Know-how for Horticulture™

Improved protein bait formulations for fruit fly control - Revised year 3 proposal

Dr Annice Lloyd
QLD Department of Primary Industries and Fisheries

Project Number: AH00012

AH00012

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Project Number AH 00012

Final Report (November 2003)

Improved Protein Baits for Fruit Fly Control

Annice Lloyd *et al.*
Department of Primary Industries
Agency for Food and Fibre Sciences, Queensland

NSW Agriculture

Department of Agriculture
Government of Western Australia

Horticulture Australia

Queensland Government
Department of Primary Industries

PROJECT DETAILS

Horticulture Australia Project Number: **AH 00012**

Project Title: **Improved protein baits for fruit fly control**

Report Date: **November 2003**

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Commercial Collaborators

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Sue Cross, Aventis CropSciences Pty Ltd
Dan Papacek, Integrated Pest Management (Bugs for Bugs) - Year 3

Project Objectives:

Years 1 & 2 - DPI, NSW Ag, Ag WA.

- To test two new bait products (GF-120 based on spinosad) and Bactrogel (based on fipronil) against both Queensland fruit fly and Mediterranean fruit fly on a range of host commodities in a variety of locations across three states to obtain the efficacy and residue data required for registration application for both products (DPI, NSW Ag, Ag WA).
- To undertake research to improve generic bait formulations by modifying bait components to increase efficacy and longevity (NSW Ag).

Year 3 - DPI only

Additional research on bait improvement

- To assistance the commercial partners to finalise registration applications.
- To test new bait station formulations in collaboration with USDA.
- To test the effects of new baits on beneficial insects.
- To undertake field evaluation of generic bait improvement by the addition of thickener.
- To evaluate phytotoxicity of new baits on a wider range of crops.
- To make recommendations for application methods for new fruit fly baits.

Year 3 - Project extension – DPI only

Preliminary research related to area-wide management of fruit fly in the Central Burnett district in Queensland.

- To monitor fruit fly populations in the Central Burnett to evaluate the effectiveness of the first stage of the introduction of Male Annihilation Technology (MAT) in some orchards.
- To determine the most effective MAT carrier for Central Burnett conditions.
- To determine fruit fly infestation levels in summer hosts in town areas so future area-wide management treatments can be targeted at breeding sites.

Project Funding:

- Dow AgroSciences Aust. Ltd (Years 1&2)
- Aventis CropSciences Pty Ltd.(Years 1&2)
- Bugs for Bugs (Year 3)
- Queensland Fruit and Vegetable Growers (Year 3)

Report Authors:

This Final Report was compiled by the DPI research team.

PARTS A, B, C were written and/or edited by **Annice Lloyd, Dan Smith, Ed Hamacek, Chris Neale and Rosemary Kopittke**, all DPI Qld.

PART D was prepared by **Andrew Jessup *et al*** NSW Agriculture.

Confidential Reports on trials in WA (**PART A** -but not included in this report) were prepared by **Francis de Lima** and **Sonya Broughton**, Agriculture WA.

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- Two new fruit fly bait products GF-120 (now called Naturalure Fruit Fly Bait Concentrate produced by Dow AgroSciences) and Bactrogel-P (now called Amulet Fruit Fly Gel produced by BASF) were evaluated against a current standard bait in a range of fruit fly host crops (citrus, pome fruit, custard apples, passionfruit, blueberries, mangoes and stone fruit) in Queensland, New South Wales and Western Australia.
- The new baits were shown to be attractive and toxic to both Qfly and Medfly and both provided effective field control. Results are now being used by the respective commercial partners to apply for registration of these products in Australia.
- In laboratory tests, the new baits which contain "soft" insecticides (spinosad in Naturalure and fipronil in Amulet Gel) were much less toxic to five species of beneficial insects than the currently used bait/insecticide formulations. This means the new products will be highly compatible with Integrated Pest Management programs. Naturalure recently obtained organic certification in Australia which will provide many benefits in commercial production and in eradication programs, particularly in urban areas.
- Generic bait performance was shown to be significantly improved by the addition of thickeners. An inexpensive, commercially available xanthan gum (Keltrol) at 0.5% increased the efficacy of generic baits by approximately 25% at 6 days after application. This will provide significant benefits for growers at times of high fruit fly pressure when weekly baiting may not provide acceptable control.
- Extensive testing of new baits and of thickened generic baits on foliage and /or fruit of fifteen different hosts showed that thickened baits (particularly GF-120) are more likely to cause phytotoxicity in sensitive crops such as mangoes, custard apples, and stone fruit.
- In glasshouse trials, abamectin was shown to have potential as an alternative toxicant for fruit fly baits. At very low concentrations (4.5ppm as currently registered for mite control in citrus) with thickener and standard yeast protein, abamectin performed as well as current standard baits without thickener. Abamectin at this level has minimal impact on beneficial insects in IPM programs. The addition of ammonium acetate and sucrose to generic baits improved bait attractancy under laboratory conditions and glasshouse tests showed Hy-Mal and yeast concentrations in generic bait formulations could be reduced without loss of bait efficacy. These bait modifications require testing under field conditions.
- The new baits and generic baits with and without thickener at different application rates were evaluated in large scale field trials carried out by DPI in stone fruit at Mundubbera.
- DPI researchers collaborated with growers to devise the most effective bait application equipment for thickened baits in both small and larger orchards. Details of equipment and recommendations for use are provided.
- In a Year 3 extension to the original project, DPI carried out preliminary studies on several issues related to the implementation of an area-wide fruit fly management program in the Central Burnett. This research evaluated the effects of preliminary Male Annihilation Technology (MAT) implementation, compared two types of MAT carriers and six different trap designs, and quantified fruit fly infestation in untreated backyard fruit trees in the towns of Gayndah and Mundubbera. The results will be important in planning AWM strategies.

2 MEDIA SUMMARY

PROJECT AH00012: Improved protein baits for fruit fly control

This project was initiated to meet a recognised national need for improvements in baiting technology for controlling fruit flies. More effective, longer lasting, lower toxicity baits were required for field control in endemic areas and for eradication and suppression programs in fruit fly exclusion areas in southern Australia. The project involved collaboration between fruit fly researchers from three state departments of agriculture - Department of Primary Industries Queensland, New South Wales Agriculture, and Agriculture Western Australia. USDA researchers with specialist expertise in fruit fly bait formulation were involved in a technical advisory capacity.

The project involved testing two newly developed bait products to obtain residue and efficacy data for registration application for these products in Australia. These new baits were GF-120 (marketed as Naturalure Fruit Fly Bait Concentrate by Dow AgroSciences) and Bactrogel-P (now Amulet Fruit Fly Gel marketed by BASF). These new baits contain thickeners designed to enhance the longevity of baits and incorporate new generation low toxicity insecticides (spinosad in Naturalure bait and fipronil in the Amulet Gel). These new insecticides are many times less toxic than those currently used in baits which means this new bait technology will provide environmental advantages and consumer health benefits.

The new baits were tested against a current standard bait in a range of fruit fly host crops (citrus, pome fruit, custard apples, passionfruit, blueberries, mangoes and stone fruit) against Queensland fruit fly in Queensland and New South Wales and against Mediterranean fruit fly in Western Australia. Both baits were shown to provide effective fruit fly control, and based on the project results both commercial collaborators are now applying for registration of these new products. Additional research by DPI showed that the new bait formulations are much less toxic to beneficial insects than current bait formulations which means the new baits will be highly compatible with Integrated Pest Management programs. The Naturalure bait recently obtained organic certification in Australia which will provide very significant benefits in both commercial production and in eradication and suppression programs.

Other research undertaken by DPI and NSW Ag investigated means of improving the currently used generic baits. Glasshouse trials conducted by DPI showed the addition of an inexpensive, commercially available thickener resulted in a 20-30 % improvement in bait performance six days after bait application to the foliage of host trees. This appears to be the most promising method of improving generic baits and was further shown to provide effective control in field trials in highly susceptible stone fruit in the Central Burnett. Laboratory cage tests carried out by NSW Agriculture showed that bait attractancy could be improved by the addition of ammonium acetate or sucrose and also confirmed that the longevity of baits could be improved by adding thickeners to increase moisture retention.

The phytotoxicity of new and older bait formulations was evaluated in a wide range of fruit fly host crops. Although thickened baits generally outperform standard baits, they are more likely to cause damage in sensitive crops (eg mangoes, custard apples, stone fruit) and care must be taken to avoid contact with fruit when applying bait in these crops.

In an extension to the project in Year 3, DPI carried out a range of preliminary activities related to the area-wide fruit fly management program which recently commenced in the Central Burnett citrus growing region. Data from this research such as district wide trap catches and fruit fly infestation levels in backyard trees in town areas will be used to plan treatment strategies.

3 INTRODUCTION

3.1 Background

Fruit flies are extremely damaging pests which cost Australian horticultural producers approximately $500 million per year (Colquhoun 1998). Fruit flies attack almost all fruit crops and some vegetable crops. Furthermore, they are regarded as serious quarantine pests impeding access for many commodities to markets both within Australia and overseas. In areas where fruit flies are endemic, intensive field control treatments must be used to ensure high quality produce and frequently additional postharvest treatments must be applied to meet phytosanitary requirements for many markets. In parts of Australia which are free from fruit fly, extensive monitoring, suppression and eradication programs must be undertaken to maintain area freedom status. In eastern Australia, the main pest species is the Queensland fruit fly (Qfly - *Bactrocera tryoni*) while in Western Australia, the pest species is the Mediterranean fruit fly (Medfly - *Ceratitis capitata*).

There is an increasing concern that chemicals such as dimethoate and fenthion, which have been widely used for both preharvest and postharvest fruit fly control in a variety of commodities in the past, may be subject to restrictions on their use in the future. This issue is critical for tropical and subtropical fruit industries where major production is in fruit fly endemic areas and where fruit fly field control relies heavily on chemical methods. The most commonly used alternative to chemical cover sprays for preharvest control is protein baiting. Fruit fly baits utilise a protein source (generally autolysed yeast) to attract flies, mixed with an insecticidal component (frequently malathion) to kill flies, and are applied as spot or strip treatments. Baiting is highly compatible with integrated pest management programs. It is also appropriate and very effective for eradication programs in urban areas. However there have been no significant improvements in bait formulations in Australia since baiting was first introduced more than twenty-five years ago and furthermore, there are some problems associated with the use of baits currently available in Australia.

The need to improve protein baiting for fruit fly control was one of the cross commodity priorities identified in the HRDC Report CT98024: Review and Management Strategy for Fruit Fly R&D. Colquhoun 1998. The currently used formulations may fail to provide an acceptable level of control under high fly pressure or in highly susceptible crops. These baits require frequent application, they are not rain fast, and they may cause phytotoxicity in some crops. A bait formulation which remained effective for longer periods would be more economical, would not require such frequent application and would thereby reduce the risk of phytotoxicity. Although baiting reduces overall pesticide usage compared to cover sprays, there is a need to find bait toxicants which have lower mammalian toxicity and are effective at very low concentrations. This is particularly important in the broader national arena of fruit fly incursion management where improved, longer lasting and less toxic bait formulations would provide many advantages. Such formulations would provide a more acceptable treatment option in both urban and environmentally sensitive areas. Longer lasting baits would require less frequent application and would result in cost savings for materials and labour in suppression and eradication programs.

3.2 Project initiation

In December 1999, a workshop was called by Horticulture Australia Ltd. (then HRDC) and AusHort to discuss the problems associated with fruit fly baiting and to determine the most cost effective way to improve bait technology for all Australian stakeholders. Approximately twenty representatives from almost all fruit fly research and regulatory groups in Australia and representatives from two chemical companies (Dow AgroSciences and Aventis CropScience) attended this meeting. Both of these companies had recently developed new fruit fly baits which required field evaluation under Australian conditions to provide data for

registration application for these products. The meeting was facilitated by Mr Peter Box and Dr Vic Edge was employed as a technical advisor.

The outcome of this meeting was to develop a national collaborative research project AH00012 "Improved protein baits for fruit fly control" to research fruit fly bait technology and to assist in making the latest in new bait products available in Australia. The project involved researchers from three state departments of agriculture viz, the Agency for Food and Fibre Sciences, Department of Primary Industries, Queensland (the lead organization); New South Wales Agriculture; and Agriculture Western Australia. The project aimed to utilise high levels of fruit fly expertise and extensive field control experience of researchers from across a range of fruit fly endemic areas. The proposed research addressed problems which have been identified by all groups and would complement recent and current projects involving fruit fly baiting. The project also involved international collaboration with Dr Bob Mangan and Dr Daniel Moreno, USDA-ARS, Weslaco Texas, who are world leaders in fruit fly bait technology and who developed the Dow AgroSciences bait product. Funding would be provided by HAL /AusHort and by the two commercial collaborators Dow AgroSciences and Aventis CropScience.

3.3 New bait products

The two new fruit fly bait products, independently developed by the commercial collaborators, required extensive testing under Australian conditions to obtain data to meet APVMA (Australian Pesticides and Veterinary Medicines Authority formerly the NRA - National Registration Authority) registration requirements. Both products were based on new safer insecticides. The Aventis product (Bactrogel) incorporated fipronil as the toxicant and the Dow AgroSciences product (GF- 120) incorporated spinosad as the toxicant. Both of these insecticides are already registered for other horticultural uses in Australia. Both spinosad and fipronil have high insecticidal activity for fruit flies enabling them to be used in baits at very low concentrations (eg 0.005 - 0.008%) compared to bait toxicants such as chlorpyrifos and malathion which are currently used at concentrations of approximately 0.2-0.5%. Furthermore, both spinosad and fipronil have very low mammalian toxicity compared to the insecticides currently registered for use in fruit fly baits in Australia.

The spinosad bait (GF-120) is a formulated product containing a protein source mixed with the insecticide and various other adjuvants. It has not previously been tested against Australian fruit fly species although it has been widely tested and is registered for use against other fruit fly species in the US. This product has recently been renamed "Naturalure Fruit Fly Bait Concentrate" for the Australian market but will be referred to as GF-120 in this report because this was the name used in all previous project documents.

At the beginning of this project, the Bactrogel product (fipronil mixed with a thickening agent) required mixing with a protein source (Mauri autolysed yeast) to form the complete bait. Mauri yeast has been is widely used with other registered insecticides in generic bait formulations in Australia for many years. Prior to the commencement of this project, Bactrogel bait had been used in a successful small scale fruit fly eradication program on Nauru Island (pers. comm. Richard Bull) but had not been fully evaluated under commercial conditions in Australia. During the course of the project, the Bactrogel product formulation was modified so that a dry powder protein component was incorporated into the product and the bait was renamed Bactrogel-P. In 2003, Aventis CropScience was taken over by Bayer. The rights to all fipronil based products were subsequently sold to BASF Australia. The Bactrogel fruit fly bait will now be marketed as Amulet Fruit Fly Gel but will be referred to in this report as Bactrogel because this was the name used in all previous project documents.

Registration of these new bait formulations was being sought for field control against Qfly and Medfly in a wide range of fruit fly host commodities as well as for use in suppression

and eradication programs. To ensure that project research outcomes would meet registration requirements, representatives from the research team and from the commercial partners met with the APVMA in February 2000 to discuss the specific trial work required to obtain the residue and efficacy data. The outcomes of this meeting enabled a coordinated cross state, cross commodity plan to be developed to minimise the research required to make these new technologies available as quickly as possible.

3.4 Project objectives (refer Project Details)

The primary objectives of the project in the first two years were as stated in Project Details. At the Year 2 project review meeting with all participants in May 2002, it was decided not to proceed with the original plan to undertake research on photodyes as toxicants for baits in the third year of the project. This decision was based on advice from Dr Bob Mangan that photodyes had failed to gain registration in the US and that they caused severe phytotoxicity problems in some crops. Furthermore, the commercial collaborators both advised no further trials were required for registration data and no further commercial funding was available for the third year of the project. A revised research plan proposed by DPI (refer Project Details) was approved for funding by HAL and AusHort for Year 3 (2002-2003). NSW Ag and Ag WA were not involved in Year 3 of the project

3.5 Project extension - Year 3 (refer Project Details) – DPI Qld

Preliminary research related to area-wide management for fruit fly in the Central Burnett district of Queensland.

In 2002, an extension to the project in Year 3 was approved by HAL for the DPI research team to undertake some additional activities related to a planned area wide management (AWM) program for fruit fly in the Central Burnett (**PART C** of this report). This research was funded by matched Voluntary Contributions provided by Queensland Fruit and Vegetable Growers Citrus Committee and Bugs for Bugs, Mundubbera, Queensland. A proposal for implementing the pilot AWM program was subsequently approved by HAL and this project (AH03002 lead by Dr Annice Lloyd, DPI) commenced in July 2003.

PART A - Evaluation of new commercial baits

4 Overview of confidential research to evaluate new baits for registration

4.1 Introduction

In Years 1&2 of the project, testing of the new bait products GF-120 and Bactrogel for efficacy and residue data involved cage bioassays and extensive field trials in a variety of crops. At each project milestone and at each of the project review meetings in Years 1 & 2, detailed reports of this research were provided in separate confidential reports to both commercial partners.

In Year 3, a confidential report on the effects of the new baits on beneficial insects important in IPM programs was provided to both commercial partners by DPI. In 2003, the Project Leader assisted both Dow AgroSciences and BASF (the new owner of the fipronil bait) by providing additional information relevant to their respective registration applications, which are now in progress.

A summary of the contents of the confidential reports provided to the respective commercial partners is shown in Table 1.

A non-confidential overview of the methodologies used in testing baits against Qfly is provided below. Similar methods, with some changes based on the known behaviour of Medfly, were employed in field trials carried out by project team researchers in Western Australia.

4.2 Experimental testing of new baits – laboratory bioassays

The effectiveness of GF-120 and Bactrogel against Queensland fruit fly (Qfly-*Bactrocera tryoni*) was evaluated in numerous laboratory cage experiments by the DPI research team at Indooroopilly and at the Maroochy Research Station. These experiments were designed to determine the attractancy of baits to Qfly; the effects of bait toxicants on Qfly (time to first symptoms and mortality); male, female and overall fly mortality; and longevity of baits on host foliage under both glasshouse and exposed weathering conditions.

All experiments were carried out using flies from the DPI Qfly colony at Indooroopilly. Protein deprived flies (male and female), 7-14 days old were used to maximise bait response. Standard bait as registered for use in Queensland (Mauris Pinnacle Protein Lure (hereafter referred to as Mauri yeast) 2L per 100L + Hy-Mal insecticide 435ml per 100L) was used for comparison in all tests. The new baits were tested at the mixing rates recommended by the respective commercial partners.

Tests undertaken at DPI Indooroopilly involved application of bait spots to leaves on a guava tree in the grounds of the research facility. Dosed leaves were removed from the tree at various times after application and bioassays of bait activity conducted in small laboratory cages with known numbers of male and female flies. Numbers of flies feeding on baited leaves were recorded during a three hour leaf exposure period. Mortality was recorded after 24, 48 and 72 hours. Bait longevity was followed for up to 21 days after application (in the absence of rain) but for shorter periods if rain occurred.

Tests undertaken at DPI Maroochy Research Station (carried out by Dan Smith) involved application of baits to the leaves of potted citrus trees in gauze cages in a glasshouse. Known numbers of flies were released into the cages at various times after bait application (up to 12 days). The effects of bait aging of fly mortality was determined.

4.3 Analysis of bioassay results

To maximise the value of the data, dose response curves were fitted instead of performing an ANOVA. This gave a mathematical relationship between bait age and mortality and hence enabled predictions of mortality to be made from the fitted curves as well as comparing the baits. Since mortality commenced at a plateau of 100% (usually) and then declined to zero, a variety of S-shaped curves (Probit, Logit, and Complementary log-log transformations applied to the mortality) were fitted to the data. The best model for the data was determined and then, using the curves for that model, the bait(s) were compared against Mauri yeast + Hy-Mal to see if the differences were statistically significant.

4.4 Field trials to evaluate efficacy and residues

Experimental design

The effectiveness of protein baiting in reducing fruit fly infestation in a crop cannot be reliably determined using small experimental plots. This is because of the high mobility of the pest and the attractant nature of the control treatment. Hence, the project team consulted with APVMA representatives at the beginning of this project to develop trial methodologies which would provide acceptable data for registration application. This plan involved all field trials being undertaken in commercial orchards using large treatment blocks (eg 0.5-1.0 hectare, depending on the crop). The necessity to undertake trials in commercial orchards meant that untreated control blocks could not be included. The risk of significant crop loss due to high levels of infestation, in the absence of any fruit fly controls, was unacceptable to participating growers. Furthermore, project funds were available for purchasing the required numbers of fruit for assessment but were not available for compensation to growers for crop damage. In view of this situation, all trials were aimed at determining if new bait products could provide a level of fruit fly control comparable to that obtained using the current commercial bait treatment for the crop in question.

Determination of efficacy

Bait efficacy was determined by the level of crop infestation in treatment blocks**.** Although male lure traps were used to monitor Qfly numbers in all blocks, trap catches were not specifically used as a measure of treatment efficacy. Extensive research by the DPI research team in other projects has shown that trap catches are not a reliable guide to crop infestation levels. Other factors such as the inherent susceptibility of a particular fruit and the availability of alternative hosts also determine infestation levels. However, trap catches for Medfly in Western Australia are used as a measure of risk for crop infestation.

Crop choice

In consultation with the APVMA and commercial partners, representative crops from each crop group were selected to trial the new baits. The trial crops and varieties were chosen on the basis of susceptibility to fruit fly attack, suitability for residue analysis and potential use for baiting in each crop type. The representative crops chosen were citrus (Qfly and Medfly), pome fruit (Qfly and Medfly), custard apples, passionfruit, and blueberries (all Qfly).

Phytotoxicity evaluation

Because mangoes are known to be particularly sensitive to phytotoxicity with currently used baits, preliminary evaluations of the new baits on this crop were undertaken in Queensland and in Western Australia prior to any large scale efficacy trials in commercial orchards. Both new baits and the standard bait caused damage when applied directly to fruit so no further trials were undertaken with mangoes. Symptoms of phytotoxicity in other crops were noted in the course of all efficacy trials.

Trial locations
All trials were conducted in production areas where fruit fly pressure was known to be consistently high enough to warrant the application of regular fruit fly treatments to achieve a commercially acceptable level of control**.** In all of the crops tested, baiting was the most commonly used fruit fly control method, occasionally supplemented by insecticide cover sprays if fruit fly pressure was severe.

The locations and crops for the Qfly trials were as follows:

Citrus 1 (Imperial mandarin) – Central Burnett district, Queensland- DPI
Citrus 2 (Murcott mandarin) – Central Burnett district, Queensland - DPI
Pome fruit (Gala apples) – Stanthorpe district, Queensland - DPI
Custard Apple (Pinks Mammoth) – Sunshine Coast hinterland, Queensland - DPI
Passionfruit (dark skin variety) – Sunshine Coast hinterland, Queensland - DPI
Blueberries 1&2 – northern NSW – NSW Agriculture
Mangoes – phytotoxicity test only – Mareeba Queensland- DPI

The locations and crops for the Medfly trials (Ag WA) were as follows:

Citrus 1 & 2 (Valencia orange) – south west Western Australia
Pome fruit (Packham pears) – south west Western Australia
Mangoes – phytotoxicity trial only
Stone fruit (peaches) – phytotoxicity trial only.

Trial methodology
Three properties in each production area were selected as sites for each crop trial. Fruit fly numbers were relatively uniform across each trial location and reflected normal seasonal pest pressure for the crops under test. Growers selected to participate in the trials were ones who followed good orchard management practices and hence good crop hygiene was practised in all of the trial situations.

In consultation with the cooperating growers, three comparable orchard blocks of the same variety were selected on each property. Bait treatments (standard bait of Mauri yeast + malathion, GF-120 and Bactrogel) were allocated randomly to the blocks. Bait treatments were applied by project staff or by growers themselves following specific instruction by the research team. Conducting trials in commercial orchards with grower involvement had the additional advantage of providing feedback to the research team about application methodology for the new bait products.

Sampling plans for efficacy determination
For each crop trial, it was planned to sample a minimum of 600 fruit per harvest (from each block) to enable detection of a 0.5% level of infested fruit (with 95% confidence). For most crops, the sample was taken from two harvests at normal commercial picking time. Where possible, larger numbers of fruit were sampled for assessment (eg citrus & pome fruit). With custard apples, the number of fruit sampled was limited by the nature of the crop.

A random sampling plan was individually prepared for each trial block nominating the tree and position on the tree to be sampled (only one position for each tree). Trees were drawn randomly from across the trial block, excluding buffer rows/trees at each edge of the block. Samples were drawn from ten positions on the trees to ensure all aspects/conditions were examined – N, S, E, W (inside and outside), Top Centre and Skirt. The samples were collected prior to the regular harvest to ensure the full range of fruit present for sampling. Non-biased sampling ensured that blemished and non-blemished fruit were picked for assessment. Sampling methods for custard apples, passionfruit and blueberries were modified to suit specific characteristics of the crop.

Fruit assessment
All sampled fruit were taken to the research facilities of the collaborating organization where they were held under standard controlled conditions (26°C and 60-70% Relative Humidity) to allow eggs and larvae to develop. After an appropriate holding time (7-12 days depending on the crop), fruit were individually examined for fruit fly infestation. The number of infested fruit and the numbers of larvae per fruit were recorded. For small blueberry fruit, pooled samples were held for pupal recovery.

Analysis of infestation results
To determine an upper limit of infestation (p_u) for each treatment sample the general formula for determining p_u was

$$\sum_{x=0}^{x=s} \frac{n!}{x!(n-x)!} p_u^x (1-p_u)^{(n-x)} = 1-C$$

where
n is the number of fruit sampled
s is the number of infested fruit found
C is the confidence level (used 95%)
p_u is the estimate of the upper infestation level

This equation is mathematically difficult to handle except in the simple case of no infested fruit (s=0)
To simplify the calculations for the case where there were infested fruit this was approximated by the following equation and solved for p_u

$$\sum_{x=0}^{x=s} \frac{e^{-np_u}(np_u)^x}{x!} = 1-C$$

(the approximation is good for large n and p close to zero – applicable to sample data in these trials). Where the results did not clearly demonstrate that the test bait was equal to or superior to the standard a comparison using an ANOVA (data p_u) was performed.

Sampling for residue analyses
Sampling of fruit for residue analyses was undertaken in one trial only for each crop type and all sampling was carried out in the Qfly trials conducted by DPI Queensland and NSW Ag. Procedures were according to the specific requirements of each commercial partner.
In each trial, a number of trees in each of the three treatment blocks at one orchard were designated for residue sampling. After the last bait application fruit samples were collected by project staff for residue testing. All samples were handled as required, frozen, and sent to external laboratories for analysis as instructed.

4.5 Effects of baits on beneficial insects

The effects of GF-120, Bactrogel and standard baits on beneficial insects were evaluated in cage experiments by Chris Neale of the DPI research team at Indooroopilly. These experiments were designed to determine the levels of mortality that resulted when beneficials were confined in a small space with the baits, or had the opportunity to forage freely on a citrus branch containing one dosed leaf. Secondarily the experiments aimed to determine whether the beneficials were attracted to the baits to feed.

Five species of beneficial insects were tested – the predators, *Cryptolaemus montrouzieri* and *Mallada signata* and the parasitoids, *Aphytis lingnanensis*, *Leptomastix dactylopii* and *Metaphycus luteolus*. Each beneficial was exposed to six bait treatments plus a control. GF-120 was tested at dilutions of 1:1.5 and 1:6.5. Both Bactrogel (2.5g Bactrogel, 490 ml water, 10ml Mauri yeast) and Bactrogel-P (6.7g Bactrogel-P, 500ml water) were tested with all beneficials except *C. montrouzieri*. Standard baits were tested as registered for use in Queensland - Hy-Mal + Mauri yeast (2.2ml Hy-Mal, 488ml water, 10ml yeast) and chlorpyrifos + Mauri yeast (2ml chlorpyrifos, 488ml water, 10 ml yeast). Treatments were applied to citrus leaves at a rate of 0.2ml per leaf in 8 to 12 discrete drops.

Two types of tests (exposure and attractant) were conducted. Exposure tests were designed to examine the effects on the beneficials when they were confined in a small space with the bait formulations. A known number of beneficials were confined in a plastic cup for 24 hours with a single dosed citrus leaf. Observations of the beneficials' behaviour were made at half hourly intervals for two hours, recording the number on each side of the leaf, the number feeding on bait droplets and the number dead. The leaf was removed after 24 hours and the beneficials supplied with a food source. (*A. lingnanensis* was supplied with honey for the entire duration of the test.) All species except *A. lingnanensis* were held for a total of 72 hours. *A. lingnanensis* were held for a total of 24 hours. At the conclusion of the test total mortality was recorded.

Attractant tests were designed to achieve two purposes. Firstly, to determine the level of mortality that resulted when beneficials were allowed to forage freely on a citrus branch containing one treated leaf. Secondly, to determine the level of attractiveness of the baits to the beneficials. Beneficials were confined in a perspex box (45cm x 30cm x 30cm) with an excised citrus branch which had a treatment applied to one leaf. In addition to the dosed leaf, three other leaves (lower, middle and upper parts of the branch) were also tagged. Observations of the beneficials were made at half hourly intervals for the first two hours, recording the number of individuals on each of the tagged leaves (including the dosed leaf), the total number foraging on the branch and the number dead. The branches were removed after 24 hours and the beneficials supplied with a food source. (*A. lingnanensis* was supplied with honey for the entire duration of the test.) All species except *A. lingnanensis* were held for a total of 72 hours. *A. lingnanensis* were held for 24 hours. At the conclusion of the test total mortality was recorded.

All tests were replicated at least 4 times and results analysed to compare effects of each bait formulation on each of the test insects.

4.6 Summary of confidential bait testing

The detailed results of the above experimental testing and field trials remain confidential at this stage. However, the following points provide a non-confidential summary of this component of the project.

- The new bait formulations proved to be attractive and toxic to both Qfly and Medfly.
- Both new baits provided effective field control in a range of crops and in a variety of locations with different fruit fly pressure.
- The spinosad and fipronil toxicants in the new baits are much less toxic to beneficial insects than the currently used bait insecticides.

4.7 Current situation with new bait products (Nov 2003)

- Based on the data generated by this project, both commercial collaborators have made applications to the APVMA for the new baits to be registered in Australia.

- The Dow AgroSciences bait GF-120 (now called Naturalure Fruit Fly Bait Concentrate), which has been in use overseas for some time has obtained organic certification in the US and also recently obtained organic certification by Biological Farmers of Australia.

4.8 USDA collaboration

Dr Bob Mangan and Dr Daniel Moreno USDA, ARS provided valuable input throughout the duration of this project. Dr Mangan visited Australia twice during the project to attend the project reviews in Years 1 and 2. He visited trial sites in Queensland, met with participating growers, held valuable discussions with the project team, and presented seminars for the wider scientific community. Throughout the project Dr Mangan and Dr Moreno willingly provide information on numerous fruit fly control issues in particular on bait formulations.

As part of this USDA collaboration, a number of experimental bait formulations developed by Dr Mangan's team were provided to DPI in the early stages of the project for preliminary evaluation against Qfly. Although some of these formulations were attractive to fruit fly species in the US, none was particularly effective against Qfly. A confidential report on this work was provided by DPI to the US collaborators. It had been hoped that more experimental baits would be available for testing in Year 3 but this did not eventuate due to unavoidable difficulties experienced by the US collaborators.

4.9 Confidential Reports to commercial partners

Table 1. Summary of CONFIDENTIAL REPORTS provided to commercial partners covering the testing of new bait products by DPI, NSW AG and AGWA.

DATE	REPORT TYPE	RESEARCH AGENCY	REPORT CONTENT
Dec 2000	Progress	DPI Qld	Qfly citrus 1 (Murcott) GF-120 trial
Jan 2001	Milestone 1&2	DPI Qld	Experimental cage testing of new baits with Qfly Mango phytotoxicity trial – Qld
		NSW Ag	Qfly blueberry trial 1
		Ag WA	Medfly citrus (Valencia orange) trial 1 Mango phytotoxicity trial – WA
Feb 2001	Year 1 Project Review	Project Team	Progress reports on all activities
July 2001	Milestone 3	DPI Qld	Qfly citrus 2 (Imperial) trial completed Qfly custard apple trial completed Experimental testing of bait longevity
		Ag WA	Medfly pome fruit (pear) trial completed
Jan 2002	Milestone 4	DPI Qld	Qfly pome fruit (apple) trial in progress Qfly passionfruit trial in progress
		NSW Ag	Qfly blueberry trial 2 in progress
		Ag WA	Medfly citrus (Valencia orange) trial 2 in progress
May 2002	Year 2 Project Review Sydney	Project Team	Progress reports on all activities
July 2002	Milestone 5	DPI Qld	Qfly bioassay overview Qfly field trial overview Qfly passionfruit trial completed
		Ag WA	Phytotoxicity trial in stone fruit WA completed
Oct 2002	Aventis CropSciences	NSW Ag	Final report on Qfly trials in blueberries
Jan 2003	Dow AgroSciences	NSW Ag	Final report on Qfly trials in blueberries

DATE	REPORT TYPE	RESEARCH AGENCY	REPORT CONTENT
May 2003	Dow AgroSciences BASF (new owners of fipronil bait)	DPI Qld	Final report on testing of new bait products against a range of beneficial insects important in IPM programs.
Jan- July 2003	Dow AgroSciences BASF	DPI Qld	Assistance with issues related to registration applications.

PART B – Generic bait research – DPI

5 Glasshouse and field testing of modifications to generic bait formulations

5.1 Introduction

This component of the project and the research undertaken by NSW Agriculture in Years 1&2 (**PART D** of this report by Andrew Jessup *et al*.), were aimed at evaluating modifications to generic bait products which might improve efficacy, increase longevity, enhance attractancy or reduce toxicity.

In the first year of the project 1999-2000, preliminary glasshouse testing of generic bait modifications against Queensland fruit fly at Maroochy Research Station showed up to 30% improvement in bait longevity after 4-6 days when Keltrol thickener (at 5g/L) was added to the standard bait (results not included in this report). Keltrol is a commercially available xanthan gum thickener, which costs approximately $36 per kg. When mixed with standard bait at this rate Keltrol costs only $3.60 extra per ha and with a possible 30% increase in bait efficacy at 4 days after application, its potential for commercial use needed to be investigated. The addition of thickener was potentially the most promising and cost effective method for improving generic baits but other bait modifications such as reducing the concentration of both the protein and insecticide components were also studied under glasshouse conditions.

Abamectin is currently registered at very low levels for mite control in citrus and is known to have minimal impact on beneficial insects used in citrus Integrated Pest Management programs. It would therefore be highly suitable as a toxicant to mix with generic protein baits but its effectiveness in fruit fly control had not previously been tested. Hence abamectin was included as a possible alternative insecticide in the glasshouse bait evaluation tests.

The efficacy of standard bait (with and without thickener) at reduced field application rates (7.5L per ha) similar to those recommended for the new bait products had not previously been evaluated. Phytotoxicity from bait application (from the insecticide, and/or protein or other additives) is a continuing problem. It is particularly severe in mangoes where the fruit is seriously marked from the protein component. However, phytotoxic effects can also occur on other crops manifesting as leaf speckling and burn, or as damage to fruit as in some citrus varieties. If lower application rates of standard bait (with or without thickener) could be shown to provide effective control, this might help to minimise the phytotoxicity problems which are currently being experienced in some crops. Extensive field trials in a stone fruit orchard at Mundubbera were carried out in Year 3 by DPI to evaluate the efficacy of these bait modifications.

5.2 Methods

5.2.1 Glasshouse trials

Trials were conducted in a glasshouse at Maroochy Research Station, Nambour, under ambient conditions (at temperatures varying from 20-30°C) during two periods from September 1999 to August 2002 and from August 2002 to September 2003. Protein baits containing the currently registered toxicants, chlorpyrifos (50% ec) and Hy-Mal (1150g/L maldison) and the potential bait toxicant, abamectin (Vertamec ec 18g/L) were compared

with and without Keltrol thickener (0.5%). The trials were conducted in aluminium framed fine mesh cages 1m x 1m x 0.6m. Two 0.6-1m high young potted citrus trees were placed in each cage, together with a source of water for the flies. Bait treatments were applied to 1, 2 or 10 leaves on one of the trees by dipping individual leaves into a mixture of the test bait in a small container and allowing excess bait to drain into the container. The volume of bait applied to both surfaces of an average leaf (15 cm^2 area) was approximately 0.5 ml. Fifty protein deprived flies (25 male + 25 female) *Bactrocera tryoni* aged 4-10 days old (average 8 days), fed sugar and water only from emergence, were introduced into each cage. Within each trial, treatments were replicated three times (occasionally 4 or 5 times).

Fly mortality was assessed after 48 hours exposure, and all flies (dead and alive) removed and then replaced with 50 fresh flies. Mortality was again assessed after a further 48 hours and the process repeated, usually another two times. Flies were thus exposed to the bait at 0, 2, 4 and 6 days after it was applied. A control treatment consisting of 2% Mauri yeast autolysate plus 0.5g Keltrol/L (no insecticide) was included in each trial. In some trials, additional check treatments were included (eg a chemical treatment without the addition of yeast autolysate) to determine the level of mortality due to accidental contact with the treated leaf.

5.2.2 Field evaluation of modified generic baits

Trial site

A low chill stone fruit orchard near Mundubbera, which had not been harvested from 2000-2002, was used to carry out a large field trial to evaluate generic bait modifications. The orchard consisted of approximately 120 ha of low chill peaches, nectarines and plums and was 1.5 km from the nearest citrus orchard. During 2001 male annihilation technology devices (MATs), consisting of a cotton wool wick impregnated with malathion (Maldison 500) and cue lure within a protective cup, were hung at 10 per ha in September but no bait or cover sprays for fruit fly were applied. The MATs appeared to suppress fly numbers early in the season, however, by early November, when a greater proportion of the crop was mature the whole orchard became very heavily infested (nearly 100% of the fruit). In the following season, September 2002, 30ha of the orchard, 20 ha of peaches and 10 ha of nectarines, (see Figure 1) was used for the fruit fly bait trial.

Trial layout

The trial site included both peach and nectarine blocks. The peach trial layout consisted of eight blocks. Six (1-6) were adjacent blocks of Florda Prince peaches, each block of approximately 2.5 ha comprising 25 rows, 250m long, 120 trees per row or a total of 3000 trees per block. Two additional peach blocks were included in the trial: 1 ha block of Florda Gem (Block 12) and a 2.5 ha block of Desert Red (Block 13). The Florda Prince blocks and the Florda Gem block were grouped together on the south eastern end of the orchard separated by 500m of vacant ground from the largest section of the orchard. The Desert Red block (which was baited by the orchardist) was well removed from the other trial blocks (Figure 1).

The nectarine trial layout consisted of five blocks. Four were adjacent blocks (7, 8, 9, 11) of Sun Wright nectarines of similar size and tree numbers to the Florda Prince peach blocks. A nearby fifth block of Suncoast nectarines (2ha, Block 10) was also included. The nectarine blocks were at the same eastern end of the orchard (as the peaches), partially separated from the rest of the orchard by vacant land or dead trees.

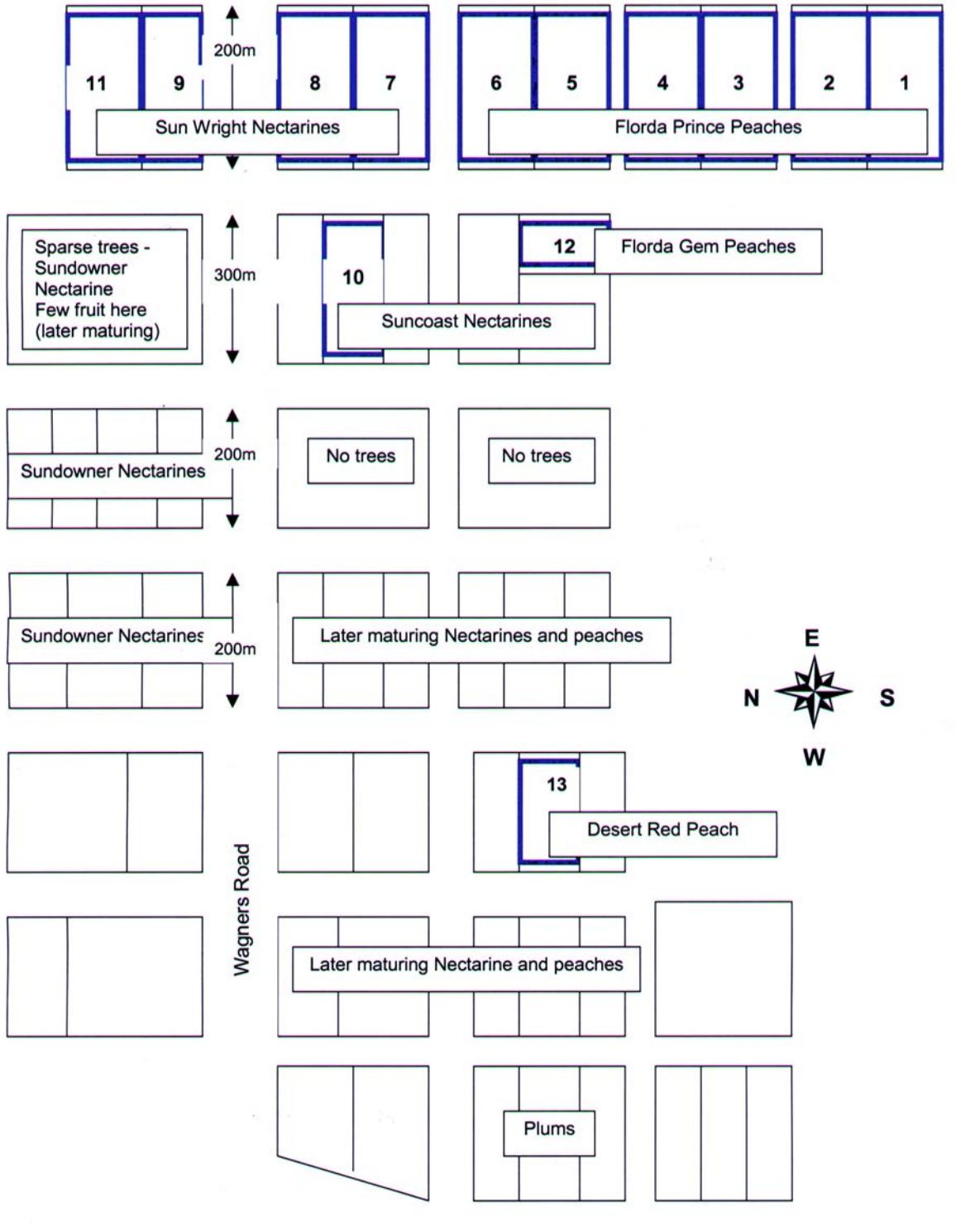

Figure 1. Plan of Treetops stonefruit orchard in Mundubbera used for field evaluation of modified bait formulations.

Fruit fly control treatments

Treatments and blocks to which they were applied are shown in Table 10 (Section 5.4). One complete block was used for each of the 13 treatments. Twelve of the trial blocks received bait treatments with one block (11) receiving MAT treatment only. Blocks 10 and

12 were also treated with MATs in addition to baiting (Block 10 with Bactrogel-P @ 5L/ha and Block 12 with GF-120 @7.5L/ha). The standard baits were all based on Mauri Yeast Autolysate @ 2L/100L with either Hy-Mal (435ml/100L) or chlorpyrifos (400ml of 50% ec/100L) as toxicants. The chlorpyrifos bait without thickener was applied to one block only at the recommended rate of 15L/ha. The Hy-Mal bait, with and without thickener, was applied to four separate blocks, two at the recommended rate (15L/ha) and two at half this rate (7.5L/ha) which is equivalent to the recommended rate for the new GF-120 bait.

The baits were applied using a quad bike carrying a 50L tank (Silvan Selecta Rakpak) with a 12 volt Shurflo fin cooled pump delivering 13.6L/min at 300kpa (45psi). The spray was delivered in a continuous band to the lower quarter of the trees by paired nozzles (adjustable in aperture and height). Bike speed was 15-20 km and the volume per ha was adjusted mainly by varying the number of rows sprayed eg for 15L/ha every pair of rows was sprayed; for 7.5L/ha every second pair of rows was sprayed. A similar unit was used by the orchardist to spray Block 13 with chlorpyrifos bait at 15L/ha. Baits were applied once per week from the beginning of September to the end of November. During the trial period there was very little rainfall so there was no need to reapply baits as per usual commercial practice. In Block 9 of the nectarines, the GF-120 bait was applied to the fork of the tree using a backpack sprayer (Sidewinder) delivering 5L per ha (20mls to every second tree in every second row).

The remainder of the orchard (90 ha not in the trial) was treated with MATs at 10 per ha and chlorpyrifos bait was applied at weekly intervals by the orchardist. There were, however, no MATs in the Block 13 (Desert Red peach) which was baited as per commercial practice and was included in the trial for comparison.

Fruit assessment and monitoring

Two cue lure male traps were hung in each trial block and emptied weekly. Weekly random picks of about 50 mature fruit were made from 25 September to 28 November (total of 10 picks). Fruit were transported to DPI Indooroopilly, Brisbane and held for 5-7 days at 25°C and 60-70% Relative Humidity prior to assessment for fruit fly larvae and pupae. Five additional on-site examinations of 50 randomly selected overripe fruit from each block were carried out during the trial period. This was done to ensure that additional infestation was not being missed by sampling mature (but not very soft, over ripe) fruit for transport to Brisbane for holding. Several random fruit samples were also collected from unsprayed home garden low chill peaches at a citrus orchard 1.5km to the east of the trial site, and from unsprayed peaches at two sites in the town areas of Mundubbera and Gayndah respectively.

5.3 Results

5.3.1 Glasshouse trials

September 1999 - August 2002 trials

This first group of trials included numerous other treatments not relevant to this study and specific ANOVAs are not shown. However summary data is presented to demonstrate the effects of adding thickener to baits (Figure 2) and to show the effectiveness of abamectin as a toxicant in fruit fly baits (Figure 3). An overall summary of combined results from all glasshouse trials (Sep 1999 - Sep 2003) is shown in Table 9.

The addition of Keltrol thickener (0.5%) generally improved mortality when added to standard 2% protein bait containing chlorpyrifos or abamectin as the insecticide (Figure 2 and Figure 3). For example, at 4 days, mortality with the abamectin bait formulations was increased by between 24.8% at 4.5 ppm (from 60.8 to 84.6%) and by 44.2% at 18 ppm (from 44.8 to 89%). Chlorpyrifos with thickener gave 97% mortality at 4 days and 65%

without thickener. However, adding Keltrol to the Hy-Mal bait did not appear to increase fly mortality at 2 or 4 days after application.

Abamectin proved to be effective as a toxicant in fruit fly baits particularly if thickener was included (Figure 3). Concentrations of abamectin are given in ppm because the levels of active ingredient in these formulations were so low (eg 4.5 ppm abamectin = 0.0004% abamectin). Mortality in the presence of thickener did not vary greatly with increasing concentration of abamectin eg after 4 days abamectin at 50 ppm averaged 96% mortality; at 25 ppm, 86%; at 18 ppm, 89%; at 9 ppm, 74% and at 4.5 ppm, 85%.

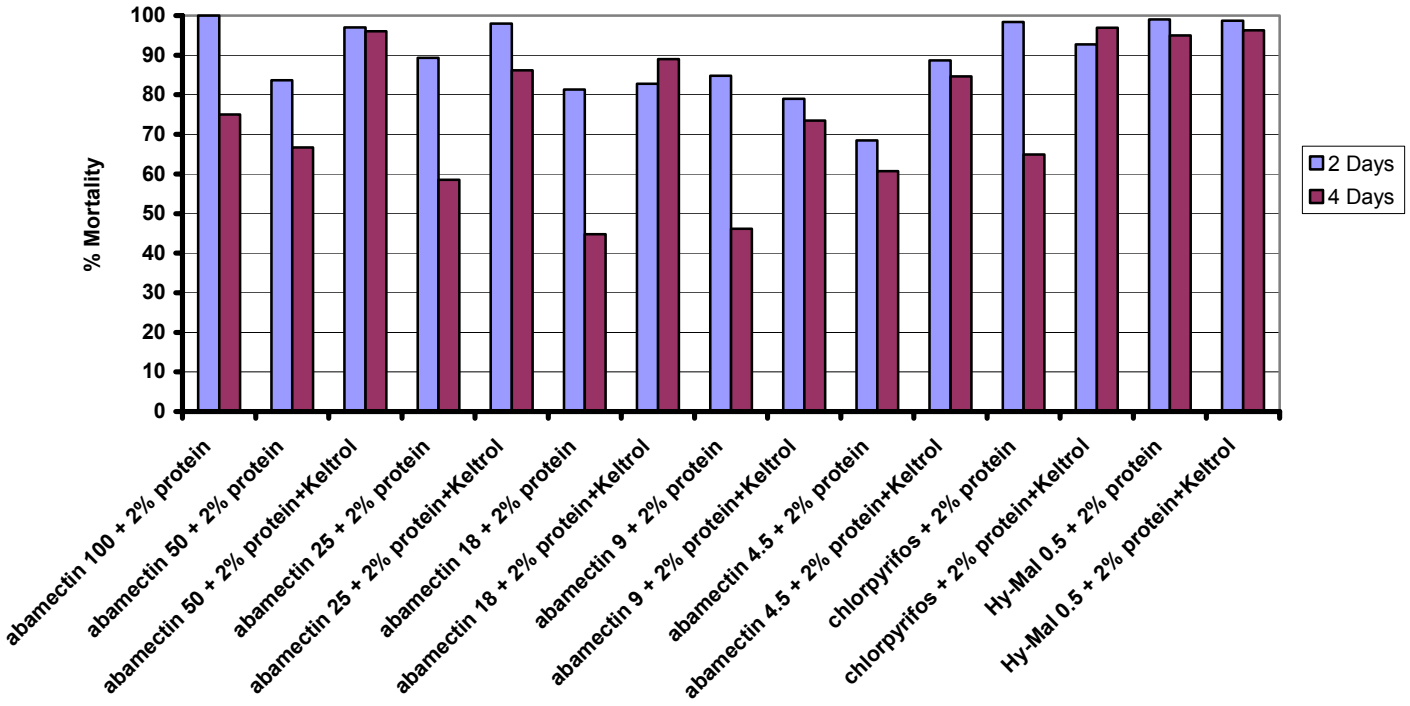

Figure 2. Sep 1999 - Aug 2002 trials. Mortality of *Bactrocera tryoni* exposed to insecticide-protein bait formulations with and without 0.5% Keltrol thickener at 2 and 4 days after bait application.

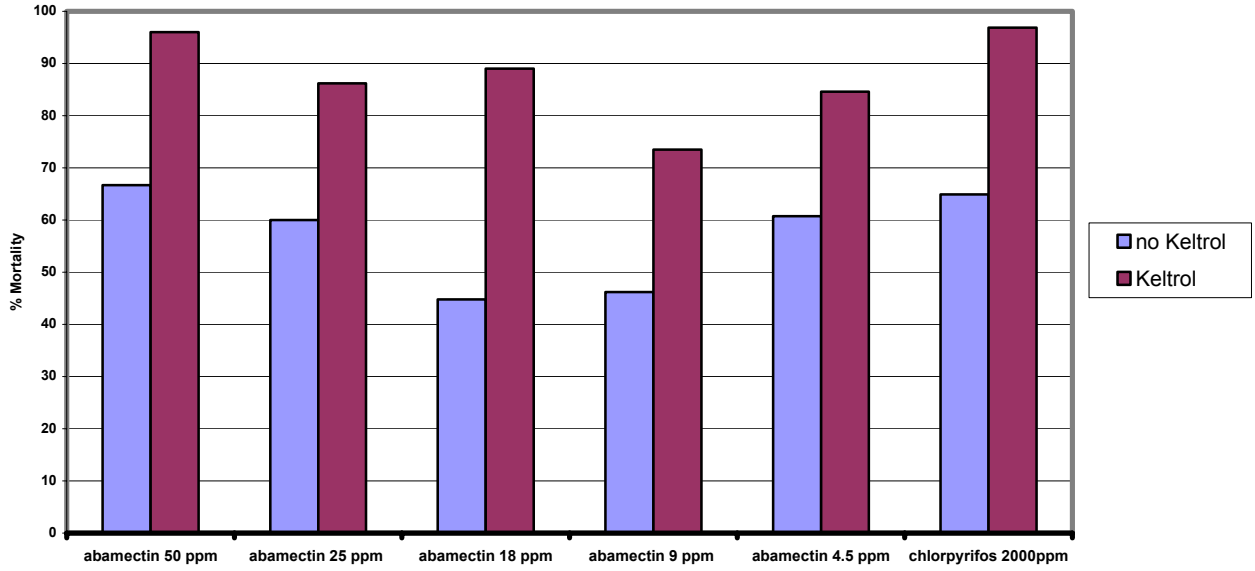

Figure 3. Sep 1999 - Aug 2002 trials. Mortality of *Bactrocera tryoni* exposed to abamectin & chlorpyrifos-protein bait formulations with & without 0.5 % Keltrol at 4 days after bait application.

August 2002 - September 2003 trials

Results from the second group of sixteen trials (August 2002 - September 2003) were analysed in more detail than the results from the first group of trials. An angular transformation was applied to the data in each of the second group trials to stabilise the variance ($x=(180)/\pi * \arcsin(\sqrt{p/100})$ where x=transformed data and p=percentage mortality, $0<p<100$). One-way ANOVAs were done for each trial, separately for each of the days after exposure. Comparisons between means were done using Fisher's Protected Least Significant Difference (LSD) test.

Results of the sixteen trials are discussed below with Tables 1-7 showing detailed results of seven representative trials (transformed means and back transformed mortality % with significant differences P=0.05).

Each series of trials had a particular focus on some modification of generic bait formulation (as described below). The standard bait (Mauri yeast 2% + Hy-Mal 0.5%) was always included for comparison and in some trials the new baits GF-120 and Bactrogel-P were also included.

Trials 1-3: In Trial 1(Table 2) baits with Hy-Mal (at 0.5% and 0.2%) with and without thickener were compared. There were no differences at 0 days, but thickened baits were significantly better after 2, 4 and 6 days. After 6 days baits with thickener gave 89% mortality (with 0.5% Hy-Mal) and 96% mortality (with 0.2% Hy-Mal) compared to 70% mortality (with 0.5% Hy-Mal without thickener) and 45% mortality (with 0.2% Hy-Mal without thickener). Hy-Mal 0.2% performed slightly better than Hy-Mal 0.5%. At 6 days, there were no significant differences between Bactrogel-P, GF-120 and the standard Hy-Mal bait without thickener. However, at 6 days the standard Hy-Mal bait with thickener produced significantly higher mortality than either of the new baits. Trials 2 and 3 gave similar results to Trial 1.

The results of these 3 trials showed no consistently significant differences between Hy-Mal rates of 0.5% and 0.2%. There were consistent differences, however, between Hy-Mal baits with and without thickener. The average % mortality (over the 3 trials) for the 4 treatments (Hy-Mal 0.5% without and with thickener, and Hy-Mal 0.2% without and with

thickener) at 6 days was respectively 71.5%, 91.7%, 60.8% and 92.81%. This represented an average 30% improvement in mortality with thickener. All of these treatments used yeast autolysate at 2% and were applied to a single leaf.

Table 2. Mortality of *Bactrocera tryoni* exposed to Hy-Mal bait formulations with and without thickener at 0, 2, 4 and 6 days after bait application.

Treatment	No. of leaves treated	Mean % mortality§ Age of bait (days)							
		0		2		4		6	
		Trans*	Back**	Trans*	Back**	Trans*	Back**	Trans*	Back**
Control (2% Mauri yeast + 0.5% Keltrol)	1	19.89a	11.57	21.68a	13.64	22.23a	14.33	22.88a	15.12
Hy-Mal 0.5% + 2% Mauri yeast	1	85.27b	99.32	72.07b	90.53	62.07b	78.00	56.80c	70.01
Hy-Mal 0.5% + 2% Mauri yeast + 0.5% Keltrol	1	90.00b	100.00	85.27c	99.32	77.22c	94.33	70.95d	89.35
Hy-Mal 0.2% + 2% Mauri yeast	1	82.61b	98.35	79.80b	96.86	60.70b	76.00	42.22b	45.15
Hy-Mal 0.2% + 2% Mauri yeast + 0.5% Keltrol	1	90.00b	100.00	90.00d	100.00	90.00d	100.00	77.97d	95.65
Bactrogel-P	1	86.15b	99.55	85.27c	99.32	81.43c	96.67	58.58c	72.74
GF-120 1:6.5	1	83.44b	98.70	77.30b	95.17	83.44c	98.00	63.63c	80.27
LSD (P=0.05)		10.81		11.54		7.94		10.23	

* Transformed means (angular transformation) ** Back transformed means

§ Treatments within a column followed by the same letter are not significantly different at the 5% level

Trials 4 –6: These trials tested the same Hy-Mal bait formulations as tested in Trials 1-3, except that an even lower concentration of Hy-Mal (0.1%) was included. All formulations included 2% yeast autolysate with and without the addition of 0.5% Keltrol. In Trial 5 (Table 3) the addition of thickener gave higher mortality in all of the Hy-Mal treatments and this continued at 2, 4 and 6 days. At 6 days the mean mortalities for the three different concentrations of Hy-Mal without thickener were 72.3%, 62.0% and 60% and for the same concentrations of Hy-Mal with thickener all treatments resulted in 100% mortality. This represented an average of 35% higher mortality when thickener was added. At 0 and 6 days, there were no significant differences in mortality with different concentrations of Hy-Mal in either thickened baits or unthickened baits (and only minor differences at the intermediate days). Similar results were obtained in Trial 6. Results of these three trials indicated improved mortality with the addition of thickener. These results were similar to those from Trials 1-3 which showed thickener enhanced Hy-Mal bait efficacy by 30%.

Table 3. Mortality of *Bactrocera tryoni* exposed to bait formulations containing different concentrations of Hy-Mal with and without thickener at 0, 2, 4 and 6 days after bait application.

Treatment	No. leaves bait sprayed	Mean % mortality§ Age of bait (days)							
		0		2		4		6	
		Trans*	Back **	Trans*	Back **	Trans*	Back **	Trans*	Back **
Untreated (2% Mauri yeast + 0.5% Keltrol)	1	23.50a	15.89	20.50a	12.26	21.33a	13.23	24.85a	17.67
Hy-Mal 0.5% + 2% Mauri yeast	1	62.10b	78.11	57.89b	71.75	67.79c	79.10	59.36b	72.33
Hy-Mal 0.5% + 2% Mauri yeast + 0.5% Keltrol	1	79.24c	96.51	90.00d	100.00	90.00d	100.00	90.00c	100.00
Hy-Mal 0.2% + 2% Mauri yeast	1	60.50b	75.75	55.20b	67.43	50.82b	60.08	52.04b	62.00
Hy-Mal 0.2% + 2% Mauri yeast + 0.5% Keltrol	1	74.86c	93.18	82.56cd	98.32	90.00d	100.00	90.00c	100.00
Hy-Mal 0.1% + 2% Mauri yeast	1	57.86b	71.70	54.75b	66.69	51.57bc	61.36	50.78b	60.00
Hy-Mal 0.1% + 2% Mauri yeast + 0.5% Keltrol	1	81.20c	97.66	80.68c	97.38	86.15d	99.55	90.00c	100.00
LSD P = 0.05		7.56		7.64		11.28		9.40	

* Transformed means (angular transformation) ** Back transformed means

§ Treatments within a column followed by the same letter are not significantly different at the 5% level

Trials 7-8: These trials were designed to determine the efficacy of abamectin as a toxicant in fruit fly baits. In Trial 7 (Table 4) abamectin at 4.5ppm (ie. 0.0004% or 25mls of Vertamec ® per 100L) with thickener was compared to Hy-Mal 0.5% with and without thickener. Again the addition of thickener resulted in a very significant improvement in the efficacy of Hy-Mal bait eg at 6 days, 77.4% mortality with thickener compared to 23% without thickener. The latter result with the standard Hy-Mal bait was much lower than that obtained for the same bait in other trials (eg 72% at 6days in Table 2). The reasons for this are not known.

On the day of application, there were no significant differences between the 4.5 ppm abamectin bait with thickener applied to 2 leaves (98% mortality), 10 leaves (100% mortality) and the Hy-Mal bait with thickener applied to one leaf (100% mortality). However, the abamectin bait applied to one leaf only was significantly less effective (95% mortality). After 6 days, mortality with 4.5ppm abamectin with thickener applied to one leaf was a low 32% compared to the 77.4% mortality with the thickened Hy-Mal bait also applied to one leaf. There were significant improvements in mortality at 6 days when the

abamectin bait was applied to 2 leaves and 10 leaves respectively. Mortality due to incidental contact with Hy-Mal in the absence of attractant protein was 16.7% at 0 days and 7.9% at 6 days.

Table 4. Mortality of *Bactrocera tryoni* exposed to bait formulations containing abamectin and thickener at 0, 2, 4 and 6 days after bait application.

Treatment	No. of leaves treated	Mean % mortality§ Age of bait (days)							
		0		2		4		6	
		Trans*	Back**	Trans*	Back**	Trans*	Back**	Trans*	Back**
Control (2% Mauri yeast + 0.5% Keltrol)	1	22.21a	14.33	22.78a	14.99	19.05a	10.67	24.34b	17.00
abamectin 4.5 ppm + 2% Mauri yeast + 0.5% Keltrol	1	77.58c	95.33	52.80b	63.44	41.91b	40.67	34.41d	32.00
abamectin 4.5 ppm + 2% Mauri yeast + 0.5% Keltrol	2	85.27d	98.00	75.25c	93.55	68.91c	86.67	50.59e	59.67
abamectin 4.5 ppm + 2% Mauri yeast + 0.5% Keltrol	10	90.00d	100.00	81.87d	98.00	78.72d	96.00	59.35f	74.00
Hy-Mal 0.5%	1	24.08a	16.67	21.94a	13.96	20.50a	12.33	16.35a	7.92
Hy-Mal 0.5% + 2% Mauri yeast	1	71.76b	90.00	48.87b	56.73	39.58b	40.67	28.65c	23.00
Hy-Mal 0.5% + 2% Mauri yeast + 0.5% Keltrol	1	90.00d	100.00	90.00e	100.00	87.29e	97.33	61.58f	77.37
LSD (P=0.05)		5.42		5.09		6.67		3.15	

* Transformed means (angular transformation) ** Back transformed means
§ Treatments within a column followed by the same letter are not significantly different at the 5% level

Trials 9-13: These trials were designed to test the effects of reducing yeast concentration on efficacy of thickened baits. Hy-Mal and abamectin baits with thickener were compared at 2%, 1% and 0.5% yeast autolysate. Results for Trial 9 (Table 5) showed that at 0 days there were no significant differences between mortalities with the different concentrations of yeast in Hy-Mal baits with thickener applied to either one leaf or two leaves. Results were variable for these baits at 2 and 4 days but at 6 days, the 2% yeast bait on one leaf produced 46.0% mortality and the same bait on two leaves produced 92.7% mortality. With 1% yeast, mortality was 60% with one leaf treated and 88.3% with two leaves treated. With 0.5% yeast, mortality was 48.0% with one leaf treated and 70.7% with two leaves treated.

Table 5. Mortality of *Bactrocera tryoni* exposed to thickened Hy-Mal and abamectin bait formulations with different concentrations of yeast protein at 0, 2, 4 and 6 days after bait application.

Treatment	No. of leaves treated	Mean % mortality§ Age of bait (days)							
		0		2		4		6	
		Trans*	Back**	Trans*	Back**	Trans*	Back**	Trans*	Back**
Control (2% Mauri yeast + 0.5% Keltrol)	1	24.60a	17.32	18.24a	10.00	16.60a	8.16	13.30a	5.33
Hy-Mal 0.5% + 2% Mauri yeast + 0.5% Keltrol	1	84.58b	99.11	72.15cd	90.00	48.08b	55.36	42.70b	46.00
Hy-Mal 0.5% + 1% Mauri yeast + 0.5% Keltrol	1	86.15b	99.55	79.60de	96.67	52.81b	63.47	50.77cd	60.00
Hy-Mal 0.5% + 0.5% Mauri yeast + 0.5% Keltrol	1	83.44b	98.70	70.44c	88.67	45.77b	51.34	43.85bc	48.00
Hy-Mal 0.5% + 2% Mauri yeast + 0.5% Keltrol	2	87.29b	99.78	85.27e	98.00	74.53d	92.89	74.32e	92.67
Hy-Mal 0.5% + 1% Mauri yeast + 0.5% Keltrol	2	84.58b	99.11	87.29e	97.33	69.66cd	87.92	69.91e	88.30
Hy-Mal 0.5% + 0.5% Mauri yeast + 0.5% Keltrol	2	90.00b	100.00	79.13de	94.67	61.66c	77.46	57.23d	70.67
abamectin 4.5ppm + 2% Mauri yeast + 0.5% Keltrol	2	82.56b	98.32	59.35b	74.00	45.00b	50.00	41.91b	44.67
abamectin 4.5ppm + 1% Mauri yeast + 0.5% Keltrol	2	83.44b	98.70	64.90bc	82.00	43.87b	48.00	38.80b	39.33
LSD (P=0.05)		9.25		8.75		9.61		8.26	

* Transformed means (angular transformation) ** Back transformed means

§ Treatments within a column followed by the same letter are not significantly different at the 5% level.

Trial 12 (Table 6) shows the effects on bait efficacy of reducing the yeast concentration in 4.5 ppm abamectin bait with thickener. There were no significant differences between baits with 2%, 1% or 0.5% yeast at 0 days or at 6 days when applied to one leaf or ten leaves. Check treatments with abamectin and thickener only (no yeast) applied to one leaf or 10 leaves showed 10.7% mortality in both cases at 0 days and 17% mortality in both case sat 6 days. A similar check treatment with Hy-Mal (no yeast and no thickener) showed mortality due to incidental contact decreased over 6 days from 16.7% to 7.9% (Table 4).

Table 6. Mortality of *Bactrocera tryoni* exposed to thickened abamectin bait formulations with different concentrations of yeast protein at 0, 2, 4 and 6 days after bait application.

Treatment	No. of leaves treated	Mean % mortality§ Age of bait (days)							
		0		2		4		6	
		Trans*	Back**	Trans*	Back**	Trans*	Back**	Trans*	Back**
Control (2% Mauri yeast + 0.5% Keltrol)	1	21.60a	13.55	21.00a	12.85	19.56a	11.21	20.76a	12.56
abamectin 4.5ppm + 2.0% Mauri yeast + 0.5% Keltrol	1	73.79bc	92.21	64.92d	82.03	55.16e	67.36	51.56c	61.34
abamectin 4.5ppm + 2.0% Mauri yeast + 0.5% Keltrol	10	79.80bc	96.86	63.00d	79.39	61.57f	77.34	45.55c	50.96
abamectin 4.5ppm + 1.0% Mauri yeast + 0.5% Keltrol	1	69.34b	87.55	49.62b	58.03	45.38cd	50.67	49.22c	57.34
abamectin 4.5ppm + 1.0% Mauri yeast + 0.5% Keltrol	10	72.73b	90.69	58.15c	72.16	47.69d	54.69	42.31c	45.31
abamectin 4.5ppm + 0.5% Mauri yeast + 0.5% Keltrol	1	52.36b	62.71	49.22b	57.33	39.60c	40.64	38.82bc	39.26
abamectin 4.5ppm + 0.5% Mauri yeast + 0.5% Keltrol	10	68.78b	86.84	53.96c	65.39	42.31c	45.32	38.82bc	39.29
abamectin 4.5ppm + 0.5% Keltrol	1	19.05a	10.65	26.08a	19.32	23.04b	15.31	24.33ab	16.98
abamectin 4.5ppm + 0.5% Keltrol	10	19.05a	10.65	24.05a	16.60	17.44a	8.98	24.05ab	16.60
Hy-Mal 0.5% + 2.0% Mauri yeast	1	80.55c	97.30	69.16e	87.55	49.24d	57.37	48.28c	55.72
LSD (P=0.05)		7.72		4.24		3.98		14.91	

* Transformed means (angular transformation) ** Back transformed means

§ Treatments within a column followed by the same letter are not significantly different at the 5% level

Trial 14: This trial was designed to further investigate the effects of reducing yeast concentration (2%, 1% and 0.5%) in both thickened and unthickened Hy-Mal and abamectin baits. All treatments were applied to one leaf only. Results in Table 7 showed treatments gave 87-95% mortality at 0 days. For the abamectin at days 2, 4 and 6 the mortality for 0.5% yeast was significantly lower than that at 2% yeast though the same differences were not reflected in the Hy-Mal results.

Table 7. Mortality of *Bactrocera tryoni* exposed to Hy-Mal and abamectin bait formulations with different concentrations of yeast protein, with and without thickener, at 0, 2, 4 and 6 days after bait application.

Treatment	No. of leaves treated	Mean % mortality§ Age of bait (days)							
		0		2		4		6	
		Trans*	Back**	Trans*	Back**	Trans*	Back**	Trans*	Back**
Control (2% Mauri yeast + 0.5% Keltrol)	1	16.41a	7.98	10.34a	3.37	12.18a	4.45	20.50a	12.33
abamectin 4.5 ppm + 2.0% Mauri yeast	1	76.70e	94.71	65.69cd	83.00	55.59b	68.07	48.90c	56.67
abamectin 4.5 ppm + 2.0% Mauri yeast + 0.5% Keltrol	1	72.56cd	91.02	71.57e	90.00	72.37d	90.83	65.96ef	83.33
abamectin 4.5 ppm + 1.0% Mauri yeast	1	76.24e	94.34	64.69c	81.67	55.38b	67.73	40.97b	43.00
abamectin 4.5 ppm + 1.0% Mauri yeast + 0.5% Keltrol	1	71.62cd	90.06	70.39e	88.67	67.63cd	85.52	63.29def	79.67
abamectin 4.5 ppm + 0.5% Mauri yeast	1	75.20e	93.48	58.50b	72.67	50.99b	60.38	44.04bc	48.33
abamectin 4.5 ppm + 0.5% Mauri yeast + 0.5% Keltrol	1	68.94c	87.08	64.43c	81.33	64.31c	81.21	58.41d	72.33
Hy-Mal 0.5% + 2.0% Mauri yeast	1	69.73cd	88.00	69.77de	88.00	66.45c	84.03	61.82de	77.67
Hy-Mal 0.5% + 2.0% Mauri yeast + 0.5% Keltrol	1	72.23d	90.69	71.62e	90.00	69.16cd	87.35	62.50de	78.67
Hy-Mal 0.5% + 1.0% Mauri yeast	1	69.16bc	87.35	66.42c	84.00	63.92c	80.68	60.00d	75.00
Hy-Mal 0.5% + 1.0% Mauri yeast + 0.5% Keltrol	1	76.24e	94.34	72.90e	91.33	74.39cd	92.76	68.42f	86.33
Hy-Mal 0.5% + 0.5% Mauri yeast	1	68.03b	86.00	67.49d	85.33	63.92c	80.68	58.71d	73.00
Hy-Mal 0.5% + 0.5% Mauri yeast + 0.5% Keltrol	1	75.28e	93.55	73.04e	91.33	68.86cd	86.99	66.06def	83.33
LSD (P=0.05)		2.95		2.78		6.81		5.27	

* Transformed means (angular transformation); ** Back transformed means.

§ Treatments within a column followed by the same letter are not significantly different at the 5% level

At 6 days the abamectin plus thickener treatments gave mortalities of 83.3% (with 2% yeast), 79.7% (with 1% yeast) and 72.3% (with 0.5% yeast). Mortalities for abamectin without thickener were significantly lower with 56.7% (with 2% yeast), 43.0% (with 1% yeast), and 48.3% (with 0.5% yeast). There was very little difference between the various Hy-Mal treatments at 6 days with mortalities ranging from 73.0% to 86.3%. The overall result demonstrated that adding thickener to bait (particularly with abamectin as the toxicant) had a far greater impact on bait efficacy than changing the concentration of yeast protein.

Trials 15 and 16: In these trials, abamectin was compared at rates from 4.5 to 45 ppm with and without thickener, with 2% or 1% yeast autolysate and against standard Hy-Mal bait. Both trials gave similar results. In Trial 15 (Table 8), addition of thickener improved all the abamectin treatments at 2, 4 and 6 days by up to 45% eg at 6 days at 4.5 ppm with thickener 84.8% versus 38.6% without thickener. There were no significant differences between yeast autolysate at 2% and 1%. Efficacy did not significantly improve as the abamectin concentration increased. At 6 days mortality with 4.5 ppm abamectin with 2% yeast autolysate and thickener was 86.8%, whereas mortality with the comparable bait mix with 22.5 ppm abamectin was 86.7% and with 45 ppm abamectin the mortality was 84.8%.

Table 8. Mortality of *Bactrocera tryoni* exposed to bait formulations with different concentrations of abamectin and yeast protein at 0, 2, 4 and 6 days after bait application.

Treatment	No. of leaves treated	Mean % mortality§ Age of bait (days)							
		0		2		4		6	
		Trans*	Back**	Trans*	Back**	Trans*	Back**	Trans*	Back**
Control (2% Mauri yeast +0.5% Keltrol)	2	15.93a	7.53	21.95a	13.97	9.67a	11.66	22.21a	14.29
abamectin 45ppm + 1% Mauri yeast	2	87.29e	99.78	76.16de	94.28	88.00c	84.00	67.06d	84.81
abamectin 45ppm + 1% Mauri yeast + 0.5% Keltrol	2	78.43 bcd	95.98	69.40c	87.62	84.00c	80.66	62.50cd	78.67
abamectin 22.5 ppm + 2% Mauri yeast + 0.5% Keltrol	2	87.29e	99.78	72.29 cde	90.75	90.67d	92.00	68.63de	86.72
abamectin 22.5 ppm + 1% Mauri yeast	2	77.84 bcd	95.56	53.18b	64.09	45.33b	87.33	37.58b	37.19
abamectin 22.5 ppm + 1% Mauri yeast + 0.5% Keltrol	2	85.27de	99.32	74.53 cde	92.89	84.67c	86.66	62.64cd	78.87
abamectin 4.5 ppm + 2% Mauri yeast + 0.5% Keltrol	2	90.00f	100.00	78.46e	96.00	96.67d	90.66	68.73de	86.84
abamectin 4.5 ppm + 1% Mauri yeast	2	84.58de	99.11	51.97b	62.05	49.33b	80.66	38.43b	38.64
abamectin 4.5 ppm + 1% Mauri yeast + 0.5% Keltrol	2	81.81 cde	97.97	73.04 cde	91.49	87.33e	85.33	67.06d	84.81
Hy-Mal 0.5% + 2% Mauri yeast	2	75.28bc	93.55	68.63c	86.72	84.00c	60.33	65.43cd	82.71
Hy-Mal 0.5% + 1% Mauri yeast	2	79.85 bcde	96.90	75.28 cde	93.55	88.00c	67.33	60.68c	76.02
Hy-Mal 0.5% + 1% Mauri yeast + 0.5% Keltrol	2	90.00f	100.00	86.15f	99.55	90.67d	68.00	72.29e	90.75
LSD (P=0.05)		8.07		6.56		6.16		4.95	

* Transformed means (angular transformation); ** Back transformed means

§ Treatments within a column followed by the same letter are not significantly different at the 5% level

5.3.2 Overview of glasshouse trials

Data from the above trials are presented graphically in Figure 4, Figure 5 and Figure 6 to highlight the effects of various bait modifications on fly mortality as the baits aged for up to 6 days under glasshouse conditions. Each point represents the mean mortality for a particular bait treatment over all trials.

Figure 4 shows comparative efficacy for a selection of bait formulations all of which contained 2% yeast and thickener at 0.5% if present. The Hy-Mal bait with thickener was the most effective over 6 days with GF-120 being the next most effective. Abamectin bait (4.5 ppm) performed as well as standard Hy-Mal bait (no thickener) provided thickener was used and the volume of bait applied was at least doubled ie 2 or 10 leaves treated instead of one leaf).

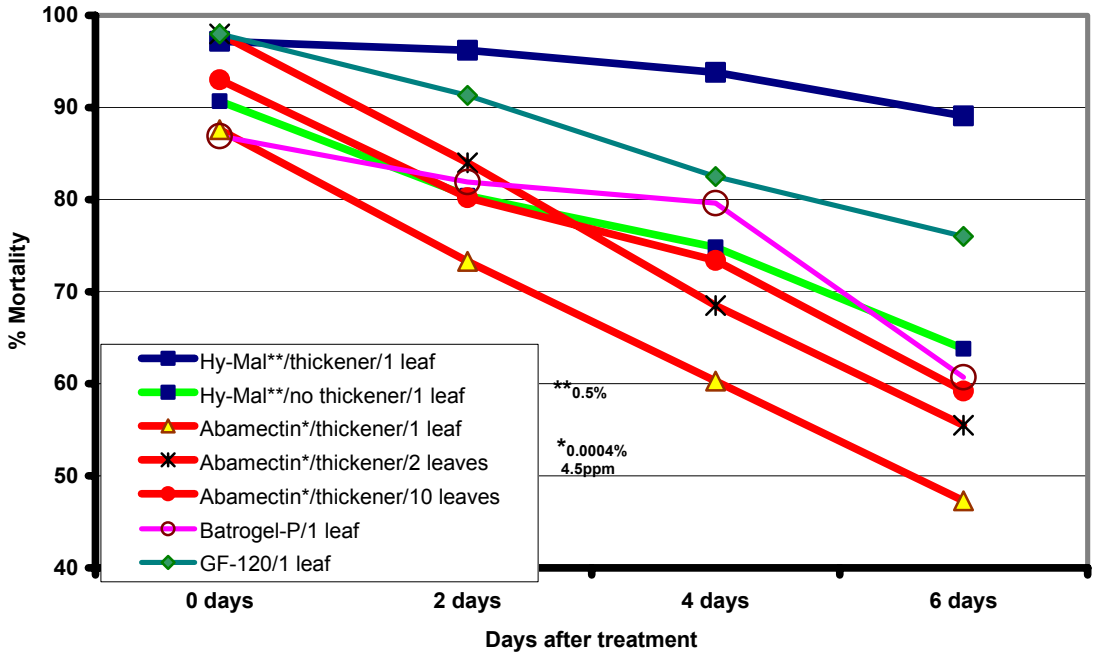

Figure 4. Aug 2002-Sep 2003 trials. Overall mean mortality of *Bactrocera tryoni* exposed to standard and modified bait formulations at 0, 2, 4 & 6 days after bait application to different numbers of leaves on potted host trees.

The effects of varying yeast concentration in abamectin and Hy-Mal formulations, both with added thickener, are shown in Figure 5. With thickened Hy-Mal baits, reducing the concentration of yeast from 2% to 1% or 0.5% did not affect mortality initially but after 4-6 days the higher yeast concentrations were more effective. With the thickened abamectin baits, there was little difference initially or over time between 2% and 1% yeast but the 0.5 % yeast bait was less effective at all times after application.

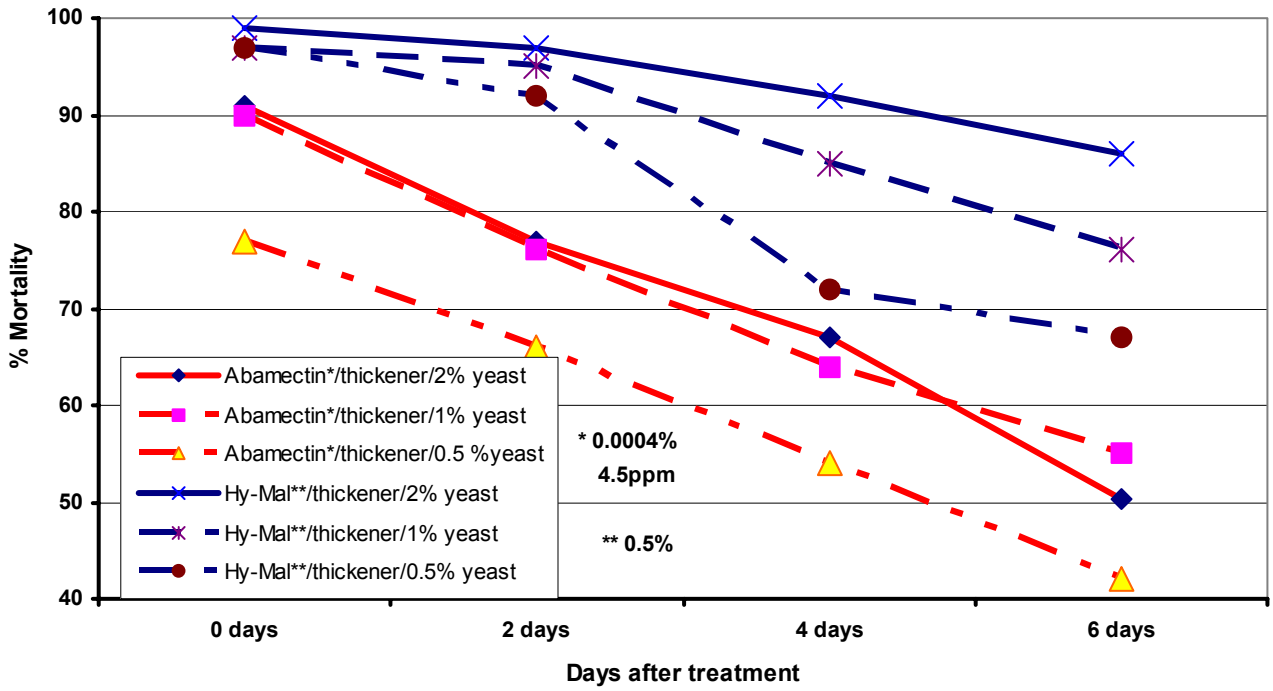

Figure 5. Aug 2002-Sep 2003 trials. Overall mean mortality of *Bactrocera tryoni* exposed to thickened abamectin and Hy-Mal baits with different concentrations of yeast protein at 0, 2, 4 & 6 days after bait application to leaves of potted host trees.

In the first group of trials reported here (Sep 1999 - Aug 2002), abamectin was shown to be effective as a toxicant in fruit fly baits but increasing the concentration of abamectin above 4.5 ppm did not improve bait efficacy (Figure 2 and Figure 3). In the second group of trials (Aug 2002 - Sep 2003), higher mortality was achieved with abamectin at 22.5 and 45 ppm in comparison with 4.5ppm providing it was used with thickener (Figure 6). This graphical presentation of fly mortality at 4 and 6 days after bait application shows the most effective abamectin formulations tested were 22.5 ppm abamectin with 1.0% yeast and 0.5% thickener and 45 ppm abamectin with 2% yeast and 0.5% thickener.

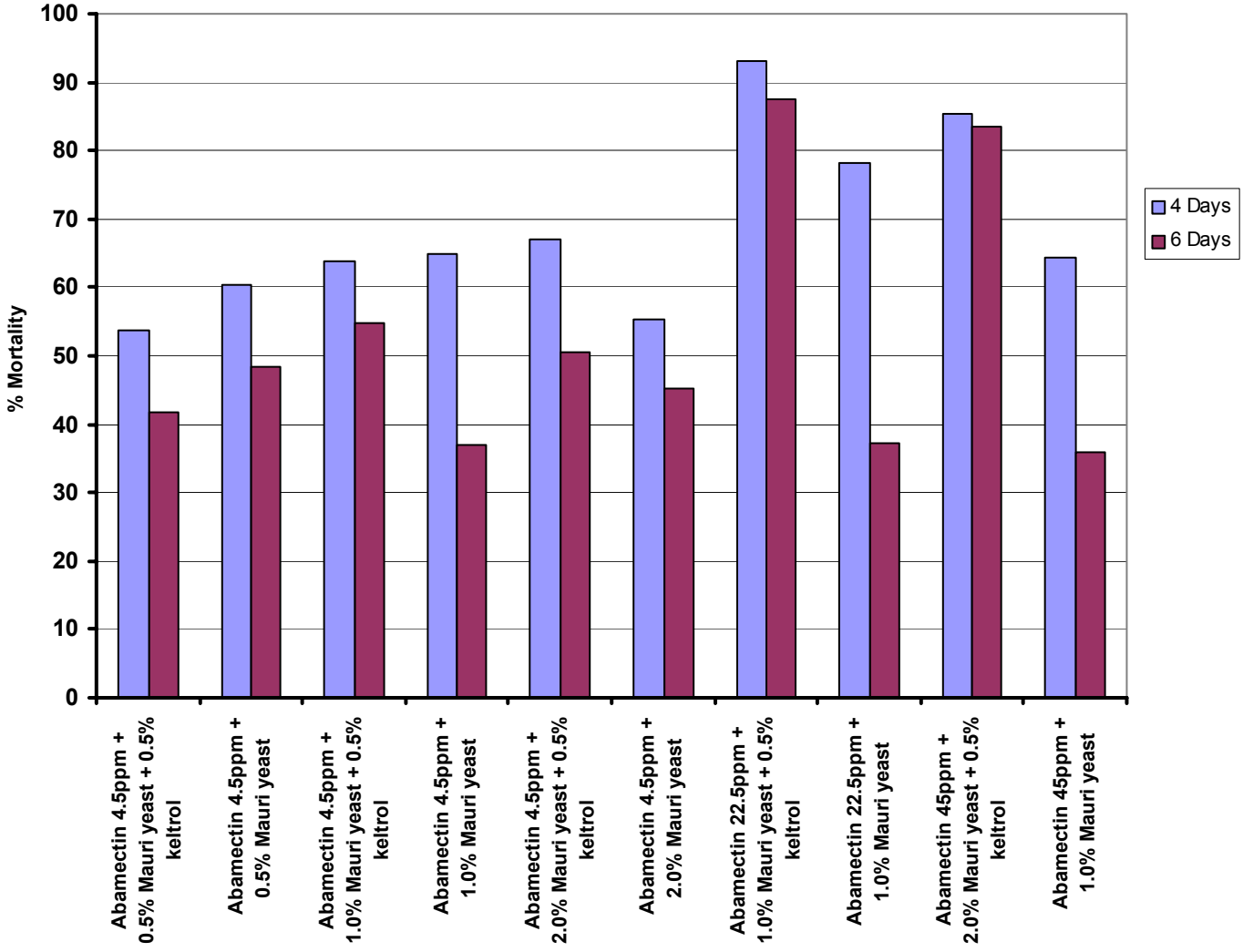

Figure 6. Aug 02-Sept 03 trials. Summary of mortality of *Bactrocera tryoni* exposed to various abamectin bait formulations at 4 & 6 days after bait application to leaves of potted host trees.

A summary of all results from glasshouse trials to evaluate bait modifications is presented in Table 9. The number of trials undertaken with each bait formulation, mean mortalities and SDs for each treatment are shown.

Table 9. Summary of all results from glasshouse trials to evaluate mortality of *Bactrocera tryoni* exposed to modified bait formulations at 0, 2, 4, 6 days after bait application to leaves of potted host trees under glasshouse conditions.

Treatment Yeast = Mauri yeast autolysate; Keltrol used at 0.5%	Mean fly age in days	No. of trials		Mortality at times after bait application			
				0 days	2 days	4 days	6 days
abamectin 100 ppm + 2% yeast	10	1	Mean	100.00	100.00	75.00	
abamectin 50 ppm + 2% yeast	8	3	Mean	98.33	83.67	66.67	
			SD	1.58	6.68	5.55	
abamectin 50 ppm + 2% yeast + Keltrol	10	1	Mean	99.00	97.00	96.00	
abamectin 45 ppm + 2% yeast + Keltrol	8	4	Mean	96.30	89.83	85.36	83.39
			SD	2.08	2.65	3.33	3.18
abamectin 45 ppm + 1% yeast	8	2	Mean	94.39	65.07	64.49	35.99
			SD	3.03	8.95	16.18	5.99
abamectin 25 ppm + 2% yeast	10	3	Mean	99.67	89.33	58.50	
			SD	1.90	14.19	12.02	
abamectin 25 ppm + 2% yeast + Keltrol	8	5	Mean	98.60	98.00	86.25	
			SD	2.06	1.30	10.83	
abamectin 22.5 ppm + 1% yeast	8	2	Mean	96.15	76.39	78.09	37.19
			SD	0.59	12.30	9.24	0.00
abamectin 22.5 ppm + 1% yeast + Keltrol	8	4	Mean	98.91	94.50	93.06	87.46
			SD	0.90	2.98	4.20	5.52
abamectin 18 ppm + 2% yeast	6	6	Mean	88.67	81.33	44.83	
			SD	12.81	19.59	6.33	
abamectin 18 ppm + 2% yeast + Keltrol	4	5	Mean	91.20	82.80	89.00	78.00
			SD	4.71	16.48		
abamectin 9 ppm + 2% yeast	6	5	Mean	91.00	84.80	46.20	
			SD	6.56	6.18	30.95	
abamectin 9 ppm + 2% yeast + Keltrol	6	2	Mean	96.00	79.00	73.50	
			SD	0.00	1.41	16.26	
abamectin 4.5 ppm	8	2	Mean	59.00	42.67	38.66	26.78
			SD	21.00	12.00	12.01	17.88
abamectin 4.5 ppm + 2% yeast	8	6	Mean	83.34	71.83	62.29	69.96
			SD	13.83	17.39	17.26	16
abamectin 4.5 ppm+ 1% yeast	8	3	Mean	96.73	76.63	64.90	37.10
abamectin 4.5 ppm + 1% yeast + Keltrol	8	15	Mean	90.08	76.37	63.76	54.74
			SD	9.06	17.11	22.27	24.39
abamectin 4.5 ppm + 0.5% yeast	8	1	Mean	93.48	72.76	60.38	48.33
abamectin 4.5 ppm + 0.5% yeast + Keltrol	8	9	Mean	76.58	65.68	53.70	41.86
			SD	13.06	15.57	17.73	12.76
abamectin 4.5 ppm +2% yeast + Keltrol	8	35	Mean	92.98	84.05	77.00	63.50
abamectin 4.5 ppm + Keltrol	8	8	Mean	18.34	18.73	15.99	19.20
			SD	8.67	5.60	3.55	7.00
chlorpyrifos 0.02% + 2% yeast	8	10	Mean	98.40	88.50	64.90	
			SD	27.61	26.63	29.98	
chlorpyrifos 0.02%+ 2% yeast + Keltrol	8	20	Mean	98.55	92.75	96.88	92.00
			SD	1.87	11.73	12.34	7.79
Hy-Mal 0.5%		3	Mean	35.01	27.81	24.55	17.93
			SD	17.55	12.72	10.21	7.70
Hy-Mal 0.5% + 0.5% yeast	8	1	Mean	86.00	85.33	80.68	73.00
Hy-Mal 0.5% + 0.5% yeast + Keltrol	8	3	Mean	97.42	91.56	71.93	67.33
			SD	2.79	2.45	15.07	14.62

Treatment Yeast = Mauri yeast autolysate; Keltrol used at 0.5%	**Mean fly age in days**	**No. of trials**	**Mortality at times after bait application**				
				0 days	**2 days**	**4 days**	**6 days**
Hy-Mal 0.5% + 1% yeast	8	3	Mean	93.89	90.30	78.45	73.91
			SD	4.63	4.46	8.32	2.30
Hy-Mal 0.5% + 1% yeast + Keltrol	8	6	Mean	98.71	96.13	81.84	88.44
			SD	1.29	3.43	13.84	2.31
Hy-Mal 0.5% + 2% yeast	8	13	Mean	92.43	79.98	70.08	61.58
			SD	7.89	14.53	17.60	
Hy-Mal 0.2% + 2% yeast	8	5	Mean	93.77	83.84	75.20	68.75
			SD	9.10	11.80	14.75	18.08
Hy-Mal 0.2% + 2% yeast + Keltrol	8	5	Mean	98.25	98.86	97.82	95.00
			SD	2.58	0.95	3.43	6.37
Hy-Mal 0.1% + 2% yeast	8	5	Mean	85.51	78.53	76.03	73.02
			SD	13.81	11.84	14.66	13.02
Hy-Mal 0.1% + 2% yeast + Keltrol	8	2	Mean	97.99	90.76	92.49	91.45
			SD	0.33	6.62	7.07	8.55
Bactrogel-P 13.4 g/L	8	3	Mean	88.20	81.93	72.67	60.65
			SD	10.42	14.96	22.11	15.21
GF-120 dilution 1:6.5	8	3	Mean	97.99	91.28	82.52	75.92
			SD	1.99	8.61	23.00	20.72
Check (2% yeast + 0.5% Keltrol)	8	52	Mean	12.28	11.56	12.73	13.64
			SD	3.19	3.38	4.03	5.74

5.3.3 Field evaluation of modified generic baits

Fruit fly monitoring

The mean weekly trap catches from each of the 26 traps distributed throughout the trial stone fruit blocks for the period 11 September – 29 November 2002 are shown in (Table 10). Fly numbers throughout the orchard were very low with the highest mean weekly catch being only 4.8 in a nectarine block treated with Bactrogel-P bait. Trapped flies represented three species: 342 *Bactrocera tryoni*, 21 *Bactrocera newmani* and 42 *Bactrocera neohumeralis*.

Table 10. Trapping data from stonefruit trial blocks at Treetops Orchard, Mundubbera. Mean weekly count per trap for each trial block from 11 Sep - 29 Nov 2002.

Trap No.	Block No.	Treatment	Variety	Mean weekly cue lure trap catches
1	1	Hy-Mal 7.5L/Ha	Peaches	3.1
2	1	Hy-Mal 7.5L/Ha	Peaches	3.2
3	2	Hy-Mal 7.5L/Ha + thickener	Peaches	1.1
4	2	Hy-Mal 7.5L/Ha + thickener	Peaches	3.5
5	3	Hy-Mal 15L/Ha	Peaches	2.9
6	3	Hy-Mal 15L/Ha	Peaches	2.1
7	4	Hy-Mal 15L/Ha + thickener	Peaches	1.1
8	4	Hy-Mal 15L/Ha + thickener	Peaches	1.7
9	5	GF-120 7.5L/Ha	Peaches	0.9
10	5	GF-120 7.5L/Ha	Peaches	2.3
11	12	GF-120 7.5L/Ha + MAT	Peaches	0.0
12	12	GF-120 7.5L/Ha + MAT	Peaches	0.3
13	6	Bactrogel P 5L/Ha	Peaches	1.0
14	6	Bactrogel P 5L/Ha	Peaches	0.6
15	13	Chlorpyrifos Bait 15L/Ha	Peaches	0.0
16	13	Chlorpyrifos Bait 15L/Ha	Peaches	0.1
17	7	GF-120 7.5L/Ha	Nectarines	1.3
18	7	GF-120 7.5L/Ha	Nectarines	3.2
19	8	Bactrogel P 5L/Ha	Nectarines	4.7
20	8	Bactrogel P 5L/Ha	Nectarines	4.8
21	9	GF-120 5L/Ha + MAT(trunk)	Nectarines	0.3
22	9	GF-120 5L/Ha + MAT(trunk)	Nectarines	0.5
23	10	Bactrogel P 5L/Ha + MAT	Nectarines	0.0
24	10	Bactrogel P 5L/Ha + MAT	Nectarines	0.1
25	11	MAT - no bait	Nectarines	0.0
26	11	MAT - no bait	Nectarines	0.0

Fruit assessment

As discussed previously in this report, large field blocks are required to effectively evaluate the efficacy of bait treatments, hence it was not possible to include replicated treatment blocks in this trial and consequently results were not able to be analysed. However, the results provided useful information on the field efficacy of various modifications to generic bait formulations.

The total numbers of mature fruit and of overripe fruit sampled and % infestation in both samples for each treatment block are shown in Table 11. The high level of infestation

(87.5%) found in untreated stone fruit from nearby town backyards showed that all treatments were providing reasonably effective control. Infestation levels in mature fruit ranged from 0.5% to 4.4% in baited peach blocks to slightly higher levels of 3.4-8.3% in baited nectarine blocks. The highest level of infestation was 14.5% in the nectarine block 11 treated with MAT and no baiting indicating that MAT alone would be unlikely to provide a commercially acceptable level of control. The second highest level of infestation (8.3%) in the nectarine block 9 indicated that trunk application of concentrated GF-120 (1:1.5 @ 5L/ha) to minimise possible phytotoxicity was not as effective as foliar application with the more dilute formulation of the same bait (1:6.5 @ 7.5L/ha) which resulted in 4.7% infestation.

Infestation levels determined by onsite examination of overripe fruit were generally lower than those in mature fruit which were held for 5-7 days prior to examination. This confirmed that infestation was not being underestimated by sampling mature (but not over ripe) fruit for transport and holding at DPI Indooroopilly. It also demonstrated that infestation determined by field inspection frequently underestimates the true level of infestation because eggs are not able to be detected with the naked eye.

Table 11. Fruit fly infestation in stonefruit from trial blocks in Treetops Orchard at Mundubbera from Sep to Nov 2002.

Treatment Fruit type	Block No.	Mature fruit samples (assessed after holding)		Overripe fruit samples (on site inspection)	
		No. fruit assessed	Percent fruit infested	No. fruit assessed	Percent fruit infested
Peaches					
Hy-Mal bait 15L/ha	3	596	1.0	250	0.7
Hy-Mal bait 15L/ha + 0.5% Keltrol	4	596	0.5	250	0.9
Hy-Mal bait 7.5L/ha	1	596	2.0	250	3.5
Hy-Mal bait 7.5L/ha + 0.5% Keltrol	2	612	1.0	250	0.3
GF-120 (1:6.5) 7.5L/ha	5	615	1.2	250	1.1
Bactrogel-P 5L/ha	6	652	3.3	250	2.2
GF-120 (1:6.5) 7.5L/ha + MAT	12	272	4.4	100	0.0
Chlorpyrifos bait 15L/ha (grower)	13	284	2.8	200	0.0
Untreated peaches (town areas)	-	297	87.5	-	-
Nectarines					
GF-120 (1:6.5) 7.5L/ha	7	637	4.7	250	3.5
Bactrogel-P 5L/ha	8	607	5.4	250	0.8
GF-120 (1:1.5) 5L/ha to trunk	9	514	8.3	250	7.6
Bactrogel-P 5L/ha + MAT	10	619	3.4	250	1.0
MAT only	11	569	14.5	250	13.3

Hy-Mal bait = 2% yeast protein + 0.5% Hy-Mal
Chlorpyrifos bait = 2% yeast protein + 0.2% chlorpyrifos

Bactrogel P used at 13.4g/L
GF-120 bait used at 1:1.5 or 1:6.5

5.4 Discussion

5.4.1 Glasshouse trials

Addition of thickener to bait formulations

These results showed that the addition of thickener significantly increased the efficacy and longevity of bait formulations applied to the foliage of potted host trees held under glasshouse conditions. The addition of thickener did not result in any significant improvements in bait efficacy immediately after application. However, improvements of 25-30% increase in mortality were demonstrated at 4-6 days after application when thickener was added to standard yeast bait using chlorpyrifos, Hy-Mal or abamectin as the toxicant. These trials were conducted in a protected glasshouse situation which excluded possible effects of external factors such as rain, dew and UV exposure on bait longevity. The results however have potentially important implications for commercial bait application in fruit fly host orchards.

The currently used standard bait formulations (eg with Hy-Mal or chlorpyrifos) show a marked reduction in efficacy 4-6 days after application in areas like the Central Burnett in Queensland. Persistency becomes important at times of maximum fruit fly pressure eg on Imperial mandarins in April and in Murcott mandarins in August – September. In such periods many growers currently are spraying twice weekly. If the addition of thickener can result in a 25-30% improvement in efficacy towards the end of the normal weekly spray interval, this would provide a significant benefit to growers and should remove the necessity to spray twice in one week. During 2002-03 the number of Central Burnett citrus growers adding Keltrol thickener to their fruit fly bait increased to about 20%. 2PH citrus and grape orchards at Emerald (1000 ha) are now trialing baits with Keltrol throughout their orchard. The rate of Keltrol being used at 2PH is 2.5 g/L (half the rate used in these trials) but this was still found to give an appropriate consistency to extend the life of the bait.

Effect of reducing protein concentration in bait formulations

With the abamectin bait formulation, 2% yeast generally performed better than 1% or 0.5% yeast baits at 0 or 6 days. However, with the Hy-Mal bait formulation, there were no consistent differences between the three yeast concentrations immediately after application or after 6 days. This suggests that yeast concentration, at least in Hy-Mal baits, could be reduced considerably without loss of effectiveness. However, the efficacy of lower yeast concentrations in baits under field conditions has not yet been fully evaluated. Commercial use of the lower 1% rate is a possibility at low-medium fly pressure but the higher rate (2%) should be used when maximum control is sought during periods of high fly pressure.

There are continual problems with phytotoxic spotting of fruit from current baiting with 2% yeast autolysate with much of the damage due to the protein component of the bait formulation (D. Smith unpublished data). The problem is not confined to Mauri yeast autolysate but also occurs with other available bait proteins. Reduction from 2% to 1% Mauri yeast content was shown to halve the fruit spotting in a field trial on Imperial mandarins at Mundubbera in 2000 and in a trial on Ellendale mandarins in 2001 (D. Papacek *pers. comm.* 2003). Whilst this research has demonstrated that lower protein concentration in baits may provide effective control, the current recommended bait formulation and application rates must be followed if preharvest baiting is included as a specific component in a quarantine protocol for fruit fly control.

Insecticidal components

Hy-Mal at 0.5% is highly toxic to beneficial insects, particularly *Aphytis* spp (see toxicity to beneficial studies in this report) which are an essential component of some IPM programs. The only reason it can be tolerated in an IPM system is because it is applied low on the tree skirt minimising the area of treated foliage. Beneficials (especially *Aphytis* spp.*)* circulate through a tree, however, and repetitive bait applications are still disruptive. This is frequently demonstrated in orchard situations by the increased occurrence of red scale infested fruit from the bait sprayed area on the trees. These glasshouse trials showed that Hy-Mal could be reduced from 0.5% to 0.1% without affecting efficacy (see Table 3). Further evaluation of lower Hy-Mal levels in bait formulations should be undertaken to determine if effective fruit fly control in the field can be achieved at insecticide levels which have minimal effect on beneficials in IPM programs.

Abamectin is currently registered in citrus for mite control at 0.00016% - 0.0004% (1.8-4.5 ppm). In these trials, it was used mostly at 4.5 ppm (ie 0.0004%), which is more than one thousandth of the current Hy-Mal rate and one tenth of the rate of spinosad in GF-120 and fipronil in Bactrogel-P. At this rate abamectin has nil toxicity to the ladybird *Chilocorus circumdatus* Gyllenhall and low to moderate toxicity to *Aphytis lingnanensis* Compere. At 4.5 ppm abamectin has been shown to cause 50% mortality when adult *A. lingnanensis* were exposed to freshly sprayed leaves but little mortality one day after application (Smith *et al.* 1998).

Mortality increased in the glasshouse trials when abamectin was applied to a greater surface area of foliage (ie 1, 2 or 10 leaves) (see Table 4). This equates with 0.5 ml, 1 ml and 5 ml of bait per trial cage. Because of the lower toxicity of abamectin to beneficials, abamectin baits have the potential to be applied to a much larger area of the host tree without seriously disrupting IPM strategies. Whether or not this would improve fruit fly control is not known. Furthermore, applying a larger volume of bait may increase the risk of phytotoxicity unless the concentration of the damaging component (most likely the protein) can be reduced at the same time. The optimum bait volume per tree or per hectare for commercial application has not been clearly defined for Qfly. Currently, Hy-Mal bait is generally applied at 15 L/ha and the new baits tested in this project are applied at much lower rates (GF-120 at 7.5L/ha and Bactrogel P at 5 L/ha). Reduction in volume applied is desirable as this means bait is less likely to hit fruit and produce phytotoxicity. Current application of unthickened baits results in considerable wastage on the ground (possibly 50-75 %) and more judicious application of thickened baits should reduce this and allow less volume to be used. Further field evaluation of some of these promising bait modifications with fine tuning of application rates to meet different crop requirements is warranted.

5.4.2 Field evaluation of modified generic baits

Fruit fly infestation pressure on low chill stone fruit is normally extremely high throughout Queensland with most fruit heavily infested every season unless controls are applied. This is true in the Central Burnett district with high fly numbers building up particularly in the town areas of Gayndah and Mundubbera from late August onwards. Breeding in untreated backyard trees such as loquats, mulberries and stone fruit, and later in the season cherry guavas and mangoes maintains high fly numbers in the district during summer. Peach samples from Mundubbera and Gayndah during October and November had infestations of 59.7% and 100% respectively with an average 12 larvae per fruit.

Fruit fly infestation in the trial stone fruit orchard could be expected to come from migrating mature female flies from Mundubbera and home garden trees in nearby orchards. There were no other fruit fly hosts in the trial orchard or in the dry sclerophyll vegetation on surrounding grazing properties. Because of the number of different peach

and nectarine varieties in the trial orchard, maturing fruit were available from September (early varieties) through to December (late varieties). The orchard had not been maintained properly in 2001 (fruit unharvested, trees unwatered and unfertilised) so fruit set was more variable than in normal commercial practice. Fruit was not being harvested commercially during the trial period which meant that large numbers of over ripe (and potentially infested) fruit were left on the trees or fallen on the ground. All of these factors could be expected to increase the fly pressure throughout the orchard. Offsetting this was the use of MATs at 10 per ha throughout the orchard and the application by the owner of weekly bait treatments to approximately 90 ha of non-trial trees. The presence of MATs throughout this area would be expected to kill many male flies (whether emigrating into the orchard or produced from fallen stung fruit). The subsequent low numbers of males should also make it very difficult for unfertilised females (produced in the orchard) to mate.

The 30 ha area used in the trial was separated for the most part by 500m from the remaining 90 ha of the orchard. The results of trapping in the trial blocks (Table 11) showed that the MATs effectively killed males throughout most of the orchard with near zero fly trap counts in blocks 10, 11, and 13. There was only one block in the trial area (and in the whole orchard) that was not baited. This was Block 11 (Sun Wright nectarines), where the only fruit fly treatment was MATs (at 10 per ha) and the mean level of infestation was 14.5%. When the nectarine crop in this block was finished, a final sample of peaches which had developed from the rootstocks was taken on 22 November. These fruit had an infestation level of 54.5%. These results indicated that MATs alone make a significant contribution to fruit fly control early in the season but in the absence of baiting (for female flies) they will not provide effective control as the season progresses. A similar situation occurred over the whole orchard in the previous season when fruit infestation was close to 100% by November in spite of the orchard wide distribution of MATs.

Efficacy of Bactrogel-P, GF-120, Hy-Mal and Chlorpyrifos baits

All four products performed very effectively in a high pressure situation. Low chill stone fruit are one of the most highly susceptible fruit fly hosts, they mature at the time of year when fruit flies are most active and the poor crop management conditions at the trial site provided excellent conditions for the build up of high fly numbers. The lowest infestation (0.5%) was with Hy-Mal bait (with thickener) in peaches at 15L/ha. GF-120 applied to the nectarine tree forks in Block 9 had an overall infestation rate of 8.3% compared to 4.7% when this bait was applied to the foliage in Block 7. This indicated that although trunk bait application gave significant control it may not provide an acceptable commercial control when used alone. There were small differences between infestation levels in other treatment blocks but no significant differences between treatments could be demonstrated due to the non-replicated nature of the trial.

Comparisons of 7.5L/ha versus 15L/ha of the Hy-Mal bait with and without thickener

Infestation levels in peaches treated with Hy-Mal bait with and without thickener applied at 7.5L/ha were 1.0% and 2.0% respectively and the corresponding infestation levels with 15L/ha treatments were 0.5% and 1.0% respectively. These results showed that all treatments provided effective control but statistical analysis was not possible on the basis of this unreplicated trial. The 15L rate was a little more effective than the 7.5L rate and the use of thickener made both of these treatments also slightly more effective. These results however clearly show that reducing the application rate of currently used bait formulations by half may not have a marked effect on bait treatment efficacy and may provide a very simple solution to the bait phytotoxicity problem currently occurring in a number of crops.

5.5 Conclusions and Recommendations

- Glasshouse testing of baits with and without thickener showed an average 25% improvement in baits with the thickener at 5g/L after 4-6 days. The thickener used was Keltrol F (available from Janbak Industries, Tingalpa, Qld). This costs only $3.60 extra per ha and prolongs the activity of the bait in the critical period towards the end of the weekly spray cycle. A simple, inexpensive method such as this for extending bait life would provide many advantages in field fruit fly control.

- Glasshouse testing showed no significant difference between baits containing Mauri yeast autolysate at the standard 2% versus 1% which suggests that currently used yeast concentrations in baits could be reduced without loss of efficacy. The lower yeast rate is likely to reduce phytotoxic damage to fruit but efficacy under field conditions needs to be confirmed.

- Glasshouse testing showed no significant difference between baits containing Hy-Mal at the currently registered rate (0.5%) and at approximately half that rate (0.2%). Hy-Mal at 0.2% will have slightly lower toxicity to beneficial insects and will reduce the impact of Hy-Mal baits on IPM programs.

- Abamectin at the rate currently registered for mite control in citrus was shown to be effective as a toxicant in fruit fly baits under glasshouse conditions. At 4.5ppm, abamectin bait with thickener gave similar control to Hy-Mal bait without thickener. Abamectin has much lower toxicity to beneficials than Hy-Mal with nil toxicity to ladybirds and low moderate toxicity to *Aphytis spp.* The efficacy of thickened abamectin bait in field situations should be evaluated.

- A large field trial in low chill peaches and nectarines at Mundubbera confirmed the efficacy of new bait formulations (Bactrogel-P and GF-120) in this crop and showed that various modifications to standard bait methodology could provide effective control. Effective control (1-5% infestation) was achieved by all the foliar applied baits in a high fly pressure situation. Bait application to the tree forks was less effective resulting in 9% fruit infestation. The use of MATs alone resulted in 14.5% infestation and much higher infestation later in the season demonstrating that MAT should be used in conjunction with baiting to ensure effective control.

6 Further evaluation of new baits for phytotoxicity

6.1 Introduction

The extensive field trials in a variety of fruit fly host commodities (described in **PART A** of this report) were designed primarily to obtain efficacy and residue data on the new baits for registration application. The new bait products were compared with a currently used generic bait. The generic bait was applied according to current commercial practices for each particular crop and the new baits were applied as recommended by the respective commercial partners. All bait applications were directed at foliage although some contact of bait with fruit was unavoidable in most situations. Qualitative observations of overall bait effects on fruit and foliage of treated crops were undertaken in the course of these trials.

In the field trials in Queensland and New South Wales with Imperial and Murcott mandarins, gala apples, passionfruit, custard apples and blueberries, no serious phytotoxic effects were observed under commercial application conditions with the standard bait or with either of the new baits. However, standard bait is known to cause significant speckling on citrus fruit in some situations and GF-120 caused some spotting on foliage (eg custard apples). In the trials in Western Australia, GF-120 caused serious sooty mould development in Valencia oranges and discolouration of both leaves and fruit in the pome fruit trial (pears).

Additional smaller experimental trials were undertaken to specifically assess phytotoxicity in crops which are known to be highly susceptible to bait damage (eg mangoes and stone fruit). These trials were carried out in mangoes at Mareeba in north Queensland and at Carnarvon in Western Australia and in stone fruit in south west Western Australia. Baits were applied directly to tagged fruit or branches so that closer observations of phytotoxic effects could be undertaken. Results confirmed that mangoes and stone fruit were particularly sensitive to phytotoxic damage with all baits causing severe, commercially unacceptable damage if sprayed directly on to fruit.

In light of the adverse bait effects with some crops, it was deemed necessary to conduct further studies on a larger number of fruit fly host crops in the third year of the project. Crops studied were custard apple (African Pride and Hilary White), banana, avocado (Edranol and Hass), coffee, mango, passionfruit, persimmon, carambola, strawberry, lychee and three varieties of citrus (Hickson mandarin, Late Valencia orange and Washington Navel orange).

6.2 Materials and methods

6.2.1 Bait formulation and application

The four bait and insecticide formulations used in the phytotoxicity study were GF-120 (1L bait + 6.5L water), Bactrogel-P (67g/5L), standard bait (Mauri yeast 20ml/L + Hy-Mal 4.35ml/L) and standard bait + Keltrol thickener (5g/L). Bactrogel-P with powdered protein in the formulation was used in all tests. Treatments were applied on a weekly basis using a 5L backpack sprayer (Sidewinder banana injector with adapted handheld nozzle), which delivered 15ml per pump (Figure 7).

Figure 7. Sidewinder backpack sprayer

All crops, except the African Pride custard apples, were tested at the DPI Maroochy Horticultural Research Station, Nambour in either experimental blocks or as potted plants in a glasshouse, between 25 March and 3 June 2003 (Table 12). Tests were conducted concurrently with all crops experiencing the same climactic conditions (either field or glasshouse). Each week for 11 weeks either 15ml or 30ml of bait was sprayed onto either a $0.1m^2$ or $0.25m^2$ area of the plant (see Table 12). In the case of the three citrus varieties and Hass avocado, the treated area included both foliage and fruit. Other crops were not fruiting at the time of testing. Each of the four bait treatments was replicated between four and ten times (see Table 12) and an untreated control was included in each replicate. Each treatment was applied to a separate plant.

The African Pride custard apples were tested at a commercial block at Woombye, between 18 March and 20 May 2003. The custard apple block consisted of twelve rows of 13-16 trees each. Rows ran east west and baits were applied on the south side. Each week for 10 weeks, 30ml of bait was sprayed onto a $0.25m^2$ area of foliage 1m above the ground. The same area of foliage was treated on each occasion. Each of the four bait treatments was applied to a complete row of trees and was replicated three times. Treatment order within the block was not randomised. Untreated controls were not included as it was unacceptable to the grower to leave a proportion of trees without fruit fly control. To compensate for lack of control trees, unsprayed areas of treated trees were examined for the presence of damage.

Table 12. Details of the bait phytotoxicity studies

Crop	Number of replicates	Plant Size	Study location	Volume bait applied/week	Number of weekly bait applications	Total area of plant treated	Parts of plant treated
Custard Apple (African Pride)	3	5m	Commercial block (Woombye)	30ml	10	0.25m^2	Mature foliage
Custard Apple (Hilary White)	10	3m	Experimental block*	30ml	11	0.25m^2	Mature and semi-mature foliage
Banana	4	1-1.5m	Experimental block*	30ml	11	0.25m^2	Young foliage
Avocado (Edranol)	4	1m	Glasshouse*	15ml	11	Whole plant	Mature foliage
Avocado (Hass)	5	5m	Experimental block*	30ml	11	0.25m^2	Mature foliage and ¾ developed fruit
Coffee	4	1m	Glasshouse*	15ml	11	Whole plant	Mature foliage
Mango (Kensington Pride)	10	4m	Experimental block*	30ml	11	0.25m^2	Young foliage
Passionfruit	4	Mature vines	Experimental block*	15ml	11	0.1m^2	Mature foliage
Persimmons	4	3m	Experimental block*	30ml	11	0.25m^2	Mature foliage
Carambola	4	5m	Experimental block*	30ml	11	0.25m^2	Mature foliage
Lychee	4	2m	Experimental block*	15ml	11	0.1m^2	Young foliage
Strawberry	4		Glasshouse*	15ml	11	Whole plant	All foliage
Citrus (Hickson mandarin)	5	3m	Experimental block*	30ml	11	0.25m^2	Mature foliage, green fruit
Citrus (Late Valencia orange)	5	2m	Experimental block*	30ml	11	0.25m^2	Foliage and fruit
Citrus (Washington Navel)	8	3m	Experimental block*	30ml	11	0.25m^2	Foliage and fruit

* Maroochy Horticultural Research Station, Nambour

6.2.2 Evaluation of bait effects

The rating system used to evaluate overall effects of the baits on the plants is shown in Table 13. Based on previous studies, possible bait effects included stickiness, sooty mould accumulation, black spotting and leaf burn.

Table 13. Rating system used to evaluate bait effects

Rating	Percentage of foliage in sprayed area showing bait effects
0	Zero effect
1	1-10%
2	11-25%
3	26-50%
4	> 50%

6.2.3 Statistical analyses

Trials conducted at Maroochy Research Station

The ratings were analysed as a randomised block design in Genstat 6. In several cases the ratings of one or more treatments resulted in a zero rating in each of the replicates. Though strictly speaking these treatments should be excluded from the analysis (they have zero variance and hence break the assumption of all treatments having the same variance), they have been retained in the analyses to provide complete comparisons between treatments. Their retention in the analyses did not result in residual plots that were unsatisfactory and hence they were judged not to have adversely affected the analysis. Comparison between the treatments was done using a Fisher's Protected Least Significant Difference (LSD) test.

African Pride custard apple field trial

The ratings were analysed as an unbalanced, randomised block design in Genstat 6 (using the number of trees tested as weights). Comparison between the treatments was done using a Fisher's Protected Least Significant Difference (LSD) test.

6.3 Results

Results of the evaluation of phytotoxic effects of different bait treatments after repeated applications to a range of fruit fly hosts are shown in Table 14. Generally, a rating of 1 or 2 indicated stickiness and/or accumulation of mould and a rating of 3 or 4 indicated black spotting and/or leaf burn. Photographs of bait effects used in this report were taken two months after the final treatments. Whereas most mould had washed off the foliage, the permanent effects of spotting and burn were still evident.

Table 14. Incidence of bait effects on commercial crops

Crop	Mean ratings of bait effects on commercial crops§				
	Treatment				
	Untreated	GF-120 1:6.5	Hy-Mal + Mauri yeast (no thickener)	Hy-Mal + Mauri yeast (thickener)	Bactrogel -P
Hilary White custard apple	0.000 a	2.200 d	0.400 ab	0.700 bc	1.000 c
	d.f. 4,36 F=20.13 probability† <0.001 LSD (5%) 0.5340				
Edranol avocado	0.000 a	3.500 c	0.000 a	1.000 ab	2.000 b
	d.f. 4,12 F=9.43 probability† =0.001 LSD (5%) 1.488				
Hass avocado	0.000 a	2.600 b	0.000 a	0.400 a	0.200 a
	d.f. 4,16 F=16.82 probability† <0.001 LSD (5%) 0.8100				
Kensington Pride mango	0.000 a	1.100 b	0.000 a	0.200 a	0.400 a
	d.f. 4,36 F=10.29 probability† <0.001 LSD (5%) 0.4079				
Banana	0.000 a	1.000 b	0.250 a	0.750 b	1.000 b
	d.f. 4,12 F=9.00 probability† =0.001 LSD (5%) 0.4665				
Hickson mandarin					
Leaf	0.0	0.4	0.0	0.2	0.2
Fruit	0.0	0.0	0.0	0.0	0.0
Late Valencia orange					
Leaf	0.0	1.0	0.0	0.0	0.0
Fruit	0.0	0.0	0.0	0.0	0.0
Washington navel					
Leaf	0.0	0.9	0.0	0.0	0.0
Fruit	0.0	0.0	0.0	0.0	0.0
Coffee	0.0	0.0	0.0	0.0	0.0
Passionfruit	0.0	0.5	0.0	0.0	0.0
Persimmon	0.0	1.0	0.0	0.0	0.0
Carambola	0.0	1.0	0.0	0.0	0.25
Lychee	0.0	0.25	0.0	0.0	0.0
Strawberry	0.0	0.0	0.0	0.0	0.0
African Pride custard apple		2.027 c	0.388 a	0.925 b	1.251 b
	d.f. 3,6 F=35.33 probability† <0.001 Average LSD (5%)‡ 0.4118				

§ Means followed by the same letter across a row are not significantly different at the 5% level
† F probability (treatment effect in the ANOVA): >0.05 is non significant
‡ Average LSD is given though comparisons between treatments have been made using exact LSD values

Custard apples (Hilary White)
Results of the study on Hilary White custard apples revealed significant treatment differences ($F_{4,36}$ = 20.13, P<0.001) in mean ratings for bait effects. GF-120, Hy-Mal + Mauri yeast (thickener) and Bactrogel-P had significantly higher ratings than the untreated control, with GF-120 being significantly greater than all other treatments. Bait effects observed included mould and black spotting on the treated foliage.

Edranol avocado
Results of the study on Edranol avocado revealed significant treatment differences ($F_{4,12}$ = 9.43, P<0.001) in mean ratings for bait effects. GF-120 and Bactrogel-P had significantly higher ratings than the untreated control, with GF-120 being significantly higher than all other treatments. GF-120 produced black spotting and leaf burn. Hy-Mal + Mauri yeast (thickener) and Bactrogel-P produced mould and very light spotting.

Hass avocado
Results of the study on Hass avocado revealed significant treatment differences ($F_{4,16}$ = 16.82, P<0.001) in mean ratings for bait effects. GF-120 had significantly higher ratings than the untreated control and all other treatments. GF-120 produced mould, black spotting and leaf burn (Figure 8).

Figure 8. Avocado leaves - treated with GF-120 showing permanent spotting (left) and untreated control (right).

Kensington Pride Mango
Results of the study on Kensington Pride mango revealed significant treatment differences ($F_{4,36}$ = 10.29, P<0.001) in mean ratings for bait effects. GF-120 had significantly higher ratings than the untreated control and all other treatments. Bait effects in evidence with GF-120 were mainly stickiness and mould accumulation, with some light spotting. Figure 9 shows mould and spotting on the lower surface of a leaf treated with GF-120 and an untreated control leaf. Figure 10 shows mould on the upper surface of a mango leaf treated with GF-120 and an untreated control.

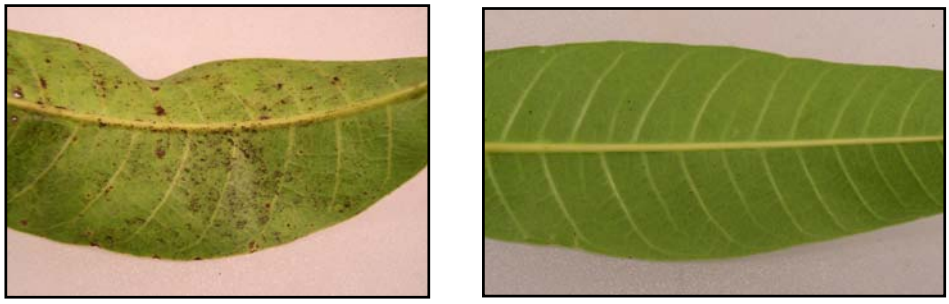

Figure 9. Lower surfaces of mango leaves – treated with GF-120 showing spotting and mould accumulation (left), untreated control (right).

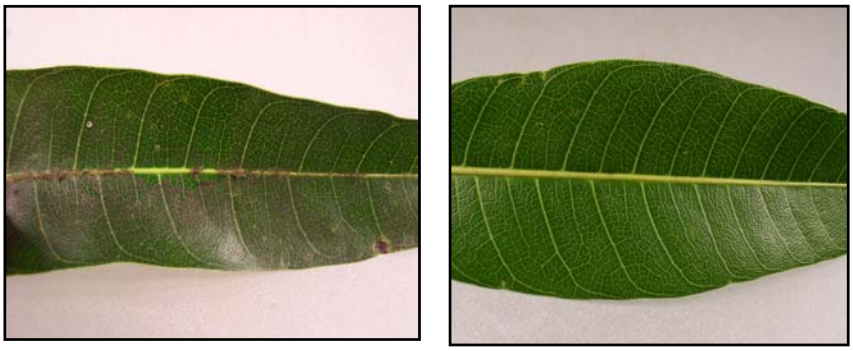

Figure 10. Upper surfaces of mango leaves - treated with GF-120 showing mould accumulation (left), untreated control (right).

Banana
Results of the study on bananas revealed significant treatment differences ($F_{4,12} = 9.00$, $P<0.001$) in mean ratings for bait effects. GF-120, Hy-Mal + Mauri yeast (thickener) and Bactrogel-P had significantly higher ratings than the untreated control. Bait effects observed were limited to mould accumulation and stickiness.

The ratings in the studies on Hickson mandarin, Late Valencia orange, Washington navel orange, coffee, passionfruit, persimmon, carambola, lychee and strawberry were not analysed because of the high number of zero ratings (ie no bait effects observed). With the exception of GF-120, only Bactrogel-P (Hickson mandarin and carambola foliage) and Hy-Mal + Mauri yeast (thickener) (Hickson mandarin foliage) showed any evidence of bait effects and in each case the effect appeared at a rating of 1 and only in a single replicate.

Hickson mandarin
Results of the study on Hickson mandarin revealed few bait effects.

Late Valencia orange
Results of the study on Late Valencia orange revealed very few bait effects. There was a slight accumulation of mould on foliage treated with GF-120.

Washington navel orange
Results of the study on Washington navel revealed very few bait effects. There was a slight accumulation of mould on foliage treated with GF-120.

Coffee
Results of the study on coffee revealed no bait effects.

Passionfruit
Results of the study on passionfruit revealed a trace of mould on foliage treated with GF-120.

Persimmon
Results of the study on persimmon revealed very few bait effects. There was a slight accumulation of mould on the foliage treated with GF-120.

Carambola
Results of the study on carambola revealed very few bait effects. There was a slight accumulation of mould on the foliage treated with GF-120.

Lychee
Results of the study on lychee revealed very few bait effects. There was a slight accumulation of mould on the foliage treated with GF-120.

Strawberry
Results of the study on strawberry revealed no bait effects.

African Pride custard apple field trial
The results of the study on African Pride custard apple revealed significant treatment differences ($F_{3,6} = 35.33$, $P<0.001$) in mean ratings for bait effects. GF-120 had a significantly higher rating than the other baits. Though the bait effect could be confounded with position in the block in this trial (treatments were not randomised within blocks), this finding is supported by the results for GF-120 found in other crops in this study. Hy-Mal + Mauri yeast (thickener) and Bactrogel-P also had a significantly higher rating than Hy-Mal + Mauri yeast (no thickener) and this too is supported by the results found in other crops. There was no evidence of damage on untreated parts of the custard apple trees. Bait effects on treated foliage included stickiness, mould accumulation, black spotting and leaf burn. Figure 11 shows a leaf treated with GF-120 with evidence of leaf burn.

Figure 11. Custard apple leaf treated with GF-120, showing permanent leaf burn.

6.4 Discussion

This series of studies has provided for the first time a quantitative assessment of the phytotoxic effects of new and existing fruit fly baits on a wide range of crops. In the varieties of custard apple, avocado and mango tested, significant differences in effects between different baits, and between baits and controls were obtained. In all these crops GF-120 produced significantly higher ratings than the other baits, indicating more extensive and severe bait effects. In studies where results were not analysed and where bait effects were evident, GF-120 also had the highest mean rating. Bait effects observed during this study were those expected from previous trials (stickiness, mould accumulation, black spotting and leaf burn) and no additional bait effects were observed on foliage or fruit (where tested).

This research demonstrated that thickened baits (particularly GF-120) were more likely to cause phytotoxicity problems than standard bait without thickener. However, in other research in this project, adding thickeners to generic baits has been shown to significantly extend bait longevity. The severity of phytotoxic effects varied significantly with different crop types. In some crops (mango, custard apple and avocado) damage may be severe and commercially unacceptable unless extra precautions are taken when applying baits. In other crops, bait effects appear to be generally minimal, but care should still be taken when applying new thickened baits.

It is possible that new thickened baits could be used in an alternative off-crop manner to provide effective protection in crops highly sensitive to bait phytotoxicity. This is currently being researched by DPI in HAL Project HG02066 (Development of non-foliar bait spot treatments for fruit fly control) where the efficacy of bait application to non-foliar materials such as plywood squares, hessian bags, and carpet squares hung in and around host trees is being evaluated under experimental and field conditions. It is hoped that the outcomes of this research will provide a practical and effective solution to overcome the bait phytotoxicity problems which still exist.

6.5 Conclusions and Recommendations

- Thickened baits (particularly GF-120) caused phytotoxic effects on mango, custard apple and avocado but had few effects on the other crops tested.

- Care should be taken in the application of all thickened baits, especially in mango, custard apple and avocado.

- Investigations should be continued into innovative bait application techniques that maximise the benefits of increased longevity of thickened formulations but minimise treatment contact with fruit and foliage in crops which are highly susceptible to phytotoxic damage.

7 Application technology for the new fruit fly baits

7.1 Introduction

The new bait products (GF-120 and Bactrogel-P) evaluated in **PART A** of this project are more viscous, may require more mixing and are applied at lower volumes per hectare than are currently used baits. This component of the project was designed to provide practical recommendations on the equipment required to prepare and deliver these new products and thickened generic baits most effectively.

Currently used baits do not usually require any special mixing techniques. These baits are applied at approximately 15L per hectare depending on the crop type. The application methodology is generally determined by the size of the orchard. For most commercial orchards (2 ha or more), growers use mechanised transport to apply baits eg. a quad bike or a small tractor drawn unit. Orchards under 5-10 ha can readily be sprayed with a hand-held spray pistol delivering the required dose (to foliage low on the tree) avoiding most large clumps of fruits. With larger orchards (25 ha or more) greater mechanisation is needed, with a system of fixed (but adjustable) spray nozzles delivering the spray in a narrow band on to the lower part of the tree.

Thickened baits require more thorough mixing to ensure that filter pumps do not become clogged. The new products (GF-120 and Bactrogel-P) are applied at 5-7L/ha (ie. 33-50% of the current bait rate). Application methods must allow baits to be applied at these lower rates while still maintaining reasonable ground speeds.

7.2 Bait preparation

GF-120 is a dark brown viscous liquid which is mixed with water at a rate of 1:6.5. Bactrogel-P is a powder formulation which is mixed with water at 13.4g per L. A commercially available powdered thickening agent (Keltrol–a xanthan gum) was tested with the current yeast protein and insecticide baits at the rate of 5g per L. All of these products can be mixed in a 20L galvanised drum using a medium-high duty electric drill with a steel commercial paint stirring attachment. Plastic drums should be avoided to prevent fine shreds of plastic from the inside of the drum being mixed with the solution. Where 100L or more of spray is to be mixed a heavier duty mixer will be required. Ideally there should be provisions to mix the bait and thickener in the actual spray tank. This can be facilitated by providing an in-tank mixing unit, run off the tractor PTO (Power Take Off) or the quad bike battery. The tank is filled with water, the yeast and toxicant added and finally the Keltrol. The tank is then agitated for 15-30 mins to thoroughly mix (Figure 12). Whatever the quantity of bait to be mixed, strong mechanical agitation for a few minutes is required to prevent the formation of lumps particularly when dealing with powdered ingredients. Complete mixing and an even consistency cannot be achieved with manual stirring. Keltrol and water can be mixed and stored for future use but protein and pesticide should be added just prior to use.

Figure 12. 1000L spray-vat, tractor drawn, for thickened fruit fly bait application. Note the in-tank stirrer for mixing the bait.

7.3 Bait application in small orchards (up to 5 ha)

The thickened baits are applied at 5-7.5L per ha so 1-2 ha can be effectively sprayed with a backpack of about 12L capacity. Conventional knapsacks (holding about 15L) are unsuitable for spraying the thickened products because the pumping mechanism and spray delivery system quickly become clogged.

The most efficient small sprayer was found to be a backpack manufactured by Sidewinder Tree Injectors (Figure 13). The equipment is a banana backpack spear injector with the spear replaced with an adjustable spraying nozzle. It is used in the banana industry for diesel injection of suckers or for bell injecting and bunch spraying. The design features a lightweight fibreglass shell together with a hand driven pump and a 5-10L spray bottle (approx. cost $600 per unit). A single pump of the spray handle comfortably delivers 15-20ml of the thickened bait spray over a distance of 2-3m.

Figure 13. Sidewinder Backpack Sprayer

7.4 Mechanised bait application for larger orchards

Growers can readily adapt existing bait sprayers, either tractor drawn or the smaller units mounted on agricultural quad bikes, to deliver thickened baits. Alterations may be necessary to replace or remove filters that are too fine for the thickened bait mixtures and to ensure that spray nozzle apertures also are not too fine. Nozzles can be adjusted to vary the coarseness and width of the spray jet.

In this project, 30ha of peaches and nectarines were sprayed weekly for three months with a Silvan Selecta Rakpak unit fitted to a quad bike (Figure 14). The fibreglass tank held 50L (tank capacities range from 14-200L). A 12 volt 'Shurflo' pump (fin cooled) with twin spray nozzles (nozzle aperture and nozzle direction adjustable) delivered a steady jet of bait 3-4m either side of the bike. The volume rate of spray per ha could be varied by the nozzle aperture, the speed of the bike, the number of rows sprayed and by use of a trigger, permitting spray to be applied in bursts rather than continuously. The pump worked at 300 kpa (45 psi) delivering 13.6L/min. Total cost of the unit was $600. Smaller Shurflo pumps eg. 8000 series with a capacity of 5.3L/min or the 2088 series with a capacity of 11.3L/min cost $257 and $313 respectively and are also sufficient for the job.

Figure 14. Quad bike with Silvan Rakpak fitted with Shurflo Pump.

To apply 7.5L of bait per ha the bike was driven between rows 1 and 2 and then back between rows 3 and 4 at 20 kph. Any foreign matter (eg. plastic shreds or pieces of wood) in the mixture tended to clog the nozzle so care was necessary when preparing the bait mixture. No problems were encountered with the pump or with spray delivery to the target during three months of use in the trial. 2PH orchards at Emerald (1000 ha of citrus and grapes) are currently trialing a 1000L tank unit with an in-tank stirring mechanism running off the tractor PTO. The thickened bait (Keltrol in this case at 2.5g/L) is pumped at low pressure into paired polythene drip hoses, which drape over the grapes or citrus as the unit drives by. This system simplifies mixing and minimises loss of bait falling to the ground and contact of bait on fruit (Figure 15). The length of the drip hoses can be varied to obtain ideal placement in the crop being baited.

Figure 15. Applicator for thickened fruit fly bait being used in grapes at Emerald. Note the 1000L tank, in-tank stirrer, paired drip hoses for dripping/wiping the bait on the vines.

7.5 Recommendations

The following equipment was determined to be the most effective for treating orchards with the new thickened fruit fly baits.

- For treating small orchards up to about 5 ha, a suitable backpack sprayer was the Sidewinder banana backpack spear injector (with the spear replaced with an adjustable hand held spray nozzle) delivering 15-20ml of bait 2-3m to the tree.

- For larger areas, a Silvan Selecta Rakpak (or comparable unit) fitted to a quad bike using a 12 volt pump with twin adjustable spray nozzles was effective. Volume output can be adjusted by varying bike speed, number of tree rows treated or by use of a trigger mechanism. Tractor driven small tank units are also suitable but adjustment may be necessary to pump filters to avoid clogging.

PART C – Year 3 project extension

8 Preliminary research related to area-wide management of fruit fly in the Central Burnett

8.1 Introduction

In 2002, Horticulture Australia Ltd (HAL) commissioned an Australia wide feasibility study on the potential for Area-Wide Management (AWM) of fruit flies in endemic areas (Final Report Project AH01016 by Keith Jorgensen, April 2002) The Central Burnett citrus production area in Queensland was given the highest rating as a potential site for implementing an effective AWM scheme. In May 2002, DPI researchers, crop consultants, citrus growers and local government authorities in the Central Burnett formed the Central Burnett Area-Wide Management Committee (CBAWMC) to develop a proposal to seek funding for implementing an area-wide management program. This proposal was subsequently approved by HAL and the Project AH03002 (Area-wide management of fruit fly- Central Burnett) lead by Dr Annice Lloyd, DPI, commenced in July 2003.

The CBAWM program is to be based on area wide application of Male Annihilation Technology (MAT) as an additional fruit fly control strategy to supplement the wide spread baiting practices that are currently employed on almost all of the seventy citrus orchards in the district. The possibility of using MAT, particularly to target peak spring fruit fly populations, was recommended by the DPI researchers in this current project who also undertook an extensive study of fruit fly issues in the Central Burnett from 1997 to 1999 (Lloyd *et al.* 2000 HRDC Final Report Project CT97036).

From mid 2001, some growers in the Central Burnett, realizing the potential benefits of MAT, began implementing this technology to supplement on-farm baiting. MAT carriers (cotton wicks dosed with cue lure and malathion) were produced and distributed by local crop management company, Bugs for Bugs, in Mundubbera. Whilst MAT for fruit fly control has been used previously in Australia (eg fruit fly exclusion zones, papaya fruit fly eradication in north Queensland), this technique has not previously been applied to Qfly under Queensland conditions. The impact of MAT in the district needed to be assessed as quickly as possible, preferably in the 2002 citrus season, and prior to the commencement of the AWM project.

By mid-2002, MAT had been introduced mainly in Gayndah orchards with minimal implementation in the Mundubbera area. In consultation with the local crop consultants, this situation was maintained during the evaluation period so that comparative data from areas with and without MAT could be obtained and compared to data from previous research activities in the district (DPI- HAL Project CT97036).

The longevity of different cue lure MAT carriers (eg dosed wicks in plastic cups or treated Cane-ite blocks) under Central Burnett conditions also needed to be evaluated prior to the commencement of any AWM program.

In 1999, a twelve month survey of fruiting crops in the Central Burnett carried out by DPI showed that there were almost no native hosts in the area but untreated cultivated fruit were likely to provide ideal breeding sites for Qfly (HAL Final Report CT97036). One of the aims of this preliminary AWM research was to obtain quantitative data on the extent of fly infestation in untreated back yard fruit trees in the towns of Gayndah and Mundubbera. This involved identifying type and numbers of fruit trees and

sampling fruit for determining infestation levels. Such information would be used for planning town treatments and would also provide baseline data for later evaluation of the AWM program.

8.2 Materials and Methods

8.2.1 Effects of MAT on trap catches in the Central Burnett.

From August 2002 to August 2003, cue lure traps were monitored at selected sites across the district. Four traps were installed on each of 4 citrus orchards in the Gayndah region and 4 citrus orchards in the Mundubbera region. The Gayndah orchards were implementing MAT and Mundubbera orchards were without MAT. As well as the orchard traps, 4 traps were installed in each of the townships of Gayndah and Mundubbera. For each property, the traps were installed by the grower and distributed across the property with one trap in or near each of 4 blocks of citrus. On properties where MAT had been implemented the traps were located no closer than 50m from the nearest MAT device to minimise competition effects. In some cases this involved hanging the trap in a suitable area outside the citrus block eg trees along the river or fence line. All traps were cleared weekly and the flies sent to DPI Indooroopilly for counting and identification.

8.2.2 Town fruit survey

Host fruit sampling

Targeted fruit collections were made in the town areas of Gayndah and Mundubbera between September 2002 and March 2003. Fruit were collected on each of seven survey dates which were planned to coincide with the peak fruiting times of known major hosts in the town areas. Sample sizes from backyard trees varied considerably depending on the size of the tree/crop and the cooperation of property owners. Fruit samples were collected in labelled brown paper bags and allocated a code number. Detailed descriptions of tree, site address, stage of maturity and condition of fruit were recorded at the time of collection.

Fruit assessment

Fruit samples were transported to the DPI laboratory at Indooroopilly for holding at 25°C and 60-70% relative humidity to allow fruit fly eggs and larvae to develop. Each sample was counted and weighed before being held on moist vermiculite in a gauze-topped plastic container. Soft fruit, which were likely to break down, were placed on gauze-topped drip containers before being placed in larger holding containers. Samples were examined for pupae after seven days and then checked approximately twice per week until no further pupae were recovered. Before discarding, each sample was cut open to determine if any larvae or pupae were still present. Pupae were held until flies emerged, and were counted and identified. The numbers of flies produced per fruit and per kilogram of fruit were recorded.

Host tree survey

A preliminary survey of the town areas of Gayndah and Mundubbera was carried out to identify and count known hosts of Qfly. A more detailed survey involving requesting permission to enter private properties to accurately determine host tree numbers will be undertaken in the AWM project.

8.2.3 Comparison of MAT carriers

Two different types of MAT carriers dosed with cue lure and malathion and exposed to natural weathering conditions in the Central Burnett were evaluated over a six month period to compare longevity and changes in chemical content.

MAT carriers

Wicks

Wick carriers produced commercially by 'Bugs for Bugs' for use in monitoring traps and as MAT devices (Figure 16) consisted of a plastic cup 6cm in diameter and 1.5cm deep with a circular groove near the margin of the underside, which accommodated a cotton dental roll. This dental roll was dosed with a mixture of 1ml cue lure and 1ml Maldison 500.

Blocks

MAT blocks consisted of small squares (50x50x12.5mm) cut from a sheet of CSR Cane-ite (unprimed). Near one corner of the block a wire was threaded and twisted through a 1.8mm hole to enable the block to be suspended (Figure 2). The blocks were dosed with a mixture of 1ml cue lure and 0.5ml Maldison ULV which equated to the same quantity of active ingredient (Malathion) as in the wicks. Maldison ULV was used in the blocks because this formulation of the insecticide had been shown to be highly effective in methyl eugenol MAT blocks used in the DPI Papaya Fruit Fly Eradication program in north Queensland in 1995-1998 (Lloyd *et al* 1998). A prepared mixture of ULV malathion and cue lure was applied to one side of the block and allowed to permeate the block overnight before use.

Figure 16. Bugs for Bugs Wick dosed with 1ml cue lure/ 1ml Maldison 500

Figure 17. Cane-ite Blocks dosed with 1ml cue lure/ 0.5 ml Maldison ULV

Weathering

120 freshly dosed wicks and 120 freshly dosed blocks were hung in exposed conditions at 'Bugs for Bugs' at Mundubbera on 17/9/2002.

Sampling

At each evaluation time, a subsample consisting of 6 blocks and 6 wicks was taken, sealed in Ziplock bags and sent by overnight courier to Indooroopilly. If, due to

weather conditions or time constraints, the test could not be commenced within 2 days of receipt then the samples were frozen until conditions for testing were suitable. Blocks and wicks were removed for testing on eleven occasions between 1 and 36 weeks after the commencement of the experiment.

Experimental design

The relative efficacy of weathered carriers to new carriers of the same type was determined in paired comparison tests in a large fruit fly proof netted enclosure over a block of mature nectarine trees at Redlands Research Station. The experimental design was a split block with 3 replicates of each carrier. Each replicate consisted of 2 days testing. Old vs. new blocks were tested on one of the days and old vs. new wicks on the other day. The testing order of wicks and blocks was determined by random selection for each replicate. Within the field cage, four positions were chosen to maximise the distance between any two treatments (Figure 19). Positions A & B were treated as a pair and positions C & D were treated as a second pair. Within a pair of positions the allocation of old and new carriers was determined by random selection.

On the day of a test, each MAT carrier was suspended over a plastic bucket, which was itself suspended over a white calico drop sheet 1.5x1.5m on an aluminium frame (Figure 18). Two cages each of ~1200 flies (2400 total) were then released, one at each of the two release points. This gave approx 1200 males at each release. The flies used were from the Indooroopilly *B. tryoni* colony. All flies were protein fed and between 10 and 18 days old at the time of the test. For each age of the MAT carriers tested, all flies were from the same cohort.

Twenty- four hours after the release, flies from both buckets and sheets were collected into labelled specimen boxes and returned to Indooroopilly for counting and recording.

Figure 18. Collection system for assessing MAT carriers. Test devices attached to bucket handle at suspension point.

Chemical analysis of MAT carriers

Chemical analyses of weathered blocks and wicks for malathion, cue lure and raspberry ketone were performed after 0, 4, 12, 20 and 28 weeks. Analyses were carried out by Graham King, Pathology and Scientific Services, Queensland Health. Raspberry ketone (also known as Willison's lure) is a closely related analogue of cue lure and can occur as a cue lure degradation product. There are conflicting reports about whether raspberry ketone is more or less attractive than cue lure (Cunningham

1989). Analysis of both types of MAT carriers for the presence of raspberry ketone was undertaken to determine if significant amounts of this compound were being formed during weathering and if these changes could account for any changes in efficacy of the carriers.

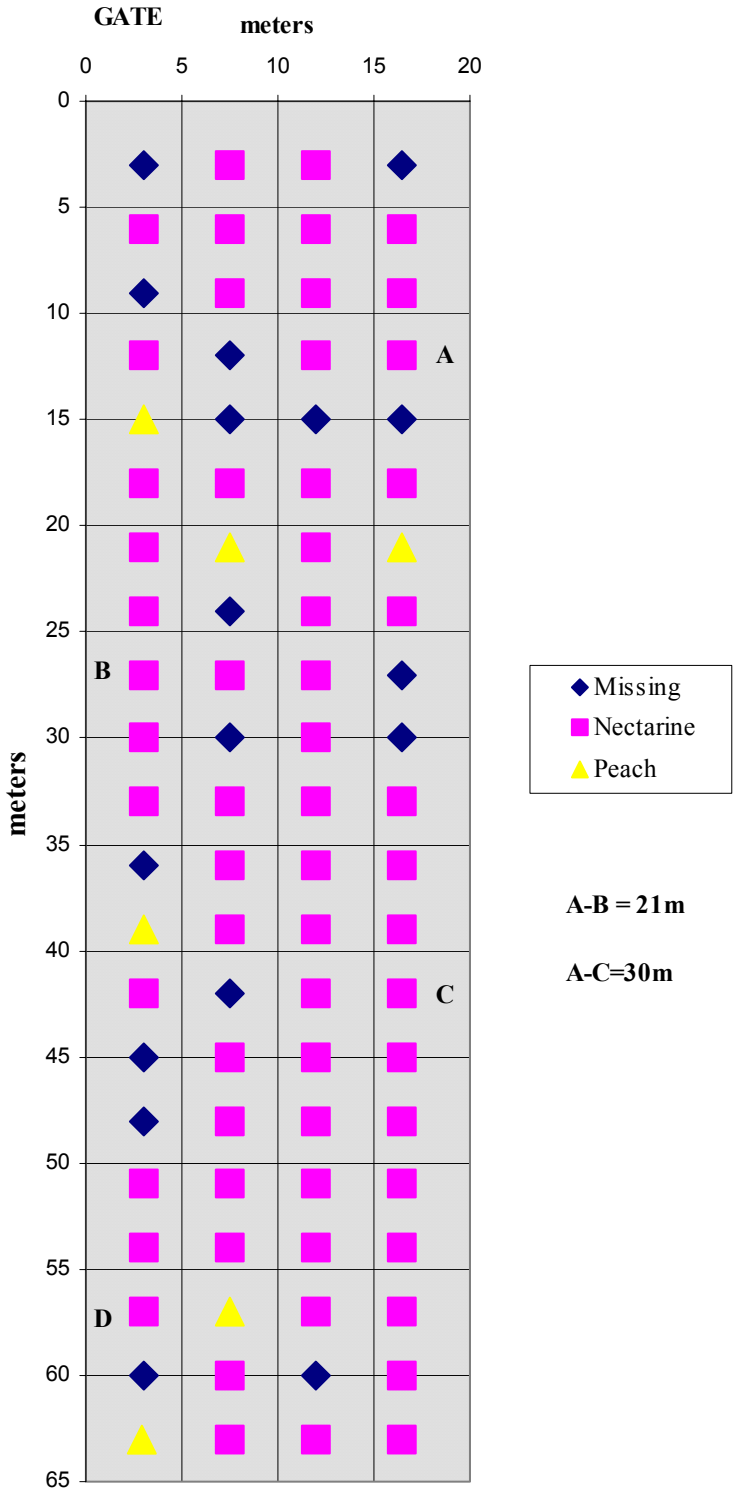

Figure 19. Experimental design for paired comparison testing of new and old MAT carriers using released flies in a large field cage at Redlands Research Station.

8.2.4 Evaluation of trap designs

Trap designs

Six different trap designs for holding cue lure/malathion dosed wicks were evaluated to determine the most effective trap type to be used in the area-wide management program in the Central Burnett. Bugs for Bugs (Mundubbera) provided samples of 4 design variations of their fruit fly trap. Each of these designs included either 2 or 4 plastic inserts (entry ports) in which holes of diameter 15mm or 30mm had been cut. A Sensus trap with 12 x 7mm holes (no entry ports) under the lid as used in South Africa and a standard Lynfield trap (4x 25mm holes, no entry ports) as used in all DPI activities in this project were included for comparison. All traps were baited with a new 'Bugs for Bugs' cue lure cup (1ml Maldison 500: 1ml cue lure) at the start of the experiment. The trap designs tested were as follows:

Figure 20. Standard Bugs for Bugs trap: -
2 x 15mm diameter holes in entry ports

Figure 21. Modified Bugs for Bugs trap: -
2x 30mm diameter holes in entry ports

Figure 22. Modified Bugs for Bugs trap: -
4 x 15mm diameter holes in entry ports

Figure 23. Modified Bugs for Bugs trap: -
4 x 30 mm diameter holes in entry ports

Figure 24. South African 'Sensus' trap: - 12 x 7mm square holes (under lid)

Figure 25. DPI Lynfield style trap: - 4 x 25mm diameter holes, no entry ports

The relative efficacy of the six trap designs was compared using released flies from the DPI *B. tryoni* colony in the fruit fly netted nectarine orchard at the Redlands Research Station. This enclosure contained 4 rows x 20 mature "Sunrise" nectarine trees. Six sites were selected in the cage to maximise the distance between any two traps. A 1.5x1.5m calico drop sheet (on an aluminium frame) was positioned under each site to catch any flies knocked down outside the trap.

On the day of the test, a trap style was randomly allocated to each position and approximately 4000 laboratory reared, 10 day old, protein fed *B. tryoni* were released with approximately half of the flies being released at each end of the cage.

The traps and sheets were cleared after 24 hours, the flies collected into separate labelled specimen boxes and returned to Indooroopilly for counting and recording. Six replications of this test were carried out on six different days, each with a new release of flies.

8.3 Results

8.3.1 Area-wide trapping

Figure 26 presents a summary of the trap catches in the 4 areas ie Gayndah town, Gayndah orchards, Mundubbera town, Mundubbera orchards. A moving average trendline was used to simplify the graph by smoothing out weekly variance in the mean trap catches. The trapping results in the Mundubbera orchards followed a similar pattern to that observed in 1999 (Figure 2) with major peaks in fruit fly activity in late August, October, late January and March coinciding with the fruiting periods of the major untreated hosts found in the towns. Trapping within the town of Gayndah was only able to continue up to the 15th January due to unforseen circumstances however, over the period up to this date the trap catches in the two towns were very similar and reflected summer breeding in untreated backyard host trees. At times of known high fruit fly activity, trap catches in Gayndah orchards with MAT were greatly reduced compared to those in Mundubbera orchards where no MAT was being implemented.

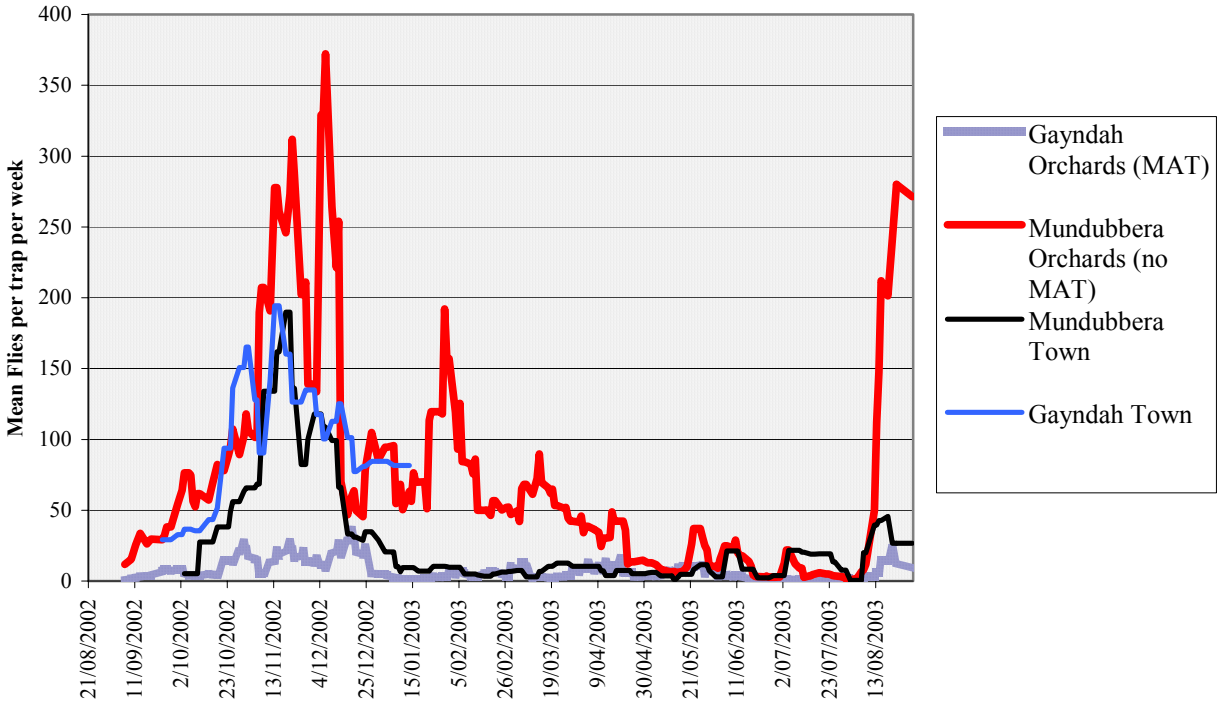

Figure 26. District trapping summary (6 period moving trendline) – Central Burnett 2002-03.

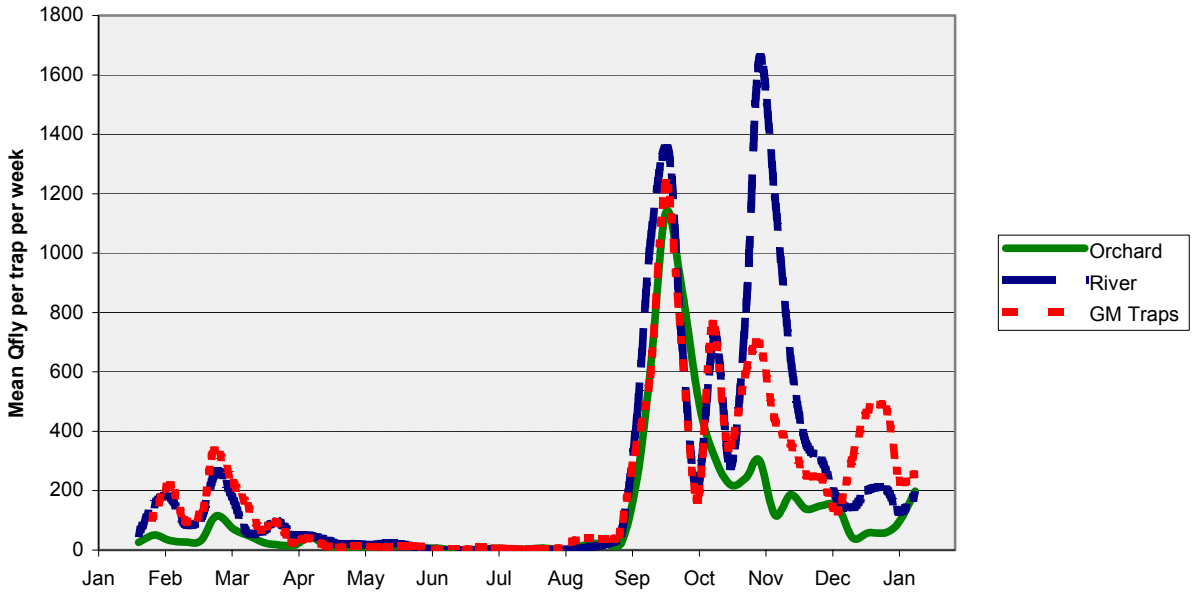

Figure 27. Comparison between Orchard, River and Town (GM) traps – 1999

Of the 79,098 flies trapped and identified, 79.3% were caught in Mundubbera orchards, 6.6% in Gayndah orchards, 6.7% in Mundubbera town, 7.4% in Gayndah town (only recorded for 4 months). *B. tryoni* represented 96% of the flies caught, *B. neohumeralis* 2% and other species (*Dacus aequalis, B. bryoniae, D. newmani, B. quadrata, B. chorista, B. jarvisi* in order) made up the remaining 2%.

8.3.2 Town fruit survey

Fruit fly Infestation

The survey of summer fruiting hosts in the towns of Gayndah and Mundubbera resulted in 92 samples weighing 81kg, equivalent to 2704 individual fruit over twelve fruit types (ie loquat, mulberry, mango, stone fruit, citrus, apple, kumquat, papaw, Brazilian cherry, cherry guava, Alexander palm fruit, *Thevetia peruviana*) (Table 15). No native potential hosts were sampled as the 1999 district survey had shown that there was no significant fruit fly breeding in the district except in commercial fruit varieties. A small number of fruit samples were taken from *Thevetia peruviana*, a common ornamental in Gayndah and Mundubbera, which was shown for the first time in Australia to be a host for *B. tryoni* in the 1999 fruit survey.

The overall levels of fruit fly infestation were 57.7% in Mundubbera samples and 65% in samples from Gayndah. The most heavily infested fruit types were mulberries, Brazilian cherries, loquats, cherry guavas and stone fruit, which showed infestation in 100% of the samples collected. No infestation was found in the small number of samples of *Thevetia peruviana*, papaw and palm fruit. A total of 5293 flies were reared from these fruit with 99.5% of the flies being *B. tryoni* and the remainder being *B. neohumeralis* (29 flies from 4 samples of mulberries, apple and 2x cherry guavas).

Host Tree Survey

The preliminary survey of properties in the town areas of Gayndah and Mundubbera identified 612 fruit fly host trees in Gayndah and 121 in Mundubbera (Table 16). Trees were counted generally without entering private properties so these figures will almost certainly significantly underestimate the actual number of host trees. On the basis of trees recorded, mangoes were the most common backyard fruit fly host in both towns with 279 trees in Gayndah and 41 in Mundubbera. Citrus were the next most common trees in both towns with 121 in Gayndah and 41 in Mundubbera.

Table 15. Summary of fruit collections in Gayndah and Mundubbera.

Mundubbera

Fruit Variety (common name)	Total samples	No. Infested samples	% Infested	No. Fruit	Total Weight Sampled (kg)	Total Pupae	Total Emergence	Flies/fruit	Flies/kg fruit
Loquat	12	11	91.7%	671	6.423	1009	859	1.4	133.8
Mulberry	6	6	100.0%	437	1.143	294	279	0.6	244.0
Mango	5	1	20.0%	16	7.575	44	23	7.2	3.0
Stone fruit	11	8	72.7%	410	10.481	649	552	2.0	52.7
Citrus	10	2	20.0%	80	9.874	17	15	0.9	1.52
Thevetia peruviana	3	0	0.0%	51	0.455	0	0	0.0	0.0
Brazilian cherry	1	1	100.0%	70	0.218	130	61	0.9	280.2
Kumquat	2	1	50.0%	54	1.029	11	10	0.4	9.7
Papaw	1	0	0.0%	1	0.346	0	0	0.0	0.0
Palm (Alexander)	1	0	0.0%	68	0.550	0	0	0.0	0.0
	52	**30**	**57.7%**	**1858**	**38.094**	**2154**	**1799**		

Gayndah

Fruit Variety (common name)	Total samples	No. Infested samples	% Infested	No. Fruit	Total Weight Sampled (kg)	Total Pupae	Total Emergence	Flies/fruit	Flies/kg fruit
Loquat	6	6	100.0%	257	4.331	849	760	3.0	175.5
Mulberry	3	3	100.0%	167	0.312	40	40	0.2	128.2
Mango	18	9	50.0%	123	26.335	960	605	8.3	23.0
Cherry Guava	3	3	100.0%	148	0.610	509	432	2.9	707.7
Stone fruit	2	2	100.0%	99	2.893	2476	1648	16.6	569.7
Citrus	6	1	16.7%	35	6.628	12	9	1.5	1.4
Apple	2	2	100.0%	17	1.856	104	42	2.5	22.6
	40	**26**	**65.0%**	**846**	**42.966**	**4950**	**3494**		

Table 16. Survey of backyard host trees in Gayndah and Mundubbera

Host fruit	No. of Trees		
	Gayndah	Mundubbera	Total
Apple	3	4	7
Apricot	1	1	2
Avocado	22		22
Brazilian Cherry	5	1	6
Cherry Guava	14		14
Citrus	121	41	162
Custard Apple	3	3	6
Date Palm	6		6
Feijoa	9		9
Fig	7		7
Guava	4	1	5
Loquat	24	13	37
Lychee	8		8
Mango	279	41	320
Mulberry	50	13	63
Persimmon	6	1	7
Sapote	1		1
Stone fruit	49	2	51

8.3.3 Comparison of MAT carriers

Relative efficacy for a carrier at a particular age was expressed as the percentage of flies caught by an aged carrier compared to a new carrier tested under the same conditions. Mean relative efficacy of wicks and blocks aged for a period of 36 weeks is shown in Figure 28. The results were not consistent with no obvious explanation from one sampling time to the next. The general trend was that there was no difference between the wicks and blocks over time. Relative efficacy decreased with time, however, due to the variability in the data, statistically this trend was very weak and could not be used reliably for prediction of relative efficacy at a particular age.

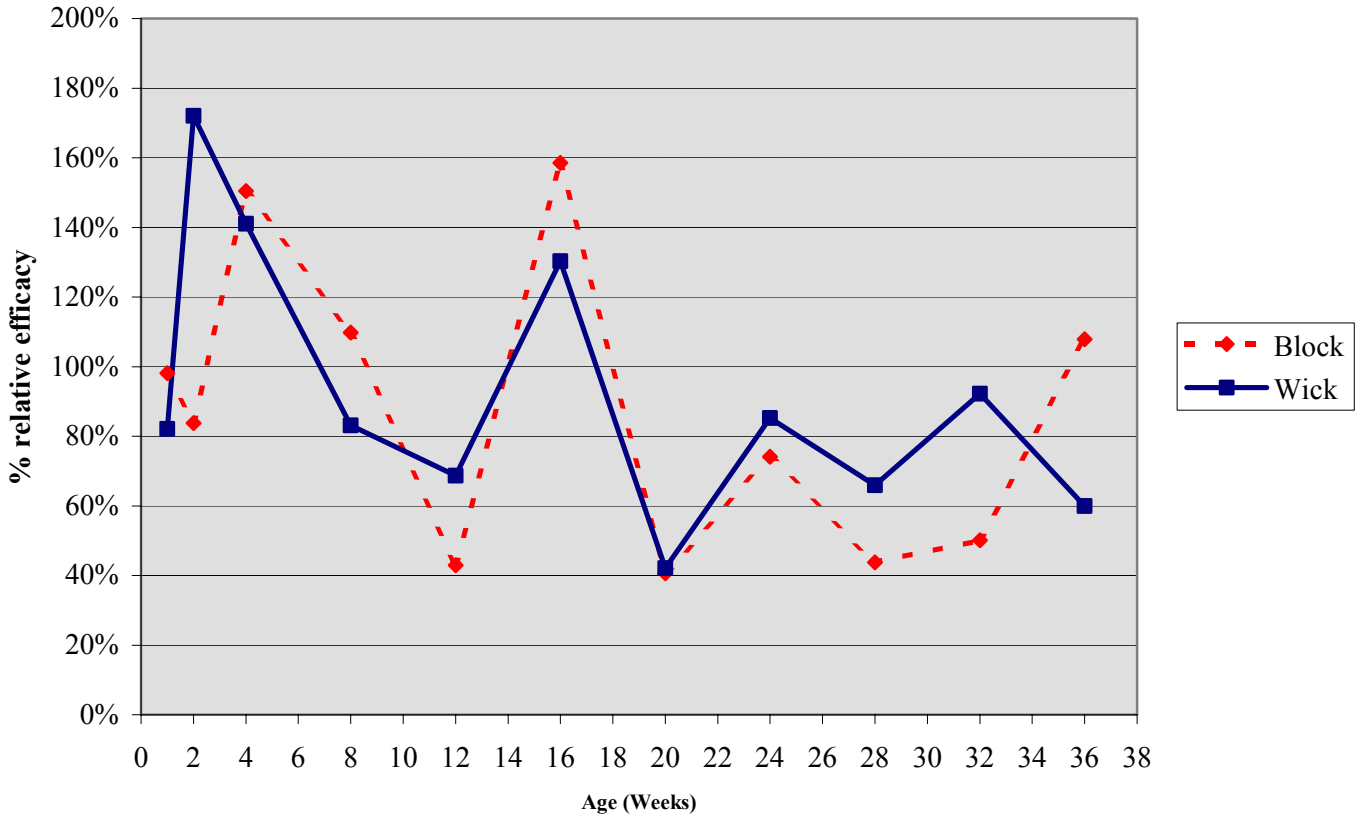

Figure 28. Mean Relative Efficacy of block and wick MAT carriers after weathering in exposed conditions in the Central Burnett.

Changes in malathion, cue lure and raspberry ketone content of wicks and blocks are shown Figure 29 & Figure 30.

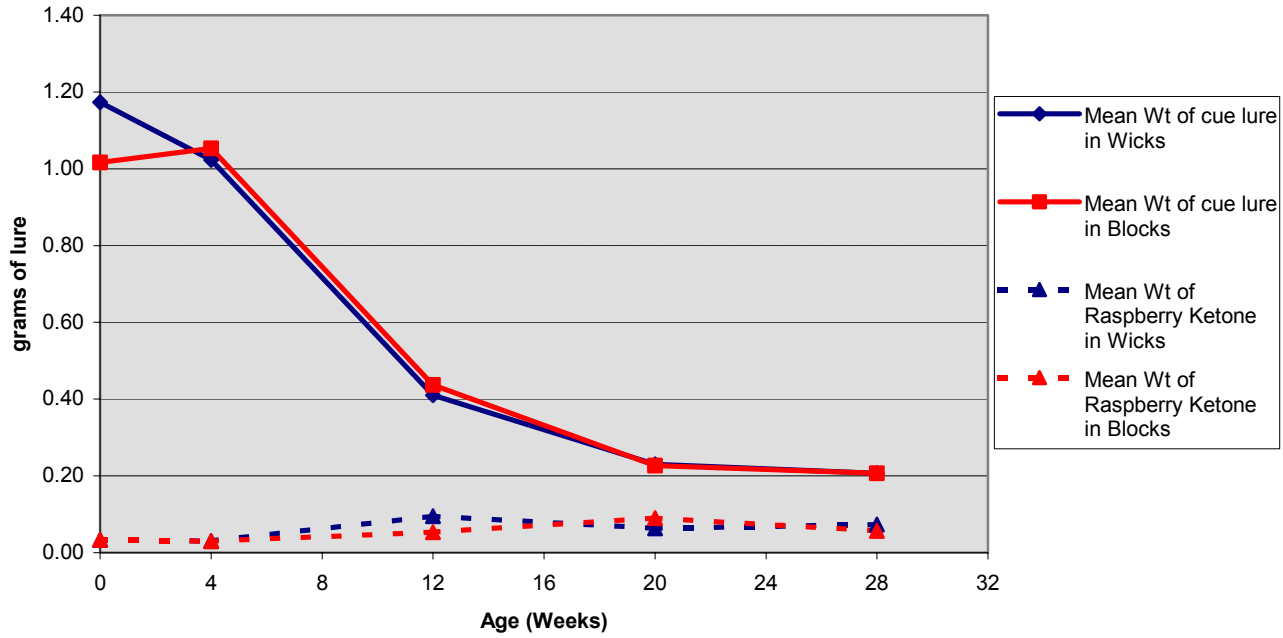

Figure 29. Effect of weathering on cue lure and raspberry ketone content of MAT wicks and blocks over time.

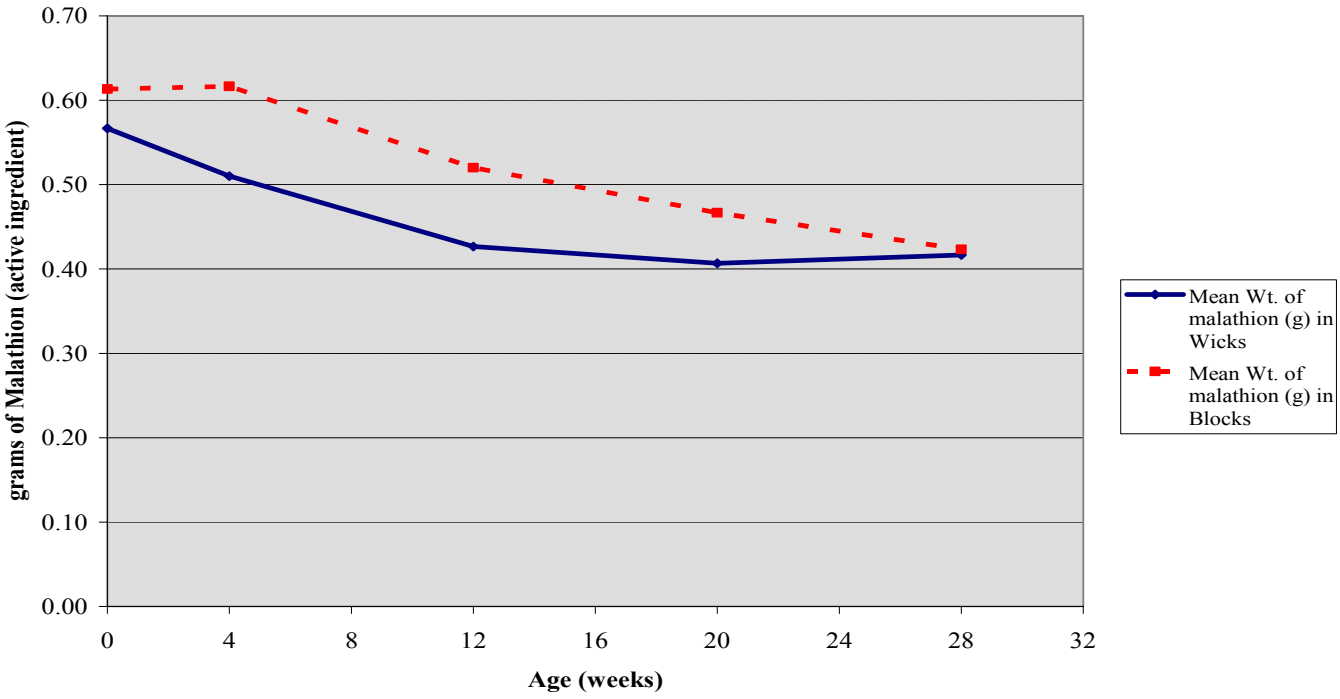

Figure 30. Effect of weathering on malathion content of MAT blocks and wicks over time.

PROJECT AH00012
(June, 2003)

Improved protein baits for fruit fly control:
Improving fruit fly baits

Andrew Jessup *et al.*
New South Wales Agriculture

New South Wales Agriculture component of
collaborative project between
Horticulture Australia,
New South Wales Agriculture,
Queensland Department of Primary Industries and
Agriculture Western Australia

AH00012

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Purpose of this Report:
This reports progress on the New South Wales Agriculture component of the larger AusHort (Horticulture Australia) project entitled "*Improved Protein Baits for Fruit Fly Control – AH00012*" . The New South Wales Agriculture component was to investigate methods of improving fruit fly baits – their efficacy in attracting and killing fruit flies. Fruit fly control using these technologies will become less reliant on extensive use of synthetic pesticides and will protect our export markets from a serious quarantine pest – Queensland fruit fly.

The authors gratefully acknowledge the financial and in-kind support provided by:

- AusHort (Horticulture Australia) for funding the project
- US Department of Agriculture, Hawaii for supply of experimental protocols, chemicals, Phloxine B and protein sources
- US Department of Agriculture, Texas for supply of information / discussion on the potential for various bait inclusions
- Dow Agro-chemicals for supply of GF120
- AVENTIS for supply of Bactrogel (Amulet® Gel)
- Bayer Australia for supply of imidacloprid and other chemicals
- AMTRADE for supply of Nu Lure and Nu Film
- Organic Crop Protectants for supply of Neem (Azamax®)
- New South Wales Agriculture for funding and supply of resources.

June, 2003

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Media Summary

As part of a collaborative project between AusHort (Horticulture Australia), New South Wales Agriculture, Queensland Department of Primary Industries, Agriculture Western Australia and Industry, New South Wales Agriculture's Horticultural Market Access Laboratory at Gosford has studied methods of improving the attractancy and efficacy of fruit fly baits.

Fruit fly baits are important tools for controlling fruit fly populations. Fruit fly baits are used to protect crops in the field and to maintain or gain markets for our fresh horticultural produce.

- Results from this work show that the bait recipe can be improved.

The addition of more attractive protein sources (fruit fly attractants), other attractants such as ammonium compounds, feeding stimulants such as sugars and new, safer chemicals in very low concentrations will increase the numbers of flies killed. Improved baits will pick flies up in the field earlier in the season than is current. The earlier in the season flies are controlled the easier it is to keep numbers down in the field later when crops are fruiting.

- Positive results from this work need to be tested in the field under commercial crop production situations.

If successful, recommendations will be combined with the those from previous research by New South Wales Agriculture at Gosford which detail optimal placement of baits for effective fruit fly control. Other research at Gosford which adds to the knowledge of the science of fruit fly monitoring and control include the sterile insect release technique of fruit fly control, improved male fruit fly traps, long-term female fruit fly traps, optimal fruit fly trap placement and colour and quarantine treatments against fruit flies for market access.

- An environmentally sensitive complete fruit fly management package will be the result.

Technical Summary

As part of a collaborative project between AusHort (Horticulture Australia), New South Wales Agriculture, Queensland Department of Primary Industries, Agriculture Western Australia and Industry, New South Wales Agriculture's Horticultural Market Access Laboratory at Gosford has studied methods of improving the attractancy and efficacy of fruit fly baits.

Fruit fly baits are important tools for controlling fruit fly populations. Fruit fly baits are used to protect crops in the field and to maintain or gain markets for our fresh horticultural produce.

This project is preliminary in nature as most of the research was carried out under controlled laboratory or field cage situations. Results from this work suggest that improvements can easily be made to fruit fly baits which will improve their attractiveness to fruit flies and improve the efficacy in killing them before they can infest fruit and vegetable crops.

The fruit fly bait recipe can be improved by using more attractive protein sources which are currently commercially available – protein hydrolysate and Nu Lure® were shown to be more attractive to fruit flies in the laboratory than Natflav® or Provesta®. Bait attractancy to fruit flies can be improved with the addition of ammonium acetate and sucrose and these baits are less prone to surface mould development than current baits. Increasing the moisture retention capacity of baits with the inclusion of various gels, such as Guar gum and Xanthan, increase the longevity of fruit fly baits.

- It must be noted, however, that these improvements need to be proven in the field under normal commercial crop production systems. This is a recommendation for future research and development.
- A great deal of similar research and development is happening overseas, too. It is recommended that this R&D be monitored by Australian researchers for potential application to Australian conditions.
- The possibilities that baits cause phytotoxic damage to crops and may attract and kill beneficial insects such as bees need to be addressed in future research.

It is anticipated that the research and development carried out under Project AH00012 will be combined with previous New South Wales Agriculture, Gosford research on

- optimal placement of baits
- improved fruit fly traps and trapping systems
- the use of the sterile insect release technique and fruit fly parasitoids for fruit fly control / eradication
- area wide management of fruit flies and
- quarantine disinfestation against fruit flies.

An environmentally sensitive complete fruit fly management package will be the result.

1. Experimental overview

1.1 Introduction

Fruit fly baits have been used in Australia and around the world for about 100 years but still fruit flies cause havoc with the production of horticultural crops. The presence of fruit flies in Australia restrict access of our fresh horticultural produce to regions both outside and within our boundaries. It is imperative that we find new ways of managing fruit flies which will enable efficient horticultural production, maintain and improve markets for our produce and reduce the adverse effects of synthetic pesticides on the crop, the environment and the consumer.

One possible way of satisfying this imperative is to examine methods of improving the baits we use so that they are more attractive and more deadly to fruit flies. If we can reduce seasonal incursions or seasonal upsurges of fruit fly populations with timely and effective baiting programs the fruit fly problem will be drastically reduced.

Techniques of field control of fruit flies using baits cover a range of issues. The main concern is to ensure the fruit fly encounters a lethal dose by either direct contact or by ingestion. But that is not all. The application must be relatively long-lasting, non-injurious to the farmer, the environment, the crop and the consumer, approved for use on the crop against fruit flies and with negligible toxic residues.

Since studies commenced on finding improved methods of the application of baits for fruit fly control, researchers around the world and in Australia have investigated new safe chemicals, long-life formulations, improved attractants and application techniques.

The reason for this surge of interest in baits is clear - the safety of the farmer, the consumer and the environment and the protection and improvement of markets for our produce. Because fruit flies are such an important and invasive pest of most fruit and vegetables grown around the world applications of toxic synthetic chemical pesticides are the norm. These chemicals are used, sometimes quite heavily (up to 7 sprays of dimethoate per season on low chill stonefruit on the North Coast of New South Wales), in areas with endemic populations of fruit flies.

These chemicals are also used, paradoxically, in production areas where fruit flies do not exist. Baits and traps in these regions are charged with lures and synthetic chemical toxicants to ensure that fruit flies do not cross the border into fruit fly free areas or to ensure that small incursions are wiped out before they become economically damaging.

The list of substances being examined by scientists in their quest for the perfect bait is large but not yet exhaustive.

For so long protein autolysate has been the mainstay. Now, however, it’s becoming quite expensive and, at times, difficult to source. New sources of protein are being tested: brewery waste, waste from sugar cane production, or from maize production, long-life encapsulation of protein hydrolysate.

Substances already approved as additives for food, cosmetics and drugs are also being studied. The so-called photo dyes, phloxine B and uranine, are approved as the dyes, D&C RED 28 and YELLOW 202-2, respectively. When an insect with an opaque abdomen ingests the dye and is subject to light the insect dies possibly as a result of free radicle formation. A formulation of dyes, sugar, yeast and water named "SureDye" is under development in the US, in use in Guatemala and Mexico and being investigated in many other countries, including Australia.

Other safe substances under detailed research and development include a range of plant extracts such as pyrethrum extract, neem, anethole (an extract from fennel) and menthol. These compounds have been shown to act in different ways. They can kill the insect or they can render the female effectively infertile or they can act as an insect repellent.

Substances of biological origin are also under investigation. Spinosad, an antibiotic insecticide prepared from broths of the bacterium, *Saccharopolyspora spinosa*, is one which is already approved in many countries as an insecticide for use on cotton, fruit trees, vegetables, etc.

We carried out trials on new baits, bait inclusions, compounds, etc and tested them against Queensland fruit fly in the laboratory and in the field. There was a large number of compounds to be tested under this project.

1.2 Basic methods

The experimental protocol was carried out at three basic levels depending on the degree of control over variability.

1.2.1 Laboratory experiments were conducted to reduce the number of variables to a quantifiable few (such as temperature, light levels, relative humidity).

1.2.2 Then treatments were tested in small field cages to introduce variables that apply to the crop (such as alternative feeds present on leaf surfaces, time taken for adult mortality may allow oviposition, etc).

1.2.3 Following this the objective was to test in semi-commercial situations to allow most variables to impact.

1.3 Rationale

Due to the large number of compounds to be tested we tested compounds against Queensland fruit fly adults either by following the experimental methods set up by the USDA in Hawaii or by comparing one or two compounds with a standard formulation. We were looking for a rapid turnover of compounds tested. Also we were interested only in demonstrable differences in response. We carried these trials out in the laboratory under tightly controlled situations so that any differences in treatment means would be due to treatment rather than being confounded by external, non-quantified variability. If a new compound was to be experimented on at the next level of complexity (i.e. in field cages) its response had to be markedly better than the control or the standard. If treatment means could be separated only by statistical analyses then the differences were

insufficient for further experimentation (unless the new compound was much cheaper than the standard). Experiments were replicated as much as possible.

2. Laboratory experiments on different toxicants in baits

A large list of toxicants are being, or have been, used or are being studied for use in baits around the world. As part of Project AH00012 we chose the chemicals described in the following pages because of their availability, low mammalian toxicity and/or their facility for use in low-volume applications. Materials and methods for laboratory experiments on baits made with these toxicants followed, predominantly, methods developed by Dr Grant McQuate and his colleagues in the US Department of Agriculture, Hilo, Hawaii. The USDA methodology was set up originally for their studies on the efficacy of photoactive dyes and spinosad as toxicants for use in fruit fly baits.

2.1 Bayer products: imidacloprid, imidacloprid analogue, spinosad analogue

2.1.1 Experiment 1. Bayer insecticides

As a result of discussions with Mr Trevor Birley of Bayer Australia I was able to obtain samples of toxicants he suggested as being suitable for use in fruit fly baits and traps. The three chemicals obtained were labelled as B1, B2 and B3 to reduce operator bias in our experiments. At a later date the 3 chemicals were identified as B1: imidacloprid (200g/L a.i.); B2: imidacloprid analogue (480g/L a.i.) and B3: a bacterial insecticide (120g/L a.i.).

Results

Data show that the imidacloprid compounds, at the concentrations of active ingredient used here (Tables 1 and 2), were more toxic than the bacterial insecticide (Table 3). Significant mortality was caused by only the highest concentration (1.0% 120g/L a.i.) of the bacterial insecticide and that occurred some 1 to 2 days after ingestion of the bait.

The two imidacloprid compounds appeared to act similarly with respect to time taken to die and the effects of concentration. Significant mortality of the 1.0% solutions occurred around 4 hours after ingestion for both imidacloprid preparations. Mortality was less in the Controls and even at 2 days after ingestion of the 0.1% solution.

These data provide us with a starting point for future development of the Bayer preparations for use in fruit fly baits.

2.1.2 Experiment 2. Commercial preparation of imidacloprid – Confidor

Commercially available imidacloprid - Confidor® (200g/L a.i.) was obtained to test as a toxicant in fruit fly baits. Five replicate experiments were carried out.

Results

Tables 4 and 5 show the effect of incorporating imidacloprid (Confidor) into the protein baits against adult Queensland fruit fly males and females in the laboratory. The data show low levels of mortality of fruit flies fed imidacloprid at 7ppm to 10ppm but even at 50ppm 100% mortality was not achieved in each replicate (Table 4). At higher doses (Table 5) males appeared to be more resistant than females. At all doses, even the highest tested here, 200ppm, the greatest level of mortality occurred between 24h and 48h after ingestion of the bait. Negligible treatment-induced mortality occurred during the first 8h after bait ingestion.

Table 1. BAYER B1: IMIDACLOPRID (200 g/L a.i.) (from 3 reps)

SEX	CONCENTRATION	MORTALITY (%)							
	(% V/V)	8.00AM	12.00 NOON	1.00 PM	2.00 PM	3.00 PM	4.00 PM	24HRS	48 HRS
FEMALE	0	0	3.3	13.3	13.3	13.3	13.3	13.3	13.3
FEMALE	0.01	0	16.7	26.7	30	33.3	33.3	33.3	50
FEMALE	0.1	0	73.3	80	73.3	83.3	86.7	96.7	100
MALE	0	0	0	0	0	0	0	0	0
MALE	0.01	0	23.3	30	33.3	43.3	56.7	56.7	66.7
MALE	0.1	0	93.3	93.3	93.3	93.3	93.3	93.3	93.3

Table 2. BAYER B2: IMIDACLOPRID ANALOGUE (480g/L a.i.) (from 3 reps)

SEX	CONCENTRATION	MORTALITY (%)							
	(% V/V)	8.00AM	12.00 NOON	1.00 PM	2.00 PM	3.00 PM	4.00 PM	24HRS	48 HRS
FEMALE	0	0	3.3	3.3	3.3	3.3	3.3	3.3	3.3
FEMALE	0.01	0	6.7	10	30	30	30	73.3	96.7
FEMALE	0.1	0	86.7	96.7	96.7	96.7	96.7	96.7	100
MALE	0	0	6.7	6.7	6.7	6.7	6.7	6.7	6.7
MALE	0.01	0	6.7	10	23.3	26.7	26.7	36.7	80
MALE	0.1	0	43.3	46.7	53.3	56.7	60	86.7	100

Table 3. BAYER B3: BACTERIAL INSECTICIDE (120g/L a.i.) (from 3 reps)

SEX	CONCENTRATION	MORTALITY (%)							
	(% V/V)	8.00AM	12.00 NOON	1.00 PM	2.00 PM	3.00 PM	4.00 PM	24HRS	48 HRS
FEMALE	0	0	0	0	0	0	0	0	0
FEMALE	0.01	0	0	0	0	0	0	0	0
FEMALE	0.1	0	0	3.3	6.7	13.3	13.3	70	90
MALE	0	0	0	0	0	0	0	0	0
MALE	0.01	0	0	0	0	0	0	0	0
MALE	0.1	0	0	0	0	0	3.3	40	80

Table 4. Percentage mortality of Queensland fruit fly adults following ingestion of baits laced with imidacloprid (corrected for control mortality by Abbott's formula) Doses 0 – 50 ppm (from 4 replicates)

	MALE								FEMALE							
CONC.	8.00am	12 noon	1.00pm	2.00pm	3.00pm	4.00pm	24hrs	48hrs	8.00am	12 noon	1.00pm	2.00pm	3.00pm	4.00pm	24hrs	48hrs
0.1ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	3.3
0.5ppm	0.0	0.0	0.0	0.0	0.0	0.0	4.4	7.1	0.0	0.0	0.0	0.0	0.0	0.0	12.5	12.5
1ppm	0.0	0.0	0.0	0.0	0.0	4.4	13.3	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.5
2ppm	0.0	0.0	0.0	0.0	4.4	4.4	14.4	33.3	0.0	0.0	0.0	0.0	0.0	3.3	10	20
4ppm	na	na	na	na	na	na	na	na	0.0	0.0	3.3	3.3	3.3	3.3	5	5
7ppm	0.0	0.0	3.3	7.1	7.1	7.1	30.4	33.7	0.0	0.0	0.0	3.3	3.3	3.3	7.5	17.5
10ppm	0.0	0.0	3.3	3.3	6.7	10	30	63.3	0.0	0.0	3.3	3.3	3.3	5.3	38.6	59.5
25ppm	0.0	0.0	0.0	0.0	0.0	4.4	50	70	0.0	0.0	0.0	3.3	7.5	7.5	51.4	74.5
50ppm	0.0	0.0	24.4	31.5	31.5	38.9	71.3	100	0.0	0.0	10.4	22.8	31.1	38.9	87.2	95

Table 5. Percentage mortality of Queensland fruit fly adults following ingestion of baits laced with imidacloprid (corrected for control mortality by Abbott's formula) Doses 0 – 200 ppm (from 1 replicate)

	MALE								FEMALE							
CONC.	8.00am	12 noon	1.00pm	2.00pm	3.00pm	4.00pm	24hrs	48hrs	8.00am	12 noon	1.00pm	2.00pm	3.00pm	4.00pm	24hrs	48hrs
50ppm	0.0	0.0	0.0	0.0	0.0	0.0	30.0	70.0	0.0	0.0	0.0	0.0	10.0	30.0	30.0	60.0
60ppm	0.0	0.0	0.0	0.0	0.0	0.0	50.0	80.0	0.0	0.0	0.0	0.0	0.0	0.0	30.0	90.0
80ppm	0.0	0.0	0.0	10.0	10.0	10.0	40.0	80.0	0.0	0.0	0.0	10.0	10.0	10.0	40.0	90.0
100ppm	0.0	0.0	0.0	0.0	0.0	0.0	30.0	70.0	0.0	0.0	0.0	0.0	0.0	0.0	30.0	70.0
120ppm	0.0	0.0	0.0	0.0	10.0	20.0	50.0	70.0	0.0	0.0	0.0	10.0	10.0	10.0	80.0	100.0
160ppm	0.0	0.0	0.0	10.0	20.0	20.0	100.0	100.0	0.0	0.0	0.0	10.0	10.0	20.0	90.0	100.0
180ppm	0.0	0.0	0.0	0.0	0.0	0.0	55.6	77.8	0.0	0.0	0.0	0.0	0.0	10.0	100.0	100.0
200ppm	0.0	0.0	0.0	0.0	0.0	0.0	60.0	80.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	100.0

2.2 Spinosad

2.2.1 Pure spinosad

A spinosad treatment was made up in a mixture with spinosad, yeast hydrolysate, fructose and water. Spinosad was obtained in the commercial formulation, “Success”, which is 120g/L a.i.

Five experimental replicates were carried out.

We called this formulation the Gosford spinosad formulation.

Results

Table 6 shows the data from this experiment. The data show high levels of mortality of baits with 25ppm and 50ppm spinosad. The 50ppm spinosad bait appeared to be quicker acting (40% mortality by 8h after ingestion) than the lower doses (24h to 48h after ingestion).

2.2.2 GF120

A supply of GF120 Naturalyte, a formulation which is now commercially available in the USA as a bait for fruit flies, was obtained through Dow Agrosciences. Experiments were carried out to compare the effects of GF120 and the Gosford spinosad formulations on fruit fly attractancy.

In this experiment only 1 concentration was used, following the GF120 label recommendations. This concentration of GF120 was compared with 130ppm of the Gosford spinosad.

Results

All flies which ate either the GF-120 or the Gosford Spinosad were affected within 1 hour of feeding. The flies did not move around or fly during this period. They were lethargic and did not react quickly to external stimuli (such as my hand or breath).

This indicates that after feeding on GF-120 or Gosford Spinosad flies would be unlikely to fly far away or to infest fruit even though death may not occur for 24-48 hours after consuming the bait.

High levels of mortality of Queensland fruit fly adults occurred after treatment with either spinosad preparation: the GF120 or the Gosford spinosad at between 24h and 48h (Table 7). 100% mortality was not achieved in all replicates. All insects were, however, affected in that they were incapable of flying. In our laboratories there was no consistent differences in rate of mortality or level of mortality between the two preparations. Significant mortality in both preparations commenced some 24h after ingestion of the bait.

Table 6. Effects on mortality of Queensland fruit fly to spinosad in a bait (from 5 reps)

	MALE								FEMALE							
CONC.	8.00am	12 noon	1.00pm	2.00pm	3.00pm	4.00pm	24hrs	48hrs	8.00am	12 noon	1.00pm	2.00pm	3.00pm	4.00pm	24hrs	48hrs
CONTROL	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0	4.0	4.0
0.1ppm	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0	2.0	2.0
0.5ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.75	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0
1ppm	0.0	0.0	0.0	0.0	0.0	0.0	12.5	12.5	0.0	0.0	0.0	0.0	0.0	0.0	2.0	10.0
2ppm	0.0	0.0	0.0	0.0	2.5	2.5	13.4	30.0	0.0	0.0	0.0	0.0	0.0	2.0	8.0	16.0
4ppm	0.0	0.0	0.0	5.0	5.0	5.0	15.0	22.5	0.0	0.0	0.0	0.0	0.0	0.0	8.0	8.0
7ppm	0.0	0.0	0.0	2.75	2.75	2.75	25.3	37.8	0.0	0.0	0.0	2.0	4.0	4.0	16.0	28.0
10ppm	0.0	0.0	2.5	10.0	10.0	15.0	47.5	77.5	0.0	0.0	0.0	0.0	6.2	6.2	60.1	73.6
25ppm	0.0	0.0	9.4	9.4	15.3	15.3	73.9	94.8	0.0	0.0	0.0	0.0	10.0	12.0	67.1	87.6
50ppm	0.0	0.0	31.5	39.0	43.3	45.5	87.3	100.0	0.0	0.0	15.1	31.5	40.0	55.6	89.8	96.0

Table 7. Comparisons of fruit fly attractancy between GF120 and Gosford spinosad in the laboratory (from 4 reps with 3 sub-samples in each rep)

	FEMALE								MALE							
Treatment	8.00am	12 noon	1.00pm	2.00pm	3.00pm	4.00pm	24hrs	48hrs	8.00am	12 noon	1.00pm	2.00pm	3.00pm	4.00pm	24hrs	48hrs
CONTROL	0.0	0.0	0.0	0.0	0.0	0.0	1.7	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
GF-120	0.0	0.8	0.8	5.0	11.7	12.5	83.3	89.2	0.0	0.8	1.7	8.4	10.9	13.4	77.5	87.5
GOSFORD	0.0	0.0	4.2	6.7	12.5	12.5	89.2	96.7	0.0	0.0	1.7	6.7	9.2	10.1	75.7	92.4

2.3 Anethole

Anethole is an extract from fennel and has been shown to be toxic to some insects.

Mixing procedures, experimental protocols and experimental timetables were the same as for the imidacloprid trials.

Results

Females - Very little bait was eaten in first 4 bait concentrations (strongest):- 50,000; 40,000; 25,000; and 20,000 ppm.

As the bait concentration weakened more of the bait was eaten. A small amount was eaten from 10,000 and 8,000ppm cages. More noticeable amounts were eaten from 5,000 and 2,500 ppm cages and quite large amounts from 1,000 ppm and control cages.

More bait appears to be eaten by females than males.

Males - Very small amounts of bait were eaten in the first six strongest bait concentrations (50,000; 40,000; 25,000; 20,000; 10,000 and 8,000ppm). A little bit of bait was consumed in the 2,500 and 5,000ppm cages. Most of the bait was consumed in the 1,000ppm and control cages.

It appears Anethole repels Queensland Fruit Fly at strong concentrations. However, flies did consume the bait at lower concentrations but at these concentration it failed to kill the flies

2.4 Pyrethrum

Results

Laboratory tests against Queensland fruit fly adults conducted on pyrethrum concentrations of 0.1 ppm to 50 ppm resulted in zero mortality after 48h at 26°C (Table 8). There was mortality when concentrations reached 10,000 ppm to 50,000 ppm but results were variable (Table 9).

Table 8. Effects on mortality of Queensland fruit fly adults following ingestion of pyrethrum in a bait: 0 – 50ppm (from 1 rep)																
	MALE								FEMALE							
CONC.	8.00am	12noon	1.00pm	2.00pm	3.00pm	4.00pm	24hrs	48hrs	8.00am	12noon	1.00pm	2.00pm	3.00pm	4.00pm	24hrs	48hrs
CONTROL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5ppm	0.0	0.0	0.0	0.0	0.0	0.0	22.2	22.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4ppm	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7ppm	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
25ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
50ppm	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 9. Effects on mortality of Queensland fruit fly adults following ingestion of pyrethrum in a bait: 0 – 50000ppm (from 2 reps)																
	FEMALE								MALE							
CONTROL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1000ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2500ppm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5000ppm	0.0	0.0	0.0	0.0	0.0	0.0	5.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	10.0
8000ppm	0.0	0.0	0.0	0.0	0.0	5.0	25.0	35.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10000ppm	0.0	0.0	5.0	5.0	5.0	5.0	30.0	40.0	0.0	0.0	5.0	5.0	5.0	5.0	15.0	20.0
20000ppm	0.0	0.0	20.0	30.0	30.0	30.0	50.0	80.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0
25000ppm	0.0	0.0	0.0	10.0	10.0	15.0	60.0	75.0	0.0	0.0	10.0	10.0	20.0	20.0	30.0	50.0
40000ppm	0.0	0.0	0.0	15.0	25.0	30.0	70.0	80.0	0.0	0.0	0.0	0.0	10.0	20.0	40.0	50.0
50000ppm	0.0	0.0	0.0	10.0	30.0	50.0	80.0	90.0	0.0	0.0	10.0	10.0	10.0	10.0	30.0	30.0

2.5 Neem

A commercial formulation of neem (Neemazal), 50000ppm azaderachtin, was tested.

Results

There was zero mortality of adult Queensland fruit fly at concentrations from 0.1 ppm to 8000 ppm. It appeared that, at concentrations of 1,000 ppm or more Queensland fruit fly adults, male and female, were repelled before they could eat the bait. At 500 ppm and less flies ate before flying away, never to return - but there was no mortality.

Neem appears to be a repellant at 250ppm or more and only partially repellant at 100ppm. Both males and females in 100ppm cages fed on the bait. The 2,000ppm cages of flies tasted the bait and flew away immediately. The 5,000ppm cages were repelled on contact. The flies came in to investigate the bait, but on contact with bait, especially mouthparts the flies are immediately repelled. It appears that some insects can tolerate the bait better than others as the same flies kept coming back for repeat feedings. Flies which couldn't tolerate the bait or were initially repulsed did not return for a second feeding.

Feeding periods in the 100ppm cages were between 10 and 30 seconds but were shorter than the control feedings.

More flies fed in the control cages than in either the 100ppm and 250ppm. There appeared to be no difference between males and females cages.

At both 25ppm and 50ppm both male and female control flies fed on the bait continuously for approximately 2 hours. There was no difference in feeding between males and females. There was zero mortality.

Overseas research suggests that ingestion of neem affects the ovipositional behaviour of the females of some fruit fly species which prevents them from laying eggs. This observation needs to be investigated for Queensland fruit fly.

2.6 Abamectin / Emamectin

We decided not to use abamectin due to its high mammalian toxicity. Some researchers (Dr R Mangan, USDA, personal communication) that abamectin is useless against some fruit flies such as Mediterranean fruit fly but that emamectin is effective against all fruit flies.

2.7 Phloxine

2.7.1 Phloxine alone

Laboratory trials were carried out on the effects of including Phloxine B in fruit fly baits as the toxicant to replace malathion. The complete experiment was carried out following the identical protocol used by researchers in the USDA, Hawaii. Fly age,

level of protein starvation, light intensity, relative humidity and temperature were set at Narara to the same levels used in Hawaii.

Results

Of the 11 dye doses tested the highest two (6.4 X 10^{-3} M and 1.28 X 10^{-2} M) were most effective in killing Queensland fruit fly males and females. Under a post-feeding light intensity of 200µE/s/m^2 most male and female flies were killed within 6 hours. The lowest doses applied, between 1.25 X 10^{-5} M and 4.0 X 10^{-4} M, had little effect on Queensland fruit fly mortality up to 48 hours post-feeding (the limit of assessments). The middle range of doses, 8.0 X 10^{-4} M, 1.6 X 10^{-3} M and 3.2 X 10^{-3} M, were of intermediate efficacy. Under these doses there appeared to be a tendency for females to be more rapidly killed than males. This could be due to the doses being marginal and the fact that females tend to eat more protein than males to optimise egg production.

The experimental light intensity of 200µE/s/m^2 is considerably less than normal Summer daylight in Gosford (where full sun light intensity is about 6000µE/s/m^2). It is anticipated that Queensland fruit fly mortality will be quicker than 6 hours for the two highest doses and higher for the other doses applied.

2.7.2 Phloxine B + Fluorescein (Uranine)

Research in the USA has shown that the combination of uranine (or Fluorescein) with phloxine B will be synergistic in the baits' adverse effects on fed fruit flies.

Results

These trials were conducted to study the gross effects of Phloxine B and Uranine on fruit flies subjected to fluorescent light and sunlight.

When Phloxine B was presented to the fruit flies on its own there was a significant adverse effect of fruit fly survival (Table 10). The rate of fruit fly mortality was quicker under sunlight than under fluorescent lighting.

A mixture of Phloxine B and Uranine did not appear to offer a synergistic improvement in fruit fly mortality (Table 10).

Conditions have been variable between the preliminary trials. The variables that have the most impact, it seems, on fruit fly survival are: - age of fly, the energy status of the fly prior to feeding on the dye and the amount of light incident on the flies.

Table 10. Comparison between percentage survival of adult Queensland fruit fly under fluorescent light or sunlight after ingestion of baits

BAIT	PHLOXINE B				PHLOXINE B + URANINE			
Exposure to	Fluoro	Fluoro	Sunlight	Sunlight	Fluoro	Fluoro	Sunlight	Sunlight
light (min.)	Male	Female	Male	Female	Male	Female	Male	Female
0	100.00	98.33	100.00	100.00	98.33	98.33	98.33	100.00
5			20.00	10.00			70.00	75.00
10			2.50	0.00			41.67	46.67
15			2.50	0.00			33.33	31.67
20			2.50	0.00			16.67	15.00
25			2.50	0.00			10.00	10.00
30	88.33	65.00	2.50	0.00	76.67	91.67	0.00	3.33
60	80.00	46.67	2.50	0.00	68.33	78.33	0.00	3.33
90	51.67	18.33			53.33	45.00		
120	33.33	8.33	2.50	0.00	46.67	38.33	0.00	1.67
150	25.00	5.00			46.67	36.67		
180	23.33	5.00	0.00	0.00	25.00	18.33	0.00	1.67
210	10.00	3.33			25.00	18.33		
240	6.67	1.67			25.00	16.67		
1440	0.00	0.00			20.00	15.00		

2.8 Malathion

Concerns have been expressed as to perceived differences in attractancy between the emulsifiable concentrate (EC) formulation of malathion used in baits, traps and killer pads and the wettable powder (WP) formulation of malathion. The EC form is the active ingredient dissolved in a hydrocarbon while the WP is in a form that will dissolve in water. The EC is more or less used exclusively these days while the WP is available to the grower only as a separate component of a home garden fruit fly trap kit.

The hydrocarbon component of the malathion EC may repel Queensland fruit fly. If hydrocarbons repel fruit flies this will reduce the efficiency of any bait, trap or killer pad based on EC toxicants.

We carried out some trials to test for differences in attractancy between baits based on either the EC or WP formulations of malathion. Four experiments were carried out by exposing 20 males or 20 females to one of three baits:

1. protein bait with no toxicant
2. protein bait with wettable powder form of malathion
3. protein bait with emulsifiable concentrate of malathion

Each experiment consisted of 6 fly cages each housing either 20 male flies or 20 female flies. One pair of cages, randomly placed outside, in the sunshine, were allocated to each of the three treatments. Mortality of flies was assessed at varying intervals.

Results can be seen in Tables 11 to 14 and Figure 1.

Conclusions

Attraction to baits depends on the maturity of fruit flies and their protein (energy) status. This aspect of bait efficacy has been known for a long time. Baits, if they are to be efficacious, need to be very attractive - not only to allow flies to sense and approach the bait but to feed on it sufficiently to ingest an insecticidal dose of toxicant.

The Emulsifiable Concentrate was initially less attractive / poisonous to Queensland fruit fly used in these experiments than the Wettable Powder formulation of malathion. This difference lasted up to between 150 minutes and 230 minutes of exposure at which there was little difference between the two treatments.

There may be an initial repellence of the EC when compared to the WP but this lasts only two to four hours at around 25°C in the sunshine. It is likely that the hydrocarbons used to dissolve active ingredient in the EC formulation are repellent to Queensland fruit fly. Once these hydrocarbons have evaporated from the bait the baits are equally attractive and effective in killing flies.

Table 11.Cumulative mortality (no. of flies dead) of Queensland fruit fly adults exposed to Malathion-based baits (Experiment 1)

Treatment / Minutes	5	10	15	25	35	45	60	80	140
Control Males	0	0	0	0	0	0	0	0	0
Control Females	0	0	0	0	0	0	0	0	0
Wettable P Males	4	5	7	8	11	14	14	17	17
Wettable P Females	0	1	1	1	2	4	6	7	7
EC form Males	2	2	2	2	2	2	4	4	6
EC form Females	0	0	0	0	0	1	3	3	5

Table 12. Cumulative mortality (no. of flies dead) of Queensland fruit fly adults exposed to Malathion-based baits (Experiment 2)

Treatment / Minutes	5	10	15	25	30	45	60	75	105	150	240
Control Males	0	0	0	0	0	0	0	0	0	0	0
Control Females	0	0	0	0	0	0	0	0	0	0	0
Wettable P Males	5	5	6	6	8	10	10	11	11	11	12
Wettable P Females	0	1	2	4	4	7	8	10	10	11	11
EC form Males	0	0	0	1	1	2	3	5	7	8	8
EC form Females	4	4	4	4	4	8	9	10	12	12	14

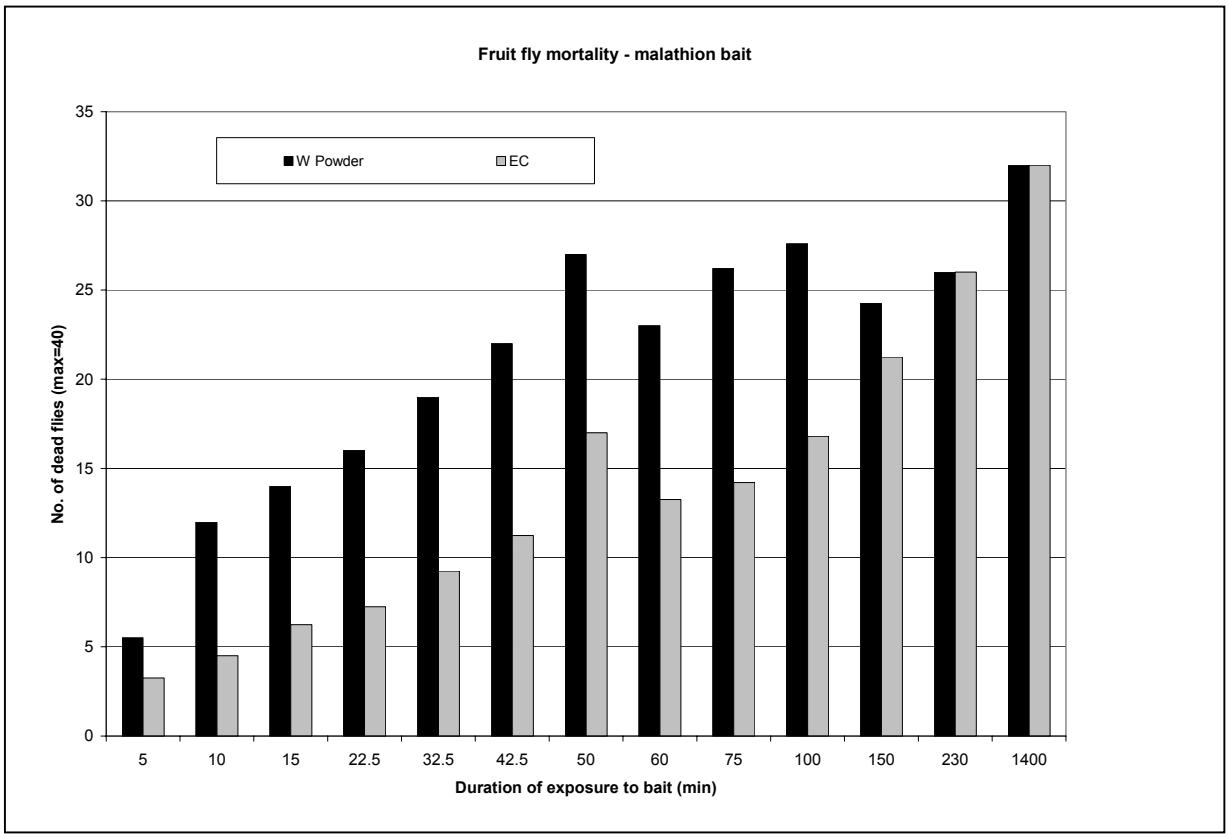
Table 13. Cumulative mortality (no. of flies dead) of Queensland fruit fly adults exposed to Malathion-based baits (Experiment 3)

Treatment / Minutes	5	10	15	20	30	40	50	60	70	80	90	100	110
Control Males	0	0	0	0	0	0	0	0	0	0	0	0	0
Control Females	0	0	0	0	0	0	0	0	0	0	0	0	0
Wettable P Males	2	7	7	7	8	9	10	10	11	11	11	11	11
Wettable P Females	6	15	16	18	20	20	20	20	20	20	20	20	20
EC form Males	1	1	1	2	2	2	3	3	3	4	4	4	4
EC form Females	3	6	6	6	8	7	8	8	9	9	9	9	9

Table 14. Cumulative mortality (no. of flies dead) of Queensland fruit fly adults exposed to Malathion-based baits (Experiment 4)

Treatment	Minutes	5	10	15	20	30	40	50	60	80	110	140	170	200	260	1295	1535
Control Males		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control Females		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wettable P Males		3	7	10	13	14	14	15	15	15	15	15	15	15	15	16	16
Wettable P Females		2	7	7	7	9	9	9	9	9	9	10	11	12	13	16	16
EC form Males		1	2	5	7	13	15	15	15	15	16	16	16	16	16	17	17
EC form Females		2	3	7	7	7	8	8	8	9	10	11	11	11	13	15	15

Figure 1. Effects of malathion formulation on attract and kill of protein bait



2.9 Conclusions – Different toxicants for fruit fly baits

Preliminary results described in *2. Laboratory experiments on different toxicants in baits* show that there are several other toxicants available for inclusion in fruit fly baits which have the potential to be as effective as malathion. High levels of mortality (greater than 90%) of adult Queensland fruit fly males and females were induced by about 50ppm active ingredient of imidacloprid, Bayer's bacterial insecticide (un-named) and Dow's bacterial insecticide (Spinosad). 100% mortality was reached at a dose of around 120ppm for imidacloprid (Confidor®).

Baits with pyrethrum, anethole or neem were not effective in attracting and killing Queensland fruit fly in laboratory cages. There was a high mortality rate (about 90%) of Queensland fruit fly females fed very high doses of pyrethrum (50,000ppm). Males showed only 50% mortality at this rate. There may be some potential for pyrethrum in baits but it is unlikely to become popular due to the high application rate.

Anethole and Neem, at high rates (Anethole: 20,000ppm to 50,000ppm; Neem 250ppm to 8,000ppm), repelled flies. Flies consumed bait with lower concentrations but there was no treatment induced mortality. There may be potential for these compounds / mixtures for use as repellents but not as toxicants against Queensland fruit fly.

The food dye, Phloxine B, is an effective bait inclusion. Under fluorescent lighting a concentration of 6.4×10^{-3} M in a protein bait killed 95% of flies within about 200 minutes. Under direct sunlight it took about 5 minutes to achieve around 95% mortality. The addition of uranine (fluorescein) did not improve efficacy. Phloxine B has a high level of potential for future research and development for use in fruit fly baits.

Concerns about the repellent effects of the emulsifiable concentrate of malathion compared with the wettable powder formulation are real – but are not significant in practice. Experiments show that there is a repellent effect although it lasts only about 2 to 4 hours after which there is no difference in efficacy against Queensland fruit fly. For field application of malathion based protein baits there would, in reality, be little difference between the two formulations.

Further research and development on emamectin as a fruit fly toxicant should be progressed. The closely related abamectin is not effective against some fruit flies e.g. Mediterranean fruit fly whereas emamectin affects all fruit flies (Dr R. Mangan, USDA – personal communication).

3. Field cage experiments on different toxicants in baits

3.1 Fipronil

Introduction

Tests were carried out comparing standard malathion + protein autolysate baits with the new Bactrogel bait in small field cages. In these experiments cages measuring 450

mm X 710 mm X 1000 mm high and placed outside under light (30% shade) shade cloth. Tomato plants with fruit were placed in the cages. Each cage housed 5 male and 5 female Queensland fruit fly. Flies were 14 days old and protein-starved for 3 days prior to treatment. There were 3 cages per treatment:

1. Protein autolysate (Natflav®) and no toxicant (Control)
2. Protein autolysate (Natflav®) + Bactrogel
3. Protein autolysate (Natflav®) + Malathion

Baits were made up according to label instructions. The experiment was replicated once a week for five weeks – i.e. 5 replicates.

We were interested in testing the Bactrogel bait in controlled outdoor conditions with fruiting fruit fly host plants to determine if the observed delay in mortality after bait ingestion allowed flies time to oviposit into fruit before their death.

Results

The results, shown in Table 15, show that there was a delay in mortality when adult Queensland fruit fly were exposed to the Bactrogel bait when compared with the standard malathion bait. Total adult mortality was not different between the two baits.

Under the conditions set by this experiment the Bactrogel bait, while killing over 50% of the flies, did not protect the fruit from infestation when compared with the Control (un-baited) fruit (Table 16). This observation needs to be explored more fully.

Conclusion

Data from this experiment show that, under the conditions experienced in small field cages at Narara, there was incomplete mortality of adult Queensland fruit fly, male or female, within three days of exposure to baits based on commercial application rates of malathion or Bactrogel reaching only just under 60% mortality. Under these conditions baits were not completely effective in preventing infestation of the fruit. The malathion based bait reduced infestation rate to 24% of the Controls whereas the Bactrogel reduced infestation rates to an average of only 81% of the Controls.

Conditions for this experiment would give results biased towards toxicant efficacy as flies had been protein-starved and were enclosed in a relatively small volume with no other protein source apart from native sources on the tomato plants. The survival of equal percentages of flies baited with malathion and Bactrogel implies that there was equal attract and kill potential over 3 days. The higher number of insects found infesting tomatoes in the Bactrogel-baited fruit compared with the malathion-baited fruit implies that the malathion incapacitates flies quicker than the Bactrogel does.

Table 15. Percentage mortality of adult Queensland fruit fly after exposure to fruit fly baits on fruiting tomato plants in small field cages (from 5 replicates of 3 sub-samples in each replicate).

Time after initial exposure (h)	0	0.17	0.33	0.5	0.67	0.83	1	1.5	2	2.5	3	24	48	72
Control ave %	0	0	0	0	0	0	0	0	0	0	0	8.02	18	30
Malathion ave %	0	0	0	0	1.32	1.32	2.66	3.32	3.32	3.32	3.32	28	44.7	57.3
Bactrogel ave %	0	0	0	0	0	0	0	0	0	0	0	18	41.3	57.3

Each data point is the average number of insects found dead in the cage at each sampling time from 3 sample cages over 5 replicates in time.

Table 16. Total number of insects infesting tomato fruits after exposure to fruit fly baits (from 5 replicates of 3 sub-samples in each replicate).

Control	277
Malathion	65
Bactrogel	224

Each data point is the total number of insects (pupae) recovered from 3 tomato fruits per cage from 3 sample cages over 5 replicates in time.

3.2 Phloxine

The above mentioned experiment (*3.1 Fipronil*) was repeated but with a protein hydrolysate bait with 1% Phloxine B as the toxicant instead of Bactrogel. Fruiting capsicum plants were used here instead of tomatoes.

In this experiment we tested the Phloxine B bait when applied as:

1. a cover spray (5mL / plant)
2. a splash bait (5mL / plant) applied to one spot in the canopy on the shady side of the plant
3. a bait station of 3 dental wicks in a Petrie dish on the ground (5mL / plant)
4. Control (no baits)

Results

Queensland fruit fly exposed to the Phloxine B solution applied as either a cover spray or as a splash bait were adversely affected within an hour of exposure but only on sunny days (Table 17). When there was cloud cover, although flies fed on the bait immediately they were presented with the bait, it took upwards of three hours for the first mortality to occur. All flies were dead by the third day after exposure.

The bait station application was slow to induce mortality. Not all flies fed on the bait station as soon as they were introduced into the cage. It was thought that food placed on the ground is not attractive to fruit flies. It would be better to place the bait station at tree level or within the canopy.

Although fruit flies were killed by the Phloxine B bait relatively quickly they still had the opportunity to infest fruit (Table 18). There were survivors from fruit treated with the bait station and the splash bait. There were no survivors following the cover pray application.

Table 17. Percentage mortality of Queensland fruit fly exposed to 1% Phloxine B solution applied in three different ways to caged capsicum plants.

Application type	Time after first exposure to bait solution (h)						
	0	1	2	3	24	48	72
Control	0	0.25	0.25	0.25	0.5	0.5	0.5
Cover spray	0	25	40	50	100	100	100
Splash bait	0	12	25	50	97	100	100
Bait station	0	0.5	10	17	75	75	100

Table 18. Infestation of capsicums treated with 1% Phloxine B solution applied in three different ways.

Application type	Total number of surviving insects (pupae) from 4 replicates of two fruit per treated plant
Control	712
Cover spray	0
Splash bait	8
Bait station	112

3.3 Conclusions - Field cage experiments on different toxicants in baits

Preliminary results from experiments carried out under *3. Field cage experiments on different toxicants in baits* showed some results that need further examination. At least we should test for the reality of these observations in the field. Both the Bactrogel bait and the Phloxine B bait did not protect fruit from infestation when applied to caged fruiting plants. The Phloxine B bait killed all flies when applied as a cover spray or as a splash bait. The Bactrogel bait killed about half of the flies only. If this is a real scenario then there should be ample opportunity for research and development into better attractants and feeding stimulants.

Within the limitations of correct statistical procedure it is reasonable to compare the two experiments (i.e. the Bactrogel and Phloxine B experiments) at least from a macro point of view. The results suggest that a splash bait with Phloxine B + protein hydrolysate gives greater control of fruit flies than a splash bait of Bactrogel (with protein autolysate). Also comparisons suggest that the Phloxine B bait is more effective in controlling adult flies and fruit fly infestations than the Bactrogel bait. These hypotheses need to be tested further.

The two experiments described above differed, also, in the type of protein used in the baits. It is possible that there is a difference in the attractancy of baits depending on the type of protein used. The protein source in the commercially manufactured mixture, Bactrogel, is protein autolysate. In Australia the protein autolysate generally used is Natflav® or Mauri Pinnacle®. In our experiments we used the Natflav® protein autolysate. In the Phloxine B experiments we used protein hydrolysate supplied by ICN.

We tested the possibility that some protein sources were more attractive to fruit flies than others (these experiments are described in the next section of this report).

4. Laboratory experiments on differential attractancy of various protein sources

Preliminary trials were undertaken at Gosford to test for major differences in the attractancy of various commercially-available protein sources to adult Queensland fruit fly. This work was done to test for gross differences in attractancy to enable future, more tightly targetted, experiments in Year 3 of Project AH00012.

Three experiments were carried out in the laboratory at 26°C and 65% relative humidity using small cages housing 20 flies each (10 males and 10 females). Baits made up as below (4.1 to 4.3) were presented to the flies. No toxicants were added to

these baits. Assessments of the number of flies feeding on the baits were made at regular intervals (1, 2, 3, 4, 5, 10 and 15 minutes) following initial exposure.

These experiments were replicated 3 times – once a week for three weeks.

4.1 Trial 1. Natflav vs. yeast hydrolysate:

- YEAST HYDROLYSATE - 21g OF PROTEIN IN 50ML WATER
- NATFLAV - 21g OF PROTEIN IN 50 ML OF NATFLAV
- BOTH WERE THEN DILUTED IN 50 ML OF WATER TO MAKE 100ML SOLUTION WITH A FINAL CONCENTRATION OF 210g/L (= 20% W/W SOLUTIONS)

4.2 Trial 2. Provesta vs. yeast hydrolysate

- 20% W/W SOLUTIONS FOR BOTH PROTEIN SOURCES

4.3 Trial 3. Yeast hydrolysate vs. yeast hydrolysate + preservatives (sodium benzoate and citric acid)

- YEAST AND YEAST + PRESERVATIVES WERE AGED ON THE LABORATORY BENCH FOR 1, 2, 3, 4, 7 OR 10 DAYS AT 20°C TO 25°C AND THEN PRESENTED TO ADULT QUEENSLAND FRUIT FLY.

Results

Under the laboratory conditions at Narara Natflav® is a very poor fruit fly attractant when compared with protein hydrolysate (Table 19).

Provesta® is also a poor attractant when compared with protein hydrolysate (Table 20). Results suggest that it loses its attractancy after about 4 minutes in the laboratory.

The addition of a preservative to the yeast hydrolysate bait does not improve the bait's attractancy to Queensland fruit fly over the period tested in these experiments nor does it detract from its attractancy (Table 21).

Table 19. Comparison of the attractancy of baits, without toxicant, based on yeast hydrolysate or Natflav, to adult Queensland fruit fly (from 3 replicates in time).

Time (min)	% female flies feeding	
	Yeast hydrolysate	Natflav
1	33	0
2	33	0
3	45	10
4	30	0
5	25	0
10	0	0
15	10	0

Table 20. Comparison of the attractancy of baits, without toxicant, based on yeast hydrolysate or Provesta, to adult Queensland fruit fly (from 3 replicates in time).

Time (min)	% female flies feeding	
	Yeast hydrolysate	Provesta
1	45	15
2	20	20
3	35	22
4	30	10
5	25	0
10	25	0
15	15	0

Table 21. Comparison of the attractancy of baits, without toxicant, based on yeast hydrolysate or yeast hydrolysate plus preservative, to adult Queensland fruit fly
(average number of flies feeding per observation – 5 observations 2 minutes apart, commencing 9:30 a.m. each day).

	% female flies feeding	
	Bait	Bait + preservative
Day 1	32	26
Day 2	20	18
Day 3	18	44
Day 4	52	26
Day 7	14	16
Day 10	16	10

4.4 Films - Nu Film

Nu Film is advertised as an ingredient that will maintain water moisture in fruit fly baits by, in effect, covering the bait with a film which is semipermeable to water. Nu Lure is a commercially available fruit fly attractant based on protein autolysate.

Baits were made up with protein hydrolysate (Controls) or Nu Lure bait with or without Nu Film according to manufacturers' recommendations or standard New South Wales Agriculture methods (Controls).

Treatment 1: yeast hydrolysate bait
Treatment 2: Nu Lure bait
Treatment 3: Nu Lure + Nu Film bait

Two cages of adult flies (one with 20 males and the other with 20 females) were allocated to each treatment. They were set up in the fly room at 26°C 60% relative humidity and 14h:10h day:night. Flies were 10 days old and protein-starved for 3 days prior to commencement of the experiment.

Assessments were made on the number of flies feeding on the baits and on the amount of mould development on the bait.

Results

Baits with added Nu Film showed a reduction in weight loss, which is good, but those baits also appeared to repel adult Queensland fruit fly (Table 22). Nu Lure, on its own, without Nu Film appears to be as attractive as protein hydrolysate in these experiments.

Table 22. Attractancy of adult Queensland fruit fly to bait

Experiment 1 (20 flies per cage)

Treatment	1 h exposure		2 h exposure		18 h exposure		24 h exposure	
	Males	Females	Males	Females	Males	Females	Males	Females
1	1	4	1	2	2	1	0	2
2	2	3	1	1	0	2	0	2
3	0	0	0	0	0	0	0	0

Experiment 2 (20 flies per cage)

Treatment		45 h	45 h 10 min	45 h 15 min	46 h	47 h	48 h	72 h	72 h 15min	73 h
1	Males	4	5	3	1	1	0	0	0	1
1	Females	6	3	2	1	0	0	1	0	0
2	Males	2	1	0	0	1	0	0	0	0
2	Females	8	4	1	1	0	1	1	1	1
3	Males	0	1	0	0	0	0	0	0	0
3	Females	0	0	0	0	0	0	0	0	0

4.5 Conclusions - Laboratory experiments on differential attractancy of various protein sources

Preliminary trials suggest that protein hydrolysate and Nu Lure are more attractive than Natflav® and Provesta®. These trials were carried out in the laboratory so should be extended to the field. Also it must be noted that some practitioners say that protein hydrolysate will cause unacceptable phytotoxic damage to some crops due to a high salt content. Because protein hydrolysate is such a good attractant the salt content issue, if real, should be investigated.

There are many more sources of protein available for trial against fruit flies and these should be investigated.

5. Bait modifications - other than protein

5.1 Ammonium acetate

A yeast hydrolysate bait similar to that described in 4.1 was made up with or without additional ammonium acetate. Ammonium acetate has been described as a potent attractant for several fruit fly species and is an inclusion in some commercial bait preparations such as Mago-Vial®, Hycase®, the Pherocon® Trap and Biolure®. It is not used in Queensland fruit fly baits nor is it included in Bactrogel.

Results

Fig. 2 shows that the addition of ammonium acetate to the bait was beneficial in attracting more adult Queensland fruit fly to the bait.

Other ammonium compounds will be tested later and compared with ammonium acetate.

5.2 Feeding stimulants

If flies are attracted to the bait and then stimulated to feed as much as possible more toxicant will be ingested and the bait will, therefore, be more effective in attract and kill. The addition of sugar as a feeding stimulant is used in some commercially available fruit fly baits such as PIB-7 (Staley Protein Insecticide Bait No. 7) and GF120.

Protein hydrolysate baits were made up as in 4.1 with or without additional ammonium acetate, sodium tetraborate (to adjust bait pH), sucrose or fructose.

Table 23. Recipe for baits

Treatment	Water (g)	Yeast (g)	Ammonium Acetate (g)	Sodium tetraborate (g)	Sucrose (g)	Fructose (g)
1	40	3	1	3	6	0
2	40	3	1	3	0	6
3 (Base)	40	3	1	0	6	0
4	40	3	1	0	0	6
5 (Control)	56	4.2	0	0	0	0

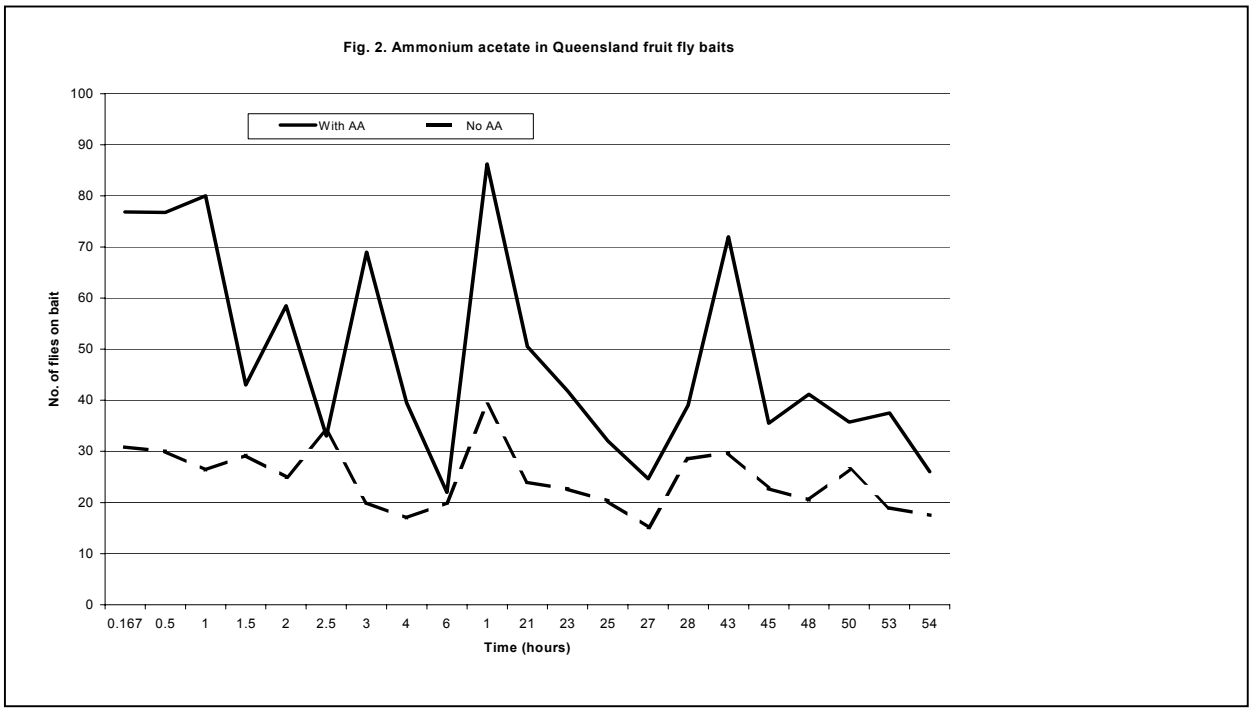
Results

There was a beneficial effect of added sucrose and ammonium acetate to the bait when compared with the Control with respect to attractancy of Queensland fruit fly. The addition of sucrose was more attractive to adult flies than fructose. Sodium tetraborate did not improve attractancy (Table 24).

Table 24. Attractancy of adult Queensland fruit fly to bait (50 flies per cage, separate cages for males and females, experiment replicated 3 times, a, b and c are significantly different at P<0.05)

Treatment	pH	Attraction								Cumulative Attractancy
		15 min exposure		1 h exposure		2 h exposure		64 h exposure		
		male	female	male	female	male	female	male	female	
1	8	8	14	6	3	3	5	0	2	41[c]
2	7	24	19	9	1	8	2	3	5	71[b]
3 (Base)	7	26	19	16	12	5	6	7	9	100[a]
4	7	21	17	9	5	3	3	6	2	66[b]
5 (Control)	7	23	12	11	5	6	5	7	3	72[b]

Figure 2. Ammonium acetate in Queensland fruit fly baits



5.3 Mould development on baits

5.3.1 Inclusion of preservatives

Baits should last in the field without fungal and bacterial degradation. Several baits (see Table 25) were assessed for the level of fungal and bacterial contamination over time at 26°C and 55% to 65% relative humidity. The gels Guar and Xanthan were used in the baits. These compounds are used in commercial baits such as Bactrogel and are used to lengthen the field life and attractancy of the bait.

Results

Table 25 shows that baits made with Guar were more protected from mould development than baits made with Xanthan (Xan). Baits with ammonium acetate (AA) were less affected by mould than baits without ammonium acetate. Baits with

Sucrose only performed the worst with respect to mould development. The best bait was made with ammonium acetate and sucrose.

Table 25. Comparison of mould development in test baits following 3 days at 26°C and 55% to 65% relative humidity

Bait	Experiment 1			Experiment 2			Experiment 3			AVE
	Rep1	Rep 2	Ave	Rep1	Rep2	Ave	Rep1	Rep2	Ave	
Guar	4*	4	4	8	9	8.5	7	6	6.5	6.3
Guar + AA**	6	2	4	8	6	7	2	6	4	5.0
Guar + Sucrose	6	7	6.5	7	9	8	7	9	8	7.5
Guar + AA + Sucrose	3	2	3.5	4	4	4	8	5	6.5	4.7
Xan	8	4	6	8	6	7	10	9	9.5	7.5
Xan + AA	5	5	5	5	5	5	8	7	7.5	5.8
Xan + Sucrose	9	10	9.5	10	10	10	10	9	9.5	9.7
Xan + AA + Sucrose	8	6	7	5	5	5	3	4	3.5	5.2

* Score for mould development from 1 (zero mould development) to 10 (100% of bait is covered in mould)
** Ammonium acetate

5.3.2 Sodium benzoate

I used sodium benzoate (0.5 g in 160 g of bait) in the bait and found that it did not attract as many fruit flies as did the bait without sodium benzoate.

5.3.3 Acetic acid

Acetic acid was used to adjust the bait's pH from 6 to 4. It was thought that acetic acid would be attractive to fruit flies (based on overseas literature) and that a pH more closely related to fruit pH would also be more attractive.

Results

The addition of acetic acid to adjust bait pH from 6 to 4 appeared to repel Queensland fruit fly adults (Table 26).

Table 26. Effects of pH (inclusion of acetic acid) in baits on attractancy of Queensland fruit fly to baits

Bait	Exposure time (h)							
	0.25	1	2	3	44	46	48	50
pH 6 Rep1	10*	7	7	7	15	8	6	5
pH 6 Rep2	13	8	5	6	26	11	8	7
pH 4 Rep1	1	0	0	0	0	0	0	0
pH 4 Rep2	0	0	0	1	1	0	0	0
pH 4 Rep3	1	0	0	0	0	0	0	0
pH 4 Rep4	0	0	0	0	0	0	0	3

* Number of Queensland fruit fly on bait during 20 seconds of observation.

5.4 Effects of dehydration on bait efficacy

5.4.1 Attractancy of GF120 with varying moisture content

A batch of GF120 was made up each day for five days according to label directions. Each batch was divided into three sub-samples, weighed and then placed into an incubator set at a relatively low temperature (30°C) to ensure no protein denaturation. On Day 5 each sub-sample was weighed to determine percentage water loss and then presented to a cage of 20 adult flies (10 days old and protein-starved for 24h prior to exposure to the bait). The Day 5 batch was the non-dried Control (no dehydration).

Observations of feeding on the bait were made at 10 minute intervals. Baits were re-weighed after the 90min observation and compared with the original Control weight.

These experiments were conducted in the fly room which is maintained at a temperature of 26°C ± 1°C and a relative humidity of 60% ± 5%.

Results

Table 27 shows that bait attractancy was low for flies following exposure to the bait dehydrated by 45%, 77% and 84%. Attractancy returned to these baits after about 30min to 40min presumably due to slight rehydration and due to the flies being protein-starved. Some rehydration occurred in the driest baits (Table 28).

Table 27. Effects of bait dehydration on fruit fly attractancy

	Average number of flies feeding on bait (n=3) after:								
Ave % moisture loss	10min	20min	30min	40min	50min	60min	70min	80min	90min
0 (Control)	11.0	12.3	15.3	14.3	5.3	5.7	3.7	4.3	4.0
23.4	10.7	10.7	15.0	15.3	7.0	5.0	5.0	4.7	4.3
45.2	2.0	4.7	10.7	12.0	11.7	7.7	4.3	3.7	2.7
77.1	1.7	2.3	6.7	7.7	11.0	6.7	6.7	4.0	3.3
83.9	0.7	2.7	4.3	7.3	10.3	6.7	3.7	2.0	2.3

Table 28. Weight of baits after the 90min exposure in the fly room (temperature of 26°C and relative humidity of 65%)

Ave % moisture loss before experiment	Ave % moisture loss after experiment
0 (Control)	19.0
23.4	22.9
45.2	42.9
77.1	62.4
83.9	70.2

Conclusions

The drier the bait the less likely flies are to feed from it. If it re-hydrates attractancy may improve. It appears from the data in Table 27 that the three driest baits were most attractive at about 40 to 50 minutes after initial exposure to the bait. This could be as a result of some rehydration. The baits were dry but the protein content is quite hydrophilic so would have absorbed some atmospheric moisture (see Table 28). After an initial feeding, within the first 30 to 40 minutes, flies did not return to the baits, even the moist baits, within the next 60 minutes.

5.4.2 Field trials of trap with long-term female bait

A series of 7 experiments were carried out to develop a technique to demonstrate that the retention of bait moisture content would improve bait efficacy. An additional benefit would be to design a female bait that will remain hydrated, and efficacious, for a period of several weeks. These experiments were conducted in the field in the mixed orchards at the Narara Research Station from May, 2001 to January, 2002.

Results

Samples of bait stored in the laboratory at ambient temperature (15°C to 25°C) and relative humidity (45% to 65%) decreased in weight from an average of 22g to 3.61g over a period of 6 weeks. Samples of bait in the trap in the field decreased in weight from an average of 22g to 16.71g. The trap collected or retained (or both) water which reduced bait dehydration.

Table 29 shows that the trap, with no toxicant, was attractive for at least 22 days in October, 2001. The technology developed in these experiments should be further developed.

Table 29. Flies trapped in one Narara trap charged with Narara bait

From	To	Males trapped	Females trapped	Total flies trapped
29 Sept 2001	8 Oct 2001	41	31	72
8 Oct 2001	15 Oct 2001	28	21	49
15 Oct 2001	22 Oct 2001	49	47	96
TOTAL (over 22 days)		118	99	217

The above field experiment was repeated using the same Narara bait mixture in McPhail traps. Results were compared with flies trapped in McPhail traps charged with a standard GF120 bait. Similar concentrations of protein were present in both the GF120 traps and the Narara bait traps. We were mindful of the fact that GF120 did not kill trapped flies quickly so fly loss from the trap was likely. A small square (about 6mm X 6mm X 2mm) of Dichlorvos-impregnated clothes moth pest strip was placed in each trap to ensure that flies entering the trap were killed quickly. Three traps of each were placed in loquat trees at the Narara Research Station and cleared after 4 weeks (November, 2001).

Results

Table 30. Average number of flies trapped in one McPhail trap charged with Narara bait (n=3 traps)

From	To	Males trapped	Females trapped	Total flies trapped
02 Nov 2001	09 Nov 2001	51	56	107
09 Nov 2001	16 Nov 2001	68	85	153
16 Nov 2001	23 Nov 2001	56	61	117
23 Nov 2001	30 Nov 2001	49	57	106
TOTAL (over 29 days)		224	259	483

Table 31. Average number of flies trapped in one McPhail trap charged with GF120 bait (n=3 traps)

From	To	Males trapped	Females trapped	Total flies trapped
02 Nov 2001	09 Nov 2001	24	48	72
09 Nov 2001	16 Nov 2001	10	35	45
16 Nov 2001	23 Nov 2001	0	6	6
23 Nov 2001	30 Nov 2001	0	0	0
TOTAL (over 29 days)		34	89	123

Conclusions

The GF120 bait in a trap did not perform as well in trapping Queensland fruit fly as the Narara bait. Of course the GF120 is not designed to last more than 1 week as the label recommends that re-application of the bait should be on a weekly basis. This experiment was not designed to prove a deficiency with FG120 but to demonstrate that maintenance of moisture in a protein bait improves the long-term efficiency of the bait in attracting Queensland fruit fly.

5.5 Conclusions - Bait modifications - other than protein

Preliminary studies on other aspects of bait improvement were carried out in *5. Bait modifications - other than protein.* The following conclusions can be made from this work. On the basis of laboratory experiments the inclusion of ammonium acetate and sucrose should be tested in the field. Concern, however, has been raised about the

possibility that sucrose will attract bees into the traps to be killed by toxicants. Ammonium acetate has been shown to be repellent to bees (Dr R. Mangan, USDA, personal communication) so the two inclusions are mutually beneficial. Ammonium acetate is a by-product of microbial action on the protein source in baits and is a potent fruit fly attractant so the addition of extra ammonium acetate to the bait will extend the bait's attractancy. By the time the added ammonium acetate has been driven out of the bait natural microbial emissions of ammonium acetate will have commenced. The sucrose inclusion will entice the trapped fly to feed more readily on the bait and hence die more rapidly thereby reducing the potential to escape from the bait and oviposit into the crop.

In the laboratory the inclusion of ammonium acetate and sucrose reduced fungal and bacterial contamination of the bait while maintaining bait attractancy. This implies that the spread of rot fungi such as *Rhizopus* is checked while the bacteria which feed on the protein to produce fruit fly attractants are not. The inclusion of the preservative sodium benzoate and adjustment of pH to 4 did not improve bait attractancy. It is likely that the sodium benzoate sterilized the bait, reduced bacterial degradation and reduced production of ammonia compounds.

In both the laboratory and the field maintaining bait moisture content improved both bait attractancy and bait longevity. More laboratory and field work should be done on this to complete preliminary studies commenced here. This is quite important if GF120 and Bactrogel are approved for use on crops grown in dry climates such as the MIA in NSW, the Riverland in South Australia, Sunraysia in Victoria and Western Australia.

An additional potential benefit to maintenance of moisture content in baits would be the potential of inventing a long-term female fruit fly trap, none of which exist for *Bactrocera* species of fruit fly at present. A long-term female fruit fly trap is the most sought-after tool for fruit fly control. More research and development on this needs to be carried out.

6. Technology transfer

Data from this project were collected under controlled laboratory and field cage situations, in the main. While it was anticipated that this project would cover field tests it turned out that it had to be terminated before completion. Consequently the authors can make recommendations, only, that more research and development on the findings from this project should be carried out in the field.

Technology transfer has occurred in utilising some of our findings on bait and trap research and development in Fruit Fly Area Wide Management programs on the Mid-North Coast of NSW.

People involved in overseas fruit fly management have asked for, and been given, advice on fruit fly bait and trap placement based on some of the outcomes from the research reported here.

7. Recommendations

- Field testing of the findings from the research reported here should be under taken
- Field tests should assess
    - the efficacy of new baits to attract and kill fruit flies
    - their effects on beneficial insects
    - phytotoxicity
    - cost
    - longevity of efficacy
- Eventual incorporation of this project's outcomes into a complete fruit fly management package.

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- Bayer Australia for supply of imidacloprid and other chemicals
- AMTRADE for supply of Nu Lure and Nu Film
- Organic Crop Protectants for supply of Neem (Azamax®)
- New South Wales Agriculture for funding and supply of resources.

8.3.4 Evaluation of trap designs

Figure 31 shows the mean trap catches for the 6 trap designs. Although the 4 x 30mm hole trap appeared to slightly outperform the other designs, analysis of the results using a one way ANOVA showed no significant difference between designs for trap catch and overall catch. There was some difference however in the sheet catch between trap designs. Results of the analyses were as follows:

Trap catch

Results showed no significant trap design effect (p=0.483) however, if an unprotected LSD test was used, the Bugs for Bugs 4 x 30mm hole trap caught significantly more flies than the Sensus trap.

Sheet catch

With all trap designs, a small number of flies were collected from the sheets indicating that some flies died outside the traps and would normally be lost. There was a significant trap design effect (p<0.001) with the Bugs for Bugs 4 x30mm hole trap resulting in a significantly greater external knockdown than the other trap designs. The external knockdown with the Bugs for Bugs 4 x 15mm hole trap was significantly greater than that of the Lynfield trap (LSD 5% = 5.178).

Total catch

When total trap catches were analysed, there was no significant trap design effect (p=0.357). However, if an unprotected LSD test was used the Bugs for Bugs 4 x 30 mm hole trap caught significantly more flies than the Sensus trap.

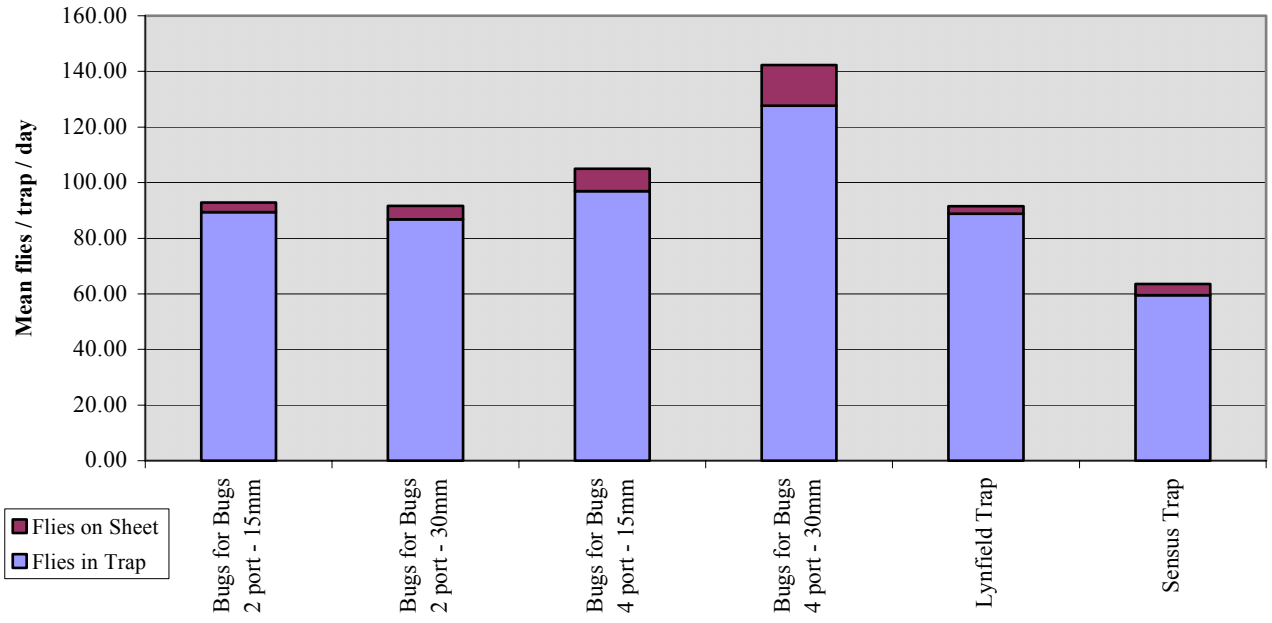

Figure 31. Mean trap catch of 6 replicates comparing 6 trap designs

8.4 Discussion

8.4.1 Effects of first stage MAT on trap catches

Previous research (DPI- HAL Project CT97036) provided extensive trapping data during a district wide survey in 1999 which showed the seasonal distribution of fruit flies in baited orchards, in town areas and along the Burnett River (Figure 27). This data reflected the effects on fruit fly numbers of seasonal conditions and a high level of baiting in orchards during the citrus season from January to early September. No male annihilation activity was taking place in the district at this time.

Results of the district wide trapping survey from August 2002- August 2003 clearly showed the effects that the implementation of MAT could have on fly populations in an area. Trap catches in the Gayndah orchards with MAT were very low compared to those in the other three test areas. Whether this reduction in trap catch represented a real reduction in the male population or purely a competition effect between traps and MATs is difficult to determine. Monitoring traps were intentionally placed at least 50m from the nearest MAT devices, but care must still be taken when interpreting male trap catches in the early stages of male annihilation control because the effects of mating disruption on the female population are difficult to measure when an effective female trap is not available. Under these circumstances, the most reliable indicator of MAT effectiveness is reduction in fruit infestation. However, quantitatively demonstrating a reduction in infestation in commercial citrus in the Central Burnett would require sampling very high numbers of fruit.

DPI research in recent years has shown the existing orchard baiting programs together with climatic and other production factors result in extremely low levels of infestation (0.02-0.04% at 95% confidence) throughout most of the citrus season (DPI- HAL project CT97036). The greatest risk of infestation is in early September when the late Murcott crop (a highly susceptible variety) is exposed to the normal spring peak in fruit fly numbers, which occurs every year in the district. In the 2003 season, with the beginnings of MAT implementation in some Gayndah orchards, growers reported a significantly lower incidence of damage in late Murcotts. This was unexpected, particularly after a relatively warm winter as was experienced during this trial. These results showed the positive initial impacts of MAT implementation in the Central Burnett and indicted the potential for improved area wide control with the commencement of the new area-wide management program (DPI-HAL Project AH03002) in July 2003.

8.4.2 Fruit survey in town areas

Previous studies by DPI researchers have shown that there are almost no wild fruit fly hosts in the Central Burnett citrus growing district. From late September at the end of the commercial citrus season, until early varieties are maturing at the beginning of the next citrus season in February, alternative commercial hosts are likely to be mangoes and stone fruit, which are grown to a much smaller extent than citrus. However, during the off-citrus season, large numbers of untreated backyard fruit trees in the town areas are likely to provide ideal breeding conditions for flies which can subsequently move into citrus orchards when fruit becomes available in late summer- autumn. In the course of collecting town fruit samples, property owners were questioned about the fruit fly control measures they applied. A small number occasionally used insecticide sprays but generally no controls were applied, backyard fruit was claimed to be heavily infested and fallen fruit were allowed to lie rotting on the ground.

Results of the 2002-2003 host fruit survey in Gayndah and Mundubbera town areas supported the theory that untreated town backyard host trees were potentially breeding “hot spots” for fruit flies in the Central Burnett. A range of highly susceptible hosts was identified

and high levels of infestation were found in many common fruit types. Even a preliminary estimate of the numbers of various host trees in both towns indicated the large numbers of fruit flies which could be produced in town areas from summer fruit. Collection data showed that infested cherry guavas (14 trees in Gayndah) and stone fruit (49 trees in Gayndah) could be producing 707 and 569 flies per kg of fruit respectively. Mangoes were less heavily infested (20% in Mundubbera and 50% in Gayndah) producing 3-23 flies per kg of fruit. However the large number of mango trees (a total of 320) and the fact that many of these trees, particularly in Gayndah, are very large bearing many hundreds of fruit highlighted the potential impact this could have on fruit fly populations in the district.

The data generated from this study will be used in planning town treatment strategies in the AWM program which commenced in the Central Burnett in July 2003. The overall results have confirmed the need for town treatments to be an important component of any AWM program. Furthermore, the identification of type and location of backyard hosts will enable protein bait treatments to be focused on particular properties when known hosts are fruiting. When all of this information is recorded on a database as part of the AWM project, it will enable operational activities to be carried out in the most efficient manner with subsequent savings in time and labour.

8.4.3 Comparison of MAT carriers

Although every effort was made to minimise variability by designing appropriate methodology to test the relative efficacy of MAT carriers, results were so variable that a decay curve could not be fitted to the data. On some occasions weathered wicks and blocks performed better than new ones resulting in relative efficacies higher than 100%. The overall conclusions from this experiment were that there was no difference in efficacy between wicks and blocks, efficacy decreased slowly with time but within the recommended 3-4 month time for replacement of cue lure MAT carriers there was relatively little loss in efficacy.

These results were in sharp contrast to those obtained with methyl eugenol (ME) – malathion dosed blocks used in the Papaya Fruit Fly Eradication Program. Research undertaken in this program by Lloyd et al (1998) showed that relative efficacy of the ME blocks (dosed with 18g of 3:1 ME and malathion a.i.) decreased by 50% after 8 weeks exposure in north Queensland. After 30-40 weeks ME blocks retained only about 10% efficacy. However, the cue lure dosed blocks tested here (which contained only 1ml of lure plus 0.5ml of malathion a.i.) maintained their efficacy much better over time. Although results were very variable, relative efficacy of a weathered block to a new block did not fall below 40% after 12-36 weeks exposure.

Chemical analyses of carriers weathered for up to 28 weeks, showed that the loss of cue lure from both wicks and blocks followed a very similar pattern. Approximately two thirds of the cue lure from both carriers was lost after 12 weeks. Loss of malathion was much slower with approximately one third being lost after 28 weeks exposure. In the early stages of weathering, malathion was lost more rapidly from the wicks than from the blocks. The levels of raspberry ketone in both MAT carriers were very similar with the mean content increasing slightly from 0.03g to 0.065g per carrier during 28 weeks exposure. It is unlikely that such low levels of this compound were having any significant positive or negative effect on the efficacy of the MAT carriers.

This component of the preliminary AWM research showed that the commercially available MAT cups performed satisfactorily for 3-4 months in the Central Burnett. There was no advantage to be gained by using Cane-ite block carriers. The MAT cups had the additional advantage that the plastic cups could be retrieved and recycled with new wicks added and the

cups could be produced in different colours so that different MAT treatment cycles could be easily identified and replaced with new cups when required.

8.4.4 Evaluation of trap designs

These results showed that modification of the standard Bugs for Bugs trap in the form of number and/or diameter of entry port holes had no significant effect on total trap catch but the 4x30mm trap design significantly increased the number of flies knocked down outside the trap. The greater external knockdown from this design could be due to an increased concentration of attractant fumes in the entry port. Flies could then be dying in the port and falling out rather than entering the trap fully. The Sensus trap design performed less efficiently than the other designs although the difference was only significant when compared to the best performing design (4 x 30mm ports).

As the design modifications did not produce any worthwhile improvement in trap efficacy, the selection of trap design for a particular situation is likely to be based on other factors such as cost, life expectancy in the field, ease of manufacture, ease of installation and ease of servicing. For experimental trials DPI uses Lynfield traps because they are generally in service for a relatively short period and they are inexpensive to produce, having no entry port inserts. Entry ports are used to minimise the entrance of water into traps and hence they are frequently used in high rainfall areas. Any of the Bugs for Bugs designs with entry ports would be suitable in such situations.

Although the results of this trial showed no significant improvement in trap catch by modifying the design "Bugs for Bugs" changed their standard trap to the 2 x30mm hole version as the larger entry surface area could potentially provide a larger 'plume' of lure which may have the advantage of attracting flies over a greater distance in the field. This modification was done with minimal cost.

8.5 Conclusions

- Preliminary implementation of MAT in some orchards in the Gayndah area in 2001-2002 had a significant effect in reducing fruit fly numbers.
- Detailed data on infestation levels and numbers of trees showed the high level of fruit fly breeding in untreated back yard fruit trees in the towns of Gayndah and Mundubbera.
- There was very little difference in performance as MAT carriers between plastic cups containing cue lure/malathion wicks and Cane-ite blocks impregnated with the same chemicals over a period of 36 weeks when both carriers were exposed to natural weathering conditions in the Central Burnett. MAT cups have the advantages that they can be easily colour coded, are retrievable and recyclable.
- Changes to trap design in the form of entry ports and increasing the number and size of holes did not significantly improve trap performance when used with cue lure/ malathion wicks for monitoring Qfly.

8.6 Recommendations

- Results of the above research should be incorporated into developing targeted treatment strategies for the area-wide fruit fly management program in the Central Burnett which commenced in July 2003.
- Some of the above outcomes are generally applicable to any area-wide management program for Qfly and could be incorporated into similar programs which are being initiated in other fruit fly endemic areas in Australia.

PART D – Generic bait research – NSW Agriculture

9 Improving fruit fly baits

The following section is a self-contained report prepared by Andrew Jessup *et al.* on the generic bait research carried out by NSW Agriculture in Years 1 and 2.

10 TECHNOLOGY TRANSFER

10.1 Research collaboration

This project was initiated at a national level and involved researchers from three state government organisations: DPI Qld, NSW Agriculture and Agriculture Western Australia. Researchers from the USDA-ARS with particular expertise in fruit fly control and fruit fly bait formulation were also involved. Team members communicated frequently and met with HAL Program Managers and commercial partner representatives at project review meetings in Years 1 and 2.

10.2 Industry consultation

At the beginning of the project an Industry Reference Committee with representatives from each participating state was established. To ensure that research outcomes were quickly made available to growers, copies of the non-confidential Milestone Reports were sent to committee members.

The members of the Industry Reference Committee were as follows:

Queensland: Mr Craig Meyer**,** commercial citrus grower
Mr Mark Panitz**,** Industry Development Manager
Queensland Fruit and Vegetable Growers

New South Wales: Mrs Nell Snaidero, Secretary Tri-State Fruit Fly Committee
Mr Gary Wright, Farm Manager, Blueberry Farms of Australia

Western Australia: Mr Jeff Fawcett, commercial citrus grower,
WA citrus industry rep on HAL board

All large scale field trials to test the new bait products were undertaken in commercial orchards with the assistance of participating growers. This facilitated grower feedback to the research team about the advantages and disadvantages of the new baits. Queensland growers were particularly helpful in contributing to the development of modified spray equipment to effectively deliver the new thickened baits. This ongoing industry involvement in research activities was an important component of technology transfer in the project.

10.3 Commercial partner involvement

The involvement of two commercial partners (Dow AgroSciences and Aventis CropScience) with two potentially competing bait products being tested in the same project presented some challenges to the project team. However, confidentiality requirements of both partners were successfully met. All field trials involved both new baits being tested against an industry standard at the same time. Confidential reports to each commercial partner contained the results for the standard bait and their respective product only. The only exception to this was with the DPI report on the effects of baits on beneficial insects where both commercial partners gave permission for a combined report to be prepared.

Since Aventis CropScience was taken over by Bayer and the fipronil insecticide group of products (including the Bactrogel bait) was sold to BASF in 2003, the Project Leader has met with representatives of this company to discuss details of the project related to the fipronil bait (now known as Amulet Fruit Fly Gel).

The DPI project team were involved in ongoing collaboration with Bugs for Bugs during Year 3 when MAT carriers and traps produced by this company were evaluated for use in the CBAWM program.

10.4 Technology transfer activities

Project team members in all three states have been involved in a wide range of activities to report research outcomes. These have included grower and industry meetings, seminars, workshops, field days, public displays and conference presentations. A wide variety of media articles related to the project have been published in industry journals, grower newsletters, and regional newspapers.

The outcomes from the preliminary AWM research in the Central Burnett (**PART C**), in particular the results of the town fruit survey, have been included in a large number of communication activities related to the CBAWM project which has recently commenced. Data to demonstrate high levels of fruit fly infestation in town backyards has been highlighted in mail outs to town dwellers, in numerous press releases and in displays organised by the project team at the Mundubbera Show and the Gayndah Orange Festival in early 2003. This information is especially relevant to obtaining a high level of cooperation from town property owners in implementing AWM treatments.

Details of technology transfer activities for each state organisation are given below.

DPI Qld

Media articles

Queensland Country Life, 26 April 2001.
South Burnett Times, 1 May 2001.
"New fruit fly baits trialled in national project" – *Good Fruit & Vegetables*, July 2001.
"Researchers test new fruit fly baits" – *The Custard Apple*, January 2002.
"Feature-Foiling the Fruit Fly!" – *Australian Society for Horticultural Science Newsletter*, September 2002.
Australian Citrus News
Numerous local press releases in the Central Burnet related to the AWM program.

Meetings, Seminars, Workshops, Presentations

- Bundaberg and District Orchardists Association, October 2000.
- Visit by Dr Bob Mangan to citrus trial sites and meeting with growers in the Central Burnett, February 2001
- Central Burnett Horticulture Committee meeting, March 2001.
- Central Burnett Horticulture Committee meeting, September 2001.
- Custard apple growers meeting – Nambour, December 2001.
- Custard apple growers meeting - Alstonville, December 2001.
- Visit by Dr Bob Mangan to Queensland fruit fly pome fruit trial site, Stanthorpe, meeting with local QDPI entomologists and growers, May 2002.
- Dr Bob Mangan presented a seminar on the latest fruit fly control strategies in the US, attended by researchers from CSIRO, Griffith University, QDPI, crop consultants, May 2002.
- Central Burnett Horticulture Committee meeting, Gayndah, March 2002.

- Meeting with AQIS, Biosecurity Australia, APHIS Agriculture Attaché to the US Embassy, June 2002.
- Central Burnett Horticulture Committee meeting, Gayndah, September 2002.
- Meeting with Bundaberg Orchardists Association, October 2002.
- Meeting with Biological Farmers of Australia Co-op. Pty Ltd, 2002.
- Central Burnett Horticulture Committee meeting, Mundubbera, March 2003.
- Meeting with Central Burnett Area Wide Management Committee, March 2003.
- Meeting with Citrus Industry Advisory Committee, March 2003.
- Presentation by Annice Lloyd to the Queensland Entomological Society meeting, September 2003.
- Presentation by Annice Lloyd and Ed Hamacek to local grower group at St George in south west Queensland interested in starting an AWM program, October 2003.

Public communication of CBAWM research outcomes

- Display at Mundubbera Show, May 2003.
- Display at Gayndah Orange Festival, June 2003.
- Display material provided for presentations by local crop consultants at Gayndah and Mundubbera Shire Councils, local schools, Gayndah Garden Show.
- Mail outs and press releases related to AWM program in the Central Burnett.

Conference papers to be published

- Lloyd *et al*. Australian Custard Apple Growers Conference, Ballina, July 2003.
- Lloyd *et al*. Queensland Organics Conference, Cairns, July 2003.

Agriculture WA

Media articles

- Quarterly Newsletter Industry Reference Committee (Mr Jeff Fawcett)

Meetings, Seminars, Workshops, Presentations

- Workshop for stonefruit and pomefruit growers in the Perth Hills, 16 May 2001.
- Entomology Discipline meeting, 26 June 2001.
- "Now and into the Future" – Citrus Industry Development Project Seminar, Canning Vale, 27 November 2001.

Field Days

- Balingup Field Day, September 2000
- Manjimup Field Day, September 2000
- Perth Hills Field Day, September 2000
- Western Australia Citrus Improvement Group Field Day, December 2000.
- Western Australia Citrus Improvement Group Field Day, 12 December 2001.

NSW Agriculture

Media Articles

- ABC radio interview with Renee Du Preez on Radio National.
- *Fruit Wise* – NSW Agriculture publication.

Meetings, Seminars, Workshops, Presentations

- Low Chill Stone Fruit Workshop, 28 March 2001.
- Citrus Industry Strategic Development Plan (through Mr Lou Relevant).

Field Days

- Queensland fruit fly information day for coastal fruit and vegetable growers.

11 BIBLIOGRAPHY

Colquhuon, E. 1998. HRDC Final Report Project CT97024. Review and management for R&D on control of fruit fly in the field.

Cunningham, R.T. 1989. Parapheromones. In World Crop Pests, Fruit Flies, Their Biology, Natural Enemies and Control. Volume 3A. Edited by A.S. Robinson and G. Hooper. Elsevier. 221-230.

Jorgensen, K. 2002. HAL Final Report Project AH01016. Area-wide Management of fruit fly in endemic areas- A feasibility study

Lloyd, A., Hamacek, E., Smith, D. and Kopittke, R. 2000. HAL Final Report Project CT97036. Evaluation of protein baiting and inspection on the packing line as a quarantine treatment for fruit fly in citrus.

Lloyd, A., Leach, P. and Kopittke, R. 1998. Effects of exposure on chemical content and efficacy of male annihilation blocks used in the eradication of *Bactrocera papayae* in north Queensland. General and Applied Entomology. 28: 1-8.

Smith, D., Smith N. J. and Smith, K. M. 1998. Effect of abamectin on citrus rust mite *Phyllocoptruta oleivera* and brown citrus mite *Tegolophus australis* and the scale natural enemies *Aphytis lingnanensis* and *Chilocorus circumdatus* on oranges. Plant Protection Quarterly 13(3), 136-139.

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The following growers participated in DPI field trials:
Citrus: **Rod Baker, Robert Geery, Neville Harris, Ken Harris, Brian Ahern.**
Custard apples: **Bill Thompson, Keith Paxton, Greg Taylor.**
Passionfruit: **Noel Day, Don Hartley, Keith Paxton.**
Pome fruit: **Corrado Rizzato, Ugo Tomasel, Marcel Veens.**

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Western Australia

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Pome fruit: **Ric Owen, Cirino Licciardello, Tony Compagnone**.
Mango & Stone fruit phytotoxicity: **John Martin, Ramona & Eric Altinier**

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