Cooperative Research Centre
for National Plant Biosecurity

Final Report

CRC30022

Developing female lures for improved market access

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December 2010

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1. Executive Summary

Fruit flies are significant pests of horticultural crops worldwide. In Australia there are two fruit fly species of economic concern; the introduced species *Ceratitis capitata* (Mediterranean fruit fly; Medfly), which is established in the south-west of Western Australia, and the endemic species *Bactrocera tryoni* (Queensland fruit fly; Qfly), which is found along the coastal fringe of the eastern states (Queensland, New South Wales and Northern Victoria). Australia maintains a number of certified fruit fly free areas, including South Australia, Tasmania and the fruit Fly Exclusion Zone on the Victoria/New South Wales border. Maintaining these areas free from fruit fly and keeping exotic species of fruit fly out of Australia is critical to retaining access to our export markets. Surveillance using fruit fly traps is the principal tool used in defence against invading pests, but there are some fruit fly species that do not respond to the male lures typically used in surveillance programmes. Development of improved lures for detection of female fruit flies would improve our surveillance capability and was the primary aim of this project.

Two prototype lures were developed: a gel lure and a dry lure. The efficacy of these lures was investigated in comparison to the standard liquid protein lure (for *Bactrocera* species) and the three-component BioLure® (for *C. capitata*). Results from the trials indicated that there are significant advantages to be gained by replacing the standard liquid protein lure with a gel lure for surveillance purposes. The gel lure developed and tested in this project was found to maintain its attractancy under a range of climatic conditions for a period of 6-12 weeks, compared to only one week for the liquid lure. It was easily dispensed in traps, did not have an unpleasant odour and captured much fewer unwanted insects, such as blowflies. The condition of fly specimens removed from traps containing the gel lure was markedly better than those removed from traps containing the liquid lure. However, the efficacy of the gel lure compared to the liquid lure was variable depending on the climate, fly species or crop. Sometimes trap captures were comparable (*e.g. D. pornia* captures at Kulnura, NSW) and sometimes significantly fewer flies were captured in traps containing the gel lures (*e.g. B. tryoni* captures at The Rock). Similar results were found with comparisons of the gel lure with BioLure® in WA. Unfortunately, the prototype dry lure consistently performed poorly, probably as a result of rapid volatilisation of attractants.

Experiments conducted in WA demonstrated that the three-component BioLure® is the most effective attractant for *C. capitata*, while orange ammonia lure performed best for *Bactrocera* spp. A significant finding was the effectiveness of 70% propylene glycol (PG) as a killing agent for use in traps with dry lures, such as three-component BioLure® or any of the male lures, including Capilure® or Cuelure. Greater numbers of flies were repeatedly found in traps containing 70% PG as the killing agent compared to traps containing malathion and/or dichlorvos (DDVP). The increased attractiveness of the lures when combined with 70% PG could be due to the presence of liquid, particularly in dry climates where flies need to seek out water. PG is a preferred killing agent, as it has relatively low toxicity compared to the commonly used organophosphate insecticides, and could be used in organic orchards.

Further improvements to the ingredients in the gel lure, or perhaps the development of a more effective trap that disperses lure odour over a greater distance, could see liquid lures being replaced in future. In the meantime, the superior efficacy of the liquid protein lure or orange ammonia lure means that these probably still remain the preferred lures for use in surveillance systems for *Bactrocera* species, and three-component BioLure® the preferred lure for use in surveillance systems for *C. capitata*. Where liquid protein lures continue to be used for surveillance, it is recommended that a stainless steel mesh insert

and DDVP pest strip be used with the McPhail trap to improve the serviceability of traps and preserve the integrity of fruit fly specimens. Where three-component BioLure® is used in Chempac/Suterra traps, propylene glycol may be a suitable alternative to DDVP pest strips, reducing the risk of accidental poisoning where traps are used in urban areas and facilitating the use of traps in organic orchards.

2. Aims and objectives

Globally, fruit flies are one of the most significant horticultural pests because they severely damage fruit, are difficult to control and create major barriers to market access. In Australia, there are two economically important species of fruit fly that are of quarantine concern to our trading partners: the introduced species *Ceratitis capitata* (Mediterranean fruit fly; Medfly), which is present mainly in the south-west of Western Australia, and the endemic species *Bactrocera tryoni* (Queensland fruit fly; Qfly), which is found along the coastal fringe of the eastern states (Queensland, New South Wales and Victoria). Both of these fruit fly species obstruct trade with other countries and, because of their discrete distribution within Australia and the maintenance of fruit fly free areas, they also interfere with trade between states and territories.

Australia is able to export horticultural produce that is host to these fruit flies by application of quarantine treatments before or during transit, or by proving area freedom. Likewise, Interstate Certification Assurances (ICAs) allow the movement of fruit within Australia. Where an area is accepted as fruit fly free, it is continuously monitored in order to confirm that the area remains free of the pest species and for the prompt detection and reporting of invading species as required by protocol through codes of practice under national and international agreements (MCOP 2006; Code of Practice for the Management of Queensland Fruit Fly 1996; FAO 1999).

We have been able to manage these two species of fruit flies effectively for many decades. However, there are a number of fruit fly species that are not present in Australia, some of which could have devastating effects on our local industries should they be introduced. Specific examples include *Bactrocera papayae* (Papaya fruit fly), *Bactrocera dorsalis* (Oriental fruit fly) and *Bactrocera cucurbitae* (Melon fruit fly).

Detection and monitoring of fruit flies is important for demonstrating area freedom in specific regions, and for maintaining secure borders against exotic fruit fly incursions. Trapping of fruit flies relies heavily on the detection of male flies, since the lures with the greatest efficacy are primarily attractive to males of the species. These include Cuelure, methyl eugenol and Capilure (trimedlure). However, some fruit fly species are not attracted to any of these male lures, for example, *Anastrepha suspensa* (Caribbean fruit fly) and *Bactrocera latifrons* (Malaysian fruit fly), both of which are exotic to Australia.

DDVP has been used as a killing agent in fruit fly traps for many years, and is highly efficacious. For the purposes of surveillance it is critical that the killing agent used has a rapid knockdown effect, ensuring that flies are unable to escape the trap once captured. The advantage of DDVP is its ability to act as a fumigant, which ensures that flies are killed as soon as they enter the trap. Existing liquid lures (*e.g.* yeast autolysate or orange ammonia lure) rely on the flies contacting the lure and being killed through drowning. The key disadvantage of DDVP is its toxicity, which can potentially cause adverse health effects through acute or chronic exposure and eliminates its use in certain situations, such as organic orchards. A series of experiments were conducted to determine if there is potential to replace DDVP with a safer killing agent.

Lures that are attractive to female fruit flies would improve our ability to detect exotic incursions, particularly of non-lure responsive species, and also help protect our pest free areas from *B. tryoni* and *C. capitata*.

C. capitata is a common pest in many countries world-wide and consequently more research has been targeted at developing better lures for its detection. Synthetic male attractant lures that are currently in use in surveillance programs are discussed in detail by Cunningham (1989). Capilure® (Trimedlure + extender) is the male attractant lure for *C. capitata* (Drew 1989; White and Elson Harris 1992). However it could be argued that this lure captures only a small percentage of the population. Release and recapture studies by Lance and Gates (1994) found a recapture rate of 0.6% in two tests with 25,600 and 12,800 released *C. capitata*, with a trap density of 10 traps per km^2 in suburban California. The incidence of death in released populations, however, was not accounted for and could have contributed to this result.

The development of a commercially available dry lure was a major advancement for the detection of *C. capitata.* The three-component BioLure® consisting of ammonium acetate, putrescine and trimethylamine (Heath *et al.* 1997), and the two-component BioLure® containing ammonium acetate and putrescine only (Heath *et al.* 1995) are available pre-packaged as separate dispensers. There are also modified versions of this lure available commercially where the three components are combined into a single dispenser such as the Unipak. However, since *B. tryoni*, and other *Bactrocera* species present in northern Australia are endemic to only Australia, fewer resources have been directed at the development of improved female lures. Presently, two lures for the detection of female fruit flies are published in the Code of Practice for Management of Queensland Fruit Fly (1996), both of which are dispensed in McPhail traps (Vickers, 1999, Dominiak and Nicol, 2009). Of the two, a 2% liquid protein (yeast autolysate) lure is most commonly used due to its suitability for the climatic conditions typical of the fruit fly exclusion zone (FFEZ). There is also the option of using an orange juice/ammonium lure, which is better suited for use in humid, tropical climates. However, liquid lures have a number of significant disadvantages (Dominiak, 2006, Dominiak *et al*., 2003), including:

- the need to be replenished every week; the lures putrefy making them smelly and messy to work with, resulting in high labour costs;
- not being specific to fruit flies, thereby capturing a lot of non-target insects, which increase decomposition rates of fruit flies and increase labour costs with regard to sorting trap captures;
- killing the flies by drowning; this can damage the flies through decomposition, making identification difficult.

This project aimed to develop improved lures that overcome these disadvantages and aid in the detection of female fruit flies. These lures could be used for quarantine and monitoring purposes by government agencies and primary producers.

3. (a) Key findings. Part One: New South Wales

METHODS AND MATERIALS

Experimentation by Andrew Jessup (I&I NSW) prior to the funding of this project produced two prototype lures. These lures were prepared as follows:

Gel lure
100g white sugar
9g agar
0.12g calcium propionate
1000mL water

Mix ingredients and bring to the boil, then cool to 40° C. Then add:
15g ammonium acetate
40g yeast hydrolysate

Dry lure
50% (w/w) yeast hydrolysate
50% (w/w) silica gel

The initial phase of this project aimed to optimise and adapt these prototype lures through a series of laboratory experiments using Queensland fruit fly (*B. tryoni*), as described here.

Calcium propionate

Calcium propionate is commonly used as a preservative to inhibit the growth of moulds in bread and other processed foods. To prevent the gel lure deteriorating prematurely due to mould, calcium propionate had been incorporated into the gel lure formulation. Despite the inclusion of 0.12g L^{-1} calcium propionate, mould growth had been observed on the prototype gel lure. An experiment was conducted to determine the optimum concentration of calcium propionate required to inhibit the growth of mould on the gel lure if stored at ambient temperatures for 4-6 weeks.

Individual gel lures containing a range of calcium propionate concentrations (0g L^{-1}, 0.12g L^{-1}, 0.18g L^{-1}, 0.24g L^{-1}, and 0.36g L^{-1}) were prepared. The molten solutions were poured into 90mm plastic Petri dishes to set. The five treatments were arranged in a Latin square design (thereby yielding five replicates of each treatment) on a bench in a well ventilated area that was subject to ambient daytime and night-time temperatures. The lids were left off the Petri dishes for 66 hours to expose them to any mould spores in the air, after which the lids were replaced to reduce desiccation. The lures were inspected and photographed weekly for mould development over five weeks.

Wind tunnel experiments and procedure

The effects of ammonium acetate concentration and sugar type on lure attractiveness were evaluated using a wind tunnel. Lure longevity following field weathering was also evaluated using this method.

The wind tunnel used for these experiments was a custom-built Perspex tunnel with a 1.5m x 0.6m x 0.6m chamber fitted with a blower fan on one end and an extraction fan and vent at the opposite end (Figure 1). The tunnel was housed within a 26°C temperature controlled room and the extraction fan was run continuously throughout the trial to draw air out of the tunnel and expel it outside the treatment room. The blower fan drew air from inside the treatment room and directed it down the tunnel. The combined

action of blower fan and extraction fan created a uni-directional air current that carried the lure volatiles.

Figure 1. The wind tunnel, showing trap placement, used to optimise attractiveness of the gel lure.

Each treatment evaluation (run) started on the hour and took 30 minutes to complete. Ten minutes before each run began; wind speed readings were taken with a digital anemometer from twelve points inside the wind tunnel to ensure that wind speeds were comparable between runs. Typical wind speeds within the tunnel were around 1km hr^{-1}. The tunnel was flushed with fresh air for 30 minutes by operating both fans in the absence of any lure between each run to reduce the likelihood of residual scent contaminating subsequent runs. For this reason, treatments were also administered using a Latin square design, to further reduce the possibility that the order in which treatments were performed affected the outcome. The wind tunnel was cleaned with hot water at the beginning and end of each day.

To quantify the number of flies attracted to the lure, a trap unit was used in the wind tunnel (Figure 2.). The trap was prepared by placing 10mL of lure in a plastic cap and positioning it in the base in a bed of talc powder, which prevented the flies escaping the trap once captured. Once the lure was in position, the top of the trap was placed over the base containing the lure. The trap was always positioned at the same point within the wind tunnel. A container of 50 female *B. tryoni* was placed at the downwind end of the tunnel.

At the end of each run the trap was removed from the wind tunnel, any captured flies counted and the trap cleaned and dried. Any flies remaining in the wind tunnel at the end of a run were removed using a vacuum cleaner.

Selection of flies for use in wind tunnel experiments

All flies used to test experimental lures in the wind tunnel were female *B. tryoni* from the experimental colony housed at Gosford Primary Industries Institute. Three separate trials were run using flies of three different nutritional and reproductive statuses: protein-deprived unmated, protein-fed unmated and protein-fed mated. All units of flies used for the testing of lures were experimentally naïve.

a) Protein-deprived, unmated flies

Approximately 9g of *B. tryoni* pupae were placed in a 10L plastic tub containing water and white sugar. The pupae were kept in the *B. tryoni* colony room, which is maintained at 26°C and 55-65% relative humidity, until eclosion. When flies were between 1-4 days old, they were chilled to 3°C for sorting. Six test units of 50 female flies were sorted into modified plastic containers and returned to the *B. tryoni* colony room to recover. *B. tryoni* are presumed to be unmated within the first 5 days of emergence (Perez-Staples *et al.*, 2007). Flies in these containers were provided with water and white sugar and used in the wind tunnel when they were 2-7 days old.

b) Protein-fed, unmated flies

Pupae and flies were treated identically to protein-deprived, unmated flies, except that flies were provided with yeast hydrolysate as well as white sugar and water.

c) Protein-fed, mated flies

Approximately 6g of *B. tryoni* pupae were placed into two 10L plastic tubs containing water, white sugar and yeast hydrolysate. The pupae were kept in the *B. tryoni* colony room until eclosion. After this the flies were kept in these tubs for three weeks and fed yeast hydrolysate twice a week. It is presumed that flies were able to mate during this time. Three weeks after emergence, flies were chilled to 3°C for sorting. Six test units of 50 female flies were sorted into modified plastic containers and returned to the *B. tryoni* colony room to recover. Flies in these containers were provided with water and white sugar and used in the wind tunnel when they were 19-22 days old.

Figure 2. The trap unit used in the wind tunnel. An entry hole was drilled in the side (top) and the cap of lure placed in a bed of talc powder in the base of the trap (bottom).

Ammonium acetate

Ammonium products are released during the breakdown of proteins and are believed to be the basis for attraction of fruit flies to proteinaceous lures. Because of this process, liquid protein lures become more attractive to fruit flies as they putrefy and degrade under field conditions. Ammonium acetate is a component of multilures (*e.g.* BioLure®) used for *Ceratitis capitata* and *Anastrepha* spp., so it is a good source of ammonium volatiles. This series of experiments aimed to determine if ammonium acetate was attractive to *B. tryoni* and to optimise the concentration of ammonium acetate in the gel lure to obtain maximum effectiveness.

Gel lures containing six different concentrations of ammonium acetate were presented to female *B. tryoni* in a wind tunnel. Lures were evaluated in three separate Latin square designs using protein-deprived unmated flies, protein-fed unmated flies and protein-fed mated flies. Lures were prepared by boiling 10g white sugar, 0.9g agar and 100mL water, cooling to below 40°C and then adding 0.4g yeast hydrolysate and the required amount of ammonium acetate. Ammonium acetate was added to the lures at the following concentrations: 0g L^{-1}, 7.71g L^{-1}, 38.54g L^{-1}, 77.08g L^{-1}, 154.17g L^{-1} and 385.41g L^{-1}, to yield 0M, 0.1M, 0.5M, 2M and 5M solutions of ammonium acetate in the complete lure. Once mixed, the liquid lure was dispensed into plastic caps as 10mL samples using a syringe. Lures were left to set and then refrigerated until the morning of the wind tunnel trials. Experimental lures were warmed to 26°C for 30 minutes prior to the start of the trials.

A 6 x 6 Latin square experimental design was used. This design accounted for variation in two dimensions: variation in time (replicate) and variation in the order of presentation of the ammonium acetate treatments (order) in the wind tunnel. The effect of ammonium acetate treatment on the proportion of flies caught was tested using a generalised linear model with binomial errors and logit link function. The model also included replicate and order effects.

Sugar

Sugars are usually added to lures as feeding stimulants if the lure contains a toxicant required to kill the flies inside the trap. This series of experiments aimed to determine if the type of sugar used affected attractiveness of the lure.

Lures were prepared by boiling 10g sugar source, 0.9g agar and 100mL water, cooling to below 40°C and then adding 0.4g yeast hydrolysate and 15.417g ammonium acetate (2M). The sugar sources tested were white sugar, brown sugar, raw sugar, molasses, fructose and a control (no sugar) in a Latin square design. Once mixed, the liquid lure was dispensed into plastic caps as 10mL samples using a syringe. Lures were left to set and then refrigerated until the morning of the wind tunnel trials. Experimental lures were warmed to 26°C for 30 minutes prior to the start of the trials.

The experimental design was a 6 x 6 Latin square which accounted for variation in two dimensions: variation in time (replicate) and variation in the order of presentation of the sugar treatments (order). The effect of sugar treatment on the proportion of flies caught was tested using a generalised linear model with binomial errors and logit link function.

Development of a dry lure

Attempts to reproduce the correct consistency of a dry lure using the original formulation described by Andrew Jessup were unsuccessful. Yeast hydrolysate is highly anhydrous and absorbs moisture from the air very quickly. Mixing the yeast hydrolysate with the silica gel was not sufficient to stop the yeast from setting rock hard in a very short time, even when greater ratios of silica gel were used. Because the lure set so quickly, it made it unmanageable in terms of preparation and deployment in traps. An alternative dry lure was developed by preparing the gel lure formulation with the omission of agar, and applying the resultant liquid lure to Grade 1 vermiculite. The lure emitted a strong ammonium aroma.

Lure longevity

Two trials to determine the longevity of gel and dry lures under different climatic conditions were conducted between February and May 2009, and May and August 2009. Trial sites were in Cairns, QLD (tropical); Alstonville, NSW (sub-tropical); Narara, NSW (sub-temperate); and Wagga Wagga, NSW (temperate). Three dry lures and three moist lures were deployed in McPhail traps at each site for a total of 12 weeks. Samples of lure (approximately 10mL) were collected from the traps at 2, 4, 6, 8, 10 and 12 weeks and mailed to Gosford Primary Industries Institute, where they were presented to protein-deprived, unmated female *B. tryoni* to evaluate attractancy over time in the wind tunnel. All procedures were the same as described previously. The traps were placed in evergreen trees at Cairns QDPI, NSW Centre for Tropical Horticulture (Alstonville), Gosford Primary Industries Institute (Narara) and Wagga Wagga Agricultural Institute. Data loggers (Tinytag Ultra 2) were also deployed at each site to monitor temperature and humidity. For each trial site, the effects of lure treatment, lure age and their interaction on the proportion of flies caught was tested using a generalised linear model with binomial errors and logit link function.

Field experiment – Kulnura

Kulnura is located on the Central Coast of NSW. The trial site was a commercial orange orchard (GPS 33°13'51°S 151°12'48°E) operated under an integrated pest management programme and no cover sprays for fruit flies were applied. Fruit were present on the trees for the majority of the trapping period. The gel lure, dry lure and liquid protein lure were compared in this trial.

The liquid protein lure consisted of 2% yeast autolysate (Natflav) in water and is the standard female lure described in the Code of Practice for Management of Queensland Fruit Fly. All lures were deployed in McPhail traps containing metal mesh inserts to keep dead flies separated from lures, and a 2cm x 2cm dichlorvos (DDVP) impregnated strip (Killmaster Zero Pest Strip; 186g kg^{-1} DDVP; Barmac). The DDVP acted as a fumigant inside the McPhail trap, killing insects as they entered. Traps containing the different lures were deployed in four 3 x 3 Latin square designs within the orchard (Figure 3). Traps were placed in every eighth tree within treatment rows, providing an approximate 15m interval between traps. This is consistent with published literature where similar experiments have been conducted with trap spacings of around 10-20 metres (Epsky *et al.*, 1999).

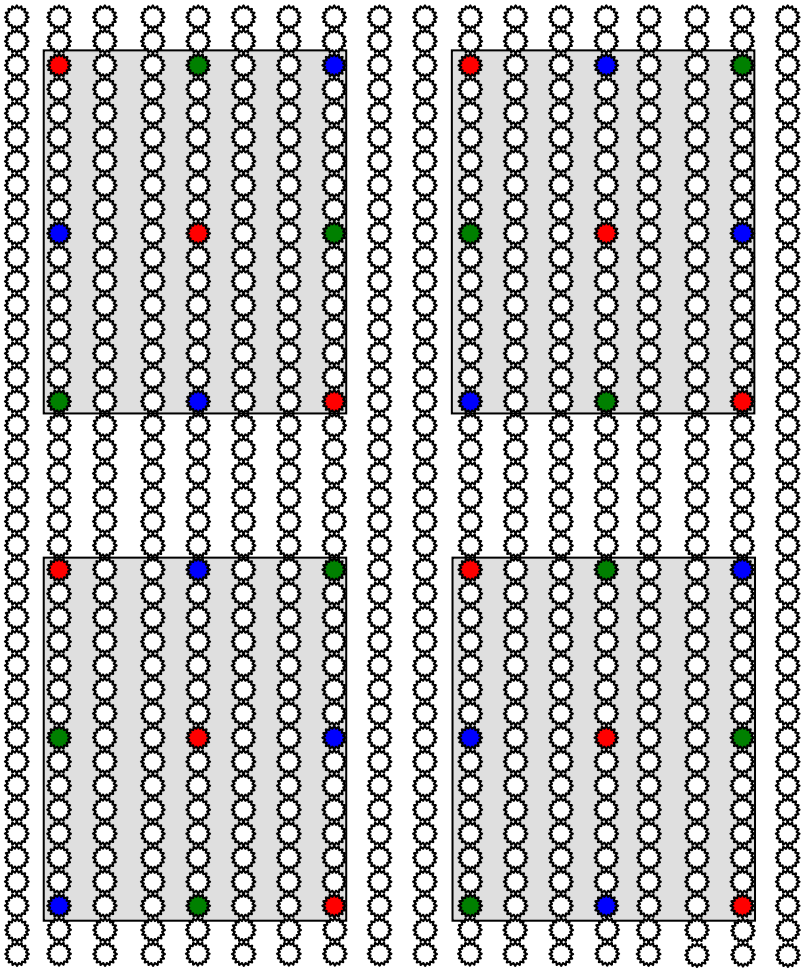

Figure 3. Aerial schematic map showing trap deployment within the citrus orchard in Kulnura. Individual trees are colour-coded to indicate traps containing liquid lure (blue), gel lure (red) and dry lure (green). Traps were deployed in four 3 x 3 Latin square blocks, as indicated by shading.

Traps were emptied weekly to remove flies. Liquid protein lures were emptied and replaced with fresh protein lure each week (as per Code of Practice) and gel and dry lures were replaced every six weeks. DDVP strips were also replaced in all traps every six weeks. Trap captures were sorted to remove any non-target insects and retain tephritid species. Flies were identified to species level, counted and sexed by technical staff at Gosford Primary Industries Institute. Due to the time consuming nature of determining the sex of flies, samples of *Dirioxa pornia* were not sexed at all collection times. All *Bactrocera* samples were sexed. If the identification of any flies was in doubt, the sample

was sent to the Insect Collections Unit at Orange Agricultural Institute (NATA accreditation number 14488) for formal identification.

Total counts of *D. pornia*, *B. tryoni* and *B. cacuminata* over the 23 week trial period were calculated and the effect of lure type on each insect species was tested using analysis of variance (ANOVA). Treatment means were compared using least significant differences at P=0.05. A linear mixed model with cubic smoothing splines was then fitted to the weekly $\log_e$(count+1) of *D.pornia* in order to model the response over time. Due to low capture data obtained for *B. tryoni* and *B. cacuminata*, further similar modelling was not appropriate for these two species.

Field experiment – The Rock

The Rock is located 32km south-west of Wagga Wagga, NSW (GPS 35°16'S 147°06'E). Consistent with the Kulnura trial, the gel lure, dry lure and liquid protein lure were compared. McPhail traps containing the lures, metal mesh inserts and a 2cm x 2cm DDVP impregnated strip were deployed in front and backyards across an urban grid (Figure 4). Citrus trees were used to hang traps wherever possible, but evergreen non-host trees were used on two occasions (Table 1). Traps were emptied weekly to remove flies, which were mailed to Gosford for counting and identification. Liquid protein lures were emptied and replaced with fresh protein lure each week and gel and dry lures were replaced every six weeks. DDVP strips were also replaced every six weeks. Flies were identified to species level, counted and sexed.

Total counts of *B. tryoni* over the 26 week trial period were $\log_e$ transformed and then analysed using ANOVA to determine treatment effects. Treatment means were compared using least significant differences at P=0.05. A linear mixed model with cubic smoothing splines was fitted to the weekly $\log_e$(count+1) of *B.tryoni* to model the response over time. Due to low capture data obtained for *D. porniai* and *B. cacuminata* no statistical analysis was possible.

Table 1. Tree type used to hang McPhail traps containing liquid, gel or dry lures in urban blocks at The Rock.

Block	Trap Type	Tree type
1	Liquid	Orange
1	Dry	Orange
1	Gel	Orange
2	Gel	Orange
2	Liquid	Orange
2	Dry	Orange
3	Dry	Lemon
3	Gel	Grapefruit
3	Liquid	Lemon
4	Liquid	Orange
4	Gel	Orange
4	Dry	Orange
5	Dry	Orange
5	Liquid	Orange
5	Gel	Lemon
6	Liquid	Orange
6	Gel	Orange
6	Dry	Lemon
7	Dry	Kurrajong (*Brachychiton populeneum*)*
7	Liquid	Privet (*Ligustrum* spp.)*
7	Gel	Loquat

* Non-host tree

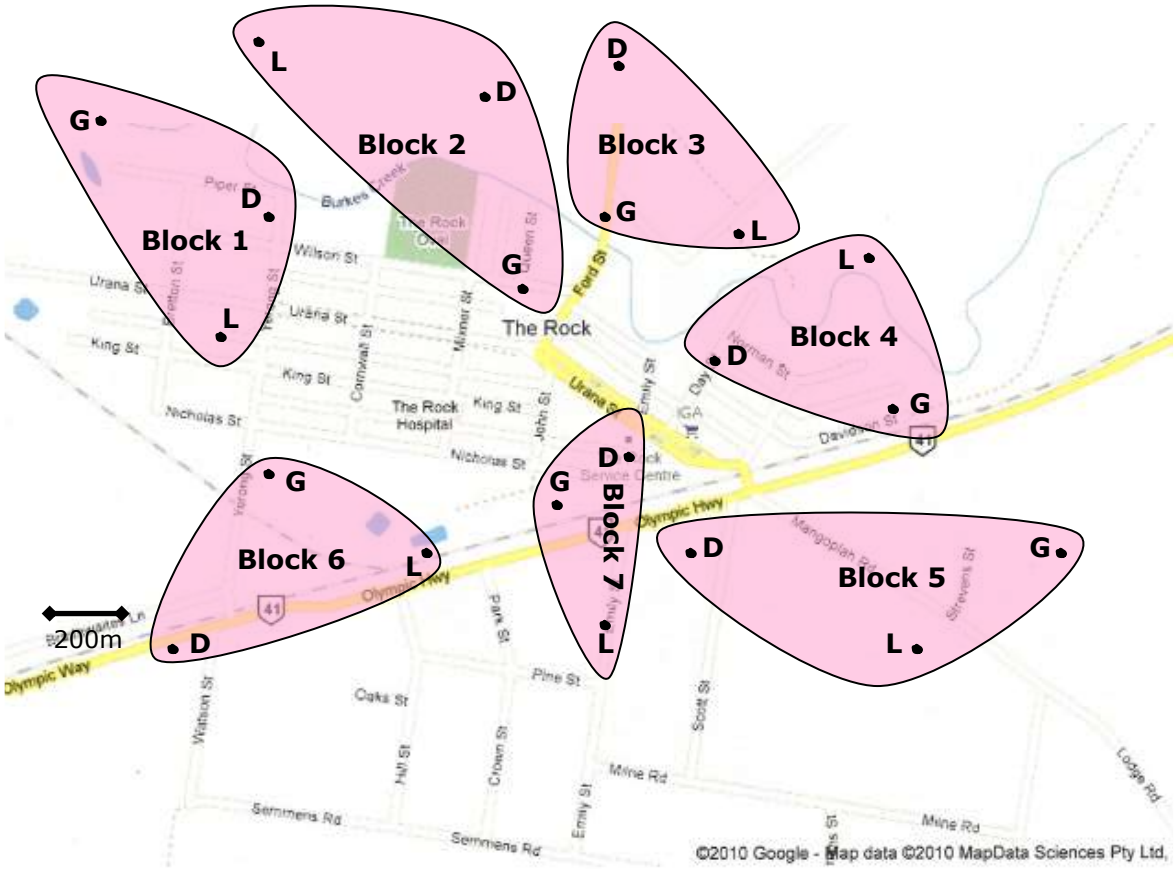

Figure 4. Map showing location of traps containing different lures within the block structure of the experiment. Lure types are labelled as liquid (L), gel (G) and dry (D).

Field experiment – Bathurst

Bathurst is located approximately 200km west of Sydney on the Central Tablelands of NSW (GPS 33°25'S 149°34'E). A *B. tryoni* release-recapture experiment was conducted in an organic pear and apple orchard at Bathurst Primary Industries Institute. McPhail traps containing either liquid lure, dry lure or gel lure with a DDVP strip were arranged in a randomised block design in a 50m radius around a central fly release point (Figure 5). Fresh dry and gel lures were used at the start of each replicate and the liquid protein lure was topped up with additional liquid as required, as evaporation was a problem with the liquid lure. Four Lynfield traps containing Cuelure were positioned in each corner of the orchard, beyond the McPhail traps to determine the number of flies dispersing to the edge of the orchard. The scope of this experiment extended beyond the basic aim of testing the efficacy of the protein lures. Although only data pertaining to lure efficacy will be presented in this report, a brief explanation of the other aims is necessary to put the methodology in context.

For the purposes of Sterile Insect Technique (SIT), *B. tryoni* can be released as adult flies or as pupae left to eclose in a release box, the latter of which was used in this experiment. Sterilised pupae are coated with a fluorescent powder dye to distinguish them from pupae that have not been sterilised by irradiation. As the adult fly emerges from the puparium, it collects a small amount of dye in the ptilinum (an eversible head pouch used to break open the pupal case), which allows adult sterile flies to be distinguished from wild flies. One of the aims outside the scope of this project was to evaluate the "fitness" of sterile flies compared to non-sterile (fertile) flies, and dyed flies compared to undyed flies. Because of these additional aims, three types of fruit fly were released at each release date: Sterile (irradiated) undyed flies, sterile dyed flies and fertile dyed flies. Approximately 60 000 of each fly type were used, meaning that around 180 000 flies were released on each release date. For the purpose of lure evaluation, all types of flies captured were pooled for analysis. All traps were checked and emptied of flies every three days until no more flies were captured for nine days. The next batch of flies was then released. Total counts of *B. tryoni* and counts of each of the different dyed flies were analysed using ANOVA to determine significant effects.

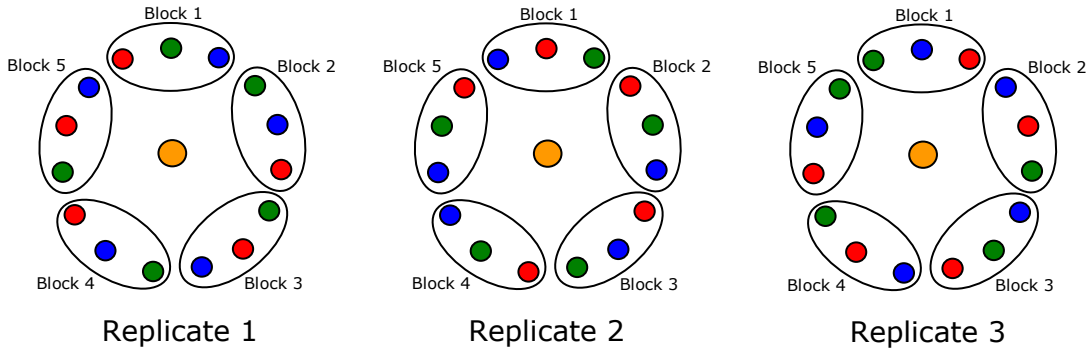

Figure 5. Configuration of trap placement in each replicate over time. Blue = liquid lure; Red = gel lure; Green = Dry lure. The yellow marker indicates a central pupal release point.

Narara – protein source

A range of protein sources were compared in McPhail traps in a mixed citrus orchard at Gosford Primary Industries Institute Narara, NSW, in November 2010. The 9 treatments were 2% aqueous solutions of yeast autolysate (NatFlav), yeast autolysate (Bugs for Bugs), yeast hydrolysate (enzymatic), torula yeast, bakers yeast, brewers yeast, full cream milk powder and water was used as a control. Traps were deployed at approximately 15m intervals (~every fourth tree) on the northern side of trees. Each trap contained 100mL of liquid and a DDVP strip as a toxicant. The 2 dimensional experimental design consisted of three blocks, each with 8 rows. Traps were emptied, flies collected, the lures replaced and the treatments re-randomised within the blocks each week for four weeks. The re-randomisation allowed each week to be considered a replicate. Randomisation was such that treatments were never in the same tree twice. The design was chosen to take into account the differences between blocks, differences within a block (down a row) and differences from week to week.

To stabilise the variance, insect counts were increased by 1 then $\log_e$ transformed prior to analysis. A linear mixed model (REML) was used to test the effect of the protein treatments on insect count. This model accounted for the random effects of the 3 blocks, 8 rows and 4 weeks.

RESULTS AND DISCUSSION

All statistical analyses were carried out using GenStat (Genstat Committee 2009), R (R Core Development Team 2008) and ASReml-R (Butler, Cullis Gilmour and Gogel 2009) with a significance level of P=0.05.

Calcium propionate

Mould development on the gel lure was not consistently affected by the addition of calcium propionate at any concentration. No mould growth was visible on any treatment until after one week and at two weeks incubation at ambient temperatures all treatments had some mould development (Figure 6). After five weeks incubation at ambient temperatures, many of the dishes had prolific mould growth and no consistent effect of calcium propionate concentration was evident (Figure 7). Based on these results, the addition of calcium propionate as a preservative in the gel lure at concentrations less than 0.36g L^{-1} cannot be justified.

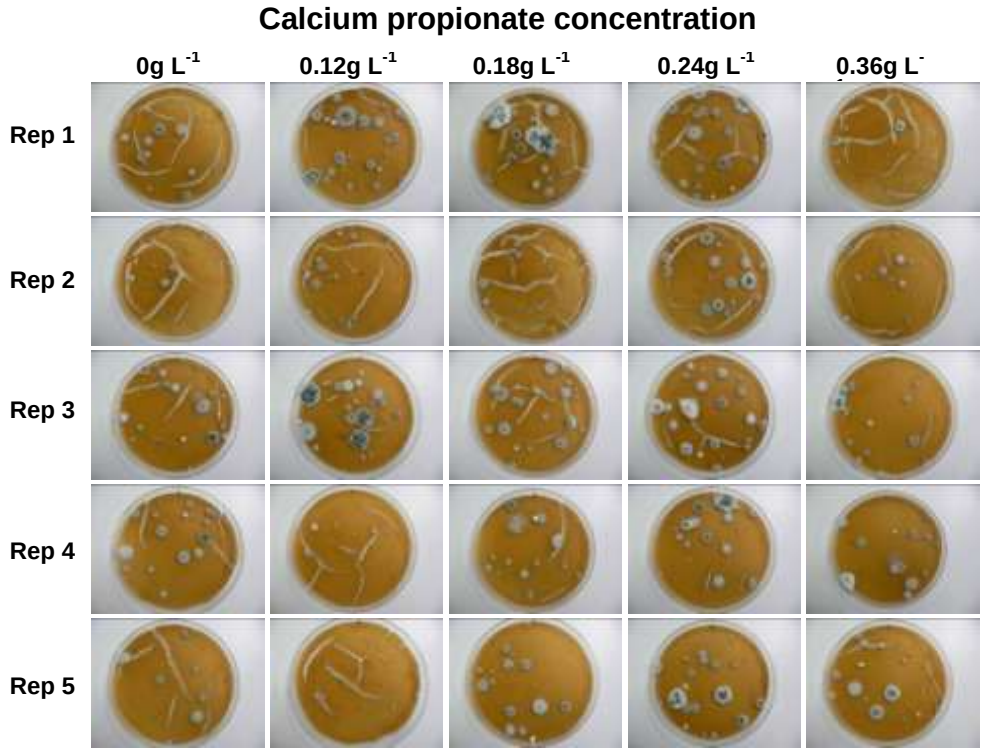

Figure 6. Mould development on gel lure with differing concentrations of calcium propionate after two weeks of incubation at ambient temperatures.

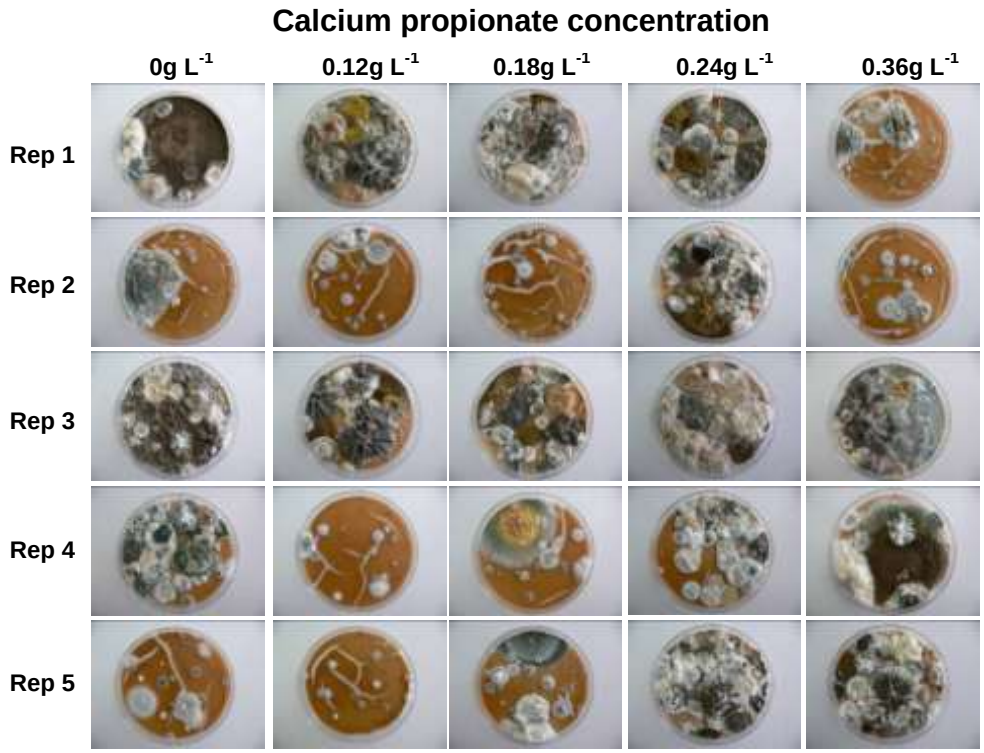

Figure 7. Mould development on gel lure with differing concentrations of calcium propionate after five weeks of incubation at ambient temperatures.

Ammonium acetate

Percent captures of protein-deprived, unmated *B. tryoni* are shown in Table 2. Replicates were included in the generalised linear model but since the effect of order of presentation of the treatments was small, the order effect was omitted. There was a significant treatment effect ($P<0.001$) with the lowest percentages of flies caught in the traps containing lure with no ammonium acetate added (control) and 0.1M ammonium acetate lure. Lures containing all other concentrations of ammonium acetate were significantly more attractive (Table 2).

Table 2. Percent protein-deprived, unmated female *Bactrocera tryoni* placed in the wind tunnel and captured in a trap containing gel lure with differing concentrations of ammonium acetate. Values are back-transformed means of six replicates; values followed by different letters are significantly different based on maximum least significant difference (5%) of pair-wise comparisons.

Ammonium acetate concentration (M)	Percent flies captured
0.0	5.00 (a)
0.1	7.00 (a)
0.5	17.00 (b)
1.0	17.67 (b)
2.0	18.33 (b)
5.0	19.00 (b)

Overall, the percentage of protein-deprived flies captured by any of the lures was low, with an observed maximum mean capture of 19% (Table 2). In this experiment, flies had to be captured inside a trap to be considered attracted to the lure. While this may have impacted on the data to an extent, protein/ammonium compounds are reported in the

published literature to have a relatively low attractancy to other fruit fly species. For example, only 14% of blueberry maggot (*Rhagoletis mendex*) were attracted to undiluted yeast autolysate bait (Barry & Polavarapu, 2004) and no more than 17.5% of Mexican fruit flies (*Anastrepha ludens*) made contact with BioLure® panels in wind tunnel experiments (Robacker, 1999). Based on these data reported previously, it would be fair to assume that the results obtained in this current study are not atypical.

The numbers of flies captured in the experiments using protein-fed mated or unmated female *B. tryoni* were too low to analyse statistically, but the results are summarised in Table 3. Although this experiment did not involve the dissection of mated and unmated flies to determine if sperm was present in the spermathecae, it was assumed that flies were mated or unmated based on the timing of separation from male flies. There are no apparent differences in the attractiveness of the lures between mated and unmated females of *B. tryoni*. However, the percent captures indicate that female *B. tryoni* that have not been fed protein prior to exposure to the lure are more likely to be attracted to a protein/ammonium compound lure. This carries implications in the field that perhaps the majority of female flies captured in traps containing these lures would be female flies that are newly-emerged and requiring protein for gamete formation.

From a technical perspective, lure with 5M ammonium acetate impeded the ability of the gel to set in a solid form. Also, observational data indicated that 2M ammonium acetate was sufficient to inhibit the growth of moulds and bacteria on the lure, overcoming the problem of using a preservative such as calcium propionate. Therefore, 2M ammonium acetate was selected as the concentration used for the gel lure.

Table 3. Percent female *Bactrocera tryoni* of different assumed reproductive status placed in the wind tunnel and captured in a trap containing gel lure with differing concentrations of ammonium acetate. Values are the means of six replicates.

Ammonium acetate concentration (M)	% Protein-fed mated flies	% Protein-fed unmated flies
0.0	0.33	0.00
0.1	0.00	0.33
0.5	0.33	1.00
1.0	0.00	2.67
2.0	0.00	1.00
5.0	0.67	1.00

Sugar

There was no significant effect of the addition of any sugar source to the gel lure in terms of attractancy to protein-deprived female *B. tryoni* (P=0.308, Table 4). Similar to the ammonium acetate trial, the number of protein-fed unmated flies and protein-fed mated flies captured during the assays were too low to analyse (Table 5).

Table 4. Percent protein-deprived, unmated female *B. tryoni* captured in a trap containing 2M ammonium acetate gel lure formulated with different sugar sources within a wind tunnel. Values are back-transformed means of six replicates; values followed by different letters are significantly different based on maximum least significant difference (5%) of pair-wise comparisons.

Sugar source (10g/lure)	% Protein-deprived unmated flies
No Sugar	17.33 (a)
White sugar	17.33 (a)
Raw sugar	14.67 (a)
Brown sugar	16.00 (a)
Fructose	17.33 (a)
Molasses	15.00 (a)

Despite the addition of any sugar having no effect on attractancy of the lure, there were no adverse effects. Sugars are usually added to lures or baits to stimulate feeding of fruit flies, to ensure that they ingest enough of the toxicant. For this reason white sugar was retained as an ingredient in the gel lure, mostly in the event that if a toxicant was added to the lure at a later date, the feeding stimulant would already be included in the lure.

Table 5. Percent female *B. tryoni* of different assumed reproductive status captured in a trap containing 2M ammonium acetate gel lure formulated with different sugar sources within a wind tunnel. Values are the means of six replicates.

Sugar source (10g/lure)	% Protein-fed mated flies	% Protein-fed unmated flies
No Sugar	1.00	2.33
White sugar	0.67	1.00
Raw sugar	1.00	2.00
Brown sugar	0.33	2.00
Fructose	2.00	1.67
Molasses	2.33	1.33

Lure longevity

Generally, there were greater effects of lure age on attractiveness to *B. tryoni* in the longevity trial conducted between February and April, than the trial conducted between May and August. This is most likely due to the higher temperatures experienced in late summer/ early autumn. When exposed to higher temperatures it is possible that the gel lure develops more ammonium volatiles over time as the yeast hydrolysate degrades. Conversely, heating the dry lure may have caused rapid volatilisation of ammonium acetate, resulting in a reduced release of ammonium volatiles over time.

Climatic conditions at all sites in the first trial conducted between February and April are summarised in Table 6. During the first trial, significant effects of lure type and age interaction on attractiveness to *B. tryoni* in the wind tunnel were observed for lures aged at Cairns ($P=0.120$) or Wagga Wagga ($P=0.001$) (Figure 8). In Cairns, the gel and dry lure remained similar in their attractiveness until after eight weeks. After that time, the gel lure became more attractive and the dry lure became less attractive. The percentage of *B. tryoni* captured in the wind tunnel ranged between 6 – 30%. Gel and dry lures aged in Wagga Wagga were very different in their attractancy after two weeks of exposure to climatic conditions. Gel lures tended to increase in attractancy (up to 35% *B. tryoni* captured), while dry lures lost attractancy very quickly (as low as 3%). Wagga Wagga was the only trial site to have hot, dry conditions, which probably increased the volatilisation of ammonium acetate in the dry lure. All other sites had higher levels of humidity, which may have helped preserve the lure. For lures aged at Alstonville there were significant main effects of lure type ($P=0.001$) and lure age ($P<0.001$) on attractiveness of the lures to B*. tryoni* in the wind tunnel (Table 7), but the interaction effect was not significant ($P=0.500$). The attractiveness of lures aged at Narara was only significantly affected by lure age ($P<0.001$), although the percentage flies captured was in the range of 10-30% (Table 8).

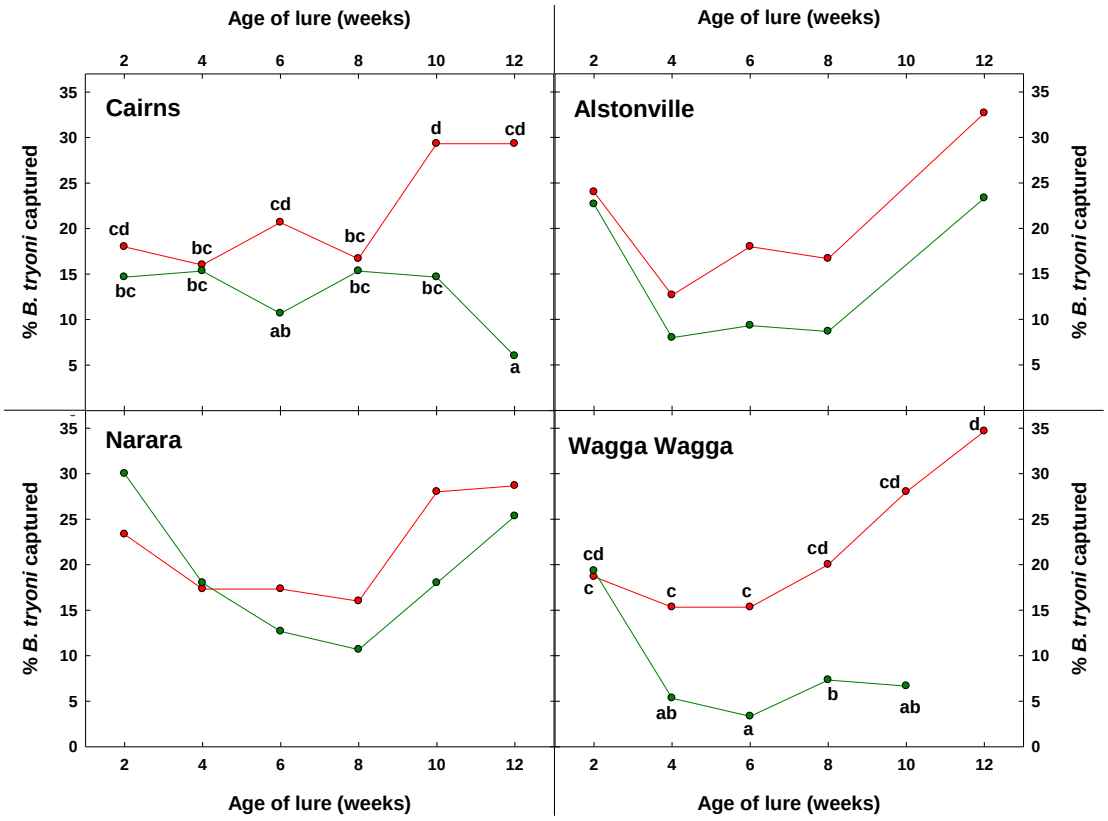

Figure 8. Percent protein-deprived, unmated female *Bactrocera tryoni* placed in the windtunnel and captured in a trap containing gel lure (red) or dry lure (green) previously aged in a McPhail trap at four locations for 12 weeks between February and April 2009. Values are back-transformed means of three replicates. For locations where a significant interaction between lure type and age was found, values followed by the same letters are not different based on the mean least significant difference (5%) of pair-wise comparisons.

Table 6. Mean (minimum – maximum) temperatures and relative humidity at each site where lures were aged during Trial 1 (February – April).

Site	Month	Temperature (°C)	Relative humidity (%)
Cairns	February	26.6 (21.5 – 34.2)	86.0 (40.6 – 99.8)
	March	25.8 (17.2 – 32.3)	82.5 (47.7 – 99.8)
	April	24.9 (17.0 – 31.7)	82.8 (26.4 – 99.8)
Alstonville	February	23.5 (16.6 – 34.3)	82.8 (32.2 – 99.8)
	March	22.3 (14.9 – 37.4)	81.2 (30.6 – 99.8)
	April	19.8 (12.5 – 28.0)	74.6 (28.7 – 99.8)
Narara	February	21.9 (13.6 – 38.7)	82.5 (27.6 – 99.9)
	March	20.4 (10.1 – 32.3)	79.1 (30.5 – 99.9)
	April	18.2 (9.8 – 28.9)	82.8 (30.5 – 99.9)
Wagga Wagga	February	26.9 (10.3 – 45.6)	36.0 (7.5 – 90.8)
	March	22.3 (8.8 – 36.4)	45.6 (12.5 – 95.8)
	April	18.3 (5.0 – 33.6)	53.9 (22 – 99.8)

Table 7. Percent protein-deprived female *Bactrocera tryoni* captured in a trap containing gel or dry lure aged in the field in Alstonville for up to 12 weeks between February and April 2009. Values are the means of three replicates; values followed by different letters are significantly different for each main effect, based on mean least significant difference (5%) of pair-wise comparisons.

	% *B. tryoni* captured
lure type	
Gel lure	20.8 (a)
Dry lure	14.4 (b)
lure age (weeks)	
2	23.3 (b)
4	10.3 (a)
6	13.7 (a)
8	12.7 (a)
10	no data
12	28.0 (b)

Table 8. Percent protein-deprived female *Bactrocera tryoni* captured in a trap containing gel or dry lure aged in the field in Narara for up to 12 weeks between February and April 2009. Values are the means of three replicates; values followed by different letters are significantly different based on mean least significant difference (5%) of pair-wise comparisons.

Lure age (week)	% *B. tryoni* captured
2	13.3 (c)
4	8.8 (ab)
6	7.5 (ab)
8	6.7 (a)
10	11.5 (bc)
12	13.5 (c)

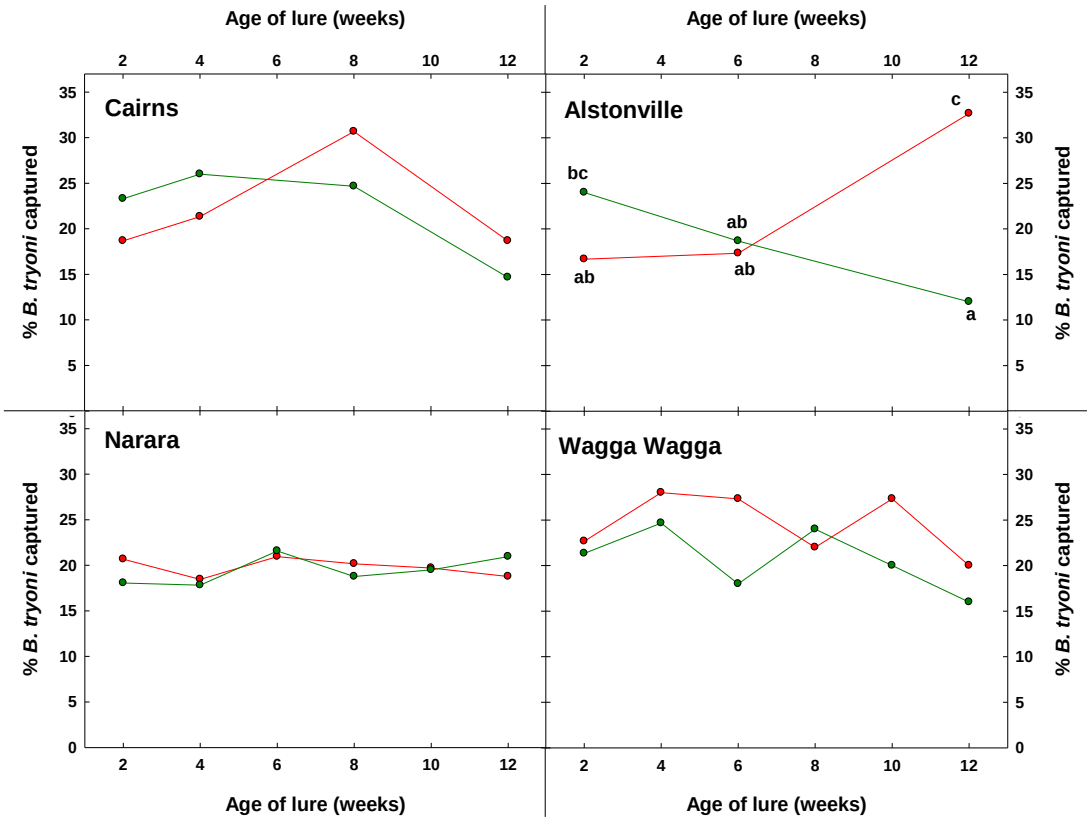

Figure 9. Percent protein-deprived, unmated female *Bactrocera tryoni* placed in the windtunnel and captured in a trap containing gel lure (red) or dry lure (green) previously aged in a McPhail trap at four locations for 12 weeks between May and August 2009. Values are back-transformed means of three replicates. A significant effect of the lure type and age interaction on lure attractiveness was found for lures aged at Alstonville. Values followed by different letters are significantly different based on the mean least significant difference (5%) of pair-wise comparisons.

Climatic conditions at all sites in the second trial conducted between May and August are summarised in Table 9. The second longevity trial (conducted between May and August) did not show such dynamic changes in the attractiveness of the lures to *B. tryoni* in the wind tunnel (Figure 9). There were no significant effects of lure type, age or their interaction on the attractiveness of the lures, with the exception of lures aged at Alstonville (Figure 9). Lure samples were only collected three times from Alstonville and this may have had some impact on the outcome, but the general trend suggests that the gel lure increased in attractancy, while the dry lure deceased in attractancy over time. Temperatures were notably lower during this trial period compared with the first trial (February to May), so this most likely the reason why the lures, particularly the dry formulation was not affected by time in the field.

Table 9. Mean (minimum – maximum) temperatures and relative humidity at each site where lures were aged during Trial 2 (May – June).

Site	Month	Temperature (°C)	Relative humidity (%)
Cairns	May	21.3 (12.4 – 27.9)	85.8 (41.3 – 99.8)
	June	20.9 (12.2. – 31.2)	83.2 (49.2 – 99.8)
	July	19.4 (8.8 – 30.5)	75.5 (29.9 – 99.8)
Alstonville	May	16.5 (11.2 – 24.1)	79.1 (36.2 – 99.8)
	June	14.4 (5.1 – 25.7)	76.8 (40.4 – 99.8)
	July	14.1 (6.9 – 25.7)	70.6 (27.7 – 99.8)
Narara	May	14.0 (5.3 – 22.3)	80.2 (41.0 – 99.9)
	June	11.3 (3.5 – 19.7)	78.4 (39.8 – 99.9)
	July	10.4 (1.6 – 23.5)	76.0 (37.6 – 99.9)
Wagga Wagga	May	13.6 (2.8 – 23.8)	66.2 (32.4 – 99.8)
	June	10.2 (-0.5 – 18.4)	84.8 (47.2 – 99.8)
	July	8.9 (-0.8 – 21.6)	82.0 (48.0 – 99.8)

Field experiment – Kulnura

Numbers of *B. tryoni* and other *Bactrocera* species were quite low during the trapping period, despite the trial being conducted in an orchard where minimal insecticides were being used. The most frequent captures were of Island fly (*Dirioxa pornia*), a native tephritid species of little economic concern, that lays eggs into decaying or fallen fruit, particularly citrus. Sufficient capture data were collected for *D. pornia*, *B. tryoni* and *B. cacuminata* (wild tobacco fly) to allow statistical analysis.

Figures 10, 11 and 12 show actual trap captures over the trial period for these three species of fruit fly. *D. pornia* was relatively common throughout the entire trial, as fruit was present on the tree. The peak in numbers observed during February ground (Figure 10) coincides with the time after harvest, when increased numbers of fruit are fallen to the. *B. tryoni* was more common in November-December (Figure 11). Although citrus trees are a good source of shelter and food for *B. tryoni*, citrus fruit, particularly oranges, are generally accepted a not being a preferred host of *B. tryoni*. This peak could therefore be due to the presence of alternative hosts (stonefruit) growing in the vicinity. *B. cacuminata* numbers were highest in January-February (Figure 12), coinciding with the fruiting of its host, the wild tobacco plant (*Solanum mauritianum*), although no plants were found growing in the immediate vicinity of the orchard.

Other tephritid species captured at Kulnura were *B. nigra* (one female fly, captured in a gel lure trap in March 2010) and *B. halfordiae* (one male fly, captured in a dry lure trap in March 2010). Both species are not responsive to male lures (Cuelure or methyl eugenol) and this is the first record of *B. nigra* in NSW. Both specimens were identified by trained staff at the Insect Collections Unit at Orange Agricultural Institute (NATA accreditation number 14488).

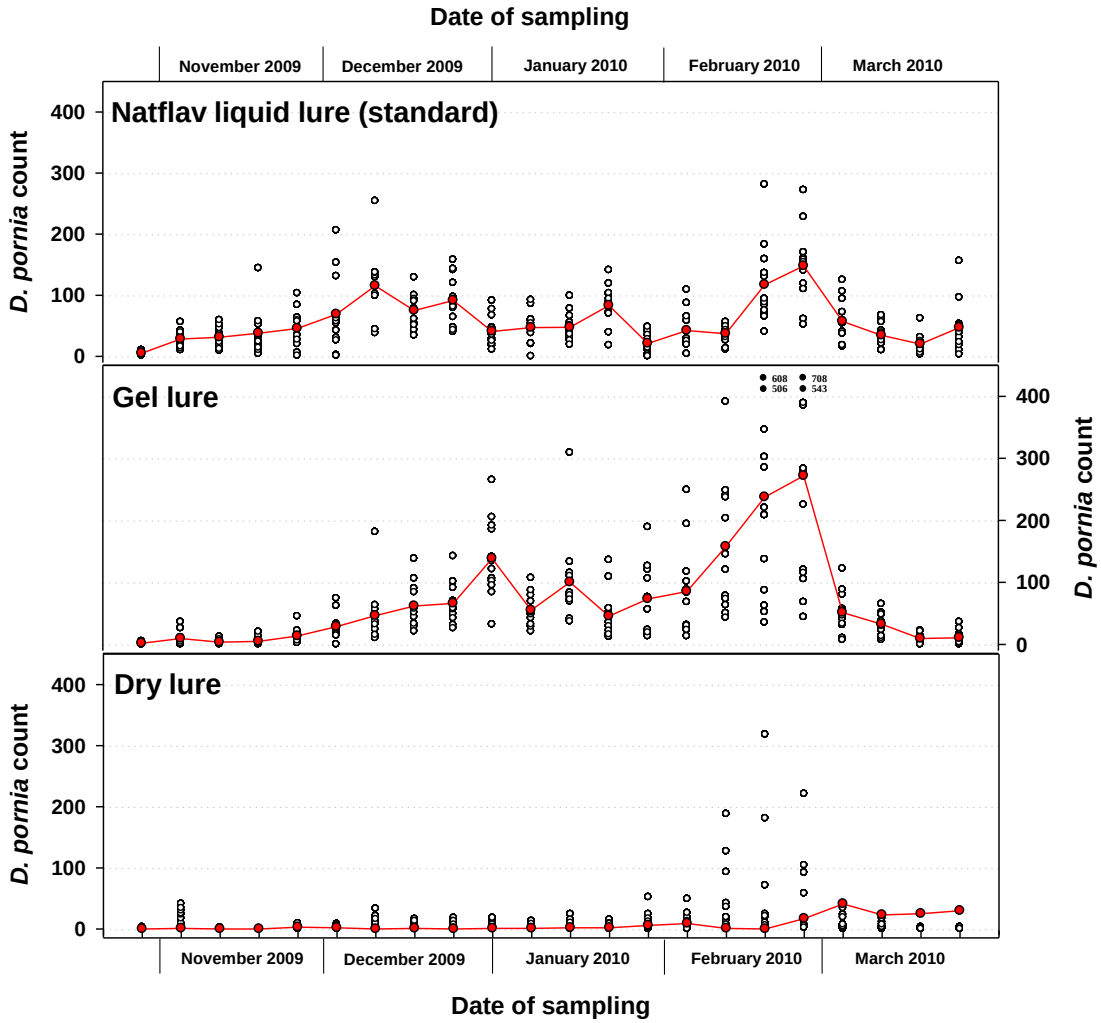

Figure 10. Actual numbers of *Dirioxa pornia* captured per week in a citrus orchard at Kulnura. White datum points represent actual captures per trap; red datum points the mean number of *D. pornia* per sample date. Black datum points (gel lure) are those beyond axis scale and are labelled with the corresponding datum value.

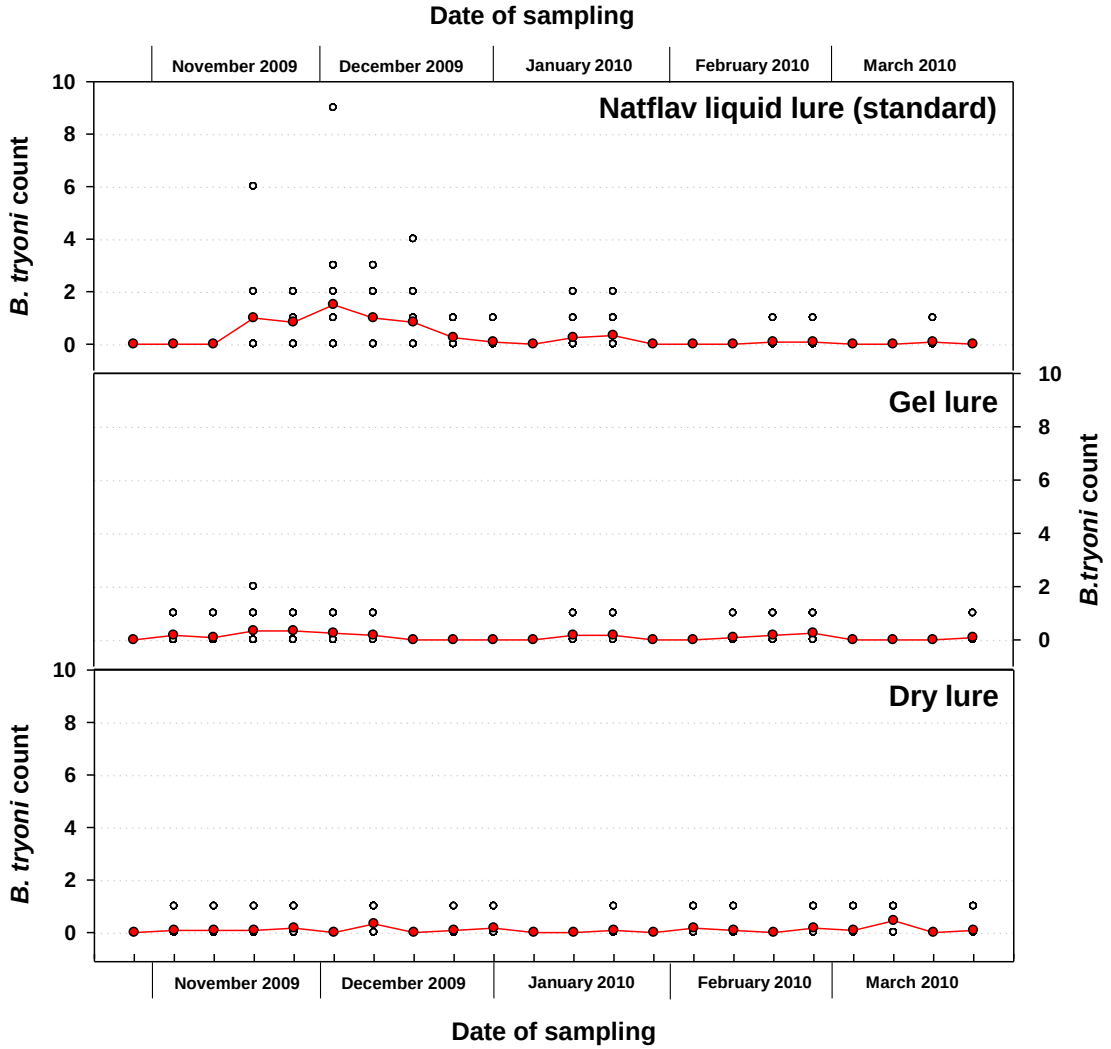

Figure 11. Actual numbers of *Bactrocera tryoni* captured per week in a citrus orchard at Kulnura. White datum points represent actual captures per trap; red datum points the mean number of *B. tryoni* per sample date.

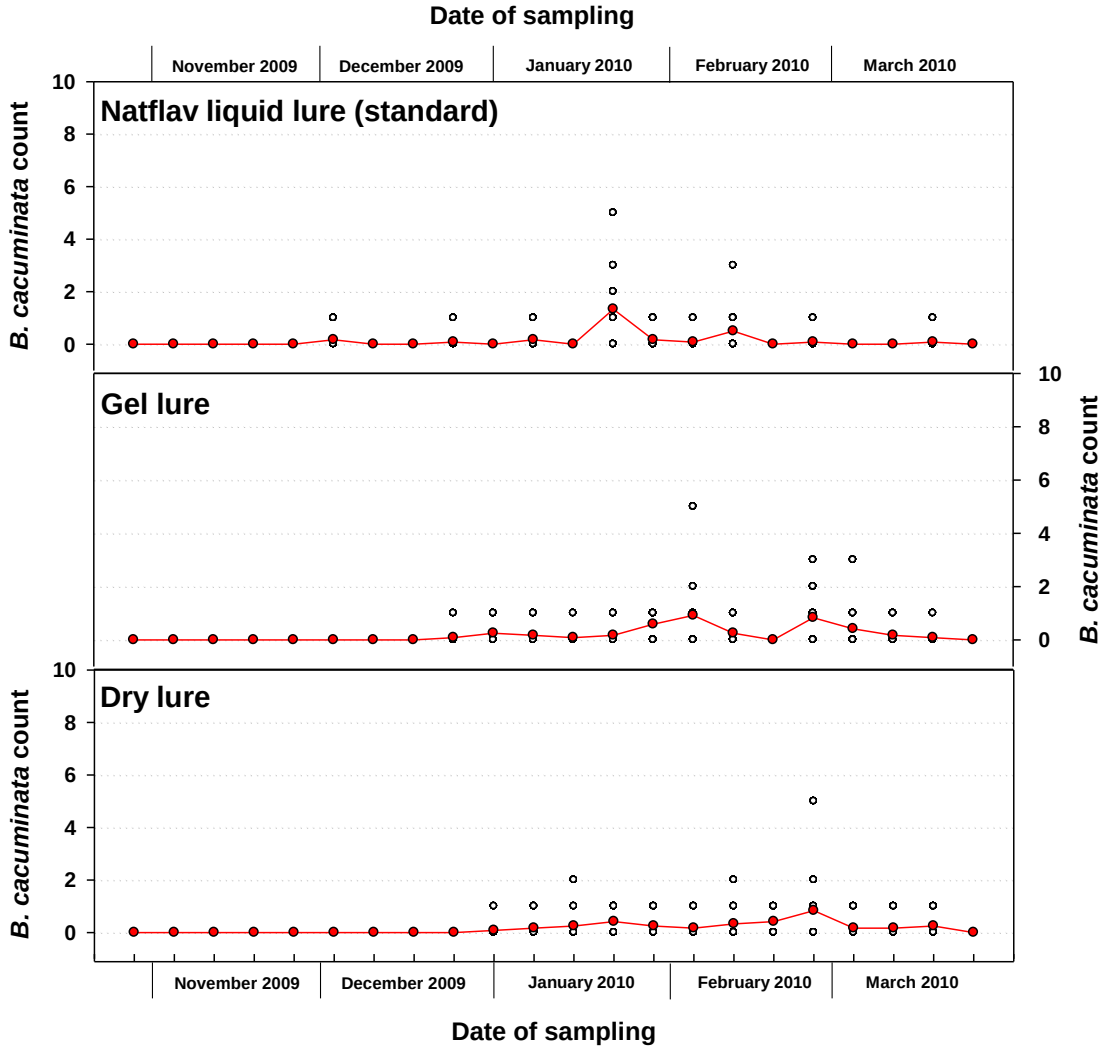

Figure 12. Actual numbers of *Bactrocera cacuminata* captured per week in a citrus orchard at Kulnura. White datum points represent actual captures per trap; red datum points the mean number of *B. tryoni* per sample date.

Total counts of *D. pornia*, *B. tryoni* and *B. cacuminata* over the 23 week trial period were analysed using ANOVA to determine significant treatment effects. Where a significant effect of lure type was found, least significant differences (l.s.d.'s) were calculated at P=0.05. There was a significant treatment effect on the number of *D. pornia* captured ($p<0.001$). Overall, the liquid and gel lure were similarly attractive to *D. pornia* whereas the dry lure was less effective.(Table 10). There was a significant treatment effect on the number of *B. tryoni* captured ($p=0.037$). The gel and dry lures were less effective at attracting *B. tryoni* compared to the liquid lure (Table 11). No significant effect ($p=0.31$) of lure type was observed for *B. cacuminata* (Table 12).

Table 10. Mean capture of *Dirioxa pornia* over 23 weeks in traps containing liquid, gel or dry lures placed in a citrus orchard at Kulnura. Values followed by different letters are significantly different to each other (l.s.d. 5% = 417).

Lure type	Mean capture
Natflav liquid lure (standard)	1253 (b)
Gel lure	1520 (b)
Dry lure	252 (a)

Table 11. Mean capture of *Bactrocera tryoni* over 23 weeks in traps containing liquid, gel or dry lures placed in a citrus orchard at Kulnura. Values followed by different letters are significantly different to each other (l.s.d. 5% = 3.49).

Lure type	Mean capture
Natflav liquid lure (standard)	6.25 (b)
Gel lure	2.25 (a)
Dry lure	2.08 (a)

Table 12. Mean capture of *Bactrocera cacuminata* over 23 weeks in traps containing liquid, gel or dry lures placed in a citrus orchard at Kulnura.

Lure type	Mean capture
Natflav liquid lure (standard)	3.50
Gel lure	4.00
Dry lure	2.08

A mixed linear model with cubic smoothing splines was fitted to the weekly $\log_e$ transformed counts of *D. pornia* (Figure 13). This analysis demonstrated that the dry lure was the least effective of the three lures at virtually all times. The liquid lure was more effective at attracting *D. pornia* than the gel lure during spring and autumn (weeks 1-7, 20-22). However, the gel lure was generally more effective than the liquid lure during the height of summer (weeks 8-19). Due to the low numbers of *B. tryoni* and *B. cacuminata* captured at Kulnura, this type of statistical modelling was not appropriate for these data.

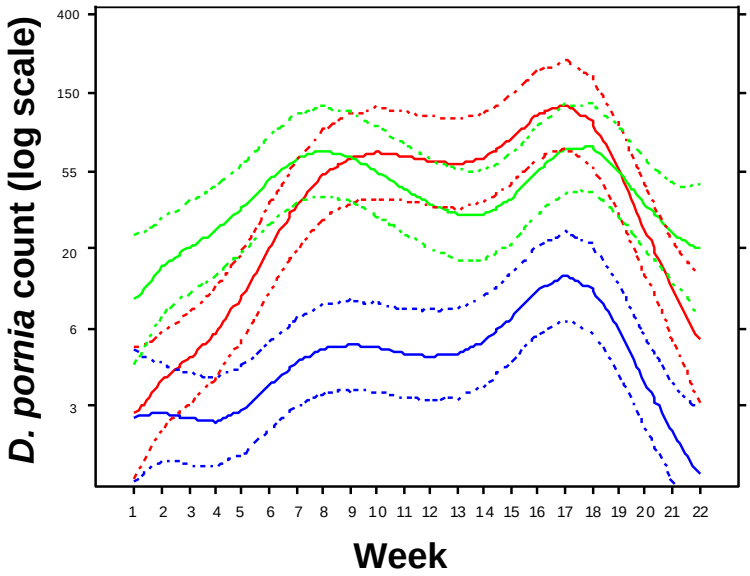

Figure 13. Predicted insect counts in traps (solid line) with 95% confidence bands (dotted line) fitted for *Dirioxa pornia* in a citrus orchard at Kulnura. Traps contained either the standard liquid (Natflav) lure (green), gel lure (red) or dry lure (blue).

Table 13. Counts of flies of each sex captured in traps deployed in a citrus orchard at Kulnura. Counts are over 23 weeks for *Bactrocera tryoni* and *B. cacuminata*, and over 13 weeks for *Dirioxa pornia*. If the abdomen was missing or damaged, flies were categorised as not sexed. The percentage of total catch that was female is shown in parentheses following the counts of female flies.

Dirioxa pornia				
Lure type	**Female**	**Male**	**Not sexed**	**Total**
Liquid (Natflav)	4817 (68%)	1488	828	7133
Gel	3907 (62%)	2342	71	6320
Dry	491 (69%)	192	27	710
Bactrocera tryoni				
Lure type	**Female**	**Male**	**Not sexed**	**Total**
Liquid (Natflav)	64 (83%)	8	5	77
Gel	21 (78%)	4	2	27
Dry	15 (60%)	9	1	25
Bactrocera cacuminata				
Lure type	**Female**	**Male**	**Not sexed**	**Total**
Liquid (Natflav)	22 (69%)	10	0	32
Gel	33 (67%)	16	0	49
Dry	23 (55%)	19	0	42

The gel and dry lures attracted noticeably fewer non-target insects, such as blowflies, compared to the liquid lure. The photographs in Figure 14 show a typical trap capture for each of the lure types. *Bactrocera* spp. have quite strong and resilient exoskeletons and are able to withstand a relatively high level of decomposition without becoming unidentifiable due to loss of body parts. *D. pornia* is a much more delicately structured fly and was highly affected by the rate of decomposition within the traps containing liquid lure. This is reflected in the high number of flies that were not able to be sexed due to missing or damaged abdomens (Table 13).

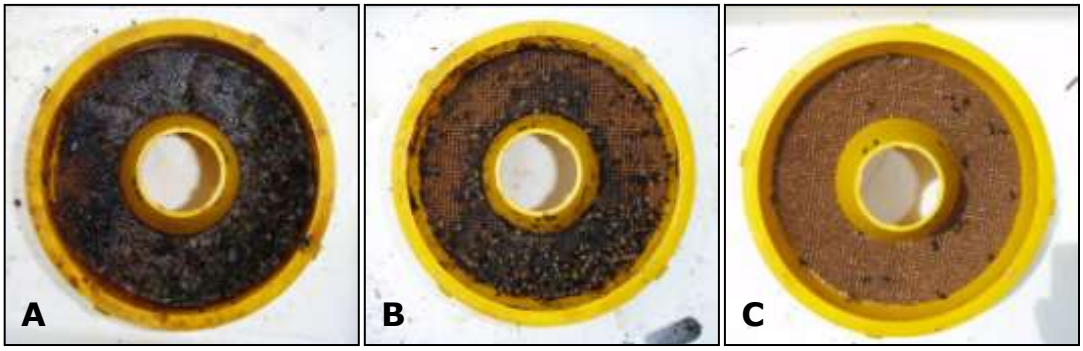

Figure 14. Typical trap captures and specimen condition after one week in a citrus orchard in Kulnura. Traps contained different lure types: liquid (A), gel (B) and dry (C).

Field experiment – The Rock

B. tryoni was the primary tephritid species captured at The Rock. Flies were not detected until December 2009, but then steadily increased until May 2010, which is when the sampling period ended (Figure 15). The time of first detection was later than what was observed at Kulnura, and is typical of the inland areas of NSW.

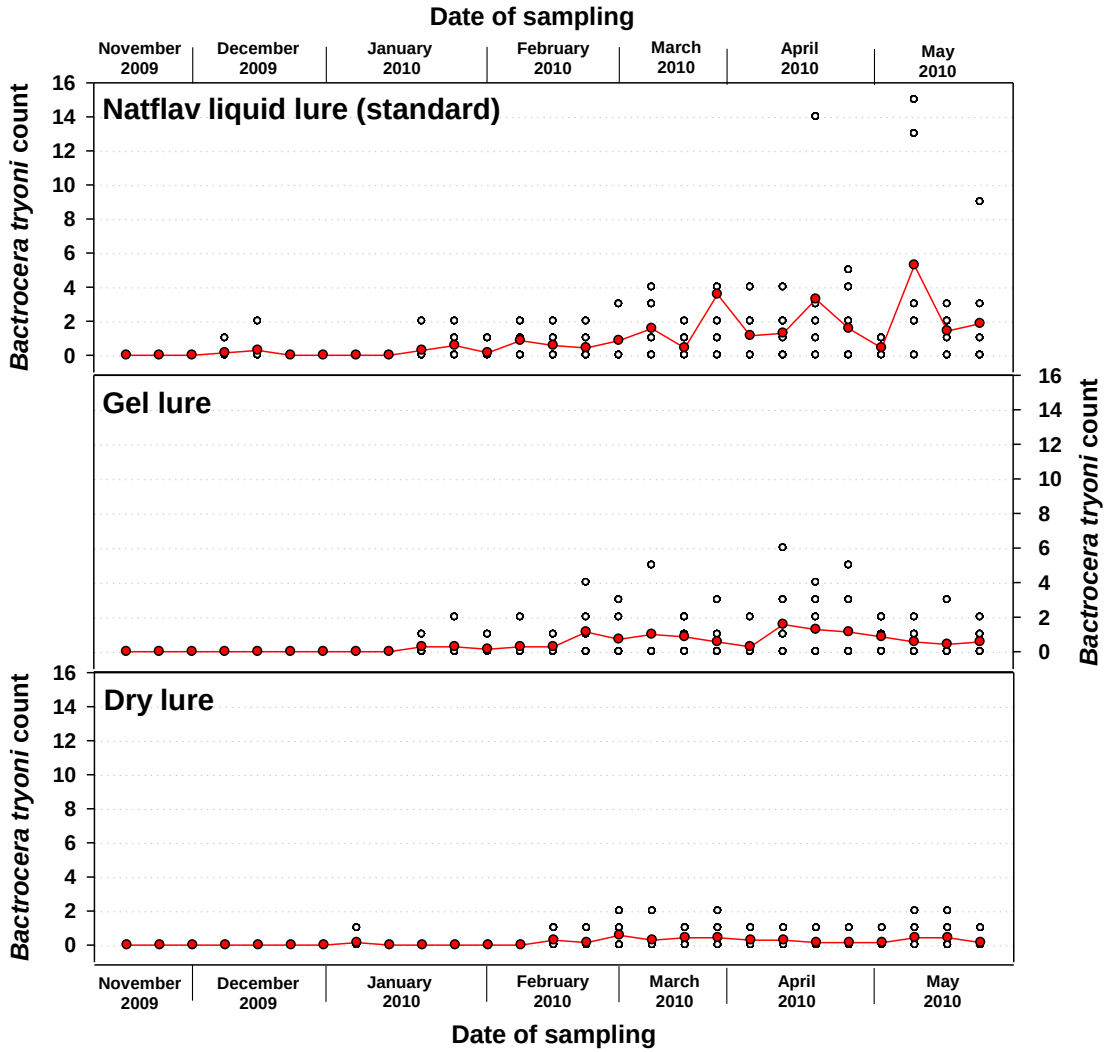

Figure 15. Actual numbers of *Bactrocera tryoni* captured per week at The Rock. White datum points represent actual captures per trap; red datum points the mean number of *B. tryoni* per sample date.

Total transformed counts ($\log_e$(count+1)) of *B. tryoni* over the 26 week trial period were analysed using ANOVA to determine significant treatment effects. Least significant differences were calculated at P=0.05. There was a significant treatment effect on the number of *B. tryoni* captured ($p<0.001$) The standard liquid lure captured more *B. tryoni* than the gel or dry lures, and the dry lure captured less *B. tryoni* than the gel or liquid lures (Table 14).

Table 14. Back-transformed mean capture of *Bactrocera tryoni* over 26 weeks in traps at The Rock containing liquid, gel or dry lures Values followed by different letters are significantly different to each other (P=0.05).

Lure type	Mean capture
Natflav liquid lure (standard)	19.3 (a)
Gel lure	8.2 (b)
Dry lure	3.2 (c)

For all three lures tested at The Rock, the majority of *B. tryoni* captured in traps were female (Table 15).

Table 15. Counts of *Bactrocera tryoni* of each sex captured in traps deployed at The Rock over 26 weeks. If the abdomen was missing or damaged, flies were categorised as not sexed. The percentage of total catch that was female is shown in parentheses following the counts of female flies.

Lure type	Female	Male	Not sexed	Total
Liquid (Natflav)	137 (75%)	44	1	182
Gel	64 (74%)	21	1	86
Dry	25 (83%)	5	0	30

A mixed linear model with cubic smoothing splines was fitted to the weekly $\log_e$(count+1) transformed counts of *B. tryoni* (Figure 16). The catch rate for all treatments increased from January onwards. However, the catch rate of the standard liquid lure increased faster between February and May than that of the gel lure. Similarly the rate of capture by the gel lure increased faster throughout this period than that of the dry lure. Note the low number of insects collected over the November 2009 –May 2010 time period.

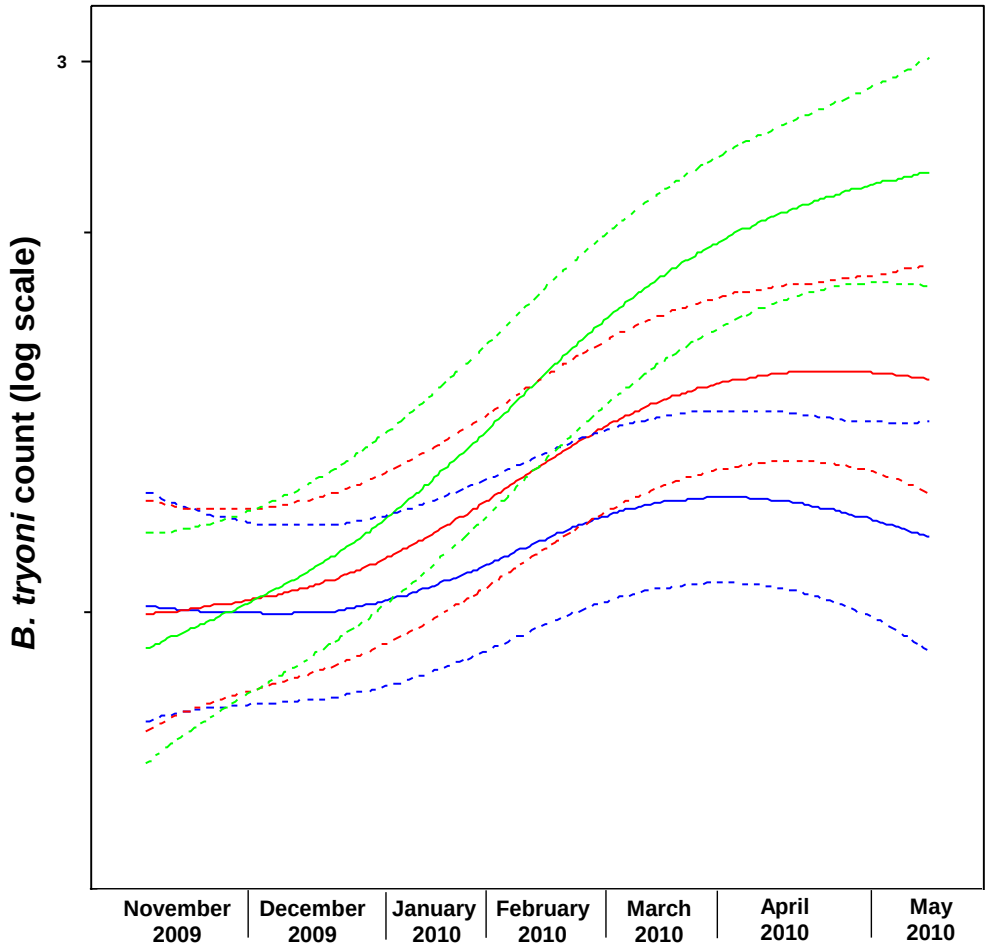

Figure 16. Predicted insect counts in traps (solid line) with 95% confidence bands (dotted line) fitted for *Bactrocera tryoni* at The Rock. Traps contained either the standard liquid (Natflav) lure (green), gel lure (red) or dry lure (blue).

Other tephritid species captured at The Rock were *B. cacuminata* (two female flies, captured in a liquid lure trap in February 2010) and *D. pornia* (one female fly, captured in a gel lure trap in December 2009 and one female fly, captured in a dry lure trap in January 2010).

Field experiment – Bathurst

Looking at the raw data, recapture rates differed greatly between each of the replicates, and there was also variation in recapture rates within treatments (Figure 17). Total recapture data was analysed by ANOVA and the effect of lure type was not significant at the 5% level of confidence (P=0.097). Analysis of recapture data for each of the fly types separately only showed a significant effect of lure type on recapture of sterile (irradiated), undyed flies. In this case, dry lure captured significantly less (p=0.035) *B. tryoni* compared to the gel and liquid lures (11.0 *c.f.* 21.4 and 19.3, respectively). However, the fact that the effect of lure type was significant in this case is possibly more likely to have occurred by chance rather than for a specific biological reason.

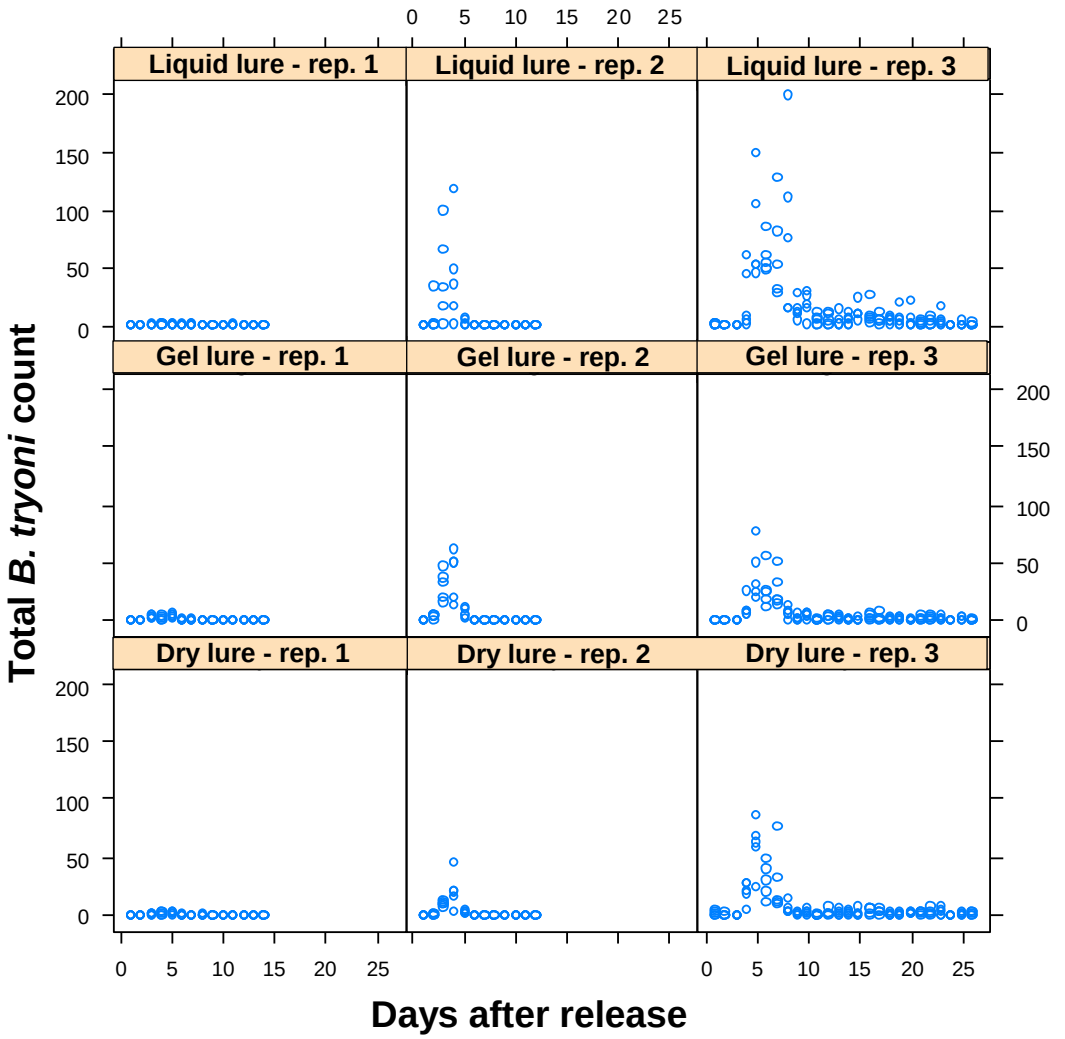

Figure 17. Recapture of *Bactrocera tryoni* in traps containing either liquid (Natflav) lure, gel lure or dry lure at Bathurst in 2009-2010. Replicates are the result of pupal release events.

Narara – protein source

The majority of flies captured during the trials at Narara were *D. pornia* (Figure 18) although reasonable numbers of *B. tryoni* were also recorded (Figure 19). Variability was high amongst most of the treatments and observational data suggests that this variability is partly due to climatic conditions. Regular rainfall occurred during the trapping period and temperatures remained relatively low. The week where the highest incidences of capture were recorded included a weekend where temperatures reached almost 40°C. Under these conditions, fruit flies need to actively seek water to survive, and protein lures rapidly degrade, releasing volatiles. The need for water, combined with the visual attractiveness of the McPhail trap is likely to account for the captures in traps containing water only.

There was a significant treatment effect of protein source on *D. pornia* ($P<0.001$). All protein sources were significantly more attractive than water. Milk powder was less

attractive than NatFlav yeast. The remainder of the protein treatments were as good at attracting *D. pornia* as the NatFlav yeast (Table 16).

A significant treatment effect of protein source on capture of *B. tryoni* was not detected (P=0.243)

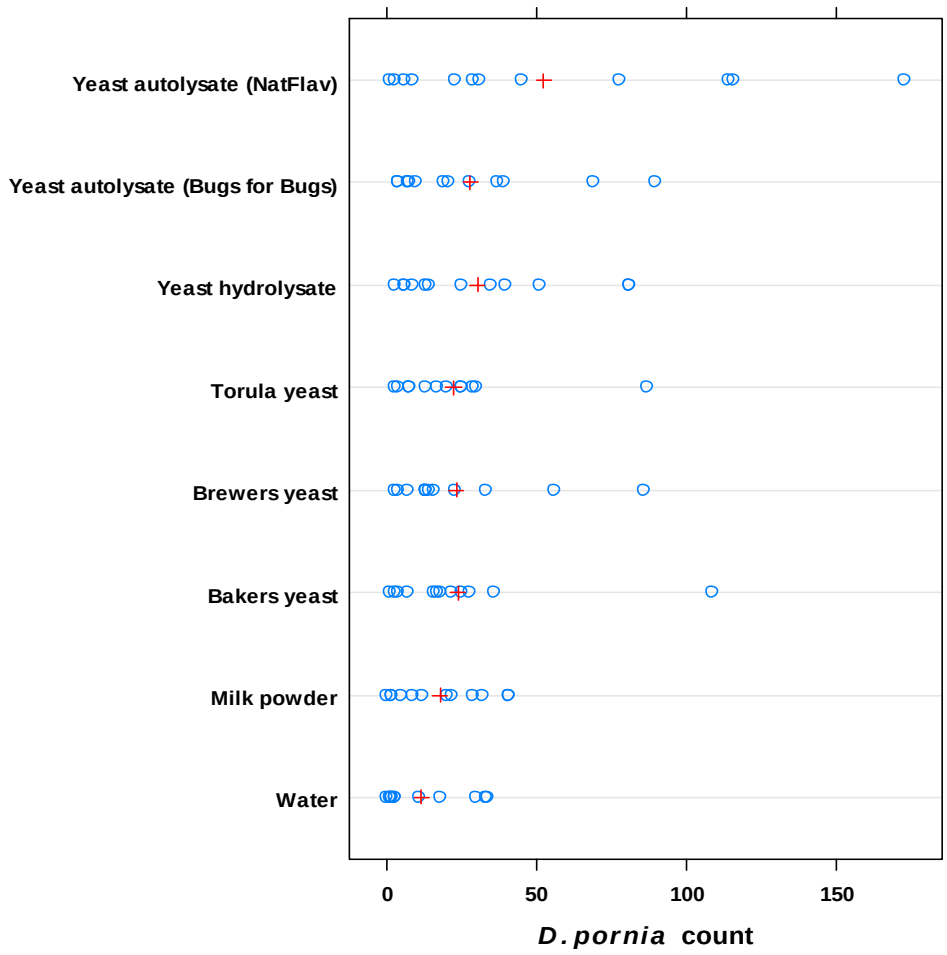

Figure 18. Actual numbers of *Dirioxa pornia* captured over four weeks at Narara. Datum points (open circles) represent actual captures per trap; red crosses represent the mean number of *D. pornia* over the sampling period.

Table 16: Back-transformed mean capture of *D. pornia* in traps containing different protein sources in Narara for 4 weeks. Values are the mean of 12 replicates; values followed by the same letter are not significantly different from each other, p=0.05.

Protein source	Mean capture
Yeast autolysate (NatFlav)	21.7 c
Yeast autolysate (Bugs for Bugs)	17.3 bc
Yeast hydrolysate	18.9 bc
Torula yeast	15.9 bc
Brewers yeast	15.3 bc
Bakers yeast	19.8 c
Milk powder	11.3 b
Water	4.6 a

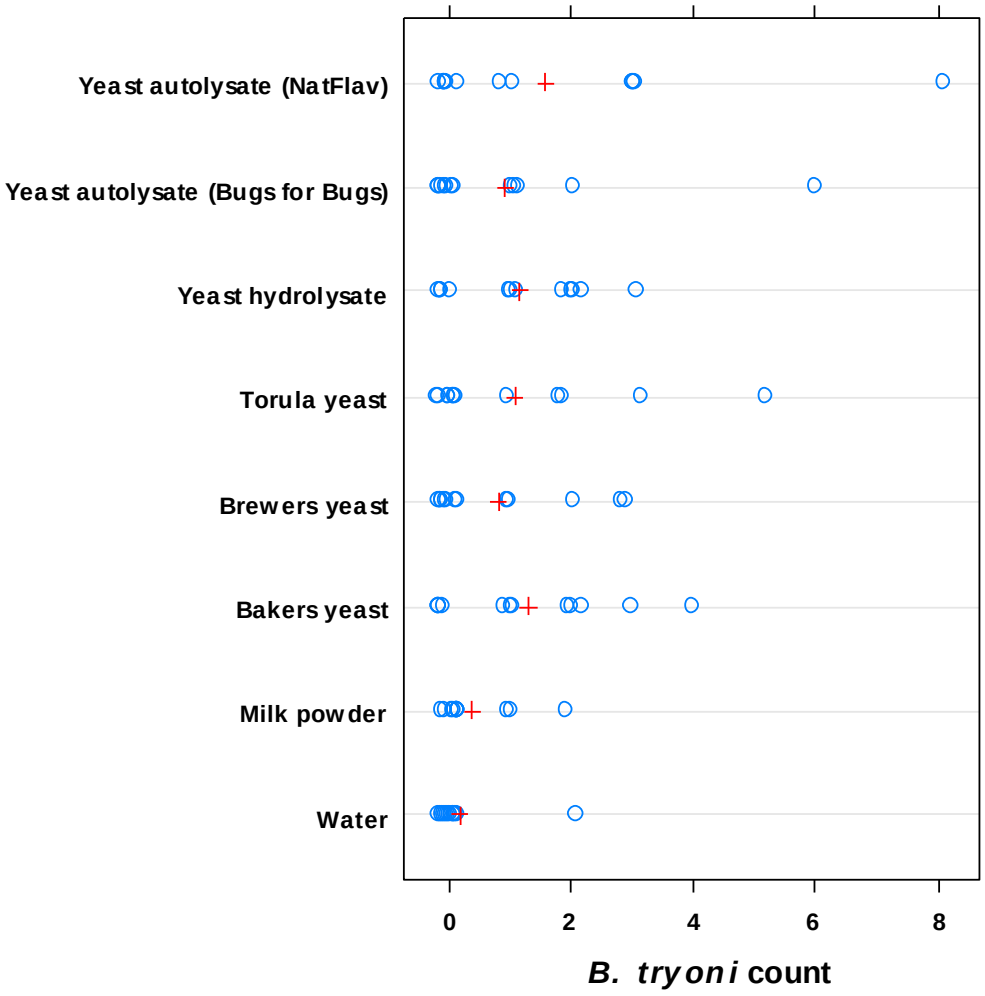

Figure 19. Actual numbers of *Bactrocera tryoni* captured over four weeks at Narara. Datum points (open circles) represent actual captures per trap; red crosses represent the mean number of *Bactrocera tryoni* over the sampling period.

3. (b) Key findings. Part Two: Western Australia

METHODS AND MATERIALS

Cage trial designs

Laboratory cages – description

Laboratory cages (50 x 50 x 50cm; 0.125m^3) were covered in mesh on all sides and the top, and fitted with a solid base. The temperature in the controlled environment was 26°C±1°C and experiments were carried out under natural light. Protein-deprived, experimentally naive flies (*C. capitata* or *B. jarvisi*) were used in all experiments and flies were provided sugar and water during the course of experiments. Flies were collected by aspirator, released into each cage and allowed to settle for one hour prior to traps being placed in cages. Twenty pairs of male and female flies were released into each cage (with the exception of 1 experiment with spinosad (ID 28) where 50 pairs of flies were used).

Laboratory cage trials: *no choice design*

All experiments conducted were no-choice trials, with only one treatment per cage. The number of treatments was equal to the number of cages used. The experiment was re-run on a number of dates and at each date treatments were re-randomised to different cages in such a way that at the end of the experiment all treatments had been placed in all cages the same number of times (or almost the same number of times depending on the number of dates) to account for any cage variability.

Outdoor cages – description

Outdoor cages (2 x 2 x 2m; 8m^3), were covered in white synthetic mesh cloth on all sides and the top was shaded with insulating material to avoid direct sunlight on traps. Each cage contained nine potted cumquat plants, 2-4 years old. Flies were collected by aspirator, released into each cage and allowed to settle for one hour prior to traps being placed in cages. Fifty pairs of male and female *C. capitata* or 30 pairs of male and female *B. jarvisi* were released into each cage. Protein-deprived, experimentally naive flies were used in all experiments.

Outdoor cage trials:

Two experimental designs were used to trial treatments in outdoor cages, a no-choice design and a choice design.

No-choice design: experiments were designed exactly as described for the laboratory cages in the previous section. As there were 3 outdoor cages, a maximum of three treatments were compared in any one experiment.

Choice design: All treatments were placed within each of the cages spaced at 1m. Each experiment was re-run on a number of dates and all 3 cages were used on each date. On each date treatments were allocated to different positions within each cage in such a away that, within each cage, at the end of the experiment, all treatments had been placed in all positions the same number of times (with the exception of the aged vs fresh Unipak lure experiment, Table 41) to account for any positional variability. Choice experiments are generally a better indicator of preference for a particular trap type.

Analysis of cage trials

A linear mixed model was used to examine the effects of treatment and sex and their interaction on insect counts for both laboratory and outdoor cages. The model included the effects of cage, position within cage and date of collection as random effects and allowed for different experimental variance for each sex and correlations between counts for each sex within the same trap. The models were simplified by removing non significant random effects where possible. In the simplest cases this meant that the model was equivalent to a 2-way analysis of variance in randomised blocks (replicates were dates for non-choice trials and a combination of dates and cages for choice trials). For consistency, all counts were transformed using a log transformation prior to analysis. Residual plots were used to confirm that the distributional assumptions were appropriate. In trap comparisons where results for control treatments were very different to other treatments, they were excluded from analyses. Results were presented as means and least significant differences ($p<0.05$; LSD 5%) on the transformed scale, and retransformed means. In addition, when an F-test has indicated there is a significant effect, means have been annotated to indicate where there are significant differences. When LSDs are presented in a lighter shade it indicates there is no difference between the associated means as indicated by an F-test.

Cage trial treatments

Trap comparison

The efficiency of three trap types, Chempac/Suterra, Lynfield and McPhail, were investigated in both laboratory and outdoor cages in experiments with the following treatments:

1) Lynfield
2) McPhail
3) Chempac/Suterra
4) Control (no lure)

The female-targeted Chempac/Suterra Bucket trap (Agrisense/Suterra) is similar to the Tephri trap (Broughton & De Lima 2002). It consists of a 5.5cm white opaque lid, that fits into a yellow bucket shaped invaginated base 12cm in height. When assembled the trap is 16.4cm in height and 14cm in diameter. Three 2cm diameter entry holes are positioned equidistant around the trap 5.5cm from the top of the trap. There are clear plastic valves inside the entry holes intended to reduce exit of flies inside the trap. The Lynfield trap (locally made) and the McPhail trap (commercially available plastic trap) were used as in previous studies (Wijesuriya & De Lima 1995; Broughton & De Lima 2002).

All traps were charged with three-component BioLure® and a $1cm^2$ DDVP strip as a killing agent.

Laboratory cage trial: Conducted with *C. capitata* this trial consisted of twelve replicates in time, with each replicate running for 2 hours (ID1, Table 16).

Outdoor cage trials: No-choice and choice experiments were conducted with *C. capitata*, and a no-choice experiment was conducted with *B. Jarvisi* (ID2, ID3 and ID4).

Lure comparisons

The efficacy of a number of lures, and the effect of lure age on lure attractiveness was investigated in both laboratory and outdoor cages. Prototype lures (gel lure; dry lure) were prepared as previously described. Synthetic lures were sourced from Agrisense/Suterra via Evergreen Marketing Pty Ltd. These were the three-component

BioLure® (ammonium acetate, trimethylamine and putrescine as three separate packages that adhere to internal walls of trap), two-component BioLure® (ammonium acetate and putrescine as two separate packages) and Unipak® (ammonium acetate, trimethylamine and putrescine in a single package but with each component in a separate segmented compartment). Orange ammonia lure is made by combining 280mL freshly squeezed orange juice, 25g ammonium carbonate, 1g potassium sorbate and 600mL of water. The solution is left overnight in the refrigerator and then diluted one part concentrate to ten parts water. 200mL of the resultant mixture is used per trap.

All lure comparison trials and lure age trials were conducted in Chempac/Suterra traps charged with a $1cm^2$ DDVP strip as the killing agent, with the exception of orange ammonia lure which did not contain a toxicant.

Prototype lure tests

1) three-component BioLure®
2) gel lure
3) dry lure
4) no lure (control)

Laboratory cage trials: A total of four replicates were conducted over time for each fly species (ID5 and ID9).

Outdoor cage trials: No-choice and choice experiments were conducted with *C. Capitata* (ID6, ID7 and ID8), and a no-choice experiment was conducted with *B. Jarvisi* (ID10). Lure types were tested without the no-lure treatment in no-choice tests. A lure volume of 120mL gel and dry lure per trap was used for trials ID6, ID7 and ID10 while a volume of 180mL gel and dry lure per trap was used for trial ID8.

Other lures - Two-component BioLure®, three-component BioLure®, orange ammonia lure and Unipak®.

TWO COMPONENT VS THREE COMPONENT BIOLURE®VS ORANGE AMMONIA LURE
The efficacy of two lure types was investigated in laboratory and outdoor cages:

1) Two-component BioLure®
2) Three-component BioLure®
3) no lure (control)
4) orange ammonia lure

Laboratory cage trials: A total of twenty replicates were conducted over time with *C. Capitata,* each replicate was run for 2 hours (ID11). This trial did not include the orange ammonia lure.

Outdoor cage trials: No-choice and choice experiments were conducted with *C. Capitata ID12 and ID13).* The no-choice experiment did not include the orange ammonia lure.

UNIPAK® VS THREE COMPONENT BIOLURE®
The efficacy of two lure types was investigated in laboratory and outdoor cages:

1) Unipak®
2) Three-component BioLure®
3) no lure (control)

Laboratory cage trials: A total of twelve replicates were conducted over time with *C. Capitata,* each replicate was run for 2 hours (ID14).

Outdoor cage trials: In a no-choice experiment with *C. capitata* the two lures were tested along with a control (ID15).

Lure ageing

Aged lures were compared with freshly made or opened lures to investigate the effect of ageing on fly capture with *C. capitata*. All lures were aged and tested in Chempac/Suterra traps with DDVP as the killing agent.

Lure age – prototype lures

Field aged lures were compared in outdoor cages (gel lure and dry lure).

Outdoor cage trials: In a choice test effectiveness of lure was investigated where fresh lure and field aged lures, aged 5 and 6 months over summer (September to March), were presented to flies in each cage.

1) Fresh lure
2) Lure aged 5 months
3) Lure aged 6 months

Each lure type (gel lure and dry lure) was tested in a separate trial (ID16 and ID17).

Lure age – Other lures - Two-component BioLure®, three-component BioLure® and Unipak®,

TWO COMPONENT VS THREE COMPONENT BIOLURE®

The effect of age on the attractancy of lure was investigated in laboratory and outdoor cages with *C. Capitata*.

Laboratory cage trials: Lures aged outdoor at South Perth for 4.5 months, between July and October 2008, were compared with freshly opened lure:

1) Two-component BioLure®
2) Three-component BioLure®
3) Two-component BioLure® aged 4.5 months
4) Three-component BioLure® aged 4.5 months

This trial was repeated four times, with each replicate running for 2 hours (ID18).

Outdoor cage trials: In a choice experiment (ID19) field aged lures, aged 2 and 4 months over summer (October to March), were compared with freshly opened lure:

1) Fresh lure
2) Lure aged 2 months
3) Lure aged 4 months

THREE COMPONENT BIOLURE®

The effect of age on the attractancy of lure was investigated in outdoor cages with *C. Capitata*.

Outdoor cage trials: In a choice experiment (ID20) field aged lures, aged 5, 6 and 7 months over spring and summer (August to March), were compared with freshly opened lure:

1) Fresh lure
2) Lure aged 5 months
3) Lure aged 6 months
4) Lure aged 7 months

UNIPAK® VS THREE COMPONENT BIOLURE®

The effect of age on the attractancy of lure was investigated in outdoor cages with *C. Capitata*.

Outdoor cage trials: In a choice experiment (ID21) field aged lures, aged 1 and 6 months over summer and autumn (October to May), were compared with freshly opened lures:

1) Fresh three-component BioLure®
2) Fresh Unipak®
3) One month old Unipak®
4) Six month old Unipak®

Killing agent comparison

Several killing agents were investigated to examine their effectiveness at retaining flies that enter traps. Dichlorvos (DDVP) pest strips that are currently used in traps used to detect *C. capitata* are easy to handle because they are dry lures and have a fast knock down effect due to its fumigant action but are highly toxic to humans. Therefore safer alternatives that had been shown to be promising in the literature were investigated.

All killing agent comparison trials were conducted in Chempac/Suterra traps. Standard Three component Biolure® was used in all treatments unless specifically stated as 'No lure'.

Propylene glycol (PG) and white oil

The efficacy of PG was investigated with *C. Capitata* in laboratory and outdoor cage trials.

Laboratory cage trial: This trial (ID22) compared:

1) BioLure® with 100mL 70% PG
2) BioLure® with 100mL 70% PG and 30mL white oil
3) BioLure® with DDVP strip
4) No lure with DDVP strip

Outdoor cage trials: No-choice and choice experiments were conducted with *C. capitata*.

a) no-choice experiment, where lure types in the laboratory trial were tested above without the no-lure treatment (ID23)
b) no-choice experiment, where each of the 3 Biolure treatments was paired with a trap that contained the same killing agent without Biolure®.
    1) BioLure® with 100mL 10% PG (pair 1)
    2) No lure with 100mL 10% PG (pair 1)
    3) BioLure® with 100mL 10% PG and 30mL white oil (pair 2)
    4) No lure with 100mL 10% PG and 30mL white oil (pair 2)
    5) BioLure® with DDVP strip (pair 3)
    6) No lure with DDVP strip (pair 3)

    Traps within pairs were spaced at 1m within a cage and each pair was placed in a separate cage (ID24).
c) no-choice experiment (ID25), where the efficacy of three concentrations of PG (100mL) was compared.
    1) 10% PG
    2) 20% PG
    3) 70% PG

d) choice experiment (ID26), where four concentrations of propylene glycol (PG 100mL) with and without white oil (WO 30mL) were presented to flies in each cage.
    1) 100% PG
    2) 70% PG
    3) 50% PG + WO
    4) 20% PG + WO

Phloxine B and spinosad

Spinosad offered as liquid drops to Western cherry fruit fly (*Rhagoletis indifferens*) achieved 100% mortality within three days (Yee & Alston 2006). In addition, spinosad at 20mg L^{-1} (20ppm) offered in drinking water to *C. capitata* adults produced 80% mortality after one day (Adan *et al.* 1996). This level of reported mortality suggests that spinosad may be a suitable alternative killing agent to DDVP.

Two trials were conducted to determine the effectiveness of phloxine B and spinosad as killing agents. In each case, the required quantity of toxicant was added to 10% Flavex® (protein hydrolysate) and 4mL volume was pipetted onto a cotton dental wick that was placed in the base of the trap.

Laboratory cage trials:

a) in the first trial (ID27) treatments were presented to 20 pairs of protein-deprived *C. capitata* in laboratory cages:
    1) Flavex® only
    2) Flavex® + 40ppm spinosad
    3) Flavex® + 40ppm spinosad + 1% fluorescein sodium salt
    4) Flavex® + 1% phloxine B
b) in a second trial (ID28), treatments were presented to 50 pairs of protein-deprived *C. capitata*:
    1) Flavex® only
    2) Flavex® + 1ppm spinosad
    3) Flavex® + 2ppm spinosad
    4) Flavex® + 3ppm spinosad

Diclorvos, Propylene glycol, spinosad and Talcum powder

Efficacy of four killing agents was tested in outdoor cages with *C. capitata*. All treatments except Spinosad were used with Biolure®, Spinosad was used with Flavex® protein as described in the laboratory trial above. PG and Talcum powder were poured into the base of the trap, spinosad was pipetted on to wick containing 4 cotton dental rolls and placed in the trap base.

Outdoor cage trial: A choice experiment (ID29) was conducted, with *C. capitata*:

1) DDVP (standard)
2) 70% PG® 150mL
3) Spinosad (40ppm)
4) Talcum powder – 30mL

Essential oils

The scientific literature indicates that the effect of essential oils as insect killing agents is variable, depending on the type of insect and the oil used. Tunc & Sahinkaya (1988) examined the vapour toxicity of four essential oils to two green house pests, *Aphis gossypii* (cotton aphid) and *Tetranychus cinnabarinus* (carmine spider mite) and found that a minimum dose of 0.5µL L^{-1} achieved 99% mortality with 2-3 days of exposure to cumin,

oregano and anise oils, but eucalyptus oil required a much longer exposure time. In a separate study, 30 essential oils were tested against two stored product pests, *Sitophilus oryzae* (rice weevil) and *Callosobruchus chinensis* (southern cowpea weevil) (Kim *et al*. 2003). Several of the oils were effective fumigants, including cinnamon oil, horseradish oil and mustard oil, all of which caused 100% mortality of both species within one day of exposure. In another trial, rosemary oil was found to be toxic to mites in the greenhouse (Miresmailli & Isman 2006). Although a majority of these studies were conducted in enclosed spaces, the knockdown effect of six essential oils that were locally available (anise, cinnamon, clove, eucalyptus, lavender, rosemary) were evaluated using *C. capitata.*

Six plant essential oils were compared with DDVP as killing agents for *C. capitata* over two laboratory cage trials, using three-component BioLure® as an attractant. Essential oils (1mL) were mixed with PG (1mL) and pipetted onto cotton dental wicks, which were placed in the base of traps. *Laboratory cage trials:*

a) in the first trial (ID30) treatments were presented to 20 pairs of protein-deprived *C. capitata*:
    1) DDVP (standard)
    2) cinnamon oil
    3) clove oil
    4) eucalyptus oil

a) in the second trial (ID31) treatments were
    1) DDVP (standard)
    2) anise oil
    3) lavender oil
    4) rosemary oil

Ethyl formate

This experiment (ID32) was conducted with twenty pairs of protein-deprived *C. capitata* in laboratory cages. One millilitre of 99.5% ethyl formate was applied to a cotton dental wick and placed in the bottom of trap with either three-component BioLure® or no lure. Due to the extremely poor response observed, this trial was not repeated.

Sticky card

In this experiment (ID33) three treatments were evaluated, which compared yellow sticky card (9 x 10cm) placed in the base of the trap, against the invaginated entry, facing the inside wall and DDVP strip.

1) Biolure® + sticky card
2) No lure + sticky card
3) Biolure® + DDVP

Table 16. Details of all experiments conducted in Western Australia

Expt ID	Trial	Cage	Choice/ no-choice	No. runs	Reps	Running time	Result table
1	Trap comparison: *C. capitata*	Lab	no choice	12	12	2 hours	18
2	Trap comparison: *C. capitata*	Outdoor	no choice	5	5	2 hours	
3	Trap comparison: *C. capitata*	Outdoor	choice	5	15	4 & 24 hours	
4	Trap comparison: *B. jarvisi*	Outdoor	no choice	3	3	4 hours	19
5	Prototype lure comparison: *C. capitata*	Lab	no choice	4	4	4 & 24 hours	20
6	Prototype lure comparison: *C. capitata* 120mL	Outdoor	no choice	9	9	4 & 24 hours	
7	Prototype lure comparison: *C. capitata* 120mL	Outdoor	choice	4	12	4 & 24 hours	
8	Prototype lure comparison: *C. capitata* 180mL	Outdoor	choice	6	18	4 hours	
9	Prototype lure comparison: *B. jarvisi* 120mL	Lab	no choice	4	4	4 hours	21
10	Prototype lure comparison: *B. jarvisi* 120mL	Outdoor	no choice	12	12	4 & 24 hours	
Other lures – all with *C. capitata*							
11	Two component Biolure vs Three component Biolure	Lab	no choice	20	20	2 hours	22
12	Two component Biolure vs Three component Biolure	Outdoor	no choice	6	6	2 hours	
13	Two component Biolure and Orange Ammonia vs Three component Biolure	Outdoor	choice	8	24	4 hours	
14	Unipak vs Three component Biolure	Lab	no choice	12	12	2 hours	23
15	Unipak vs Three component Biolure	Outdoor	no choice	6	6	2 hours	
Lure ageing – with *C. capitata*, with DDVP							
16	Prototype lures – Gel lure	Outdoor	choice	3	12	4 hours	24
17	Prototype lures – Dry lure	Outdoor	choice	3	12	4 hours	
18	Fresh and Aged Two component Biolure vs Three component Biolure	Lab	no choice	4	4	2 hours	25
19	Two component Biolure	Outdoor	choice	3	12	4 hours	
20	Three component Biolure	Outdoor	choice	4	16	4 hours	
21	Fresh and Aged Unipak vs Three component Biolure	Outdoor	choice	3	9	4 hours	26

Killing agent comparisons with *C. capitata* using Biolure unless otherwise stated							
22	70%PG, white oil and DDVP	Lab	no choice	5	5	2 hours	27
23	70%PG, white oil and DDVP	Outdoor	no choice	4	4	24 hours	
24	10%PG, white oil and DDVP with and without Biolure	Outdoor	no choice	4	4	24 hours	
25	PG at different concentrations	Outdoor	no choice	3	3	24 hours	
26	PG concentrations vs DDVP	Outdoor	choice	4	12	4 hours	
27	Phloxine B and spinosad (with Flavex)	Lab	no choice	1	1	24 & 48 hours	28
28	Spinosad at different concentrations (with Flavex)	Lab	no choice	1	1	24 & 48 hours	
29	DDVP, 70%PG, Talc and spinosad (with Flavex)	Outdoor	choice	4	12	4 hours	29
30	Essential oil A	Lab	no choice	3	3	24 hours	30
31	Essential oil B	Lab	no choice	3	3	24 hours	
32	Ethyl formate (with and without Biolure)	Lab	no choice	2	2	1 & 18 hours	31
33	Sticky card (with and without Biolure)	Lab	no choice	3	3	6 hours	32

Orchard trial designs

Field trials were conducted to evaluate lures for *C. capitata* in south-west WA and for *Bactrocera* spp. in Kununurra in the state's north.

Donnybrook

Donnybrook is located 180km south of Perth (33°S, 115°E). Trials were conducted in an orange orchard in 2009 and 2010, and in a pear orchard in 2010. The orange orchard (~100m x 140m) is a commercial orchard located on Bendall Road. Trees were 3-3.5m high at the time of the trial and although the orchard is intermittently bait-treated, no fruit fly treatments were applied during the trial. At the commencement of the lure comparison trials, trees contained mature green fruit.

The pear orchard (~200m x 60m) was planted alongside a creek on Irishtown Road. Trees were around 4m in height, with 5m spacing between trees and between rows. Pears were planted in staggered rows with a small orange and apple orchard adjoining another pear orchard on one side. At the commencement of the lure comparison trial a few green fruit remained but most had been harvested. This was a commercial orchard that was regularly baited for *C. capitata*.

Harvey

Harvey is located 140km south of Perth (33°S, 115°E). Trials were conducted in orange orchards at Harvey in 2009 (Young Street) and 2010 (Calder Grove). Mature citrus trees

in this orchard (~130m x 80m) were 3-3.5 m high, with 3m spacing between trees and rows. On Young street there was an adjoining citrus orchard (orange and mandarin) on one side of the orchard. Other fruit trees such as pear and stone fruit were present in small numbers. There were an assortment of other fruiting trees on one side of the orchard including avocado, white sapote, pear, nectarine and fig. This was a commercial orchard that was regularly baited for *C. capitata*. Trees contained mature green fruit at the commencement of the trial.

Kununurra

Kununurra is located 2220km north-east of Perth (15°S, 128°E). Trials were conducted in two separate carambola orchards in 2009 and 2010, respectively, a mango orchard in 2009, and a sandalwood plantation in both 2009 and 2010. *B. aquilonis* and *B. jarvisi* are the primary fruit fly species trapped in this region.

In 2009, the carambola orchard used was about 150m x 100m in area, trees were 3.5-4m tall and canopy diameter was 4-5m. The distance between trees was around 3.5m. Trees were healthy but short on water, which caused flower drop and severe reduction in fruit set. Adjoining the orchard on one side was bare land, which had been previously planted to pumpkin; on other sides were native vegetation, vegetable garden and pawpaw. In 2010, a different carambola orchard was used on the same property. This orchard was about 90m x 35m in area, trees were 3.5-4m tall and canopy diameter was 3-4m. The distance between trees and rows was about 4m. Trees were healthy, harvest was nearing completion and some fruit were on the ground when the trial was set up. Adjoining the orchard on one side was bare land, another side was planted with pumpkin; on other sides there was native vegetation. Vegetable garden, some mango and pawpaw were nearby.

The mango orchard used in 2009 was 150m x 100m in area, trees were about 4m tall and canopy diameter was 5m. Trees contained green fruit, both immature and nearing maturity. The distance between trees was about 5m. There was a row of large grapefruit trees on one side and several rows of taller, older mango trees. A house garden bordered on another side and there was fallow land on the two remaining sides.

The same sandalwood plantation was used in both 2009 and 2010 and was approximately 2000m x 1000m in area, trees were 2-3m tall and canopy diameter was 1-1.5m. Trees were in various stages of flowering and fruiting, with a majority of trees containing ripe fruit. Sandalwood trees were interspersed with *Sesbania* and *Cathomian* species planted as hosts for sandalwood which are semi-parasitic. Traps were placed in a corner of the plantation bordered by native vegetation on one side and continuing sandalwood plantation on the other side across from an access road. There was an area (50m x 50m) of exotic fruit planting about 500m from this site containing grapefruit, sapote, star apple, sapodilla and avocado, quite a few of which contained mature or immature fruit.

Layout

Replicate blocks were set up, with all treatments randomised within each replicate, in each orchard. For all trials apart from the lure age trial at Harvey (described below), traps within a replicate were 10m apart and replicates were 25m apart. Flies were collected once a week and traps were sequentially rotated through positions within each replicate at each collection time.

Analysis of orchard trials

A linear mixed model was used to examine the effects of treatment and sex and their interaction on insect counts from orchard trials. The model included the effects of block and position within block and date of collection as random effects and allowed for different experimental variance for each sex and correlations between counts for each sex within

the same trap. The models were simplified by removing non significant random effects where possible. In the simplest case this meant that the model was equivalent to a 2-way analysis of variance in randomised blocks (replicates were a combination of blocks, positions and dates). For consistency, all counts were transformed using a log transformation prior to analysis. Residual plots were used to confirm that the distributional assumptions were appropriate. Results were presented as means and least significant differences ($p<0.05$; LSD 5%) on the transformed scale and retransformed means. In addition, when an F-test has indicated there is a significant effect, means have been annotated to indicate where there are significant differences. When LSDs are presented in a lighter shade it indicates there is no difference between the associated means as indicated by an F-test.

In the case of the lure ageing trial in Harvey, which consisted of 3 female attractant lures and 4 ages, and a male attractant lure which was not aged, the fixed model included a comparison between the male and female attractant lures, the effect of female lure type, the effect of ageing on female lures, the effect of sex and all interactions (Table 36).

Orchard trial treatments

Lure comparisons in south-west WA

The Chempac/Suterra trap was used for all trials.

Prototype lures – 2009

Identical experiments (ID34 and ID35, Table 17) were conducted in orange orchards in Donnybrook and Harvey. Three types of female targeted attractants were tested with DDVP as killing agent, with a 4th treatment that contained PG as killing agent. Traps with PG did not contain a toxicant.

1) Three component Biolure®
2) Gel lure: 120mL
3) Dry lure: 120mL
4) Three component Biolure® with 100mL of 70% PG®

Prototype lures – 2010

Identical experiments (ID36, ID37 and ID38) were conducted in the pear and orange orchards in Donnybrook, and the orange orchard in Harvey. Three types of female targeted attractants were tested with DDVP as a killing agent, along with male lure (Capilure with 2 types of killing agents: DDVP or PG). Traps with PG did not contain any toxicant. Flies were strained out of the PG and the liquid was returned to the trap. Gel and Dry lures were replaced with freshly made lure for the second rotation.

1) Three component Biolure®
2) Gel lure: 180mL
3) Dry lure: 180mL
4) Capilure - 3mL with DDVP
5) Capilure - 3mL with 150mL of 70% PG®

Two and three component lures – 2010

This trial (ID39) was conducted in an orange orchard in Harvey 2010. All traps contained DDVP as a killing agent.

1) Two-component BioLure®
2) Three-component BioLure®

Effect of lure age in the south-west WA - 2010

The lure aging trial (ID40) was conducted in the orange orchard in Harvey. Lures aged in traps in the field during summer months were compared with fresh lures. All traps contained DDVP as a killing agent. Male lure (Capilure) was also included as a measure of population level as well as for comparison with female lures.

1) Biolure®: 3 component lure – Fresh
2) Biolure®: 3 component lure – Aged 1 month
3) Biolure®: 3 component lure – Aged 2 months
4) Biolure®: 3 component lure – Aged 3 months
5) Dry lure: 120mL – Fresh
6) Dry lure: 120mL – Aged 1 month
7) Dry lure: 120mL – Aged 2 months
8) Dry lure: 120mL – Aged 3 months
9) Gel lure: 120mL – Fresh
10) Gel lure: 120mL – Aged 1 month
11) Gel lure: 120mL – Aged 2 months
12) Gel lure: 120mL – Aged 3 months
13) Capilure – 3mL (fresh)
14) Capilure – 3mL (fresh)
15) Capilure – 3mL (fresh)

Three replicate blocks were set up. Each replicate block was a row, with traps 6m apart (every other tree) in mature trees with fruit; distance between replicates was 25m. Flies were collected once a week for 5 weeks and traps were rotated within a replicate following an incomplete Latin square design (Youden square) at each collection time.

Lure comparisons in Kununurra

The Chempac/Suterra trap was used for all trials. Within each orchard, traps were placed in trees of similar size at distances ≥10m. In 2009 distance between replicates in all orchards was 25m, but in 2010 in the carambola orchard this distance was 15m.

Prototype lures – 2009

Identical trials (ID41 (a: *C. capitata* and b: *B. jarvisi*), ID42 and ID43) were conducted in carambola, mango and sandalwood orchards in 2009. Three types of female targeted attractants were tested along with male lure (Cuelure with 2 types of killing agents: malathion or PG:

1) Three component Biolure®
2) Gel lure: 120mL
3) Dry lure: 120mL
4) Cuelure - 4mL with Hymal® (malathion)
5) Cuelure - 4mL with 100mL of 70% PG®

All treatments other than (5) were tested as dry traps with a square of DDVP in the base of the trap. In treatment (4) the DDVP was to prevent predation by ants. Flies were strained out of the PG and the liquid was returned to the trap and was topped up as necessary.

Prototype lures – 2010

Identical trials (ID44 and ID45) were conducted in carambola and sandalwood orchards in 2010. Five types of female targeted attractants were tested along with a control treatment with no lure:

1) Three component Biolure®
2) Gel lure: 210mL
3) Dry lure: 210mL
4) Natflav®: 200mL
5) Orange ammonia: 200mL
6) No lure (control)

All treatments other than (4 and 5) were tested as dry traps with a square of DDVP in the base of the trap. Orange ammonia lure and Natflav were replaced weekly.

Killing agent comparisons in Kununurra – 2010

Traps containing male lure (Cuelure with 2 types of killing agents) were deployed on either side of the blocks containing the female lure trials (carambola and sandalwood) in 2010.

The Chempac/Suterra trap was used for both trials (ID46 and ID47).

1) Cuelure + Hymal® (8:1) 4mL on 4 cotton dental rolls.
2) Cuelure (4mL) with 150mL of 70% PG®.

Treatments were set up as pairs with 10 m between traps and 25m between replicates. In treatment (1) the DDVP was to prevent predation by ants. Flies were collected twice a week for 3 weeks and traps were rotated between the positions within each pair at collection time.

Table 17. Details of all experiments conducted in Western Australia in orchard trials.

Expt ID	Trial	Year	Locality and crop	No. runs	Reps	Collection time	Result table
Orchard trials with DDVP except in treatments with PG or orange ammonia							
34	DDVP, PG (with Biolure) vs prototype lures	2009	Donnybrook (DBK) orange	3 blocks x 4 dates	12	weekly	33
35	DDVP, PG (with Biolure) vs prototype lures	2009	Harvey (HAR) orange	3 blocks x 4 dates	12	weekly	
36	DDVP, PG (with Capilure) & Biolure vs prototype lures	2010	DBK pear	4 blocks x 10 dates	40	weekly	34
37	DDVP, PG (with Capilure) & Biolure vs prototype lures	2010	DBK orange	4 blocks x 10 dates	40	weekly	
38	DDVP, PG (with Capilure) & Biolure vs prototype lures	2010	HAR orange	4 blocks x 10 dates	40	weekly	
39	Two component Biolure vs Three component Biolure	2010	HAR orange	6 blocks x 6 dates	36	weekly	35
40	Lure age: Biolure vs prototype lures and capi	2010	HAR orange	3 blocks x 5 dates	15	weekly	36
41 A	Malathion and PG (with Cuelure) and Biolure vs prototype lures	2009	Kununurra carambola - *B. aquilonis*	4 blocks x 10 dates	40	weekly	37
41 B	Malathion and PG (with Cuelure) and Biolure vs prototype lures	2009	Kununurra carambola - *B. Jarvisi*	4 blocks x 10 dates	40	weekly	
42	Malathion and PG (with Cuelure) and Biolure vs prototype lures	2009	Kununurra mango- *B. aquilonis*	4 blocks x 10 dates	40	weekly	38
43	Malathion and PG (with Cuelure) and Biolure vs prototype lures	2009	Kununurra sandalwood- *B. aquilonis*	4 blocks x 10 dates	40	weekly	39
44	Biolure vs orange ammonia, and prototype lures	2010	Kununurra carambola - *B. aquilonis*	4 blocks x 6 dates	24	twice weekly	40
45	Biolure vs orange ammonia, and prototype lures	2010	Kununurra sandalwood - *B. aquilonis*	4 blocks x 6 dates	24	twice weekly	41
46	Malathion vs PG	2010	Kununurra carambola - *B. aquilonis*	2 blocks x 6 dates	12	twice weekly	42
47	Malathion vs PG	2010	Kununurra sandalwood - *B. aquilonis*	3 blocks x 6 dates	18	twice weekly	43

RESULTS AND DISCUSSION

Trap comparison

Three trap types were compared in laboratory cages and in outdoor cages with both *C. capitata* and *B. jarvisi*.

C. capitata

The no-choice laboratory cage trials (ID1) showed that, after 2 hours, the Chempac/Suterra trap was not different from the McPhail trap, whereas the Lynfield trap captured less flies than both McPhail and Chempac/Suterra traps (P<0.001) (Table 18). Overall, more males were captured than females (P=0.003) and a significant interactive effect was observed in that McPhail traps captured less males than the other traps while less females were captured in the Lynfield trap compared to other traps (P<0.001).

Table 18. Effect of trap type, sex, and their interaction on the number of *Ceratitis capitata*. Values are means of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID1 in laboratory cages, 20 male and 20 female flies were the test subjects; and experiments ID2 and ID3 in outdoor cages 50 male and 50 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).

Trap type	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID1 (2 hours)			
Lynfield	1.268 (88%) a	1.311 (98%) b	1.225 (79%) a
McPhail	1.294 (94%) b	1.282 (91%) a	1.306 (96%) b
Chempac/Suterra	1.308 (97%) b	1.315 (99%) b	1.302 (95%) b
LSD 5%	0.018	0.026	
NB: control treatment has been excluded from analysis.			
Expt. ID2 (2 hours)			
Lynfield	1.450 (54%)	1.504 (62%)	1.395 (48%)
McPhail	1.568 (72%)	1.590 (76%)	1.546 (68%)
Chempac/Suterra	1.555 (70%)	1.605 (78%)	1.506 (62%)
LSD 5%	0.141	0.169	
Expt. ID3 (4 hours)			
Lynfield	1.000 (18%) b	1.013 (19%)	0.987 (17%)
McPhail	0.758 (9%) a	0.733 (9%)	0.783 (10%)
Chempac/Suterra	1.052 (21%) c	1.058 (21%)	1.047 (20%)
LSD 5%	0.102	0.144	
Expt. ID3 (24 hours)			
Lynfield	1.047 (20%) b	1.032 (20%)	1.063 (21%)
McPhail	0.800 (11%) a	0.751 (9%)	0.848 (12%)
Chempac/Suterra	1.102 (23%) b	1.078 (22%)	1.127 (25%)
LSD 5%	0.098	0.138	

The no-choice experiment conducted in outdoor cages (ID2) found no significant effect of trap type, sex or their interaction after 2 hours (Table 18). However, significant effects

were found when the traps were used in a choice experiment (Table 18: ID3). The Chempac/Suterra and Lynfield traps were preferred to the McPhail trap after both 4 and 24 hours of exposure (P<0.001). After four hours exposure the Chempac/Suterra trap had captured significantly more flies than the Lynfield trap but this was no longer the case after 24 hours. There was no significant effect of sex on trap captures at either 4 or 24 hours (P=0.918 and P=0.144, respectively) or interactions between sex and trap type (P=0.742 and P=0.782, respectively).

B. jarvisi

A no-choice experiment (ID4) showed no significant difference in trap captures between trap types (P=0.966) and there were no significant effects of sex (P=0.420) or the trap type by sex interaction (Table 19; P=0.362).

Table 19. Effect of trap type, sex, and their interaction on the number of *Bactrocera jarvisi*. Values are the mean of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID4 in outdoor cages 30 male and 30 female flies were the test subjects.

Trap type	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID4 (4 hours)			
Lynfield	1.261 (34%)	1.234 (32%)	1.288 (37%)
McPhail	1.278 (36%)	1.304 (38%)	1.251 (34%)
Chempac/Suterra	1.288 (35%)	1.348 (43%)	1.227 (32%)
LSD 5%	0.178	0.214	

Many trap designs have been used in the history of fruit fly trapping in combination with protein and male attractant lures (Steiner 1957; Harris *et al.* 1971; Epsky *et al.* 1999; Robacker & Thomas 2007; Navarro-Llopis *et al.* 2008). Among these are the well known McPhail and Tephri traps used with wet lures and Steiner, Lynfield and Jackson sticky trap used with dry lures. The combination of the trap design, the lure and the toxicant can alter the attractancy of the trap. In WA, according to the Medfly Code of Practice, the McPhail trap or equivalent baited with three-component BioLure® (Broughton & De Lima 2002) is used for trapping females, and the Lynfield trap (Cowley *et al.* 1990; Wijesuriya & De Lima 1995) is used with Capilure® to trap male flies. The Chempac/Suterra trap used in our trials was similar in design to the Tephri trap but slightly larger.

Overall, the trials conducted here have demonstrated that the Chempac/Suterra trap is more effective for trapping *C. capitata* compared to the Lynfield and McPhail traps when used with three-component BioLure®. This is probably due to a combination of the visual attractiveness of the trap, the ease of entry into the trap and possibly better lure dispersion through the side entry holes. The invaginated base makes the trap compatible for use with both liquid and dry lures. Traps of similar design (*e.g.* Tephri trap) have been shown in previous studies to outperform the McPhail trap in the capture of male *C. capitata* although there was no difference in the capture of female flies (Broughton & De Lima 2002).

For *B. jarvisi*, trial results indicated that trap type was not an important factor for attraction of flies to the lure. The McPhail trap has historically been used for trapping of female *Bactrocera* spp., but these results indicates that the Chempac/Suterra trap could be equally as effective.

Because of the results found here, the Chempac/Suterra trap was subsequently used for all other trials in the WA component of this project.

Lure comparisons

Prototype lures

The prototype gel and dry lures were compared to the standard three-component BioLure® in laboratory cages and in outdoor cages with both *C. capitata* and *B. jarvisi*.

C. capitata

After two hours exposure, both the gel and dry lures were as effective as three-component BioLure® in laboratory cages (P<0.001) (Table 20: ID5). Overall, more females were captured than males (P=0.023) and there was a significant interactive effect of sex and treatment as more females than males were captured in the control trap but not in other traps (P=0.001). This indicates a possibility that the trap itself may be visually attractive to female *C. capitata*, even in the absence of a lure.

After 24 hours, capture data were similar to that observed at four hours (Table 20: ID5). All lures captured more flies than the control (P<0.001), more females were captured than males (P=0.011) and more females than males were captured in the control trap containing no lure (P=0.005). However, numbers in control traps were noted to be higher than after 4 hours. This suggests that small cage trials may not be suitable for comparative tests of lures, particularly over a longer time period.

A no-choice experiment was conducted in outdoor cages to evaluate relative lure attractancy. Dry and gel lures were dispensed as 120mL lures. After four hours of exposure (Table 20: ID6), the dry lure was significantly less attractive than the gel lure and three-component BioLure® (P=0.001). Overall, more females were captured than males (P<0.001) but there was no significant interactive effect observed (P=0.293).
After 24 hours of exposure, the trend in lure attractancy was similar to that observed at four hours (Table 20: ID6). The dry lure was still significantly less attractive than the gel lure and three-component BioLure® (P=0.005). Overall more females were captured than males (P=0.023) but no significant interactive effect was observed (P=0.840).

The results of a choice experiment using the same lure types (Table 20: ID7) were similar to those of the no-choice experiment after 4 hours and 24 hours (ID6). After both exposure times three-component BioLure® and gel lure were equally attractive and the dry lure was significantly less attractive (P<0.001) (Table 20: ID7). All lures were more effective than traps without lures. There was no significant difference between the numbers of male and female flies captured (P=0.116 and P=0.570, respectively) and the interaction of lure type and sex was not significant (P=0.090 and P=0.097, respectively).

To determine whether using a larger volume of the gel and dry lures affected attractancy, the choice experiment in outdoor cages was repeated using 180mL of dry and gel lure (Table 20: ID8). Results after four hours exposure were similar to that shown previously using 120mL of lure in that dry lure was less effective than both gel lure and three component Biolure®. In addition however, three-component BioLure® was the most attractive lure, followed by the gel lure and then the dry lure (P<0.001). There was no difference between the capture of males and females (P=0.486) and there was no interactive effect (P=0.505).

Table 20. Effect of lure type, sex, and their interaction on the number of *Ceratitis capitata*. Values are means of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID5 in laboratory cages, 20 male and 20 female flies were the test subjects; and experiments ID6, ID7 and ID8 in outdoor cages 50 male and 50 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).

Lure type	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID5 (2 hours)			
Three-component BioLure®	1.253 (85%) b	1.257 (85%) b	1.250 (84%) b
Gel lure	1.269 (88%) b	1.256 (85%) b	1.283 (91%) b
Dry lure	1.196 (74%) b	1.233 (81%) b	1.159 (67%) b
No lure (control)	0.439 (9%) a	0.226 (3%) a	0.652 (18%) a
LSD 5%	0.190	0.215	
Expt. ID5 (24 hours)			
Three-component BioLure®	1.314 (98%) b	1.305 (96%) b	1.322 (100%) b
Gel lure	1.314 (98%) b	1.306 (96%) b	1.322 (100%) b
Dry lure	1.303 (96%) b	1.312 (98%) b	1.294 (94%) b
No lure (control)	1.152 (66%) a	1.100 (58%) a	1.203 (75%) a
LSD 5%	0.047	0.056	
Expt. ID6 (4 hours)			
Three-component BioLure®	1.587 (75%) b	1.543 (68%)	1.631 (84%)
Gel lure	1.529 (66%) b	1.508 (61%)	1.555 (70%)
Dry lure	1.425 (51%) a	1.366 (45%)	1.483 (59%)
LSD 5%	0.072	0.088	
Expt. ID6 (24 hours)			
Three-component BioLure®	1.616 (81%) b	1.590 (76%)	1.641 (86%)
Gel lure	1.580 (74%) b	1.565 (71%)	1.596 (77%)
Dry lure	1.501 (61%) a	1.472 (57%)	1.529 (66%)
LSD 5%	0.062	0.076	
Expt. ID7 (4 hours)			
Three-component BioLure®	1.120 (24%) c	1.145 (26%)	1.095 (23%)
Gel lure	1.129 (25%) c	1.119 (24%)	1.140 (26%)
Dry lure	0.780 (10%) b	0.675 (7%)	0.885 (13%)
No lure (control)	0.115 (1%) a	0.090 (0%)	0.140 (1%)
LSD 5%	0.102	0.114	
Expt. ID7 (24 hours)			
Three-component BioLure®	1.197 (30%) c	1.227 (32%)	1.168 (27%)
Gel lure	1.198 (30%) c	1.199 (30%)	1.197 (29%)
Dry lure	0.860 (12%) b	0.780 (10%)	0.940 (15%)
No lure (control)	0.203 (1%) a	0.215 (1%)	0.190 (1%)
LSD 5%	0.092	0.130	
Expt. ID8 (4 hours)			
Three-component BioLure®	1.202 (30%) c	1.210 (32%)	1.195 (28%)
Gel lure	1.068 (21%) b	1.062 (21%)	1.074 (21%)
Dry lure	0.949 (16%) a	0.924 (15%)	0.975 (17%)
LSD 5%	0.068	0.088	

B. jarvisi

When tested in laboratory cages with *B. jarvisi*, both the gel and dry lures were as effective as three-component BioLure® (Table 21: ID9). All lures captured more flies than the control (P=0.002) and overall, more females were captured than males (P=0.004) and there was no interactive effect (P=0.365).

After four hours in the no-choice outdoor cage experiment with *B. jarvisi* (Table 21: ID10) there was no difference in the capture of flies between the lures (P=0.088), there was no difference between the capture of males and females (P=0.117) and there was no interaction (P=0.546). After 24 hours (Table 21: ID10), gel lure recorded higher numbers of *B. jarvisi* (P=0.025) than Biolure, but there was no difference between the dry lure and other lures. Overall more males were captured than females (P=0.003. However, there was no significant interaction between sex and treatment (P=0.545).

Table 21. Effect of lure type, sex, and their interaction on the number of *Bactrocera jarvisi*. Values are means of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID9 in laboratory cages, 20 male and 20 female flies were the test subjects; and experiments ID10 in outdoor cages 30 male and 30 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).

Lure type	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID9 (4 hours)			
Three-component BioLure®	1.295 (94%) b	1.278 (90%)	1.311 (98%)
Gel lure	1.289 (93%) b	1.271 (89%)	1.306 (96%)
Dry lure	1.300 (95%) b	1.295 (94%)	1.306 (96%)
No lure (control)	0.997 (45%) a	0.965 (41%)	1.029 (49%)
LSD 5%	0.121	0.125	
Expt. ID10 (4 hours)			
Three-component BioLure®	1.357 (44%)	1.380 (46%)	1.333 (41%)
Gel lure	1.406 (49%)	1.425 (51%)	1.387 (47%)
Dry lure	1.373 (45%)	1.374 (45%)	1.373 (45%)
LSD 5%	0.044	0.062	
Expt. ID10 (24 hours)			
Three-component BioLure®	1.405 (49%) a	1.433 (52%)	1.377 (46%)
Gel lure	1.457 (55%) b	1.489 (60%)	1.425 (51%)
Dry lure	1.437 (51%) ab	1.450 (54%)	1.425 (51%)
LSD 5%	0.038	0.054	

The gel and dry lures were equally attractive to *C. capitata* as the standard three-component BioLure® when tested in laboratory cages. The differences in attractancy became apparent when lures where compared in outdoor cages. Using a no-choice experiment the gel lure and three-component BioLure® performed equally when assessed after four and 24 hours, while the dry lure underperformed. A choice experiment allowed all three lures plus a trap with no lure (acting as control) to be evaluated for preferential

attractancy in a single cage. All lures were more attractive than a trap containing no lure. Overall, three-component BioLure® was indicated to be the most attractive lure, followed by the gel lure, then the dry lure. These results suggest that three-component BioLure® probably remains the best option for use as a female lure for *C. capitata* in preference to the prototype gel and dry lures. This was tested further in a series of field trials using wild populations of flies in south-west WA.

No significant differences were observed between any of the lures when tested in laboratory cages with *B. Jarvisi* but gel lure captured more flies in outdoor cages than the three-component Biolure®. The relative attractiveness of the lures to *Bactrocera* spp. was tested further in field trials with wild *B. aquilonis* and *B. jarvisi* populations in north-west WA, as with *B. tryoni* and *B. cacuminata* populations in NSW.

Other lures - Two-component BioLure®, three-component BioLure®, orange ammonia lure and Unipak®,

TWO COMPONENT VS THREE COMPONENT BIOLURE®
A no-choice experiment in laboratory cages showed that after 2 hours the two- and three-component BioLure® lures captured significantly more flies than the control trap (P<0.001) (Table 22: ID11). However, attractancy of the two-component lure did not differ from the three-component lure. Overall, more females were captured than males (P=0.017), particularly in control traps (P<0.001), again suggesting that the trap itself may act as a visual cue for female *C. capitata*.

The same effect of lure type on fly captures was observed in no-choice experiment conducted in outdoor cages (Table 22: ID12). While both the two- and three-component BioLure® lures captured more flies than the control (P<0.001), there was no difference in fly capture between the lure types after two hours. There was no significant difference between the capture of males and females (P=0.761) however, there was a significant interactive effect observed where more males than females were captured in control traps in contrast to traps with lures (P=0.002). This result also contrasts to the laboratory cage where more females were captured in control traps than males.

Orange ammonia lure was compared with two- and three-component BioLure® in a choice experiment conducted in outdoor cages (Table 22: ID13). While all lures were more effective than the control after 4 hours, the three-component BioLure® captured the greatest number of flies, followed by the orange ammonia lure, then two-component BioLure® (P<0.001). Overall, marginally more male flies were captured than females (P=0.006), particularly in orange ammonia lure and control treatments (P=0.001).

Based on the no-choice laboratory cage and outdoor cage experiments conducted with *C. capitata*, it appears that two- and three-component BioLure® are much the same in their ability to attract flies of both sexes. However, with the choice test in outdoor cages (Table 22: ID13), it becomes clear that there is a strong preference for three-component BioLure®.

Table 22. Effect of lure type, sex, and their interaction on the number of *Ceratitis capitata*. Values are means of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID11 in laboratory cages, 20 male and 20 female flies were the test subjects; and experiments ID12 and ID13 in outdoor cages 50 male and 50 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).

Lure type	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID11 (2 hours)			
Two-component BioLure®	1.269 (88%) b	1.265 (87%) b	1.272 (89%) b
Three-component BioLure®	1.288 (92%) b	1.289 (93%) b	1.288 (92%) b
No lure (control)	0.529 (12%) a	0.435 (9%) a	0.623 (16%) a
LSD 5%	0.044	0.034	
Expt. ID12 (2 hours)			
Two-component BioLure®	1.547 (68%) b	1.493 (60%) b	1.600 (78%) b
Three-component BioLure®	1.585 (75%) b	1.563 (71%) b	1.607 (79%) b
No lure (control)	0.245 (2%) a	0.310 (2%) a	0.180 (1%) a
LSD 5%	0.156	0.160	
Expt. ID13 (4 hours)			
Two-component BioLure®	0.595 (6%) b	0.624 (6%) b	0.566 (5%) b
Three-component BioLure®	1.366 (44%) d	1.324 (40%) d	1.408 (49%) d
Orange Ammonia	0.976 (17%) c	1.030 (19%) c	0.921 (15%) c
No lure (control)	0.294 (2%) a	0.384 (3%) a	0.203 (1%) a
LSD 5%	0.088	0.110	

UNIPAK® VS THREE-COMPONENT BIOLURE®

Comparison of Unipak® and three-component BioLure® in a no-choice experiment conducted in laboratory cages demonstrated that after 2 hours both lures outperformed the control, where no lure was used (P<0.001) (Table 23: ID14). However, the lures were similar in their attractancy. More females were captured than males (P=0.023) and as observed previously, more female flies were captured in the control trap compared to male flies (P<0.001).

In a no-choice experiment conducted in outdoor cages, there was no difference in attractancy observed between three-component BioLure® and Unipak® after 2 hours exposure (Table 23: ID15). Both lures performed significantly better than the control, which had no lure (P<0.001). Overall, more females were captured than males (P<0.010) and marginally more females were captured in control traps than males (P=0.051).

In no-choice laboratory cage and outdoor cage experiments Unipak® performed as well as three-component BioLure®, a finding similar to that reported by Holler *et al.* (2009) who found similar recapture rates in experiments conducted with sterile *C. capitata* in Florida. However, when the two lures were compared in a choice test in outdoor cages (Table 26 - see next section) three-component BioLure® was preferred by *C. capitata*. A similar product to Unipak®, Econex Trypak®, was found to be less effective than the standard three-component BioLure® (Navarro-Llopis *et al.* 2008).

Table 23. Effect of lure type, sex, and their interaction on the number of *Ceratitis capitata*. Values are the mean of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID14 in laboratory cages, 20 male and 20 female flies were the test subjects; and experiments ID15 in outdoor cages 50 male and 50 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).

Lure type	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID14 (2 hours)			
Unipak®	1.259 (86%) b	1.279 (90%) b	1.238 (82%) b
Three-component BioLure®	1.290 (93%) b	1.296 (94%) b	1.283 (91%) b
Control	0.643 (17%) a	0.529 (12%) a	0.757 (24%) a
LSD 5%	0.079	0.098	
Expt. ID15 (2 hours)			
Unipak®	1.434 (52%) b	1.429 (52%) b	1.440 (53%) b
Three-component BioLure®	1.556 (70%) b	1.506 (62%) b	1.586 (75%) b
Control	0.344 (2%) a	0.206 (1%) a	0.483 (4%) a
LSD 5%	0.166	0.195	

The results from the current project indicate that three-component BioLure® is the preferred lure for *C. capitata* and is likely to have the greatest impact when used in a field situation. Interestingly, while choice experiments clearly demonstrate preference for a particular lure but when no choice is offered, lures generally perform equally. However, how this translates to a field setting is not obvious. For example, if only one type of lure is used, it may perform as well as other lures despite it not being the preferred lure as demonstrated by choice outdoor cage experiments. Or conversely, the lures' relative attractancy under choice testing may be reflected in the open environment where it needs to compete with naturally-occurring sources of protein.

Analysis of the control treatments, which consisted of a Chempac/Suterra trap with no lure, indicated that often more female flies were caught in these traps compared with males. This trap type is obviously visually appealing to female *C. capitata* and should thereby enhance the effect of any lures they are baited with.

Lure age – prototype lures

A choice experiment conducted in outdoor cages with prototype lures demonstrated that after 4 hours exposure gel lures were more effective when they were fresh compared to when they were five or six months old (P<0.001) (Table 24: ID16). There was no significant difference between the capture of male and female flies (P=0.191), and there was no significant interactive effect (P=0.737).

A choice experiment conducted in outdoor cages to compare the effect dry lure age on attractiveness to *C. capitata* (Table 24: ID17) showed that after 4 hours exposure the dry lure was most attractive when fresh but demonstrated a significant loss in attractancy over time (P<0.001). There was no significant difference between the capture of male and female flies (P=0.844) and there was no significant interactive effect (P=0.244).

Table 24. Effect of lure age, fly sex, and their interaction on the number of *Ceratitis capitata*. Values are means of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID16 (gel lure) and in experiment ID17 (dry lure) in outdoor cages 50 male and 50 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).

Lure type and age	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID16 (4 hours) Gel lure			
Fresh	1.185 (29%) b	1.144 (26%)	1.226 (32%)
Five months	1.040 (20%) a	1.007 (18%)	1.074 (22%)
Six months	0.977 (17%) a	0.924 (15%)	1.030 (19%)
LSD 5%	0.071	0.087	
Expt. ID17 (4 hours) Dry lure			
Fresh	1.136 (25%) c	1.054 (21%)	1.218 (31%)
Five months	0.833 (12%) b	0.869 (13%)	0.797 (11%)
Six months	0.514 (5%) a	0.543 (5%)	0.486 (4%)
LSD 5%	0.188	0.237	

Lure age –Other lures - Two-component BioLure®, three-component BioLure® and Unipak®

TWO COMPONENT VS THREE COMPONENT BIOLURE®

In a no-choice laboratory cage experiment, after 2 hours exposure no difference in attractancy between two- and three-component BioLure® lures was observed when they were freshly opened (Table 25: ID18). However, two-component BioLure® aged for four months was somewhat less attractive than three-component BioLure® of the same age (P=0.069). No overall significant differences in attractancy were observed between the sexes (P=0.075) and there was no significant interactive effect (P=0.268). This suggests that the three-component BioLure® may be more practical for use in the field as attractancy may be sustained for a longer period, reducing the cost of lures and trap servicing.

A choice experiment conducted in outdoor cages (Table 25: ID19) with two-component BioLure® of different ages showed that the freshly opened lure was significantly more attractive after 4 hours exposure than lures that had been aged for 2 or more months (P<0.001). There was no significant difference in the number of male and female flies captured (P=0.134) and there was no significant interaction observed (P=0.327).

A choice experiment was conducted in outdoor cages with three-component BioLure® of different ages (Table 25: ID20). Freshly opened three-component BioLure® was significantly more attractive after 4 hours exposure than three-component BioLure® which had been aged for five months (P<0.001). After six months, the attractancy of the lure is again significantly reduced. There was no significant difference observed in the capture of male and female flies (P=0.221) and there was no interaction (P=0.071). An orchard trial testing aged lures showed that three component Biolure aged 2-3 months captured about 13% less flies than freshly opened lure (see lure ageing trial Table 36: ID40)

Table 25. Effect of lure type/age in two- and three component BioLure®, fly sex, and their interaction on the number of *Ceratitis capitata*. Values are means of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID18 in laboratory cages, 20 male and 20 female flies were the test subjects; and experiments ID19 and ID20 in outdoor cages 50 male and 50 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).

Lure type and age	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID18 (2 hours)			
Fresh two-component BioLure®	1.256 (85%)	1.229 (80%)	1.283 (91%)
Two-component BioLure® aged 4 months	1.219 (78%)	1.223 (79%)	1.216 (77%)
Fresh three-component BioLure®	1.247 (84%)	1.235 (81%)	1.259 (86%)
Three-component BioLure® aged 4 months	1.295 (94%)	1.289 (93%)	1.300 (95%)
LSD 5%	0.049	0.058	
Expt. ID19 (4 hours) Two component BioLure®			
Fresh	1.165 (27%)	1.123 (25%)	1.206 (30%)
Two months	0.906 (14%)	0.913 (14%)	0.899 (14%)
Four months	0.952 (16%)	0.925 (15%)	0.980 (17%)
LSD 5%	0.108	0.126	
Expt. ID20 (4 hours) Three component BioLure®			
Fresh	1.215 (31%) c	1.210 (30%)	1.219 (31%)
Five months	0.874 (13%) b	0.889 (13%)	0.859 (12%)
Six months	0.732 (9%) a	0.706 (8%)	0.757 (9%)
Seven months	0.734 (9%) a	0.839 (12%)	0.629 (7%)
LSD 5%	0.113	0.152	

UNIPAK® VS THREE-COMPONENT BIOLURE®

In a choice experiment conducted in outdoor cages (Table 26: ID21), Unipak® did not perform as well as three-component BioLure®, regardless of age (P<0.001). No difference in the capture of male and female flies was observed (P=0.265) and there was no interactive effect (P=0.156).

In these studies, attractiveness of all lures, with the exception of Unipak®, was significantly affected by age. For two-component BioLure®, effectiveness was reduced after two months, while three-component BioLure® was affected after five months. In no-choice laboratory experiments, three-component BioLure® seemed to be equally effective after four months, but a reduction in effectiveness was seen in the field trial. Although Unipak® wasn't affected by age, it was less attractive than three-component BioLure® when fresh. The prototype lures were also less effective after five months in the field.

Table 26. Effect of lure type/age in Unipak®**and three component** BioLure®, **fly sex, and their interaction on the number of *Ceratitis capitata*. Values are the means of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID21 in outdoor cages 50 male and 50 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).**

Lure type and age	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID21 (4 hours)			
Fresh three-component BioLure®	1.156 (27%) b	1.123 (25%)	1.190 (29%)
Fresh Unipak®	0.888 (13%) a	0.936 (15%)	0.840 (12%)
One month-old Unipak®	0.895 (14%) a	0.950 (16%)	0.840 (12%)
Six month-old Unipak®	0.938 (15%) a	0.940 (15%)	0.938 (15%) a
LSD 5%	0.102	0.136	

Killing agent comparison

Propylene glycol (PG) and white oil

In a no-choice laboratory experiment, three-component BioLure® used with both DDVP and PG retained similar numbers of flies in traps after 2 hours exposure and the addition of white oil to PG did not make any difference to the mortality rate (Table 27: ID22). Traps with lure recorded significantly higher numbers than the control trap (P<0.001). However, there was no difference between the killing agents where traps contained BioLure®. There was no difference between the sexes (P=0.239) and the interaction was not significant (P=0.222).

The same results were obtained when the treatments were tested in a no-choice experiment in outdoor cages (Table 27: ID23). The only treatment to be significantly different after 24 hours exposure was the control, which contained no lure (P<0.001). There was no significance between the capture of male and females (P=0.173) and no interactive effect (P=0.294).

A choice experiment in outdoor cages demonstrated that after 24 hours exposure similar numbers of flies were captured in traps containing 10% PG and DDVP (Table 27: ID24). However, the addition of white oil to 10% PG reduced the number of flies killed (P<0.001). Although the number of flies captured in the DDVP and 10% PG traps were similar, there was a difference in mortality in that flies exposed to DDVP died rapidly, while many of the flies in the 10% PG were swimming and managed to escape at times. There was no significance between the capture of male and females (P=0.876) and no interactive effect (P=0.376).

The effectiveness of different concentrations of PG was investigated in no-choice experiments conducted in outdoor cages (Table 27: ID25). Of the concentrations tested, there was no significant difference in fly capture (P=0.695). However, the water in the 10% PG and 20% PG solutions evaporated over time and many live flies were observed in traps containing these lower concentrations of PG, especially when traps were checked before 24 hours had elapsed. There was no significance between the capture of male and females (P=0.156) and more males were detected in 20% and 70% PG treatments whereas more females were recorded in the 10% PG treatment (P=0.047).

Table 27. Effect of killing agent, sex, and their interaction on the number of *Ceratitis capitata*. Values are means of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID22 in laboratory cages, 20 male and 20 female flies were the test subjects; and experiments ID23, ID24, ID25 and ID26 in outdoor cages 50 male and 50 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).

Killing agent type and concentration	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID22 (2 hours)			
Lure + 70% PG	1.311 (98%) b	1.315 (99%)	1.307 (97%)
Lure + 70% PG + white oil	1.281 (91%) b	1.266 (88%)	1.296 (94%)
Lure + DDVP	1.310 (97%) b	1.317 (99%)	1.304 (96%)
No lure + DDVP	1.136 (64%) a	1.100 (58%)	1.172 (70%)
LSD 5%	0.076	0.088	
Expt. ID23 (24 hours)			
Lure + 70% PG	1.625 (82%) b	1.625 (82%)	1.624 (82%)
Lure + 70% PG + white oil	1.610 (79%) b	1.605 (79%)	1.615 (80%)
Lure + DDVP	1.611 (80%) b	1.598 (77%)	1.625 (82%)
No lure + DDVP	0.260 (2%) a	0.151 (1%)	0.369 (3%)
LSD 5%	0.128	0.182	
Expt. ID24 (24 hours)			
Lure + DDVP	1.500 (61%) d	1.486 (59%)	1.537 (67%)
No lure + DDVP	0.272 (2%) a	0.380 (3%)	0.186 (1%)
Lure + 10% PG	1.397 (48%) d	1.422 (51%)	1.358 (44%)
No lure + 10% PG	0.589 (6%) b	0.564 (5%)	0.601 (6%)
Lure + 10% PG + white oil	1.126 (25%) c	1.041 (20%)	1.202 (30%)
No lure + 10% PG + white oil	0.536 (5%) b	0.504 (4%)	0.560 (5%)
LSD 5%	0.226	0.196	
Expt. ID25 (24 hours)			
Lure + 10% PG	1.637 (85%)	1.617 (81%) a	1.657 (89%) b
Lure + 20% PG	1.636 (85%)	1.656 (89%) a	1.616 (81%) ab
Lure + 70% PG	1.609 (79%)	1.648 (87%) a	1.570 (72%) a
LSD 5%	0.063	0.076	
Expt. ID26 (4 hours)			
Lure + PG 100%	1.102 (23%) b	1.045 (20%)	1.158 (27%)
Lure + PG 70%	1.261 (34%) c	1.281 (36%)	1.241 (33%)
Lure + PG 50% + white oil	0.427 (3%) a	0.386 (3%)	0.468 (4%)
Lure + PG 20% + white oil	0.371 (3%) a	0.310 (2%)	0.432 (3%)
LSD 5%	0.150	0.188	

A choice experiment in outdoor cages showed that that 70% PG was more effective than 100% PG (Table 27: ID26) after 4 hours of exposure. Lower concentrations of PG combined with white oil reduced the number of flies captured compared to both 100% PG

and 70% PG (P<0.001). Based on the results presented in Table 27: ID25, it could be assumed that this reduced capture rate is due to the addition of white oil, as PG concentration did not significantly affect retention of flies in the trap in that trial. There was no significance between the capture of males and females (P=0.088) and no interactive effect (P=0.452).

Published studies examining the use of PG as a killing agent and attractant for a number of fruit fly species have yielded positive results. PG is considered to be one of the safer killing agents and has been investigated as an alternative to ethylene glycol, which has been used in the past (Spencer 2005). Uchida *et al*. (2007) found that addition of PG to methyl eugenol baited traps (also containing DDVP) increased capture of *B. dorsalis*. Hall *et al*. (2005) looked at PG as an attractant for *Anastrepha suspensa* in combination with the *C. capitata* two-component BioLure® and the *A. suspensa* two- and three-component lures. PG was shown to improve capture and preservation of *A. suspensa* when compared to Vapona pest strips (Thomas *et al.* 2001). Since PG is a diol it acts as an emulsifier, breaking the surface tension of water, which inhibits the ability of flies to escape from the water once trapped. The alcohol properties of PG are also responsible for the improved preservation of trapped specimens. In this project, PG was examined for its suitability as an alternative killing agent to DDVP in traps containing three-component BioLure®. No-choice experiments conducted in laboratory and outdoor cages indicated that 70% PG was equal to DDVP in the retention of *C. capitata* in traps (Tables 27: ID22 and ID23).

While the concentration of PG did not appear to significantly affect the retention of *C. capitata* lower concentrations (10-20%) were observed to drown the flies more slowly, and in some cases allow flies to escape. These lower concentrations of PG also allowed the water to evaporate more quickly than higher concentrations.

When white oil was added to 10% PG in attempt to reduce the evaporation of water, fewer flies were captured (Table 27: ID24). Similarly, low PG solutions containing white oil were also less effective in choice experiments in outdoor cages (Table 27: ID26). Interestingly, white oil did not appear to affect trap captures when it was in combination with 70% PG. It is possible that the white oil is having a repellent effect on the fruit flies as horticultural mineral oil (an ultra-pure fraction of white oil) has been shown to repel *B. tryoni* in laboratory trials (Nguyen *et al*. 2007). However, it isn't clear how this effect would be overcome by the addition of 70% PG.

100% PG was less effective than 70% PG (Table 27: ID26), which suggests that the presence of water also attracts flies to the trap. Attraction of flies to liquid traps in higher numbers during hot weather was also an observation made in the preferred protein source trial conducted in Narara, NSW (Figures 18 and 19).

Phloxine B and spinosad

Investigative laboratory cage experiments indicated that spinosad had potential as a toxicant at 40ppm (Table 28: ID27) and continued to be effective down to concentrations as low as 1-3ppm (Table 28: ID28) (Chi squared tests; P<0.001). Most flies were killed within 24 hours; however, spinosad is a slow acting toxicant and a number of flies were found dead on the floor of cages rather than inside traps. Because of this action, spinosad is not suitable for consideration as a killing agent in traps intended for surveillance. As a matter of interest, however, examination of dead flies under UV light did not indicate ingestion of the fluorescein sodium salt (FSS) which suggests that flies were killed by contact with spinosad rather than ingestion.

Phloxine B is a photoactive dye that oxidises when exposed to light. If it has been ingested by an insect this oxidation will occur *in situ*, causing death. It has been combined experimentally with protein bait sprays for use against *C. capitata* in coffee plantations and while it was equally effective as spinosad, it was not as effective as malathion (Peck & McQuate 2000). Thomas (1997) found that concentrations of 0.1-5.0% caused 85% mortality within five hours. Lower concentrations of 0.05% caused 100% mortality in 48 hours. However, investigative laboratory cage experiments conducted in this current project indicated that phloxine B was not a suitable killing agent for use in surveillance traps (Table 28: ID27).

Table 28. Effect of killing agent on the number of *Ceratitis capitata*. In experiment ID27 in laboratory cages, 20 male and 20 female flies were the test subjects; and experiments ID28 also in laboratory cages 50 male and 50 female flies were the test subjects. Values are the result of a single replicate in each experiment and % of total flies is given in parentheses.

Expt. ID27				
Treatment	24hr		48hr	
	Male (%)	Female (%)	Male (%)	Female (%)
Flavex® only	4 (20%)	0 (0%)	11 (55%)	5 (25%)
Flavex® + 40ppm spinosad	19 (95%)	19 (95%)	20 (100%)	20 (100%)
Flavex® + 40ppm spinosad + 1% FSS	20 (100%)	18 (90%)	20 (100%)	20 (100%)
Flavex® + 1% Phloxine	5 (25%)	2 (10%)	5 (25%)	3 (15%)
Expt. ID28				
Treatment	24hr		48hr	
	Male (%)	Female (%)	Male (%)	Female (%)
Flavex® only	0 (0%)	0 (0%)	1 (2%)	1 (2%)
Flavex® + 1ppm spinosad	41 (82%)	50 (100%)	48 (96%)	50 (100%)
Flavex® + 2ppm spinosad	44 (88%)	47 (94%)	48 (96%)	50 (100%)
Flavex® + 3ppm spinosad	46 (92%)	45 (90%)	48 (96%)	49 (98%)

Diclorvos, Propylene glycol, spinosad and Talcum powder

In outdoor cage experiments, PG and DDVP captured similar numbers of flies ($P<0.001$), while talcum powder was a poor killing agent in comparison (Table 29: ID29). Spinosad used with Flavex® was not as effective as DDVP or PG used with three-component BioLure®, but did capture more flies than talcum powder used with three-component BioLure®. In the spinosad treatment although the lure type used may have had an effect on trap captures, there is also the possibility that flies may have escaped from traps before they were killed as suggested by observations in laboratory cage experiments, reducing the actual count data for that treatment. There was no significant difference between the capture of males and females ($P=0.191$). Significantly more female flies were captured in traps containing talcum powder, DDVP and 70% PG ($P<0.001$).

Table 29. Effect of killing agent, sex, and their interaction on the number of *Ceratitis capitata*. Values are the means of transformed counts (back-transformed counts as % total flies in parentheses). In experiment ID29 in outdoor cages 50 male and 50 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).

Killing agent type and concentration	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID29 (4 hours)			
DDVP	1.096 (23%) c	1.066 (21%) b	1.126 (25%) b
70% propylene glycol	1.238 (33%) c	1.252 (34%) c	1.224 (31%) b
40 ppm spinosad	0.809 (11%) b	0.883 (13%) b	0.735 (9%) a
Talcum powder	0.476 (4%) a	0.322 (2%) a	0.629 (7%) a
LSD 5%	0.153	0.184	

Essential oils

The efficacy of essential oils as killing agents, with three component Biolure® as attractant, was assessed over two experiments in laboratory cages. In the first experiment comparing cinnamon oil, eucalyptus oil and clove oil to DDVP (Table 30: ID30), flies were observed moving in and out of traps without any immediate adverse effect. At the end of the 24 hour period, 13-30 flies remained alive in cages containing traps with essential oils compared to 0-2 flies remaining alive in the cage with DDVP (P<0.001). Overall, more males were captured than females (P=0.003) and less females than males were captured with the trap containing cinnamon oil (P=0.001).

Similarly in the second experiment comparing anise, lavender and rosemary oils to DDVP (Table 30: ID31), flies were moving in and out of traps without any immediate adverse effect. After 24 hours, 14-28 flies remained alive in cages containing essential oils compared to 1-4 flies remaining alive in the cage with DDVP (P<0.001). Overall, more males were captured than females (P=0.026) and but there was no significant interaction between sex and type of killing agent (P=0.163).

Table 30. Effect of killing agent on the number of *Ceratitis capitata*. Values are the means of transformed counts (back-transformed counts as % total flies in parentheses). In experiments ID30 and ID31 in laboratory cages, 20 male and 20 female flies were the test subjects. Values followed by different letters within each column are significantly different from each other (P<0.05).

Killing agent type and concentration	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID30 (24 hours)			
DDVP	1.309 (97%) b	1.312 (98%) b	1.305 (96%) c
Cinnamon oil	0.503 (11%) a	0.715 (21%) a	0.291 (5%) a
Clove oil	0.638 (17%) a	0.751 (23%) a	0.524 (12%) ab
Eucalyptus oil	0.646 (17%) a	0.751 (23%) a	0.541 (13%) b
LSD 5%	0.219	0.243	
Expt. ID31 (24 hours)			
DDVP	1.290 (93%) c	1.301 (95%)	1.278 (90%)
Anise oil	0.788 (26%) b	0.823 (28%)	0.752 (23%)
Lavender oil	0.410 (8%) a	0.460 (10%)	0.360 (7%)
Rosemary oil	0.729 (22%) b	0.923 (37%)	0.534 (12%)
LSD 5%	0.262	0.3108	

Ethyl formate

Ethyl formate is used as a quarantine fumigant against a range of insects. While longer exposure times are generally required in comparison to methyl bromide, ethyl formate has significantly lower toxicity to humans. In this investigative trial, ethyl formate did not have an adverse effect on flies when used inside traps (Table 31: ID32). Although the three-component BioLure® attracted flies into the trap, with up to 30 flies in the lured trap at the end of the 18 hour period, they were largely unaffected.

Table 31. Effect of killing agent on the number of *Ceratitis capitata*. Values are mean counts of two replicates. In experiment ID32 in laboratory cages, 20 male and 20 female flies were the test subjects.

Expt. ID32 (1 and 18 hours)				
Treatment	1hr		18hr	
	male	female	male	female
No lure + ethyl formate	0	1	0	1
BioLure® + ethyl formate	0	0	0	0

Sticky card

With three-component BioLure® as an attractant, sticky cards proved to be an effective killing agent in this study (Table 32: ID33). However, as the card is a physical trap and doesn't act as a toxicant, the flies remain alive on the card for some time. As the flies struggle to escape the adhesive, they were often damaged. Matched with the handling difficulties associated with collection and identification of flies, the sticky card may not be appropriate for use in traps used for surveillance. However, it may be useful in situations where less toxic alternatives are sought, such as backyards and orchards.

Table 32. Number of *Ceratitis capitata* captured by sticky card placed in traps. In experiment ID33 in laboratory cages, 20 male and 20 female flies were the test subjects, % of total flies captured are given in parentheses.

Expt. ID33	
Treatment	**Mean captured (% of total)**
BioLure® + DDVP	34 (85%)
Three-component BioLure® + sticky card	33 (82.5%)
No lure + sticky card	6 (15%)

Lure comparisons in orchards in south-west WA

Prototype lures – 2009

Three-component BioLure® outperformed both the gel and dry lures when tested in an orange orchard in Donnybrook where *C. capitata* were present (Table 33: ID34; Figure 20a) (P<0.001). Similar trap captures were observed if three-component BioLure® was used with either DDVP or 70% PG as the killing agent. There was no significant difference in the capture of male and female flies (P=0.301) and no interaction between lure type and sex (P=0.146).

Although fewer numbers of *C. capitata* were captured in the orange orchard in Harvey, similar results were observed as in the Donnybrook orchard (Table 33: ID35; Figure 20b). Three-component BioLure® outperformed the gel and dry lures (P=0.018) and three-component BioLure® performed equally as well with either DDVP or 70% PG as the killing agent. However, there was no difference in the capture of males and females (P=0.660) and there was no interaction between lure type and gender (P=0.873).

Table 33. Effect of lure type, sex, and their interaction on the number of *Ceratitis capitata* captured. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05). Experiment ID34 was conducted in an orange orchard in Donnybrook between April 15 and May 14, 2009 and ID35 was conducted in an orange orchard in Harvey during the same period.

Lure type and killing agent	Mean male & female (% of flies)	Mean male (% of males)	Mean female (% of females)
Expt. ID34 (Donnybrook 4 weeks)			
Three-component Biolure®	1.402 (28.6) b	1.426 (25.7)	1.378 (22.9)
Gel lure	0.429 (3.4) a	0.412 (1.6)	0.447 (1.8)
Dry lure	0.318 (2.2) a	0.249 (0.8)	0.387 (1.4)
Three-component Biolure® + 70% PG	1.287 (36.8) b	1.289 (18.5)	1.285 (18.3)
LSD 5%	0.160	0.180	
Expt. ID35 (Harvey 4 weeks)			
Three-component Biolure®	0.422 (3.2) b	0.426 (1.7)	0.418 (1.6)
Gel lure	0.141 (0.8) a	0.108 (0.3)	0.173 (0.5)
Dry lure	0.145 (0.8) a	0.146 (0.4)	0.145 (0.4)
Three-component Biolure® + 70% PG	0.364 (2.6) b	0.362 (1.3)	0.367 (1.3)
LSD 5%	0.188	0.212	

Prototype lures – 2010

In 2010, the lures were tested in orange and pear orchards. In a pear orchard in Donnybrook, three-component BioLure® captured more flies than the either the gel or dry lures (Table 34: ID36) (P<0.001). This difference in efficacy was most apparent in late autumn as flies were dispersing in search of overwintering sites (Figure 20c). Three-component BioLure® captured similar numbers of flies to the male lure, Capilure®. In traps containing Capilure®, PG was a more efficient killing agent than DDVP.

Table 34. Effect of lure type, sex, and their interaction on the number of *Ceratitis capitata* captured. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05). Experiment ID36 was conducted in a pear orchard in Donnybrook between March 24 and June 9, 2010 and ID37 was conducted in an orange orchard in Donnybrook during the same period. Experiment ID38 was conducted in an orange orchard in Harvey between March 10 and May 19, 2010.

Lure type and killing agent	Mean male & female (number)	Mean male (number)	Mean female (number)
Expt. ID36 (Donnybrook 10 weeks)			
Three-component BioLure®	0.324 (2.2) bc	0.225 (0.7) b	0.423 (1.7) c
Gel Lure	0.136 (0.8) a	0.120 (0.3) ab	0.152 (0.4) b
Dry lure	0.066 (0.4) a	0.053 (0.1) a	0.080 (0.2) ab
Capilure®	0.274 (1.8) b	0.516 (2.3) c	0. 033 (0.1) a
Capilure® + 70% PG	0.401 (3.0) c	0.780 (5.0) d	0.023 (0.1) a
LSD 5%	0.078	0.011	
Expt. ID37 (Donnybrook 10 weeks)			
Three-component BioLure®	0.287 (1.8) b	0.154 (0.4) a	0.419 (1.6) c
Gel Lure	0.119 (0.6) a	0.120 (0.3) a	0.120 (0.3) b
Dry lure	0.095 (0.4) a	0.087 (0.2) a	0.103 (0.3) ab
Capilure®	0.147 (0.8) a	0.262 (0.8) b	0.031 (0.1) ab
Capilure® + 70% PG	0.307 (2.0) b	0.598 (3.0) c	0.015 (0.0) a
LSD 5%	0.068	0.096	
Expt. ID38 (Harvey 10 weeks)			
Three-component BioLure®	0.655 (7.0) d	0.584 (2.8) d	0.725 (4.3) c
Gel Lure	0.295 (2.0) bc	0.233 (0.7) b	0.359 (1.3) b
Dry lure	0.083 (0.4) a	0.084 (0.2) a	0.083 (0.2) a
Capilure®	0.240 (1.4) b	0.468 (1.9) c	0.012 (0.0) a
Capilure® + 70% PG	0.373 (2.8) c	0.746 (4.6) e	0.000 (0.0) a
LSD 5%	0.082	0.112	

Because larger numbers of males were attracted by Capilure®, less female flies were captured overall than males (P<0.001). A significant interaction was observed in that more males than females were captured in Capilure® and more female flies than male flies were captured with three-component BioLure® (P<0.001).

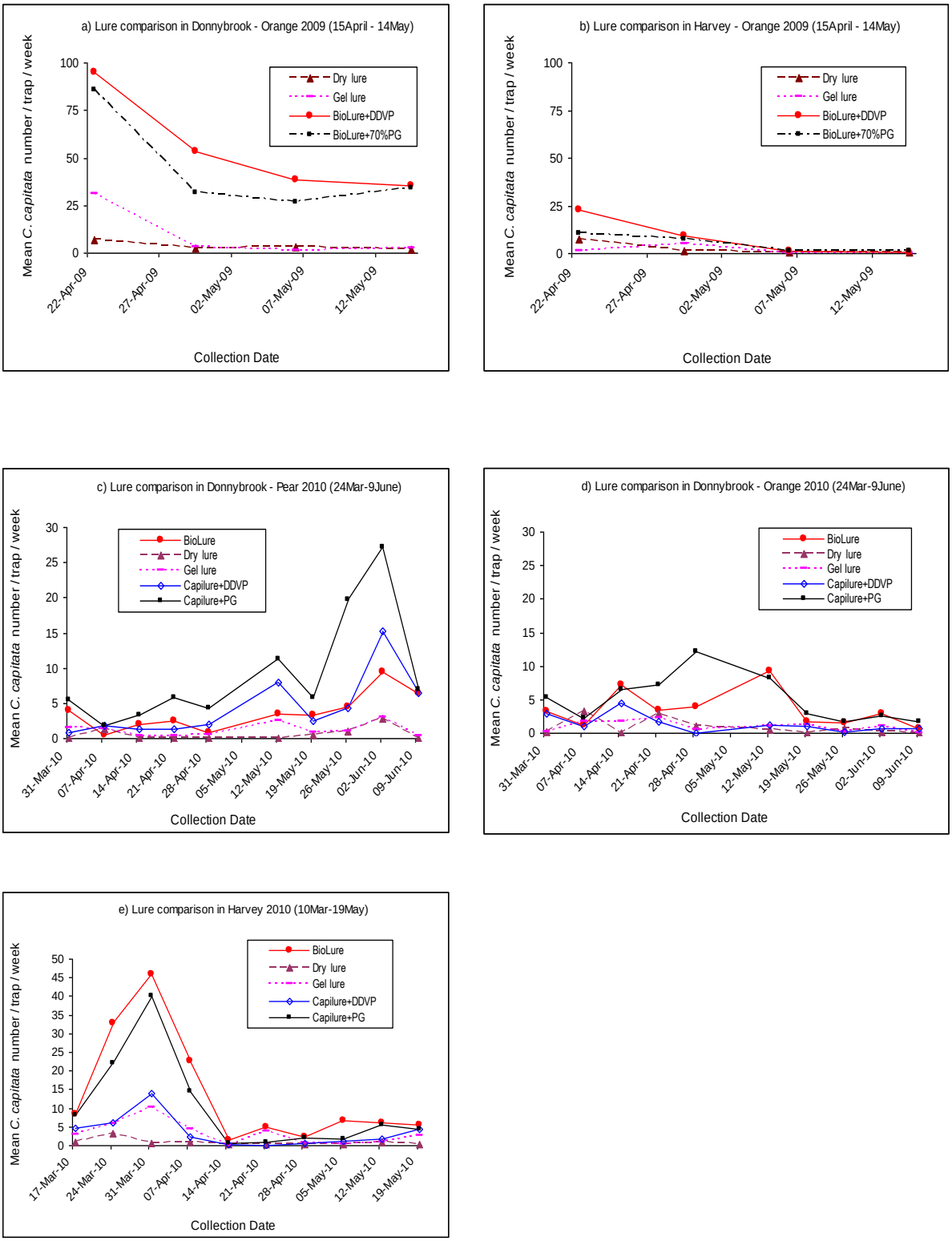

Figure 20. Comparison of prototype lures and killing agents in orchard trials conducted in south-west WA in 2009-2010.

An identical trial was conducted simultaneously in an orange orchard in Donnybrook (Table 34: ID37). Again, three-component BioLure® captured more flies than the either the gel or dry lures (P<0.001), with this difference being most apparent during May (Figure 20d). Significantly more flies were captured in Capilure® traps containing PG as a killing agent compared to DDVP. The inclusion of Capilure® as a treatment caused a sex bias in the capture data (P<0.001) and there was the same interactive effect with more female than male flies in three-component BioLure® and more male than female flies in Capilure® (P<0.001). There were notably fewer flies captured in this orchard in 2010 compared with 2009 (Table 35: ID34).

The same lure treatments were compared in an orange orchard in Harvey in 2010 (Table 34: ID38; Figure 20e). As observed in the trials at Donnybrook, three-component BioLure® performed better than the gel and dry lures, with the dry lure being the least effective treatment (P<0.001). In the Capilure® traps, more flies were in the traps containing 70% PG compared to traps containing DDVP. Overall, less females were captured than males (P<0.001), and more females were captured in three-component BioLure® and more males in Capilure® (P<0.001).

Two and three component lures – 2010

Experiments conducted in laboratory and outdoor cages had indicated that three-component BioLure® was superior to two-component BioLure® for attraction of *C. capitata*, however, a trial was conducted at Harvey to confirm that this was the case under field conditions. The three-component BioLure® captured nearly twice as many flies as the two-component BioLure® (Table 35: ID39, Figure 21) (P=0.009). There were no significant differences in the sex of flies captured (P=0.681) or the interactive effect of lure type and sex (P=0.964).

Table 35. Effect of lure type, sex, and their interaction on the number of *Ceratitis capitata* captured. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05). Experiment ID39 was conducted in an orange orchard in Harvey between March 31 and May 12, 2010.

Lure type	Mean male & female (number)	Mean male (number)	Mean female (number)
Expt. ID39 (6 weeks)			
Two-component BioLure®	0.236 (2.0) a	0.228 (1.0)	0.243 (1.1)
Three-component BioLure®	0.384 (4.0) b	0.378 (1.9)	0.390 (2.1)
LSD 5%	0.094	0.11	

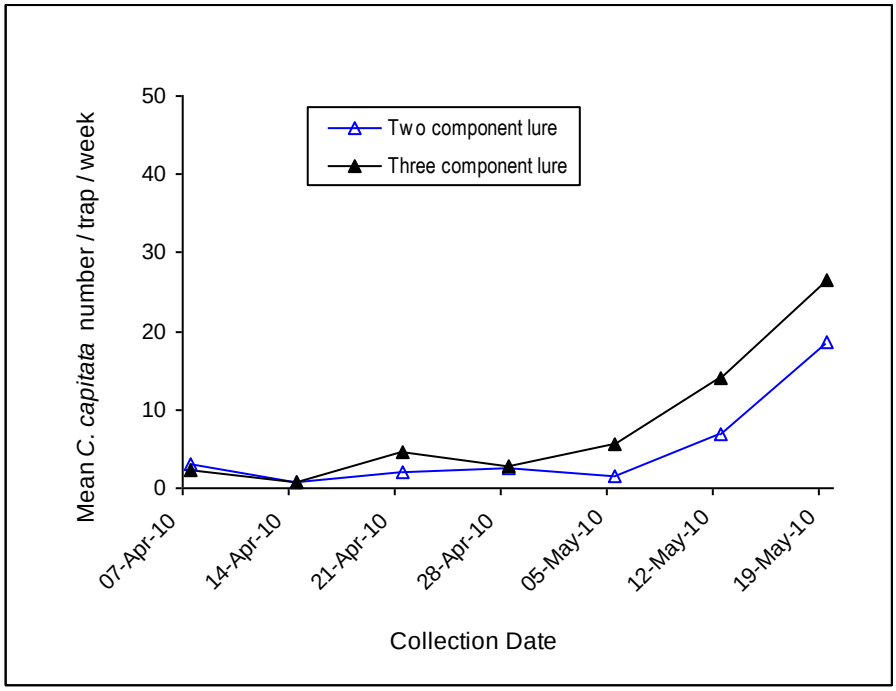

Figure 21. Comparison of two- and three-component BioLure® in an orange orchard in Harvey in 2010 (31st March and 12th May).

Trials conducted in orange and pear orchards in Donnybrook and Harvey clearly demonstrated that the gel and dry lures are not as attractive to *C. capitata* as three-component BioLure®. Whilst the dry lure had been shown to be a poor performer in cage trials with *C. capitata* (Table 20), the gel lure was also not able to attract as many flies as three-component BioLure® in field trials.

An interesting result was the consistently higher number of flies captured in Capilure® traps when 70% PG was used as a killing agent instead of DDVP. This suggests that the standard killing agent used with Capilure® should be reviewed.

Effect of lure age in the south-west WA - 2010

Investigation of lure efficiency with ageing in an orange orchard in Harvey (Table 36: ID40) indicated a significant lure type by sex interaction (P=0.003) and a significant lure type by lure age interaction (P<0.001) was found. Age effect is significant for Biolure and not for Dry or Gel lures. For Biolure every month of ageing decreases performance by about 13%.

Table 36. Effect of lure type/age, sex, and their interaction on the number of *Ceratitis capitata*. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05), †indicates a significant difference from Capilure. Experiment ID39 was conducted in an orange orchard in Harvey over a five week period between February 17 and March 24, 2010.

Lure type and age at beginning of the trial	Mean male & female (number)	Mean male (number)	Mean female (number)
Expt. ID40 (6 weeks)			
Fresh BioLure®	0.748 (9.2) d†	0.568 (2.7) cd	0.929 (7.5) d†
One month BioLure®	0.682 (7.6) d†	0.485 (2.1) c†	0.880 (6.6) d†
Two month BioLure®	0.499 (4.4) c†	0.335 (1.2) bc†	0.662 (3.6) c†
Three month BioLure®	0.375 (2.8) bc	0.264 (0.8) b†	0.486 (2.1) bc†
Gel lure (average of 4 ages)	0.266 (1.6) b	0.190 (0.5) b†	0.341 (1.2) b†
Dry lure (average of 4 ages)	0.098 (0.6) a†	0.054 (0.1) a†	0.142 (0.4) a
Fresh Capilure® (average of 3 reps)	0.326 (2.2) b	0.642 (3.4) d	0.009 (0.0) a
LSD 5%	Max[1] LSD: 0.144 Min LSD: 0.072	Within sexes: Max LSD: 0.203 Min LSD: 0.102	

[1] *Since means are averages over varying numbers of traps there are a large number of LSDs for treatment comparisons which have been summarised by presenting the minimum and maximum LSD's*

Lure comparisons in Kununurra

Prototype lures – 2009

Carambola

In the carambola orchard, gel and dry lures captured around one *B. aquilonis* per week, three-component BioLure® up to three flies per week, while Cuelure captured small numbers through to several hundred flies per week (Figure 22a). A total of 169 *B. jarvisi* were recorded in the carambola orchard, 118 of which were captured in traps containing Cuelure (Figure 22d). Three *Dacus newmani* flies and one *Bactrocera tenuifascia* were captured in traps containing Cuelure.

Analysis of data showed that the three-component BioLure®, gel lure and dry lure were significantly less effective at capturing *B. aquilonis* compared to the male lure, Cuelure (Table 37: ID41) (P<0.001). Although not significant in this analysis, three-component BioLure® generally attracted more *B. aquilonis* than the gel and dry lures. Using PG as a killing agent with Cuelure instead of malathion significantly increased the number of male flies that were captured. As Cuelure is a male attractant lure, significantly less females were captured than males (P<0.001). A significant interaction of effects was observed for the same reason (P<0.001).

More *B. jarvisi* were captured in traps containing gel lure than traps containing three-component BioLure® (Table 37: ID41) (P<0.001), confirming observations in outdoor cage trials (Table 21: ID10). The greatest number of *B. jarvisi* was captured in traps containing Cuelure with 70% PG as the killing agent. *B. jarvisi* is not strongly attracted to Cuelure, so the numbers seen here maybe only an indication of the potential population. Overall,

significantly more males were captured than females (P=0.024) and the effectiveness of Cuelure as a male attractant when combined with 70% PG caused a significant interaction of lure type and sex (P<0.001).

Table 37. Effect of lure type (and killing agent with male lure), sex, and their interaction on the numbers of *Bactrocera aquilonis* and *Bactrocera jarvisi*. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05). Experiment ID41 was conducted in a carambola orchard in Kununurra between September 30 and December 9, 2009.

Lure type and killing agent	Mean male & female (number)	Mean male (number)	Mean female (number)
Expt. ID41 (10 weeks) *B. aquilonis*			
Three-component BioLure®	0.061 (0.30) a	0.021 (0.05) a	0.101 (0.26) a
Gel lure	0.016 (0.08) a	0.008 (0.02) a	0.023 (0.05) a
Dry lure	0.020 (0.10) a	0.008 (0.02) a	0.032 (0.08) a
Cuelure + malathion	0.658 (7.0) b	1.220 (15.6) b	0.095 (0.2) a
Cuelure + propylene glycol	0.886 (13.4) c	1.713 (50.6) c	0.059 (0.1) a
LSD 5%	0.078	0.110	
Expt. ID41 (10 weeks) *B. jarvisi*			
Three-component BioLure®	0.019 (0.08) a	0.007 (0;.02) a	0.031 (0.07) a
Gel lure	0.083 (0.42) b	0.053 (0.13) a	0.114 (0.30) b
Dry lure	0.042 (0.20) ab	0.014 (0.03) a	0.070 (0.18) ab
Cuelure + malathion	0.043 (0.20) ab	0.077 (0.19) a	0.009 (0.02) a
Cuelure + propylene glycol	0.157 (0.88) c	0.290 (0.95) b	0.024 (0.06) a
LSD 5%	0.056	0.076	

Mango

In the mango orchard, no *B. aquilonis* were captured in traps containing gel or dry lures (Table 38: ID42, Figure 22b). Traps containing three-component BioLure® captured three *B. aquilonis* on one occasion. All female lures were markedly inefficient compared to the Cuelure, which captured up to 29 flies per week when malathion was used as the killing agent , and up to 95 flies per week when 70% PG was used as the killing agent (P<0.001). Like the trial conducted in carambola, a similar main effect of sex (P<0.001) and interaction of sex and lure type (P<0.001) were observed.

No *B. jarvisi* or other tephritid species were captured in traps in the mango orchard.

Table 38. Effect of lure type (and killing agent with male lure), sex, and their interaction on the number of *Bactrocera aquilonis*. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05). Experiment ID42 was conducted in a mango orchard in Kununurra between September 30 and December 9, 2009.

Lure type and killing agent	Mean male & female (number)	Mean male (number)	Mean female (number)
Expt. ID42 (10 weeks)			
Three-component BioLure®	0.008 (0.04) a	0.015 (0.04) a	0.000 (0.00) a
Gel lure	0.000 (0.00) a	0.000 (0.00) a	0.000 (0.00) a
Dry lure	0.000 (0.00) a	0.000 (0.00) a	0.000 (0.00) a
Cuelure + malathion	0.461 (3.78) b	0.914 (7.2) b	0.008 (0.02) a
Cuelure + propylene glycol	0.641 (6.74) c	1.274 (17.8) c	0.008 (0.02) a
LSD 5%	0.060	0.084	

Sandalwood

In sandalwood, the gel lure captured up to four *B. aquilonis* per week and three-component BioLure® up to three flies per week, while Cuelure® captured up to 2000 flies per week in the same period (Figure 22c). An average of one *B. jarvisi* per week was recorded in Cuelure® traps and a single *Dacus newmani* was recorded in a Cuelure® trap.

The vast majority of *B. aquilonis* were captured in Cuelure® traps (Table 39: ID43) (P<0.001). Of the female attractant lures, the three-component BioLure®, gel lure and dry lure captured similar numbers of flies. There was a significant effect of sex (P<0.001) and interactive effect of sex and lure type (P<0.001). Although more flies on average were captured in Cuelure® traps baited with 70% PG compared to malathion, the difference was not significant.

Table 39. Effect of lure type (and killing agent with male lure), sex, and their interaction on the number of *Bactrocera aquilonis*. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05). Experiment ID43 was conducted in a sandalwood orchard in Kununurra between September 30 and December 9, 2009.

Lure type and killing agent	Mean male & female (number)	Mean male (number)	Mean female (number)
Expt. ID43 (10 weeks)			
Three-component BioLure®	0.076 (0.38) a	0.080 (0.20) a	0.072 (0.18) a
Gel lure	0.063 (0.32) a	0.042 (0.10) a	0.085 (0.21) a
Dry lure	0.023 (0.10) a	0.030 (0.07) a	0.015 (0.04) a
Cuelure® + malathion	1.218 (31.0) b	2.436 (272.0) b	0.000 (0.0) a
Cuelure® + propylene glycol	1.280 (36.2) b	2.560 (361.7) b	0.000 (0.0) a
LSD 5%	0.104	0.146	

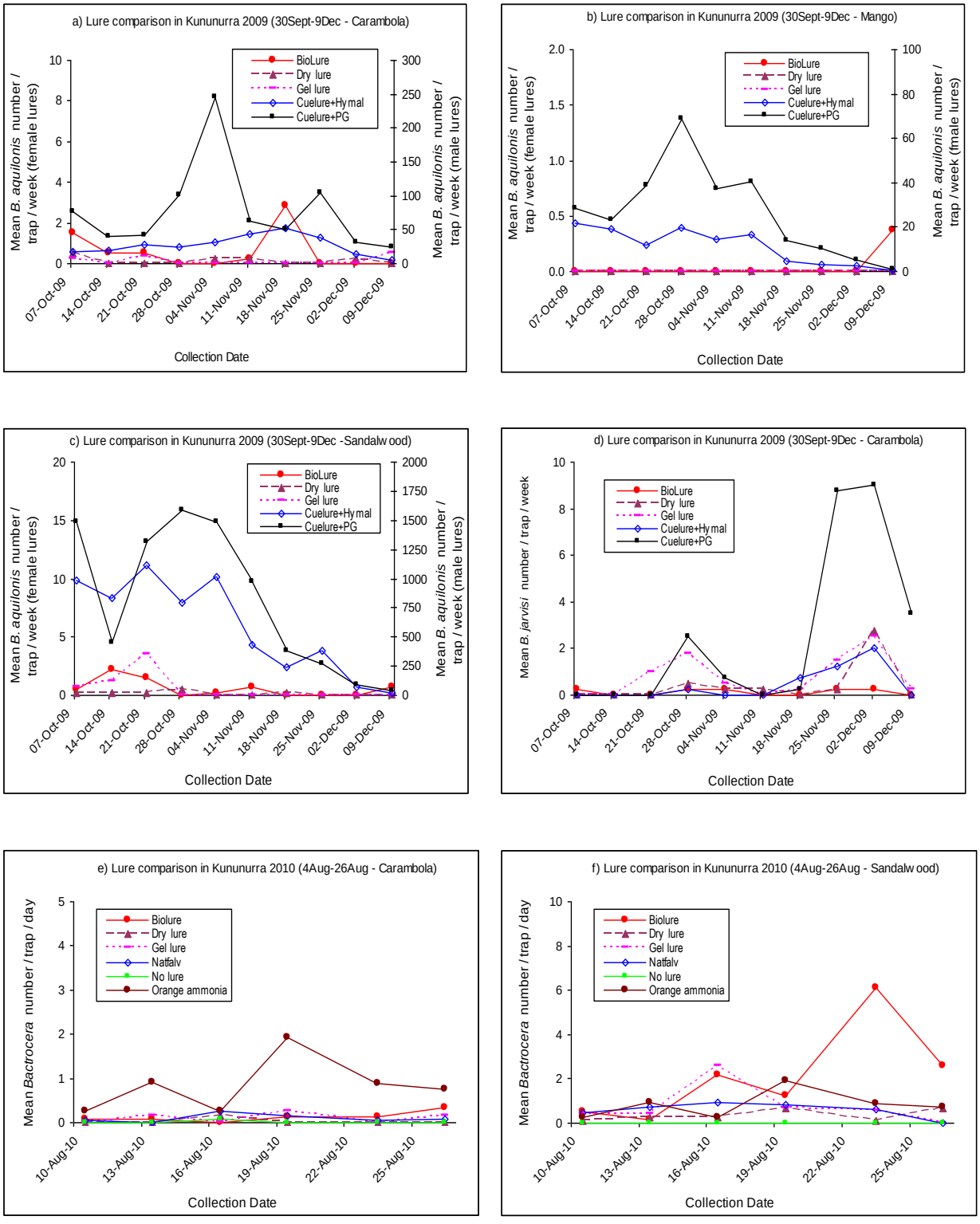

Figure 22. Mean trap captures of *Bactrocera* spp. in carambola, mango and sandalwood orchards in field trials conducted in Kununurra in 2009 and 2010. *B. aquilonis* numbers in Cuelure treatments in the 2009 trial are plotted against the secondary y-axis.

Prototype lures – 2010

Carambola

In 2009, Cuelure® was included as a treatment in the field trials, but because of its high efficacy, it tended to skew the data and due to the small numbers captured in the female lures the differences between female lures were difficult to ascertain. To overcome this, the male lure was not included in field trials in 2010. Instead, five types of female-targeted attractants were tested along with a control treatment containing no lure. Traps containing the male lure, Cuelure® with either 70% PG or malathion as killing agents, were deployed on either side of the block containing the female lure trial.

Overall, the majority of tephritids caught in the carambola orchard in 2010 were *B. aquilonis.* Other detections included 5 *B. jarvisi*, 19 *B. tenuifascia*, 1 *D. newmani* and 2 *B. cucumis*. All *Bactrocera* (and *Dacus)* species captured were combined for statistical analyses of lure attractancy (Figure 22e).

As with the previous season the fly population was low in this orchard compared to that of the sandalwood plantation. Even in the absence of male lure within the treatment block all female lures captured very small numbers (Figures 22e and 23). Numbers captured by male lures adjacent to this trial are given in Table 42 (ID46). Except for the orange ammonia lure, all other lures captured similar numbers of flies as the control treatment (Table 40: ID44) (P<0.001). The orange ammonia lure captured more flies than all other lures which had similar numbers to the control treatment (Table 40: ID44) (P<0.001). Significantly less males were captured than females (P=0.001). There was no significant interactive effect (P=0.074)

Table 40. Effect of lure type, sex, and their interaction on the number of *Bactrocera aquilonis*. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05). Experiment ID44 was conducted in a carambola orchard in Kununurra between August 4 and August 26, 2010.

Lure type and killing agent	Mean male & female (number)	Mean male (number)	Mean female (number)
Expt. ID44 (3 weeks)			
Three-component Biolure®	0.025 (0.12) a	0.009 (0.02)	0.041 (0.10)
Gel lure	0.017 (0.08) a	0.010 (0.02)	0.024 (0.06)
Dry lure	0.005 (0.02) a	0.000 (0.00)	0.010 (0.02)
Natflav	0.019 (0.08) a	0.000 (0.00)	0.037 (0.09)
Orange ammonia lure	0.115 (0.60) b	0.076 (0.19)	0.154 (0.42)
No lure (control)	0.003 (0.02) a	0.005 (0.01)	0.000 (0.00)
LSD 5%	0.028	0.040	

Sandalwood

An identical trial was conducted in the sandalwood plantation in 2010 (Table 41: ID45). Single flies of *B. jarvisi*, *B. tenuifascia* and *D. newmani* were recorded and the *B. aquilonis* population was higher in the sandalwood plantation compared to the carambola orchard. Overall, the orange ammonia lure and three-component BioLure® captured similar numbers of flies and they performed better than the other lures tested (P<0.001). All lures, with the exception of the dry lure, performed better than the control treatment. Slightly lower numbers of females were captured than males (P=0.090). A significant interaction was observed with the three-component BioLure® and the gel lure capturing more male *Bactrocera* sp. than females, and the orange ammonia lure capturing markedly

more female flies than male flies (P<0.001). As in Carambola, the number of flies captured by female lures in this host was much lower than those captured in male traps placed adjacent to this trial (Table 43: ID47).

Table 41. Effect of lure type, sex, and their interaction on the number of *Bactrocera aquilonis*. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05). Experiment ID46 was conducted in a sandalwood plantation in Kununurra between August 4 and August 26, 2010.

Lure type and killing agent	Mean male & female (number)	Mean male (number)	Mean female (number)
Expt. ID45 (3 weeks)			
Three-component Biolure®	0.162 (0.90) c	0.311 (1.04) c	0.014 (0.03) a
Gel lure	0.088 (0.44) b	0.141 (0.38) b	0.034 (0.08) ab
Dry lure	0.046 (0.22) ab	0.068 (0.17) ab	0.025 (0.06) ab
Natflav	0.097 (0.50) b	0.065 (0.16) ab	0.130 (0.35) b
Orange ammonia lure	0.195 (1.14) c	0.100 (0.26) b	0.290 (0.95) c
No lure (control)	0.000 (0.00) a	0.000 (0.00) a	0.000 (0.00) a
LSD 5%	0.066	0.094	

Trials conducted in carambola and sandalwood orchards/plantations in Kununurra clearly demonstrated that the gel and dry lures are not as attractive as the orange ammonia lure or the three-component BioLure® to the native *Bactrocera* species in Western Australia. Moreover, attractancy of female lures was much weaker than the attractancy of male lures.

Killing agent comparisons in Kununurra – 2010

Carambola

Overall, significantly higher numbers of *B. aquilonis* were captured in traps containing PG compared to those containing malathion, (Table 42: ID46, Figure 23) (P<0.001), despite low overall numbers of flies being present in the orchard. However, the improvement seen through the addition of 70% PG appeared to vary at each collection time with such a low fly population (Figure 23).

Table 42. Effect of killing agent, sex, and their interaction on the number of *Bactrocera aquilonis*. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05). Experiment ID45 was conducted in a carambola orchard in Kununurra between August 4 and August 26, 2010 (adjacent to experiment ID44).

Expt. ID46 (3 weeks)	
Killing agent	Mean male fly (number)
Malathion	0.478 (2.0) a
Propylene glycol	0.680 (3.8) b
LSD 5%	0.094

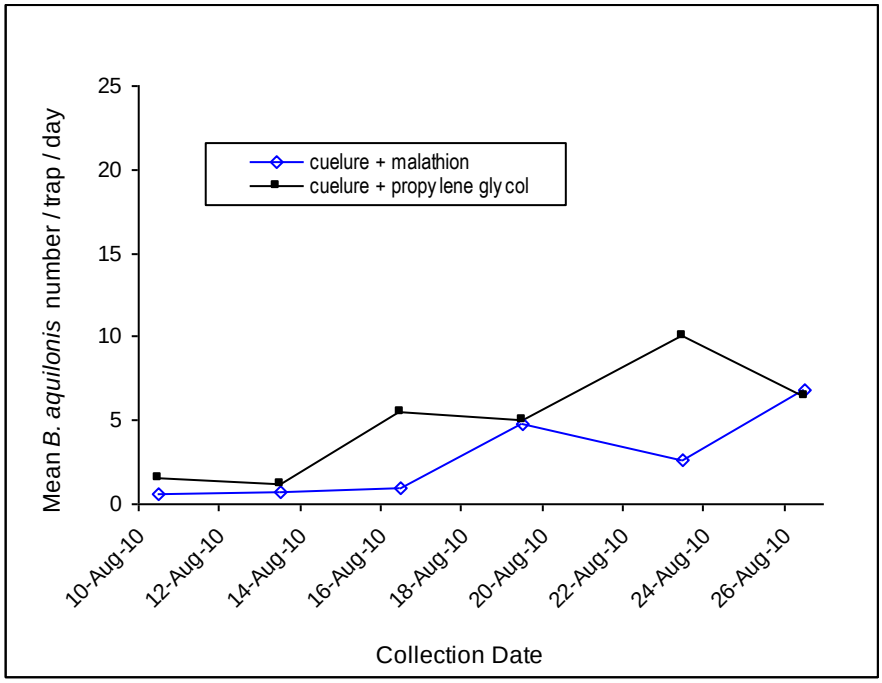

Figure 23. Mean captures of *Bactrocera aquilonis* in Chempac/Suterra traps containing Cuelure® and either 70% propylene glycol or malathion as killing agent. Traps were placed in a carambola orchard in Kununurra in 2010 (4Th and 26th of August).

Sandalwood

Compared to malathion, significantly higher numbers of *B. aquilonis* were captured in traps containing PG (Table 43: ID47, Figure 24) (P<0.001). Fly numbers were higher adjacent to the sandalwood orchard compared to the carambola orchard, so the effect of including 70% PG in the trap as a killing agent is significant under a range of population densities. At these higher densities, the improved effect of the addition of 70% PG is apparent at all collection times (Figure 24).

Table 43. Effect of killing agent, sex, and their interaction on the number of *Bactrocera aquilonis*. Values are the means of transformed counts (back-transformed values in parentheses). Values followed by different letters within each column are significantly different from each other (P<0.05). Experiment ID47 was conducted in a sandalwood orchard in Kununurra between August 4 and August 26, 2010 (adjacent to experiment ID46).

Expt. ID47 (3 weeks)	
Killing agent	Mean male fly (number)
Malathion	1.256 (17.0) a
Propylene glycol	1.820 (65.1) b
LSD 5%	0.158

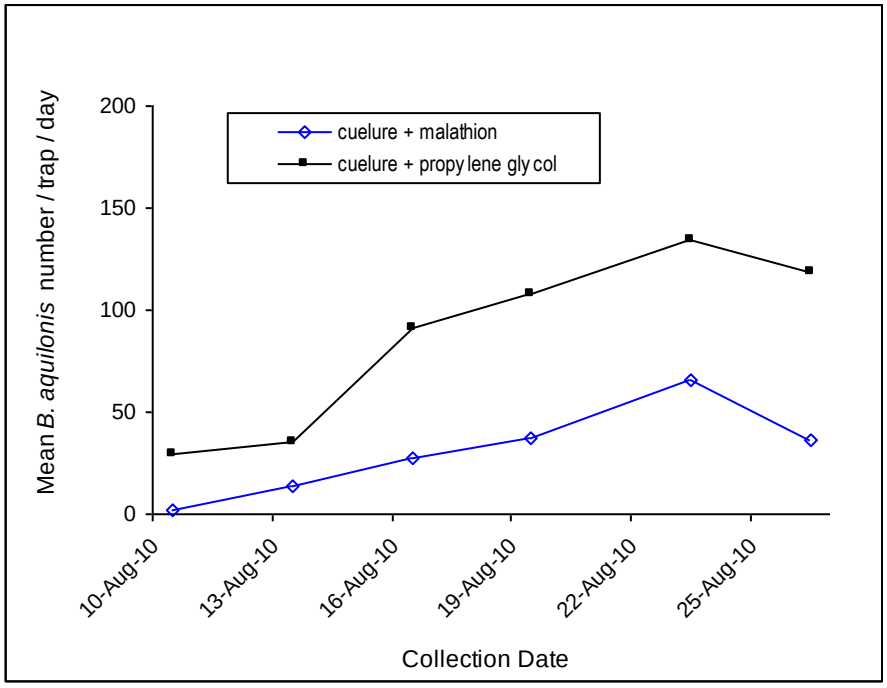

Figure 24. Mean captures of *Bactrocera aquilonis* in Chempac/Suterra traps containing Cuelure® and either 70% propylene glycol or malathion as killing agent. Traps were placed in a sandalwood plantation in Kununurra in 2010 (4Th and 26th of August).

Consistently higher numbers of flies were captured in Cuelure® traps when 70% PG was used as a killing agent instead of DDVP in both 2009 and 2010. A significant difference was observed even in the mango orchard in the 2009 trial where the population was relatively low (Table 38: ID42). This suggests that the standard killing agent used with Cuelure® should be reviewed in surveillance traps.

The major implication of these findings is that the use of 70% PG as a killing agent in combination with male lures can immediately improve the sensitivity of existing surveillance grids, particularly where low populations are likely to be detected (*e.g.* shipping ports, Torres Strait islands and other areas covered by the Northern Australia Quarantine Strategy).

3. (c) Acknowledgments

Mrs. Jane Speijers (DAFWA) provided invaluable guidance in the planning of orchard trials and in statistical analyses of the Western Australia component of the project.

Thanks are due to the following DAFWA staff who assisted with field work at various stages of the project: Ms Charlotte Butler, Ms Helen Collie, Ms Linda Fernihough, Ms Emma Mansfield, Ms Valentina Mella, Ms Sandra Wellington and Ms Candice Wong with work in the south west. Thanks are due to the DAFWA staff in Kununurra including Penny Goldsmith, and specifically Ms Natalie Bort who assisted in the set up and experimental work in Kununurra with considerable diligence invaluable for the completion of this work.

In NSW, appreciation is shown to I&I NSW staff who assisted with field trials: Mr Matthew Pearse, Mr David Cruickshank, Mr Vincent van der Rijt and Ms Dorothy Evans. Mr Ross Hitchcock is also acknowledged for his generosity in allowing us to use his orchard for field trials. Ms Jane Royer of DEEDI, Cairns and Mr Phillip Wilk of I&I NSW, Alstonville co-operated in the conduct of the lure longevity studies.

The NSW authors would like to thank Prof. Phillip Taylor of Macquarie University for valuable advice and Dr Gus Campbell of I&I NSW for the conduct of the release-recapture experiment conducted at Bathurst.

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4. Implications for stakeholders

The most critical implication of the data produced in this project is that the food-based attractants for female fruit flies, particularly *Bactrocera* spp., are not consistently effective lures. The laboratory data produced here indicates that generally less than 20% of protein-deprived *Bactrocera tryoni* are attracted to a protein lure. It is unclear why so few flies were attracted, given that they had not previously been fed protein and have a nutritional requirement for protein in order to become sexually mature. It was clear, however, that once flies had been fed protein, they were even less attracted to a protein lure, with typical captures of less than 3%. This means that the likelihood of capturing a female fruit fly in a trap with a protein-based lure is very low, particularly if the population is low, as would be expected with an exotic incursion. Under the experimental conditions used here, the proportion of *C. capitata* attracted to three-component BioLure®, which is based on food attractants, was considerably higher, up to 75%. In this instance it is clear that this lure could be used effectively for surveillance of areas free from *C. capitata*, such as eastern Australia, and as a tool to reduce female populations in affected areas.

The low attractancy of protein to *Bactrocera* spp. has other impacts on fruit fly management. Apart from being used for lures, protein is also incorporated with insecticide and used as fruit fly splash baits. Baits are applied to crop foliage or surrounding vegetation to attract the fruit flies to feed. The data produced here indicates that the flies are likely to find the baits by chance, particularly since baits are applied to sites where fruit flies are known to rest and forage.

The sensitivity of surveillance lures can be greatly enhanced through the addition of 70% propylene glycol (PG) as a killing agent. Data clearly showed that combining PG with male lures increased the number of flies captured. This small adaptation can immediately improve the sensitivity of existing surveillance grids, particularly where low populations are likely to be detected (*e.g.* shipping ports, Torres Strait islands and other areas covered by the Northern Australia Quarantine Strategy). However, because of the liquid nature of PG, this change would increase the level of maintenance that these traps would require.

The modification of McPhail traps to include a stainless steel mesh insert when using liquid lures would improve the serviceability of these traps and markedly improve the quality of fly specimens. However, this modification would require the use of DDVP as a killing agent.

5. Recommendations

Based on the data produced from this project, the following recommendations can be made:

- For *Bactrocera* spp. the liquid protein lure or orange ammonia lure continue to be used in either McPhail, modified McPhail or Chempac/Suterra traps.

- Where liquid protein lures continue to be used for surveillance, it is recommended that a stainless steel mesh insert and DDVP pest strip be used with the McPhail trap to improve the serviceability of traps and preserve the integrity of fruit fly specimens.

- Further research be conducted to improve the efficacy of the gel lure, or to develop a trap that disperses the lure volatiles over a greater distance.

- Three-component BioLure® is used in Chempac/Suterra traps for surveillance of *C. capitata*.

- Where male lures are used, Cuelure or Capilure®, propylene glycol is used as an alternative to DDVP pest strips. Benefits of this are reduced exposure to toxic chemical and an improved trap capture. Use propylene glycol with Biolure where there is a need to minimise toxic exposure to public and/or field workers as there was no advantage in fly capture with the alternative to DDVP.

6. Abbreviations/glossary

Insert list of abbreviations of acronyms (for example)

ABBREVIATION	**FULL TITLE**
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
DAFWA	Department of Agriculture and Food Western Australia
DDVP	Dichlorvos
FFEZ	Fruit fly exclusion zone
I&I NSW	Industry & Investment NSW
PG	Propylene glycol
SIT	Sterile insect technique
WO	White oil

7. Plain English website summary

CRC project no:	CRC30022
Project title:	Developing female lures for improved market access
Project leader:	Dr Katina Lindhout
Project team:	Dr Francis De Lima, Ms Shirani Poogoda, Mr Scott Dalton, Mr Andrew Jessup, Dr Olivia Reynolds, Ms Lorraine Spohr, Ms Anne Harris
Research outcomes:	Fruit flies are significant pests of horticultural crops worldwide. In Australia there are two fruit fly species of economic concern; the introduced species *Ceratitis capitata* (Mediterranean fruit fly; Medfly), which is present mainly in the south-west corner of Western Australia, and the endemic species *Bactrocera tryoni* (Queensland fruit fly; Qfly), which is found along the coastal fringe of the eastern states (Queensland, New South Wales and Victoria). Australia maintains a number of certified fruit fly free areas, including South Australia, Tasmania and the fruit Fly Exclusion Zone on the Victoria/New South Wales border. Maintaining these

	areas free from fruit fly and keeping exotic species of fruit fly out of Australia is critical to retaining access to our export markets. Surveillance using fruit fly traps is the principal tool used in defence against invading pests, but there are some fruit fly species that do not respond to the male lures typically used in surveillance programmes. Development of improved lures for detection of female fruit flies would improve our surveillance capability and was the primary aim of this project. Two prototype lures were developed: a gel lure and a dry lure. The efficacy of these lures was investigated in comparison to the standard liquid protein lure (for *Bactrocera* species) and the three-component BioLure® (for *C. capitata*). Results from the trials indicated that there are significant advantages to be gained by replacing the standard liquid protein lure with a gel lure for surveillance purposes. The gel lure developed and tested in this project was found to maintain its attractancy under a range of climatic conditions for a period of 6-12 weeks, compared to only one week for the liquid lure. It was easily dispensed in traps, did not have an unpleasant odour and captured much fewer unwanted insects, such as blowflies. The condition of fly specimens removed from traps containing the gel lure was markedly better than those removed from traps containing the liquid lure. However, the efficacy of the gel lure compared to the liquid lure was variable depending on the climate, fly species or crop. Sometimes trap captures were comparable (*e.g. D. pornia* captures at Kulnura, NSW) and sometimes significantly fewer flies were captured in traps containing the gel lures (*e.g. B. tryoni* captures at The Rock). Gel lure did not compare favourably with BioLure® in field trials in WA for *C. capitata* or native *Bactrocera* species other than *B. jarvisi*. Unfortunately, the prototype dry lure consistently performed poorly, probably as a result of rapid volatilisation of attractants. Experiments conducted in WA demonstrated that the three-component BioLure® is the most effective attractant for *C. capitata*, while orange ammonia lure performed best for *Bactrocera* spp. A significant finding was the effectiveness of 70% propylene glycol (PG) as a killing agent for use in traps with dry lures, such as three-component BioLure® and male lures, Capilure® and Cuelure. Greater numbers of flies were repeatedly found in traps containing 70% PG as the killing agent compared to traps containing malathion and/or dichlorvos (DDVP). The increased attractiveness of the lures when combined with 70% PG could be due to the presence of liquid, particularly in dry climates where flies need to seek out water. PG is a preferred killing agent, as it has relatively low toxicity compared to the commonly used organophosphate insecticides, and could be used in organic orchards.

	Further improvements to the ingredients in the gel lure, or perhaps the development of a more effective trap that disperses lure odour over a greater distance, could see liquid lures being replaced in future. In the meantime, the superior efficacy of the liquid protein lure or orange ammonia lure means that these probably still remain the preferred lures for use in surveillance systems for *Bactrocera* species, and three-component BioLure® the preferred lure for use in surveillance systems for *C. capitata*. Where liquid protein lures continue to be used for surveillance, it is recommended that a stainless steel mesh insert and DDVP pest strip be used with the McPhail trap to improve the serviceability of traps and preserve the integrity of fruit fly specimens. Where three-component BioLure® is used in Chempac/Suterra traps, propylene glycol may be a suitable alternative to DDVP pest strips, reducing the risk of accidental poisoning where traps are used in urban areas and facilitating the use of traps in organic orchards.
Research implications:	The most critical implication of the data produced in this project is that the food-based attractants for female fruit flies, particularly *Bactrocera* spp., are not consistently effective lures. The laboratory data produced here indicates that generally less than 20% of protein-deprived *Bactrocera tryoni* (Queensland fruit fly) are attracted to a protein lure. If flies had been fed protein prior to the trial, they were even less attracted to a protein lure, with typical captures of less than 3%. This means that the likelihood of capturing a female fruit fly in a trap with a protein-based lure is very low, particularly if the population is low, as would be expected with an exotic incursion. Under the experimental conditions used here, the proportion of *Ceratitis capitata* (Mediterranean fruit fly) attracted to three-component BioLure®, which is based on food attractants, was considerably higher, up to 75%. In this instance it is clear that this lure could be used effectively for surveillance of areas free from *C. capitata*, such as eastern Australia, and as a tool to reduce female populations in affected areas. The low attractancy of protein to *Bactrocera* spp. has other impacts on fruit fly management. Apart from being used for lures, protein is also incorporated with insecticide and used as fruit fly splash baits. Baits are applied to crop foliage or surrounding vegetation to attract the fruit flies to feed. The data produced here indicates that the flies are likely to find the baits by chance, particularly since baits are applied to sites where fruit flies are known to rest and forage. The sensitivity of surveillance lures can be greatly enhanced through the addition of 70% propylene glycol (PG) as a killing agent. Data clearly showed that combining PG with male lures increased the number of flies captured. This small

	adaptation can immediately improve the sensitivity of existing surveillance grids, particularly where low populations are likely to be detected (*e.g.* shipping ports, Torres Strait islands and other areas covered by the Northern Australia Quarantine Strategy). However, because of the liquid nature of PG, this change would increase the level of maintenance that these traps would require. The modification of McPhail traps to include a stainless steel mesh insert when using liquid lures would improve the serviceability of these traps and markedly improve the quality of fly specimens. However, this modification would require the use of dichlorvos (DDVP) as a toxicant.
Research publications:	
Acknowledgements:	Mrs. Jane Speijers (DAFWA) provided invaluable guidance in the planning of orchard trials and in statistical analyses of the Western Australia component of the project. Thanks are due to the following DAFWA staff who assisted with field work at various stages of the project: Ms Charlotte Butler, Ms Helen Collie, Ms Linda Fernihough, Ms Emma Mansfield, Ms Valentina Mella, Ms Sandra Wellington and Ms Candice Wong with work in the south west. Thanks are due to the DAFWA staff in Kununurra including Penny Goldsmith, and specifically Ms Natalie Bort who assisted in the set up and experimental work in Kununurra with considerable diligence invaluable for the completion of this work. In NSW, appreciation is shown to I&I NSW staff who assisted with field trials: Mr Matthew Pearse, Mr David Cruickshank, Mr Vincent van der Rijt and Ms Dorothy Evans. Mr Ross Hitchcock is also acknowledged for his generosity in allowing us to use his orchard for field trials.