Project title	Identifying prey preferences of earwigs in an apple orchard as a prerequisite for assessing their biocontrol potential
Project number:	TF 185
Project leader:	Dr Jean Fitzgerald East Malling Research New Road East Malling, Kent ME19 6BJ
Report:	Final report, March 2009
Previous report	None
Key staff:	Jean Fitzgerald Nick Harvey Mike Easterbrook
Location of project:	East Malling Research
Project coordinator:	
Date project commenced:	1 April 2008
Date project completed (or expected completion date):	31 March 2009
Key words:	Earwigs, *Forficula auricularia*, predation, molecular detection of predation, prey preferences, biocontrol

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 Jean D Fitzgerald
Project Leader
East Malling Research

Signature ..... Date .....

Dr Christopher J Atkinson
Head of Science
East Malling Research

Signature ..... Date . 31 March 2009

CONTENTS

Grower Summary

Headline

- Molecular techniques successfully identified the types of food consumed by earwigs in apple orchards
- A major constituent of the earwig diet throughout the season was algae and fungi associated with lichens but they were also shown to consume apple leaf midge larvae and rosy apple aphid

Background and expected deliverables

The common earwig, *Forficula auricularia*, is present in large numbers in many orchards and in windbreak trees. These insects are nocturnal and their numbers are often underestimated in orchards. Previous research at East Malling Research has shown that large numbers of earwigs use artificial refuges in orchard trees as shelter sites during the day and that releasing earwigs into pear trees significantly reduced numbers of pear psyllids on pear trees. Other research has also reported that this predator consumes psyllids and woolly apple aphid in orchards. To develop reliable integrated control measures that include this predator more information is needed about its feeding habits and preferences in orchards.

Some, but not all, food types consumed by earwigs may be detected in the gut if visually identifiable remains can be found in gut dissections. The use of molecular techniques provides an opportunity to assess earwig feeding patterns by directly identifying DNA from different food sources within the gut of individual earwigs. This is much more direct and definite evidence of predation than that gained by drawing inferences from changing pest numbers in the orchard.

Expected deliverables from this project were:

- The development of a molecular technique to identify food consumed by earwigs in orchards
- An initial assessment of feeding preferences of earwigs

Summary of the project and main conclusions

Earwigs were collected from artificial refuges in apple trees and from tap sampling in apple orchards at EMR between May and September 2008. The guts of individual earwigs were removed and the contents subjected to PCR (polymerase chain reaction) amplification of the constituent DNA using both universal primers that amplify DNA from a range of different species, and species specific primers for apple leaf midge and rosy apple aphid. Using the universal primers a total of 69 PCR products (different DNA sequences and thus potentially different food types) were cloned, sequenced and matched with published sequences in databases. One product recorded from almost all samples was *Metschnikowia,* a yeast symbiont which has been reported from a variety of other insects such as plant hoppers, lacewings and beetles. Other products present that were sequenced were:

- green algae associated with lichen (*Trebauxia* /*Chlorella*-like)
- fungi associated with lichen (*Capronia*-like)
- yeasts and rots associated with fruit (*Candida* /*Pichia*-like; *Monolinia fructigena*; *Rhizopus, Mucor*)
- soil/leaf litter fungi (*Fusarium*-like; *Cladosporium*-like; *Davidiella*-like *Mellassezia*-like)
- insect associated fungi (*Cryptococcus* yeast)

No apple DNA was found within the earwig gut, indicating that earwigs were not inflicting any damage to the trees or fruit.

Using the species specific primers, only two of 164 earwigs screened (1%) were shown to have consumed apple leaf midge in an infested orchard. This low level of predation on this pest is not surprising as the larvae are protected within leaf curls that earwigs would find difficult to access. The two earwigs found to have consumed apple leaf midge larvae may have found them on the ground when they fell to pupate in the soil. At least six earwigs were positive for rosy apple aphid consumption in samples collected in June (13% of June collection); this is when populations of the aphid were at their highest and before they left apple for their summer host plant.

This project showed that:

- a major constituent of the earwig diet throughout the season was algae and fungi associated with lichens
- apple DNA was not found within the earwig gut indicating that earwigs were not inflicting damage to the trees or fruit

- arthropod DNA was not detected using universal ITS primers and subsequent sequencing
- arthropod DNA was detected when specific primers for apple leaf midge and rosy apple aphid were used in the PCR
- the lichen and fungal material in the earwig gut masked the presence of arthropod DNA
- future molecular studies on this pest will need to be targeted to a particular prey/food source

Financial benefits

There are no immediate financial benefits to growers. Additional studies using species specific primers to amplify DNA from different potential prey species are required before it is possible to confirm the effectiveness of this predator as a biocontrol agent for specific pests.

Action points for growers

- Although the major dietary content in the earwig gut was shown to be lichen and fungi, apple pest DNA was also detected
- There was no evidence from this study to suggest that earwigs were causing damage to trees or fruit
- Providing refuges for earwigs in orchards may increase biocontrol of pests and should have no deleterious effect on fruit quality

Science Section

Introduction

The common earwig, *Forficula auricularia*, is present in large numbers in many orchards and in windbreak trees (Fitzgerald & Solomon, 1996; Solomon *et al*., 1999). Females tend their young in nests on the ground until after their first moult, in around mid April. The nymphs then begin to forage for themselves, and move into the tree canopy (Phillips, 1981). These insects are nocturnal and their numbers are often underestimated in orchards. Work at EMR showed that large numbers of earwigs used artificial refuges in orchard trees as shelter sites during the day (Fitzgerald & Solomon, 1996; Solomon *et al*., 1999), and that releasing earwig into pear trees significantly reduced numbers of pear psyllids on the trees (Solomon *et al*., 1999). Other research (e.g. Phillips, 1981; Noppert *et al*., 1987; Ravensburg, 1981) reported that this predator consumes psyllids and woolly apple aphid in orchards. Information in the scientific literature on the mainly circumstantial evidence of predation by earwigs was reviewed by Solomon et al. (2000).

Some food types consumed by earwigs can be detected in the gut if visually identifiable remains can be found in gut dissections. A study of this kind by Phillips (1981) showed that the food consumed by earwigs included algae and fungi, and Buxton (1974) and Sunderland (1975) identified aphid remains. Earwigs are popularly blamed for causing feeding damage on the fruit. However, in laboratory experiments at EMR with earwigs caged on ripe apples there was no evidence of earwig feeding except on over ripe fruit or on fruit where damage caused by other factors was already present (Fitzgerald, unpublished results). It is not clear if earwigs require different food types at various stages in their development, or if they have preferences for particular prey.

The use of molecular techniques provides an opportunity to assess earwig feeding patterns by directly identifying prey DNA within the gut of individual earwigs. This is much more direct and definite evidence of predation than that gained by drawing inferences from changing pest numbers in the orchard as described, e.g., by Ravensburg, 1981 and Solomon et al., 1999. In an investigation undertaken at EMR in 2005, a molecular technique was assessed for its potential to identify earwig gut contents. This technique used primers that amplify DNA from a particular variable region of the genome of a wide number of species, giving rise to products of different size for these species after amplification in a polymerase chain reaction (PCR). Amplification products were removed and sequenced and these sequences compared with published data in databases to identify what the earwig had consumed. At the time the samples were taken in September the gut contents were identified as mostly fungi and algae (Harvey and Lo, unpublished data).

Database matching of PCR products as described above enables a range of different species present in an insect gut to be identified, but is relatively expensive as each product has to be cloned and sequenced. Using a different strategy at EMR we have designed primers specific to particular pest species including aphids, which each produce a band of a particular size. This strategy enables us to assess the proportion of a particular predator population that has consumed the prey of interest without cloning and sequencing (Fitzgerald, 2006; 2007). This HDC-funded pilot project has enabled us to extend the molecular technique to assess earwig predation preferences and to investigate the potential of earwigs as part of a biocontrol strategy for pests in apple.

Materials, methods and results

Collection of samples

Artificial refuges were made from 2 litre plastic drinks bottles by removing the bottoms of the containers and packing them with rolls of corrugated cardboard. The refuges were then attached to the trunk or branches of apple trees (Figure 1).

Figure 1. Apple tree containing an artificial refuge

Previous work at EMR has shown that large numbers of earwigs can often be found in these refuges as they are used as daytime sheltering sites (Fitzgerald & Solomon, 1996). Thirty refuges were placed in three orchards at EMR; earwigs were sampled from the refuges by tapping the containers over a collecting dish. Earwigs were also collected by tap sampling on trees that had infestations of *Dysaphis plantaginea* (rosy apple aphid) and/or *Dasineura mali* (apple leaf midge), between June and September from two orchards at EMR. Samples of potential food sources on the trees such as lichens, fresh and rotting leaves and fruit, moss, liverwort, rosy apple aphid and apple leaf midge larvae, were collected as controls for the experiment. The presence of other potential arthropod prey species on the trees was also recorded. The earwigs and potential prey species were held at -80ºC until analysed.

PCR analysis

Earwigs were thawed at room temperature for 5 minutes. Their guts were removed by dissection and the contents washed out with 100 µl sterile water. These water samples were pipetted individually into 500 µl of 5% w/v chelex solution (BioRad) containing 0.2 µg/µl proteinaseK and held at 55ºC overnight to release DNA for analysis. Other samples were prepared by macerating each potential food source directly in 500 µl of chelex/proteinaseK solution. The PCR technique used primers that amplify DNA from the variable ITS region of the ribosomal RNA. Because of the variability in this region these primers were expected to give rise to products of different sizes for each potential food source species after the DNA has been amplified in a polymerase chain reaction (PCR). This size variability of PCR products was visualised as bands on agarose gels after separation by electrophoresis. DNA present in different sized PCR products was removed from the gel, cloned and sequenced and these sequences compared with published sequence data to identify what the earwig had consumed.

Preliminary gut content analysis

This was undertaken on the gut contents of six earwigs to assess the degree of food source diversity present and to identify the optimal method to be used in studying the earwig populations. Two sets of universal primers that amplify the ITS region of the species genome, one specific for bacteria and one for fungi, animals and plants, were used in the PCR amplification of DNA in the gut content samples. DNA from the ITS region of potential food sources was also amplified.

An example of DNA from six earwig gut contents replicated twice and amplified using bacterial ITS-specific primers is shown in Figure 2 which is a photograph of an agarose gel on which DNA products derived from PCR amplification are separated on the basis of

product size. Samples run in lanes from top to bottom of the gel, and each lane shows one particular sample. Smaller products run through the gel more quickly and so are found towards the bottom of the gel.

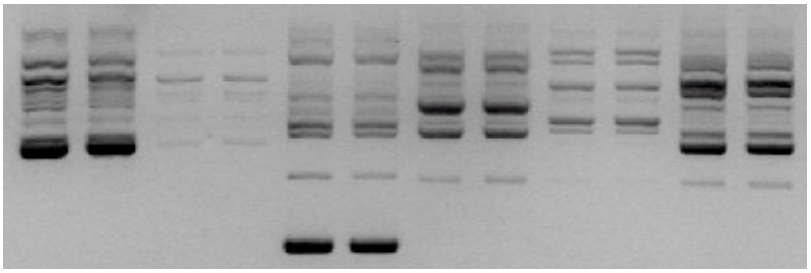

Figure 2. Earwig gut content amplified with bacteria-specific ITS primers

The bacterial diversity present in the earwig guts was extensive. To identify the species present 25 of the products were cloned and sequenced. Sequences were then compared with published sequences in DNA databases; many bands were identified as common micro-organisms living inside the earwig gut e.g. *Candidatus* types which are endosymbionts that have been found in the gut of sap-feeding insects such as psyllids and aphids, and other *Erwinia* / *Serratia* / *Salmonella*-like entrobacteriaceae.

The amplification of non-bacterial DNA from the six earwig guts (Figure 3) gave 4 products (bands). After cloning and sequencing a major microbial presence was detected, with the major common band representing the symbiotic yeast *Metschnikowia* (lowest band) which has been reported from a variety of other insects such as plant hoppers, lacewings and beetles. There was also evidence of feeding on lichen (top two bands) and a common leaf-litter mould *Cladosporium* (middle band).

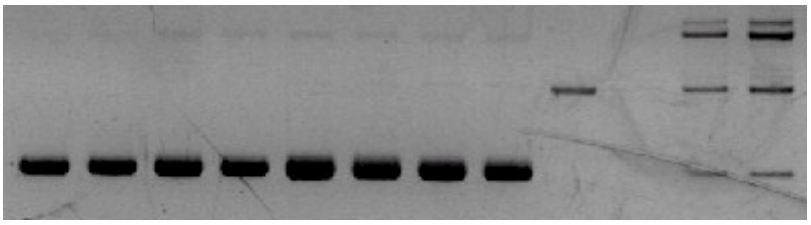

Figure 3. Earwig gut content amplified using ITS primers for fungi, animals and plants

An example of results from PCR amplification of the ITS region from selected potential food sources is shown in Figure 4.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23

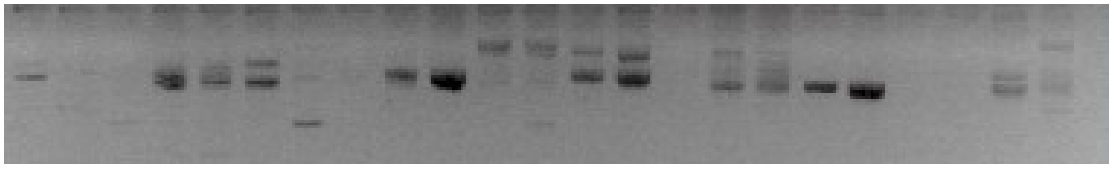

Figure 4. DNA amplification of the ITS region of potential earwig food sources

Key: Lanes 1-2 *Eriosoma lanigerum*; 3-6 *Dysaphis plantaginea*; 7 *Aphis pomi*; 8-10 *Dasineura mali*; 11-15 lichens; 16 and 17 liverwort; 18-19 rotten apple; 20-21 apple skin; 22 apple leaf; 23 moss

No consistent pattern of bands (DNA amplification products) was seen within species, and several bands from different species were of similar sizes (found in the same relative positions on the gel) so they could not be used as diagnostic markers for particular species. It was thus clear that representative bands amplified from the earwig gut content would need to be cloned and sequenced in order to be able to identify the constituents of earwig diet.

Screening of earwig gut content

PCRs were done on samples of earwigs collected between June and September. A total of 164 earwigs were analysed using the ITS primers that amplify fungi, animals and plants. The dietary profiles obtained were complex (an example is shown in Figure 5) and difficult to compare across gels and months. As the bands were usually very faint and tightly bunched it was necessary to cut bands in groups of similar size for cloning to obtain sufficient DNA for identification and to assess length variation of DNA products within species. Sequences obtained from cloned DNA were matched to database records of sequences from a wide range of species.

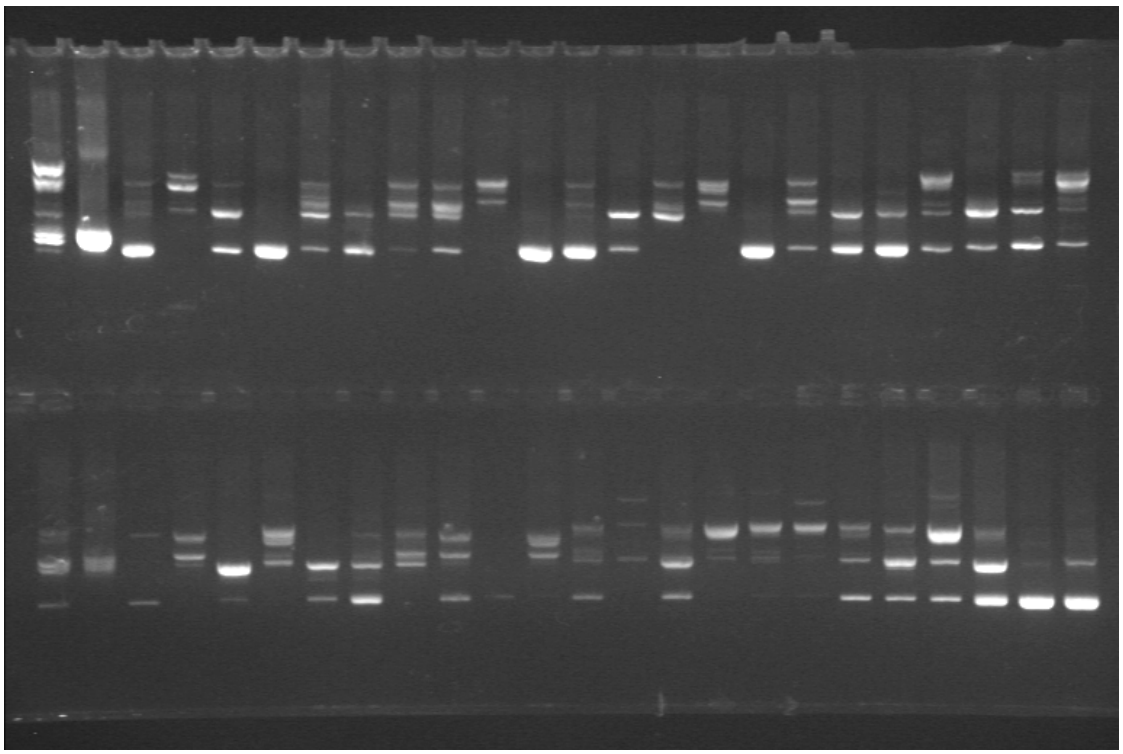

Figure 5. Two rows of DNA amplifications (48 individual earwigs) showing the diversity of the detected dietary profiles using the universal primers. Amplification products were subsequently extracted, cloned and identified by database matching

In total 69 variable bands were cloned, sequenced and database matched. The common lower band recorded from almost all samples was *Metschnikowia* which had also been found in the preliminary analysis. Sequencing revealed that at least 11 variants of this yeast symbiont were present in the earwigs tested in this study.

The other products sequenced represented multiple length variants of five main groups of organisms:

- green algae associated with lichen (9 variants - mainly *Trebauxia* / *Chlorella*-like)
- fungi associated with lichen (4 variants – *Capronia*-like)
- yeasts and rots associated with fruit (6 variants *Candida* / *Pichia*-like; *Monolinia fructigena*; *Rhizopus, Mucor*)
- soil/leaf litter fungi (*Fusarium*-like; *Cladosporium*-like; *Davidiella*-like *Mellassezia*-like)
- insect associated fungi (*Cryptococcus* yeast)

There was also a single match in the 69 bands cloned with a spider and with a plant (Fat hen - *Chenopodium album*). No apple DNA was found within the earwig gut contents indicating that earwigs were not inflicting any damage to the trees or fruit.

Use of species specific primers

Since the demonstrated complexity of the earwig diets might mask the identification of predated arthropods it was decided that in addition to the screening of earwig gut contents with universal primers, the presence of two particular arthropod pests, rosy apple aphid and apple leaf midge would be assessed directly. Specific primers that had been designed to amplify DNA from rosy apple aphid and apple leaf midge were therefore employed in a PCR of the gut content of earwigs screened with the universal ITS primers described above. The rosy apple aphid primers were microsatellite primers developed for a Defra-funded population study (HH 3103 TTF). The apple leaf midge primers were ITS primers developed in a Defra-funded project to assess predation on this particular pest (HH 3121 SSF).

Only two of the 164 earwigs screened (1%) were shown to have consumed apple leaf midge; these two earwigs had been collected by tap sampling trees in August. This low level of predation on this pest is not surprising as the larvae are protected within leaf curls that earwigs would find difficult to access. The two earwigs that were positive for apple leaf midge may well have consumed them on the ground when they fell to pupate in the soil.

Earwigs were only found to have consumed rosy apple aphid in the samples collected in June; at least six earwigs were positive for rosy apple aphid consumption from these samples (13%), but bands were very faint. It is not surprising that rosy apple aphid was only found in the gut content of earwigs collected in the June samples as this is when populations of the aphid are at their highest and before they leave apple for their summer host plant. Earwigs can often be found in the curled up leaves of rosy apple aphid infested trees.

Discussion

The molecular technique based on cloning PCR products and matching the sequences obtained with published sequences was refined in this project and successfully amplified DNA from earwig guts. Results confirmed that earwigs are omnivorous and have a substantial gut microbiota. To what extent these microorganisms are beneficial symbionts or dietary-intake, remains to be studied. The gut contents of many earwigs contained lichens; it is this that makes the dietary profiles so complex, as lichens produce multiple bands, being a mixture of fungi and algae. Apple trees are often covered with extensive lichen growth composed of many species. No arthropod DNA was detected in the initial screening programme with universal ITS primers. There are several possible reasons for this, including:

- the abundant lichen and fungal diet components may have been masking amplification of arthropod DNA in the PCR
- arthropods may be consumed during an active foraging period during the night and this DNA may be digested rapidly in the gut
- the earwigs may be consuming very few arthropods in their diet

Subsequent use of arthropod specific primers in PCR demonstrated that rosy apple aphid and apple leaf midge were consumed by earwigs. This indicates that any future molecular assessment of earwig diet will need to be targeted and designed to determine if a particular species has been consumed throughout the season rather than trying to assess the whole dietary intake. At no time was apple DNA found within the earwig gut indicating that earwigs were not inflicting damage to the trees or fruit.

Conclusions

- A molecular technique for assessing earwig gut content was successfully developed
- A major constituent of the earwig diet throughout the season was algae and fungi associated with lichens
- Apple DNA was not found within earwig gut contents indicating that earwigs were not inflicting damage to the trees or fruit
- Arthropod DNA was not detected using universal ITS primers and subsequent sequencing
- Arthropod DNA was detected when specific primers for apple leaf midge and rosy apple aphid were used in the PCR
- The abundant lichen material in the earwig gut masked the presence of arthropod DNA
- Future molecular studies on this pest will need to be targeted to a particular prey/food source

Technology transfer

Harvey, N & Fitzgerald, J.D. Earwigs: exploitable biocontrol agents? Poster presentation at National Fruit Show 2008.

References

BUXTON, J.H. (1974). The biology of the European earwig *Forficula auricularia* L. with reference to its predatory activities on the damson hop aphid *Phorodon humuli* (Schrank). PhD thesis. Wye College, University of London.

FITZGERALD, J.D. (2006). Final report to Defra on project HH3103TTF.

FITZGERALD, J.D. (2007). Final report to Defra on project HH3121SSF.

FITZGERALD, J.D. & SOLOMON, M.G. (1996). Artificial refuges for enhancing populations of natural enemies of pests in apples and pear orchards. *APRC News* **12**, pp 16-17.

NOPPERT, F., SMITS, J.D. & MOLS, P.J.M. (1987). A laboratory evaluation of the European earwig (*Forficula auricularia* L.) as a predator of woolly apple aphid (*Eriosoma lanigerum* Hausm.). *Mededelingen van de Faculteit Landbouuwwetenschappen, Rijksuniversiteit Gent* **52**, 413-422.

PHILLIPS, M.L. (1981). The ecology of the common earwig *Forficula auricularia* in apple orchards. PhD Thesis, University of Bristol.

RAVENSBURG, W.J. (1981). The natural enemies of the woolly apple aphid, Eriosoma lanigerum, and their susceptibility to diflubenzuron. *Mededelingen van de Faculteit Landbouuwwetenschappen, Rijksuniversiteit Gent* **46**, 437-441.

SOLOMON, M.G., FITZGERALD, J.D. and JOLLY, R.L. (1999). Artificial refuges and flowering plants to enhance predator populations in orchards. *IOBC/WPRS Bulletin* **22** (7), 31-37.

SOLOMON, M.G., CROSS, J.V., FITZGERALD, J.D., CAMPBELL, C.A.M., JOLLY, R.L., OLSZAK, R.W., NIEMCZYK, E. and VOGT, H. (2000). Biocontrol of pests of apples and pears in Northern and Central Europe: 3 Predators. *Biocontrol Science and Technology* **10**, 101-138.

SUNDERLAND, K.D. (1975). The diet of some predatory arthropods in cereal crops. *Journal of Animal Ecology* **12** (2) 507-515.

