Studentship Project: Annual Progress Report
Sept/2022 to Sept/2023

Student Name:	Hayden Tempest	AHDB Project Number:	C-300147
Project Title:	Radio tagging earwigs to understand the breakdown in successful woolly apple aphid, *Eriosoma lanigerum* (Hausmann), control		
Lead Partner:	Harper Adams University		
Supervisor:	Tom Pope, Michelle Fountain, Ed Harris		
Start Date:	28.09.2020	End Date:	28.09.2024

1. Project aims and objectives

The woolly apple aphid (*Eriosoma lanigerum*, Hausmann) (WAA) is a colony forming aphid species which infests apple trees worldwide. WAA attacks the woody tissue of apple trees and causes galling in the roots, trunk, and branches. These galls interfere with the xylem and phloem of the tree causing nutrient cycling deficiencies and reducing overall growth. As the use of broad-spectrum insecticides has become more tightly regulated (and consequentially declined), WAA has become an increasing issue for apple growers. In the UK, the chemical insecticide Batavia (Bayer Crop Science Limited; active ingredient, spirotetramat) is currently the only chemical control option available to growers. While most growers consider Batavia to be effective, it is also expensive and must be applied while sap is flowing. The promotion of generalist predators in apple orchards could reduce the need for chemical insecticides and improve economic returns for growers.

Images of the common European earwig (Forficula auricularia) and the woolly apple aphid (Eriosoma lanigerum)

The common European earwig (*Forficula auricularia*, Linnaeus) has been studied as a natural enemy of WAA that might have the potential to provide effective biocontrol. Previous studies have shown that with high population densities of earwigs, usually achieved through augmentative release programs, WAA populations can be kept to acceptable levels. However, there have been a few studies that have found a failure of earwigs to provide effective control of WAA. Importantly, apple orchards often contain earwigs naturally, but their distribution is patchy and poorly explained. If it can be understood why some orchards contain naturally high numbers of earwigs it may help more growers benefit from earwig predation of aphids. The key aim of the last two years of work has been to identify environmental and management factors which contribute to high earwigs populations in orchards, and test if this provides control of WAA. Most importantly, this has been investigated in orchards under organic or conventional management. Additionally, molecular gut content analysis has been used to determine the proportion of earwigs feeding on woolly apple aphid in commercial growing conditions, and possibly identify factors which might reduce or enhance earwigs' consumption of woolly apple aphid.

Another goal of the project is to assess the viability of radio frequency identification (RFID) tagging as a means of gathering information on earwig behaviour, such as foraging. RFID (as applied in ecology) is a method of remote sensing involving short range radio transmissions from a tag attached to an animal, which produces a unique identity code and timestamp upon coming into range and being detected by an RFID antennae. RFID is used in entomology because the tags are one of the smallest and lightest ways of remotely tracking an animal, and thus RFID is one of the only methods of its kind applicable to small insects. Remote monitoring of earwigs is desirable because they are nocturnal insects, rarely fly, are positively thigmotactic so seek small shelters and crevices, and are (somewhat) cryptic. All these traits make them difficult for humans to find and study in the field, and even in the laboratory it can be difficult to gather information on them while they would most naturally be active (night-time).

2. Key messages emerging from the project

Determining the impact of earwigs on WAA *in the field*

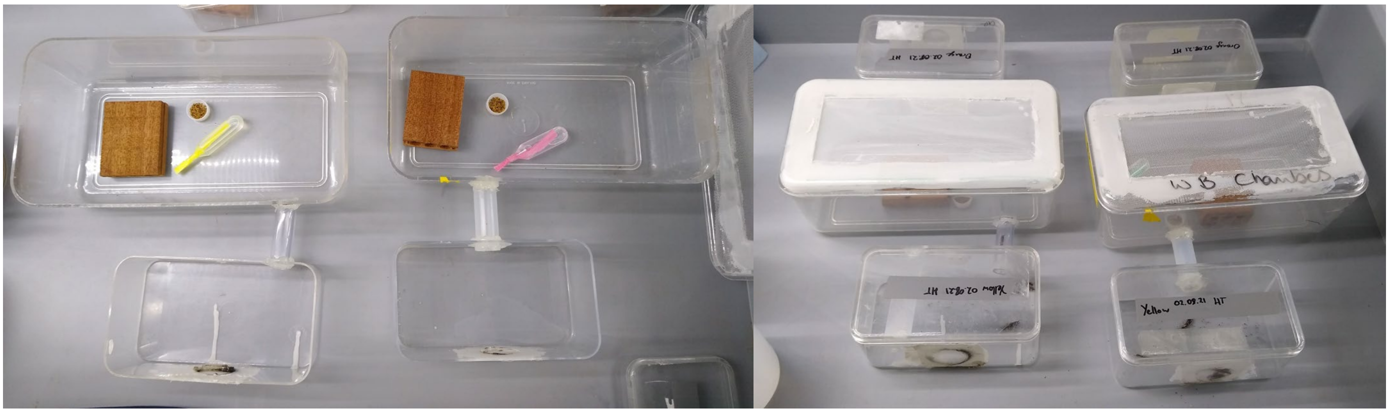
Fieldwork in the first year highlighted that in trees with or without artificial earwig refuges, earwig refuges increased the number of earwigs foraging on trees but this did not result in a reduced number of WAA colonies. Earwigs may provide valuable control without completely eliminating WAA from a given tree, so data collected in the first year which focused on numbers of colonies rather than number of aphids per colony may have missed any reduction in colony sizes. Nevertheless, the data showed no evidence for earwig control of WAA. Data from surveys in the second year found more evidence of biocontrol. There was a significant negative interaction between the presence of earwigs and the presence of WAA. However, the negative interaction effect occurred only in the conventionally sprayed orchards in the study. On the organic farm there was no relationship, suggesting a lack of control.

WAA DNA was detected in the guts of earwigs on 7 of 77 trees, using a WAA specific primer, which provided more evidence for earwigs predating on WAA. This rate of DNA detection may underrepresent the actual level of earwig consumption, work this year has established an estimate for the half-life of detectability for WAA DNA in earwig guts, and from this it has become clear that earwigs should be collected early in the morning to minimize the chance of DNA decay.

Overall, earwig populations did not appear to respond strongly to any of the factors measured during the season. They were unaffected by the presence or abundance of moss or algae as alternative food sources and showed no clear preference for older or younger orchards. There was a slightly lower chance of finding earwigs in the orchards of the single organic farm surveyed, which was unexpected given previous research. However, given only one organic grower was surveyed, this result may not apply more broadly to organic management.

Practicability of RFID for remote monitoring of earwigs

RFID may be practical for the study of earwig behaviour in laboratory studies, provided experimental arenas can be designed with the limitations of RFID antennae in mind, and a methodology can be developed for tagging earwigs which does not substantially alter their health and behaviour. As a result of preliminary work an experimental arena design has been developed, which can be used to detect earwig movement in response to changes in light levels. RFID technology is poorly suited to monitoring earwigs in the field and functions best on colony forming species, or insects with clear focal points in their daily routines. Earwigs are generalists with a broad diet so the choice of food, and by extension the location of feeding, is unpredictable. While earwigs show a tendency to aggregate when finding shelter during the day, this a) does not lead to aggregations similar to colony forming species in size (eusocial bees being the best known example of RFID technology applied to insects), and b) does not mean earwig individuals will select the same shelter/aggregation on a consistent basis. Because of these two facts, any shelter chosen for the housing of an RFID antennae will be in 'competition' with other shelters to be selected as a refuge by tagged earwigs individuals. Given that both RFID antennae and tags are expensive, it was not financially viable both to provide enough RFID-antennae-enabled shelters, or to release enough tagged earwigs such that an appropriate proportion of tagged individuals will be redetected consistently.



Images of prototype experimental arenas designed to capture information from RFID tagged earwigs. RFID antennae can be placed around the tubes which connect the two plastic boxes, to detect earwigs travelling from one box to the other.

Development of RFID tagging methodology

Preliminary work on adhesives for tagging suggested that Araldite epoxy was the best option for attaching RFID tags to earwigs. Cyanoacrylate glues were toxic to earwigs, while many other non-toxic glues are too weak for long-lasting tagging. The long-term acute and chronic effects of the glues needs to be confirmed using larger sample sizes, and this work is planned for the final year of the PhD.

3. Summary of results from the reporting year

During the 2023 field season 20 orchards belonging to 10 different growers were surveyed for earwigs and WAA abundance, using similar methods to last year. This expanded sample size included a greater number of organic orchards to try and better identify if organic management effects earwigs and WAA interactions. A number of facets of organic management could impact earwig populations, such as pesticide use, nutrient additions, mulch use, *etc.*. *Dysaphis plantaginea* (Passerini), rosy apple aphid, was more common this year, and can therefore be included in analysis for any interaction effect with earwig predation of WAA. Ground cover assessments have been carried out, which may provide a better understanding of earwig habitat preferences. In addition, assessments of the grass-alley vegetation were taken and pitfall traps have been installed in the orchards to see if earwigs are foraging on the ground rather than in the trees of some orchards. The data from 2023 remains to be analysed.
WAA DNA was detectible from field collected earwig guts, though only in a small number of samples. The half-life of detectability for the consumption of a single aphid was estimated to be between 9-10 hours, meaning data from last year may underrepresent the true level of predation. This year earwigs have been collected for sampling early in the morning, to try and reduce the impact of DNA digestion on detection.

4. Key issues to be addressed in the next year

Analyse the field data collected 2023.
Perform dissections, extractions, and PCR analysis of earwigs collected from 20 orchards in 2023 for molecular gut content analysis.
Expand the replicates on adhesives for use with RFID tags.
Revisit and update literature review on earwigs as biological controls of WAA.

Begin to draft Thesis Chapters.
Complete industrial placement.

5. Outputs relating to the project

(events, press articles, conference posters or presentations, scientific papers):

Output	Detail
Harper Adams PhD colloquium	1 Page abstract: Project introduction 30.11.22
AHDB Crops PhD Conference	3 minute presentation: Project introduction and immobilising earwigs for RFID tagging. 25.01.21 - 27.01.21
Harper Adams research conference	Poster: Earwig foraging and RFID. 07.09.21
AHDB Tree fruit technical day	10 minute presentation: Artificial refuges and earwig control of WAA. 24.02.22
Hereford Cidermakers UK orchard walk	Poster: earwigs and woolly apple aphid - artificial refuges and insecticides. 05.07.22 - 06.07.22
International conference of entomology Helsinki	Poster: earwigs and woolly apple aphid - artificial refuges and insecticides. 18.07.22 - 22.07.22
SCI visit	5 minute presentation: Artificial refuges and insecticides impact on earwigs and WAA. 23.09.22
HARUG! meeting	30 minute presentation: Using mixed effects in generalized linear models. 16.11.22
NIAB PhD meeting	1 hour presentation: Using mixed effects in generalized linear models. 23.11.22

6. Partners (if applicable)

Scientific partners	Harper Adams University, NIAB
Industry partners	World Wide Fruit, The National Association of Cider Makers
Government sponsor	