Double knock low dose fenthion treatment of zucchinis as a quarantine treatment against cucumber fly

Andrew Jessup
NSW Department of Primary Industries

Project Number: VG13066

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Project VG13066

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FINAL REPORT

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Purpose of Report
The use of fenthion as a post harvest dip treatment for fresh horticultural commodities that are host to pest fruit flies has been restricted due to health concerns with residues. The restricted treatment is a single dip, or flood spray or spray of fenthion (obsolete Interstate Certification Assurance Operational Procedures ICA-01, ICA-02 and ICA-03, respectively) at a rate of 412.4mg/L fenthion for 60s or more.

The focus of this report is to determine the effect of a reduced rate (100mg/L instead of 412.5mg/L) of fenthion on the mortality of Cucumber fly (*Bactrocera cucumis*, French) (CF) applied twice (the second dip applied 24h after the first) in Australian-grown zucchinis at four different fly developmental stages; eggs, first instar larvae, second instar larvae and third instar larvae.

This project was funded through a voluntary contribution from Hannay-Douglas Pty Ltd facilitated by HAL. The Australian Government provides matched funding for all HAL's research and development activities. NSW Department of Primary Industries provided on-going support and contributed significantly to the outcomes of this project.

Media summary

Market access is a key priority for the Australian vegetable industry. The presence of quarantine pests such as Cucumber fly in some growing areas requires fruit to be treated and/or certified as being free from live quarantine pests before accessing important pest-free markets.

Up until recently a single postharvest dip, spray or flood treatment of fresh Australian zucchinis in/with a 412.4mg/L solution of fenthion was the approved quarantine treatment for access of these fruit to New Zealand and other fruit fly sensitive parts of Australia (e.g. Tasmania, Western Australia and South Australia). Rulings by the Australian Pesticides and Veterinary Medicines Authority (APVMA) restricted the use of fenthion on human food due to public health concerns with residues.

A new method of applying fenthion to acquire quarantine security was devised by Hannay-Douglas Pty Ltd using a much lower concentration of fenthion (100mg/L) but applied twice (24h apart). This ensures residues that are well below the maximum residue limit stipulated by the APVMA.

This report describes the results of efficacy tests of this prototype treatment carried out on the main quarantine fruit fly pest of zucchinis, the Cucumber fly (*Bactrocera cucumis*, French).

Tests carried out showed that eggs, first instar larvae, second instar larvae and third instar larvae dominated larval populations within infested fruit at certain times after initial egg laying (oviposition). Infested fruit were dipped in the 100mg/L fenthion solution for 60s at each of these larval development times (and the treatment repeated 24h later) so that there was assurance that each life stage that would be likely to be found infesting fresh zucchinis was treated. Survival of insects from Control fruit that were infested but not fenthion dipped (they were water dipped) was used to estimate the number of insects treated in the fenthion dipped fruit and, hence, treatment induced mortality.

Experiments were replicated three times in accordance with normal procedures used for the development of internationally accepted quarantine treatments.

Over 30,000 insects (total from three replicates in time) at each of the four immature life stages were treated.

This information can be used by industry to support an application for the use of the double knock low dose fenthion treatment of zucchinis as a quarantine treatment against cucumber fly as a quarantine treatment for market access.

NOTE: Research on residue analyses of zucchinis treated using this new treatment were commissioned by Hannay_Douglas Pty Ltd and showed residues were well below maximum residue limits set by the APVMA in all cases.

Technical summary

The use of fenthion as a post harvest dip treatment for fresh horticultural commodities that are host to pest fruit flies has been restricted due to health concerns with residues. The restricted treatment is a single dip, flood spray or spray of fenthion (obsolete Interstate Certification Assurance Operational Procedures ICA-01, ICA-02 and ICA-03, respectively) at a rate of 412.4mg/L fenthion for 60s or more.

The focus of this report is to determine the effect of a reduced rate (100mg/L instead of 412.5mg/L) of fenthion on the mortality of Cucumber fly (*Bactrocera cucumis*, French) (CF) applied twice (the second dip applied 24h after the first) in Australian-grown zucchinis at four different fly developmental stages; eggs, first instar larvae, second instar larvae and third instar larvae.

Survival of insects from Control (untreated) infested fruit gave an estimate of the number of insects exposed to the test treatment in fenthion dipped fruit. Over 30,000 insects (total from three replicates in time) at each of the four immature life stages were treated. The findings were that all double dip fenthion treatments caused 100% CF mortality in zucchinis. The outcome of this experiment was that two 1 minute dips of fenthion, at a rate of 100mg/L of fenthion in water, separated by 24 hours in storage at 10°C caused 100% mortality of CF eggs and larvae in fresh zucchinis. The temperature of the dip solution was 20°C.

This information can be used by industry to support an application for the use of the double knock low dose fenthion treatment of zucchinis as a quarantine treatment against cucumber fly as a quarantine treatment for market access.

NOTE: Research on residue analyses of zucchinis treated using this new treatment were commissioned by Hannay-Douglas Pty Ltd and showed residues were well below maximum residue limits set by the APVMA in all cases.

Abbreviations

ANOVA	analysis of variance
APVMA	Australian Pesticides and Veterinary Medicines Authority
°C	Celsius (degree)
CF	Cucumber fly (*Bactrocera cucumis*, French)
F	ANOVA F-statistic
g	gram
h	hour/s (as in 24h = 24 hours)
l.s.d.	least significant difference
mg	milligram
mL	millilitre
MRL	Maximum Residue Limit
NSW DPI, NSWDPI	New South Wales Department of Primary Industries
OHS	Occupational Health and Safety
p	probability
s	second/s (as in 60s = 60 seconds)

Low dose double knock fenthion dip for *Bactrocera cucumis* (French) (cucumber fly) in fresh zucchini

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Abstract

The use of fenthion as a post harvest dip treatment for fresh horticultural commodities that are host to pest fruit flies has been restricted due to health concerns with residues. The restricted treatment is a single dip, or flood spray or spray of fenthion (obsolete Interstate Certification Assurance Operational Procedures ICA-01, ICA-02 and ICA-03, respectively) at a rate of 412.4mg/L fenthion for 60s or more.

The focus of this report is to determine the effect of a reduced rate (100mg/L instead of 412.5mg/L) of fenthion on the mortality of Cucumber fly (*Bactrocera cucumis*, French) (CF) applied twice (the second dip applied 24h after the first) in Australian-grown zucchinis at four different fly developmental stages; eggs, first instar larvae, second instar larvae and third instar larvae.

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Introduction

Bactrocera cucumis (French) – The Cucumber fly

Figure 1 *Bactrocera cucumis (CF)*

The Cucumber fly (*Bactrocera cucumis*, French) (CF), shown in Figure 1, is a native insect found from Cape York, in the north of Australia, to the south of the southern border of Queensland. They are coastal and sub-coastal flies. The CF is ~8mm long with a wasp-like body, they are coloured yellow-brown with yellow marks on the side of the thorax and a yellow line on the centre of the dorsal surface between the wings. CF is a major pest in the north Rockhampton area. It targets cucurbits and papaya and can cause extensive damage to zucchinis. It is found in the field that the CF prefer to lay their eggs in damaged or ripe fruit.

From previous studies it was found that, at the optimum survival temperature of 26°C to 27℃, the CF egg hatches at about 24h after infestation. CF eggs are white and have a banana-like shape as seen in Figure 2. After 24h from oviposition, eggs hatch and the larva emerges as a first instar larva.

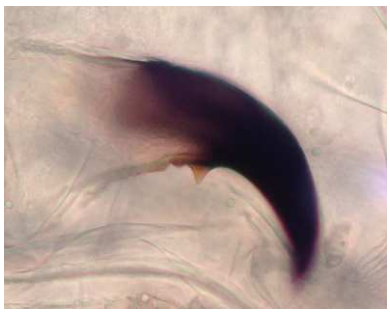
Figure 2 CF eggs

First instar larval mouthhooks are light brown in colour showing little to no sclerotisation (blackening) and have a secondary (preapical) tooth on each of the two mouthhooks (Figure 3).

Figure 3 CF first instar

At ~62 hours after being laid the larvae have moulted into the second instar where they have increased in size. The bases of the mouth hooks have darkened showing with sclerotisation. The preapical tooth on the mouthhook is retained in the second instar (Figure 4).

Figure 4 CF second Instar



At ~86 hours, the larvae have moulted to the third instar and have completely darkened. The preapical tooth has decreased in size relative to the mouthhook but is still retained (Figure 5).

Figure 5 CF third instar

Fenthion

Fenthion, with a molecule weight of 278D, is a broad spectrum organophosphate which acts on the enzyme acetyl cholinesterase which is used by the nervous system and this causes death of the insect. Organophosphates break down rapidly by hydrolysis when exposed to air, soil and sunlight. The figure to the right shows the chemical formula of fenthion. Fenthion is an insecticide used in horticulture within Australia. On the 11th of September 2012, its use for gardening was suspended and currently fenthion is under restrictions which control its use.

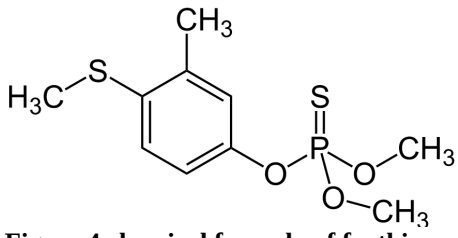


Figure 4 chemical formula of fenthion

Method

Rationale for the experimental procedure used in these experiments

There is international acceptance that treatment efficacy against fruit flies can be demonstrated in laboratory experiments by proving that there were zero survivors from three replicate trials on 10,000 insects treated at the most treatment-tolerant life stage that is likely to be found infesting exported fruit.

In the trials reported here we did not carry out the experiments where the most treatment-tolerant life stage was determined. Instead, we carried out 3 X 10,000 insect trials on all immature life stages of *Bactrocera cucumis* (cucumber fly) that are likely to infest exported zucchinis – namely eggs, 1st instar larvae, 2nd instar larvae and 3rd instar larvae.

The reason we did this was to reduce the time taken to carry out these trials and also to exploit the ease that CF were able to infest, and survive in, zucchinis. We believe that the results obtained this way are more robust in that all likely infesting life stages are tested separately and to the fullest extent as approved by our international trading partners.

Dose Rate: How dose rate was determined

1. Rate to be used: 100mg/L of fenthion

2. Active ingredient (a.i.) of commercially available fenthion: 550g/1L

3. 50L of solution was made up to fill a 70L container by the following working:
   100mg/L=0.1g/L
   0.1g in 50L= 5g in 50L
   5g/0.550L=9.09g/50L

So therefore 9.09 grams of the commercially available fenthion was mixed with 50L of water for the dipping treatments to produce a dose of 100mg/L of fenthion a.i.

The fruit

Fresh zucchinis were purchased from Sydney Markets and were grown and packed by Wisemans Organic Produce, Farm 528, Coleambally NSW 2707 (ph 02 69544339) (Wise Choice – Naturally Organic) (see Figure 10 at the end of this report). The fruit was purchased in 4 batches for the different experiments to ensure the fruit was fresh for the experiment on the following dates 2 January 2014, 8 January 2014, 16 January 2014 and 6 March 2014. Organic zucchinis were used to minimise the chance of pesticide residue on the samples. Samples from all batches were test infested to check for the absence of pesticides. The zucchinis were refrigerated at 5°C until 24 hours before infestation.

Different batches were infested on the following dates:

- 6 January 2014 (from fruit purchased on 2 January 2014; for treatment at the egg stage)

- 13 January 2014 (from fruit purchased on 8 January 2014; for treatment at the second instar larval stage)
- 17 January 2014 (from fruit purchased on 16 January 2014; for treatment at the third instar larval stage) and
- 11 March 2014 (from fruit purchased on 6 March 2014; for treatment at the first instar larval stage).

This experiment was divided into four parts where insects were treated in infested zucchinis at the following different immature life stages:

- Eggs
- First instar larvae
- Second instar larvae and
- Third instar larvae.

Fruit to be treated when insects were at the egg stage were infested early on 6 January 2014. Infested fruit were held at 26°C for incubation and then first-dipped 6h after infestation. The second dipping was done 24 hours after the first dip. Prior to the first dip eggs and larvae were dissected from a sample of five infested fruit and checked for which life stage was present in the fruit to be dipped. Results showed that the infesting population was 100% at the egg stage prior to the first dip (Appendix 1).

Fruit to be treated when insects were at the second instar larval stage were infested on the 13 January 2014 and incubated at 26°C for 48h prior to the first dip on 15 January 2014. The second dip was done 24h after that. Fruit dissection prior to the first dip showed that insects to be treated were a mixture of first instar larvae and second instar larvae but second instar larvae predominated (Appendix 2).

Fruit to be treated when insects were at the third instar larval stage were infested on 17 January 2014 and incubated at 26°C until 20 January 2014 when they were first-dipped. The second dip took place 24h after the first dip. Prior to the first dip dissection of insects from sample infested zucchinis showed that a mixture of second instar larvae and third instar larvae were present but third instar larvae predominated (Appendix 3).

Fruit to be treated when insects were at the first instar larval stage were infested on 11 March 2014 and incubated for 24h at 26°C when they were then given their first dip (i.e on 12 March 2014). Dissection of sample infested fruit revealed that the larval population was 100% first instar (Appendix 4).

Infestation

Fruit that had been warmed up from 5°C (storage temperature) to 26°C (optimal temperature for survival of CF) were placed on top of cages holding mature, gravid CF for 30 minutes. Fruit were removed when fruit had obvious signs of infestation. Fruit with no sign of infestation was left on the cage for an additional 5 minutes and was then removed. Zucchinis being infested by CF can be seen below in Figure 7.

These fruit were not punctured artificially. Even though this causes some unevenness in infestation rates between individual fruit, due to the contagious nature of infestation by this species of fruit fly, it was thought best that artificial holes may allow extra

penetration of fenthion solution into the fruit which would bias the test in favour if the treatment. It was decided to allow the flies, themselves, to puncture the fruit to maintain as natural an approach as possible.

Figure 5 Zucchinis being infested by CF

Dipping

Fruit was placed in mesh fruit bags of 9 fruit except for the experiment on third instar larvae which was double bagged (i.e. 18 fruit per bag). Bags were placed on trays, they were then dipped in solution and forced under the surface for a duration of 60s. The temperature of the dip solution was 20°C. They were then removed from solution and placed in new trays. After all treatments were completed they were all placed in a 10°C room for 24 hours where they were removed, dipped in a new batch of solution for 60s and allowed to drip dry for 10 mins, then placed on vermiculite in the usual manner and placed in the 26°C incubation room.

Figure 7 Dipping of zucchinis in fenthion

Figure 6 Larval rearing tray with moist vermiculite and mesh insert

Rearing, sieving and counting surviving larvae and pupae

The fruit was placed on a mesh insert, suspended over moist vermiculite in a larval rearing tray. As seen in Figure 9, the larval rearing tray had 3L of sieved vermiculite with 700ml of water mixed together. The tray was wrapped in a mesh bag to stop contamination with other fruit flies and *Drosophila* species and then placed in a 26°C incubation room to allow CF to develop normally.

After about 1 week from the second dipping infested fruit were removed from the 26°C incubation room. The vermiculite within each larval rearing tray beneath each mesh tray of infested fruit was removed and replaced with new, clean damp vermiculite as described in the above paragraph. Trays of infested fruit with new vermiculite were returned to the 26°C incubation room.

The removed vermiculite was sieved through a 1.6mm sieve which allowed the vermiculite through but not fruit fly pupae. All pupae surviving the treatment and the Controls were collected and counted.

The criterion for post-treatment survival was the formation of a normal puparium.

The above sieving process was repeated one week after the first sieve. A third and final sieve was carried out at three weeks after the second dip.

Larval stage sampling and assessment

Before dipping occurred, 5 zucchinis were selected randomly and the life stage of 100 insects randomly found within the fruit was used to determine the average life stage at the time of first dipping. Life stages were identified using the information presented in the introduction to this report. The results from this can be seen in Appendices 1 to 4.

Replication of experiments

Dipping treatments for each CF immature life stage (i.e. eggs, first instar larvae, second instar larvae and third instar larvae) consisted of Control fruit (infested and dipped in water) and Treated fruit (infested and dipped, twice [24h apart] in 100mg/L a.i. aqueous fenthion solution).

The reason the Control fruit were dipped in water was to account for the possibility that some insects in the fruit may have been washed out of the fruit during the dip treatment and, therefore, would not be counted in the final assessment for post-treatment larval survival. We decided to treat the Controls in the same way as the Treated fruit except that they were dipped in water rather than fenthion solution.

Within each life stage dipping treatment three replicate treatments were carried out. Although the fruit and the infesting insects were from the same cohort within each life stage treatment three separate water baths (for the Controls) and three newly made up dip solutions (for the Treated fruit) were used. Hence, replication was based on dip setup (i.e. replication in space).

The experimental design was therefore in the following format:

4 immature insect life stages (eggs, 1st instar, 2nd instar and 3rd instar larvae)
X
2 treatments (Control vs. Treated)
X
3 replicates (newly made up water dips [Control] and fenthion dips [Treated])

Results

Summary of survival of insects treated at the Egg Stage

The raw data to determine the insect stage that was treated can be found in Appendix 1 and the raw data from experiments testing for insect survival from the treatment can be seen in Appendix 5.

Table 1. Survival of *Bactrocera cucumis* (cucumber fly), when treated at the EGG STAGE, from a first dip treatment in fenthion solution at 100mg/L for 60s followed, 24h later (stored at 10°C in the meantime), by a second dip treatment in fenthion solution at 100mg/L for 60s and subsequent storage at 26°C – the Low Dose Double Knock Fenthion treatment.

Infested on 6 January 2014 First-dipped on 6 January 2014 Second-dipped on 7 January 2014	**No. of surviving pupae**
Control 1 (36 zucchinis)	12,874
Treatment 1 (36 zucchinis)	0
Control 2 (36 zucchinis)	24,516
Treatment 2 (36 zucchinis)	0
Control 3 (36 zucchinis)	15,367
Treatment 3 (36 zucchinis)	0
TOTAL (Control)	**52,757**
TOTAL (Treated)	**0**
ED (at 95% Confidence)	99.9943

There was zero survival of CF from over 52,000 CF treated at the EGG STAGE following treatment with the Low Dose Double Knock Fenthion treatment. This is in excess of the internationally approved criterion for acceptance of experimental data for new quarantine treatments.

Summary of survival of insects treated at the Second Instar Larval Stage

The raw data to determine the insect stage that was treated can be found in Appendix 2 and the raw data from experiments testing for insect survival from the treatment can be seen in Appendix 6.

Table 2. Survival of *Bactrocera cucumis* (cucumber fly), when treated at the SECOND INSTAR LARVAL STAGE, from a first dip treatment in fenthion solution at 100mg/L for 60s followed, 24h later (stored at 10°C in the meantime), by a second dip treatment in fenthion solution at 100mg/L for 60s and subsequent storage at 26°C – the Low Dose Double Knock Fenthion treatment.

Infested on 13 January 2014 First-dipped on 15 January 2014 Second-dipped on 16 January 2014	**No. of surviving pupae**
Control 1 (36 zucchinis)	13,789
Treatment 1 (36 zucchinis)	0
Control 2 (36 zucchinis)	13,217
Treatment 2 (36 zucchinis)	0
Control 3 (36 zucchinis)	14,016
Treatment 3 (36 zucchinis)	0
TOTAL (Control)	**41,022**
TOTAL (Treated)	**0**
ED (at 95% Confidence)	99.9927

There was zero survival of CF from over 41,000 CF treated at the SECOND INSTAR LARVAL STAGE following treatment with the Low Dose Double Knock Fenthion treatment. This is in excess of the internationally approved criterion for acceptance of experimental data for new quarantine treatments.

Summary of survival of insects treated at the Third Instar Larval Stage

The raw data to determine the insect stage that was treated can be found in Appendix 3 and the raw data from experiments testing for insect survival from the treatment can be seen in Appendix 7.

Table 3. Survival of *Bactrocera cucumis* (cucumber fly), when treated at the THIRD INSTAR LARVAL STAGE, from a first dip treatment in fenthion solution at 100mg/L for 60s followed, 24h later (stored at 10°C in the meantime), by a second dip treatment in fenthion solution at 100mg/L for 60s and subsequent storage at 26°C – the Low Dose Double Knock Fenthion treatment.

Infested on 17 January 2014 First-dipped on 20 January 2014 Second-dipped on 21 January 2014	**No. of surviving pupae**
Control 1 (36 zucchinis)	18,252
Treatment 1 (36 zucchinis)	0
Control 2 (36 zucchinis)	16,383
Treatment 2 (36 zucchinis)	0
Control 3 (36 zucchinis)	17,753
Treatment 3 (36 zucchinis)	0
TOTAL (Control)	**52,388**
TOTAL (Treated)	**0**
ED (at 95% Confidence)	99.9943

There was zero survival of CF from over 52,000 CF treated at the THIRD INSTAR LARVAL STAGE following treatment with the Low Dose Double Knock Fenthion treatment. This is in excess of the internationally approved criterion for acceptance of experimental data for new quarantine treatments.

Summary of survival of insects treated at the First Instar Larval Stage

The raw data to determine the insect stage that was treated can be found in Appendix 4 and the raw data from experiments testing for insect survival from the treatment can be seen in Appendix 8.

Table 4. Survival of *Bactrocera cucumis* (cucumber fly), when treated at the FIRST INSTAR LARVAL STAGE, from a first dip treatment in fenthion solution at 100mg/L for 60s followed, 24h later (stored at 10°C in the meantime), by a second dip treatment in fenthion solution at 100mg/L for 60s and subsequent storage at 26°C – the Low Dose Double Knock Fenthion treatment.

Infested on 11 March 2014 First-dipped on 12 March 2014 Second-dipped on 13 March 2014	**No. of surviving pupae**
Control 1 (27 zucchinis)	27,829
Treatment 1 (27 zucchinis)	0
Control 2 (27 zucchinis)	16,012
Treatment 2 (27 zucchinis)	0
Control 3 (27 zucchinis)	11,626
Treatment 3 (27 zucchinis)	0
TOTAL (Control)	**55,467**
TOTAL (Treated)	**0**
ED (at 95% Confidence)	99.9946

There was zero survival of CF from over 55,000 CF treated at the FIRST INSTAR LARVAL STAGE following treatment with the Low Dose Double Knock Fenthion treatment. This is in excess of the internationally approved criterion for acceptance of experimental data for new quarantine treatments.

Conclusions

The results determined through these series of experiments were that the Low Dose Double Knock Fenthion treatment caused 100% mortality of eggs and larvae of *Bactrocera cucumis* (Cucumber fly) (CF). The treatment was a 60s dip in 100mg/L fenthion, storage at 10°C for 24h and then a second 60s dip in 100mg/L fenthion. There were no survivors from greater than 40,000 insects treated at each immature life stage that is likely to be found in fresh zucchinis grown in the subtropical to tropical regions of eastern Australia.

With the high success rate demonstrated by the experiments reported here this dipping treatment should allow zucchinis to have access to markets currently restricted because of CF.

In conclusion, it was determined that the Low Dose Double Knock Fenthion treatment caused 100% mortality in CF infesting zucchinis where over 30,000 insects were treated at each immature life stage, thus satisfying international requirements for such experiments.

This schedule forwarded to trading partners, both domestic and international, through the Australian Department of Agriculture for approval as a quarantine treatment to control *Bactrocera cucumis* (cucumber fly) in fresh zucchinis exported interstate and overseas.

Recommendations

It is recommended that the data included in this report and the data from a third party which reports on residue analyses following the double knock fenthion treatment be compiled into a submission for approval by Government regulatory authorities (Domestic Quarantine Working Group) for interstate trade and to the Commonwealth Department of Agriculture for international trade review negotiations targeting, in the first instance, trade with New Zealand.

Acknowledgments

This project came about due to the innovative thinking of Hannay-Douglas Pty Ltd. I would like to thank, in particular, Alastair Scott (Managing Director) and Douglas Smith (Export Manager) for sharing the concept, their voluntary contribution to the research reported here and for advice and leadership throughout the process of the research.

I would like to thank, also, the project team at NSW Department of Primary Industries for their expert technical assistance; Dr. Barbara Blades, Dr Shashi Satyan, John Archer, David Cruickshank and Christine Cruickshank for their technical guidance and assistance.

This project was funded through a voluntary contribution from Hannay-Douglas Pty Ltd facilitated by HAL.

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NSW Department of Primary Industries provided on-going support and contributed significantly to the outcomes of this project.

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Appendix 1. Raw data – Eggs – Confirmation of target life stage

Experiment	**Eggs**				
Date	**6 January 2014**				
Sample #	insect life stage	Sample #	insect life stage	Sample #	insect life stage
1	Eggs	42	Eggs	83	Eggs
2	Eggs	43	Eggs	84	Eggs
3	Eggs	44	Eggs	85	Eggs
4	Eggs	45	Eggs	86	Eggs
5	Eggs	46	Eggs	87	Eggs
6	Eggs	47	Eggs	88	Eggs
7	Eggs	48	Eggs	89	Eggs
8	Eggs	49	Eggs	90	Eggs
9	Eggs	50	Eggs	91	Eggs
10	Eggs	51	Eggs	92	Eggs
11	Eggs	52	Eggs	93	Eggs
12	Eggs	53	Eggs	94	Eggs
13	Eggs	54	Eggs	95	Eggs
14	Eggs	55	Eggs	96	Eggs
15	Eggs	56	Eggs	97	Eggs
16	Eggs	57	Eggs	98	Eggs
17	Eggs	58	Eggs	99	Eggs
18	Eggs	59	Eggs	100	Eggs
19	Eggs	60	Eggs		
20	Eggs	61	Eggs		
21	Eggs	62	Eggs		
22	Eggs	63	Eggs		
23	Eggs	64	Eggs		
24	Eggs	65	Eggs		
25	Eggs	66	Eggs		
26	Eggs	67	Eggs		
27	Eggs	68	Eggs		
28	Eggs	69	Eggs		
29	Eggs	70	Eggs		
30	Eggs	71	Eggs		
31	Eggs	72	Eggs		
32	Eggs	73	Eggs		
33	Eggs	74	Eggs		
34	Eggs	75	Eggs		
35	Eggs	76	Eggs		
36	Eggs	77	Eggs		
37	Eggs	78	Eggs		
38	Eggs	79	Eggs		
39	Eggs	80	Eggs		
40	Eggs	81	Eggs		
41	Eggs	82	Eggs		

Appendix 2. Raw data - Second instar larvae – Confirmation of target life stage

Experiment	**Second instar larvae**				
Date	**15 January 2014**				
Sample #	insect life stage	Sample #	insect life stage	Sample #	insect life stage
1	2	42	2	83	2
2	1	43	2	84	2
3	2	44	2	85	2
4	2	45	2	86	2
5	2	46	2	87	2
6	2	47	2	88	2
7	2	48	2	89	2
8	2	49	2	90	2
9	1	50	2	91	2
10	2	51	2	92	2
11	2	52	2	93	2
12	2	53	2	94	2
13	2	54	2	95	2
14	2	55	2	96	2
15	2	56	2	97	2
16	2	57	2	98	2
17	2	58	2	99	2
18	2	59	2	100	2
19	2	60	2		
20	2	61	2		
21	2	62	2		
22	2	63	2		
23	2	64	2		
24	1	65	2		
25	2	66	2		
26	2	67	2		
27	2	68	2		
28	2	69	2		
29	2	70	2		
30	1	71	2		
31	2	72	2		
32	2	73	2		
33	1	74	2		
34	2	75	2		
35	2	76	2		
36	2	77	2		
37	2	78	2		
38	2	79	2		
39	2	80	2		
40	2	81	2		
41	2	82	2		

Appendix 3. Raw data – Third instar larvae – Confirmation of target life stage

Experiment	**Third instar larvae**				
Date	**20 January 2014**				
Sample #	insect life stage	Sample #	insect life stage	Sample #	insect life stage
1	3	42	3	83	3
2	3	43	3	84	3
3	3	44	3	85	3
4	3	45	3	86	3
5	3	46	3	87	3
6	3	47	3	88	3
7	3	48	3	89	3
8	3	49	3	90	3
9	3	50	3	91	3
10	3	51	3	92	3
11	3	52	3	93	3
12	3	53	3	94	3
13	3	54	3	95	3
14	3	55	3	96	3
15	3	56	3	97	3
16	3	57	3	98	3
17	3	58	3	99	3
18	3	59	3	100	3
19	3	60	3		
20	3	61	3		
21	3	62	3		
22	3	63	3		
23	3	64	3		
24	3	65	3		
25	3	66	3		
26	3	67	3		
27	3	68	3		
28	3	69	3		
29	3	70	3		
30	3	71	3		
31	3	72	3		
32	3	73	3		
33	3	74	3		
34	3	75	3		
35	3	76	3		
36	3	77	3		
37	3	78	3		
38	3	79	3		
39	3	80	3		
40	3	81	3		
41	3	82	3		

Appendix 4. Raw data – First instar larvae – Confirmation of target life stage

Experiment	**First instar larvae**				
Date	**12 March 2014**				
Sample #	insect life stage	Sample #	insect life stage	Sample #	insect life stage
1	1	42	1	83	1
2	1	43	1	84	1
3	1	44	1	85	1
4	1	45	1	86	1
5	1	46	1	87	1
6	1	47	1	88	1
7	1	48	1	89	1
8	1	49	1	90	1
9	1	50	1	91	1
10	1	51	1	92	1
11	1	52	1	93	1
12	1	53	1	94	1
13	1	54	1	95	1
14	1	55	1	96	1
15	1	56	1	97	1
16	1	57	1	98	1
17	1	58	1	99	1
18	1	59	1	100	1
19	1	60	1		
20	1	61	1		
21	1	62	1		
22	1	63	1		
23	1	64	1		
24	1	65	1		
25	1	66	1		
26	1	67	1		
27	1	68	1		
28	1	69	1		
29	1	70	1		
30	1	71	1		
31	1	72	1		
32	1	73	1		
33	1	74	1		
34	1	75	1		
35	1	76	1		
36	1	77	1		
37	1	78	1		
38	1	79	1		
39	1	80	1		
40	1	81	1		
41	1	82	1		

Appendix 5. Raw data – Eggs – Treatment survival counts

First-dip at EGG STAGE				
Infested on 6 January 2014				
Sieve #	1	2	3	Total
	#pupa	#pupa	#pupa	#pupa
Control 1 A (18 zucchinis)	2960	62	0	3022
Control 1 B (18 zucchinis)	8545	407	0	8952
Control 2 A (18 zucchinis)	9441	303	0	9744
Control 2 B (18 zucchinis)	14571	201	0	14772
Control 3 A (18 zucchinis)	4471	18	0	4489
Control 3 B (18 zucchinis)	10811	67	0	10878
Treatment 1 A (18 zucchinis)	0	0	0	0
Treatment 1 B (18 zucchinis)	0	0	0	0
Treatment 2 A (18 zucchinis)	0	0	0	0
Treatment 2 B (18 zucchinis)	0	0	0	0
Treatment 3 A (18 zucchinis)	0	0	0	0
Treatment 3 B (18 zucchinis)	0	0	0	0

Appendix 6. Raw data – Second instar larvae – Treatment survival counts

First-dip at SECOND INSTAR LARVAL STAGE				
Infested on 15 January 2014				
Sieve #	1	2*	BAG*	Total
	#pupa	#pupa	#pupa	#pupa
Control 1 A (18 zucchinis)	4662	-	3188	7850
Control 1 B (18 zucchinis)	2751	-	3188	5939
Control 2 A (18 zucchinis)	4477	-	3188	7665
Control 2 B (18 zucchinis)	2364	-	3188	5552
Control 3 A (18 zucchinis)	4118	-	3188	7306
Control 3 B (18 zucchinis)	3522	-	3188	6710
Treatment 1 A (18 zucchinis)	0	0	0	0
Treatment 1 B (18 zucchinis)	0	0	0	0
Treatment 2 A (18 zucchinis)	0	0	0	0
Treatment 2 B (18 zucchinis)	0	0	0	0
Treatment 3 A (18 zucchinis)	0	0	0	0
Treatment 3 B (18 zucchinis)	0	0	0	0

* Due to the very slurpy nature of the insect damaged zucchinis found in this experiment many larvae escaped from the larval rearing trays and moved into the fine mesh bag in which the 6 Control trays had been placed. Larvae remaining in the slurpy remains of the zucchinis were dead and we considered that they may have been killed by drowning and not necessarily by the fenthion dips. We decided to discard these dead larvae from the assessment of treatment efficacy. We collected all surviving pupae and counted them. We then allocated equal quantities to each tray.

Appendix 7. Raw data – Third instar larvae – Treatment survival counts

First-dip at THIRD INSTAR LARVAL STAGE				
Infested on 20 January 2014				
Sieve #	1	2	BAG*	Total
	#pupa	#pupa	#pupa	#pupa
Control 1 A (18 zucchinis)	3261	18	5290	8569
Control 1 B (18 zucchinis)	4350	43	5290	9683
Control 2 A (18 zucchinis)	2170	66	5290	7526
Control 2 B (18 zucchinis)	3511	56	5290	8857
Control 3 A (18 zucchinis)	4177	18	5290	9485
Control 3 B (18 zucchinis)	2956	22	5290	8268
Treatment 1 A (18 zucchinis)	0	0	0	0
Treatment 1 B (18 zucchinis)	0	0	0	0
Treatment 2 A (18 zucchinis)	0	0	0	0
Treatment 2 B (18 zucchinis)	0	0	0	0
Treatment 3 A (18 zucchinis)	0	0	0	0
Treatment 3 B (18 zucchinis)	0	0	0	0

* Due to the moist nature of the insect damaged zucchinis many larvae escaped from the larval rearing trays and moved into the fine mesh bag in which the 6 Control trays had been placed. And dead larvae found in the fruit were not assessed in this experiment because it was uncertain if they had been killed as a result of drowning in the slurpy remains of some (not all) of the zucchinis used in this experiment or as a result of the fenthion dips. We collected all pupae and counted them. We then allocated equal quantities to each tray.

Appendix 8. Raw data – First instar larvae – Treatment survival counts

First-dip at FIRST INSTAR LARVAL STAGE				
Infested on 12 March 2014				
Sieve #	1	2	3	Total
	#pupa	#pupa	#pupa	#pupa
Control 1 A (9 zucchinis)	14854	20	0	14874
Control 1 B (9 zucchinis)	5512	54	0	5566
Control 1 C (9 zucchinis)	7342	47	0	7389
Control 2 A (9 zucchinis)	5339	194	0	5533
Control 2 B (9 zucchinis)	4701	293	0	4994
Control 2 C (9 zucchinis)	5284	114	0	5485
Control 3 A (9 zucchinis)	3807	201	0	4008
Control 3 B (9 zucchinis)	4547	337	0	4884
Control 3 C (9 zucchinis)	2287	447	0	2734
Treatment 1 A (9 zucchinis)	0	0	0	0
Treatment 1 B (9 zucchinis)	0	0	0	0
Treatment 1 C (9 zucchinis)	0	0	0	0
Treatment 2 A (9 zucchinis)	0	0	0	0
Treatment 2 B (9 zucchinis)	0	0	0	0
Treatment 2 C (9 zucchinis)	0	0	0	0
Treatment 3 A (9 zucchinis)	0	0	0	0
Treatment 3 B (9 zucchinis)	0	0	0	0
Treatment 3 C (9 zucchinis)	0	0	0	0

Figure 10. Showing supplier of certified organic zucchinis