Studentship Project: Annual Progress Report 28/09/2021 to 28/09/2022

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Project Title:	**Radio tagging earwigs to understand the breakdown in successful woolly apple aphid, *Eriosoma lanigerum* (Hausmann), control**		
Lead Partner:	NIAB EMR		
Supervisor:	Bethan Shaw, Tom Pope, Ed Harris, Michelle Fountain		
Start Date:	28.09.2020	**End Date:**	28.09.2024

1. Project aims and objectives

The woolly apple aphid (*Eriosoma lanigerum*, Hausmann) is a colonial aphid species which infests apple trees worldwide. *Eriosoma lanigerum* 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), *E. lanigerum* has become an increasing issue for apple growers. In the UK, the chemical insectide Batavia (Bayer crop science limited) (active ingredient spirotetramat) is currently the only method of chemical control. While most growers consider Batavia effective, it is also expensive and needs to a timely application while sap is flowing in the trees. The promotion of generalist predators in apple orchards could reduce the need for chemical insecticides and improve economic returns for growers.
The common European earwig (*Forficula auricularia*, Linnaeus) has been studied as a natural enemy of *E. lanigerum* that might have the potential to provide effective biocontrol. Previous studies have shown that with high population densities of *F. auricularia*, usually achieved through augmentative release programs, *E. lanigerum* populations can be kept to acceptable levels. However, there have been a few studies that have found a failure of *F. auricularia* to provide effective control of *E. lanigerum*. Importantly, apple orchards often contain *F. auricularia* naturally, but their distribution is patchy and poorly explained. If it can be understood why some orchards contain high numbers of *F. auricularia* naturally it may help other growers benefit from *F. auricularia* too.
Another goal of the project is to assess the viability of radio frequency identification (RFID) tagging as a means of gathering information *F. auricularia*'s foraging and behaviour. 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 *F. auricularia* is desirable because they are nocturnal insects, do not fly, are positively thigmotatic so seek small shelters and crevices, and (somewhat) cryptic. All of these traits make

them difficult for humans to find and study in the field, and even in the lab it can be difficult to gather information on them while they would most naturally be active (nightime).

2. Key messages emerging from the project

Determining the impact of *F. auricularia* on *E. lanigerum* in the field: Fieldwork in first and second years has highlighted the importance of taking the correct measurements. *Forficula auricularia* may provide valuable control for example, without completely wiping out *E. lanigerum* colonies or completely eliminating *E. lanigerum* from a given tree, so *E. lanigerum* colony count (as measured in the first year) for a tree without measuring colony size can be misleading. Surveys in the future will focus more on the early season, the point at which most growers will react to *E. lanigerum* if it is present, and on distinguishing trees as above or below the action threshold of most growers. Surveys so far had mixed results regarding *F. auricularia*'s effect on *E. lanigerum* populations, with year 1 showing no effect, while the survey in year 2 suggested *F. auricularia* may help control *E. lanigerum*.
Practicability of *F. auricularia* augmentation for growers: Augmenting *F. auricularia* populations seems eminently doable, based on the fieldwork completed so far the provisioning of shelters has a tangible benefit on the number of *F. auricularia* foraging in trees. Said shelters are cheap and easy to produce, although a full cost estimate of implementation should be carried out.
Practicability of RFID for remote monitoring of *F. auricularia*: RFID may be usable on *F. auricularia* in lab 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. Preliminary work has generated an experimental arena design which detected *F. auricularia* movement in response to changes in light levels. RFID technology is poorly suited to monitoring *F. auricularia* in the field, or rather, *F. auricularia* is poorly stuited to being monitored via RFID. RFID works best on colonial species, or insects with clear focal points in their daily routines. *Forficula auricularia* is a generalist with a broad diet so its choice of food, and by extension the location of feeding, is unpredictable. While *F. auricularia* shows a tendency to aggregate when finding shelter during the day, this a) does not lead to aggregations rivalling colonial species in size (eusocial bees being the preeminent example of RFID applied to insects), and b) does not mean *F. auricularia* 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 *F. auricularia* individuals. Given that both RFID antennae and tags are expensive, it is not financially viable both to provide enough RFID-antennae-enabled shelters, or to release enough tagged *F. auricularia* such that an appropriate proportion of tagged individuals will be redetected consistently.
Development of RFID tagging methodology: Preliminary work on adhesives for tagging suggested that Araldite epoxy may be the best option for attaching RFID tags to *F. auricularia*. Cyanoacrylate glues appear to be toxic to *F. auricularia*, while many other non-toxic glues are too weak for long-lasting tagging. The long term acute and chronic effects of the glues need to confirmed with larger sample sizes.

3. Summary of results from the reporting year

During the field season 11 orchards were surveyed on two occasions for *E. lanigerum*, *F. auricularia*, algae, and moss. *E. lanigerum* was assessed visually, using an acetate grid to estimate colony size, and counts of individuals and colonies. *F. auricularia* was assessed using refuge trapping, with artificial shelters created using plastic bottles containing rolls of corrugated cardboard. Algae and moss were both scored qualitiatively on a scale from 0 to 3. In addition, information about the orchards was gathered with help from World Wide Fruit and the growers who owned the orchards, such as their age, whether they were organic or conventional, etc. This information was used to create and fit generalized hierarchical mixed effect models using a binomial link function to model the presence or absence of both *E. lanigerum* and *F. auricularia*.
The following results were obtained from the field work:
Forficula auricularia appeared less often in organic orchards when compared to conventional ones, a result which is surprising and goes against the results of some previous studies. And *E. lanigerum* was found more often in multi-row orchards, suggesting they prefer denser plantings of apple trees. However, these conclusions may have been an artefact of the limited sample size for these factors
Forficula auricularia presence was negatively correlated with *E. lanigerum* presence in conventional orchards but not in organic orchards. This could be an indicator of predation, although this cannot be concluded outright from the current data. The interaction is in line with previous results that have suggested *Forficula auricularia* work best at controlling *E. lanigerum* when *F. auricularia* are present early in the season and *E. lanigerum* populations have not grown too large.

Forficula auricularia presence did not seem to be affected by the level of moss or algae on apple trees. Orchard age did not show an effect on the presence of either species.
In addition, spray records and soil compaction measurements are still being gathered from this survey, but have not been analysed at the time of recording.

Lab work was carried out before the field season in order to try and improve the *F. auricularia* tagging procedure and evaluate the prototype RFID arenas that had been assembled. In total there were 90 attempted tagging events, during which some variations in the information gathered and the tagging methodology were tried. In all cases, a *F. auricularia* individual was knocked out by one minute exposure to CO_2, was sexed and weighed, had their elytra cleaned using a cotton swab soaked in ethanol, were then immobilised using a wooden ring containing a grid made of fishing line placed upon their back while resting on a sponge, had a drop of araldite epoxy glue applied to their elytra, before having a tag applied to the glue, and then weights applied to the top of the ring. They were then closely monitored for up to 20 minutes until the first signs of movement and recovery, at which point the ring was removed. The earwigs were then moved to container with no heavy objects in it for 24 hours, before being moved into a box containing a Wignest (Russell IPM) for refuge. The length of time earwigs remained immobile, and the date on which their tag was discovered to have dettached, were also recorded.
Tagging *F. auricularia* with RFID tags remains difficult due to the high rate of tag loss, even using the best performing glue so far, Araldite epoxy. Footage was captured showing that *F. auricularia* work actively to remove tags glued to their backs. Of the 90 tagging events, 30 resulted in the tag being lost on the first day post-tagging, another 30 resulted in the tag being lost on second day. Even more of the remaining tags were lost in the following days. This poor success rate represents a strong limitation on the ability to generate ample sample sizes, particularly when time is limited.
RFID can work for tracking earwig movements and activity. Experimental design is limited by the detection range of the readers, and reader-reader interference when placed in proximity to one another. Experimental arenas must be designed to encourage or force *F. auricularia* to move through a narrow aperture in order for detection to take place, current prototypes work by separating shelter and food with a plastic length of tube being the only means of travelling between the two of them (this is where the RFID antenna can be mounted). Differences were found between prototype arenas, probably due to the diameter of the tubing's aperture connecting the two sides of the arena. Larger diameter apetures were preferred as this reduced the likelihood of individuals choosing to shelter in the tube itself, which generates large amounts of spurious RFID detection data. While this setup did generate detections, *F. auricularia* were not strongly motivated to pass between the two sides of the arena. This may have reflected the experiments being carried out in winter when *F. auricularia* typically stops feeding, thus reducing their desire to find food. This can be corrected by carrying out any RFID experiments in the spring or summer. There may also have been a reduced impetus for *F. auricularia* to seek out shelter due to the dim light in the experimental environment. A 16:8 light:dark cycle was used, along with a 20°c temperature to try and simulate summertime conditions, however, the lighting in the room may not have been strong enough for earwigs to respond to it as sunlight and seek shelter.

4. Key issues to be addressed in the next year

Identification of biotic and abiotic drivers of *F. auricularia* distribution and density.
Improvement of the RFID tagging procedure of *F. auricularia*, including a revisit of the best adhesive to use.
Identification of possible sublethal effects of tagging on *F. auricularia*.

5. Outputs relating to the project

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

Output	**Detail**
NACM Hereford growers walk	Poster: Earwigs and woolly apple aphid – refuges and insecticides
ICE 2020 Helsinki	Poster: Earwigs and woolly apple aphid – refuges and insecticides (same as Hereford growers walk)
Tree fruit technical day	10 minute presentation: Radio tagging earwigs to understand the breakdown in successful woolly apple aphid, Eriosoma lanigerum (Hausmann), control

SCI visit	10 minute presentation: Radio tagging earwigs to understand the breakdown in successful woolly apple aphid, Eriosoma lanigerum (Hausmann), control

6. Partners (if applicable)

Scientific partners	Harper Adams University, NIAB East Malling
Industry partners	Loraine Boddington (Cidermakers UK), Charnee Butcher (World Wide Fruit)
Government sponsor	