Project title:	Radio tracking earwigs to understand the breakdown in successful woolly apple aphid, *Eriosoma lanigerum* (Hausmann), control
Project number:	C-3000147
Project leader:	Dr Tom Pope, Harper Adams University
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Previous report:	N.A.
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Location of project:	NIAB EMR
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Date project commenced:	28 September 2020

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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.

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CONTENTS

GROWER SUMMARY

Headline

This project aims to provide ways for growers to enhance the natural biocontrol of woolly apple aphid, and utilise the widespread UK insect, the common European earwig, in apple orchards.

Background

Common European earwigs (*Forficula auricularia*, Linnaeus) (unless specified 'earwig' refers to this species) are generalist predators that live in many UK apple orchards and consume a wide variety of other invertebrates, as well as plant matter. Research has shown that they may be particularly important for controlling the woolly apple aphid (*Eriosoma lanigerum*, Hausmann) (WAA). WAA has previously been controlled as a side effect of insecticide spraying for codling moth (*Cydia pomonella*, Linnaeus). However, as broad-spectrum insecticide use is being reduced due to regulatory restriction, WAA is of increasing concern to apple growers.

WAA attacks the woody tissue of apple trees. Compounds in the aphid's saliva cause the tissue of apple trees to swell up into galls which disrupt the plants internal transport systems. These galls can also split or become pulpy, providing an opportunity for pathogens to infect the tree. Gall formation happens both on the branches and trunks of apple trees, but also on the roots where WAA colonies form to avoid cold winters.

Studies have shown that biocontrol can keep WAA populations low enough that they are not a problem for apple growers, often when earwigs are present alongside other natural enemies of WAA such as the parasitoid wasp, *Aphelinus mali* (Haldeman). However, successful biocontrol often required high numbers of earwigs (more than five per tree), for earwigs to be present early in the growing season, or for somewhat WAA resistant varieties of apple to be used.

Earwig foraging behaviour is poorly understood due to their nocturnal lifestyle. Remote monitoring using passive radio frequency identification (RFID) tags attached to earwigs may help fill this gap in our understanding. Better information on how earwigs hunt for WAA has the potential to provide new ways for apple growers to use this species as a biocontrol agent for WAA.

Experimental approach:

A survey was conducted to map the spatial distribution of WAA within a commercial apple orchard, with the aim of targeting future research in areas that were more likely to contain WAA.

A second survey was conducted in an experimental apple orchard to investigate if corrugated cardboard refuges tied to the trunks of trees increased the number of earwigs foraging in those trees, and if this led to a decrease in WAA colonies. The effect of historic applications of broad-spectrum insecticides on the populations of WAA and earwigs was also investigated.

An experiment was carried out where 80 earwigs marked with fluorescent powder were released into a commercial apple orchard, and then their positions recorded with the use of a UV torch at night. This experiment aimed to measure the earwigs' dispersal from their release point. The direction of their dispersal was also measured. This was done to test the effectiveness of using fluorescent powder to measure the speed of earwig dispersal. The experiment provided early data on the speed of earwig dispersal along planting rows, as well as the frequency at which earwigs cross between rows.

Summary

Two orchards were identified which had populations of WAA present. These will be used for future field trials.

The addition of corrugated cardboard shelters in orchards increased earwig numbers. However, this did not lead to a decrease in the number of WAA colonies found on trees.

The historical application of broad-spectrum insecticides had a long-term impact on the number of WAA colonies found on trees. In contrast, earwig numbers had either recovered or were not significantly affected.

Releasing fluorescent earwigs showed that they dispersed within the orchard quickly. Marking with fluorescent powder was a suitable way of finding earwigs, and this method could be repeated to learn about how different orchard conditions affect their dispersal.

Financial Benefits

This project cannot currently provide this kind of information.

Action Points

This project cannot currently provide this kind of information.

SCIENCE SECTION

Introduction

The following is a review of the key information available that describes the potential of *F. auricularia* as a biocontrol agent for WAA.

The suitability of earwigs as biological control agents of WAA, or as a component of biological control programs, has been previously investigated by many research groups. Earwigs possess several traits which make them attractive as biocontrol options for WAA. Firstly, WAA occur in apple orchards; one of the agricultural systems where earwigs are not considered an important pest. Secondly, earwigs already occur in many apple orchards, so many growers do not need to purchase or deliberately introduce earwigs. In situations where growers want to introduce or enhance earwig numbers, *F. auricularia* can be trapped and transported using artificial refuges, providing a simple way for growers to gather and release them. Finally, as generalist predators, earwigs have the potential to contribute to the control of not only WAA, but also other common apple pests such as rosy apple aphid, *Dysaphis plantaginea* (Passerini).

Caroll *et al.* (1985) primarily looked at earwigs as a control method for green apple aphid (*Aphis pomi* (de Greer)), but part of their study involved caging earwigs on apple rootstock stool beds, after the application of insecticides. They found that the earwigs prevented the resurgence of green apple aphid but had no significant effect on the WAA colonies already established on the rootstocks.

Muller *et al.* (1988) examined three plots of apple trees with different densities of earwig (monitored by refuge trapping). They found that in the high (≈ 6 earwigs per tree) and intermediate (≈ 3 earwigs per tree) earwig density plots there were significantly fewer WAA colonies than in the third plot, where earwigs were excluded. They also showed that artificially introduced colonies of WAA were located and destroyed in significantly less time when earwigs were not excluded. It is worth noting that *A. mali* was present in the orchards used for this experiment which may have contributed to the reduction in WAA.

Nicholas *et al.* (2005) compared orchards under an IPM spraying regime that used codling moth mating disruption (MD) (using sex pheromone dispensers), with orchards that combined fenoxycarb (an insect growth regulator) and codling moth mating disruption (FMD), and orchards that used the broad-spectrum insecticide azinphos-methyl (an organophosphate) alongside the mating disruption (AMD). They also used sticky bands around the base of the trunk to stop crawling predators from entering some of the trees in the MD and FMD treatments (they ensured these were infested with WAA) and then compared the extent of

WAA on these trees to the unbanded ones. They found that while WAA numbers started out similar in all three treatments, they increased later in the season and stayed significantly higher in the AMD orchards. They found a strong negative correlation between the number of earwigs taking refuge in a tree and the level of WAA infestation. Also, when earwigs were excluded using the sticky bands, WAA infestation levels were significantly higher, a result also found in similar trials by Orpet *et al.* (2019b). As well as earwigs, *A. mali* was found to benefit from the lack of broad-spectrum insecticides. *A. mali* is likely to have played an important role in controlling WAA in the MD and FMD orchards. However, it would not have been excluded by the sticky bands, so earwigs were considered the key species responsible for WAA control in the two IPM strategies. The authors found a significant interaction between apple cultivar and the ability of earwigs to effectively control WAA, as red delicious, a more susceptible variety, was not effectively cleared of WAA in all seasons.

Gontijo *et al.* (2015) looked at the suppression of WAA in a series of exclusion-cage trials. While earwigs were not explicitly studied, they were included in the 'generalist predators' guild in the study. The authors found evidence for a low level of antagonism between predators and the parasitoid *A. mali*, which proved insignificant for the control of WAA. Instead, there appeared to be a great deal of complementarity between the predators and *A. mali*, with the effective extermination of WAA colonies only being found in plots where neither guild was excluded. *A. mali* alone was capable of significantly slowing WAA population growth but not actually reducing their number.

Quarrell *et al.* (2017) looked at a variety of organic, IPM, and conventional orchards, and monitored several different ecological groups within the insect community, as well as WAA numbers. They found that, after management type, earwig numbers early in the season were the best predictor of WAA infestation scores. If 15 or more earwigs were trapped per tree in the first 7 weeks, then WAA scores remained below acceptable levels. If earwig numbers dropped below this, then *A. mali* numbers became important, but on its own *A. mali* was not sufficient to control WAA.

Happe *et al.* (2018) studied the impacts of orchard management and landscape factors on both earwig and WAA abundance in Spanish and German apple orchards. They found in both countries *F. auricularia* abundance was similar in IPM and organic orchards (in Spain the earwig *F. pubescens*, Gené, was significantly more abundant in organic orchards). In one German orchard, for one month, there was a significant negative correlation between earwig abundance and WAA infestation, but in all other months there was no significant correlation. Interestingly, this study also found a negative effect of woody habitats on earwig abundance in German IPM orchards specifically, which the authors noted runs counter to some previous studies. Happe *et al.* (2018) suggest the older orchards used in their study already have established earwig populations and so do not require woody habitats to act as migration corridors; instead, these habitats may act as more attractive areas that earwigs migrate to. No other landscape factor influenced earwig abundance in either country. For WAA, organic orchards had higher levels of infestation in Spain, while in Germany having larger proportions of apple orchard in the surrounding 1km was the only significant factor for WAA abundance.

Orpet *et al.* (2019a) used video recordings of WAA colonies to assess the levels of predation by different predators. They found that earwigs made the most attacks and were present in orchards to attack WAA before other predator species arrived. *Coccinellid* larvae (unidentified species) spent the longest cumulative time attacking WAA colonies, due to their longer attack duration when compared with earwigs. There was no assessment in this study of the impact each predator on WAA, so the number of attacks and time spent attacking are the only indication of which species might be most important for WAA control. Earwigs were never observed antagonising other predators; however, they themselves were often antagonised by ants (*Formica* and *Myrmica* species). When this occurred, the earwigs would move away, and the number of these ant-earwig interactions was negatively correlated with the number of earwig attacks on WAA colonies.

Marshall and Beers (2021) tested the effects of full-block net enclosures designed to stop codling moth. They found that enclosed apple trees had higher WAA levels than control trees, despite earwigs being unaffected by the nets, and *A. mali* abundance was higher inside the netted orchard blocks. The authors noted that this result was unexpected but stated that the exclusion of lacewings and syrphids may have been responsible for this finding. Due to the way the data is presented, it is difficult to know if the earwig numbers inside the cages met the recommended densities prescribed by Nicholas *et al.* (2005) or Quarrel *et al.* (2017).

In summary, there are mixed results on the efficacy of earwigs in controlling WAA. While there have been clear positive results in some studies, others have shown limited changes in WAA populations because of earwigs. Currently it is not well understood why such inconsistencies occur, and there are several possible explanations for this. Earwig population densities are highly variable, and there are several potential reasons for this. Earwigs tend to aggregate, which will naturally lead to a patchy distribution. They are sensitive to insecticide spraying regimes (Jana *et al.*, 2020). Soil conditions may affect the ability of females to create and maintain brood chambers (Moerkens *et al.*, 2012). As well as this, hedgerows and woodland may act as reservoirs or sink habitats for earwigs (Happe *et al.*, 2018). All these factors contribute to variations in earwig density, which in turn make their efficacy as control agents uncertain. Studies conducted on their gut contents have also shown that earwigs have a broad diet that often consists more of algae and pollen than of insects. It is therefore still an open question how common earwig predation on WAA colonies is in the field, and the answer to this question likely changes depending on the availability of other insect prey, algae, and pollen, as well as the abundance of ants (which antagonise earwigs) and other generalist predators (which compete with earwigs for resources).

This report summarises the findings of three studies carried out in apple orchards in Kent (UK). The first of these was a survey aimed at investigating the spatial distribution of WAA within an orchard. If this study had identified relevant microhabitats associated with WAA presence, then these would have provided conditions to target in future studies. The second was a pair of assessments, one looking at WAA and the other looking at earwigs. This study investigated whether the historical application of broad-spectrum insecticides and/or the addition of artificial earwig refuges impacted the abundance of either species, or if there was a correlation between the two species. Finally, the third study was an attempt at monitoring earwig dispersal using fluorescent powder by locating them at night when they are foraging.

Additional details are provided in the methods section about the field collection and husbandry of earwigs which were used in preliminary work, the results of which are not presented here.

Materials and methods

Earwig collecting

Adult earwigs were collected from a mix of deciduous trees in Torkington Park (Hazel Grove, Stockport) (53°22'38.1"N 2°06'48.8"W), deciduous garden vegetation (Poynton, Cheshire) (53°20'48.0"N 2°06'28.0"W) and blackcurrant bushes (NIAB EMR) (51°17'17.5"N 0°26'58.3"E) in late summer and autumn 2020. Earwigs were captured by hand (Hazel Grove) and using refuge traps (Poynton and NIAB EMR). Some of the earwigs collected at NIAB EMR had previously been used in studies investigating the effects of entomopathogenic fungi before being used in this study.

For the fluorescent release experiment, earwigs were captured by hand on 27.07.21, on strawberry plants grown at NIAB EMR (51°17'18.1"N 0°27'13.8"E).

Earwig husbandry

Earwigs were kept in plastic containers (Figure 1). All containers had ample ventilation and refuges were provided for earwigs to shelter in. Ventilation occurred through fine mesh, which allowed air flow while containing earwigs. Initially, torn cardboard was used for refuges, however this was replaced with commercially available artificial refuges called Wignests (Trademark Russel IPM) (Figure 1) once these became available. To maintain humidity, pipette bulbs were filled with distilled water, and then plugged with foam. This prevented earwigs from drowning and facilitated the constant but slow evaporation of water to regulate humidity. The modified pipette bulbs were replaced as needed. For nutrition, the earwigs were provided with a constant supply of ground dried cat food (Purina, St Louis, USA and Iams, Mason, USA), which was supplied in small plastic trays. The cat food was replaced as soon as signs of mould were identified. The cat food was supplemented with sliced raw carrot and dried insects (Wilko, Worksop, England). Before 15.12.2020, the earwigs were kept indoors at ambient room temperature and lighting was not controlled. From 15.12. 2020 the earwigs were kept within an insectary at NIAB EMR at a constant 17°C and a 12-hour light and 12-hour dark regime.

Earwigs used for the fluorescent release experiment were collected on 27.07.2021 and housed in containers as above, at room temperature, with an uncontrolled light cycle, for 6 days.

Figure 1: A) A typical container used to house earwigs. In this image small trays of ground cat food can be seen, along with supplementary carrot. Pipette bulbs plugged with blue foam were filled with distilled water to provide water and control humidity. At the top of the image, the lid of the container can be seen, including the mesh which provided ventilation. This image also shows carboard substrate used to provide shelter, which was later replaced by wignests (Trademark Russel IPM) such as those depicted in B and C.

B) An assembled wignest ready to be suspended on a tree using the blue plastic hook.

C) A disassembled wignest showing the interior of the two wooden sections which slot together to provide three grooves which act as refuge for earwigs.

Clock House WAA assessment

A royal gala apple orchard at Clock House Farm, Maidstone, UK (51°15'41.4"N 0°28'40.6"E), was divided into eight squares, four of which consisted of 20 rows of 13 trees, and four of which consisted of 21 rows of 13 trees (in this case 'row' is used to refer to the line along which trees were planted, which is used as an X coordinate for this study). Eleven trees in each square (88 trees overall) were selected for sampling using a regular patter designed to give good coverage of the orchard's area.

WAA colony counts were carried out at Clock House Farm on 03.06.21, 08.06.21, and 10.06.21. Trees were searched thoroughly for colonies of WAA, which can be easily identified by their white wool, and the number and location of colonies were recorded. These searches began at the base of the tree with the rootstock and then moved up the trunk and branches on both sides. Because the number of WAA colonies varied greatly, and these colonies were immobile, searches were not conducted in a fixed time. Instead, the search was considered exhaustive and an accurate measure of the true number of WAA colonies in the tree. Nevertheless, most trees took approximately five minutes to search when accounting for time spent recording and travelling between rows. Searches were finished when both the top of the tree had been reached and no new colonies were detected for approximately 30 seconds. Colony locations were classified as being either on the rootstock, the trunk, or the branches of the trees. Colony size was not recorded.

These spatial data were analysed using Moran's I test. WAA colony counts were tested for spatial autocorrelation (clustering) with row and tree acting as X and Y coordinates respectively.

Wiseman WAA assessment

Royal gala apple trees were selected for sampling at Wiseman apple orchard at NIAB EMR, East Malling, UK. Wiseman apple orchard currently contains nine 12-by-12 blocks of fully-grown trees, with hedgerows separating the blocks. Royal gala apple trees are found in four of these blocks (Blocks 3, 5, 6, and 7), in each case consisting of three rows of trees (Figure 2). Twenty-two trees from each block were selected for sampling (88 trees in total). All four of the blocks containing royal gala apple trees were treated with the same spray program from February of 2021 until 14.05.21 (approximately 3 months before the WAA searches), at which point all spraying stopped. This spray program included the use of targeted insecticides (Chlorantraniliprole for codling moth, and Flonicamid for rosy apple aphid). However, prior to this, Blocks 6 and 7 had broad spectrum insecticides applied to them while blocks 3 and 5 never received broad spectrum insecticides. To investigate these historic differences in spray regime, Blocks 6 and 7 were labelled ‘Conventional’ while Blocks 3 and 5 were labelled ‘IPP’ (for Integrated Plant Protection). The trees in Blocks 5 and 7 were also fitted with corrugated cardboard bands, which are known to act as an artificial refuge for earwigs. These bands were 10 cm wide and 40 cm long. They were tied around the trunks of trees 40 cm above the ground and fixed in place using electrical tape. Block 7 was banded in May 2021 (three months before the first WAA assessment took place) and Block 5 was banded on the 15.08.21. Because Block 5 was banded after the WAA assessments and on the same day as the earwig assessment there, it has been considered unbanded for the purpose of this analysis. This means that for this analysis there is no block which had the IPP spray regime and banding added.

1 No Gala	2 No Gala	**3** IPP No banding
4 No Gala	**5** IPP No banding (late)	**6** Conventional No banding
7 Conventional Banding	8 No Gala	9 No Gala

Figure 2: A graphic illustrating the layout of the nine orchard blocks present in Wiseman orchard. Blocks have been numbered left to right and top to bottom. Grey blocks with “No Gala” indicate that these blocks contained no royal gala apple trees. Blocks 3, 5, 6, and 7 contained royal gala apple trees. Blocks 3 and 5 used an Integrated Plant Protection spray regime which used targeted insecticides only, while Blocks 6 and 7 were under a conventional spray regime which used broad spectrum insecticides. In Block 7, the royal gala apple trees had bands of corrugated carboard tied around the trunks to provide artificial refuges for earwigs. In Blocks 3 and 6, the trees were not banded. In Block 5, similar corrugated carboard bands were applied less than 12 hours before the block was surveyed for earwigs, too late for them to affect the results of the woolly apple aphid and earwig surveys conducted in the block. It was therefore also considered not to be banded for the purposes of this study.

WAA colony counts were carried out at Wiseman apple orchard at NIAB EMR, on 27.07.21, 29.07.21, and 01.08.21, using the same methodology as the survey at Clock House Farm's apple orchard. Due to the lack of a full factorial design, the effects of spray regime and the addition of artificial earwig refuges could not be analysed together in a mixed effects general linear model. Instead, spray regime and artificial refuge presence were each analysed separately, using a Wilcoxon signed rank test, because the colony count data were skewed. A similar test was also conducted comparing blocks 6 and 7 only.

Wiseman earwig assessment

Earwig counts were carried out at Wiseman apple orchard on the same royal gala apple trees that were used for the WAA colony counts. Blocks 3 and 6 were searched on the 13.08.21 and Blocks 5 and 7 were searched on the 15.08.21. For the earwig search completed on 15.08.21, Block 5 had only been banded for approximately 8 hours, which was not considered long enough for the earwig population to react to the presence of the new refuges. It was therefore considered unbanded for this analysis.

On each night, two searches were performed per tree, one along each side of the row. Each search lasted one minute and thirty seconds, for a total of three minutes per tree, and was conducted using a handheld torch. Earwigs were categorized as male, female, or immature (no immature earwigs were recorded to date), based on their appearance. All earwig searches were completed after sunset (earliest search 22:15, latest search 02:20).

Like the WAA colony count data, the earwig count data were analysed using Wilcoxon signed rank tests to see if the spray regime applied before this study and the presence or absence of artificial earwig refuges had any effect. A third Wilcoxon test was also conducted to compare Blocks 3 and 5 to Block 6, to avoid conflating the effect of artificial refuges with spray regime. The earwig count data were also plotted against the WAA colony count data to investigate any possible correlation between the two.

Clock House fluorescent earwig release

The rows of a royal gala apple orchard owned by Clock House Farm (51°15'37.5"N 0°28'38.8"E) were assigned a letter from A to Z, as there were 26 rows of trees within the orchard (Figure 3). Rows L, M, N, and O were selected for their central position. The tree in the middle of each of these 4 rows was designated as 'tree 0', with the trees along the row in the uphill direction (NNE) designated as '1, 2, 3, etc.' and trees along the row in the downhill direction (SSW) designated as '-1, -2, -3, etc.'. Earwigs captured by hand on 27.07.21 at NIAB EMR were divided into four groups of 20, giving 80 earwigs in total.

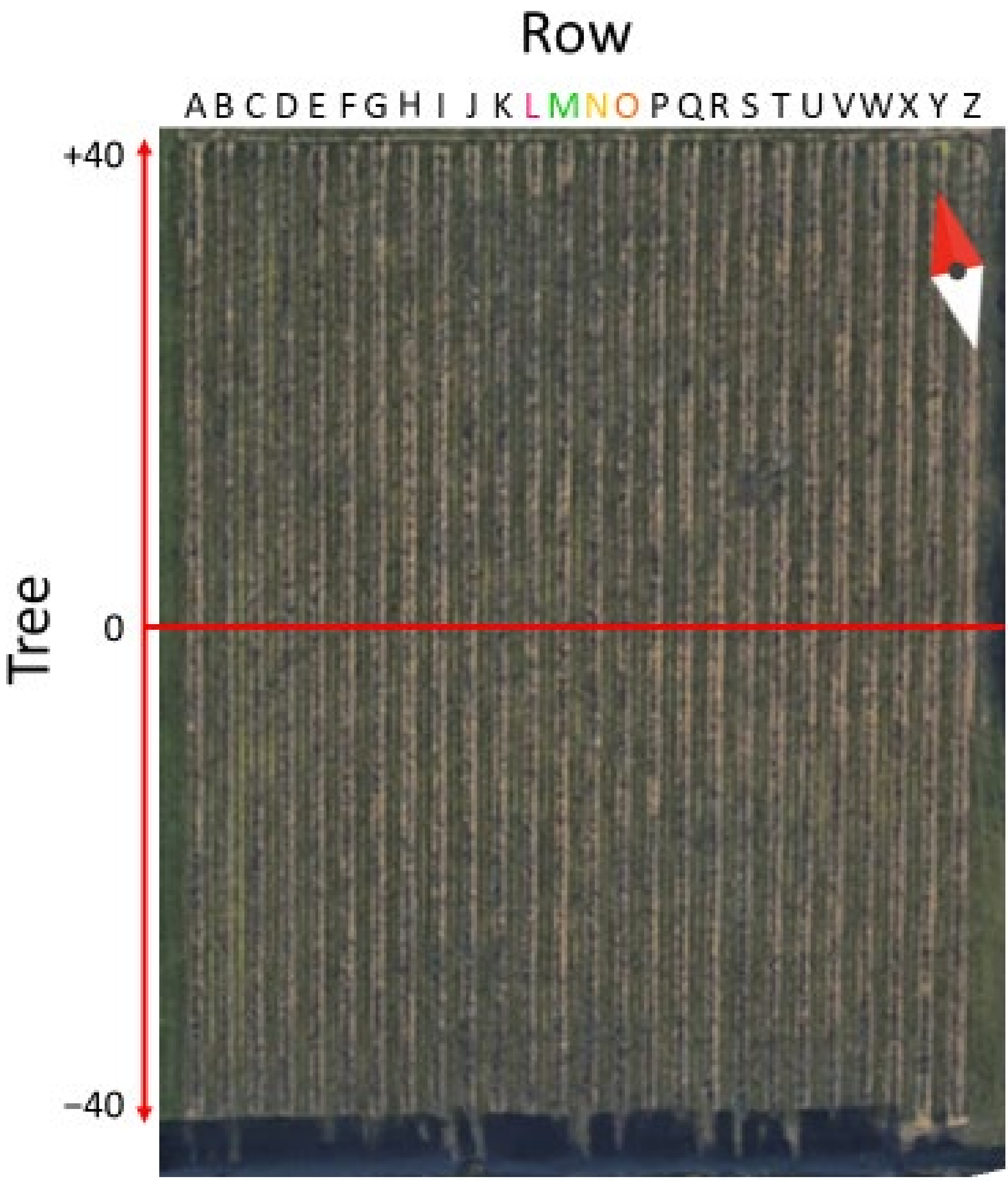

Figure 3: An aerial view of one of the orchards owned by Clock House Farm, which was used to study the dispersal of earwigs (taken from google earth™). Earwigs were marked with fluorescent powder and released into the orchard on rows L, M, N, and O. The letters designating these rows of apple trees have been coloured to show the colour of fluorescent powder used to mark the earwigs released onto that row, pink earwigs were released on row L, green on row M, yellow on row N, and orange earwigs were released on row O. Earwigs were released onto tree 0 of their assigned row. In the positive direction the ground sloped uphill, while in the negative direction the ground sloped downhill. The red arrow of the compass needle indicates north. The rows are 3.5 meters apart, the trees are 1.5 meters apart.

Earwigs were housed at NIAB EMR as detailed in the earwig husbandry section, from the 27.08.19 to the 03.09.21. Each of these groups was assigned a colour of dye, and one of the selected rows. The earwigs assigned to row L were given pink, earwigs assigned to row M were green, earwigs assigned to row N were yellow, and earwigs assigned to row O were orange. On 02.09.21 each earwig was immobilized using 1-minute exposure to CO_2, and then had a small dab of shellac (70g of Liberon (New Romney, England) lemon shellac flakes mixed with 500ml of ethanol) mixed with fluorescent powder (50ml of shellac mixed with 0.5g of powder) of the appropriate colour, applied to its elytra with a cotton-tipped bud. Then, on the 03.09.21, the earwigs were transported from NIAB EMR to Clock House Farm, then placed in their groups of 20 into plastic bags containing fluorescent powder of the same colour already applied to each earwig. The dry powder and coloured shellac were used together to provide a clear, long-lasting mark. The earwigs were shaken gently to dust them in the powder before they were released onto the leaves of tree 0 of their assigned row. One green earwig assigned to row M had died after being painted with shellac, so only 19 earwigs were released onto tree 0 of row M. For all other rows, 20 earwigs were released. The earwig release occurred from 16:30 to 17:16, during daylight. On the day of release, it was sunny with a temperature of 19°C.

On 03.09.21, starting at 22:20, the first earwig search was conducted 3 hours after the release of earwigs. Rows K, L, M, N, O, and P were searched from tree zero to ±15, with a search carried out on both sides of the trees. A UV torch was used during the search so that the fluorescent powder with which the earwigs had been coated in (and painted with) would fluoresce. The location and colour of any earwigs found was recorded, in order to determine the distances they had travelled since being released. These distances were calculated using the planting grid of the orchard. Similar searches were carried out on 04.09.21 and 05.09.21, each starting at or shortly after 22:20. On these two subsequent searches, only 1 earwig was detected each night, so the data from these searches were not used in analysis and only the data from the first night were considered.

A chi-squared test was conducted on the data from the 13 earwigs which had moved from tree 0 of their respective row to determine if the earwigs preferred dispersing uphill or downhill. Yate's continuity correction was applied due to the low sample size.

Results

Clock House WAA assessment

Moran's I test showed that the distribution of WAA colonies in the sampled trees at Clock House farm was not significantly different from random (N = 88, Moran's Index observed = 0.003, expected Index = -0.011, standard deviation: 0.015, $p > 0.05$) (Figure 4).

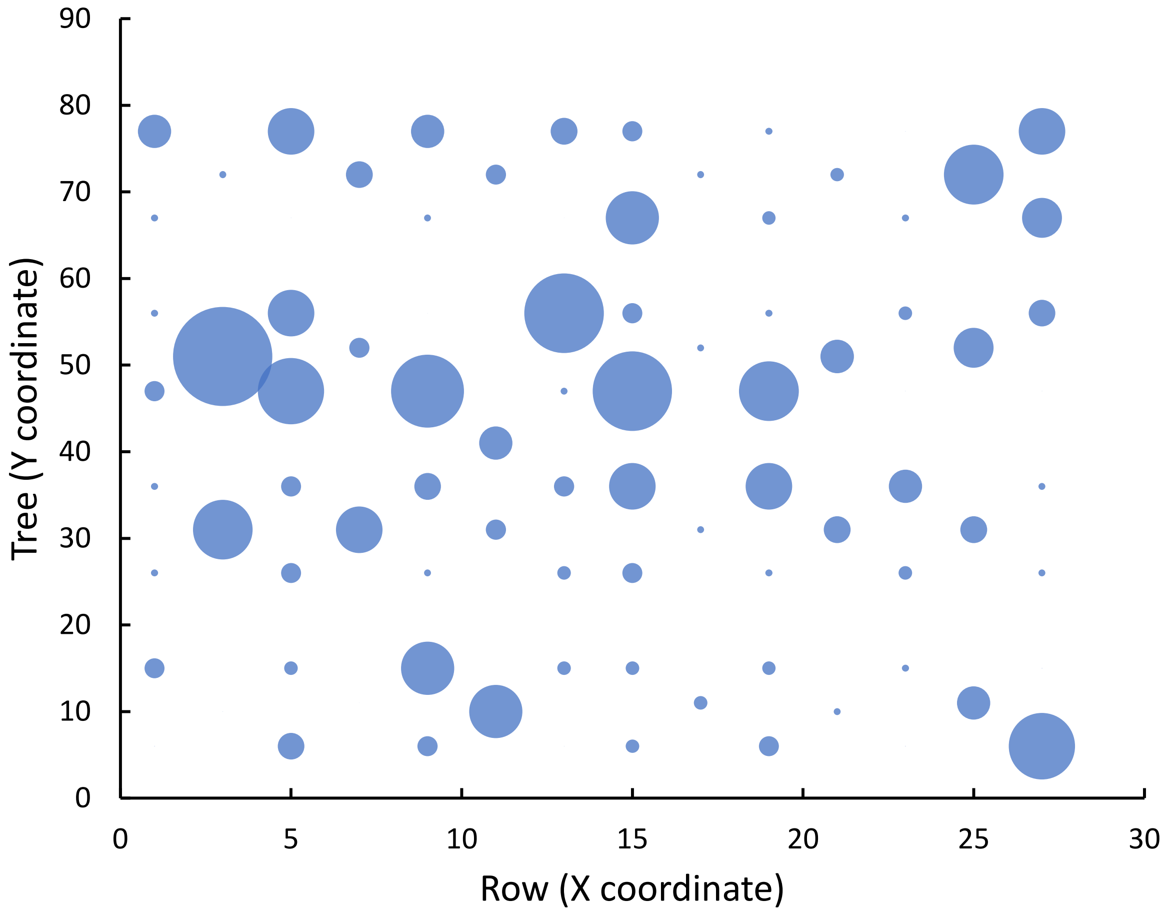

Figure 4: Bubble chart displaying the distribution of woolly apple aphid colonies on 88 trees in a commercial royal gala apple orchard. The planting rows of the orchard were converted from their original letter designations to numbers, used to provide the X coordinates. The position of trees along the rows were used to provide the Y coordinates, starting from the top of the orchard (NNE) and working down the slope of the hill (towards SSW). The diameter of the circles are proportional to the number of colonies on the sampled tree. The smallest circles represent 0 woolly apple aphid colonies, while the largest circle represents 15.

Wiseman WAA assessment

A Wilcoxon's signed-rank test suggested that the presence or absence of artificial refuges for earwigs (banding with corrugated cardboard) had a significant effect on the number of WAA colonies (N no refuge = 66, N refuge = 22, W = 1081.5, $p < 0.001$) (Figure 5). A similar test comparing blocks which had previously been under an IPP spraying regime to blocks which had previously been under a conventional spraying regime also suggested that the spraying regime had a significant effect on the number of WAA colonies, even though spraying had ceased almost 3 months before the study was conducted (N IPP = 44, N Conventional = 44, W = 316.5, $p < 0.0001$) (Figure 6). These results must be considered carefully, as the only block containing artificial refuges, Block 7, was a conventional block, and thus the effect of refuges on the data is nested within the effect the conventional spraying regime. To account for this, a third Wilcoxon's test was conducted to compare only Blocks 6 and 7. Figure 7 shows that Block 7 had similar numbers of WAA colonies to Block 6, and the test confirmed there was no significant difference between them (N Block 6 = 22, N Block 7 = 22, W = 254.5, $p > 0.05$). This suggests that the significant difference between the block with artificial refuges and the blocks without them (Figure 5) is due to the differences in spray regime and not because of the artificial refuges.

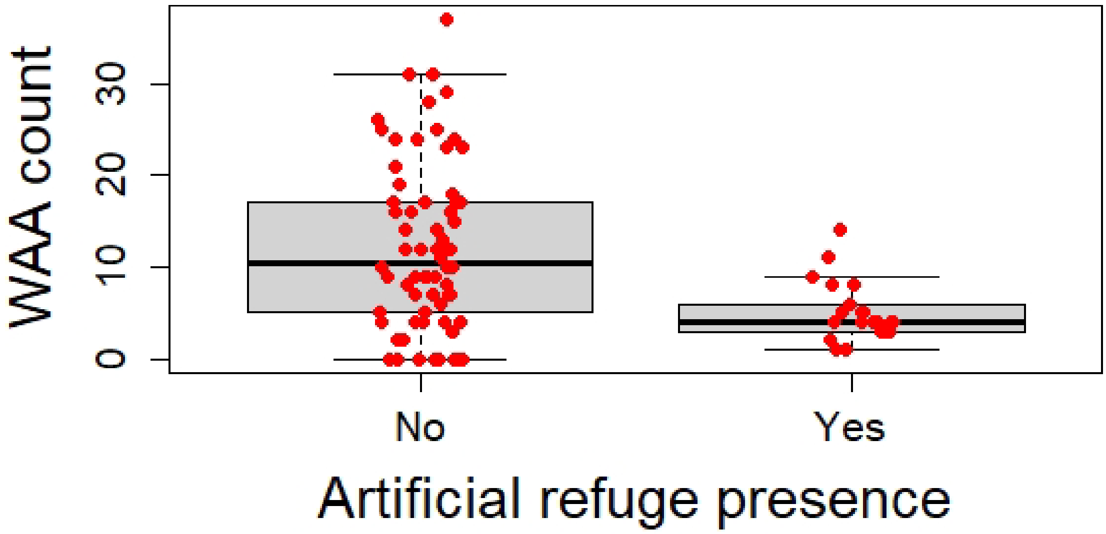

Figure 5: Boxplot of woolly apple aphid colony counts from royal gala apple trees in orchard blocks which either did (N = 22) or did not (N = 66) have artificial refuges for earwigs. The datapoints are superimposed in red.

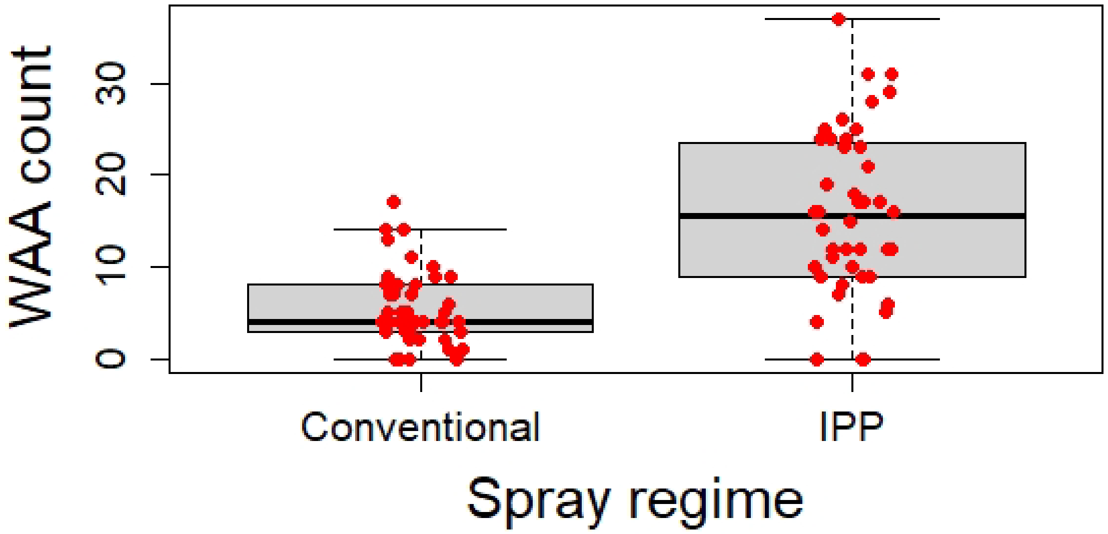

Figure 6: Boxplot of woolly apple aphid colony counts from royal gala apple trees in orchard blocks which were either conventionally sprayed using broad spectrum insecticides (N = 44) or were sprayed with an Integrated Plant Protection scheme that used targeted pesticides only (N = 44). The datapoints are superimposed in red.

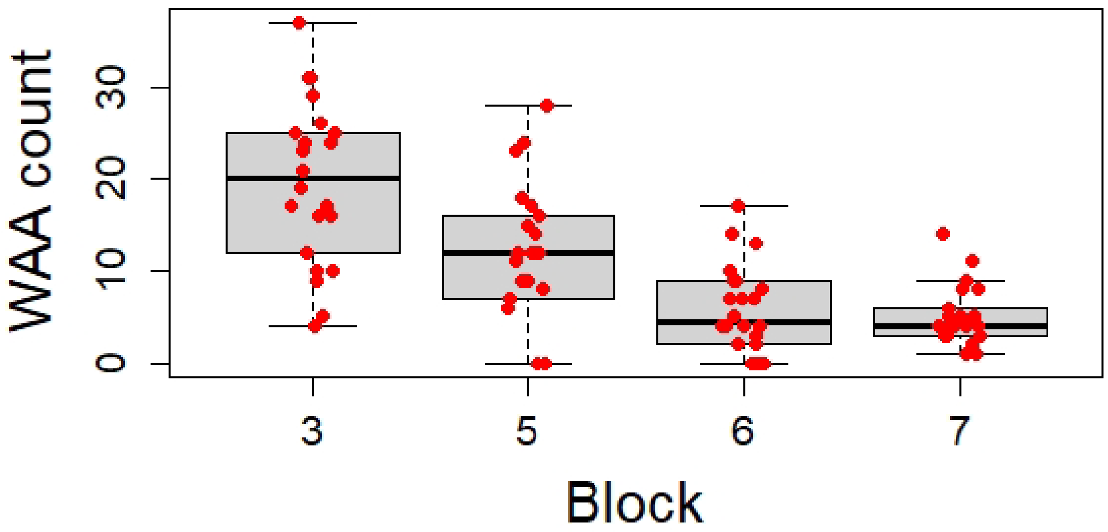

Figure 7: Boxplot of woolly apple aphid colony counts from royal gala apple trees in four different orchard blocks. Blocks 3 and 5 had targeted insecticides applied, while blocks 6 and 7 had broad spectrum insecticides applied. Block 7 also had artificial earwig refuges. 22 trees were sampled from each block. The datapoints are superimposed in red.

Wiseman earwig assessment

A Wilcoxon's signed rank test showed that the presence of artificial refuges significantly increased earwig numbers (N no refuge = 66, N refuge = 22, W = 214.5, $p < 0.0001$) (Figure 8). A similar result was obtained when investigating if the spray regime affected earwig numbers (N IPP = 44, N Conventional = 44, W = 1208, $P < 0.025$) (Figure 9), but this result is misleading. When looking at all the blocks individually (Figure 10), only Block 7 has high numbers of earwigs, while Block 6 is very similar to Blocks 3 and 5. This suggests the significantly higher numbers of earwigs in conventionally sprayed blocks is due to the presence of the artificial refuges in Block 7, rather than the spray regime. To provide more evidence for this, another Wilcoxon's test was carried out comparing Block 6 to Blocks 3 and 5, which found no significant difference (N Blocks 3 and 5 = 44, N Block 6 = 22, W = 395.5, $p > 0.05$). Figure 11 shows the earwig counts for each tree plotted against the WAA colony count for the same tree. A linear trendline has been plotted, with an R^2 value of 0.0206, indicating there was no strong linear correlation between the abundance of earwigs and WAA in the 88 trees surveyed at Wiseman orchard.

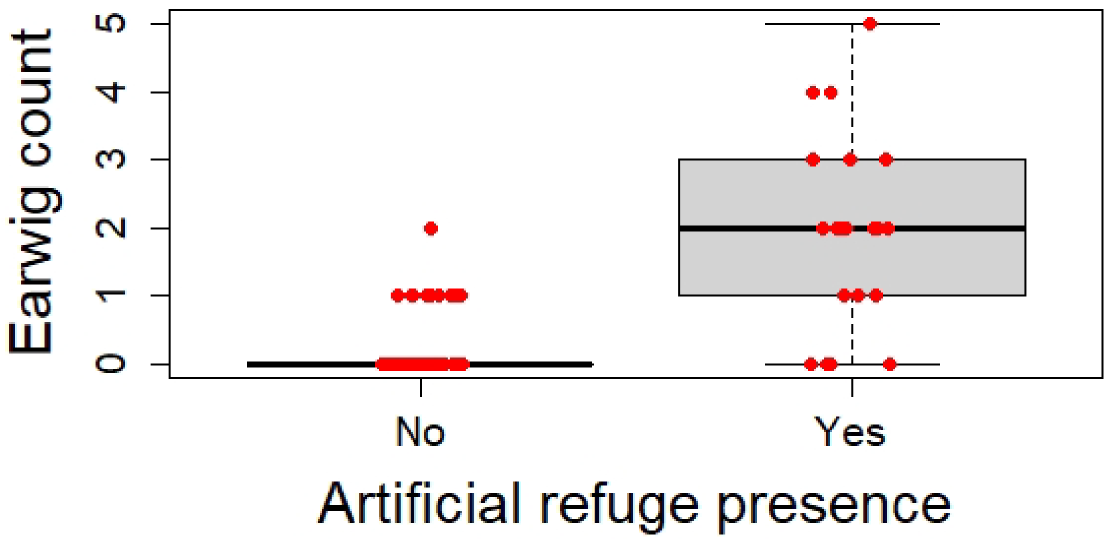

Figure 8: Boxplot of earwig counts from royal gala apple trees in orchard blocks which either did (N = 22) or did not (N = 66) have artificial refuges for earwigs. The datapoints are superimposed in red.

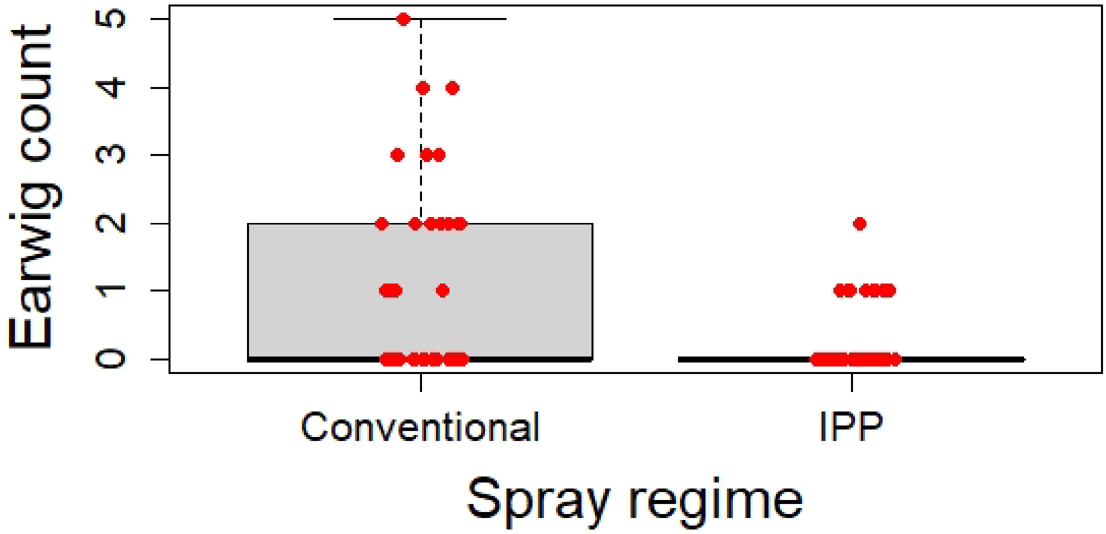

Figure 9: Boxplot of earwig counts from royal gala apple trees in orchard blocks which were either conventionally sprayed using broad spectrum insecticides (N = 44) or were sprayed with an Integrated Plant Protection scheme that used targeted pesticides only (N = 44). The datapoints are superimposed in red.

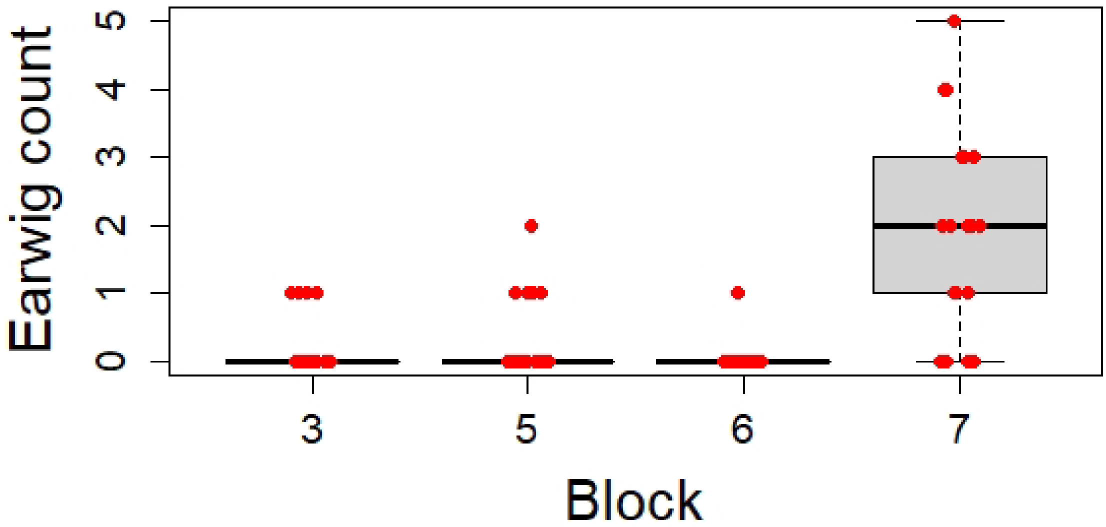

Figure 10: Boxplot of earwig counts from royal gala apple trees in four different orchard blocks. Blocks 3 and 5 had targeted insecticides applied, while Blocks 6 and 7 had broad spectrum insecticides applied. Block 7 also had artificial earwig refuges. 22 trees were sampled from each block. The datapoints are superimposed in red.

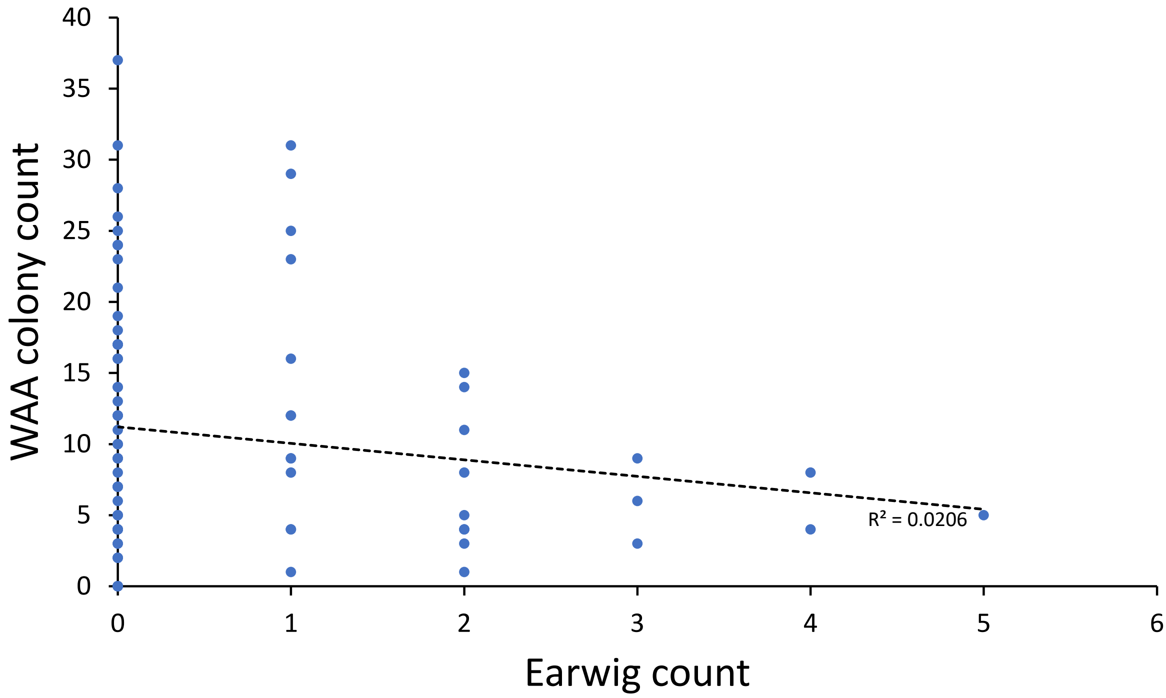

Figure 11: Scatterplot of woolly apple aphid colony counts against earwig counts for 88 royal gala apple trees. A linear trendline has been plotted with a displayed R^2 value.

Clock House fluorescent earwig release

Figure 12 shows the straight-line distances travelled by the 21 earwigs redetected on the first night of searching, 3 hours after they had been released, while Figure 13 shows the movements of the earwigs specifically in the Y direction along the rows of trees, including the direction of movement. A chi-squared test shows that earwigs' choice to disperse uphill or downhill was not significantly different from choosing randomly (N Uphill = 8, N Downhill = 5, Df = 1, X^2 (Yate's continuity correction) = 0.3, $P > 0.05$).

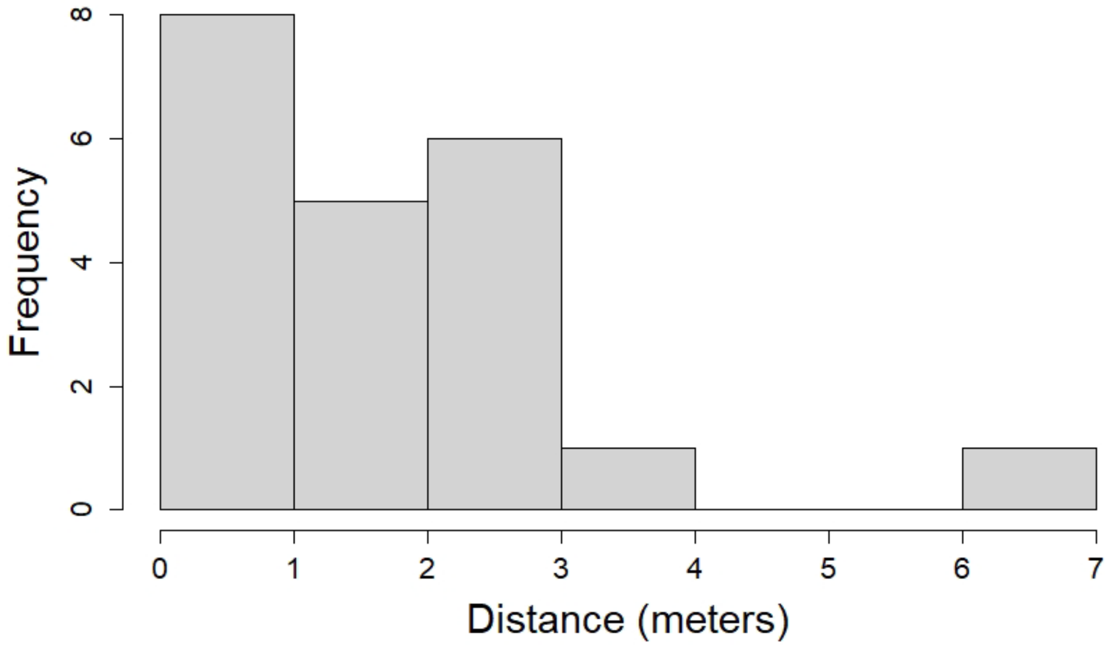

Figure 12: Histogram of the estimated straight-line distances 21 earwigs had travelled in three hours, after being coated in fluorescent powder and released on a royal gala apple tree in the middle of an orchard. Distances were calculated using the orchard’s planting grid.

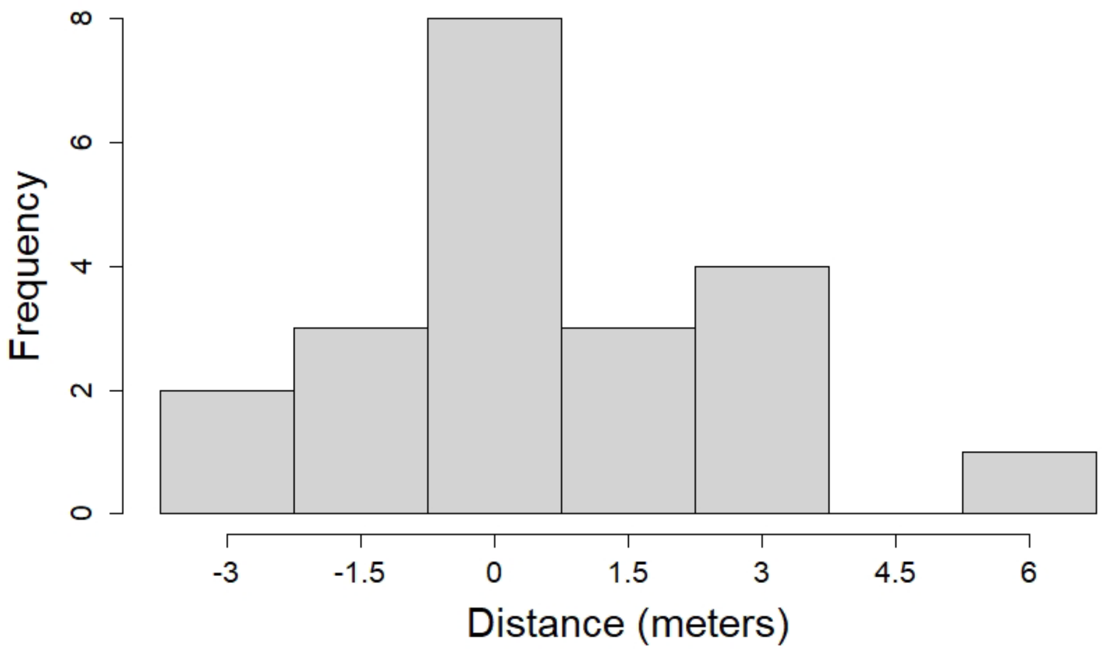

Figure 13: Histogram of the distances 21 earwigs had travelled along planting rows three hours after being coated in fluorescent powder and released on a royal gala apple tree in the middle of an orchard. Positive numbers indicate movement uphill (NNE) while negative numbers indicate movement downhill (SSW). Distances were calculated using the orchard’s planting grid.

Discussion

Overall, the surveys carried out in this study are a good foundation for further research. At Clock House Farm, WAA did not show significant autocorrelation. This implies a lack of important microhabitat factors influencing their distribution in this orchard, in other words, from the point of view of WAA, the orchard might be considered homogenous. Importantly, this means that the WAA colonies did not apparently suffer from an edge effect when near hedgerows. The effect of hedgerows on WAA has been investigated before. Peñalver-Cruz *et al.* (2020) similarly found a lack of edge effects when measuring WAA abundance at different distances from *Pyracantha coccinea* hedgerows. However, Alspach and Bus (1999) found that WAA were not randomly distributed in a mixed apple orchard in New Zealand. Shelter from wind appeared to be important, but they also showed a tendency for clumping over small distances (eight meters) which this study might have missed. Careful thought is therefore needed to determine if individual trees or orchards are the more appropriate sampling unit for a given study.

The data from the different blocks at Wiseman is interesting, but the lack of a full factorial design means the conclusions which can be drawn from the current data set are weakened. When taken as a whole, Figures 5, 6, and 7 and the respective Wilcoxon's tests suggest that the historic difference between spray regimes may have significantly impacted the number of WAA colonies, but the presence of artificial refuges (and resulting rise in earwig abundance) did not. Block 7 had similar WAA colony counts to Block 6, which indicates that increased earwig numbers did not provide any additional control of WAA when combined with previous applications of broad-spectrum insecticides. Figure 11 further supports this, as a strong negative correlation between earwig and WAA abundance would be expected if earwigs were providing effective biocontrol. However, the possibility of a non-linear correlation in these data has not yet been investigated. While some studies have found earwigs provided effective control of WAA, often a high density of earwigs is required. Nicholas *et al.* (2005) found a minimum average of five earwigs per tree was required, while Quarrell *et al.* (2017) suggested a minimum of 15. Even for Block 7, the block with the highest number of earwigs in this study, the mean number of earwigs per tree was just 1.9. Another important factor to consider is that in many successful cases of WAA suppression by earwigs, *A. mali* has been present (Muller *et al.*, 1988; Nicholas *et al.*, 2005; Gontijo *et al.*, 2015; Quarrell *et al.*, 2017). The lack of *A. mali* as an additional biocontrol agent, or the low numbers of earwigs even when refuges were provided, may explain why this study showed no significant effect of earwigs on WAA populations.

Given the sensitivity of earwigs to foliar insecticides, a strong interaction between spray regime and refuge presence might be expected, where artificial refuges only enhance earwig numbers in the absence of broad-spectrum insecticides (Shaw and Wallis, 2010). These current data would seem not to suggest this; however, the flaws of the current dataset must be considered. While the Wilcoxon's test showed significantly higher numbers of earwigs in the conventionally sprayed plots (Figure 9), Figure 10 strongly indicates this is an artefact of the effect of artificial refuges being nested within the conventionally sprayed plots. Under an active spray regime which includes broad-spectrum insecticides, the benefit of artificial refuges to earwigs may be reduced (Nicholas *et al.*, 2005). To test this hypothesis, earwig and WAA assessments should be repeated under an active spraying regime with refuges in both the IPP and conventionally sprayed blocks.

The fluorescent earwig release experiment is currently a preliminary piece of work but these data are still useful in informing future studies. The purpose of this methodology was to attempt to create a time series of earwig dispersal over several days, which was not possible due to the negligible detection rate after the first night. However, the redetection rate 3 hours after release was high, and may well be a useful method to repeat in order to try and discern factors which effect earwigs' short-term dispersal. The majority of information about earwig dispersal has been gathered through traditional mark-release-recapture studies targeting the daytime refuges of earwigs as the trapping point (Moerkens *et al.*, 2010). Therefore, this experiment is unusual in attempting to gather data on earwigs as they are foraging at night. Potentially, traditional refuge trapping could be combined with this technique in order to gather information on average foraging distances (although this would probably require the ability to identify individuals). Using fluorescent powders provides a rapid and simple way to relocate marked earwigs at night, when they are most active, and allows large areas to be searched with minimal effort. Similar releases could be carried out in orchards with different environmental conditions to see if any of these conditions alter the behaviour of the earwigs.

The methodology for fluorescent powder application has room to be refined further. Painting the earwigs with fluorescent shellac was done with the hope of increasing the longevity of the marking, but in this case clearly provided no measurable benefit to the long-term detection rate. Future repeats of this experiment could therefore skip this step and would require less handling of the earwigs. There would also be no need to sedate the earwigs with CO2, something which may have affected earwig dispersal in this study (Bartholomew *et al.*, 2015).

An explicit aim of this mark-release experiment was that it would be compared to a future release of earwigs in an orchard with corrugated carboard banding to provide refuges for earwigs. Thus, no artificial refuges could be added to the plot of trees the earwigs were released into. Because of this, the earwigs were released loose onto tree 0 of their respective row, during the daylight - a situation they would usually avoid. It was assumed earwigs would rapidly disperse from their release point towards the nearest refuge, remain there until nightfall, and then resume their natural pattern of nocturnal foraging and hiding during daylight. However, this disruption could also have caused more long-term changes to their behaviour, such as a desire to move rapidly away from the site of their release, or perhaps a decrease in their likelihood of emerging to forage for several nights. Regardless of whether the daytime release led to such a behaviour change, the low detection rate after the first night was a surprising result. Moerkens *et al.* (2010) found that 99% of double-brood earwigs (earwigs which produce two lots of eggs per year, rather than one) travelled less than 11 meters in a month. Earwigs have been shown to behave as more-or-less sedentary provided they have ample food and refuge (Lamb, 1975; Phillips, 1981) and, in Moerkens *et al.*'s (2010) study, artificial refuges were provided. So, if food was not limiting, their dispersal may have been lower than in more typical orchard conditions. The Clock House farm orchard used in this study showed very low numbers of earwigs (personal observation) so potentially one or both of these resources (refuge or food) may have been lacking in the study plot, and could explain why so few earwigs were redetected after the first night. Earwig dispersal in Clock House Farm may also have been higher if earwigs detected harmful insecticide residue from foliar sprays and attempted to avoid this. Another possibility is that the marked earwigs were still present in the search area but were not foraging during the searches. Earwigs are known to occasionally skip foraging on some nights (Lamb, 1975; Phillips, 1981), presumably if they are still satiated after a previous night's foraging. The earwigs released in this study had access to an excess of food for 6 days while they were maintained in the lab. This might explain the lack of activity on the second and third nights of searching, but would still require an additional explanation of the high redetection rate on the first night. Earwigs are also known to avoid foraging in the rain (Philips, 1981); however, the weather was similar on all three nights when the searches were performed. To test these possible explanations, the study could be repeated with artificial refuges present (as planned), or with earwigs starved for 24 hours before release. Information should be obtained about the insecticides used at Clock House Farm, and a comparison between Clock House Farm and an unsprayed orchard would also be useful.

In regards to directionality, the released earwigs showed no preference for travelling up or downhill. Moerkens *et al.* (2010) similarly found a lack of directionality in the movement of marked earwigs; however, they described earwig movement as completely random in direction. While this was not analysed due to the size of the dataset, only two of the 13 earwigs which moved in this study had crossed to a different tree row. Potentially the more-or-less continuous tree canopy running along the planting rows provides a natural corridor for earwigs to disperse along while foraging. If this observation proved to be statistically significant in a larger study, it would run counter to the results of Moerkens *et al.* (2010). However, far more data must be collected before this question can properly be addressed.

Conclusions

- WAA distribution at Clock House Farm appeared to be random
- Artificial refuges made from corrugated cardboard significantly increased the number of earwigs found in night-time searches of Wiseman orchard
- The historical application of broad-spectrum insecticides has significantly decreased the number of WAA colonies in parts of Wiseman orchard
- More earwigs did not lead to a decrease in WAA colonies, and earwigs showed no long-term effects from historical broad spectrum insecticide applications in Wiseman orchard
- Earwigs did not prefer dispersing up or downhill when released at Clock House Farm

Knowledge and Technology Transfer

Presentations given:

- CTP Autumn event, digital, November 2020
- AHDB Crops PhD conference, digital, January 2021
- CTP Summer event, digital, July 2021

Posters presented:

- Harper Adams University research conference, Shropshire, September 2021

Other events attended:

- Harper Adams University PhD colloquium, digital, November 2020
- RES ento careers day, digital, March 2021
- RES Aphid special interest group, digital, April 2021
- AHDB Selection and use of biological control agents in the production of ornamental crops - Aphids and whiteflies, digital, May 2021
- RES Ento21, digital, August 2021

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