Alternative fruit fly treatment for interstate market access for strawberries: winter window option

Brendan Missenden The Department of Agriculture, Fisheries and Forestry, Qld

Project Number: BS09022

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Horticulture Australia Project Number BS09022 Final Report (March 2014)





Great state. Great opportunity.

PROJECT DETAILS

Horticulture Australia Ltd Project Number: BS09022

Project Title: Alternative fruit fly treatment for interstate market access for strawberries: Winter Window option

Report Date: March 2014

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Project Objective:

To provide further data to support the Winter Window option for interstate market access for strawberries from Southeast Queensland and to provide scientific data to extend the geographic area covered by ICA-34 to include the Bundaberg region.

Funding:

This project has been funded by HAL using voluntary contributions from Queensland Strawberry Growers Association and Bundaberg strawberry growers and matched funds from the Australian Government.

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1 Technical Summary

Building on the research project 'Alternative fruit fly treatment for interstate market access for strawberries' (BS06022), the Market Access Team of Queensland Department of Agriculture, Fisheries and Forestry carried out further trials to assess the risk of fruit fly infestation in untreated strawberries harvested from Southeast Queensland during the Winter Window period. This data would be used to support the Winter Window option for interstate market access for strawberries from Southeast Queensland

From monitoring fruit fly activity this study found that the population and abundance of Queensland fruit fly (*Bactrocera tryoni*) and lesser Queensland fruit fly (*B. neohumeralis*) on strawberry farms is very low from May until mid August. Also, from assessment of strawberry samples we have demonstrated that the risk of fruit fly infestation in untreated strawberries produced in Southeast Queensland during the winter period was negligible.

On 16 January 2011 a new operational procedure (ICA-34 '*Pre-harvest field control and inspection of strawberries*') came into effect, which allowed market access for Southeast Queensland strawberries to enter restricted quarantine markets based on data collected in this project and the project BS06022. This procedure details a systems approach, which uses a combination of control tools coupled with a low fruit fly population during winter to obtain control and meet quarantine entry requirements for interstate trade. In November 2011, this project was extended to collect scientific data to expand the geographic region covered by ICA-34 to include the Bundaberg region.

From monitoring fruit fly activity in the Bundaberg region it was found that the population and abundance of Queensland fruit fly (*Bactrocera tryoni*) and lesser Queensland fruit fly (*B. neohumeralis*) on strawberry farms was very low, particularly during the winter months from May to 10 August. Also, the risk of fruit fly infestation in packed strawberries during this time was negligible. During the trials in 2012 and 2013 a number of fruit fly detections occurred, however these were most likely caused by non-conformances of the operational procedures.

In trails conducted in the Bundaberg region, it was found there was a greater risk of infestation in strawberries after 10 August. It is likely that additional measures are required to ensure the protection of the crop and to meet interstate quarantine requirements. This may include, but is not limited to, the use of shorter reapplication intervals for currently available cover spray chemicals, as well as the use of bait sprays after 10 August until the end of harvesting. In addition, investigation of alternative cover sprays that are effective against fruit flies may also be useful to provide more options to growers.

2 Media Summary

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3 Introduction

Strawberries (*Fragaria ananassa*) are grown all year round in Australia, with peak production times varying between each state (Keogh et al., 2010). In Queensland, strawberries are grown in subtropical areas, namely along the east-coast from Caboolture to Eumundi, and in recent years Bundaberg has become a significant production area (Queensland Government, 2014). In the 2012/13 season the Queensland strawberry industry was forecast to be worth \$125 million (Queensland Government, 2012). The most recent information from the 2010/11 season suggests that Queensland accounts for 35% of the total national production, producing 24 million kilograms or 96 million punnets each year (Strawberries Australia, 2012). It is estimated that 30% of Queensland Government, 2011).

Strawberries are recognized as a host of Queensland fruit fly (*Bactrocera tryoni*) (Hancock et al., 2000), however when grown on the ground under commercial practices, particularly during winter months, strawberries pose a very low risk of infestation by fruit flies (Gu et al., 2010, Greer, 2002). However, because Queensland strawberries are grown in areas that are endemic to Queensland fruit fly, growers are required to implement control programs in order to meet market access entry requirements. Prior to 2010, in order for Queensland strawberry growers to access restricted markets in South Australia and Victoria, growers used a range of treatment options to ensure fruit flies were controlled (Queensland Government, 2011). These included:

- Harvesting of fruit during a Winter Window period from June until 19 September, where low fruit fly pressure reduced the risk of fruit fly infestation, which allowed an exemption from treatment
- 2) a systems approach under the Interstate Certification Assurance Scheme (ICA-11), which required the pre-harvest treatment of strawberries with dimethoate, as well as the in-field and pack-house inspection
- 3) or methyl bromide fumigation (ICA-04)

In 2009, the Winter Window option was withdrawn by Victoria and South Australia due to the detection of live larvae in two consignments of strawberries in late August. These were the first reported interceptions of Queensland fruit flies since the negotiation and commencement of trade in 1996 (Queensland Government, 2011). The detection of these fruit flies during August 2009 followed a period of unseasonably high temperatures (Gu et al., 2010).

Following the withdrawal of the Winter Window option, in order for Queensland strawberry growers to access southern markets they relied on the use of ICA-11, which meant growers were applying dimethoate cover sprays throughout the entire season. Growers found that the early use and continual application of dimethoate disrupted the Integrated Pest Management systems that were established to control mites and other insect

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pests. Adding to this, the long term availability of dimethoate was uncertain because it was being reviewed by the Australian Pesticides and Veterinary Medical Authority (APVMA). Subsequently, the use of dimethoate in strawberries was suspended in October 2011 following the release of the Dimethoate Residues and Dietary Risk Assessment Report in August 2011(APVMA, 2011). This report found that the use of dimethoate in strawberries could exceed the recommended public health standard (APVMA, 2011).

In 1998-2001 the Better Berries project made preliminary investigations looking at an alternative to dimethoate cover sprays, which involved bait spraying trap crops (i.e. lupins) (Greer, 2002). Requests made to interstate quarantine authorities to modify the protocols for dimethoate based on the results of the Better Berries project were rejected due to the requirement for more quantitative efficacy data suitable for market access negotiations. Since 2002, the Queensland Department of Agriculture, Fisheries and Forestry (DAFF QLD) in conjunction with the Queensland Strawberry Growers Association (QSGA) have been investigating alternative treatments to replace dimethoate and to gain access to interstate quarantine markets.

During 2006-2009 the HAL project BS06022 "Alternative fruit fly treatment for interstate market access for strawberries" gathered guantitative data that could be used to support the development of an interstate guarantine protocol for Queensland strawberries. This project aimed to develop a suitable method for bait spray application and determine whether the same level of protection as dimethoate cover sprays could be provided by bait spraying. This project found that the risk of fruit fly between May and early August was negligible and that in-field application of bait sprays to black plastic mulch was not efficacious and needed to be improved by using other methods, such as applying bait to perimeter/ border crops. This project also found that fruit fly infestation in elevated (1.2 m) hydroponically grown strawberries was slightly higher than in ground grown strawberries, but the statistical differences were not significant (Gu et al., 2010). During this time another project funded by the Cooperative Research Centres (CRC) Plant Biosecurity program (Project Number CRC40088) ran in parallel with the HAL project BS06022. The project aim was to attempt to analyse the spatial and temporal foraging patterns of Queensland fruit flies in strawberry and apple crops. This research confirmed previous non-empirical observations that more flies were found on the edge of the strawberry blocks early in the season compared to the interior of the block (Balagawi et al., 2013). This result provides justification for the use of control measures targeting the edge or boundary of strawberry crops (Balagawi et al., 2013).

In 2010-2012 the project BS09022 "Alternative fruit fly treatment for interstate market access for strawberries - Winter Window option" was undertaken. This project was developed to monitor the population and abundance of fruit flies in strawberries during winter in Southeast Queensland, as well as quantify the risk of fruit fly infestation during this period. This project aimed to collect data that would enable the reinstatement of a Winter Window option after it was revoked in 2009. This would remove the requirement for growers to apply

dimethoate cover sprays during winter. Following the completion of two years of field trials, Biosecurity Queensland in collaboration with the DAFF (QLD) Market Access Team and QSGA developed ICA-34 based on scientific data collected from the three projects mentioned above (i.e. BS06022, BS09022, CRC40088). ICA-34 was approved on 16 January 2012, which included a modified Winter Window period and alternative cover spray chemicals to replace dimethoate. Due to the detections in August 2009, the new Winter Window period was changed to end on 10 August, which coincided with the collection of the first gravid female after winter. This new Winter Window period did not require growers to apply insecticide cover sprays from planting until 10 August. However, in order to satisfy interstate market access requirements, growers were required to apply bait sprays to trap crops planted on the edge of the strawberry blocks and use male fruit fly lures (i.e. Male Annihilation Technique) to further mitigate the risk of fruit fly infestation during the winter period. Following 10 August, growers were required to apply cover sprays using an approved chemical, as well as implement additional control measures such as protein baiting depending on the chemical label or permit requirements. A field hygiene program and an inspection and culling program during harvest, packing and grading also formed components of ICA-34 (Queensland Government, 2013).

In November 2011, the project BS09022 was extended to collect scientific data to broaden the geographic area covered by ICA-34 to include the Bundaberg region. Data previously collected from Southeast Queensland (Sunshine Coast and Caboolture regions) were not accepted to substantiate fruit fly control for the Bundaberg region due to possible differences between the regions.

On 1 July 2013, Victoria changed its quarantine entry conditions for host fruit and vegetables of Queensland fruit fly. Certification of produce was no longer required to access this market, except when produce was consigned to areas within the Greater Sunraysia Pest Free Area. This meant that several ICAs for Queensland produce, including strawberries (i.e. ICA-34), were now no longer required to enter Victoria. While entry conditions to Victoria have been relaxed both Victoria and New South Wales have reiterated that it continues to be an offence to sell fruit fly infested produce, and that businesses may be prosecuted if found not to comply.

In response to the Bundaberg strawberry industry requesting access to use ICA-34 in order to have access to interstate markets, the DAFF (QLD) Market Access Team have been implementing ICA-34 over the 2012 and 2013 season on strawberry farms in Bundaberg to monitor the fruit fly population and quantify the risk of fruit fly infestation throughout the season.

This report details the research conducted as part of the HAL project BS09022 to provide further data to support the Winter Window option for interstate market access for strawberries from Southeast Queensland and to provide scientific data to extend the geographic area covered by ICA-34 to include the Bundaberg region.

Part A- Southeast Queensland (2010-2012)

4 Field trials to evaluate the risk of fruit flies in Southeast Queensland

4.1 Introduction

Field trials in Southeast Queensland were conducted during the strawberry seasons of 2010 and 2011 to investigate the risk of fruit fly infestation in strawberries prior to and during the Winter Window period. Prior to 2009, strawberries harvested between 1 June and 20 September were exempt from requiring a treatment to control fruit flies. However, due to two separate detections of live fruit fly larvae in strawberries, South Australia and Victoria withdrew this option. The aim of these trials was to provide further field trial data to support the use of the Winter Window option for strawberries from Southeast Queensland to access markets in South Australia and Victoria.

4.2 Materials and Method

4.2.1 Trial blocks

The field trials of both production seasons in 2010 and 2011 were carried out on the same three strawberry farms located at Wamuran (27°01S, 152°53E), Bellmere (27°04S, 152°53E) and Chevallum (26°41S, 152°59E). The three farms are referred to as Properties A, B and C respectively. The details of the variety, block size and season are shown below (Table 1).

Droportu	Looption	2010 sea	ison	2011 sea	son
Property	Location	Block size (Ha)	Variety	Block size (Ha)	Variety
A	Wamuran	1.6	Ruby Gem	1.0	Ruby Gem
В	Bellmere	1.5	Festival	1.6	Fortuna
С	Chevallum	0.2 0.3	Early Blush Festival	0.3	Festival

Table 1 Strawberry variety and block size for Southeast Queenslandfield trials

Field trials in both years consisted of (1) monitoring fruit fly activity and (2) assessing fruit fly infestation in strawberries from late May to early September in 2010 and from early June to the end of August in 2011.

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On Property C during the 2010 season, fruit was collected from one block until it was sprayed out in early August. For the remainder of the season, a later cropping variety was harvested from another block of a similar size (Table 1).

During the trial period, all trial blocks remained untreated with no control measures for fruit flies applied.

4.2.2 Monitoring fruit fly activity

In the 2010 season, four cue-lure traps and two McPhail traps were installed on each property to monitor fruit flies. Two cue-lure traps and one McPhail trap were placed inside the trial block, with the remaining traps installed on the outside or edge of the trial block. Monitoring started from 21 May to 9 September. In the 2011 season, eight to ten cue-lure traps and four McPhail traps were installed on each property. Two cue-lure traps and one McPhail trap were placed inside the trial block, with the remaining traps installed on the outside or edge of the trial block. McPhail traps were baited with an orangeammonia solution and targeted female fruit flies, whereas cue-lure traps targeted male flies. An additional four Cera traps were set up on each property outside the trial block, which aimed to catch female fruit flies. Trap distribution on Properties A, B, and C in the 2011 season is shown in Figure 1, 2 and 3, respectively.

All traps were installed and serviced by the project team on a weekly basis. The trap catches were brought to the Market Access Team (MAT) laboratory for species identification. Female fruit flies (*Bactrocera tryoni* and *B. neohumeralis*) caught in McPhail traps were dissected to determine the stage of reproductive maturity. Reproductive maturity was based on methods detailed by Fletcher (Fletcher, 1975), where five stages of ovarian development were recognized. Female flies with Stages 1-4 ovaries were considered as immature, whereas females with Stage 5 ovaries were considered mature. Although trap counts were made weekly, the data are expressed as mean flies/ trap/ day.

Daily trap catches of male fruit flies were analyzed in relation to changes in temperature, using the meteorological data recorded at a nearby weather station at Beerburrum (26°96S, 152°96E).

Within this report two pest fruit fly species, Queensland fruit fly (*B. tryoni*) and the lesser Queensland fruit fly (*B. neohumeralis*) are reported on. These species have similar host ranges and are both treated equally in regards to quarantine requirements by interstate trading partners.

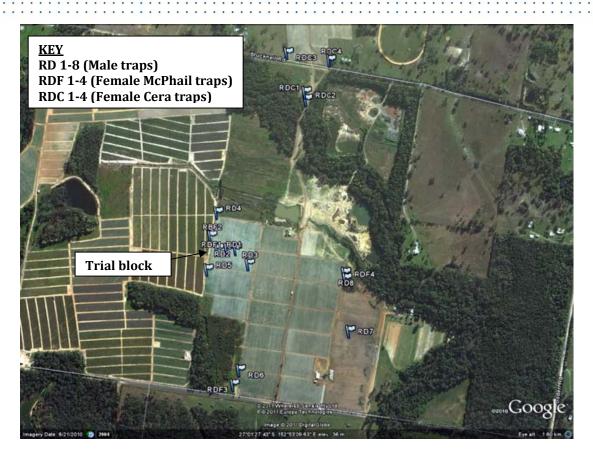


Figure 1 Trap locations on Property A in 2011



Figure 2 Trap locations on Property B in 2011

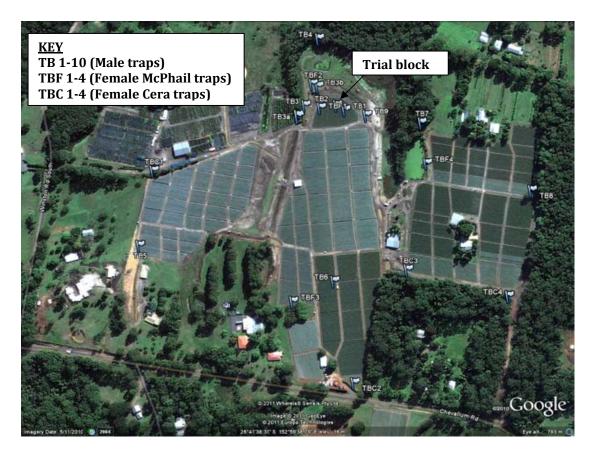


Figure 3 Trap locations on Property C in 2011

4.2.3 Assessing fruit fly infestation in strawberries

In 2010, fruit fly infestation was assessed from late May at Property B and C, which had early fruiting varieties. Fruit fly infestation in June, July and August was assessed from Properties A, B and C. In each of these three winter months, samples were taken at 7-14 day intervals, with 2 collections each month. In September only one sample was taken from each trial block.

In 2011, assessment of strawberries commenced in early June from all three trial blocks and continued until the end of August. In each of the three winter months, samples were taken at an interval of approximately 14 days, with 2 collections each month. In August 2011, a final sample at the end of August was not obtained from Property C because operation on this farm had ended.

All fruit samples were taken from packed first grade fruit following inspection and culling of suspect fruit during the harvest and packing process. Samples were transported to the DAFF (QLD) Market Access Team's laboratory and received at a maximum of two days following harvest. Individual fruit were held on egg cartons and then enclosed in plastic boxes with a fine mesh cover (Figure 4). Fruit samples were incubated in controlled environment rooms under standard conditions of 26°C and 70% Relative Humidity (RH) for approximately 7 days. At assessment, fruit samples were individually cut into 47 slices and checked for the presence of fruit fly larvae and pupae (Figure 5). Any larvae or pupae found in fruit samples were reared through to adult emergence for species identification.



Figure 4 Set-up of strawberry samples for incubation



Figure 5 Examination of fruit fly infestation in strawberry samples

The upper percentage infestation (with 95% confidence) was calculated for the assessed strawberry samples, using the formulas of (Couey and Chew, 1986). All calculations were performed using CQT_STATS (Liquido et al., 1997).

4.2.4 Monitoring fruit fly activity

4.2.4.1 Trap catches in 2010

In total, 212 male fruit flies were caught from the 12 cue-lure traps installed on the three commercial farms where field trials were conducted. These trap catches included two pest species: Queensland fruit fly, *Bactrocera tryoni* (187) and the lesser Queensland fruit fly, *B. neohumeralis* (10]). Two non-pest species were also trapped: *B. bryoniae* (14) and *Dacus aequalis* (1]). *B. neohumeralis* was not caught until early September. The percentage of the species captured from the three properties during the trial period from 21 May to 9 September 2010 is shown in Figure 6.

Trap catches of male fruit flies (*B. tryoni* and *B. neohumeralis*) between late May and mid August in the 2010 season were consistently lower than 0.3 flies/ trap/ day for most of this period, with a maximum of 0.6 flies/ trap/ day being captured (Figure 7), however trap catches were consistently lower than 0.3 flies/ trap/ day for most of this period. From late August, fruit fly numbers increased on Property A and C and by the end of sampling in September trap catches had reached the highest levels, with a daily mean of 1.2 and 1.9 flies/ trap/ day respectively. On Property B, trap catches remained at a low level throughout the sampling period, averaging between 0.0 and 0.3 flies/ trap/ day. The daily trap catches of male fruit flies were found to be significantly correlated with daily maximum temperature (r = 0.5017, P < 0.001). This finding is consistent with other studies which found that temperature is one component that influences the population of Queensland fruit flies (Muthuthantri et al., 2010, Drew and Hooper, 1983).

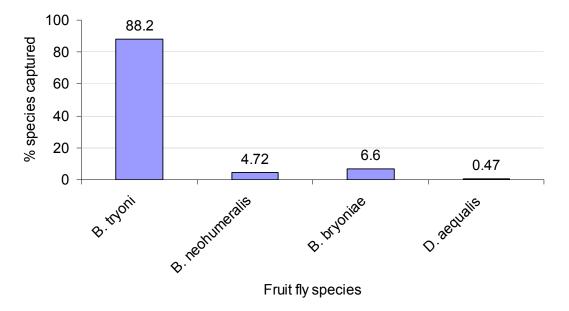


Figure 6 Percentage of fruit fly species caught from male traps on all three strawberry farms between late May and early September 2010

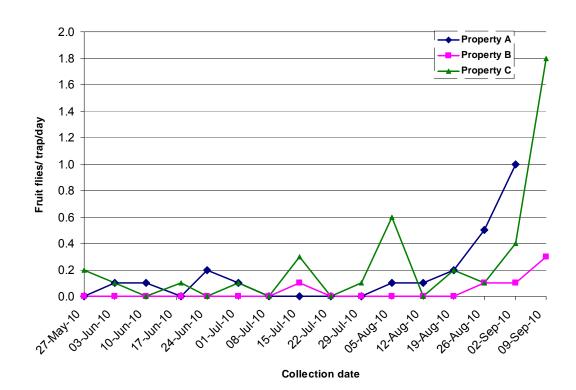


Figure 7 Male Fruit fly (*B. tryoni* and *B. neohumeralis*) trap catches from late May to early September 2010

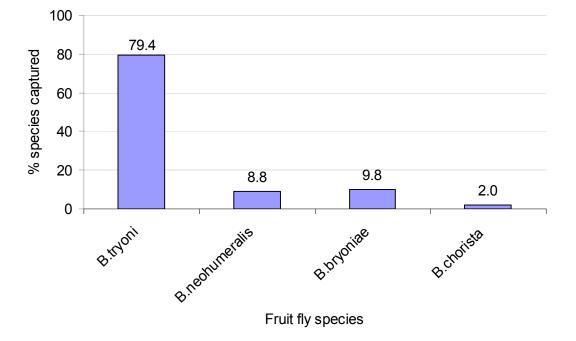
During the winter months of May, June and July no female fruit flies were caught. Over the entire season, only two female fruit flies were caught from a total of six McPhail traps installed over the three trial properties. The first female fly was caught on Property C at Chevallum between 13-19 August. This female did not contain mature eggs. The second fly, which contained mature eggs, was caught on Property A at Wamuran between 3-9 September.

4.2.4.2 Trap catches in 2011

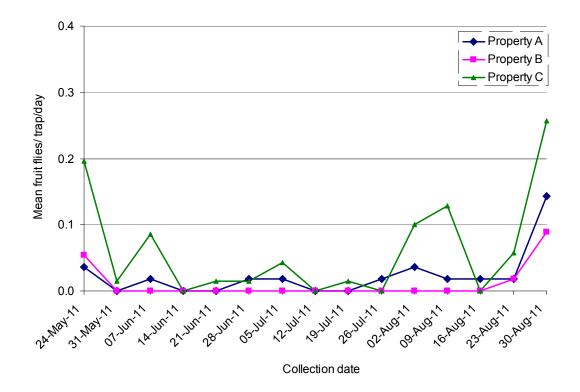
In total, 102 male fruit flies were caught from the traps installed on the three strawberry farms where field trials were conducted. A total of 101 flies were caught in cue-lure traps and one fly was caught in a Cera trap. These trap catches included two pest species: *B. tryoni* (81) and *B. neohumeralis* (9). Two non-pest species were also trapped: *B. bryoniae* (10) and *B. chorista* (2). The percentage of each of these species caught on the three properties during the trial period is shown in Figure 8.

Trap catches of male fruit flies (*B. tryoni* and *B. neohumeralis*) between late May and the end of August in the 2011 season were consistently lower than 0.2 flies/ trap/ day for most of this period (Figure 9). Trap catches showed a tendency to increase at the end of August. This increase is likely due to rising temperatures as the daily trap catches were found to be significantly correlated with the daily maximum temperature (r = 0.5443, p = 0.036).











A combined total of six *B. tryoni* female flies were caught in the orangeammonia traps from all three properties over the trial period (Figure 10). Of these female flies, one was caught from Property A on 9 August, three from Property B on 30 August, and two from Property C on 30 August. The first female fruit fly caught at the start of August on Property A was found to be reproductively mature with four mature eggs, while two of the five females caught on 30th August were reproductively mature with 1 and 33 mature eggs, respectively (Figure 11).

No female fruit flies (*B. tryoni* and *B. neohumeralis*) were caught in Cera traps on any of the three properties over the trial period.

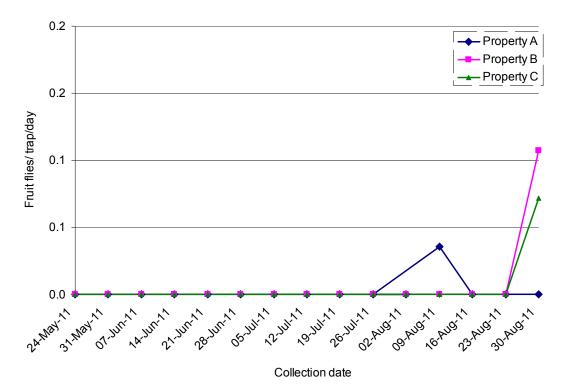


Figure 10 Trap catches of female fruit flies (*Bactrocera tryoni*) on three commercial strawberry farms from late May to end August 2011

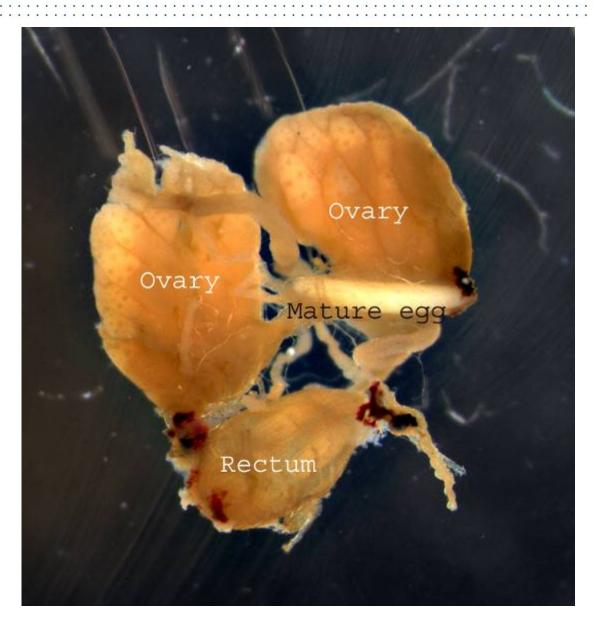


Figure 11 Dissection of female fruit fly ovaries showing 1 mature egg from female collected in orange-ammonia trap on 30 August 2011

4.2.5 Assessing fruit fly infestation in strawberries

4.2.5.1 Fruit assessment in 2010

Table 2 shows the assessment results for fruit fly infestation from strawberry samples over the 2010 season (May-September). In total, 12,889 strawberries (260.2 kg) were sampled and assessed. None of these fruit samples were found to be infested by fruit flies, and the upper infestation level with 95% confidence is estimated to be 0.023%.

Table 2 Assessment for fruit fly infestation in strawberries harvested fromlate May to early September 2010

Date picked	Property	Variety	Number of fruit	Weight fruit (kg)	Number infested fruit	Number of larvae and pupae	Upper infestation level % (95% confidence)
29/05/2010	С	Early Blush	992	16.3	0	0	0.3020
25/05/2010	Α	Ruby Gem	1040	19.7	0	0	0.2880
May Subtotal			2032	36.0	0	0	0.1474
8/06/2010	А	Ruby Gem	500	10.3	0	0	0.5991
14/06/2010	С	Early Blush	430	8.2	0	0	0.6967
18/06/2010	В	Festival	620	12.6	0	0	0.4832
22/06/2010	Α	Ruby Gem	520	10.0	0	0	0.5761
22/06/2010	С	Early Blush	440	8.2	0	0	0.6808
24/06/2010	В	Festival	500	12.1	0	0	0.5991
28/06/2010	С	Early Blush	358	8.2	0	0	0.8368
June Subtotal			3368	69.6	0	0	0.0895
7/07/2010	С	Festival	453	8.7	0	0	0.6613
8/07/2010	В	Festival	499	12.6	0	0	0.6003
13/07/2010	А	Ruby Gem	545	9.8	0	0	0.5497
26/07/2010	С	Festival	360	8.3	0	0	0.8321
27/07/2010	A	Ruby Gem	585	9.8	0	0	0.5121
29/07/2010	В	Festival	447	12.5	0	0	0.6702
July Subtotal			2889	61.7	0	0	0.1037
10/08/2010	А	Ruby Gem	550	10.1	0	0	0.5447
10/08/2010	С	Festival	251	8.5	0	0	1.1935
12/08/2010	В	Festival	472	12.7	0	0	0.6347
24/08/2010	A	Ruby Gem	555	10.1	0	0	0.5398
25/08/2010	С	Festival	906	13.3	0	0	0.3307
26/08/2010	В	Festival	725	17.2	0	0	0.4132
August Subtotal			3459	71.9	0	0	0.0866
7/09/2010	А	Ruby Gem	601	9.9	0	0	0.4985
9/09/2010	В	Festival	540	11.1	0	0	0.5548
September Subtotal			1141	21.0	0	0	0.2626
	Window To 30 Septem		10857	224.2	0	0	0.0276
	and Total sample per	iod)	12889	260.2	0	0	0.0232

4.2.5.2 Fruit assessment in 2011

Table 3 shows the assessment results for fruit fly infestation from strawberry samples over the 2011 season (June-August). In total, 11,765 strawberries (258.93 kg) were sampled and assessed. None of these fruit samples were

found to be infested by fruit flies. The upper infestation level with 95% confidence is estimated to be 0.0255%.

Date picked	Property	Variety	Number of fruit	Weight fruit (kg)	Number infested fruit	Number of larvae and pupae	Upper infestation level % (95% confidence)
7/06/2011	A	Ruby Gem	1018	20.4	0	0	0.2943
7/06/2011	В	Fortuna	497	12.8	0	0	0.6028
7/06/2011	С	Festival	602	13.3	0	0	0.4976
20/06/2011	А	Ruby Gem	855	17.6	0	0	0.3504
20/06/2011	В	Fortuna	486	12.7	0	0	0.6164
20/06/2011	С	Festival	482	12.6	0	0	0.6215
June Subtotal			3940	89.3	0	0	0.0760
4/07/2011	А	Ruby Gem	675	13.2	0	0	0.4438
4/07/2011	В	Fortuna	514	12.7	0	0	0.5828
4/07/2011	С	Festival	528	12.4	0	0	0.5674
18/07/2011	A	Ruby Gem	753	13.3	0	0	0.3978
19/07/2011	В	Fortuna	502	12.7	0	0	0.5968
18/07/2011	С	Festival	602	12.5	0	0	0.4976
July Subtotal			3574	76.7	0	0	0.0838
1/08/2011	С	Festival	599	12.3	0	0	0.5001
2/08/2011	А	Ruby Gem	578	9.9	0	0	0.5183
2/08/2011	В	Fortuna	495	12.6	0	0	0.6052
15/08/2011	В	Fortuna	418	12.7	0	0	0.7167
15/08/2011	С	Festival	599	12.7	0	0	0.5001
18/08/2011	А	Ruby Gem	580	10.0	0	0	0.5165
30/08/2011	А	Ruby Gem	567	10.0	0	0	0.5283
30/08/2011	В	Fortuna	415	12.7	0	0	0.7219
August Subtotal			4251	92.9	0	0	0.0705
-	rand Total sample per	iod)	11765	258.9	0	0	0.0255

Table 3 Assessment for fruit fly infestation in strawberries harvested fromearly June to end August 2011

4.3 Discussion

Trap catches of fruit flies (*B. tryoni* and *B. neohumeralis*) from the three strawberry farms in Southeast Queensland were very low during the trial period of 2010 and 2011, particular during winter months (June-August). These low fruit fly trap counts show that the active fruit fly population within these environments is low, and therefore poses very low risk of infesting strawberries during this period. Fruit fly numbers began increasing towards the end of August. This period between the end of August and the beginning of September has historically been associated with a peak in the fruit fly population. This has been observed in other cropping systems such as citrus grown in the Central Burnett region in Queensland (Lloyd et al., 2010).

Very small numbers of female fruit flies were caught during 2010 and 2011. At this stage, there are currently no strong, long lasting attractants to monitor female Queensland fruit flies. However, during the trial very low numbers of reproductively mature females were caught towards the end of the Winter Window period, in mid to late August. This shows that mature females capable of infesting strawberries are present and that control measures may be required to control the population to minimize risk and meet market access entry requirements.

The low risk of fruit fly infestation is further supported by results of fruit assessments during the 2010 and 2011 season. No fruit fly infestation was found in any of the strawberry samples, from any of the collection dates, taken from all three trial blocks. Since these strawberry blocks were not treated with any chemical insecticides to control fruit flies, these results further demonstrate that the risk of fruit fly infestation in the strawberries produced in Southeast Queensland during the Winter Window period is negligible. Therefore, this data provides further scientific evidence to support the Winter Window option for market access for strawberries in Southeast Queensland.

Studies have been conducted to determine the ability of Queensland fruit fly to develop and mature eggs during winter months. Pritchard (1970) and Fletcher (1975) conducted research on the wild populations of Queensland fruit flies from Sydney. These studies showed that the ovaries of female flies in these populations would not develop below a threshold temperature of 13.5°C. For the maturation of ovaries to occur they found that female flies required 1.6 day-degrees above this threshold. Between 1.6 day-degrees and 1.0 day-degrees resorption of eggs occurred. A day degree is the mean daily temperature minus the development threshold of 13.5 °C.

A day-degree model based on temperature records from Beerburrum meteorological station, showed several time periods during the Queensland strawberry winter growing months that were less than 1.6 day-degrees above the 13.5 °C threshold (Figure 12 and 13). However, during these months there were several sustained periods of time where day-degrees exceeded 1.6 daydegrees above the 13.5°C threshold. According to Pritchard (1970) and Fletcher (1975), these times where day-degrees are above the threshold of 13.5°C, ovarian development could occur, and hence may be times of possible risk of infestation in the strawberry crop. From fruit assessment results in 2010 and 2011, fruit fly infestation during these times has not been observed. This may be attributed to differences in ovary development thresholds between the fruit fly populations of Sydney and Southeast Queensland. This means that populations of Queensland fruit flies in Southeast Queensland may require relatively higher temperatures for ovarian development than populations from Sydney. Such geographic differences in thermal requirements for insect development have been documented elsewhere in a number of species from different insect orders (Honek, 1996, Rae and Death, 1991, Umeya and Yamada, 1973). For instance, investigations into climatic adaptation of the potato moth (Phthorimaea operculella) found that the lower threshold of temperature for development ranged from 9.5°C to 13.7°C in populations from different geographic regions

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(Broodryk, 1971, Briese, 1986, Briese, 1980). In addition, although maturation can occur above 13.5°C there currently is no data on maturation rates at different temperatures. This means that it is difficult to determine how long it takes for a clutch of eggs to develop at temperatures that are marginally above the threshold.

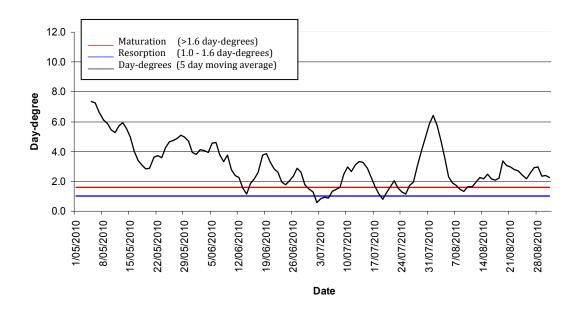


Figure 12 Day-degree model from Beerburrum temperatures from 1 May to 31 August of 2010. Ovarian development occurs at 1.6 day-degrees above 13.5 °C.

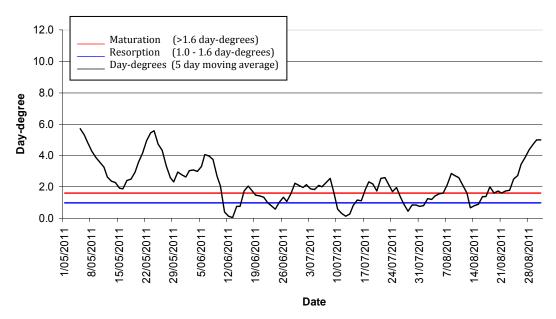


Figure 13 Day-degree model from Beerburrum temperatures from 1 May to 31 August of 2011. Ovarian development occurs at 1.6 day-degrees above 13.5 °C.

4.4 Conclusions and recommendations

In conclusion, from monitoring fruit fly activity this study found that the population and abundance of Queensland fruit flies on strawberry farms in Southeast Queensland is very low from May until mid August. Also, from assessment of strawberry samples we have demonstrated that the risk of fruit fly infestation in strawberries produced in Southeast Queensland during the winter period is negligible.

Although there were several days when the day-degree model exceeded 1.6 day-degrees above the threshold of 13.5 °C and therefore theoretically suitable for female ovarian development, no infested fruit were found. We propose that due to the lack of infestation found in the crop, populations of Queensland fruit flies in Southeast Queensland may require higher temperature thresholds compared to populations from Sydney or extended periods above the day-degree threshold to induce ovarian development. It is necessary to further investigate this hypothesis and model the process of reproductive maturation in Queensland fruit fly populations from this region. This research may provide further scientific evidence for acceptance of a fruit fly risk-free Winter Window for strawberries from Southeast Queensland and other production regions in Australia.

Due to the presence of mature females in mid to late August and the increase in fruit fly pressure seen from male trap catches, it is suggested that in order to reduce the risk of infestation and to control the population of fruit flies, additional control measures are required towards the end of the Winter Window period from mid August. These measures may include but are not limited to (1) protein baiting of trap crops around the boundary of the strawberry blocks, (2) Male Annihilation Technology and (3) insecticide cover sprays.

Part B- Bundaberg field trials (2012-2014)

5 Field trials to evaluate the risk of fruit flies in the Bundaberg region

5.1 Introduction

On 16 January 2011 a new operational procedure (ICA-34 '*Pre-harvest field control and inspection of strawberries*') came into effect, which allowed market access for Southeast Queensland strawberries to enter restricted quarantine markets in South Australia, Victoria, New South Wales, Western Australia and Tasmania. This procedure was developed from scientific data collected over several years of field trials conducted between 2008 and 2010. The procedure details a systems approach, which uses a combination of control tools coupled with low fruit fly population during winter to obtain control and meet quarantine entry requirements for interstate trade.

In November 2011, the current project (BS09022) was extended to collect scientific data to expand the geographic region covered by ICA-34 to include the Bundaberg region. Interstate trading partners did not accept data collected from Southeast Queensland (Sunshine Coast and Caboolture regions) to substantiate fruit fly control in the Bundaberg region due to possible differences between the regions. Bundaberg was granted interim approval to use ICA-34 for the 2012 and 2013 season, which allowed access only to Victoria. However, entry to Victoria was conditional and depended on field trials being conducted to determine the associated risk of fruit flies within this region.

This report details the research conducted over the 2012 and 2013 season as part of the HAL project BS09022 to provide scientific data to extend the geographic area covered by ICA-34 to include the Bundaberg region.

5.2 Materials and Method

5.2.1 Trial blocks

Field trials in 2012 were conducted over three commercial farms located across the major production areas of Bundaberg. Farms were located at Bundaberg North (24°50S, 152°18E), Bundaberg East (24°53S, 152°27E) and Alloway (24°57S, 152°25E) (Figure 14). These three locations represented different geographic areas where strawberries are grown in Bundaberg and are referred to as Property D, E and F respectively. Each of these properties controlled fruit flies by following the operational procedures of ICA-34.

In 2013, field trials were conducted on four commercial properties, of which two were used previously in 2012 (Property E and F), and an additional two

properties that are referred to as Property G and H. Property G was located at Sharon (24°52S, 152°16E) and Property H was located at Rubyanna (24°48S, 152°24E) in the Bundaberg region (Figure 14). During the 2013 season, Properties E, F and G controlled fruit flies by following the operational procedures of ICA-34. Property H served as an untreated control site, which did not receive any treatments to control fruit flies.

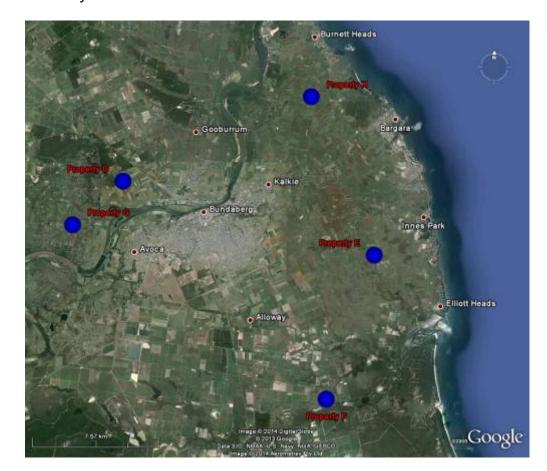


Figure 14 Geographic location of strawberry farms where field trials were carried out in the Bundaberg region in 2012 and 2013

The details of the variety, block size and season are shown below (Table 4). The project research over the 2012 and 2013 season consisted of (1) monitoring fruit fly activity and (2) assessing fruit fly infestation in packed strawberry samples.

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Bronortu	Location	2012 s	eason	2013 se	eason
Property	Location	Block size (ha)	Variety	Block size (ha)	Variety
D	Bundaberg North	2.0	Festival and Fortuna	-	-
E	Bundaberg East	2.6	Festival and Camarosa	1.9	Camarosa
F	Alloway	2.2	Pamela, Ruby Gem and Festival	2.2	Festival
G	Sharon	-	-	3.4	Festival
Н	Rubyanna	-	-	2.3	Ruby Gem, Festival and Camarosa

Table 4 Strawberry variety and block size for Southeast Queenslandfield trials

5.2.2 Monitoring fruit fly activity

In both the 2012 and 2013 season, ten cue-lure traps and four McPhail traps were installed on each property. Two cue-lure traps and one McPhail trap were placed inside the trial block, with the remaining traps installed on the outside or edge of the trial block. McPhail traps were baited with an orange-ammonia solution and targeted female fruit flies, whereas cue-lure traps targeted male flies. Monitoring commenced on 14 May until 3 September in the 2012 season. In 2013, monitoring started on 9 April until the end of harvesting, which at the latest was 24 September. Trap distribution on Properties D, E, F, G and H in the 2012 and 2013 season are shown below (Figure 15-21)

All traps were installed and serviced by the project team on a weekly basis. The trap catches were brought to the Market Access Team (MAT) laboratory for species identification. Female fruit flies (*B. tryoni* and *B. neohumeralis*) caught in McPhail traps were dissected to determine the stage of reproductive maturity. Reproductive maturity was based on methods detailed by Fletcher (Fletcher, 1975), where four stages of ovarian development were recognized. Female flies with Stages 1-4 ovaries were considered as immature, whereas females with Stage 5 ovaries were considered mature. Although trap counts were made weekly, the data are expressed as mean flies/ trap/ day. As described in section 4.2.4 trap counts include two pest fruit fly species, *B. tryoni and B. neohumeralis*.

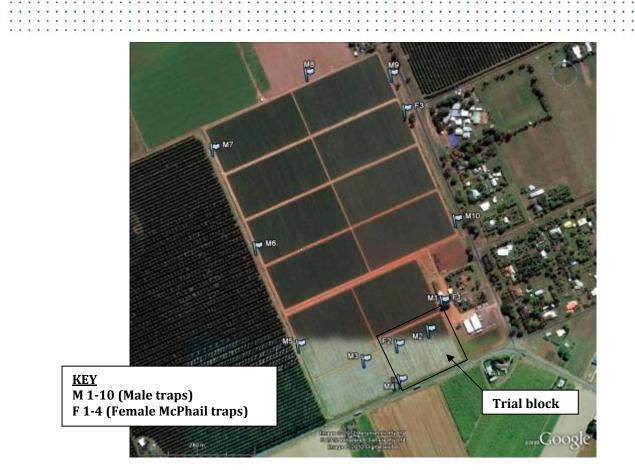


Figure 15 Trap locations on Property D in 2012

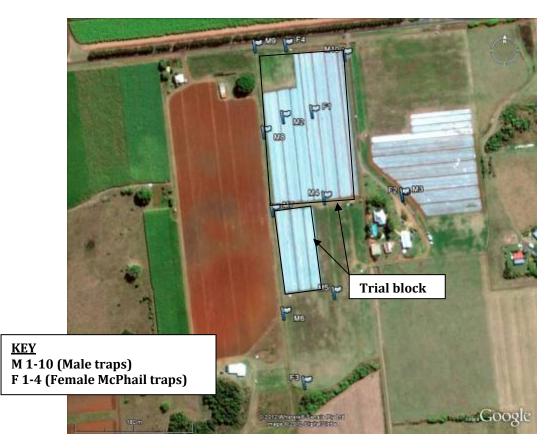


Figure 16 Trap locations on Property E in 2012

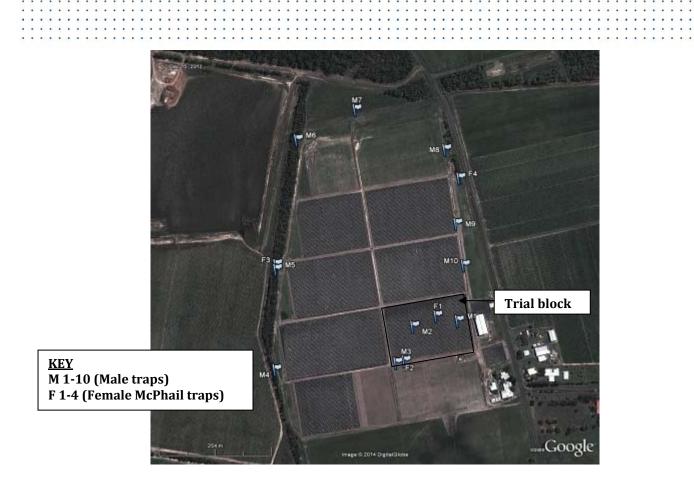


Figure 17 Trap locations on Property F in 2012

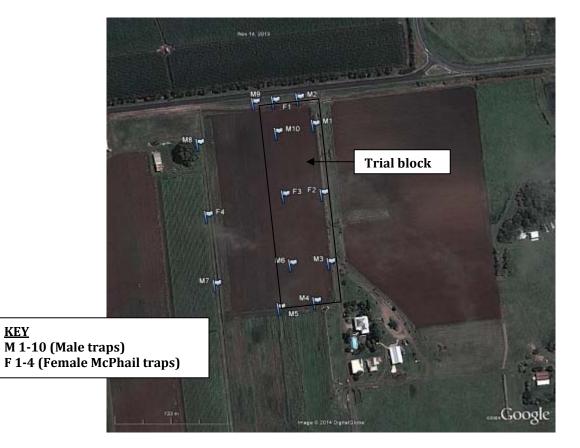


Figure 18 Trap locations on Property E in 2013

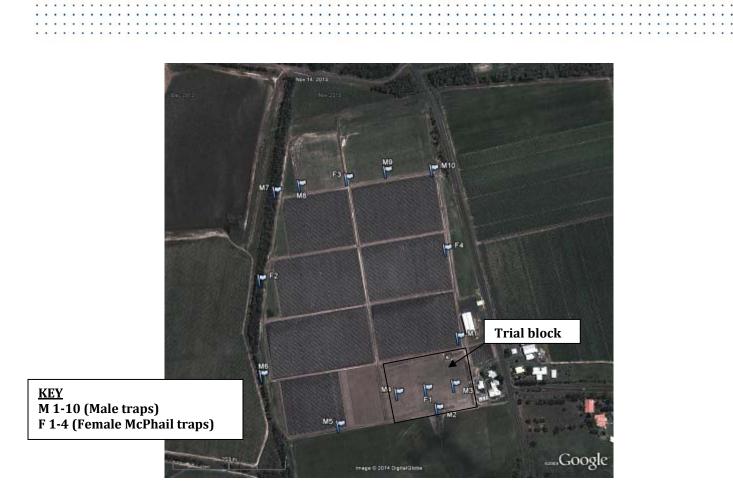


Figure 19 Trap locations on Property F in 2013



Figure 20 Trap locations on Property G in 2013

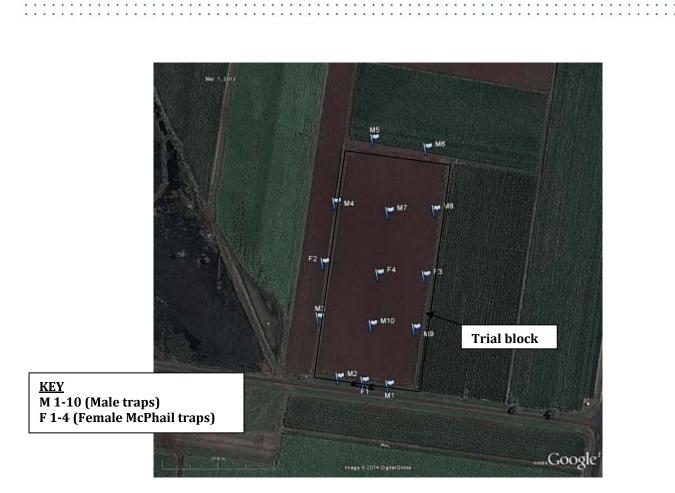


Figure 21 Trap locations on Property H in 2013

5.2.3 Defining the management tools of ICA-34 systems approach

Growers participating in the field trials of the current project were required to manage their farms and blocks in accordance with the operational procedures of ICA-34. Growers needed to comply with the following requirements (Queensland Government, 2013):

- 1. Implement a pre-harvest field control program consisting of
 - a) A program of **Male Annihilation Technique** (MAT) devices consisting of
 - i. MAT devices being placed on the **perimeter of the source property**;
 - ii. from the time of planting and renewed every three months until all plants are removed;
 - iii. at **20 metre** intervals; and
 - iv. attached to available **vegetation** (for example, trees and woody shrubs) or **artificial structures**.
 - b) Implement a perimeter bait spray program consisting of
 - i. **Naturalure™** Fruit Fly Bait Concentrate:

- a) shall be applied to the perimeter of all strawberry blocks at a rate of 1 L per hectare. The bait spray mixture can be applied as either –
 - a strip spray with a diluted solution (1 part Naturalure™ mixed with 6.5 parts water); or
 - a spot spray with a diluted solution (1 part Naturalure™ mixed with 6.5 parts water) applied as coarse spots of 50 mL per spot of 1 m² with 150 spots per hectare; or
 - a spot spray with a concentrated solution (1 part Naturalure[™] mixed with 1.5 parts water) applied as coarse spots of 20 mL per spot of 1 m² with 125 spots per hectare;
 - b) applied to the fruit fly resting sites on the perimeter of all strawberry blocks on the source property;
 - c) at a maximum interval of every seven days, reapplying sooner if rain washes off the deposit;
 - d) from the time of planting, or 1 May for ratoon crops;
- ii. **Maldison** bait spray (NB- this option only available to growers in 2013 season):
 - a) A program of bait sprays consisting of
 - 700 mL of a 440 g/L product per 100 L of water; or
 - 500 mL of a 1000 g/L product per 100 L of water; or
 - 435 mL of a 1150 g/L product per 100 L of water; and
 - b) applied in combination with a product containing either hydrolysate or a yeast autolysate protein lure and in accordance with manufacturer's label instruction;
 - c) as a low pressure coarse spray in a strip or as spots of 50
 100 mL/Ha, up to 15 20 L/Ha;
 - **d)** at intervals of every four (4) to ten (10) days, reapplying sooner if rain washes off the deposit;
 - e) commencing at least six (6) weeks prior to harvest.

The bait sprays must be continued until either:

- the **completion of harvest** of all fruit for certification from the source property; or
- all fruit has been removed from the block; or
- the plants have been sprayed out or removed from the block; or
- the pre-harvest cover spray program has commenced.
- c) Implement a pre-harvest cover spray program, commencing prior to 10 August, to allow for certification of strawberries from 10 August and consisting of either –
 - i. A **trichlorfon** mixture applied:
 - a) in a high volume application containing 250 mL of a 500 g/L product per 100 L of spray mixture;
 - b) thoroughly to the fruit to the point of run-off;

- c) for a maximum of three (3) applications per crop per
 -) for a maximum of three (3) applications per crop per season with a minimum treatment interval of 7 days between applications.
 - OR
 - ii. A **Maldison** mixture applied:
 - a) in a high volume application containing either -
 - 140 mL of a 440 g/L product per 100 L water; or
 - 60 mL of a 1000 g/L product per 100 L water; or
 - 55 mL of a 1150 g/L product per 100 L water;
 - b) thoroughly to the fruit to the point of run-off;
 - c) for a maximum of six (6) applications per crop per season with a minimum treatment interval of 7 days between applications.

OR

- iii. A **spinetoram** mixture applied:
 - a) as a thorough foliar cover spray after flower set applied at a maximum rate of 400 mL of a 120 g/L product per hectare in 250 to 1000 L of water;
 - b) not exceeding a maximum of four (4) applications per season with a minimum of 7 - 14 days between consecutive (repeat) sprays; and
 - c) in conjunction with a continued perimeter bait spray program listed in 1 b) above.
- iv. The pre-harvest cover spray program must be applied:
 - a) to each block of strawberries grown on the property intended for certification from 10 August (a cover spray must have been applied prior to 10 August for certification to occur from 10 August);
 - b) following APVMA permit and label directions; and
 - c) ending at the completion of harvest.
- d) A field hygiene program whereby
 - i. during the harvest period, nonconforming fruit will be disposed of in an approved manner; and
 - ii. abandoned or spent strawberry blocks must receive either a chemical cover spray treatment (including a combination of herbicide and insecticide according to the approved use instructions), or have all fruit removed from the block.
- 2. **Inspection** of suspect fruit during harvest and during the grading and packing process, and found free of live fruit fly infestation.

In 2012 and 2013, growers coordinated the application of each of these control measures. They were responsible for establishing suitable fruit fly resting sites, which consisted of forage sorghum, corn, sugar cane, bamboo, tall grass, ornamental shrubs and/or adjacent native bushland vegetation (Figure 22). Although the height of the resting site was not stipulated in the

requirements of the operational procedures of ICA-34, the procedure stated that to improve efficacy, bait sprays should be applied to fruit fly resting sites at a height of 1 metre or above (Queensland Government, 2013).



Figure 22 Fruit fly resting sites consisting of sorghum used as a trap crop for bait spraying

In 2013, in addition to the requirements of ICA-34, participating growers were asked by the project team to implement additional bait spray treatments following 10 August. This additional component to the systems approach aimed at reducing fruit fly populations during spring and to further reduce the risk of infestation over and above what was stipulated in ICA-34.

Following the release of the first version of the operational procedures of ICA-34 on 16 January 2012, revisions were made regarding the wording for reapplication of sprays and published in the second version. In the first version the operational procedures prescribed that bait sprays and cover sprays must be reapplied if rain sufficient to cause run-off from the leaves occurred within two hours of spraying. It also stated that where prolonged periods of rainfall inhibit the application of sprays, pre-harvest application of sprays must be reapplied immediately following the rainfall event. In the second version, this wording was changed so that it would take into account all rain events, not just those that occurred within two hours of application. Current wording of the protocol now states to- " apply chemicals at the specified interval, reapplying sooner if rain washes off the deposit" (Queensland Government, 2013).

In 2012, growers selected a regime of cover sprays that suited their cropping system. All three properties had different regimes, however they all complied

with the requirements of ICA-34. Below details the combination of chemicals used for both bait sprays and cover sprays:

Property D

- Naturalure applied at 7 day intervals from planting
- Maldison cover sprays commencing from 8 August, with 3 applications applied at 8-10 day intervals

Property E

- Naturalure applied at 7 day intervals from planting
- Spinetoram cover sprays commencing from 9 August, with 4 applications applied at 10 day intervals
- 3 Maldison cover sprays (after Spinetoram applications ceased), applied at 6-7 day intervals.

Property F

- Naturalure applied at 7 day intervals from planting
- Spinetoram cover sprays commencing from 9 August, with 3 applications applied at 10 day intervals

In 2013, growers again selected a regime of cover sprays that suited their cropping system, which all complied with the requirements of ICA-34. Below details the combination of chemicals used for both bait sprays and cover sprays:

Property E

- Naturalure applied at 7 day intervals from planting
- Spinetoram cover sprays commencing from 11 August, with 3 applications applied at 10-14 day intervals

Property F

- Naturalure or Maldison/yeast bait spray applied at 7 day intervals from planting, except on one occasion (28/4/13) where the interval was 14 days.
- Maldison cover sprays commencing from 8 August, with 3 applications applied at 7 day intervals

Property G

- Naturalure or Maldison/yeast bait spray applied at 3-10 day intervals from planting
- Spinetoram cover sprays commencing from 8 August, with 4 applications applied at 9 day intervals
- 2 Maldison cover sprays (after Spinetoram applications ceased), applied at 9 day intervals.

Property H

• No chemical cover sprays or bait sprays applied

A complete list of spray records for each treated block is included in the appendix (section 8.1). Only chemicals that were applied for the control of fruit flies and in the implementation of ICA-34 have been included.

5.2.4 Assessing fruit fly infestation in strawberries

In 2012, fruit infestation was assessed from late May at Property D and F, which had early fruiting varieties. Fruit fly infestation in June, July and August was assessed from all three trial blocks. Fruit was sampled at approximately 14 day intervals, with fruit samples sourced from the same trial blocks throughout the season. An exception to this in 2012 was at Property F, where fruit was sampled from other ICA-34 treated blocks on the property because fruit from the trial block had not been set aside. All blocks from which fruit was sampled had undergone treatment in accordance with ICA-34. Samples from Properties D, E and F taken at different dates may have consisted of different varieties, however each sample date consisted of the same strawberry variety.

In 2013, fruit infestation was assessed from late May at Property F, G and H, which all had early fruiting varieties. Fruit fly infestation in June, July and August was assessed from all four trial blocks. During September, fruit was sampled only from Properties E, G and H, because Property F did not have late fruiting varieties. Fruit was sampled at approximately 14 day intervals, with fruit samples sourced from the same trial blocks throughout the season. On Property H, different varieties were sampled throughout the season from the same block. Where this occurred, each sample date consisted of the same strawberry variety.

All fruit samples were commercially picked and packed under standard operating procedures, which included culling, inspection and packaging, in accordance with ICA-34. Samples were transported to the DAFF (QLD) Market Access Team's laboratory and received at a maximum of two days following harvest. Individual fruit were held on egg cartons and then enclosed in plastic boxes with a fine mesh cover. Fruit samples were incubated in controlled environment rooms under standard conditions of 26°C and 70% Relative Humidity (RH) for approximately 7 days. At assessment, fruit samples were individually cut into 4-7 slices and checked for the presence of fruit fly larvae and pupae. Any larvae or pupae found in fruit samples were reared through to adult emergence for species identification.

Infestation was calculated for each property individually due to the differences in bait spray and cover spray treatments. The upper percentage infestation (with 95% confidence) was calculated for the assessed strawberry samples, using the formulas of (Couey and Chew, 1986). All calculations were performed using CQT_STATS (Liquido et al., 1997).

5.3 Results

5.3.1 Monitoring fruit fly activity

5.3.1.1 Trap catches in 2012

In total, 140 male fruit flies were caught in cue-lure traps and 17 fruit flies caught in orange-ammonia baited McPhail traps installed on the three strawberry farms between May and September. Trap catches of flies caught in cue-lure traps included two pest species: Queensland fruit fly, *B. tryoni* (119) and the lesser Queensland fruit fly, *B. neohumeralis* (13). Four non-pest species were also trapped: *B. bryoniae* (3), *Dacus aequalis* (1), *B. cucumis* (3) and *B cacuminata* (1). Trap catches of flies caught in McPhail traps included two pest species: Queensland fruit fly, *B. tryoni* (10) and the lesser Queensland fruit fly, *B. tryoni* (10) and the lesser Queensland fruit fly, *B. neohumeralis* (3). Two non-pest species were also caught in McPhail traps: *B. cucumis* (3) and *B cacuminata* (1). The percentage of each of these species caught during the trial period is shown in Figure 23.

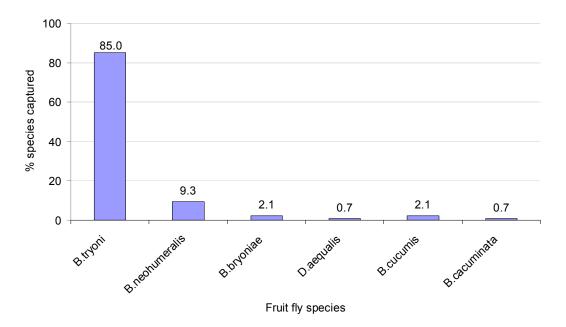


Figure 23 Percentage of fruit fly species caught from cue-lure and McPhail traps on all three strawberry farms in the Bundaberg region from 14 May to 3 September 2012

Male fruit flies of the two pest species (*B. tryoni* and *B. neohumeralis*) were caught throughout the trial period (Figure 24). Trap catches between May and September were very low, with an average of less than 0.25 male flies/ trap/ day. The trap catches did not show any obvious tendency of seasonal increase, which is usually expected during spring. This may be because sampling finished before any changes were detected.

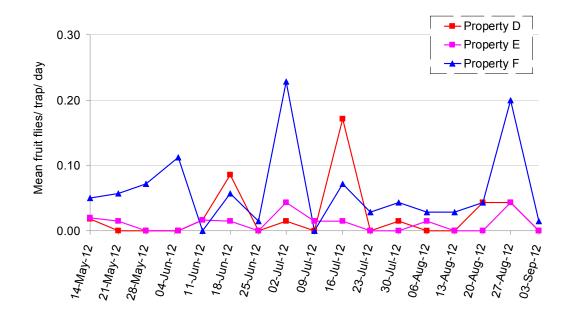


Figure 24 Trap catches of male fruit flies (*Bactrocera tryoni* and *B. neohumeralis*) on three strawberry farms in the Bundaberg region from 14 May to 3 September 2012

A total of 11 *B. tryoni* and one *B. neohumeralis* female flies were caught in the McPhail traps on all three properties over the period from 14 May to 3 September 2012. Six of these female flies were found to have ovaries containing mature eggs (ovarian maturation Stage 5), while the other 6 flies did not contain mature eggs in their ovaries (ovarian maturation Stage 1-4).

S	stage in 20)12			
te cted	Property	Maturity	Ovarian Stage	Number of mature eggs (Stage 5 only)	Tot numb

Table 5 Female fruit fly collections showing the ovarian maturation
stage in 2012

Date collected	Property	Maturity	Ovarian Stage	Number of mature only		Total number of eggs
			(Stages 1-5)	1st ovary	2nd ovary	
14-May	D	Mature	5	3	5	8
25-Jun	E	Immature	1	0	0	0
20-Aug	F	Immature	3	0	0	0
20-Aug	F	Immature	2	0	0	0
27-Aug	D	Mature	5	3	2	5
27-Aug	D	Mature	5	3	0	3
27-Aug	D	Mature	5	Not able to count	No able to count	41
27-Aug	D	Mature	5	6	5	11
27-Aug	D	Immature	3	0	0	0
3-Sep	F	Mature	5	13	11	24
3-Sep	D	Immature	1	0	0	0
3-Sep	D	Immature	3	0	0	0

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5.3.1.2 Trap catches in 2013

In total, 1644 male fruit flies were caught in cue-lure traps and 4 fruit flies caught in orange-ammonia baited McPhail traps installed on the 3 treated and 1 untreated control property between April and September. Trap catches of flies caught in cue-lure traps included two pest species: Queensland fruit fly, *Bactrocera tryoni* (1338) and the lesser Queensland fruit fly, *B. neohumeralis* (245). Three non-pest species were also trapped: *B. bryoniae* (4), *D. aequalis* (56) and *D. signatures* (1). Trap catches of flies caught in McPhail traps included one pest species: Queensland fruit fly, *B. tryoni* (4). No other species of flies were caught in McPhail traps. The percentage of each of these species caught during the trial period is shown in Figure 25.

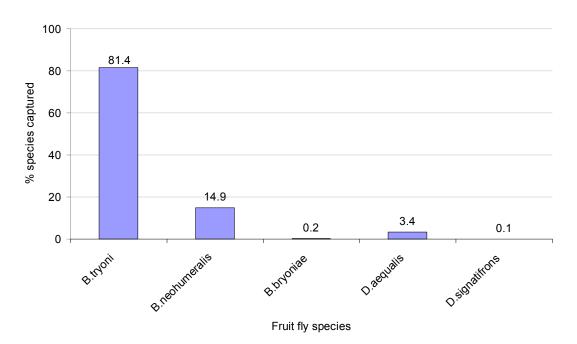


Figure 25 Percentage of fruit fly species caught from cue-lure and McPhail traps on all four strawberry farms in the Bundaberg region from 9 April of 24 September 2013

Male fruit flies of the two pest species (*B. tryoni* and *B. neohumeralis*) were caught throughout the trial period (Figure 26). Trap catches were very low, with an average of less than 0.5 male flies/trap/day from April until mid August. The exception to this was on Property F, where on two occasions (early June and early July) Trap catches of \approx 1.25 flies/trap/day were obtained for a single trap collection each time. Trap catches after these two occasions returned to an average of less than 0.5 male flies/trap/day. The trap catches did show a slight tendency of seasonal increase, however this was not consistent over each of the trial properties. After mid August there was an increase on Property E and G, with a very large increase in the population to 3.83 flies/trap/day on 17 September on Property E. This seasonal increase was not observed on Property H, which served as an untreated control site.



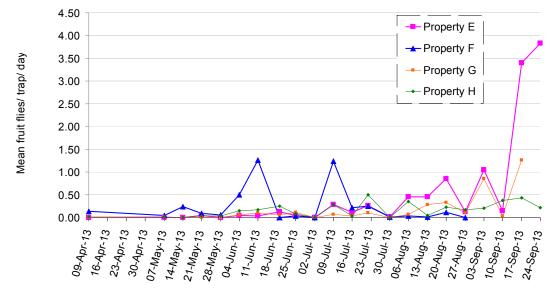


Figure 26 Trap catches of male fruit flies (*Bactrocera tryoni* and *B. neohumeralis*) on three strawberry farms in the Bundaberg region from 9 May to 24 September 2013

A total of 4 *B. tryoni* female flies were caught in the McPhail traps during the 2013 season, with all flies containing mature eggs (ovarian maturation Stage 5) (Table 6). All flies were collected from the same property (Property E). No female flies were collected from the other 3 properties.

Date collected	Property	Maturity	Ovarian Stage	Number of mature only		Total number of eggs
			(Stages 1-5)	1st ovary	2nd ovary	
24 Sept	E	Mature	5	*	*	*
24 Sept	E	Mature	5	*	*	*
24 Sept	E	Mature	5	*	*	*
24 Sept	E	Mature	5	*	*	*

Table 6 Female fruit fly collections showing the ovarian maturationstage in 2013

* Egg counts for female fruit fly dissections were not recorded for samples in 2013

5.3.2 Assessing fruit fly infestation in strawberries

5.3.2.1 Fruit assessment in 2012

In total, 10735 individual strawberries (206.8 kg) were assessed for fruit fly infestation from the start of the season from May until 10 August and 1414 strawberries (31.2 kg) assessed after 10 August over all properties (Tables 7-9)

On Properties E and F, no fruit fly infestation was detected between May and 10 August. During this period a total of 3725 strawberries and 3437 strawberries were assessed from Properties E and F respectively. After 10 August, no infestation was found in the samples harvested from these

properties, from which a total of 476 and 499 strawberries were sampled respectively.

On Property D during the period between May and 10 August, a total of 3573 strawberries were assessed and one single strawberry was found to be infested with fruit fly (collected on 2 July). After 10 August, 439 strawberries were assessed and a second fruit was found to be infested with fruit fly. Each of these infested fruit contained one Queensland fruit fly (*B. tryoni*) larva.

Table 7 Assessment of	fruit fly	infestation	in	strawberries I	harvested
from Property D in 2012.					

Property	Variety	Date picked	No. fruit	Weight fruit (Kg)	No. infested	No. larvae or pupae	Upper infestation level % (95% confidence)
D	Festival	20/05/2012	753	9.4	0	0	0.3984
Total (May)			753	9.4	0	0	0.3984
D	Festival	6/06/2012	760	9.4	0	0	0.3942
D	Festival	17/06/2012	600	8.9	0	0	0.4993
Total (June)			1360	18.3	0	0	0.2203
D	Fortuna	2/07/2012	388	9.2	1	1	1.2224
D	Fortuna	16/07/2012	361	9.6	0	0	0.8298
D	Fortuna	30/07/2012	314	9.7	0	0	0.9540
Total (July)			1063	28.5	1	1	0.4462
D	Fortuna	7/08/2012	397	9.9	0	0	0.7546
D	Fortuna	20/08/2012	439	9.2	1	1	0.6824
Total (August)			836	19.1	1	1	0.5674
Total May- 10 August			3573	66.1	1	1	0.1327
Total Post 10 August			439	9.2	1	1	1.0804

Table 8 Assessment of fruit fly infestation in strawberries harvested from Property E in 2012.

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Property	Variety	Date picked	No. fruit	Weight fruit (Kg)	No. infested	No. larvae or pupae	Upper infestation level % (95% confidence)
E	Festival	5/06/2012	524	10.7	0	0	0.5717
E	Festival	16/06/2012	540	11.5	0	0	0.5548
Total (June)			1064	22.2	0	0	0.2816
E	Festival	1/07/2012	436	12.4	0	0	0.6871
E	Festival	14/07/2012	494	12.0	0	0	0.6064
E	Festival	30/07/2012	870	13.4	0	0	0.3443
Total (July)			1800	37.8	0	0	0.1664
E	Camarosa	7/08/2012	861	14.1	0	0	0.3479
E	Camarosa	19/08/2012	476	12.5	0	0	0.6293
Total (August)			1337	26.6	0	0	0.2241
Total May- 10 August			3725	74.1	0	0	0.0804
Total Post 10 August			476	12.5	0	0	0.6293

Table 9 Assessment of fruit fly infestation in strawberries harvestedfrom Property F in 2012.

Property	Variety	Date picked	No. fruit	Weight fruit (Kg)	No. infested	No. larvae or pupae	Upper infestation level % (95% confidence)
F	Pamela	20/05/2012	454	10.3	0	0	0.6598
Total (May)			454	10.3	0	0	0.6598
F	Ruby Gem	5/06/2012	577	9.6	0	0	0.5192
F	Ruby Gem	17/06/2012	540	9.2	0	0	0.5548
Total (June)			1117	18.8	0	0	0.2682
F	Festival	1/07/2012	478	9.0	0	0	0.6267
F	Ruby Gem	16/07/2012	453	9.6	0	0	0.6613
F	Ruby Gem	29/07/2012	455	9.2	0	0	0.6584
Total (July)			1386	27.8	0	0	0.2161
F	Pamela	7/08/2012	480	9.7	0	0	0.6241
F	Festival	20/08/2012	499	9.5	0	0	0.6003
Total (August)			979	19.2	0	0	0.3060
Total May- 10 August			3437	66.6	0	0	0.0872
Total Post 10 August			499	9.5	0	0	0.6003

5.3.2.2 Fruit assessment in 2013

In total, 11369 individual strawberries (202.6 kg) were assessed for fruit fly infestation from the start of the season from May until 10 August and 6330 strawberries (93.0 kg) assessed after 10 August over all properties (Tables 10-13).

On Properties E and G, which used the operational procedures of ICA-34 to control fruit flies, no fruit fly infestation was detected between May and 10 August. During this period a total of 1518 and 4523 strawberries were assessed from these properties respectively. On Property H, which served as an untreated control site, no fruit fly infestation was detected between May and 10 August from a total of 2904 strawberries.

On Property F, between May and 10 August three samples of fruit (harvested 8 July, 23 July and 6 August) contained a very low level of infested fruit (0.30%). A total of 8 individual fruit from a total of 2694 fruit sampled from this period were found to be infested, with 3 fruit infested from both samples in July and 2 fruit infested in August.

After 10 August, fruit fly were detected from most sampling dates from all properties. Over this period Property E had the highest rate of fruit fly infestation (2.39%), followed by Property G (0.77%), Property H (0.56%) and Property F (0.17%). All fruit flies reared from these samples were confirmed as *B. tryoni* or *B. neohumeralis*.

Property	Variety	Date picked	No. fruit	Weight fruit (Kg)	No. infested	No. larvae or pupae	Upper infestation level % (95% confidence)
E	Camarosa	9/07/2013	615	12.8	0	0	0.4871
E	Camarosa	23/07/2013	547	9.2	0	0	0.5477
Total July			1162	22	0	0	0.2578
E	Camarosa	6/08/2013	356	9.4	0	0	0.8415
E	Camarosa	20/08/2013	550	9.3	2	3	1.1446
Total August			906	18.7	2	3	0.6948
E	Camarosa	2/09/2013	360	9.3	2	5	1.7486
E	Camarosa	16/09/2013	594	8.9	32	46	7.2362
Total September			954	18.2	34	51	4.7449
Total May- 10 August			1518	31.4	0	0	0.1973
Total Post 10 August - Last pick			1504	27.5	36	54	3.1609

Table 10 Assessment of fruit fly infestation in strawberries harvested from Property E in 2013

Table 11 Assessment of fruit fly infestation in strawberries harvested from Property F in 2013

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Property	Variety	Date picked	No. fruit	Weight fruit (Kg)	No. infested	No. larvae or pupae	Upper infestation level % (95% confidence)
F	Festival	28/05/2013	555	9.3	0	0	0.5398
Total May			555	9.3	0	0	0.5398
F	Festival	11/06/2013	440	9.4	0	0	0.6808
F	Festival	25/06/2013	527	9.5	0	0	0.5684
Total June			967	18.9	0	0	0.3098
F	Festival	8/07/2013	400	9.3	3	3	1.9384
F	Festival	23/07/2013	432	9.5	3	5	1.7948
Total July			832	18.8	6	8	1.4233
F	Festival	6/08/2013	340	9.4	2	2	1.8515
F	Festival	20/08/2013	661	9.3	2	3	0.9524
F	Festival	27/08/2013	504	9.5	0	0	0.5944
Total August			1505	28.2	4	5	0.6082
Total May- 10 August			2694	56.4	8	10	0.5358
Total Post 10 August - Last pick			1165	18.8	2	3	0.5404

Table 12 Assessment of fruit fly infestation in strawberries harvested from Property G in 2013

Property	Variety	Date picked	No. fruit	Weight fruit (Kg)	No. infested	No. larvae or pupae	Upper infestation level % (95% confidence)
G	Festival	27/05/2013	789	11.1	0	0	0.3797
Total May			789	11.1	0	0	0.3797
G	Festival	11/06/2013	549	8.2	0	0	0.5457
G	Festival	25/06/2013	1168	11.2	0	0	0.2565
Total June			1717	19.4	0	0	0.1745
G	Festival	9/07/2013	490	12.6	0	0	0.6114
G	Festival	23/07/2013	515	11.2	0	0	0.5817
Total July			1005	23.8	0	0	0.2981
G	Festival	6/08/2013	1012	13	0	0	0.2960
G	Festival	20/08/2013	1238	12.6	9	15	1.2687
Total August			2250	25.6	9	15	0.698
G	Festival	2/09/2013	1343	13.3	11	16	1.3557
Total September			1343	13.3	11	16	1.3557
Total May- 10 August			4523	67.3	0	0	0.0623
Total Post 10 August - Last pick			2581	25.9	20	31	1.1260

Table 13 Assessment of fruit fly infestation in strawberries harvested from Property H in 2013

Property	Variety	Date picked	No. fruit	Weight fruit (Kg)	No. infested	No. larvae or pupae	Upper infestation level % (95% confidence)
Н	Ruby Gem	28/05/2013	489	7.3	0	0	0.6126
Total May			489	7.3	0	0	0.6126
Н	Festival	11/06/2013	670	8.2	0	0	0.4471
Н	Ruby Gem	25/06/2013	430	8.2	0	0	0.6967
Total June			1100	16.4	0	0	0.2723
Н	Ruby Gem	8/07/2013	434	8.2	0	0	0.6903
Н	Ruby Gem	23/07/2013	201	3.8	0	0	1.4903
Total July			635	12	0	0	0.4718
Н	Ruby Gem	6/08/2013	680	11.8	0	0	0.4405
Н	Ruby Gem	20/08/2013	498	8.1	2	4	1.2641
Total August			1178	19.9	2	4	0.5344
Н	Camarosa	2/09/2013	357	8	3	8	2.1718
Н	Camarosa	16/09/2013	225	4.7	1	1	2.1080
Total September			582	12.7	4	9	1.5727
Total May- 10 August			2904	47.5	0	0	0.1032
Total Post 10 August - Last pick			1080	20.8	6	13	1.0965

5.4 Discussion

In 2012, very low numbers of male and female Queensland fruit fly (*B. tryoni*) and Lesser Queensland fruit fly (*B. neohumeralis*) were trapped over the three strawberry farms from mid May to early September. Six out of the 11 female fruit flies caught in McPhail traps throughout the season had mature eggs in their ovaries. No mature females were caught between 25 June and 20 August. From assessment of packed fruit samples from the three properties only two strawberries out of 10942 sampled were found to be infested by Queensland fruit fly. This result shows that there is little risk of fruit fly infestation during the winter months up until 10 August.

Investigations of the two detections from Property D in 2012 were conducted by Biosecurity Queensland (DAFF QLD) and members of the project team. These audits aimed to determine whether the detections were due to a nonconformance of the operational procedures of the ICA or from a failure of the system. These investigations included a compliance check of the spray records and an on-site farm visit.

Investigation of the first detection, from fruit harvested on 2 July 2012, showed that all spray records were in accordance with the requirements of ICA-34. During the period preceding the harvest on 2 July, weather data from 23 June to 2 July showed that the Bundaberg region received repeated, almost daily

rainfall, with a total of 183 mm within the month of June. The total in June 2012 was almost four times the average monthly rainfall of 49.4 mm. During this period bait sprays were not reapplied by the grower and under the operational procedures of the ICA reapplication was not required. In the first version of the operational procedures of ICA-34, the procedures advised that 'if rain sufficient to cause run-off from leaves within two hours of spraying' bait sprays needed to be reapplied. These rain events did not occur within the required time frame of 2 hours after application that would have triggered reapplication of the bait sprays. Therefore, it is likely that due to the continual rainfall events, this unseasonal weather compromised the effectiveness of the bait program. This was further compromised by the operational procedures of the ICA, which did not contain measures for reapplication of bait sprays after rainfall events. As a result, the project team recommended that the operational procedures be changed and subsequently in the second version this wording was changed to- " apply chemicals at the specified interval, reapplying sooner if rain washes off the deposit" (Queensland Government, 2013).

The factors causing the second infestation on Property D in 2012 from fruit harvested on 20 August were not as clear. Infestation of strawberries occurred after plants had received two cover spray applications of Hy-Mal (Maldison) insecticide (8 and 17 August). From this detection only 1 fruit out of a total of 439 was infested with B. tryoni larvae. Records of bait spray and cover spray applications for this property were found to be correct and in compliance with the operational procedures of ICA-34. In addition, male trapping records do not show an increase in fruit fly numbers and female fruit fly collections also showed that there is very low risk. Females that were collected over winter (between 25 June and 20 August) did not contain mature eggs. One possibility that should be considered is the efficacy of Maldison cover sprays and whether a shorter spray interval is required. On this property, spray intervals used on the treatment block prior to the detection was 9 days, with a maximum of 10 days used at other times. The current permit for Maldison use in strawberries allows for a minimum spray interval of 7 days, therefore there is a possibility to reduce the spray interval to increase the efficacy of this insecticide.

As a result of these two detections during 2012, the project team suggested that after 10 August bait spray applications should be continued during the second season of field trials in 2013. This additional component aimed to reduce fruit fly populations during spring and to further reduce the risk of infestation over and above what was stipulated in ICA-34.

In 2013, 3 of the 4 participating properties had no infestation in packed fruit samples during the period from July to 10 August. This demonstrated that there was minimal risk of fruit fly infestation during the winter period. However, on one property 8 fruit from a total of 3 sample dates were infested during this period. Investigations of these detections from Property F were conducted by Biosecurity Queensland (DAFF QLD) and members of the project team. These audits determined whether the detections were due to a non-conformance of the operational procedures of the ICA or from a failure of the

system. These investigations included a compliance check of the spray records and an on-site farm visit. Investigations showed that the grower did not achieve the requirements stipulated in the operational procedures. Queensland Biosecurity officers found that fruit fly resting sites had not attained an appropriate height for effective fruit fly control, which likely compromised the efficacy of the bait spray program. Fruit fly resting sites were found to be very low growing, of less than 1 meter in height and either non-existent or very sparsely planted (Figure 27)



Figure 27 (Left) Non-conformance of operational procedures on Property F, which require fruit fly resting sites to be planted between strawberry block and shed (Right) Non-conformance of operational procedures on Property F, which require fruit fly resting sites to be well maintained and at least 1 meter in height.

In 2013, after 10 August all 4 properties contained samples with infested fruit. This result demonstrated that the risk of fruit fly infestation within the Bundaberg region using the current operational procedures of ICA-34 is greater after 10 August compared to the preceding winter months. Rates of infestation differed between properties, with the highest levels recorded from Property E at the end of September. It is likely that higher infestation rates on this property could be due to the grower's production methods, where strawberries are left on the plants until they are very ripe. This grower is noted for his very ripe strawberries, which are often softer than what is expected from large commercial operations, and therefore may be more susceptible to infestation.

In addition, detections after 10 August may be due to the decreased efficacy of the cover sprays from the use of extended spray intervals. On Property E, a maximum spray interval of 14 days was used for Spinetoram (Success Neo) for some applications dates, where the minimum allowable interval was 7 days. This was also seen on Property G, where Spinetoram and Maldison were applied at 9 day intervals, where the minimum allowable interval was 7 days.

5.5 Conclusions and recommendations

In conclusion, from monitoring fruit fly activity it was found that the population and abundance of Queensland fruit flies on strawberry farms in the Bundaberg region is very low particularly during the winter months from May to 10 August. Also, the risk of fruit fly infestation in packed strawberries during this time is negligible. Although a number of fruit fly detections occurred in 2012 and 2013, non-conformances of the operational procedures were found and were likely to have resulted in the detection of fruit fly larvae in the fruit samples.

Problems with the operational procedures for the reapplication of cover sprays and bait sprays after rainfall were highlighted, which is likely to have reduced the efficacy of the bait spray programs causing infestation in fruit. This wording has been rectified in the latest version of the operational procedures.

After 10 August there is a greater risk of infestation in strawberries within the Bundaberg region. It is likely that if greater levels of control are required additional measures are needed to ensure the protection of the crop. This may include, but is not limited to, the use of shorter reapplication intervals for currently available cover spray chemicals, as well as the use of bait sprays after 10 August until the end of harvesting. In addition, investigation of alternative cover sprays that are effective against fruit flies may also be useful to provide more options to growers.

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8 Appendix

8.1 Chemical spray records

8.1.1 Bundaberg field trials 2012

Property D

Date	Time	Product	Volume of mixture (L)	Volume of concentrate (L)	Block	Area treated (Ha)
7/04/2012	10:00:00 AM	Naturalure	225	30	All	30
14/04/2012	9:00:00 AM	Naturalure	225	30	All	30
21/04/2012	10:00:00 AM	Naturalure	225	30	All	30
28/04/2012	10:00:00 AM	Naturalure	225	30	All	30
5/05/2012	10:00:00 AM	Naturalure	225	30	All	30
12/05/2012	9:30:00 AM	Naturalure	225	30	All	30
19/05/2012	11:00:00 AM	Naturalure	225	30	All	30
26/05/2012	10:00:00 AM	Naturalure	225	30	All	30
2/06/2012	10:00:00 AM	Naturalure	225	30	All	30
9/06/2012	9:00:00 AM	Naturalure	225	30	All	30
16/06/2012	10:00:00 AM	Naturalure	225	30	All	30
23/06/2012	10:00:00 AM	Naturalure	225	30	All	30
30/06/2012	11:00:00 AM	Naturalure	225	30	All	30
7/07/2012	10:00:00 AM	Naturalure	225	30	All	30
14/07/2012	10:00:00 AM	Naturalure	225	30	All	30
21/07/2012	10:00:00 AM	Naturalure	225	30	All	30
28/07/2012	10:00:00 AM	Naturalure	225	30	All	30
4/08/2012	10:00:00 AM	Naturalure	225	30	All	30
8/08/2012	3:00:00 PM	Hy-mal	10100	5.555	A1-4,B1-4,C1-4	9.04
9/08/2012	3:00:00 PM	Hy-mal	10100	5.555	A5-8,B5-8,C5-8	9.04
11/08/2012	10:00:00 AM	Naturalure	225	30	All	30
17/08/2012	3:00:00 PM	Hy-mal	10100	5.555	A1-4,B1-4,C1-4	9.04
19/08/2012	3:00:00 PM	Hy-mal	10100	5.555	A5-8,B5-8,C5-8	9.04
27/08/2012	4:00:00 PM	Hy-mal	10100	5.555	A1-4,B1-4,C1-4	9.04
29/08/2012	3:00:00 PM	Hy-mal	10100	5.555	A5-8,B5-8,C5-8	9.04

Property E

Date	Time	Product	Volume of mixture	Volume of concentrate (ml)	Block	Area treated (HA)
25/03/2012		Naturalure			Ruby Gem	
31/03/2012		Naturalure			Ruby Gem	
7/04/2012		Naturalure			Ruby Gem and	
		Naturalure			Festival	
14/04/2012		Naturalure			All Blocks	
21/04/2012		Naturalure			All Blocks	
28/04/2012		Naturalure			All Blocks	
4/05/2012		Naturalure			All Blocks	
11/05/2012		Naturalure			All Blocks	
18/05/2012		Naturalure			All Blocks	
25/05/2012		Naturalure			All Blocks	
31/05/2012		Naturalure			All Blocks	
7/06/2012	6:00:00 AM	Naturalure				
13/06/2012	6:30:00 AM	Naturalure				
20/06/2012	6:30:00 AM	Naturalure				
27/06/2012	7:00:00 AM	Naturalure				
4/07/2012	6:30:00 AM	Naturalure				
11/07/2012		Naturalure				
18/07/2012	4:00:00 PM	Naturalure				
18/07/2012	4:00:00 PM	Success Neo				
25/07/2012	6:00:00 AM	Naturalure				
1/08/2012		Naturalure				
8/08/2012		Naturalure				
9/08/2012		Success Neo			Camarosa, Ruby Gem, Festival	
15/08/2012		Naturalure				
19/08/2012		Success Neo				
22/08/2012		Naturalure				
29/08/2012		Naturalure				
29/08/2012		Success Neo				
5/09/2012		Naturalure				
8/09/2012		Success Neo				
17/09/2012	4:00:00 PM	Maldison			North Block	
18/09/2012	5:00:00 PM	Maldison			South Block	
24/09/2012	5:00:00 PM	Maldison				
1/10/2012	5:00:00 PM	Maldison				

Property F

Date	Time	Product	Volume of mixture (L)	Volume of concentrate (L)	Block	Area treated (Ha)
23/04/2012	5:00:00 PM	Naturalure	143	22	1, 2	22
30/04/2012	12:00:00 PM	Naturalure	143	22	1, 2	22
7/05/2012	7:00:00 AM	Naturalure	143	22	1, 2	22
14/05/2012	3:00:00 PM	Naturalure	143	22	1, 2	22
21/05/2012	1:00:00 PM	Naturalure	143	22	1, 2	22
28/05/2012	1:00:00 PM	Naturalure	143	22	1, 2	22
4/06/2012	1:00:00 PM	Naturalure	143	22	1, 2	22
11/06/2012	2:00:00 PM	Naturalure	143	22	1, 2	22
18/06/2012	12:00:00 PM	Naturalure	143	22	1, 2	22
25/06/2012	1:00:00 PM	Naturalure	143	22	1, 2	22
2/07/2012	9:00:00 AM	Naturalure	143	22	1, 2	22
9/07/2012	1:00:00 PM	Naturalure	143	22	1, 2	22
16/07/2012	1:00:00 PM	Naturalure	143	22	1, 2	22
23/07/2012	9:00:00 AM	Naturalure	143	22	1, 2	22
30/07/2012	3:00:00 PM	Naturalure	143	22	1, 2	22
6/08/2012	7:00:00 AM	Naturalure	143	22	1, 2	22
9/08/2012	1:00:00 PM	Success Neo	9500	3.8	1,2,7,8	9.5
10/08/2012	2:00:00 PM	Success Neo	12500	5	3,4,5,6	12.5
13/08/2012	10:00:00 AM	Naturalure	143	22	1,2	22
19/08/2012	4:00:00 PM	Success Neo	9500	3.8	1,2,7,8	9.5
20/08/2012	9:00:00 AM	Naturalure	143	22	1,2	22
20/08/2012	5:00:00 PM	Success Neo	12500	5.94	3,4,5,6	12.5
27/08/2012	9:00:00 AM	Naturalure	143	22	1,2	22
29/08/2012	1:00:00 PM	Success Neo	12500	5.94	3,4,5,6	12.5
30/08/2012	1:00:00 PM	Success Neo	9500	3.8	1,2,7,8	9.5

8.1.2 Bundaberg field trials 2013

Property E

Date	Time	Product	Volume of mixture (L)	Volume of concentrate (L)	Block	Area treated (Ha)
25/03/2013		Naturalure			All blocks	
1/04/2013		Naturalure			All blocks	
8/04/2013		Naturalure			All blocks	
15/04/2013		Naturalure			All blocks	
22/04/2013		Naturalure			All blocks	
29/04/2013		Naturalure			All blocks	
6/05/2013		Naturalure			All blocks	
13/05/2013		Naturalure			All blocks	
20/05/2013		Naturalure			All blocks	
27/05/2013		Naturalure			All blocks	
3/06/2013		Naturalure			All blocks	
10/06/2013		Naturalure			All blocks	
17/06/2013		Naturalure			All blocks	
24/06/2013		Naturalure			All blocks	
1/07/2013		Naturalure			All blocks	
8/07/2013		Naturalure			All blocks	
15/07/2013		Naturalure			All blocks	
22/07/2013		Naturalure			All blocks	
29/07/2013		Naturalure			All blocks	
5/08/2013		Naturalure			All blocks	
11/08/2013	5:00:00 PM	Success Neo	2800	460	All Blocks	
12/08/2013		Naturalure			All blocks	
19/08/2013	3:30:00 PM	Success Neo	2800	460	All Blocks	
19/08/2013		Naturalure			All blocks	
25/08/2013	4:30:00 PM	Success Neo	2100	460	All Blocks, except Festival	
4/09/2013	5:00:00 PM	Success Neo	2100	460	All Blocks	

Property F

Date	Time	Product	Volume of mixture (L)	Volume of concentrate (L)	Block	Area treated (Ha)
7/04/2013	7:00:00 AM	Naturalure	143	22	1	22
14/04/2013	8:00:00 AM	Naturalure	143	22	1	22
28/04/2013	7:00:00 AM	Naturalure	143	22	1	22
5/05/2013	9:00:00 AM	Naturalure	143	22	1	22
12/05/2013	7:00:00 AM	Naturalure	143	22	1	22
19/05/2013	7:00:00 AM	Naturalure	143	22	1	22
26/05/2013	10:00:00 AM	Naturalure	143	22	1	22
2/06/2013	7:00:00 AM	Naturalure	143	22	1	22
9/06/2013	5:00:00 PM	Naturalure	143	22	1	22
16/06/2013	9:00:00 AM	Naturalure	143	22	1	22
23/06/2013	10:00:00 AM	Naturalure	143	22	1	22
30/06/2013	8:00:00 AM	Naturalure	143	22	1	22
7/07/2013	10:00:00 AM	Naturalure	143	22	1	22
14/07/2013	9:00:00 AM	Naturalure	143	22	1	22
21/07/2013	9:00:00 AM	Naturalure	143	22	1	22
28/07/2013	10:00:00 AM	Yeast + Maldison	165	3.3 + 0.72	1	22
4/08/2013	9:00:00 AM	Yeast + Maldison	165	3.3 + 0.72	1	22
8/08/2013	4:00:00 PM	Maldison	9500	9.5	3,4,5,6	9.5
9/08/2013	5:00:00 PM	Maldison	12500	12.5	1,2,7,8	12.5
11/08/2013	8:00:00 AM	Yeast + Maldison	165	3.3 + 0.72	1	22
15/08/2013	4:00:00 PM	Maldison	9500	9.5	3,4,5,6	9.5
16/08/2013	6:00:00 PM	Maldison	12500	12.5	1,2,7,8	12.5
18/08/2013	7:00:00 AM	Yeast + Maldison	165	3.3 + 0.72	1	22
22/08/2013	5:00:00 PM	Maldison	9500	9.5	3,4,5,6	9.5
23/08/2013	5:00:00 PM	Maldison	12500	12.5	1,2,7,8	12.5
25/08/2013	7:00:00 AM	Yeast + Maldison	165	3.3 + 0.72	1	22

Property G

Date	Time	Product	Volume of mixture (L)	Volume of concentrate (L)	Block	Area treated (Ha)
20/04/2013	7:00:00 AM	Naturalure	150	20	F	3.6
					G	4.2
					Н	4.2
					I	4
					J	3.6
					К	3.6
27/04/2013	10:00:00 AM	Naturalure	150	20	F	3.6
					G	4.2
					H	4.2
					I	4
					J	3.6
7/05/0040	0.00.00 AM	Network	450		K	3.6
7/05/2013	8:00:00 AM	Naturalure	150	20	F	3.6
					G	4.2
					Н	4.2 4
					l J	4 3.6
					K	3.6
17/05/2013	2:00:00 PM	Naturalure	150	20	K	3.6
17/05/2013	2.00.00 F IVI	Naturalure	150	20	G	4.2
					H	4.2
						4
					J	3.6
					ĸ	3.6
24/05/2013	7:00:00 AM	Naturalure	150	20	F	3.6
					G	4.2
					H	4.2
					I	4
					J	3.6
					К	3.6
1/06/2013	8:30:00 AM	Naturalure	150	20	F	3.6
					G	4.2
					Н	4.2
					I	4
					J	3.6
					K	3.6
7/06/2013	7:30:00 AM	Naturalure	150	20	F	3.6
					G	4.2
					H	4.2
					I	4
					J	3.6
44/00/0040	7.00.00 414	NI-4.	450	00	K	3.6
14/06/2013	7:00:00 AM	Naturalure	150	20	F	3.6
					G	4.2
					H	4.2
					I	4 3.6
					J K	3.6 3.6
					r.	3.0

19/06/2013	7:00:00 AM	Naturalure	150	20	F	3.6
					G	4.2
					Н	4.2
					I	4
					J	3.6
					K	3.6
26/06/2013	2:00:00 PM	Naturalure	150	20	F	3.6
					G	4.2
					H	4.2
					1	4
					J	3.6
2/07/0042	0.00.00 414	Netwolves	450		<u>K</u>	3.6
3/07/2013	8:00:00 AM	Naturalure	150	20	F	3.6
					G	4.2 4.2
					H	4.2 4
					J	4 3.6
					ĸ	3.6
9/07/2013		Success	8500	1.2	I,F,H	11.8
10/07/2013	7:30:00 AM	Naturalure	150	20	F	3.6
10/01/2010	1.00.007.00	- Tataralaro	100	20	G	4.2
					H	4.2
					I	4
					J	3.6
					К	3.6
17/07/2013	10:00:00 AM	Naturalure	150	20	F	3.6
					G	4.2
					Н	4.2
					I	4
					J	3.6
					K	3.6
23/07/2013	6:30:00 AM	Yeast Autolysate + Hymal	250	6.1	F	3.6
		,			G	4.2
					H	4.2
					I	4
					J	3.6
					К	3.6
29/07/2013	7:30:00 AM	Yeast Autolysate + Hymal	250	6.1	F	3.6
		- i giriai			G	4.2
					Н	4.2
						4
					J	3.6
					ĸ	3.6
8/08/2013	4:00:00 PM	Yeast Autolysate + Hymal	250	6.1	F	3.6
					G	4.2
					H	4.2

					к	3
8/08/2013	8:00:00 AM	Success	8000	1.2	J,K,G	1
11/08/2013	2:00:00 PM	Yeast Autolysate + Hymal	250	6.1	F	3
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17/08/2013	12:00:00 PM	Yeast Autolysate + Hymal	250	6.1	F	
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17/08/2013	6:30:00 AM	Success	8000	1.2	G,J,H	
18/08/2013	7:00:00 AM	Success	8500	1.2	I,F,K	1
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23/08/2013	1:00:00 PM	Autolysate + Hymal	250	6.1	F	
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26/08/2013	7:00:00 AM	Success	8500	1.2	G,H,J	
27/08/2013	7:00:00 AM	Success	8000	1.2	I,F,K	
28/08/2013	2:30:00 PM	Yeast Autolysate + Hymal	250	6.1	F	
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4/09/2013	4:00:00 AM	Success	8000	1.2	F,J,G	
5/09/2013	6:00:00 AM	Success	8500	1.2	H,I,K	1
6/09/2013	3:00:00 PM	Yeast Autolysate + Hymal	250	6.1	F	:
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10/09/2013	2:00:00 PM	Hymal	3000	1.62	I	
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11/09/2013	10:00:00 AM	Yeast Autolysate + Hymal	250	6.1	F	3
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11/09/2013	9:00:00 AM	Hymal	NA	1.62	K	3
12/09/2013	2:00:00 PM	Hymal	5500	1.65	G,J	7
18/09/2013	2:00:00 PM	Yeast Autolysate + Hymal	250	6.1	F	3
					G	2
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					J	3
					K	3
19/09/2013	6:00:00 PM	Hymal	6000	1.65	H,I	8
20/09/2013	5:00:00 PM	Hymal	3000	1.65	K	3
21/09/2013	10:00:00 AM	Hymal	5500	1.65	G,J	7

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