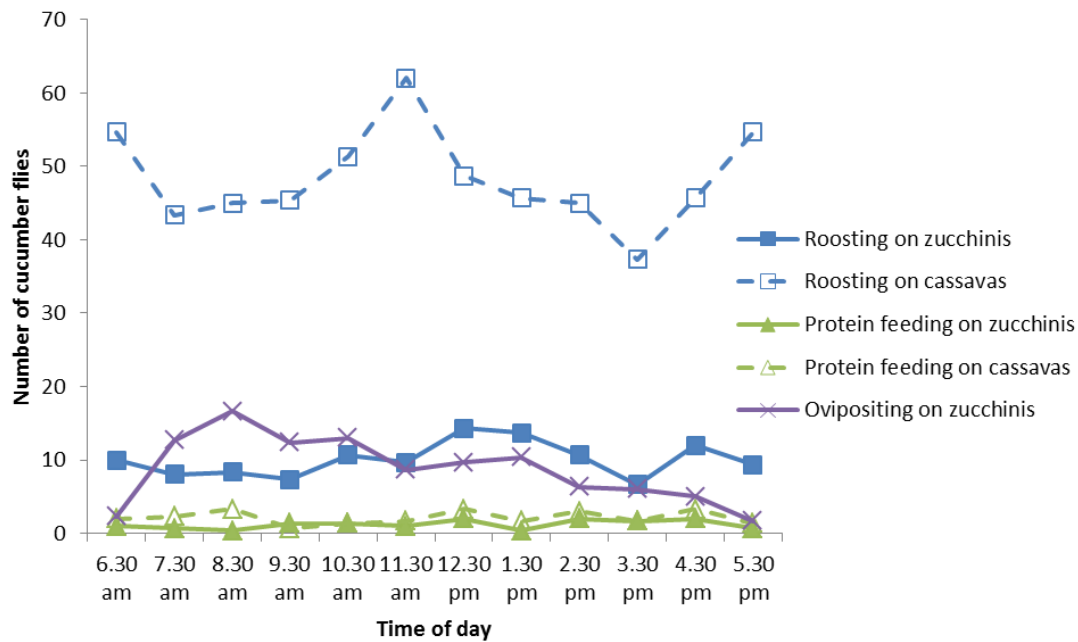
Figure 6.   Number of *B. cucumis* roosting, protein feeding and ovipositing on cassava and zucchini plants at different times of the diurnal cycle from dawn to dusk.
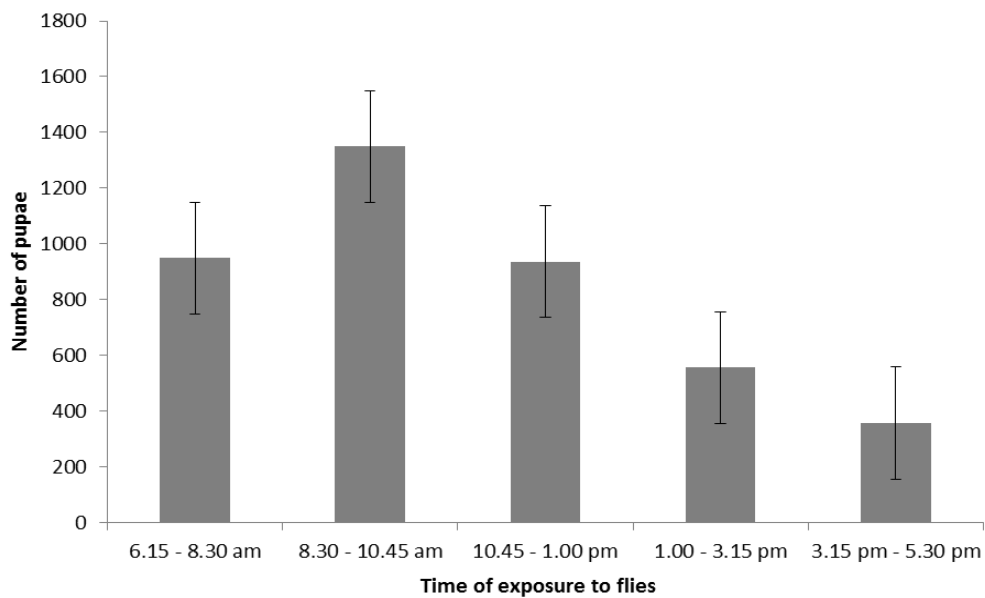

Figure 7.   Number of pupae recovered from zucchini fruit exposed to *B. cucumis* at different times of the diurnal cycle from dawn to dusk. Error bars represent the standard error of the mean.

Field trapping of *Bactrocera cucumis*

B. cucumis was trapped for various periods from 11/10/2013 to 25/3/2014 at nine sites in the Lockyer Valley, Bundaberg and Bowen (Table 2), to monitor its populations and obtain information on its behaviour in the field. A new lure for this species (Scentry Biologicals Inc, Montana, USA) was used in

combination with monitoring traps obtained from Bugs for Bugs Pty Ltd (Munduberra, QLD). The lure was suspended in the trap within a small mesh bag, and a dichlorvos insecticide block was placed in the base of the trap. Lures and dichlorvos blocks were replaced every month.

Traps were placed within cucurbit crops, hung from wire hoops at a height of approximately 30 cm above the ground. Traps were also placed in vegetation on the perimeter of the crop, at a height of approximately 1.5 m. Trapped fruit flies were collected weekly and sent to the DAF laboratories in Brisbane for identification.

Results

Total *B. cucumis* trap catches at each site are presented in Table 2. High catch numbers only occurred at one site, Lockyer Valley site A. *No B. cucumis* were trapped at any of the Bowen sites.

Table 2.   Total number of *B. cucumis* trapped at monitoring sites in the Lockyer Valley, Bundaberg and Bowen.

Monitoring site	Number of traps	Trapping period (days)	Total number of cucumber flies trapped
Lockyer Valley site A[1]	6	112	6245
Lockyer Valley site B	6	63	5
Bundaberg site A	5	165	1
Bundaberg site B	5	165	2
Bundaberg site C	2	116	0
Bowen site A	1	86	0
Bowen site B	1	86	0
Bowen site C	1	86	0
Bowen site D	1	86	0

[1]4731 flies caught at this site were from a single collection during harvest.

At the Lockyer Valley site A, collections were taken from six traps over 16 weeks. Of these 96 collections, 68 (71%) contained at least one *B. cucumis*. Of the 6245 flies trapped during the trapping period, 76% were taken in a single collection that coincided with the harvest of the pumpkin and melon crops in mid-December 2014 (Figure 8). The second highest trap catch at this site occurred in late January following a period of high rainfall.

Trap catch from each of the six traps at Lockyer Valley site A was examined separately. Two traps were placed in vegetation on the headland at the perimeter of the crop, and the remaining four were within the crop at varying distances (15 m to 140 m) from the headland. When trap catch from the harvest was excluded, most flies were caught in the trap placed in the crop 15 m from the headland. During the harvest, most flies were collected from traps placed in the crop at greater distances from the headland (55 m, 100 m, 140 m) (Figure 9).
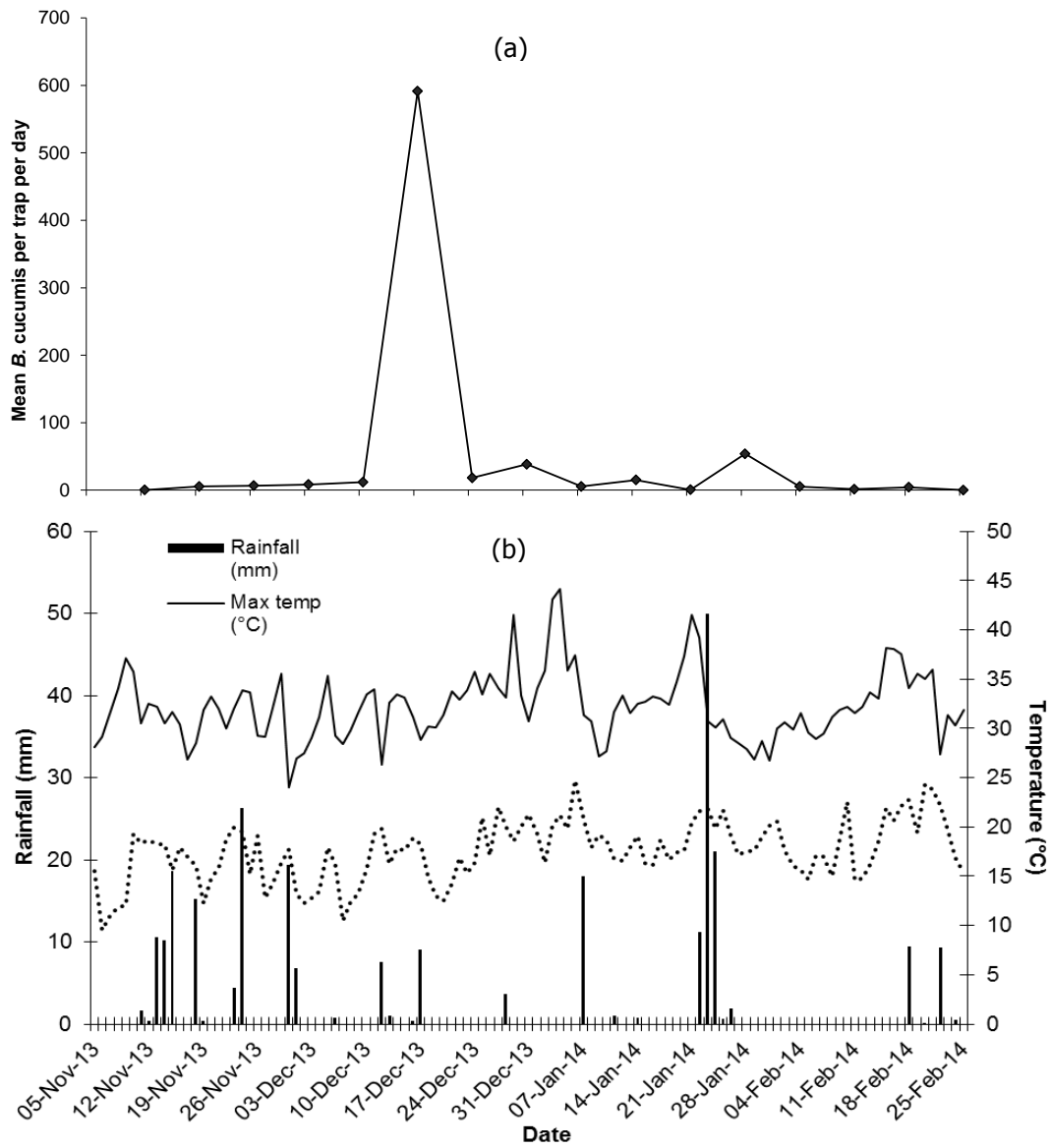
Figure 8. (a) *B. cucumis* trap catches, and (b) rainfall and temperatures over a 112 day monitoring period at Lockyer Valley site A.
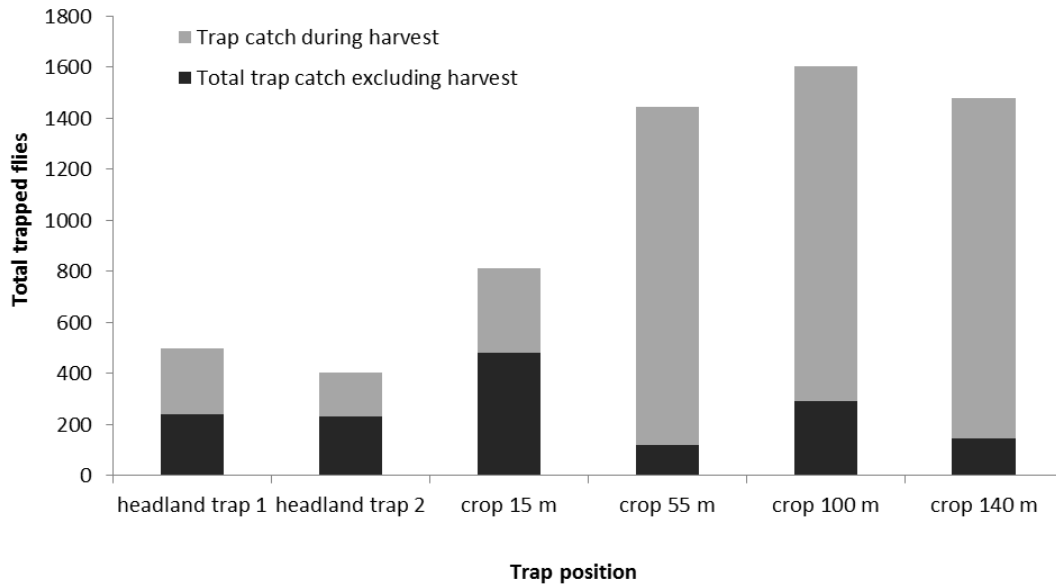
Figure 9.   Total *B. cucumis* catches over the monitoring period from traps placed in vegetation on the pumpkin and melon crop headland and within the crop at increasing distances from the headland at Lockyer Valley site A.

Assessment of trap technologies for attracting *Bactrocera cucumis*

Principal Researcher: G Lowe and S De Faveri

Cucumber lure trap technologies were tested in two trials (6/12/13 to 17/1/14 and 10/1/14 to 21/2/14) within zucchini, cucumber and pumpkin crops growing at Tolga, North Queensland. Trap technologies were a combination of trap type and toxicant treatment (Table 3).

Table 3.   Trap technologies compared in cucurbit crops at Tolga, North Queensland.

Treatment	Trap type	Insecticide treatment
McPhail	McPhail	300 ml 10% propylene glycol solution
Probodelt (DDVP)	Probodelt cone	1 cm^3 dichlorvos block
Probodelt (maldison)	Probodelt cone	Dental wick containing 1 ml maldison
Steiner (DDVP)	Steiner	1 cm^3 dichlorvos block
Steiner (maldison)	Steiner	Dental wick containing 1 ml maldison

Traps were assembled with the lure and insecticide prior to arrival at the trial site. Traps were suspended 0.5 m above the ground on wooden stakes placed at 5 m intervals along the row in the middle of the crop (Figure 10). In both trials there were four replicates of each trap. Traps were cleared weekly over the six week duration of each trial. Captured fruit flies were identified to species, counted and sexed. The number of gravid female fruit flies was also recorded.

Figure 10.   Placement of traps in cucumber crops at Tolga, North Queensland.

Mean *B. cucumis* count data (individual weeks and over the duration of the trial) were analyzed using a generalized linear mixed model assuming a Poisson distribution and a log link function. Treatment was fitted as a fixed effect and replicate as the random effect. If a significant treatment effect was found, pairwise comparisons were performed using the pairwise 95% least significant difference.

Results

Over the duration of each trial, there was a trend of higher mean numbers of *B. cucumis* caught in the McPhail traps compared with the other traps (Table 4). Higher numbers caught by this trap were not significantly different from the other trap treatments in Trial 1 (p=0.594), however they were in Trial 2 (p<0.001). In general, mean weekly catches of all traps followed a similar trend in both trials, and it was only in week three of Trial 1, and weeks three and four of Trial 2, where catches were significantly higher (p<0.001) for McPhail traps compared with the other traps (data not presented).

In both trials, all traps caught *B. cucumis* females and male. There was a consistent trend of higher numbers of each caught by McPhail trap compared with the other traps; however differences amongst traps were not significant in Trial 1 (females p=0.602; males p=0.600; and gravid females p=0.326), but they were in Trial 2 (p<0.005) (data not presented).

Table 4.   Mean numbers of *B. cucumis* caught in different trap technologies in two trials at Tolga, North Queensland.

Protein treatment	Trial 1				Trial 2			
				BT[1]				BT[1]
McPhail	2.2	a	*0.37*	8.9	2.2	a	*0.18*	9.1
Probodelt (DDVP)	1.7	a	*0.46*	5.4	0.7	b	*0.37*	2.0
Probodelt (maldison)	1.7	a	*0.46*	5.6	0.3	b	*0.47*	1.3
Steiner (DDVP)	1.1	a	*0.60*	3.1	1.0	b	*0.33*	2.6
Steiner (maldison)	1.6	a	*0.48*	4.9	0.1	b	*0.51*	1.1

Means not followed by a common letter within a column differ significantly p<0.001. Values in italics are standard errors of the transformed means. [1]Back-transformed means.