Project title:	Further development of earwig-safe spray programmes for apple and pear orchards
Project number:	TF 220
Project leader:	Dr Michelle Fountain, East Malling Research, New Road, East Malling, Kent, ME19 6BJ
Report:	Annual report, 2015
Previous report:	None
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Location of project:	East Malling Research, New Road, East Malling, Kent, ME19 6BJ
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Date project commenced:	01 April 2014
Date project completed (or expected completion date):	31 March 2017

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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Dr Michelle Fountain

Research Leader in Entomology

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Report authorised by:

Professor Peter Gregory

Chief Executive

East Malling Research

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5 March 2015

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CONTENTS

GROWER SUMMARY

Headline

- The results of this project are contributing to a growing evidence of the effects of several crop protection products on different growth stages of earwigs

Background and expected deliverables

Earwigs are important generalist predators in both apple and pear orchards. They play a key part in regulating populations of several highly damaging pests including woolly aphid and other aphid pests, mussel scale, codling moth and pear sucker. Recent laboratory tests and field experiments by EMR and experiments by other European scientists have indicated that several commonly used insecticides including thiacloprid (Calypso), indoxacarb (Steward), chlorpyrifos (various prouducts) and spinosad (Tracer) have harmful effects on earwigs and could be responsible for low populations in some orchards. However, growers need to be able to use products containing acetamaprid (Gazelle), thiacloprid (Calypso), abamectin (Agrimec) and spirodiclofen (Envidor) for control of aphids, mussel scale, weevils, capsids, pear sucker and sawfly (see Table 1 of the Science Section of this report).

This project will build on research carried out by EMR in AHDB Horticulture project TF 196, which showed that earwigs can be disrupted by routine crop protection programmes. It will test how to integrate key crop protection products into pest management programmes without causing harm to earwig populations in orchards and further investigate the sub-lethal effects (growth and reproduction) that these products have on nymph and adult earwigs in highly replicated laboratory trials.

Summary of the project and main conclusions

Twenty nymph, adult male and adult female earwigs were exposed to a dried insecticide residue on a leaf disk for 1 week in a laboratory test. The earwigs were then held in Petri dishes for at least 42 days post exposure and weighed weekly. The insecticides tested were acetamaprid (Gazelle), thiacloprid (Calypso), abamectin (Agrimec) and spirodiclofen (Envidor). All were compared to a water only control.

Earwig nymphs avoided feeding on leaves sprayed with Calypso, but Envidor appeared to stimulate adult earwig feeding. In addition, Calypso affected the growth of earwig nymphs and adult males. Nymphs were generally more sensitive to the effects of Calypso than adult earwigs. There was a high natural mortality of nymph earwigs in the laboratory; 40% died (water only treatments). Only 20% of adult males died and none of the females had died by the end of the experiment in the water only treatments.

Gazelle and Agrimec appeared to be relatively safe to earwig nymphs and adults. However, Calypso appears quite toxic in terms of behavioural effects and leads to eventual mortality and may be better replaced with Gazelle at key times in the earwigs' lifecycle in tree fruit.

The results of this project are contributing to a growing evidence of the effects of pesticides on different stages of earwigs, a key predator of tree fruit pests. By using initial laboratory screening tests we have ascertained some of the effects of insecticides on earwigs that would not be observable in the field.

Future research will test the products in apple and pear orchards in the early- and mid-growing season, and 1-2 applications. The results from the long term toxicity effects of the lab study (still ongoing) will be reported in the 2016 report.

Financial benefits

- The industry will be provided with independently obtained information on the relative safety of critical orchard insecticides on earwigs; important natural enemies of several damaging pests.
- Growers will be able to judge when best to use which insecticides for essential pest control tasks such as control of codling moth, aphids, mussel scale and pear sucker.
- There will be fewer problems with many important pests if earwig populations are allowed to thrive.

Action points for growers

- Growers should make considered choices of pesticide products based on the knowledge of important predators in the orchard at the time of spraying (see Table 4 in the Science Section of this report).
- Growers can consult agronomists to determine which products are safe to apply at key times of the earwig lifecycle.
- Gazelle may be a better control option for mussel scale, aphid, rhynchites and sawfly post blossom, when earwig nymphs start to enter the trees.

SCIENCE SECTION

Introduction

Earwigs (Dermaptera) are important predators of many pests of orchards including scale insects (Karsemeijer 1973; McLeod & Chant 1952), psyllids (Sauphanor *et al*., 1994), woolly apple aphid (Phillips, 1981; Ravensburg, 1981; Noppert *et al*,. 1987; Mueller *et al*., 1988; Solomon *et al*., 1999; Nicholas *et al*., 2005) and codling moth (Glen, 1977). Reports that earwigs are declining in some orchards (Gobin *et al*., 2008) has raised concern for this effective, natural, biocontrol agent. The earwig most commonly encountered in UK orchards is *Forficula auricularia* (Fitzgerald and Solomon, 1996; Solomon *et al*., 1999). A female *F. auricularia* lays 50 to 90 eggs in the spring (Fig. 1). She attends the first stage nymphs and then dies before midsummer. Third instar nymphs move into the tree canopy (Phillips, 1981) from May onwards and, after the fourth instar, emerge as adults (July-August) (Gobin *et al*., 2008). Earwigs are nocturnal and their numbers are often underestimated in orchards.

(a) (b)

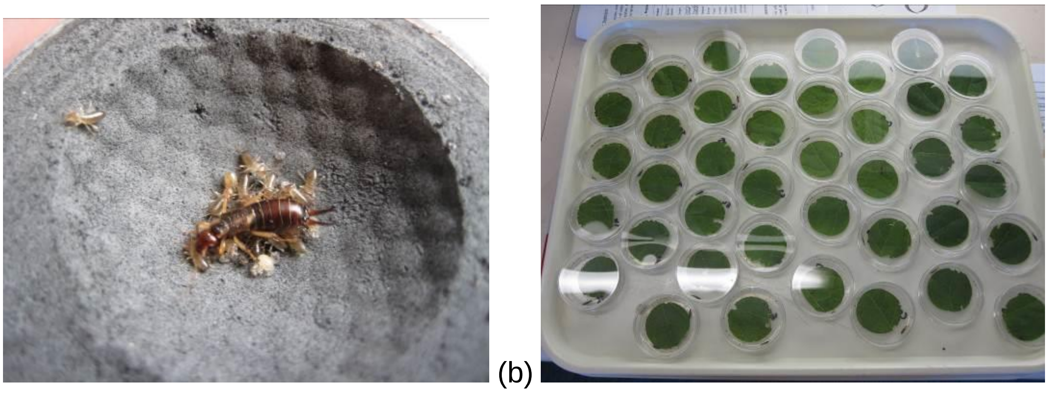

Figure 1. (a) Female earwig in artificial nest chamber with offspring, (b) leaf insecticide residue experiment

Insecticides applied between March and October are likely to have effects on earwig populations and even the slightest effects on behaviour may have consequences on populations for the rest of the year. Earwigs are exposed to spray residues whilst moving around and feeding at night in the tree canopy and on the ground (Ffrench-Constant and Vickerman, 1985). The data available for sensitivity of earwigs to many modern insecticides is building; however, growers need to apply potentially earwig harmful insecticides at certain times of the year to protect against pests such as aphids, weevils, capsids, pear sucker and sawfly. These include the neonicotinoids, acetamiprid (Gazelle) and thiacloprid (Calypso), and two products used to help manage pear sucker in the summer, abamectin (Agrimec) and spirodiclofen (Envidor). The vulnerability of the different earwig life stages to these products requires investigation in well replicated trials.

Laboratory experiments have screened adult earwigs at experimental doses of a few pesticides (Peusens & Gobin, 2008) and EMR/AHDB Horticulture project TF 196 has screened the most commonly used UK insecticides in laboratory trials (Table 1), but more research is needed on the timing of applications in real orchards and any sublethal effects of the few pesticides available for aphid, weevil, capsid, pear sucker and sawfly control.

The AHDB Horticulture project TF 196 made an excellent start in testing spray programmes on two farms, but no consideration was made to sprays of thiacloprid and abamectin. It is also not known whether acetamaprid (more water soluble than thiacloprid) would have less detrimental effects on earwigs. Evidence from studies of predatory mites suggests that these latter products differ in toxicity (Beers and Himmel 2002; Bostian *et al*. 2009).

Project aim:

To determine whether (if and when?) acetamiprid (Gazelle), thiaclorpid (Calypso), abamectin (Agrimec) and spirodiclofen (Envidor) can be used in earwig safe spray programmes on apple and pear.

Year 1 objective:

Determine the short-term and long-term sub-lethal effects of abamectin (Agrimec), acetamiprid (Gazelle), spirodiclofen (Envidor) and thiacloprid (Claypso) on feeding, development and longevity of different earwig life stages in the laboratory.

Materials and methods

Treatments

Insecticides were tested at the recommended field concentrations (Table 1). Unsprayed bean leaves grown on an allotment in West Malling, Kent were cut into discs sprayed with the pesticide, left to dry, and then placed on agar in a Petri dish. Treatments were made up to 1 litre with distilled water in graduated flasks.

Table 1. Treatments applied in the laboratory earwig toxicity trial

Trt Code	Product	Active ingredient	Mode of action	Chemical class
A	Agrimec	abamectin	chloride channel activator	Avermectin
G	Gazelle	acetamiprid	acetylcholine agonist (mimic)	Neonicotinoid
E	Envidor	spirodiclofen	lipid biosynthesis inhibition	Tetronic acid
Ca	Calypso	thiacloprid	binds to acetylcholine receptor	Neonicotinoid
U	Untreated control	-	-	-

Experimental design and statistical analyses

Earwigs collected from a minimal sprayed orchard at East Malling Research were tipped into one box for each life stage class and then selected at random to be placed into their individual Petri dishes. There were 20 replicates of each life stage for each of the five treatments. Individuals of adult males, adult females and L2-L3 instars were tested with each insecticide (three life stages x 20 replicates x five treatments = 300 Petri dishes). A control with distilled water was included (Table 1).

Treatment application

The standard tree PACE model, supported by CRD, was used to calculate the dose each leaf disk would receive. This calculated the amount of spray in litres that contacts the tree in theory. The standard model uses a tree height of 3 m and a row spacing of 3.5 m. Based on a spray volume of 300 l/ha it predicts a best case scenario of 80 l/ha of leaf (outer leaves upper surfaces unshielded), and a worst case of 40 l/ha of leaf (inner leaves/and undersides well shielded). Because this was a single sided application, the mean of these two extremes was used (60 l/ha of leaf).

Treatments were applied to bean leaf discs (4 cm diameter) using a Burkard computer controlled laboratory spraying apparatus (EMR Standard Operating Procedure 767). Treated leaf disks were placed onto a 1 % agar layer in a 5 cm Petri dish after spraying and allowed to dry. The individual earwigs were added (picking up with soft forceps) and kept at 20°C for seven days (exposure time). Trays were inserted into polythene bags to prevent rapid moisture loss. During this period the earwigs fed on the bean leaves. After seven days the earwigs were transferred to clean 5 cm Petri dishes with water and food (dried Iams cat food).

Assessments

Short-term toxicity

Earwigs were collected from the field and kept with abundant food (Iams dried cat food) and water for two days prior to testing to ensure they were in good health.

The L2-L3 earwigs were weighed on 20 May and the test began on 22 May. The nymphs were moved to a clean Petri dish on 29 May and fed dried cat food and provided with water. The test was maintained at 20°C and ended on 3 July, 42 days after the initial exposure. Live earwigs were weighed every seven days.

The adult male and female earwig test began on 29 September. Adults were exposed to insecticide for one week and were then transferred to clean living conditions, as the nymphs had been. Earwigs were weighed weekly after exposure to the insecticides which ceased 28 days after the beginning of the test (27 October).

On each assessment earwigs were scored as:

a) Healthy living earwig;
b) Affected (abnormal behaviour, convulsive movements, lethargy etc.);
c) Moribund (very little movement, unable to stand after turning over);
d) Dead.

Feeding inhibition

This was assessed by scanning the bean leaf disk after the earwigs had been housed with them for seven days. The scanned images were converted to bitmap image files. These image files were cropped and cut to create a single image file for each leaf disc. Each file was exactly 200 x 200 pixels (Fig. 2). Control discs (freshly cut, not eaten) were used for calibration. Each disk was 3.9 cm in diameter (11.94 cm^2) equating to 28963 pixels. Using Scion Image (http://scion-image.software.informer.com/) the images were analysed and the percentage and area of each disk that was eaten was calculated.

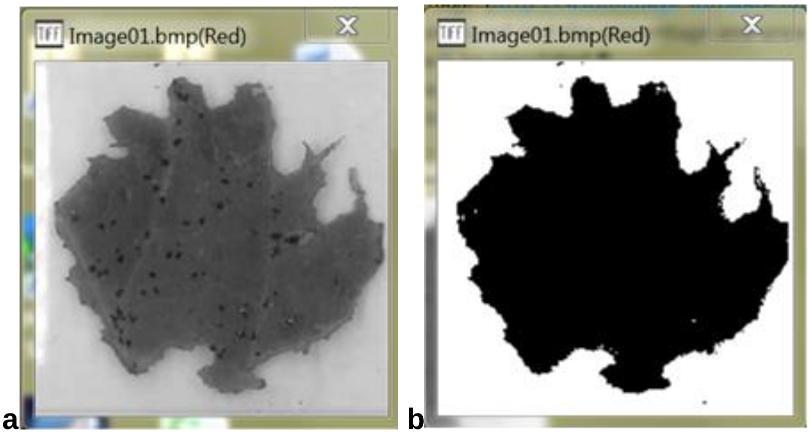

Figure 2. Example of leaf disk scan after feeding by earwig and conversion to black and white for pixel counting

Sub-lethal/ long-term toxicity

The effects of insecticides on reproduction in the adult female test earwigs that survived the short-term exposure experiment were investigated. Earwig 'couples' (males and females) were paired up from the same treatment (short-term toxicity test). Pairings were housed in clear ventilated Petri dishes with food and water. Mating in most boxes occurred within 10 minutes of introduction (Fig. 3). Food was supplemented with vegetable matter in some weeks, e.g. cabbage, carrot, apple, etc. On 3 November all pairs of earwigs were housed in Perspex boxes with a nest chamber in a 6°C room under constant darkness to mimic winter conditions. On 21 January 2015 all boxes were removed from the cold store, cleaned and earwigs refreshed with water and dried cat food (+ other vegetable matter) and placed in front of a window (natural daylight) at room temperature. Males and females in some of the replicates were observed to mate again. The numbers of eggs and successfully hatching eggs were recorded. Males were removed once eggs were laid to prevent cannibalism.

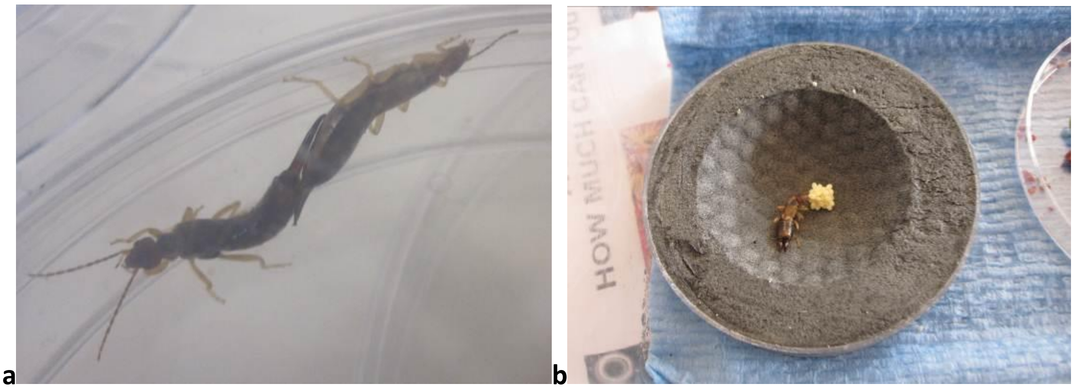

Figure 3. (a) Male and female earwig mating; (b) Female earwig in nest chamber with eggs (lid removed)

Results

Nymph short-term toxicity test

Two types of analyses were carried out:

(1) Snapshot analysesanalysed the health of the nymphs at each date separately, as affected by treatments. For the first two dates earwigs were classified as 'Healthy', 'Affected', 'Moribund' or 'Dead', whereas on all other dates the 'Healthy' or 'Dead' classifications were used. The snapshot analyses allowed all four categories to be included. Counts in each category were analysed using GLM's (generalized linear model) with a Poisson distribution and a log link, fitting the terms Treatment, followed by Health. This enabled the significance of the Treatment x Health interaction to be quantified. If this interaction is significant it implies that the percentage of earwigs in each of the four (or two) health categories differs between treatments. A pairwise comparison between individual pairs of treatments was then carried out to check for individual treatment differences.

(2) Overall analysis analysed of the number of insects that died before the first date, then in each time interval, and the number that "died" after the last time point (i.e. which survived). The counts for the overall analyses were further divided into pre-final date and the final date. For the first two dates, categories Healthy, Affected and Moribund were pooled into the Healthy category, so there were only two categories, Healthy or Dead. The analysis was carried out omitting the numbers that survived (i.e. that were still alive after the last date), which is investigating the mortality rate profile over the experiment, but is not affected by the number surviving. A further analysis was carried out on the number of survivors (GLM with a Binomial distribution and a logit link, with n = 20, the number of earwigs given each treatment).

There was evidence of an overall significant Treatment x Health category interaction at all dates ($p<0.01$). Pairwise Treatment x Health category tests showed that Calypso (thiacloprid) had a significantly different profile from any of the other treatments ($p<0.01$). Gazelle, Envidor, Agrimec and the water only treated control did not differ from each other (Fig. 4).

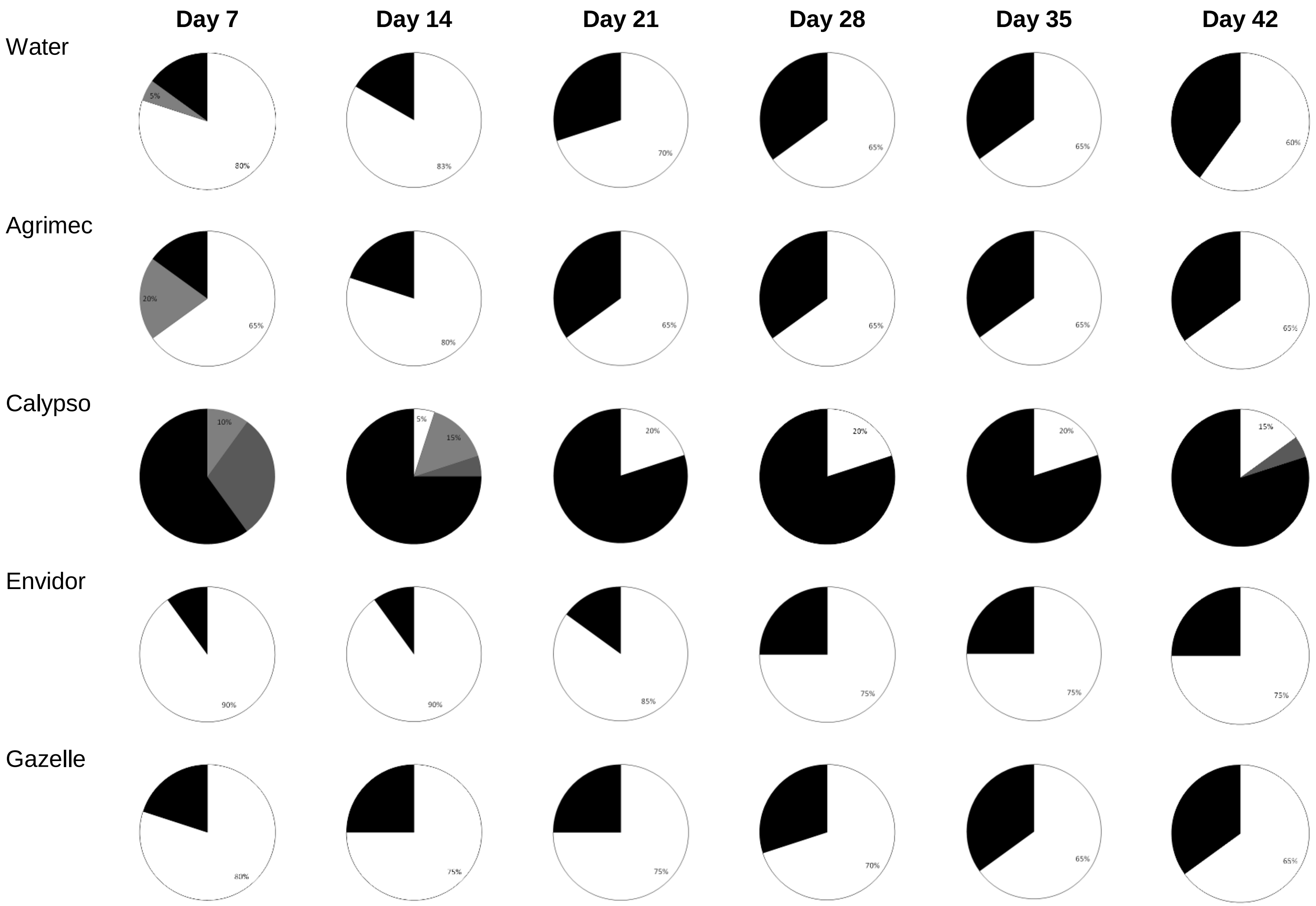

Figure 4. Assessments of earwig nymph health from beginning of test

There may have been some behavioural effects of Agrimec (abamectin) on a few individuals during the first seven days whilst on the leaf disk, but these effects disappeared once the earwigs were moved to clean Petri dishes (Fig. 4) and were not significant.

Analysis, excluding the numbers surviving beyond the last date, showed no evidence of differences between any of the treatments, implying that the time course is the same for all treatments, although the total mortality may differ between treatments. Analysis of the number of survivors (alive on the last date) showed significant Treatment differences ($p<0.01$). Pairwise treatment analyses showed that, again, Calypso had significantly higher mortality from all other treatments, but there were no differences between the other treatments, including the water only control.

Calypso induced behavioural effects on earwig nymphs during exposure and then up to one week after removal to a clean Petri dish. Many of the earwig nymphs effected did not recover, so that only 20% (compared to water only, 60%) of individuals had survived by the end of the test (Fig. 4). It was noted that the mortality of nymph earwigs was high compared to adults (see below) in water only controls. Hence, around 40% earwig nymph mortality in the field may be normal.

The earwig nymph growth data was log-transformed to stabilise the variance. Any nymph that died during the time course was excluded from this analysis. As a result there were many missing values, leaving only 57 nymphs. A repeated measures analysis was carried out using REML (restricted maximum likelihood). This assumes that dates are equally spaced, although 19 June measurements were omitted.

There was a significant Treatment x Date interaction ($p<0.001$), but no evidence of an interaction between the group Agrimec vs. Envidor vs. Gazelle x Date. All other pairwise combinations of treatments had a significant interaction with Date ($p<0.001$) i.e. the growth of the earwig nymphs was significantly slowed by exposure to Calypso (Fig. 5, Table 2). The mean end weight of these earwigs (0.023 g) was almost half the weight of earwigs in the water only treatment (0.041 g).

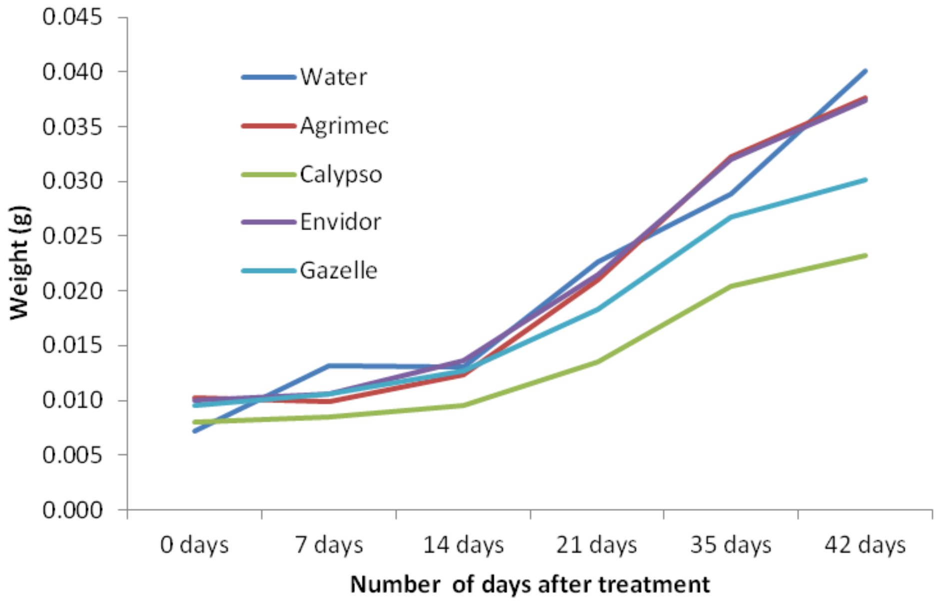

Figure 5. The mean weights (growth) of nymph earwigs after exposure to insecticides

In addition, the majority of the earwigs in the water control reached adulthood (Day 35) before the earwigs in all of the insecticide treatments (Table 2). Most of the earwigs in the Agrimec, Envidor and Gazelle treatments were adult by Day 42, but only one earwig had reached adulthood in the Calypso treatment by the end of the test.

Table 2. Mean nymph stage of earwigs for each treatment during the course of the tes days). Also numbers of males and females that resulted by the end of the test days)

Treatment	0 d	7 d	14 d	21 d	28 d	35 d	42 d	No males	No females
Water	2	2	3	4	4	Adult	Adult	3	5
Agrimec	2	3	3	4	4	4	Adult	3	6
Calypso	2	2	3	4	4	4	4	0	1
Envidor	2	3	3	4	4	4	Adult	4	11
Gazelle	3	3	3	4	4	4	Adult	5	6

Adult short-term toxicity test

There was no evidence of gender differences (Figs. 6 and 7, males and females respectively) in any form on behaviour, however, there was evidence of an overall significant Treatment x Health category interaction at all dates, especially on the first four dates ($p<0.001$). Pairwise Treatment x Health category tests showed that Calypso had a significantly different profile from any of the other treatments ($p<0.05$); which did not differ from each other (Figs. 8 and 9). Calypso caused behavioural changes in the male and female earwigs from the beginning of exposure.

Overall analysis (Healthy or Dead), excluding the numbers surviving beyond the last date, showed no evidence of differences between any of the treatments, implying that the time course is the same for all treatments. There was no evidence of any significant Gender or Treatment differences.

Adults appear to be less susceptible to Calypso than nymphs for mortality, but are equally affected in their behaviour, rendering them susceptible to other perturbations (e.g. predation).

For the growth measurements the data was log-transformed to stabilise the variance. Any adult that died during the course of the experiment was excluded from the analysis; resulting in 151 adults. A repeated measures analysis was carried out using REML, assuming that dates are equally spaced.

There was a significant Treatment x Gender x Date interaction, so the two sexes were analysed separately using AREPMEASURES.

For the male earwigs there was evidence of a Treatment x Date interaction ($p<0.001$). Pairwise Treatment x Date interactions were tested and the treatments appeared to divide into three groups (Water, Gazelle, and (Agrimec, Calypso, Envidor)). For Agrimec, Calypso and Envidor there was no evidence of interaction with Date, although Calypso vs. Envidor x Date was almost significant ($p=0.066$). All (Water vs Treatment) x Date interactions were significant, as were all (Gazelle vs Treatment) x Date interactions. The exception to this was that the (Calypso vs Water) x Date interaction was almost significant ($p=0.085$, Fig. 8).
For the females there was no evidence of a Treatment x Date interaction ($p=0.179$), or of overall treatment differences ($p=0.081$). There was a highly significant Date effect ($p<0.001$, Fig. 9).

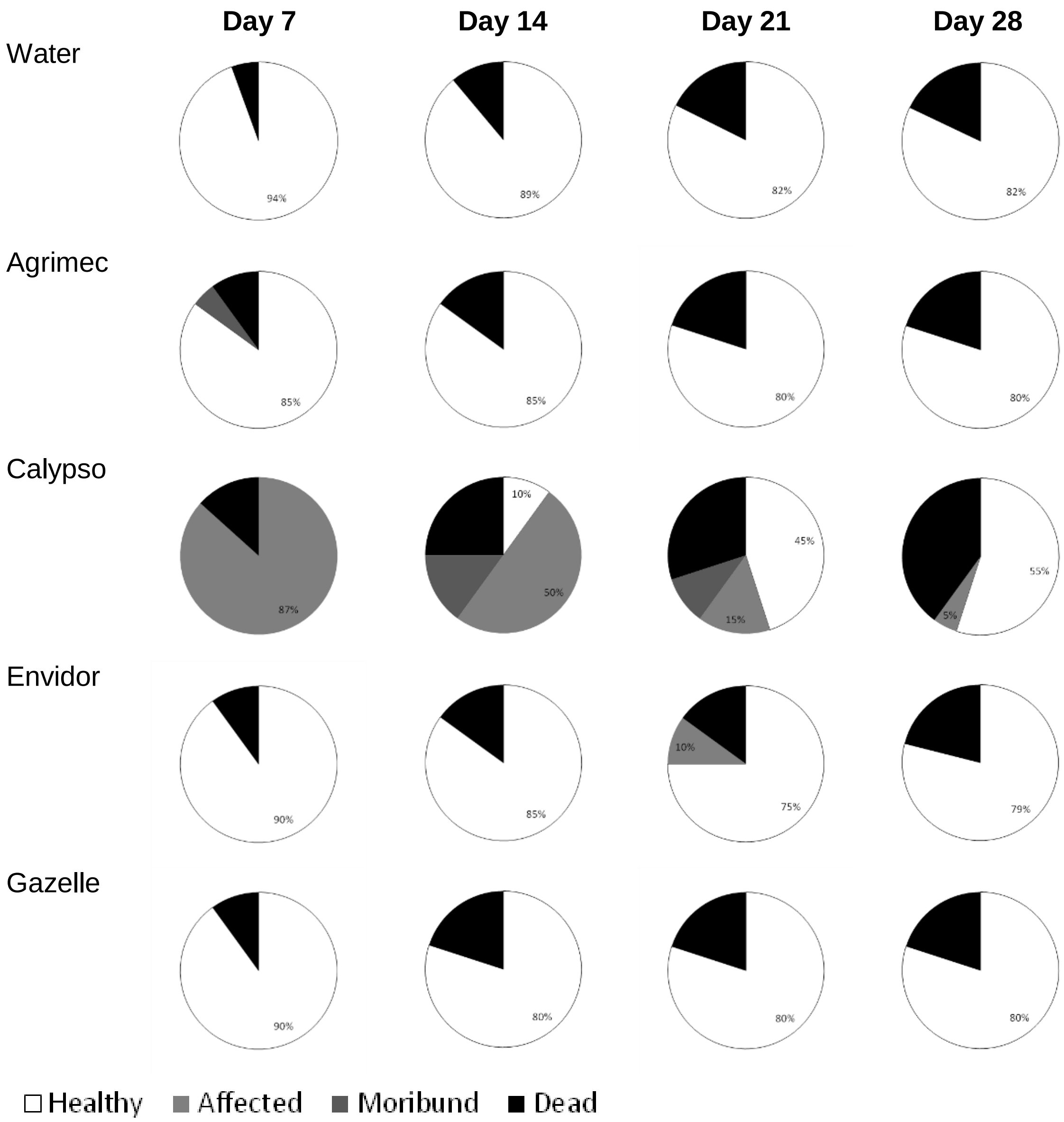

Figure 6. Assessments of adult male earwig health from beginning of test

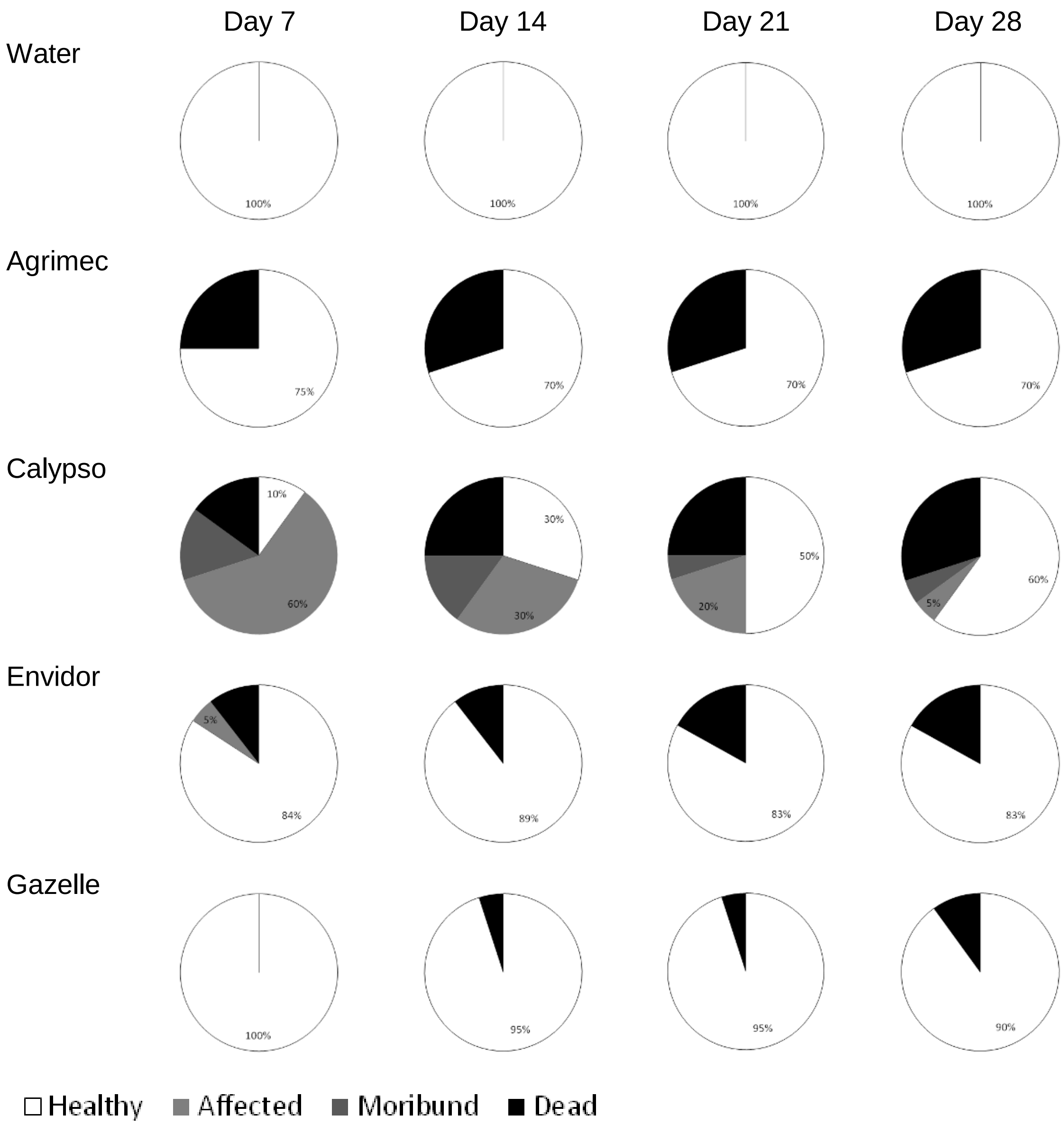

Figure 7. Assessments of adult female earwig health from beginning of test

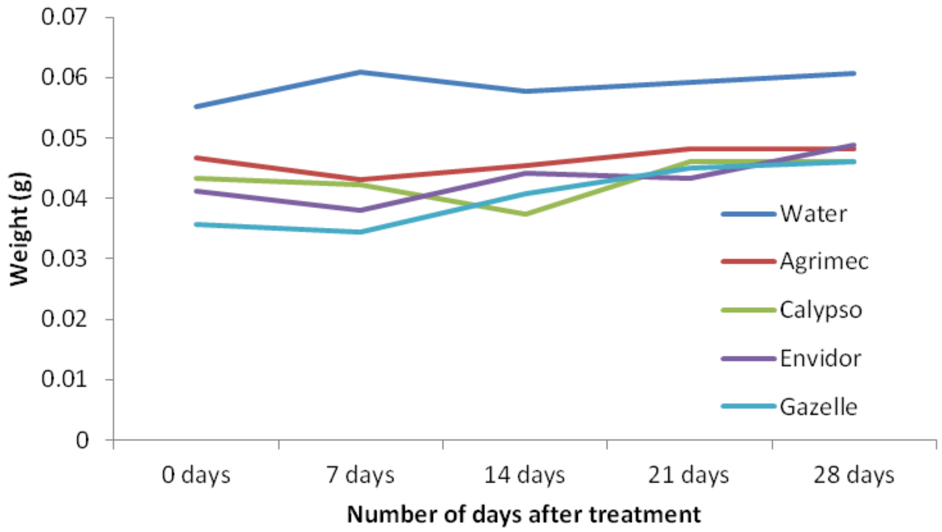

Figure 8. The mean weights (growth) of adult male earwigs after exposure to insecticides

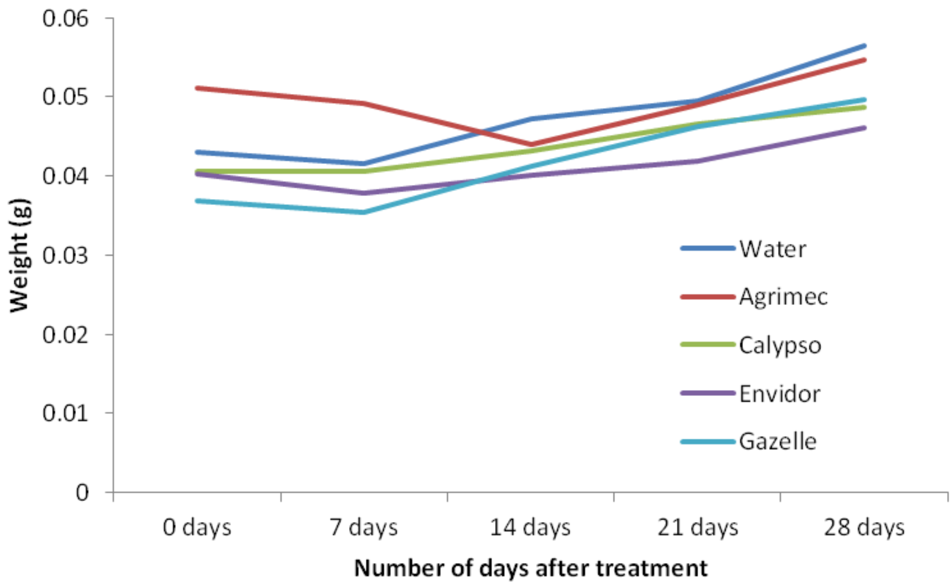

Figure 9. The mean weights (growth) of adult female earwigs after exposure to insecticides

Feeding inhibition

The amount of sprayed leaf eaten by earwig nymphs was significantly lower if sprayed with Calypso compared to all other treatments including the water only control (Fig. 10. ANOVA square root transformed data $p<0.001$, sed = 0.5523, lsd = 1.1013).

In contrast, male and female earwigs consumed more leaf if treated with spirodiclofen compared to all other treatments including the water only control (Figs. 11 and 12. ANOVA square root transformed data, male; $p = 0.001$, sed = 0.314, lsd = 0.6243, female; $p = 0.004$, sed = 0.3228, lsd = 0.642).

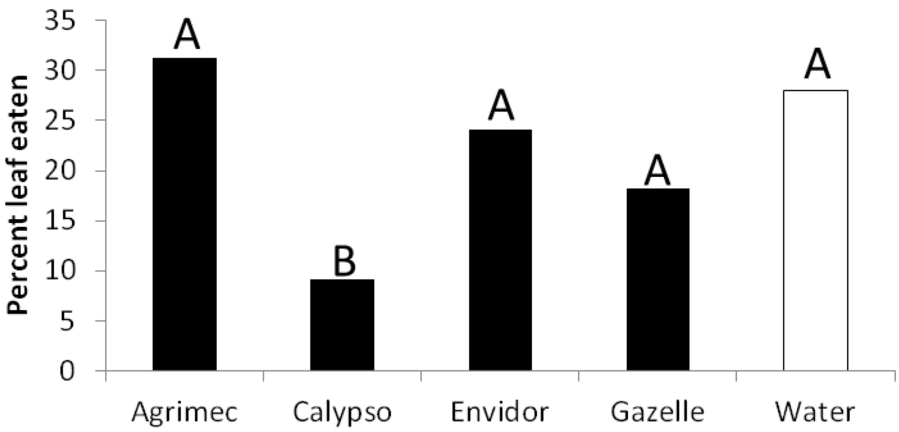

Figure 10. Mean percentage of leaf eaten by earwig nymphs

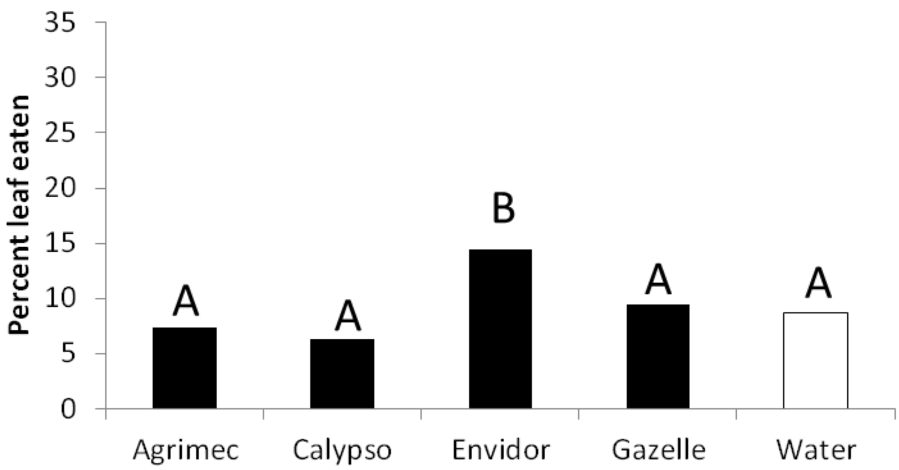

Figure 11. Mean percentage of leaf eaten by adult male earwigs

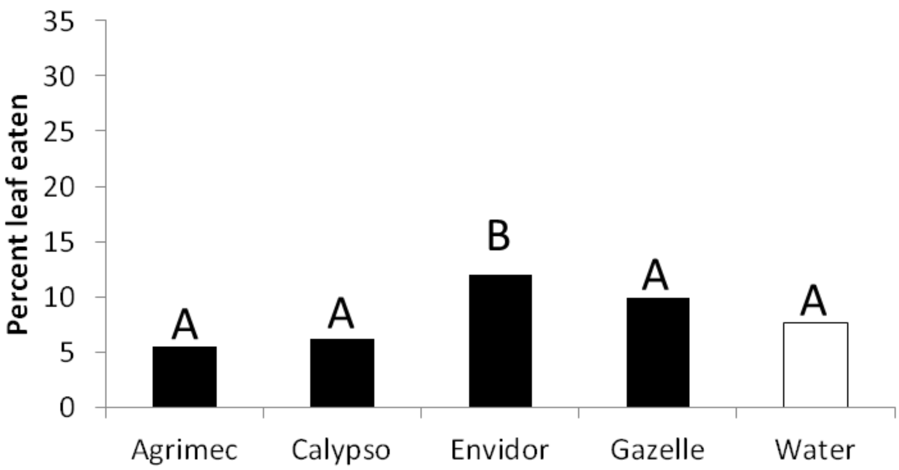

Figure 12. Mean percentage of leaf eaten by adult female earwig

Long-term reproduction test

The numbers of surviving adults that were paired up from the short term toxicity tests is shown in Table 3. At the time of writing the data for the long term reproduction tests (numbers of eggs, date eggs laid, numbers of nymphs) are still underway and will be reported in the 2016 report.

Table 3. The numbers of mated females overwintered and bought into spring conditions

Treatment	Pair	Lone mated female
Water	12	0
Agrimec	9	0
Calypso	8	1
Envidor	7	4
Gazelle	12	1

Discussion and Conclusions

Earwig nymphs avoided feeding on leaves sprayed with Calypso, but Envidor appeared to stimulate adult earwig feeding. In addition, Calypso affected the growth of earwig nymphs and male adults. Nymphs were generally more sensitive to the effects of Calypso than adult earwigs. There was a high natural mortality of nymph earwigs in the laboratory; 40% died (water only treatments). Only 20% of adult males died and none of the females had died by the end of the experiment in the water only treatments.

Gazelle and Agrimec appeared to be relatively safe to earwig nymphs and adults. However, Calypso appears quite toxic in terms of behavioural effects and eventual mortality and may be better replaced with Gazelle at key times in the earwigs' lifecycle in tree fruit.

The results of this project are contributing to a growing evidence of the effects of pesticides on different stages of earwigs, a key predator of tree fruit pests. By using initial laboratory screening tests we have ascertained some of the effects of insecticides on earwigs that would not be observable in the field.

Future research will test the products in apple and pear orchards in the early- and mid-growing season, and 1-2 applications. The results from the long term toxicity effects of the lab study (still ongoing) will be reported in the 2016 report.

Table 4. Summary of data from this project and data published by other researchers on the safety of active ingredients to earwigs

a.i.	Data from this project	Other researchers	Reference*
abamectin	Safe	Harmful	1
acetamiprid	Safe	-	
Bacillus thuringiensis	-	Safe	9
chlorantraniliprole	Safe	Safe to adults	10,12
chlorpyrifos	Harmful	Harmful	1,2
cypermethrin	-	Harmful (nymphs), knockdown	1,8
deltamethrin	-	Harmful, knockdown	1,4,7,8
diflubenzuron	-	Harmful	9,11
dimethoate	-	Harmful	1,8
flonicamid	Safe (lab) harmful (nymphs, field)	Safe, harmful	1,3,5
indoxacarb	Harmful (males), knockdown	Harmful, knockdown	1,3,4,5,10
methoxyfenozide	Harmful to nymphs	Safe to adults	4, 10
pirimicarb	-	Safe	1,8
potassium bicarbonate	-	Safe	12
spinosad	Harmful, knockdown	Harmful	1,2,3,5,6, 10
spirodiclofen	Safe	-	
thiacloprid	Harmful	Harmful	1,3,5,10

*1 Peusens and Gobin 2008; 2 Cisneros *et al*. 2002; 3 Vogt *et al*. 2010; 4 Peusens *et al*. 2010; 5 Vogt *et al*. 2009; 6 Peusens *et al* 2009; 7 Colvin and Cranshaw 2010; 8 Ffrench-Constant and Vickerman 1985; 9 Maher *et al*. 2006; 9 Sauphanor *et al*. 1993; 10 Shaw and Wallis 2010, 11 Ravensberg 1981, 12 Beliën 2012

Knowledge and Technology Transfer

9 April 2014 Michelle Fountain and Jerry Cross - Conservation of the common earwig, *Forficula auricularia*, in orchards. University of Reading Seminar

24 April 2014 Michelle Fountain and Adrian Harris - Further development of earwig-safe spray programmes for apple and pear orchards, AHDB Horticulture Tree Fruit day

8 May 2014 Michelle Fountain - Pests, Predators and Pollinators, Warwick

25 September 2014 Michelle Fountain - Pests, Predators and Pollinators, Ornamental Nursery Group, EMR

20 November 2014 Michelle Fountain, Adrian Harris - Conservation of the common earwig, *Forficula auricularia*, in orchards. AAB conference

5 February 2015 Michelle Fountain, Northern Ireland Apple Growers Association – Pollination, Pest Control and Blastobasis in Orchards

11 February 2015 Michelle Fountain Cider Growers Association – Pollination and Pest Control in Orchards

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