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GROWER SUMMARY

Headline

Growers should conserve nettles, willow and hazel trees in the vicinity of pear orchards to act as early season sources of anthocorids and other important pear sucker natural enemies and consider planting these if they are not present.

Background and expected deliverables

Pear sucker is a devastating pest of pears which cannot currently be effectively and reliably controlled by UK growers. This project aims to combine exploitation of semiochemicals, conservation biocontrol and selective physical controls to develop improved Integrated Pest Management methods for the pest. It was thought that a pear sucker sex pheromone possibly exists and could be identified providing a tool for monitoring pear sucker populations and, more importantly, a possible means of control of the pest by mating disruption, mass trapping or attract-and-kill approaches. Anthocorid bugs are known to be powerful predators of pear sucker and can naturally regulate pear sucker populations but they do not over-winter in pear orchards and their influx in spring is often inadequate or too late. There is an opportunity to improve the species composition of hedgerows/windbreaks and develop management methods for a greater, more-timely influx. Extensive underpinning research in the Netherlands has identified a number of volatile substances produced by foliage infested with pear sucker that attract anthocorid predators. Two of the compounds are inexpensive and readily available and lures containing one of these have been shown to be attractive. It may prove possible to exploit these to enhance further the influx of anthocorid predators. Growers currently use spray programmes of chemicals that are considered to act physically to control pear sucker, including high volume sprays of water and wetters, sulphur and magnesium sulphate. The treatments used are not evidence-based; life stages against which they act, their relative efficacy, optimum concentrations and, crucially, effects on anthocorids have not been determined. Careful experimental investigation through laboratory and field testing should enable the value of these treatments to be determined and selection and optimisation of treatments to avoid disruptive effects on natural enemies.

Summary of the project and main conclusions

Objective 1. Identify and exploit the pear sucker sex pheromone for pest monitoring

The project's first surprising finding was that the species of pear sucker attacking intensively managed pear orchards in the UK is *Cacopsylla pyri* and not *C. pyricola,* the species which was dominant when

surveys were last done in the 1970s. *C. pyri* has long been the dominant species in continental Europe and it may be speculated that resistant strains have spread from Europe, possibly on nursery trees. A mixture of the two species now often occurs with *C. pyricola* being predominant in less intensively managed orchards. The implications of this finding have not yet been ascertained. The two species have very similar, though not identical, life cycles and attack pears in the same ways. It is possible that *C pyri*, a slightly larger species, is more resistant to insecticides than *C. pyricola*. A detailed analysis of the life tables of the two species and their susceptibilities to insecticides is needed to better understand them.

As a consequence of this early project finding, it was decided at the outset to focus effort on identifying the sex pheromone of *C. pyri* rather than *C. pyricola*. To attempt to identify the pheromone, numerous collections of body washings of volatiles were made from groups of winter and summer-form males and females alone or feeding on pear foliage. Exhaustive chemical and electrophysiological analyses of numerous samples were done. Despite this, no sex specific compounds that might be components of a sex pheromone could be identified. Work in the USA claimed 13Me-27:H an involatile cuticular hydrocarbon, to be the sex pheromone of *C. pyricola*. This compound was found in this work to be present on the cuticle of *C. pyri* but present in similar quantities in both males and females. The fact that it was present in similar quantities in both sexes and that it is involatile indicated to us that it was probably not a sex pheromone and the US claims were erroneous. However, the compound was synthesised, dispensers made and tested in sticky traps (similar to those used in the USA to test attractancy) in pear orchards where both *C. pyricola* and *C. pyri* were present. Considerable numbers of both species were caught in traps, but this was by random chance, there being no statistical difference in the numbers of males and females caught. Furthermore, the research was unable to demonstrate attraction of males to females or vice versa. In addition, no consistent EAG responses were observed when males were exposed the proposed pheromone of *C. pyricola* 13Me-27:H or to hexane washed off *C.pyri* females. This led us to the conclusions that a sex pheromone is unlikely to exist or if it does exist it is very weak and transitory, and that the identification of 13Me-27:H in the USA is almost certainly erroneous.

Recently, acoustic signalling has been shown to be a means of sexual attraction in various species of planthopper and psyllid so finding ways of using the sounds these pests make, may offer a more fruitful avenue of research.

Objective 2. To develop conservation biocontrol methods to maximise anthocorid populations and other natural enemies of pear sucker

An important aim of this work was to identify trees and herbaceous plants that could be provided round the borders of pear orchards and which could act as sources of the pear sucker's important natural enemies, anthocorid predatory bugs. It was known that these predators did not over-winter in significant numbers in pear orchards and the aim was to identify other plants which were rich sources of them in early spring when pear sucker egg laying commences and throughout the growing season. An important realisation at the outset was that though 2 species of anthocorid (*Anthocoris nemoralis* and *A. nemorum*) occur on pear and are both important predators of per sucker, *A. nemoralis* was adapted and had a preference for feeding on psyllids which occur on certain trees early in the season, whereas A. nemorum has a preference for feeding on aphids which tend to occur slightly later.

At the outset of the project, three new experimental hedgerows over 200 m long and comprising replicate 8 m plots of 12 different native tree species were planted on the farms of three grower consortium members. While these established in the first two years of the project, arthropod faunal surveys were done on a wide range of different tree species and on stinging nettles in three old established hedgerows around existing pear orchards. In the first year, 24 different species were sampled through the season to determine the abundance of anthocorid and other predators, of other psyllids and aphids which might act as food sources for anthocorids and of other specialist predatory bugs that might compete with anthocorids for these sources of food. Grey and pussy willow, hawthorn, hazel and stinging nettle were identified as by far the best sources of anthocorids as well as being rich sources of other important pear sucker natural enemies including ladybirds, earwigs and spiders. *Alnus cordata* widely used as a windbreak was found to support very few predators. The best subjects were studied in greater detail in the later years of the project, the seasonal population dynamics of the predators and prey being determined on each. Work on the purpose planted hedgerows in the final years of the project confirmed the earlier findings, but indicated that it could probably take 6-10 years for characteristic arthropod fauna to establish after planting, though some dominant species colonised early.

Mark and capture experiments using monoclonal antibody detection methods to study the movement of anthocorid predators from nettles in hedgerows adjacent to pear orchards and within pear orchards showed the predators could disperse over a distance of at least 50 m in a single day.

Observations of predators feeding on pear sucker in orchards, clearly indicated that although anthocorids were highly mobile with a comparatively fast numerical response to pear sucker infestations, they have only a low prey consumption rate as their body size is small compared to pear sucker. Earwigs, though much less mobile and slower to increase, showed much higher consumption

rates and it suspected that pear sucker is only a problem where earwig numbers are low or where they are absent, possibly due to inappropriate pesticide use. Spiders, especially *Phylodromus* sp., and ladybirds, especially the Harlequin ladybird, were also observed to be very important pear sucker predators.

This work as a whole has led to recommendations for pear sucker management with a new emphasis on providing the correct plant species in hedgerows/windbreaks, avoiding large orchards where sources of predators are too distant, and use of only the safest pesticides to important pear sucker predators. These recommendations have been strongly communicated to UK pear growers and recognised as being of the highest importance.

Objective 3. To exploit synomones (of pear foliage fed on by pear sucker) to attract anthocorids into pear orchards in spring

Previous work in The Netherlands had shown that pear sucker infested pear trees are strongly attractive to anthocorid predators. Volatiles were collected from pear foliage infested with pear sucker adults and nymphs both in the laboratory and field. Chemical analysis (GC-MS) and link GC-electroantennagramme analysis was conducted using anthocorid antennae. Analysis of collections from foliage showed no consistent differences with, versus without pear sucker. Volatile compounds identified at the highest concentrations were ocimene, (Z)-3-hexenyl acetate, linalool oxide, copaene, linalool, caryophyllene, δ-cadinene, α-farnesene, methyl salicylate and eugenol. Of these (Z)-3-hexenyl acetate, α-farnesene and methyl salicylate had been identified in the previous Dutch research. GC-EAG analyses of synthetic compounds with *Anthocoris nemoralis* showed consistent EAG responses to decanal and methyl salicylate and occasional responses to (Z)-3-hexenyl acetate and 2-phenylethanol. GC-EAG analyses of volatiles collected from pear seedlings with *Anthocoris nemoralis* showed EAG responses to decanal, and methyl salicylate and also to δ-cadinene. Lures containing methyl salicylate, phenyl ethanol or farnesene were not attractive to anthocorids in field trials, though the sex pheromone of the vine mealy bug, lavandulyl senecioate, was weakly attractive to *Orius* sp.. This latter compound was tested on the advice of Suterra following observation where the vine mealy bug lures had been used in Europe. Methyl salicylate had been shown to be attractive to hoverfly adults in previous experiments. Regrettably no synthetic synomone attractant was identified but the scope for extensive further work investigating blends and release rates of the compounds identified was highlighted.

Objective 4. To identify the most effective physically-acting spray treatment of those used currently that is safe to anthocorid predators and to determine optimum concentration and spray cover requirements

A series of lab and field experiments were done to investigate the efficacy of magnesium sulphate, sulphur, various adjuvants and kaolin for control of pear sucker. It is common practice for growers to apply sprays of these materials, the former three in admixture, to control serious outbreaks of pear sucker. The work showed that magnesium sulphate and sulphur had little direct insecticidal effect on pear sucker but non-ionic and silicone wetters did, especially when used at high concentrations. Trials also showed that early season sprays of kaolin greatly reduced egg laying by over-wintering adults in early spring having lasting results on the populations well into the summer. Kaolin may also deter egg laying by anthocorids so later applications are not recommended. Further laboratory work showed that the above materials were relatively safe to eggs and nymphs of *Orius* and Anthocorid bugs. This work identified new safe and effective treatments for pear sucker control which are already being used in the industry.

Objective 5. To transfer the results of the research to UK pear growers in a series of workshops as part of a wider focus on improving and increasing UK pear production

Two one day pear conferences focussing on UK pear growing were organised by English Apples and Pears, Sainsbury's and East Malling Research. At each conference the results of the project were reported.

25 February 2010: 'Pear growing for the future'

Programme: The UK pear market and industry (Adrian Barlow, English Apples and Pears Ltd); Retailer / customer perspective (Theresa Huxley, Sainsbury's Supermarkets Ltd); Improving pear growing at A Scripps Ltd (James Simpson, A. Scripps Ltd); Limiting factors in UK pear growing (Tim Biddlecombe, FAST Ltd); The economics of intensive pear production (Wouter van Teeffelen, WTE Fruitadvies, NL; Visit to concept pear orchard at EMR; The Concept Pear Orchard (Francis Wheatley, Chingford Fruit Ltd, Henk Nooteboom, NL); Pear breeding for the "Alternative Variety"(Jean Paul Reynoird, Pepinieres Georges Delbard, FR); Application of water research at EMR to pear growing (Mark Else, EMR) Pear sucker research (Jerry Cross, EMR); Conclusions: Changing pear industry attitude and investment (Discussion led by Adrian Barlow, English Apples and Pears)

27 February 2012 'Profitable pear production in the UK'

Programme: The UK market and pear industry (Adrian Barlow, English Apples and Pears); Retailer/customer perspective (Theresa Huxley, Sainsbury's Supermarkets Ltd); Experiences of

growers (Clive Baxter, Tony Frankum, Michael Bentley, Tom palmer, Oliver Doubleday); The concept pear orchard at EMR (Francis Wheatley, Chingford Fruit); Economics of pear production (David Knight ARC Agriculture); Optimising the performance of Conference (Leon Jahe, Agrovista); Pear sucker management (Jerry Cross, EMR); Application of water research at EMR to pear growing (Mark Else, EMR)

Both of these conferences were very well attended with 80 and > 100 delegates, respectively. Good feedback was received after the conferences indicating that they both had a significant effect on grower's attitudes towards pear growing in the UK. At both conferences, the outcomes of this HortLINK project were strongly communicated, the presentation at the second conference setting out important requirements and changes needed for successful pear sucker management based on the findings of this project, which is vital to the future success of the UK pear industry.

Main conclusions

- Pear sucker is a man-made pest caused by monoculture and inappropriate use of pesticides that are harmful to pear sucker's natural enemies.

- A wide range of generalist predators are important for naturally regulating pear sucker populations including anthocorids, earwigs , ladybirds and spiders.

- Pear sucker is not a problem in orchards where earwigs are sufficiently abundant.

- The paramount importance of avoidance of the use of broad-spectrum pesticides is stressed and adoption of a precautionary approach of only using pesticides which are known with certainty to be safe to anthocorids and earwigs.

- Growers should conserve nettles, willow and hazel trees in the vicinity of pear orchards to act as early season sources of anthocorids and other important pear sucker natural enemies and consider planting these if they are not present. Hawthorn is also an excellent source of predators, though if it is used it should be regularly inspected and managed to avoid the risk of fireblight.

- Large orchards should be avoided. Ideally all parts of a pear orchard should be <50 m from hedgerow/windbreak sources of natural enemies.

Financial benefits

Losses to the UK pear industry due to pear sucker, which vary considerably from season to season depending on weather conditions, have not been quantified but the pest is present in every commercial pear orchard, many orchards suffering regularly. Assuming 10% of the crop is forgone as a result of these infestations, this is equivalent to 2,300 tonnes of pears, worth £2.9 m per annum. Additionally, a substantial number of young trees in newly planted orchards become infected with the pear decline phytoplasma vectored by pear sucker and a number of orchards are so badly attacked by the pest that they have become unviable and have to be grubbed. Loss and replanting of 25 ha of pear orchards per annum, directly or indirectly, as a result of pear sucker, costs the UK industry a further £1.3 m per annum. Additionally, growers typically spend £200 per ha on pesticides to control pear sucker though this amount rises steeply (to up to £500 per ha) if a problem arises. The cost of control of pear sucker to the industry is estimated to be approximately £0.5 m per annum. Thus the grand total costs of the pest to the industry are in the region of £5 m per annum.

The work is highly relevant to the UK pear industry which has suffered grievously from pear sucker which has developed resistance to most pesticides. Growers realise and have learnt from bitter experience that they cannot reliably control pear sucker with pesticides. The UK only produces less than 20% of the pears that it consumes nationally and there is substantial scope for increase in UK production, as was strongly identified and highlighted in both the Pear conferences held as part of this project. Reliable pear sucker management is a key to future investment in the UK pear industry. The findings of this project have provided growers with clear and sound guidelines on how to manage pear sucker.

Action points for growers

- Pear sucker is a man-made pest caused by monoculture and inappropriate use of pesticides that are harmful to pear sucker's natural enemies.

- A wide range of generalist predators are important for naturally regulating pear sucker populations including anthocorids, earwigs , ladybirds and spiders.

- Pear sucker is not a problem in orchards where earwigs are sufficiently abundant.

- The paramount importance of avoidance of the use of broad-spectrum pesticides is stressed and adoption of a precautionary approach of only using pesticides which are known with certainty to

be safe to anthocorids and earwigs.

- Growers should conserve nettles, willow and hazel trees in the vicinity of pear orchards to act as early season sources of anthocorids and other important pear sucker natural enemies and consider planting these if they are not present. Hawthorn is also an excellent source of predators, though if it is used it should be regularly inspected and managed to avoid the risk of fireblight.

- Large orchards should be avoided. Ideally all parts of a pear orchard should be <50 m from hedgerow/windbreak sources of natural enemies.

- Sprays of dormant season kaolin give good suppression of the first generation of pear sucker nymphs.

- *Cacopsylla pyri* is now the dominant pear sucker species in intensively managed pear orchards in the UK, whereas *C. pyricola* was the dominant species when surveys were last done in the 1970s. The implications for this for pear sucker management are not understood but it may be that *C. pyri* is more resistant to insecticides. A pear sucker species identification guide is available from Jerry Cross or Michelle Fountain at East Malling Research (Email: jerry.cross@emr.ac.uk; michelle.fountain@emr.ac.uk, Office: 01732 523748).

Further publicity of results

The results of this project have been strongly publicised to UK pear growers and especially to the five grower members of the consortium, including at the two specially organised pear conferences. It has been agreed with the HDC that a new HDC factsheet will be produced in 2012 based on the findings of the project.

SCIENCE SECTION

Introduction

Objective 1. Identify and exploit the pear sucker sex pheromone for pest monitoring

US workers have reported that for *C. pyricola* the female sex pheromone is best extracted by making whole body washes in hexane and the long-chain hydrocarbon, 13-methylheptacosane (13Me27:H) has been proposed to be the major pheromone component. Three years of air entrainment and hexane body wash analyses, of both winterform and summerform *C. pyri*, and testing 13Me27:H for attractiveness to *C. pyri* in field trials have resulted in no positive attraction of either males or females to sticky traps.

Analyses of washes from males and females showed no compounds that were present in one but not in the other sex, nor were there any significant differences in relative amounts of compounds. This was true for both winterform and summerform insects. Most of the compounds were identified as *n*-alkanes, 2- and 3-methylalkanes and long chain aldehydes.

There were, however, significant quantitative differences between the profiles from winterform and summerform insects with the relative amounts of the *n*-alkanes and aldehydes higher in the latter. 13Me27:H was detected as a minor component in all the body washes. In the winterform *C. pyri* there was a slightly higher percentage in those from males and in the summerform there was slightly more in those from females and it is considered unlikely that this is a pheromone component in *C. pyri*.

No attraction of the opposite sex of has *C. pyri* been demonstrated in the field using 13Me27:H 1 mg mL^{-1} in lures or unmated males or females as bait. Hexane washes of females also failed to attract male *C. pyri* males. In addition no consistent EAG responses were observed when males were exposed the proposed pheromone of *C. pyricola* 13Me-27:H or to hexane washed of *C.pyri* females. Male *C. pyri* showed consistent EAG response to decanal. In 2011 we tested lures provided by the US scientists who claim to attract *C. pyricola* males but we could not demonstrate attraction of either *C. pyri* or *C. pyricola*.

Objective 2. Develop conservation biocontrol methods to maximise anthocorid populations and other natural enemies of pear sucker in spring

In 2008 and 2009, established and diverse hedges adjacent to pear orchards were sampled through the season to characterise their predator and prey communities. A database of >70,000 specimens was collected and many identified to species. *C. pyri* was found to be the dominant (> 90%) pear

sucker species in most, but not all orchards. When the species compositions were last characterised in the 1980s, *C. pyricola* was universally the dominant species in the UK. This finding could have important implications for the management of pear sucker in commercial orchards in the UK.

Psyllid larvae, on which *Anthocoris nemoralis* feeds, were more numerous on trees very early in the season, with egg laying and nymphs occurring from February onwards. *A. nemoralis* is the dominant anthocorid predator of pear sucker. It is adapted to feed on psyllids (eggs and nymphs). *A. nemorum* also occurs on pear, particularly later in the season.

Plant species that had psyllid nymphs early in the season (February – April) were most likely to support *A. nemoralis* early on and be good sources of these predators for pear sucker control. During the early season the highest numbers of anthocorids were found on grey and goat willow, hawthorn and nettle. However, hawthorn may not be a favoured choice because it is susceptible to fireblight and can be a source of several pear pests, including the common green capsid. Stinging nettle may also be a source of common green capsid.

Tree species that support aphids will provide large numbers of *A. nemorum* later in the season. Aphids become more numerous from May until July. The highest numbers of aphids were found on birch, hazel and nettle. Inclusion of one or more woody aphid hosts may be beneficial as a source of late season *A. nemorum*. Nettles were host to the nettle psyllid, *Trioza urticae*, which is abundant throughout the year in the adult stage and, thus, a good source of *A. nemoralis* early in the season. It also has abundant aphids when growth commences in spring and *A. nemorum* is abundant later in the season.

In 2009, established hedgerows were sampled on 8 occasions through the season. A database of specimens was collected and the most important insect groups identified to species. Pussy willow (*Salix caprea*), hazel (*Corylus avellena*) and nettle (*Urtica dioica*) were found to be the best potential species for conservation biocontrol of pear sucker.

Sampling of the replicate tree species hedgerow plots planted in spring 2008 was done in 2010 and 2011. In 2010 a database of 5753 arthropods was made. Numbers of anthocorids collected were rather small and erratic. The seasonal dynamics of the key species have been determined, providing valuable information for exploitation for conservation biocontrol. The trees were only in their third season of growth and the characteristic aphid, psyllid and predator fauna associated with each subject had only just starting to establish. Nettles had established strongly at all three sites and were tall and

the abundant arthropod fauna of nettles was present on many subjects.

Bottle refuges were found to be poor overwintering sites for anthocorids, however, many spiders used them. In addition two years of hedgerow leaf litter collection and Tullgren funnel extraction resulted in no anthocorids being found. Anthocorids were however, recovered from pear tree tap samples in February suggesting that at least some anthocorid overwinter in the orchards.

Purpose sown flowering ground herbage in an organic pear orchard near Hereford was found to contain no anthocorids or psyllids and only a few aphids in May and July and thus appeared to have little benefit for conservation biocontrol of pear sucker.

An experiment using protein (milk and egg white) markers and monoclonal antibody detection methods demonstrated low levels of dispersal of anthocorid adults from a border strip nettle into an adjacent pear orchard. Numbers were small and no obvious difference between nettles cut to the ground and uncut was apparent. Dispersal occurred for distances > 50 m. In 2011 an additional experiment again showed that dispersal could occur within a pear orchard over at least 50 m.

Objective 3. Exploit synomones for attracting anthocorids into pear orchards

Volatiles were collected from trees infested with *C. pyri* adults during January-February, the nymphs or adults on potted trees during June-August and from individual adults on pear shoots during August 2009. Little or no material was present in collections made during January-February. Collections from nymphs on potted trees during 15-23 June showed significant quantities of 2-phenylethanol and little or no α-farnesene or methyl salicylate. Subsequently, when adults were put on potted trees from 25 June onwards, only methyl salicylate was observed. This suggested 2-phenylethanol as a good candidate for involvement in attraction of anthocorid predators to pear trees infested with pear sucker.

In field experiments there was no evidence to suggest that anthocorids were attracted by the plant volatiles used at the rates tested; very few were caught in traps and low numbers were collected in tap samples from trees containing the lures. There was also no suggestion that psyllid numbers were reduced in any treatment; if this had been recorded it might suggest that anthocorids or other predators had been attracted to the trees but were not being caught in the traps or tap samples. Hoverflies were attracted to methyl salicylate.

In 2011 more *Orius* predators were attracted by the volatile lavandulyl senecioate. No significant EAG responses were recorded from *A. nemoralis* to a range of compounds found in analyses of volatiles

from pear trees attacked by pear sucker, *C. pyri.* In 2011 consistent EAG responses of *A. nemoralis* were obtained to decanal and methyl salicylate, and responses were sometimes observed to (Z)-3-hexenyl acetate and 2-phenylethanol.To date, we have not been able to demonstrate attraction to anthocorids to the compound identified in this project or in previous Dutch work, either singly or in mixtures.

Objective 4. Efficacious, physically-acting spray treatment that is safe to anthocorid predators

Bioassays of young pear psyllid nymphs on leaves and on leaf discs indicated that even at eight times the rates commonly recommended for use in the field magnesium sulphate without a wetter did not significantly reduce psyllid numbers. At eight times the field rate psyllid numbers were significantly reduced in the sulphur treatment. Activator 90 at two-eight times field rate significantly reduced psyllid numbers. The addition of Activator 90 to sulphur and magnesium sulphate did not enhance the effect of these two products.

An effective bioassay technique was developed that caused minimal mortality to nymphs of the predator *Orius laevigatus* in control treatments. A combination of Activator 90, Sulphur SC and magnesium sulphate showed no detrimental effect on *A. nemoralis* in topical dosing bioassays. In addition no effect was seen on egg hatch of either *A. nemoralis* or *O. laevigatus*.

A 2011 field spray experiment showed that Silwet (a drift retardant/spreader/wetter) and BreakThru (a wetter) were effective at reducing the numbers of first and second instar nymphs. Activator 90 (a non-ionic surfactant/wetter) also reduced nymph numbers. In this experiment total numbers of anthocorids increased across the experimental area. In a gauzehouse experiment a single spray of the treatments Sulphur SC, Activator 90, magnesium sulphate, either alone or in combination, was not as effective at reducing the levels of pear psyllid nymphs as Dynamec.

Spray trials with Surround (kaolin) reduced numbers of pear sucker nymphs by over 75% and showed good promise for the control pear sucker early on in the season (pre bud burst).

Objective 1. Identify and exploit the pear sucker sex pheromone for pest monitoring

Introduction

US workers showed that winterform males of *C. pyricola* were attracted to pear shoots infested with post-diapause female psylla (Horton and Landolt, 2007; Horton et al., 2007), and Guedot et al. (2009) confirmed that this was due to volatiles from the insects rather than from the plants. In these experiments mated females were as attractive as virgin females, freshly-killed females were as attractive as live females and there was also evidence for male-male repellency (Horton et al., 2008; Guedot et al., 2009). These laboratory studies were confirmed in field trapping experiments by Brown et al. (2009).

In the first year of this project it was demonstrated that both *C. pyricola* and *C. pyri* are found in UK orchards with the latter tending to predominate. During the second year efforts were focussed on identifying a female sex pheromone for *C. pyri*. Volatiles were collected from psylla in the laboratory and field and hexane body washes were also made. Analyses of the various collections by GC-MS showed no apparent differences between those from males and those from females. No responses were detected from male *C. pyri* when volatile collections from female insects were analysed by GC linked to EAG recording.

In bioassay studies on *C. pyricola*, hexane body washes of females were shown to be as attractive to males in a Y-tube olfactometer as live female insects (Horton et al., 2008; Guedot et al., 2009). Recently, Guedot et al. (2010) reported 13-methylheptacosane (13Me27:H) to be the female sex pheromone of *C. pyricola*. This was based on comparison of analyses of hexane body washes from females and males.

During the third year of the project efforts were focussed on repeating the analyses of hexane body washes of both winterform and summerform *C. pyri* and on testing 13Me27:H for attractiveness to *C. pyri* in field tests. However, analyses of washes from males and females showed no compounds that were present in one but not in the other, or any significant differences in relative amounts. This was true for both winterform and summerform insects. Most of the compounds were identified as *n*-alkanes, 2- and 3-methylalkanes and long chain aldehydes. There were, however, significant quantitative differences between the profiles from winterform and summerform insects with the relative amounts of the *n*-alkanes and aldehydes higher in the latter. 13Me27:H was detected as a minor

component in all the body washes. In the winterform there was a slightly higher percentage in those from males and in the summerform there was slightly more in those from females and it is considered unlikely that this is a pheromone component in *C. pyri*.

In 2011 it was decided to test the US manufactured lures for *C. pyricola* in the UK. Also EAG responses of C. pyri were re-investigated to volatiles from conspecific insects and to synthetic compounds including 13Me-27:H.

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Horton, D.R., Guedot, C. and Landolt, P.J. 2008. Attraction of male summerform pear pyslla to volatiles from female pear psylla: effects of female age, mating status, and presence of host plant. *Canadian Entomologist*, 140:184-191.

Sub-objective 1.1. To identify the sex pheromone of pear sucker, Cacopsylla pyricola

Task 1.1.1. Establish C. pyricola rearing methods (EMR, Yr 1)

This has been done throughout the project using whole trees in glasshouses.

Task 1.1.2. Collect volatiles (EMR, Yrs 1, 2)

Done throughout project

Task 1.1.3. Conduct chemical analyses of collections (NRI, Yrs 1, 2)

Done throughout project

Task 1.1.4. Conduct GC-EAG (NRI, Yrs 1,2)

In 2011 further EAG studies have been completed. Pear psyllids were used to assess responses to potential pheromones and also to assess responses to volatiles that have been used in the field experiments as part of *Objective 3. Exploit synomones for attracting anthocorids into pear orchards.*

Materials and Methods

Gas chromatography coupled to electroantennographic recording (GC-EAG)

GC-EAG Analyses were carried out on a polar GC column (DBWax, Supelco; 30 mm x 0.32 mm i.d. x 0.25 µm film thickness) with oven temperature held at 50°C for 2 min then programmed at 10°C/min or 20°C/min to 240°C. The column effluent was split (1:1) with equal lengths of deactivated fused silica tubing leading to the flame ionisation detector and to a glass T-piece in the column oven. The contents of the T-piece were continuously flushed over the EAG preparation with humidified air (300 ml/min).

EAG recordings were made with a portable device consisting of micromanipulators, electrode holders and amplifier (INR-02; Syntech, The Netherlands) connected to the GC (HP6890, Agilent) as a second detector. Electrodes were fine glass capillaries filled with saline (0.1M KCl with 1% polyvinylpyrrolidine) and placed over silver wire electrodes. Various techniques were investigated for making EAG preparations, including whole body preparations and excising the head and inserting the base electrode into the neck; after excising the proboscis and one antenna to reduce mechanical and electrical interference, the end was removed from one antenna and inserted into the recording electrode.

Data from both EAG and GC were collected and process with EZChrom Elite software.

Results

In GC-EAG analyses of synthetic compounds, male *C. pyri* showed a consistent EAG response to decanal and generally also responded to α-farnesene and methyl salicylate (Figure 1.1.4.1). No response was observed to the proposed pheromone of *C. pyricola*, 13Me-27:H.

When collections of volatiles from female *C. pyri* were analysed by GC-EAG with a male *C. pyri* preparation, responses were observed to small amounts of the aldehydes octanal, nonanal and

decanal and to methyl salicylate (Figure 1.1.4.2). These aldehydes are often observed in collections of volatiles made on Porapak and elicit EAG responses from a wide range of insects even though then have no behavioural activity (unpublished observations). A remarkably sensitive EAG preparation gave large responses (> 1mV) to the aldehydes and also responses to (E,Z)-2,6-nonadienal, α-farnesene and methyl salicylate, presumably derived from the pear leaves in with the insects (Figure 1.1.4.3).

GC-EAG analyses of hexane washes of winterform or summerform *C. pyri* females showed no significant EAG responses from the antennae of males. Responses to the aldehydes confirmed that the preparation was responding (Figure 1.1.4.4).

GC-EAG analyses of synthetic compounds with a female *C. pyri* preparation showed responses to nonanal and decanal and also to DMNT, α-farnesene and methyl salicylate, but not (*Z*)-3-hexenyl acetate and 2-phenylethanol (Figure 1.1.4.5).

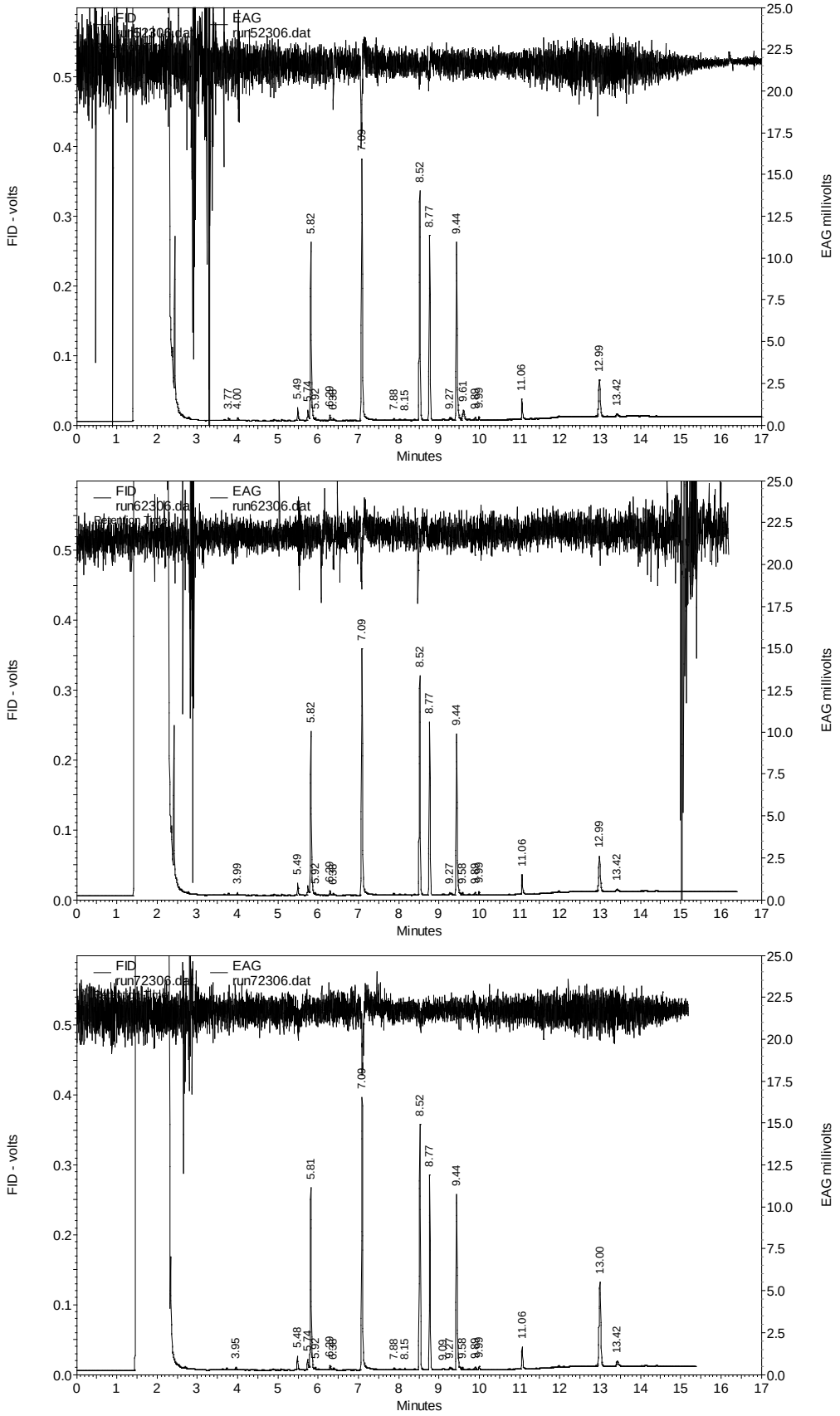
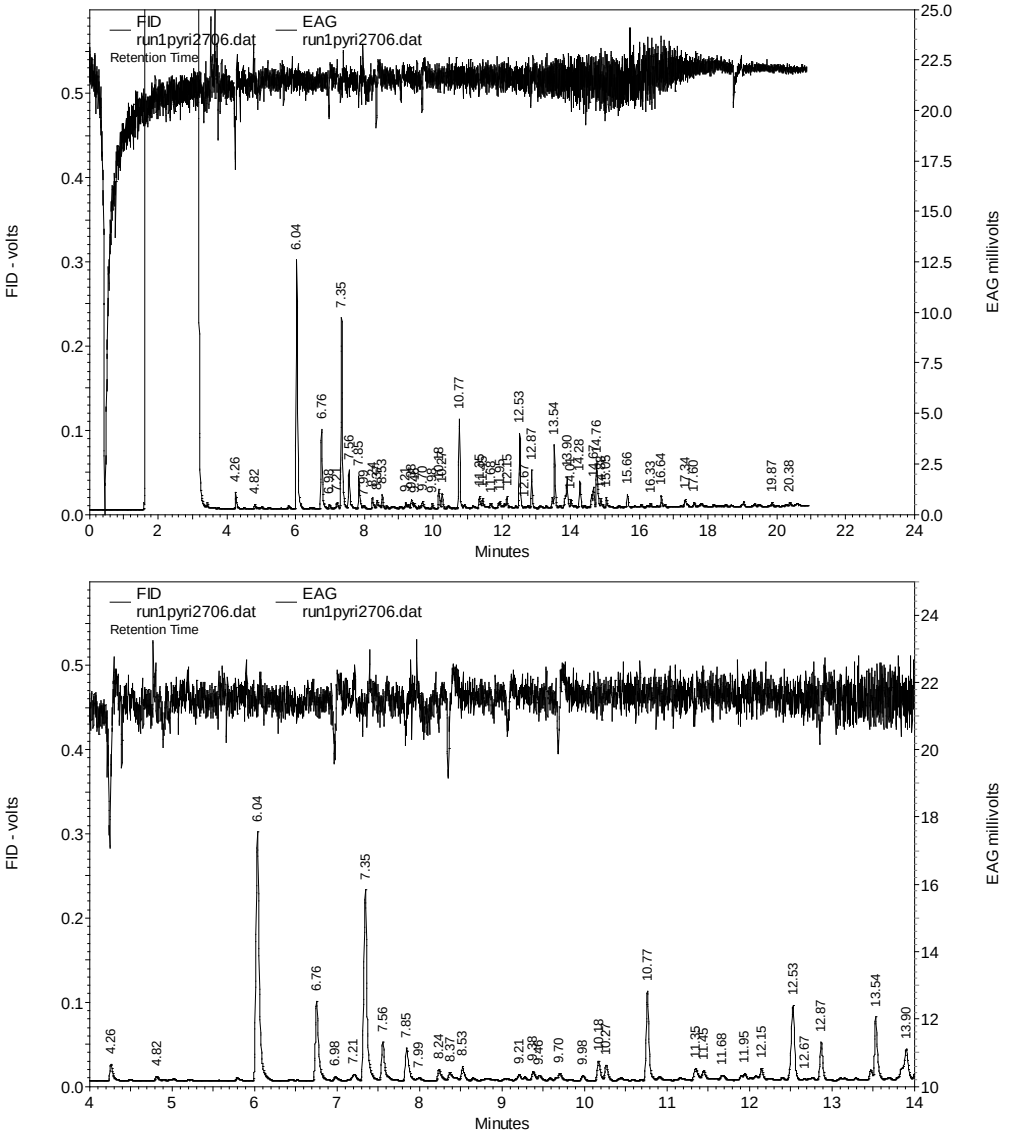


Fig. 1.1.4.1. GC-EAG analyses of standards (100 ng) and 13Me-27:H with male *C. pyri* showing response to

decanal at 7.09 min α-farnesene (8.52 min) and methyl salicylate (8.77 min) and no EAG response to 13Me-27:H at 12.99 min (other compounds (Z)-3-hexenyl acetate (5.82 min), and 2 phenylethanol 9.44 min).



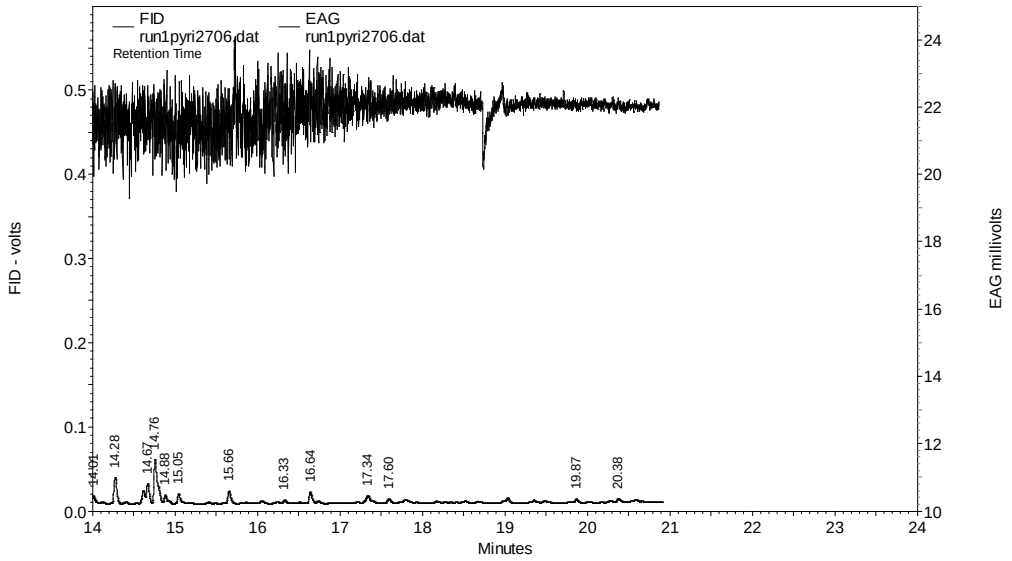
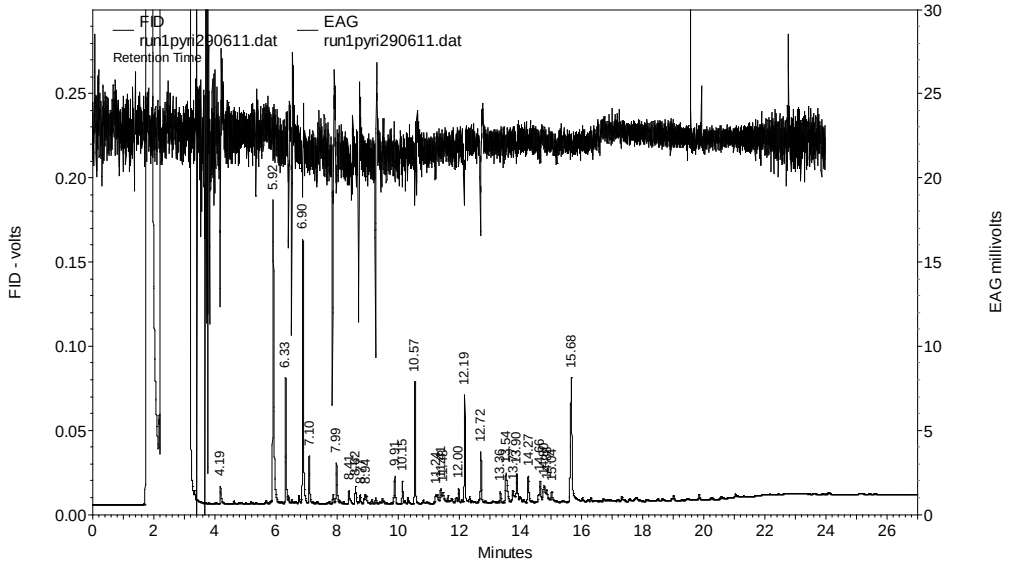


Fig. 1.1.4.2. GC-EAG analysis with male *C. pyri* preparation of volatiles from 222 females (octanal 6.98 min, nonanal 8.37 min, decanal 9.70 min, methyl salicylate 12.87 min)



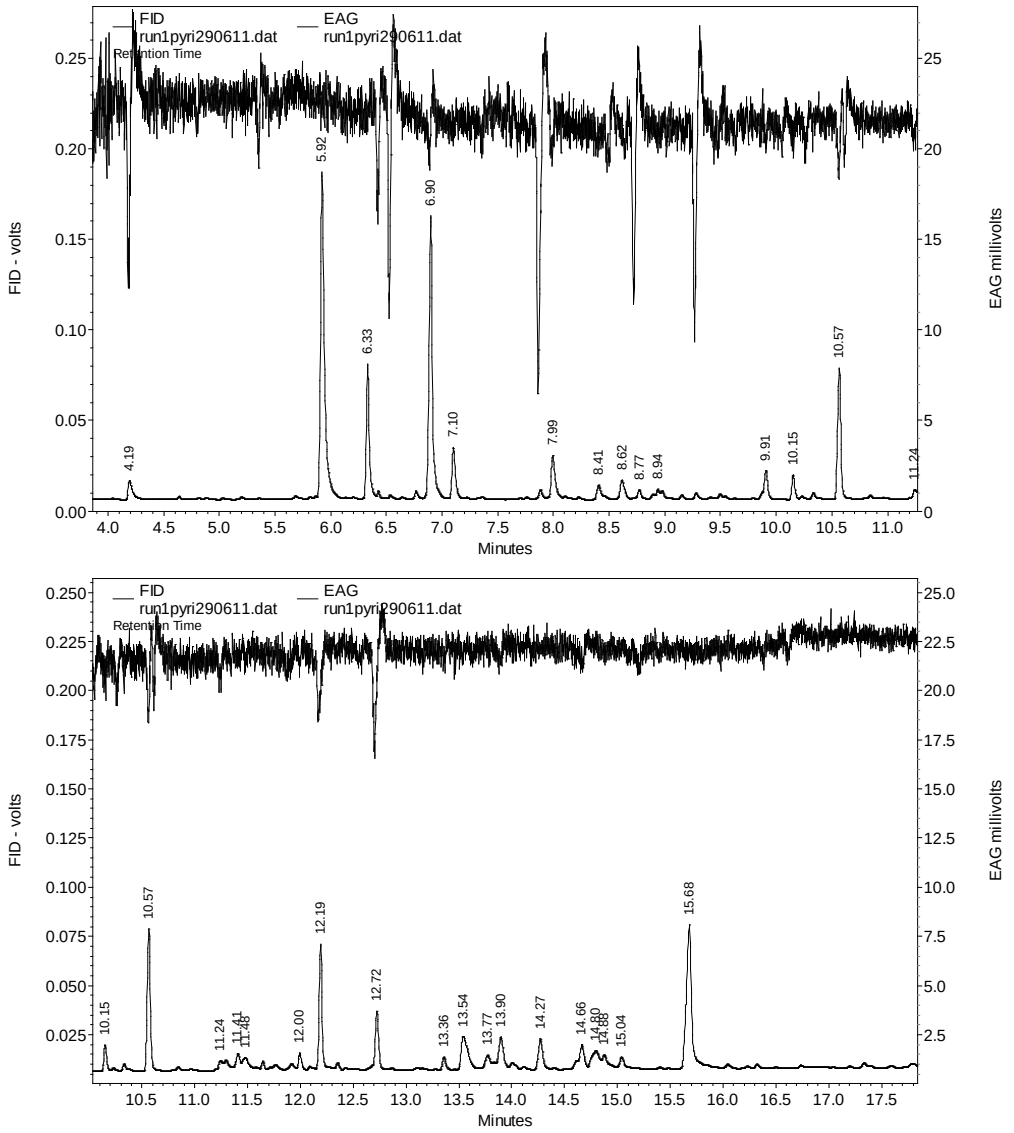


Fig. 1.1.4.3. GC-EAG analysis with male *C. pyri* preparation of volatiles from 222 females (octanal 6.53 min, nonanal 7.88 min, decanal 9.26 min, (E,Z)-2,6-nonadienal 10.57 min, α-farnesene 12.19 min, methyl salicylate 12.72 min)

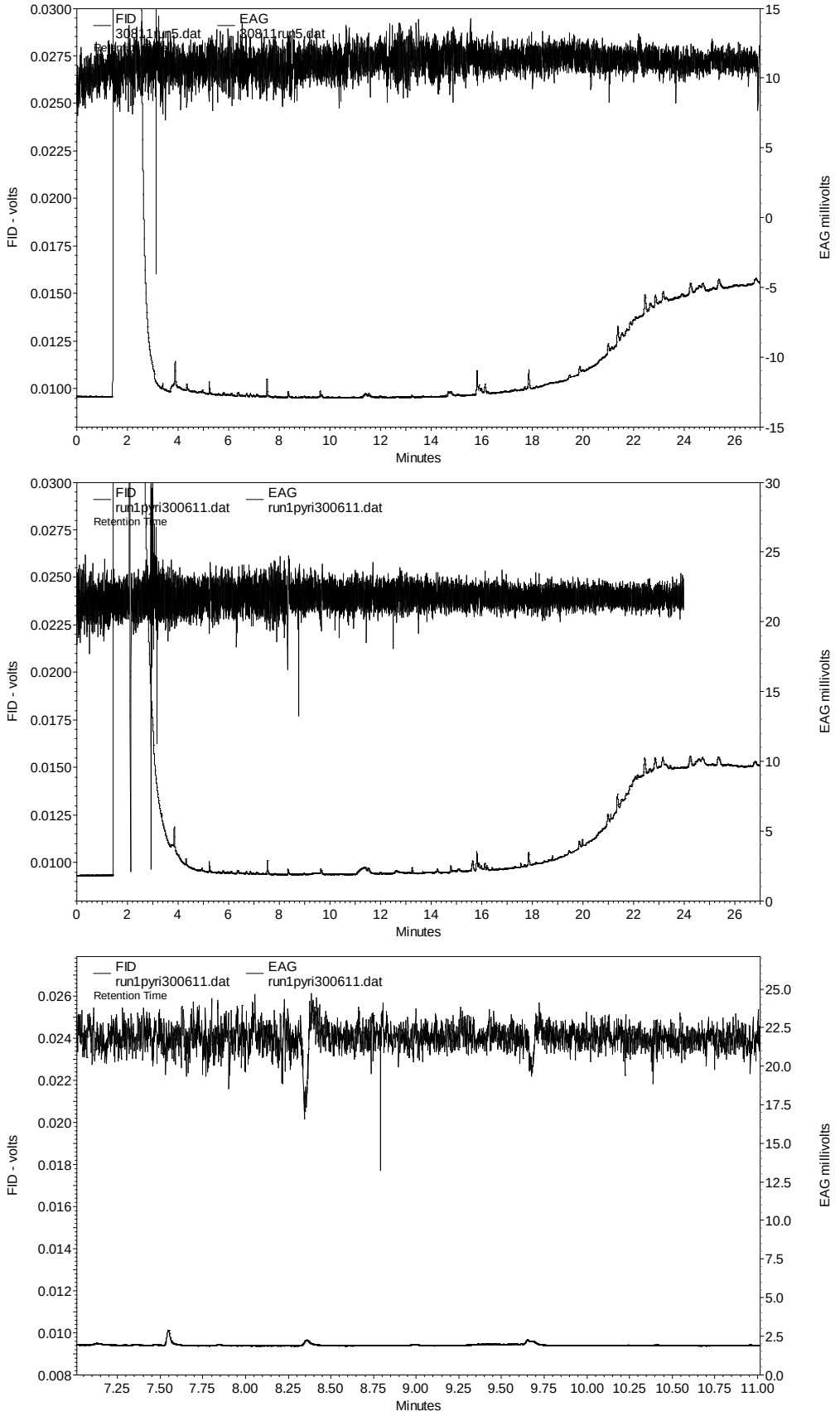


Fig. 1.1.4.4. GC-EAG analysis with male *C. pyri* preparation of hexane wash from winterform females (upper), hexane

wash from summerform females (middle) and expansion of latter showing responses to octanal at 8.35 min and nonanal at 9.68 min.

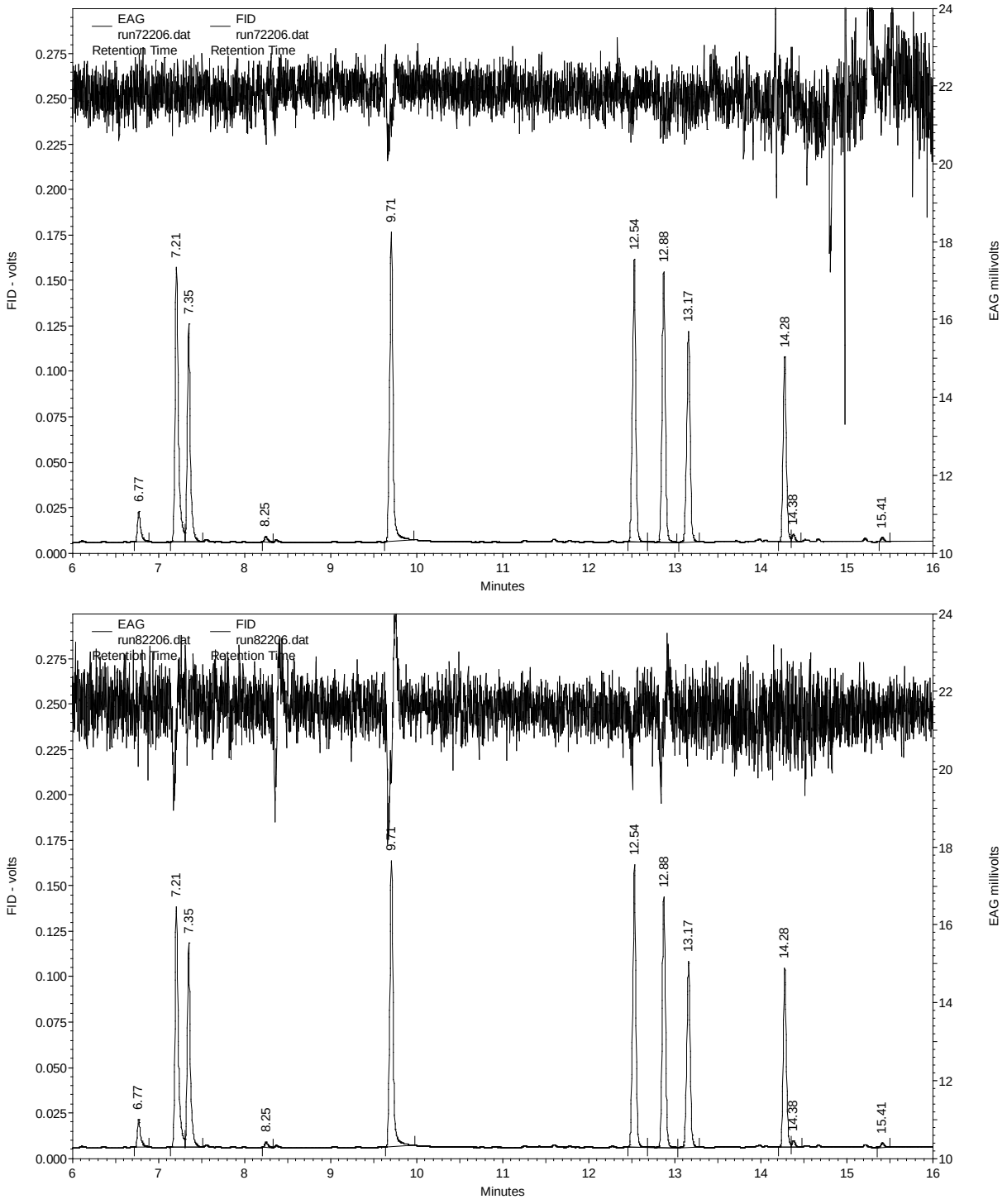


Fig. 1.1.4.5. GC-EAG Analyses of synthetic compounds with female *C. pyri* EAG preparation showing responses to DMNT (7.21 min), nonanal (8.25 min), decanal (9.71 min), α-farnesene (12.54 min) and methyl salicylate (12.88 min) (other compounds (*Z*)-3-hexenyl acetate 7.35 min and 2-phenylethanol 14.28 min).

Conclusions

In GC-EAG analyses, male *C. pyri* showed no EAG response to 13Me-27:H, the proposed pheromone of *C. pyricola*, although good responses were obtained to straight-chain aldehydes confirming that the preparations were responsive. Decanal was tested specifically, but small amounts of octanal and nonanal were also detected as impurities from solvents or Porapak in other samples. These aldehydes are often found in collections of volatiles and elicit EAG responses from a wide range of insects even though then have no behavioural activity (unpublished observations).

Male *C. pyri* also showed no responses to hexane washes from winterform or summerform female *C. pyri*. In GC-EAG analyses of volatiles collected from female *C. pyri*, responses were observed to (E,Z)-2,6-nonadienal, α-farnesene and methyl salicylate which are from the leaves present with the insects.

GC-EAG analyses of synthetic compounds with a female *C. pyri* preparation showed responses to nonanal and decanal and also to DMNT, α-farnesene and methyl salicylate, but not (*Z*)-3-hexenyl acetate and 2-phenylethanol.

Thus no likely candidate compounds for components of a female sex pheromone were detected in *C. pyri*, although EAG responses were obtained from both males and females to plant volatiles (E,Z)-2,6-nonadienal, α-farnesene and methyl salicylate.

Task 1.1.5. Determine and synthesise chemical structures (NRI, Yrs 1, 2)

Task 1.1.6 (if required). Develop pheromone bioassays (EMR, NRI, Yrs 2, 3)

Sub-objective 1.2. Demonstrate pheromone activity, develop a lure and trapping system, and calibrate in the field.

Task 1.2.1. Prepare suitable dispensers (NRI, Agrisense Yrs 2, 3)

Task 1.2.2. Demonstrate attractiveness and optimise lure and trap (EMR, Agrisense Yrs 1-3)

Task 1.2.3. Calibrate for pest monitoring purposes (EMR, Agrisense Yrs 3, 4)

Task 1.2.4. Prepare protocol for trap use by growers (EMR, Agrisense Yr 4)

Materials and Methods

In April 2011 we were supplied with 100ug of 13-methylheptacosane pre-loaded septa for field trapping pear psylla (Cacopsylla pyricola). Control lures were also supplied, containing pentane. 25 lures of each were supplied by Christelle Guédot (USDA-ARS, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA 98951, Phone: (509) 454-4446, Fax: (509) 454-5646, christelle.guedot@ars.usda.gov). The lures were tested at sites with both species of pear sucker (*C. pyricola* and *pyri)*.

Site 1. Clive Baxter, J L Baxter & Son, Westerhill Farm, Westerhill Lane, Linton, Maidstone, Kent ME17 4BS cv. Conference pear orchard, Churchfield, West Farleigh (OS ref:532 734).

Site 2. David Long, Marshgate Farm, Main Rd, Cooling, Rochester, Kent ME3 8DP.

Site 3. Mr Henry Rudge, H & E Rudge, Ballingham Hall Farm, Ballingham, Hereford HR2 6NH.

Treatments were grey rubber septa lures with either the sex pheromone (100 ug of 13-methylheptacosane) or blanks (pentane). These were tested in the field on a 30 x 30 cm, 1-2 mm, insect mesh (screen) (Fig 1.2.1). The screens had a length of bamboo cane heat sealed on two sides with wire so that they can be suspended within or between trees. They were coated with Ecotac by pressing the mesh onto a white tray coated with the glue. One septa was attached with a paper clip to the centre of the trap after the trap had been suspended from two branches within the tree canopy

Figure 1.2.1. Mesh sticky trap suspended in tree with septa lure in the centre of the trap

Every other trap in a row was a pheromone trap. There were 8 replicates of the 2 treatments at each site. Traps were more than 10 m apart.

Bags were wrapped in Clingfilm and returned to the laboratory. The numbers, sex and species of pear sucker were recorded in the laboratory.

Results

Between 11-18 May, at sites 1 and 2 (*C. pyri* dominated), there was no significant difference between the numbers of males or females trapped on the sex pheromone lure or control traps (Fig. 1.2.2). Between 25 May-7 June, at site 3 (*C. pyricola* dominated), there was no significant difference between the numbers of males or females trapped on the sex pheromone lure or control traps (Fig. 1.2.3). However, square root transformed data revealed that significantly more male *C. pyricola* were trapped on the control traps compared to the pheromone traps (P= 0.039, sed = 0.379, lsd 0.857). This result is surprising as the pheromone is reported to attract males. However, the number of males captured was low with a maximum of only 12 on a single trap the 2 week the trial was out (Fig. 1.2.3).

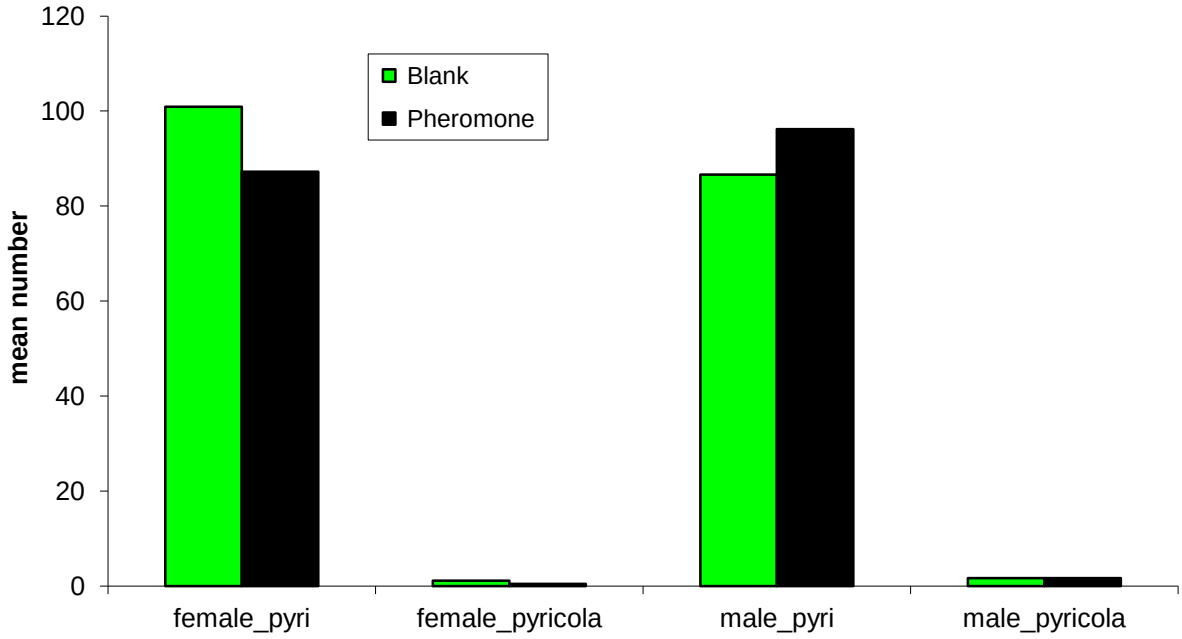


Figure 1.2.2. Numbers of male and female pear sucker trapped on the pheromone and blank (control) traps.

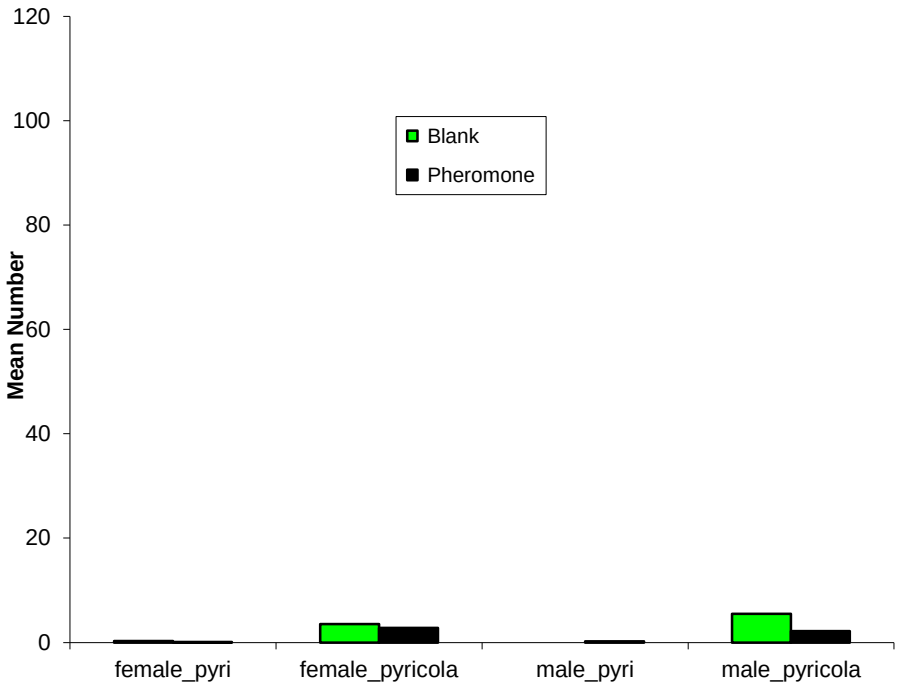


Figure 1.2.3. Numbers of male and female pear sucker trapped on the pheromone and blank (control) traps at the Hereford site.

Objective 2. Develop conservation biocontrol methods to maximise anthocorid populations and other natural enemies of pear sucker in spring

Sub-objective 2.1 Identify woody species and species mixes for hedgerows / windbreaks

Task 2.1.1. Plant, establish and manage experimental hedgerows (G H Dean, H Chapman, H Rudge Yrs 1-4)

Task 2.1.2. Survey existing hedgerows/windbreaks and identify and characterise 5 with a range of species compositions and structures to compliment purpose planted hedgerows (task 2.1.1) (EMR, WWF, grower partners, Yr 1)

Task 2.1.3. Sampling of hedgerows/windbreaks and adjacent pear crops (from 3.1.1. and 3.1.2.) for spring and summer predator and prey communities (EMR, WorldWideFruit, H L Hutchinson, UAP all years)

Introduction

Anthocorid predatory bugs are the key natural enemies of pear sucker but they often migrate into orchards too late to effect adequate natural control of pear sucker populations. The aim of this study is to determine the suitability of different native woody plant species for growing in hedgerows round pear orchards to maximise anthocorid populations in spring and foster their migration into pear orchards when pear sucker populations start to increase.

At each of three sites, in 2008, a new experimental hedgerow was planted comprising two replicate 10 m plots of different candidate woody species. Beat sampling of each plot and the adjacent pear orchard was done at three-four week intervals from the end of March to September 2010 to establish the pattern of natural enemy and prey communities on each plot and on the adjacent pear orchard.

Sites

New hedgerows comprising replicate plots of different woody species were erected in early spring 2008 at the following three sites.

Site 1: Rodmersham Court Farm, Rodmersham, Kent (kind agreement of Oliver Doubleday): Hedge length = 220 m

Site 2 Ballingham Hall Farm, Ballingham, Hereford (kind agreement of Henry Rudge)
Hedge length = 200 m

Site 3: Broadwater Farm, West Malling, Kent (kind agreement of Peter Checkley*)*

Hedge length = 160 m

Woody species evaluated are given in Table 2.1.3.1 with a plot plan for each site in Table 2.1.3.2.

Table 2.1.3.1. Woody species planted at each site in spring 2008 (note: plant spacing = 0.33 m)

Common name	**Species**	**Site (s)**
Ash	*Fraxinus excelsior*	1,2
Grey willow	*Salix cinerea*	1,2
Birch	*Betula pendula*	1,2
Blackthorn	*Prunus spinosa*	2,3
†	*Alnus glutinosa*	1,2,3
Elder	*Sambucus nigra*	1,2
Field maple	*Acer campestre*	1,3
Goat willow†	*Salix caprea*	1,2,3
Hazel	*Coryllus avellana*	1,3
Hawthorn	*Crataegus monogyna*	1,3
Lime	*Tilia cordata*	2,3
Norway maple	*Acer platanoides*	1,2

† Internal standard to be planted at every site

Table 2.1.3.2. Plot plan

Rodmersham Court (220 m)			Ballingham Hall (200 m)			Broadwater Farm (160 m)		
Plot no.	**Species**	**Block**	**Plot no.**	**Species**	**Block ‡**	**Plot no.**	**Species**	**Block**
1	Hazel	1	1	Elder	1	1	Hawthorn	1
2	F maple	1	2	Alder	1	2	Blackthorn	1
3	Grey willow	1	3	Mix†	1	3	Mix†	1
4	Alder	1	4	Ash	1	4	Lime	1
5	N maple	1	5	N maple	1	5	F maple	1
6	Ash	1	6	Birch	1	6	Alder	1
7	Goat willow	1	7	Blackthorn	1	7	Goat willow	1
8	Birch	1	8	Goat willow	1	8	Hazel	1
9	Elder	1	9	Lime	1	9	Lime	2
10	Mix†	1	10	Grey willow	1	10	Blackthorn	2
11	Hawthorn	1	11	Mix†	2	11	Goat willow	2
12	Goat willow	2	12	Lime	2	12	Hawthorn	2
13	Hawthorn	2	13	Goat willow	2	13	F maple	2
14	Ash	2	14	Grey willow	2	14	Hazel	2
15	N maple	2	15	N maple	2	15	Alder	2
16	Grey willow	2	16	Ash	2	16	Mix†	2
17	Birch	2	17	Alder	2			
18	Alder	2	18	Birch	2			
19	Hazel	2	19	Blackthorn	2			
20	Mix†	2	20	Mix	2			
21	F maple	2	21	Elder	2			
22	Elder	2						

† A random mix of all the species in the hedge

Each plot was sampled to characterise predator communities, especially anthocorids. Populations of key prey species including aphids and psyllids that are present on the woody hosts were quantified. The counting and identification was done in the field on the day of sampling. A 0.25 m^2 beating tray was used for beat sampling the plots and the adjacent pear orchard at each site. The number of beats per plot was 10 (1 per meter). After each five beats, the numbers of each target insect in the beating tray were counted and recorded.

Results

In 2010 a data base of 5753 arthropods was constructed. However, numbers of anthocorids collected were rather small. For the 2011 (3173 arthropods) data the hedgerow species were ranked in order of most predators (Figure 2.1.3.1) and ranked according to the species with the most predatory arthropod classes (Table 2.1.3.3). There were more predatory classes of arthropods on Blackthorn (5) and Hazel (5) > Hawthorn (4) and Pear (4) > Field maple (3) > Elder (2) and Goat willow (2) > Birch (1)

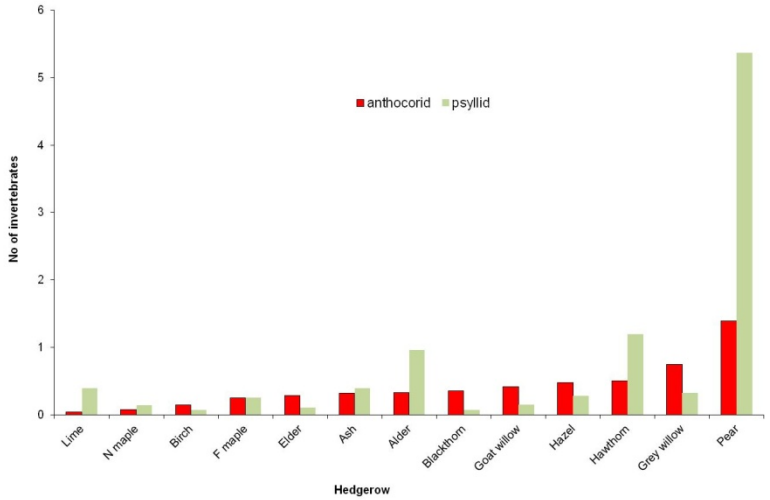
Alder (1), Grey willow (1), Lime (1) and Ash (1). However, the highest numbers of anthocorids were on pear>grey willow>hawthorn>hazel>goat willow>blackthorn. Lime, Norway maple and birch were generally the poorest performers in terms of predatory arthropods. Beat samples showed psyllid adults were most abundant on the pear trees in the adjacent orchard, with comparatively small numbers on the other hedgerow species (Figure 2.1.3.1). Aphids were very abundant on birch > field maple> hazel and blackthorn.

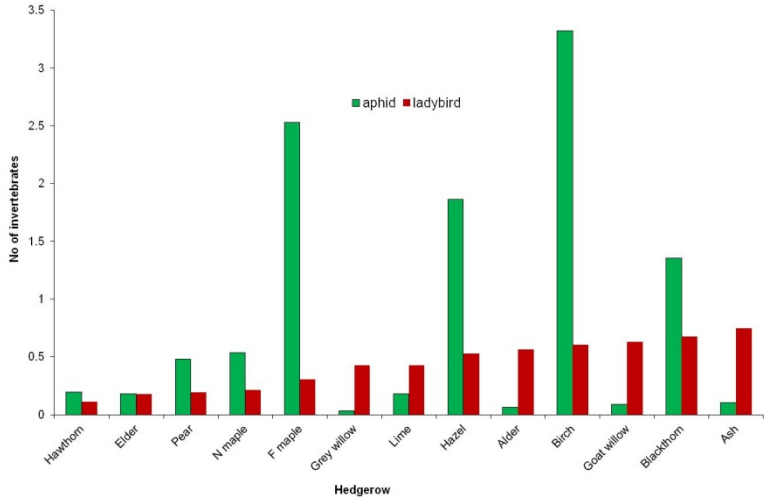
In the detailed species identification of arthropods in 2008 and 2009 it was found that the plant species that seem to be extremely reliable sources of anthocorids are those which have one or more species of aphids which have a small body size, live on the underside of the leaves, are able to reach high numbers and colonise the plants at any time and with a population dynamic less dependent on ants. These plants, and their associated aphids, are:

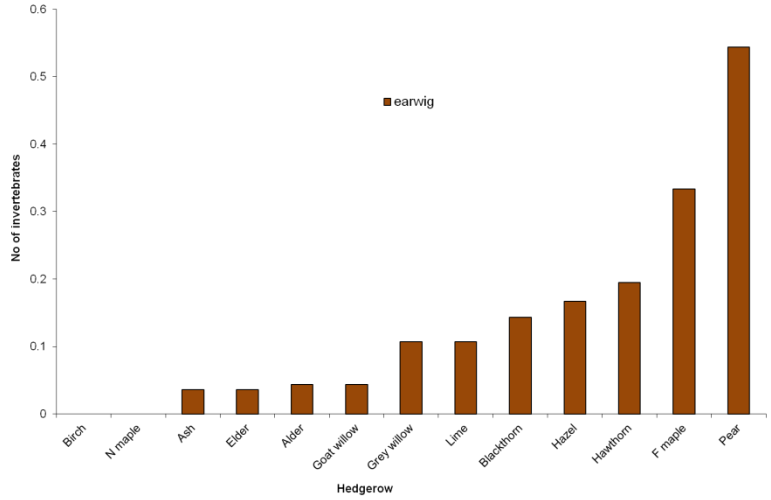
Plant common name	**Plant species**	**Aphid species**
Grey willow	*Salix cinerea*	*Chaitophorus* sp.
Common alder	*Alnus glutinosa*	*Pterocallis alni*
Goat willow†	*Salix caprea*	*Chaitophorus* sp.
Hazel	*Coryllus avellana*	*Myzocallis coryli*

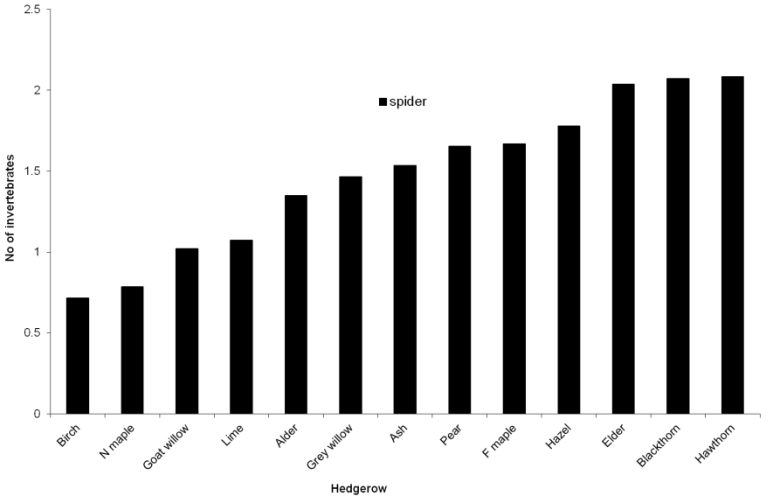
Other potentially good sources are the plants with ant visited aphid species. These aphids cannot reach large population sizes without ants:

Plant common name	**Plant species**	**Aphid species**
Field maple	*Acer campestre*	*Periphyllus testudinaceus*
Norway maple	*Acer platanoides*	*Periphyllus testudinaceus*
Hawthorn	*Crataegus monogyna*	*Aphis pomi*
Blackthorn	*Prunus spinoza*	*Brachicaudus helichrysi*
Common pear	*Pyrus communis*	*Aphis pomi*
Goat willow	*Salix caprea*	*Aphis farinosa*
Grey willow	*Salix cinerea*	*Aphis farinosa*
Elder	*Sambucus nigra*	*Aphis sambuci*









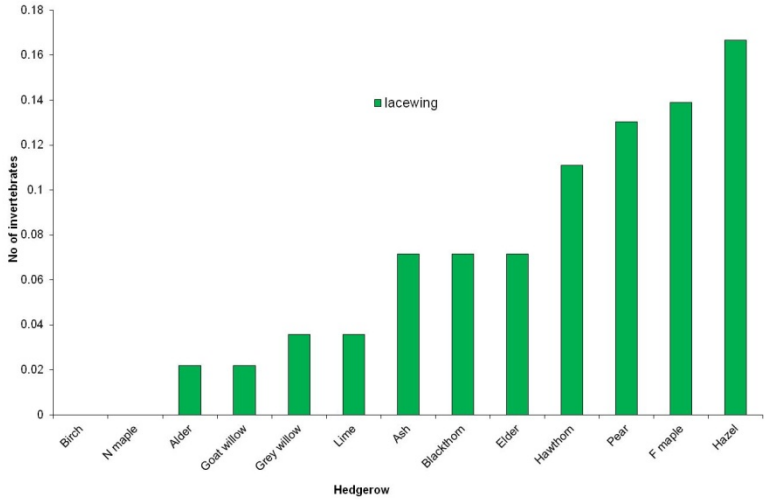
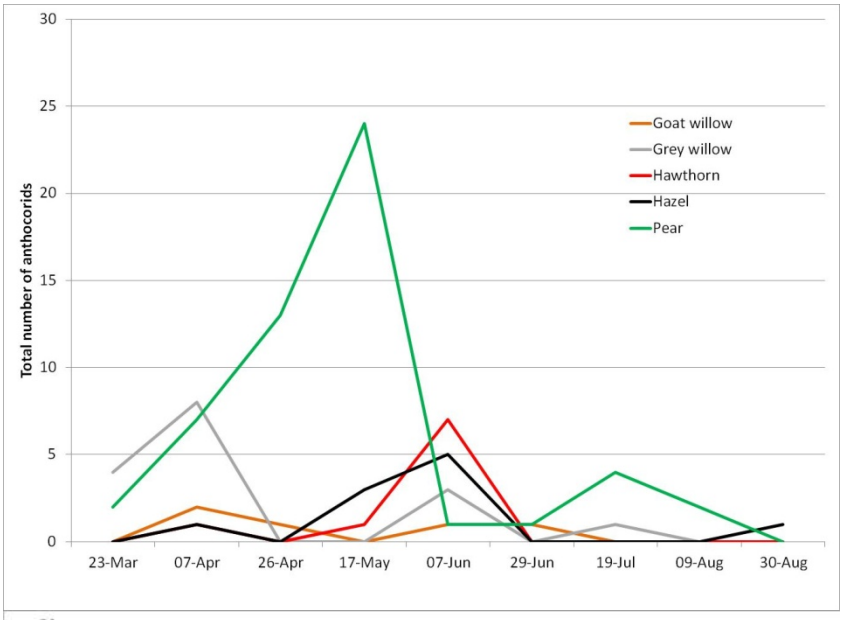


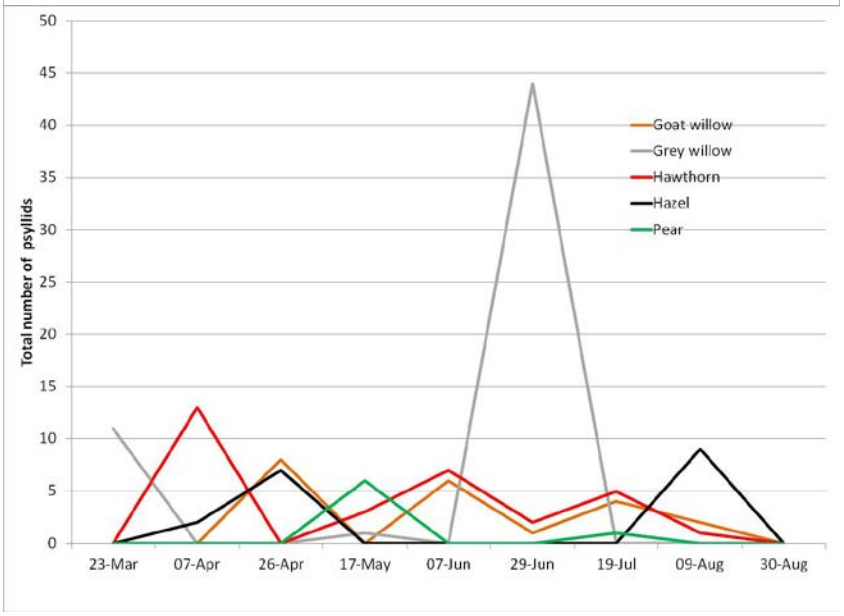
Figure 2.1.3.1. Hedgerow species ranked in order of most abundant predatory arthropods.

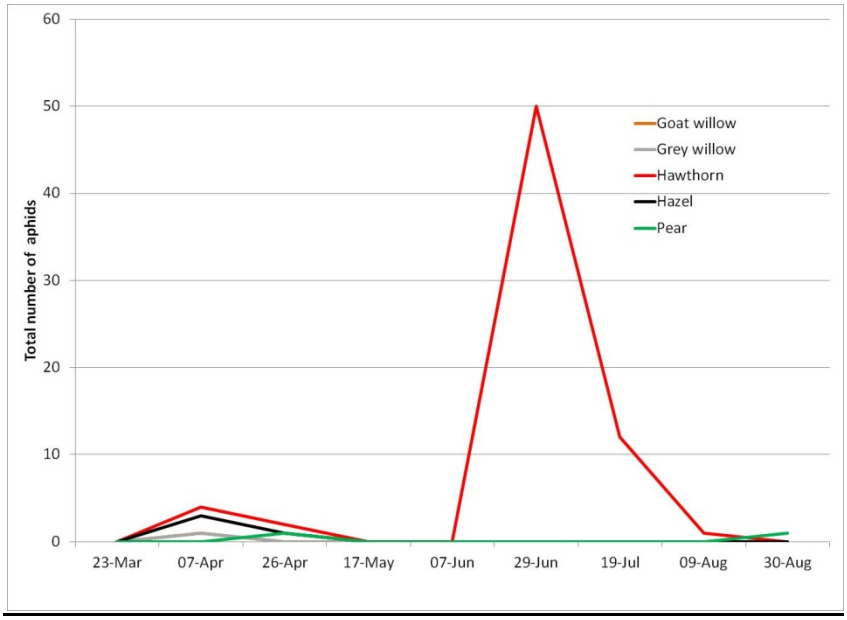
Table 2.1.3.3. Hedgerow species ranked in order of most abundant predatory groups of arthropods.

Top 6 hedgerow species	Total anthocorid	Total ladybirds	Earwigs	Spiders	Lacewings	Most predator rich woody species
1	Pear	Ash	Pear	Hawthorn	Hazel	Blackthorn (5)
2	Grey willow	Blackthorn	Field maple	Blackthorn	Field maple	Hazel (5)
3	Hawthorn	Goat willow	Hawthorn	Elder	Pear	Hawthorn (4)
4	Hazel	Birch	Hazel	Hazel	Hawthorn	Pear (4)
5	Goat willow	Alder	Blackthorn	Field maple	Elder	Field maple (3)
6	Blackthorn	Hazel	Lime	Pear	Blackthorn	Elder (2)
						Goat willow (2)
						Birch (1)
						Alder (1)
						Grey willow (1)
						Lime (1)
						Ash (1)

Rodmersham Court had the highest numbers of arthropods in the hedgerow (1451) so we have detailed the phenology of arthropods through the growing season below. Peaks of anthocorids on pear coincided with peaks of pear sucker on the trees in the orchard (Fig. 2.1.3.2).







Task 2.1.4. Investigate whether timely trimming of hedgerows can foster anthocorid influx into adjacent pear orchards (EMR yrs 3 and 4)

In 2010 it was shown that anthocorids will move from banks of nettles throughout an adjoining orchard, although it was not possible to conclude that cutting the nettles was of benefit.

The aim of the 2011 study was to determine the spatial dispersal of anthocorids. This study covers sub-objectives 2.1 and 2.3 of objective 2

Introduction and methods

Background and outline

Anthocorid predatory bugs are the key natural enemies of pear sucker. A protein (milk) monoclonal antibody-based mark and recapture method developed in the USA was used (Hagler & Naranjo, 2004; Hagler, 2006) to study the dispersal of adult anthocorids in pear orchards. When adult anthocorid numbers were at their peak in August a central area of an orchard was sprayed (20 m x 20 m approximately). Once the spray deposits had dried, the subsequent dispersal of the anthocorids to the adjacent pear trees was studied over a period of four days. Anthocorids were collected by beat sampling of the pear orchard in a regular grid pattern. Anthocorids collected were tested for presence of the protein marker in the laboratory using MAB ELISA tests.

Site

The experimental orchard was ‘Big Plantation’ cv. Conference pear orchard at Marshgate Farm, Main Rd, Cooling, Rochester, Medway ME3 8DP, UK by kind permission of David Long (Figure 2.1.4.1). ‘Big Plantation’ is the orchard to the east of the farm yard (marked A). It has a large cold store in the SE corner. The orchard was planted in 1978 on a 14’ x 10’ (= 4.26 x 3.04 m) spacing and is 3.16 ha.

Figure 2.1.4.1. Location of Big Plantation, Marshgate Farm, Cooling, the site of the experiment

Negative and positive control plots were sampled in another orchard at Marshgate Farm.

Treatments

Experimental orchard: On day 0 of the study, the plot in the centre of the orchard (M) was sprayed to run off with a 20% milk solution in water containing 0.3 g/l EDTA using a motorised air-assisted back pack sprayer (Fig. 2.1.4.2.). The plot was five rows wide and seven trees long (21.3 x 21.3 m). The remainder of the experimental orchard was not sprayed.

Figure 2.1.4.2. Location of the plot in the centre of Big Plantation pear orchard sprayed with milk solution on day 0.

Control plots: Two control plots, a positive control (CP) and a negative control (CN), each comprising 10 trees and separated by at least 50 m, were marked in another orchard at Marshgate Farm well away (>200 m) from the experimental orchard. On day 0 the positive control (CP) was sprayed to run off with a 20% milk solution in water containing 0.3 g/l EDTA using a motorised air-assisted back pack sprayer, in the same way as plot M in the experimental orchard, and as soon as possible after. The negative control was not treated.

Sampling

Pear trees: Adult anthocorids were sampled by beat sampling over a large beating tray. For each sampled pear tree, 10 sharp taps were done over a pair of large clean beating trays, each 1.0 m x 1.5 m. The numbers of anthocorids recovered from each tree were recorded. Each adult was transferred with an individual wooden disposable cocktail stick to an individual Eppendorf tube. The batches of tubes for each tree on each sampling date were held together in a bag labelled with the date, row and tree number. A list of the samples collected was made. As avoiding cross contamination of individuals was critical, the pear trees were generally sampled starting at the furthest point from the sprayed area.

Pre- and post-treatment sampling

Eight sampling transects, N1&2, E1&2, S1&2, W1&2 were marked out and labelled in the experimental orchard before spraying on day 0. Trees 1, 2, 4, 8, 15/16 (numbers from edge of sprayed plot) in each transect were labelled with the transect and tree number. Samples that were taken pre and post spraying from the experimental orchard, control plots and pear trees in the sampling grid are listed in Table 2.1.4.1.

Table 2.1.4.1. List of samples

Day	Plot	Descriptor	Label
Day 0, Pre-spraying	Overall from experimental orchard	20 adult anthocorids	APre Exp1…20
	From positive control CP	20 adult anthocorids	APre CP1…20
	From negative control CN	20 adult anthocorids	APreCN1…20
Day 0, Post-spraying	From positive control CP	20 adult anthocorids	APostCP1…20
	From negative control CN	20 adult anthocorids	APostCN1…20
Day1	From positive control CP	20 adult anthocorids	ADay1CP1…20
	From negative control CN	20 adult anthocorids	ADayt1CN1…20
	Each of 40 pear trees in sampling grid	All target anthocorids	Day1 NT1/1, NT1/2 ….WT4/16
Day 2	From positive control CP	20 adult anthocorids	ADay2CP1…20 LDay2CP1…20
	From negative control CN	20 adult anthocorids	ADay2CN1…20 LDay2CN1…20
	Each of 40 pear trees in sampling grid	All target anthocorids	Day2 NT1/1, NT1/2 ….WT4/16
Day 4	From positive control CP	20 adult anthocorids	ADay4CP1…20 LDay4CP1…20
	From negative control CN	20 adult anthocorids	ADay4CN1…20 LDay4CN1…20
	Each of 40 pear trees in sampling grid	All target anthocorids	Day4 NT1/1, NT1/2 ….WT4/16

Samples were held overnight in a fridge before ELISA testing the following day. If necessary, samples were stored in a deep freeze at -80 ˚C until processing at a later date. MAB ELISA testing on each sample was done to determine the presence of the milk tracer on each individual anthocorid collected.

ELISA assay

The protocol used was based on that described in Jones *et al*., 2006; this assay is an indirect ELISA.

Insects were collected into 2ml Eppendorf tubes, which were stored in a freezer at -20°C until the analysis. To remove the protein marker they were washed in buffer; 500 µl of coating buffer (TBS + 1.1% EDTA) was added to each tube and the tubes were shaken and mixed using a vortex. 80 µl of each sample was added to an individual well in a Nunc Maxisorp™ 96 well microplate. Outer wells of the plates were not used for the test samples, but instead were loaded with 80 µl coating buffer per well. Positive and negative controls were added. Samples were held overnight at 4°C to allow the marker to adsorb onto the plate.

Samples were removed using a multi-pipette set at 100 µl with new pipette tips per sample to prevent contamination. The plate was then washed five times with PBST (PBS + 0.09% Triton X-100). Blocker (PBS + 20% bovine serum for the whole milk protocol) was added to the wells, 250 µl per well, and then incubated for 1 hour at 37ºC. The plate was washed twice with PBST. The primary antibody for the milk, was a rabbit polyclonal antibody to casein (ab48406, Abcam, Cambridge, UK), which was diluted in PBS + 1300 ppm Silwet + 20% Bovine serum at 1:1000. This was specific to the protein marker, and were added to the wells at 80 µl per well. The plate was then incubated for 30 minutes at 37°C. Plates were washed five times with PBST.

The secondary antibody for the milk was a goat anti rabbit with a horse radish peroxidase conjugation (ab6721, Abcam, Cambridge, UK). This was diluted using the same antibody diluent at a rate of 1:1500. The secondary antibody was added to the wells at 80 µl per well and the plate was incubated for 120 minutes at 37°C. Plates were washed three times with PBS + 2.3g/L SDS and three times with PBST. The substrate was a soluble 1-Step TMB substrate (Pierce) which was added at 80 µl per well and was incubated at room temperature for 10 minutes in the dark on a rotating plate. The reaction was stopped with 2N sulphuric acid. The absorbance of the samples was read on a plate reader at 450 nm and a numeric value was given for each well.

The mean absorbance reading (colour change) of the negative control wells plus three times the standard deviation has been used as a cut-off point in other assays using this system, but this gave a high number of false positives in preliminary assays in 2009, when to reduce background noise 1.5 x greater than the mean of the negative wells was used as the threshold for the milk assay. In the 2010 experiment wells were deemed to be positive if they were 1.84 x greater than the mean of the negative wells for the milk. Whilst this may have given a conservative estimate compared to using 3 x SD, it protected against false positives which would overestimate movement. In 2011 both values were used as a lower and higher threshold.

Results and discussion

All pre-treatment samples tested (20 of each) were negative, and the post-treatment samples in the negative control (20 tested) were all negative, whilst 10 out of 16 were positive at the higher threshold in the positive control. This indicated that the insects had picked up the marker in the positive control plot, but that there was no contamination due to spraying in the negative control plot. On Day 1, there were no positive samples out of 20 tested from the negative control plot and out of 19 tested from the positive control plot 9 were positive at the lower threshold and six were positive at the higher threshold.

All anthocorids collected from the pear trees in the sampling grid were tested, and from 158 tested on Day 1, eight were positive for the presence of the milk marker using the lower threshold and five were positive at the higher threshold. Of those testing positive, three were found at tree 1, one at tree 2, two at tree 4, one at tree 8 and one at tree 16. The anthocorid found at tree 16 tested positive at both threshold values and was definitely able to move 49 metres away from the sprayed area.

On Day 2 there were no positive samples out of 10 tested from the negative control plot at the higher threshold. From the positive control plot only five out of 18 tested positive at the higher threshold and seven tested positive at the lower threshold. Of 189 anthocorids sampled, only one tested positive at the lower threshold and this was found at tree 2 on an Eastern transect.

On Day 4 there were no positive samples out of 18 tested from the negative control plot, and five out of 16 tested were positive from the positive control plot. This may indicate that unmarked anthocorids were moving into the sprayed area and were not subsequently picking up trace milk protein on the foliage, or may indicate that the milk protein had been washed/rubbed off the previously marked anthocorids, despite the fact that it had not rained during the experimental period. Of 225 anthocorids tested on Day 4, seven were positive for the presence of the milk marker using the lower threshold and four were positive at the higher threshold. Of those testing positive, three were found at tree 1 and four at tree 2. The anthocorids that tested positive on Day 1 and Day 4 were found in all directions of the grid.

This confirms the findings of the previous year that anthocorids are highly mobile and are able to move up to 50 m in a day. Movement in a field situation may be due to a reduction in food supply, or in this experiment it may have been driven by the spraying process. As the

UK is subject to variable weather conditions this marking technique seems best suited to experiments of short duration.

Task 2.1.5. Determine best choice of woody species and management practices for hedgerows/windbreaks and formulate recommendations for growers

Sub-objective 2.2. Investigate anthocorid overwintering and the benefits of artificial refuges

Task 2.2.1. Investigate anthocorid overwintering in natural habitats (EMR, Yrs 1, 2)

Task 2.2.2. Investigate anthocorid overwintering in artificial refuges (EMR, WWF, HLL, UAP, all years)

No anthocorids were found in leaf litter in years 1 or 2 of the project and none were found in corrugated cardboard-bottle traps. One *A. nemoralis* was found in a pear tree in February 2011 whilst sampling for pear sucker.

Task 2.2.3. Determine natural anthocorid overwintering and benefits of artificial refuges. Formulate recommendations for growers (EMR, Yrs 3 and 4)

Sub-objective 2.3. Investigate use and management of strips of stinging nettle versus a purpose-sown flowering ground herbage mix adjacent to hedgerow/windbreak bordering pear orchards

Task 2.3.1. Establish experimental strip plantings of stinging nettles and of a flowering herbage species mix adjacent to hedgerows/windbreaks round borders of pear orchards (G H Dean, H Chapman, H Rudge, Yr 1)

Task 2.3.2. Sampling strips for spring predator communities (EMR, Yrs 1-4)

Task 2.3.3 Investigate whether timely cutting of strips can foster anthocorid influx into adjacent pear orchards (EMR yrs 3 and 4)

Task 2.3.4. Determine whether strips of nettles or flowering herbage adjacent to hedges/windbreaks provide significant benefits and formulate recommendations for growers (EMR, Yr 4)

Sub-objective 2.4. Investigate the benefits of more diverse flowering ground herbage in pear orchard alleyways

Task 2.4.1. Establish whole orchard comparisons of diverse flowering ground herbage with standard mown alleyway herbage (A Scripps, H Rudge, Yrs 1-4)

Task 2.4.2. Monitor populations of anthocorids and other important natural enemies

of pear sucker in the 6 orchards with different alley way herbages (EMR, Yrs 1-4)

Task 2.4.3. Quantify the effects of different alleyway herbage on anthocorid and pear sucker populations in the attendant pear orchards (EMR, Yrs 1-4)

Task 2.4.4. Determine whether tall alleyway flowering herbage provides significant benefits and formulate recommendations for growers (EMR, Yr 4)

New data from previous years

A. Combined tables of Anthocoridae and Miridae

Table ND1. Numbers of identified Anthocoridae on plants collected by the beating method. A=adult, N=nymph

Tree sp.	Site	*Anthocoris nemoralis*		*Anthocoris nemorum*		*Orius (Heterorius) laticollis*	*Orius (Heterorius) majusculus*	*Orius (Heterorius) vicinus*	*Orius (heterorius)* sp.	*Orius (Orius) laevigatus*	*Orius (Orius) niger*	*Orius (Orius)* sp.	**Grand total**
		A	N	A	N	A	A	A	N	A	A	N	
Acer campestre	S1	1						2					**3**
	S3	3		1									**4**
Acer platanoides	S1								1				**1**
	S2	3		2	1								**6**
Alnus glutinosa	S1	2			1			1	1				**5**
	S2			1	2								**3**
	S3	1		1	1								**3**
Betula pendula	S1	1	10										**11**
	S2	2		6									**8**
Corylus avellana	S1	2	12	6	1			1			1	1	**24**
	S3	1		1									**2**
Crataegus monogyna	S1	5	2		1			4	3	1		1	**17**
	S3	5	1		1								**7**
Fraxinus excelsior	S1	2	3					2	2		1		**10**
	S2	4		5	3								**12**
Prunus spinosa	S2			1	2								**3**
	S3	1											**1**
Pyrus communis	S1	3	4										**7**
	S2	5		4	2								**11**
	S3	3	3								1		**7**
Salix caprea	S1		1					3	3				**7**
	S2	4		4	3	2							**13**
	S3	1	1										**2**
Salix cinerea	S1	5	12			8		15	22				**62**
	S2	4		1	1						1		**7**
Sambucus nigra	S1		4	1	1	2		6	2	1		4	**21**
	S2			5									**5**
Tilia cordata	S2			6	2		1						**9**
	S3												
Total		**58**	**53**	**45**	**22**	**12**	**1**	**34**	**34**	**2**	**4**	**6**	**272**

Table ND2. Numbers of identified Miridae (Dicyphini, Deraeocorini and Mirini) on plants collected by the beating method. A=adult, N=nymph

		Dicyphini	Deraeocorini					Mirini							
Tree sp.	Site	*Dicyphus pallicornis*	*Deraeocoris flavilinea*	*Deraeocoris lutescens*	*Deraeocoris ruber*	*Liocoris tripustulatus*	*Lygocoris pabulinus*	*Lygocoris rugicollis*	*Lygus rugulipennis*	*Phytocoris sp. (reuteri?)*	*Phytocoris tiliae*	**Grand total**
		A	A	A	N	A	A	A	N	A	N	A	N	N	
Acer campestre	S1		1	1	1									1	**4**
	S3														
Acer platanoides	S1	1													**1**
	S2														
Alnus glutinosa	S1														
	S2		2				1	1				2			**6**
	S3														
Betula pendula	S1					1									**1**
	S2		1												**1**
Corylus avellana	S1				2										**2**
	S3														
Crataegus monogyna	S1														
	S3														
Fraxinus excelsior	S1												1		**1**
	S2							23				2			**25**
Prunus spinosa	S2						2				1	1			**4**
	S3														
Pyrus communis	S1											1			**1**
	S2														
	S3							1							**1**
Salixcaprea	S1														
	S2							1	18	4					**23**
	S3														
Salix cinerea	S1											1			**1**
	S2					1		7				3			**11**
Sambucus nigra	S1														
	S2						1	21				2			**24**
Tilia cordata	S2							1	1						**2**
	S3														
Total		**1**	**4**	**1**	**3**	**1**	**1**	**4**	**55**	**19**	**5**	**12**	**1**	**1**	**108**

Table ND3. Numbers of identified Miridae (Orthotylini and Phylini) on plants collected by the beating method. A=adult, N=nymph

Tree sp.	Site	Orthotylini										Phylini							**Grand total**
		Heterotoma planicornis		*Malacocoris chlorizans*	*Blepharidopterus angulatus*		*Orthotylus marginalis*		*Harpocera thoracica*	*Plagiognathus arbustorum*		*Plagiognathus chrysanthemi*	*Phylus coryli*	*Compsidolon salicellum*	*Psallus haematodes*		*Psallus montanus*		
		A	N	A	A	N	A	N	A	A	N	A	A	A	A	N	A		
Acer campestre	S1		2						1										**3**
	S3																		
Acer platanoides	S1																		
	S2																1		**1**
Alnus glutinosa	S1				4														**4**
	S2		3		6	6													**15**
	S3				1							1							**2**
Betula pendula	S1																		
	S2																		
Corylus avellana	S1	5	2	2									1	1					**11**
	S3											1							**1**
Crataegus monogyna	S1	4	1																**5**
	S3											3							**3**
Fraxinus excelsior	S1	3																	**3**
	S2		1			1													**2**
Prunus spinosa	S2		10								1							1	**12**
	S3											2							**2**
Pyrus communis	S1																		
	S2																		
	S3																		
Salix caprea	S1	2													1				**3**
	S2							8							6	1			**15**
	S3									1									**1**
Salix cinerea	S1	1					1								1				**3**
	S2		2				5	2											**9**
Sambucus nigra	S1	7								2									**9**
	S2		9																**9**
Tilia cordata	S2																		
	S3											1							**1**
Total		**22**	**30**	**2**	**11**	**7**	**6**	**10**	**1**	**3**	**1**	**8**	**1**	**1**	**8**	**1**	**2**		**114**

Notes (apart from the ones in 2011 report):

Anthocorids:

The total numbers of anthocorids collected from the new hedges in 2010 are much lower than the ones from the old hedges in 2009. The most abundant species were *Anthocoris nemoralis*, *A. nemorum* and *O. vicinus*, respectively. Similarly to the 2009 data, the new coloniser *O. laticollis* is becoming more common, especially on willows.

Nettle problem:

The nettle effect is clearly seen in the data, especially in the case of anthocorids. The presence of nettles (as another good source of anthocorids) under nearly each tree species makes the anthocoris data confusing, because there is no evidence that the anthocorid species collected from the hedgerow plants had been originally present on the trees or on the nettles. Because of this, the anthocorid data of 2010 should be interpreted carefully.

Miridae:

A total of 21 species of the family Miridae were found on the hedgerow plants. Total numbers of specimens were much lower than in the 2008 and 2009 old hedge samples. Only the hedgerow of Site 2 (Ballingham) had more or less strong Miridae fauna, the other two sites had very few mirids. *Heterotoma planicornis* was the most abundant predacious species found on various hedgerow plant species. However, the nettle effect can be also important in the case of this species.

On some plant species, the elements of their typical mirid fauna were already present in the young hedges.

For example:

-*Blepharidopterus angulatus* on *Alnus glutinosa*
-*Lygocoris rugicollis*, *Orthotylus marginalis* and *Psallus haematodes* on *Salix caprea*
-*Malacocoris chlorizans* and *Phylus coryli* on *Corylus avellana*

The generalist, mostly phytophagous ,pest species *Lygocoris pabulinus* was found in large numbers on *Fraxinus excelsior* and on *Sambucus nigra*.

B. Anthcoridae 2009

Table ND4. Total numbers of Anthocoridae collected on the plant species at Site 1 (Foxbury)

	Life stage	*Betula pubescens*	*Corylus avellana*	*Crataegus monogyna*	*Fraxinus excelsior*	*Pyrus communis*	*Salix caprea*	*Urtica dioica*
Anthocoris confusus	A	3	1	1				
Anthocoris nemoralis	A	1	3	7	1	7	7	12
	L		1	1		1	1	3
Anthocoris nemorum	A	13	49	26	11	27	45	179
	L	15	47	10	9	12	58	309
Orius (Heterorius) vicinus	*A*	*1*	*1*		*1*			
Orius (Heterorius) sp.	*L*			*1*				*2*
Orius (Orius) laevigatus	*A*					*1*		*1*
Orius (Orius) niger	*A*							*2*
Orius (Orius) sp.	*L*						*1*	

Table ND5. Total numbers of Anthocoridae collected on the plant species at Site2 (MarshGate), Site3 (Broadwater) and Site4 (Westerhill)

		S2 Marshgate			S3 Broadwater		S4 Westerhill	
	Life stage	*Betula pubescens*	*Pyrus communis*	*Urtica dioica*	*Crataegus monogyna*	*Pyrus communis*	*Pyrus communis*	*Salix caprea*
Anthocoris confusus	A				2			1
Anthocoris nemoralis	A	3	5	44	53	7	26	64
	L		3	5	4	1	35	26
Anthocoris nemorum	A			10	32	19	14	56
	L		1	6	9	11	19	32
Orius (Heterorius) laticollis	*A*	*1*			*1*			*10*
Orius (Heterorius) majusculus	*A*							*1*
Orius (Heterorius) vicinus	*A*	*2*	*1*	*108*	*2*	*1*		*2*
Orius (Heterorius) sp.	*L*			*47*				*10*
Orius (Orius) niger	*A*			*4*				
Orius (Orius) laevigatus	*A*		*2*	*6*				*1*
Orius (Orius) sp.	*L*			*3*	*1*			

Notes (apart from the ones in 2010 report):

Five *Orius* species were found on the four sites in total. The most abundant species is *O. vicinus*, especially on nettle in Site 2 (MarshGate).

Orius species were not abundant on pear in any sites. Only one specimen of *O. laevigatus* was found in Site 1 (Foxbury), one specimen of *O. vicinus* and two specimens of *O. laevigatus* were found in Site 2 (Marshgate) and one specimen of *O. vicinus* was found in Site 3 (Broadwater) on pear trees. *Orius* specimens were not found in Site 4 (Westerhill) on pear trees. Our data from these four pear orchards suggest that *Orius* species probably cannot play an important role in pear sucker biocontrol compared with *Anthocoris* species (especially *A. nemoralis*).

Orius laticollis is a rare species in Britain, it was included to the British Heteroptera checklist in 2006 (Nau, 2006). This species was the most common one on willow trees in Site 4 (Westerhill), which suggests that this species is getting more common in South East England, especially on willows.

Perhaps we should sign the presence of this species on this map on the following website:

http://data.nbn.org.uk/gridMap/gridMap.jsp?allDs=1&srchSpKey=NHMSYS0020309559

Literature:

Nau, B. S. (2006) Current names of Southwood & Leston (1959) Heteroptera species

C. Psyllidae 2010

Table ND6. Numbers of identified Psyllids on plants collected by the beating method. Species belonging to other plants have been deleted.

Tree sp.	Site	*Baeopelma foersteri*		*Cacopsylla ambigua*	*Cacopsylla brunneipennis*	*Cacopsylla melanoneura*		*Cacopsylla peregrina*	*Cacopsylla pyri*		*Cacopsylla pyricola*		*Chamaepsylla hartigi*	*Psyllopsis fraxini*		*Psyllopsis fraxinicola*		**Grand total**
		A	N	A	A	A	N	A	A	N	A	N	A	A	N	A	N	
Acer campestre	S1																	
	S3				1	1	1	1	4									**8**
Acer platanoides	S1				1		2		1							2		**6**
	S2																	
Alnus glutinosa	S1	34	1															**35**
	S2	1																**1**
	S3						1											**1**
Betula pendula	S1								4				1					**5**
	S2	1																**1**
Corylus avellana	S1								1									**1**
	S3			1														**1**
Crataegus monogyna	S1						11	2	1									**14**
	S3						7	12	2									**21**
Fraxinus excelsior	S1								1					2		19	2	**22**
	S2										1			19	1	2	1	**24**
Prunus spinosa	S2																	
	S3						1		1									**2**
Pyrus communis	S1						2		124	3								**129**
	S2						1				19	2						**22**
	S3								79	6	33							**118**
Salix caprea	S1			1														**1**
	S2																	
	S3			10			1											**11**
Salix cinerea	S1			3					3									**6**
	S2			1														**1**
Sambucus nigra	S1								1									**1**
	S2																	
Tilia cordata	S2																	
	S3						1											**1**
Total		**35**	**1**	**16**	**2**	**1**	**28**	**15**	**222**	**9**	**53**	**2**	**1**	**21**	**1**	**23**	**3**	**433**

The most typical species were already present on the plants of Rodmersham, Ballingham and Broadwater sites, but in a low number compared to the samples of the old hedges from 2008 and 2009.

Acer campestre and *A. platanoides*:

The associated *Rhinocola aceris* was not found and the only other species found came from the other plants.

Alnus glutinosa:

One of the associated psyllid species (*Baeopelma foersteri*) was found on Rodmersham site in good numbers. On the Ballingham site only one adult specimen was found.

Betula pendula:

Only one specimen of the *Chamaepsylla hartigii* has been found so far, on the Rodmersham site.

Corylus avellana:

There is no associated species but specimens of species from other plants can be present in a low number.

Crataegus monogyna:

Both of the most dominant associated species (*Cacopsylla melanoneura* and *C. peregrina*) were found on hawthorn on the Rodmersham and Broadwater sites, but at much lower numbers compared with old hedges.

Fraxinus excelsior:

Both of the dominant associated species (*Psyllopsis fraxini* and *P. fraxinicola*) were found on Rodmersham and Ballingham sites too. Their numbers were much lower than in the old hedge.

Prunus spinosa:

The associated *Cacopsylla pruni* has not been found so far.

Pyrus communis:

The Rodmersham site was the most psyllid infested site, the Broadwater site was a bit less infested and the Bellingham site had minimal psyllid problems. *Cacopsylla pyri* was the only species at Rodmersham and the dominant species on the Broadwater site. Only *C. pyricola* was found on the Ballingham site. *C. pyricola* was also present on the Broadwater site, but in a smaller number than *C. pyri*.

Salix caprea and *S. cinerea*:

The associated *Cacopsylla ambigua* adults were found on the Rodmersham and Broadwater sites too, but in low numbers. Only one specimen of this species was found on the Ballingham site. The other common willow feeding species (*Cacopsylla brunneipennis*) has not been found on willows so far, but two summerform specimens were found on *Acer campestre* and on *A. platanoides*.

With field shoot checking method psyllid egg recording was not be effectively possible, so, 10 shoots per tree were taken to lab for checking. With this method, Rodmersham site was found the most infested, and Broadwater site was the less infested with psyllid eggs. *S. caprea* was generally more infested than *S. cinerea*.

Sambucus nigra:

There are no associated species, only guest specimens have been present.

Tilia cordata:

There are no associated species and only one specimen of *C. melanoneura* was found on *Tilia* on the Broadwater site.

General comment:

There was a serious nettle effect in the case of psyllids. All of the samples were full of the adults of the nettle psyllid (*Trioza urticae*), especially on the Rodmersham site. The exact numbers were not recorded but signed on data paper if the numbers were very large.

Objective 3. Exploit synomones for attracting anthocorids into pear orchards

Sub-objective 3.1. Establishment of blends and release rates of synomones for attracting anthocorids.

Task 3.1.1. Re-investigate attractive compounds from infested pear seedlings (NRI, EMR Yrs 1,2)

Introduction

Scuteraneau *et al.* (1997) analysed volatiles from samples of freshly-picked leaves from uninfested pear trees and leaves from trees with various degrees of infestation with the pear sucker, *Psylla pyricola*. Infestation caused enhanced production of 2-pentenal, 1-penten-3-ol, hexyl acetate, (*E*)-4,8-1,3,7-nonatriene, (*E,E*)-farnesene, hexanal, methyl salicylate, (*E*)-2-hexenal, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate, in ascending order of abundance. They reported that the anthocorid species *Anthocorus nemoralis* and *A. nemorum* were attracted to (*E,E*)-farnesene and methyl salicylate in a Y-tube olfactometer, but not to (Z)-3-hexenyl acetate.

During 2008 volatiles were collected from cut shoots and leaves in the laboratory and intact branches of pear trees in the field with and without pear sucker adults. The main compounds collected from infested leaves in the laboratory were (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, (*E,Z*)-2,6-nonadienal, 4-ethylbenzaldehyde, (*E,E*)-farnesene, methyl salicylate and (*Z*)-

jasmone. The main compounds found in samples collected from infested and uninfested branches in the field were 4-ethylbenzaldehyde, (*Z,E*)- and (*E,E*)-farnesene. Methyl salicylate was only present in very small amounts in the field samples and (*Z*)-jasmone was undetectable.

In 2009, volatiles were collected from trees infested with *C. pyri* adults during January-February, the nymphs or adults on potted trees during June-August and from individual adults on pear shoots during August 2009. Little or no material was present in collections made during January-February. Collections from nymphs on potted trees during 15-23 June showed significant quantities of 2-phenylethanol and little or no α-farnesene or methyl salicylate. Subsequently, when adults were put on potted trees from 25 June onwards, only methyl salicylate was observed. This suggested that 2-phenylethanol was a good candidate for involvement in attraction of anthacorid predators to pear trees infested with pear sucker.

During 2010 efforts were focussed on measuring EAG responses of anthocorids to compounds identified in collections from pear trees in the previous years. Commercially available *Orius laevigatus* were used to develop techniques, as well as *Anthocoris nemoralis* collected from the field. Possible EAG responses to methyl salicylate and 2-phenylethanol were recorded, but these studies needed repeating. Attempts were also made to collect and analyse volatiles in the air in pear orchards and methyl salicylate was the only compound detected at very low levels.

During 2011 further EAG studies were carried out on *Orius laevigatus* and *Anthocoris nemoralis* collected from the field.

Materials and Methods

Gas chromatography coupled to electroantennographic recording (GC-EAG)

GC-EAG Analyses were carried out on a polar GC column (DBWax, Supelco; 30 mm x 0.32 mm i.d. x 0.25 µm film thickness) with oven temperature held at 50°C for 2 min then programmed at 10°C/min to 240°C. The column effluent was split (1:1) with equal lengths of deactivated fused silica tubing leading to the flame ionisation detector and to a glass T-piece in the column oven. The contents of the T-piece were continuously flushed over the EAG preparation with humidified air (300 ml/min).

EAG recordings were made with a portable device consisting of micromanipulators, electrode holders and amplifier (INR-02; Syntech, The Netherlands) connected to the GC (HP6890, Agilent) as a second detector. Electrodes were fine glass capillaries filled with saline (0.1M

KCl with 1% polyvinylpyrrolidine) and placed over silver wire electrodes. Various techniques were investigated for making EAG preparations. The most satisfactory method involved excising the head and inserting the base electrode into the neck. After excising the proboscis and one antenna to reduce mechanical and electrical interference, the end was removed from one antenna and inserted into the recording electrode.

Data from both EAG and GC were collected and process with EZChrom Elite software.

Commercially available *Orius laevigatus* were as well as *Anthocoris nemoralis* collected from the field.

Attempts were made to record responses from both synthetic compounds and collections of volatiles from pear trees infested with psyllids. Synthetic compounds tested were (*Z*)-3-hexenyl acetate, decanal, methyl salicylate, α-farnesene, 2-phenylethanol, (*E,E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (*E,E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT).

Results

In February 2011, EAG recordings were made from 17 individuals of *O. laevigatus* to both pear tree volatiles and synthetic compounds, but no useful responses were recorded.

In June 2011, recordings were made from 20 individuals of *A. nemoralis*. In analyses of mixtures of the synthetic compounds, consistent responses were obtained to decanal (Figure 1). This is not produced naturally by pear foliage in any significant amount, but elicits a good EAG response from a wide variety of insects (unpublished observations) and serves as a convenient “internal standard” to indicate that the EAG preparation is responding.

A reasonably consistent response was also obtained to methyl salicylate with responses also sometimes observed to (*Z*)-3-hexenyl acetate and 2-phenylethanol (Figure 3.1.1.1).

In analyses of volatiles collected from pear seedlings infested with pear sucker nymphs, a response was observed to a small amount of decanal. A weak response was elicited by a much larger peak of methyl salicylate, and a stronger response was observed to a small peak eluting just before the methyl salicylate (Figure 3.1.1.2). This was identified provisionally as δ-cadinene based on its mass spectrum and comparison with literature values of retention indices on polar and non-polar GC columns (1755 and 1735 respectively).

In analyses of volatiles collected from female pear sucker on pear leaves in the laboratory, no significant and consistent responses were observed from *A. nemoralis* females (Figure 3.1.1.3).

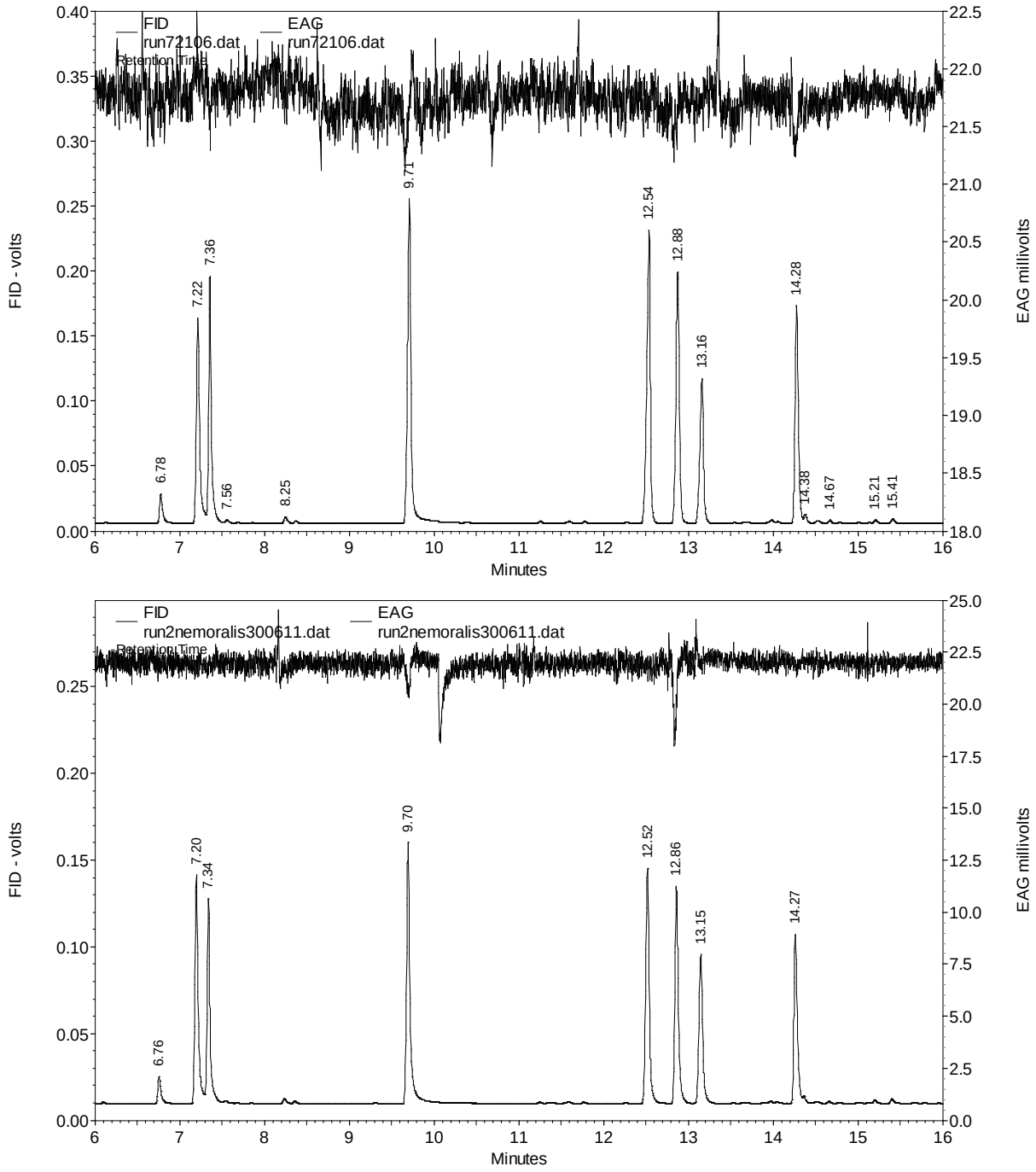


Fig. 3.1.1.1. GC-EAG Analysis of synthetic standards (100 ng) with two different *A. nemoralis* female preparations showing responses to decanal (9.71 min) and methyl salicylate (12.88 min) in both cases and (Z)-3-hexenyl acetate (7.36 min) and 2-phenylethanol 14.28 min in the upper run (other peaks DMNT 7.22 min; α-farnesene 12.88 min; TMTT 13.16 min).

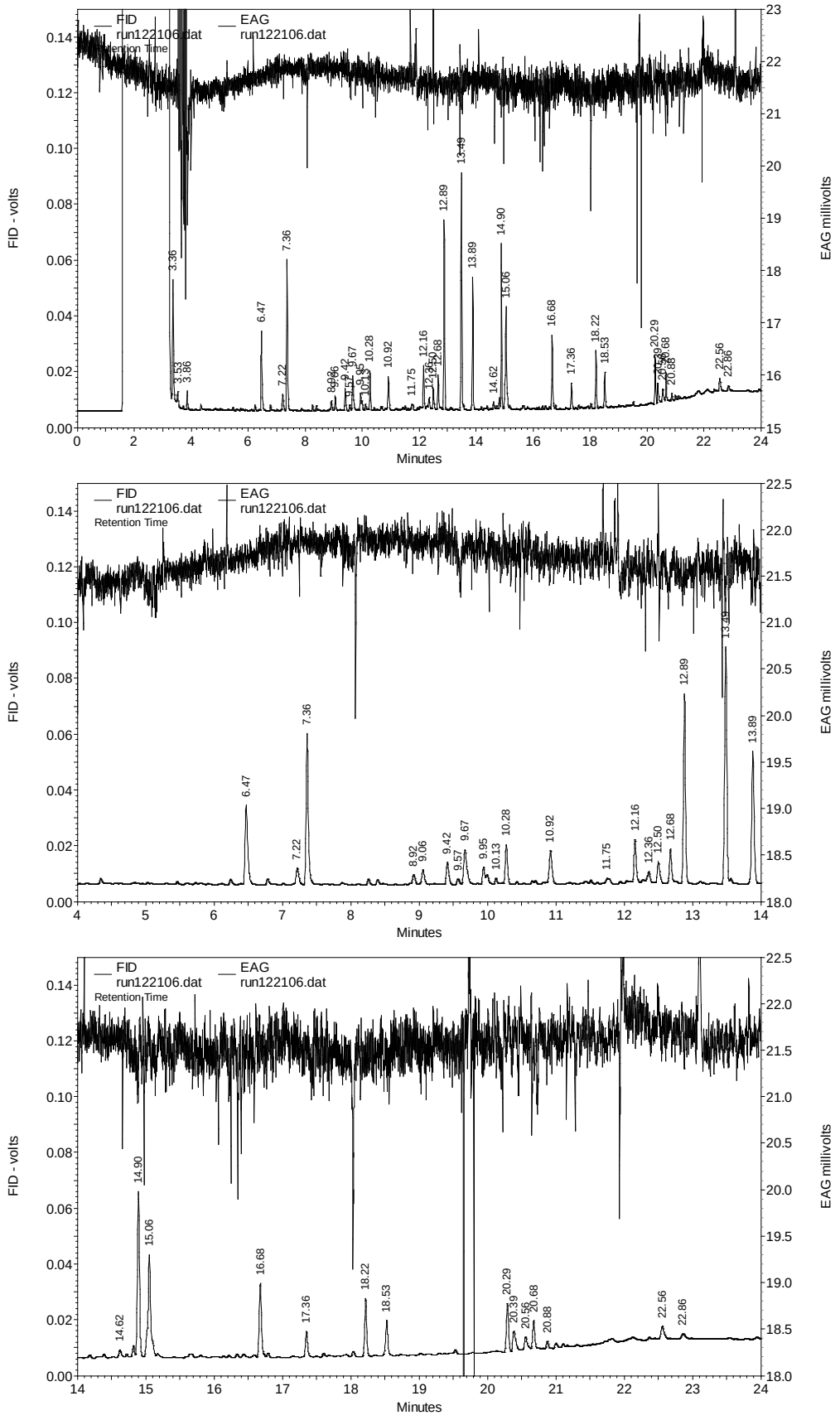


Fig. 3.1.1.2. GC-EAG analysis of volatiles from pear plant infested with approximately 20 5th instar pear sucker nymphs with female *A. nemoralis* EAG preparation with responses to decanal at 9.67 min, δ-cadinene at 12.68 min and methyl salicylate at 12.89 min.

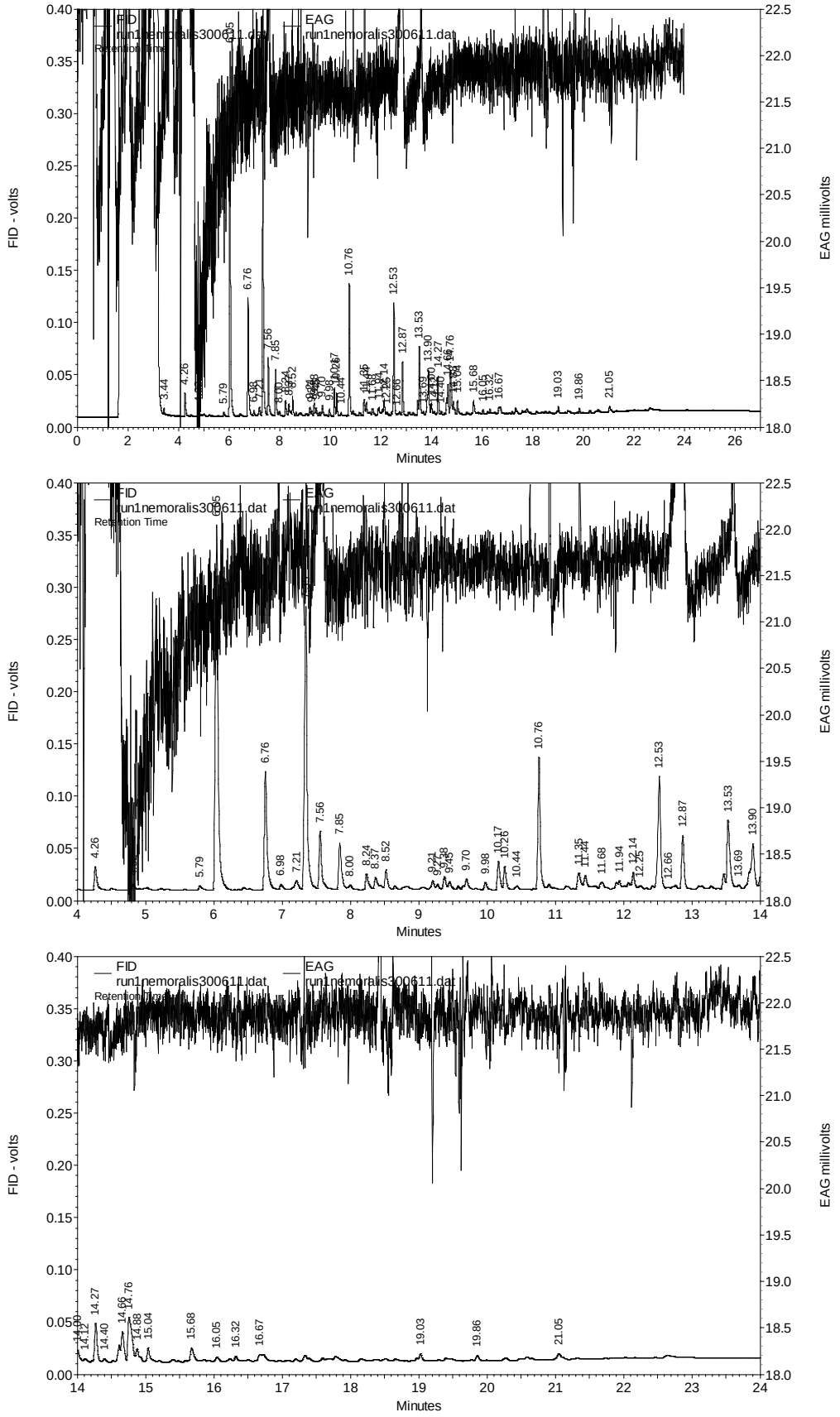


Fig. 3.1.1.3. GC-EAG analysis of volatiles from 222 female *C. pyri* on pear leaves in laboratory with female *A. nemoralis* EAG preparation.

Conclusions

Although no useful EAG responses were recorded from the model species, *O. laevigatus,* consistent EAG responses were obtained from *A. nemoralis* to decanal. In GC-EAG analyses of synthetic compounds, consistent EAG responses were also elicited by methyl salicylate and responses were also sometimes observed to (*Z*)-3-hexenyl acetate and 2-phenylethanol.

In analyses of volatiles collected from pear seedlings infested with pear sucker nymphs, a response was observed to a small amount of decanal. A weak response was elicited by a much larger peak of methyl salicylate, and a stronger response was observed to a small peak eluting just before the methyl salicylate and identified provisionally as δ-cadinene (Figure 3.1.1.4). This is a new compound identified in the pear-anthocorid complex.

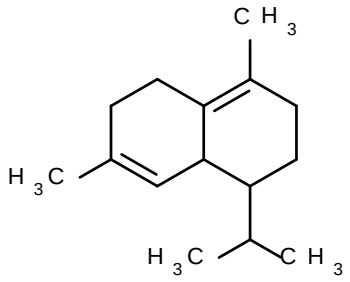


Fig. 3.1.1.4. Structure of δ-cadinene

Task 3.1.2. Development of dispensers (NRI, Agrisense Yrs 1,2)

Task 3.1.3. Field evaluation of lures (NRI, EMR, Yrs 1, 2)

Sub-objective 3.2. Development of method for deployment of synomones for attracting anthocorids into pear.

Task 3.2.1. Assess effect of synomones on anthocorid and psyllid populations in the field (NRI, EMR, Agrisense, Yrs 3, 4)

Background

Anthocorid bugs are known to be strongly attracted to psyllid-infested pear trees. Feeding by pear psylla on pear leaves triggers the release of monoterpene and phenolic volatiles to which the anthocorids have been shown to respond. A number of volatiles have been identified including methyl salicylate, (E,E)-α-farnesene, and (7E)-4,8-dimethyl-1,3,7-nonatriene. The predators have been shown to respond to these volatiles in laboratory olfactometer tests. Only limited work has been done to test and exploit synthetic compounds

for managing anthocorid populations in the field.

Methyl salicylate and technical grade α-farnesene are readily available at low cost and these volatile compounds were tested for their effects on anthocorids and other beneficial species in pear orchards during summer 2008. In 2009 these compounds were tested in spring and early summer alongside two other compounds cis jasmone and nonadienal, at different rates, to determine if effects were different at this time of year when less pear foliage was present. During 2009 entrainments from psyllid-infested and non-infested pears identified another compound that was present when psyllids were feeding. Lures containing this compound, phenyl ethanol, were compared with lures containing methyl salicylate in a pear orchard later in the season.

In all of these experiments there has been a significant increase in the number of hoverflies caught in traps containing methyl salicylate and phenyl ethanol lures on some sample dates. In an experiment using water traps instead of Delta traps in a weed field there was a significant effect of phenyl ethanol and another volatile, germacrene, on numbers of hoverflies caught, but no effects on other beneficial species. This experiment aimed to determine effects of phenyl ethanol on beneficial species throughout the season, and included methyl salicylate and a 'no-lure' control.

In 2010 high release rate dispensers of methyl salicylate and phenyl ethanol plus a 'no-lure' control were placed in white delta traps. The sticky bases of the traps were checked and changed from May to August and tap samples were taken. There was an increase in hoverflies in traps with methyl salicylate and phenyl ethanol in August, but no significant effect on anthocorids. In this experiment the dispensers were set up as point source. An alternative strategy used in 2011 was to set up higher numbers of lures in larger replicated areas, as has been done in the US (James *et. al*., 2005), with traps in the centre of these areas, to see if anthocorids can be encouraged into orchards when high levels of volatile are present. As these predators are so mobile it may be difficult to see effects in single tree experiments.

Experiment 3.2.1.1 - Field experiment to determine the effect of two volatiles on anthocorid populations in pear orchards in spring

Methods

Pear orchard site

Westerhill Farm, East Farleigh (TQ735532): J L Baxter & Son, Westerhill Farm, Westerhill Lane, Linton, Maidstone, Kent, ME17 4BS (by kind agreement Clive Baxter).

Treatments

Treatments were high release rates of methyl salicylate and phenyl ethanol plus a 'no-lure' control (Table 3.2.1.1.). Initial laboratory studies of release rates and duration of release (Table 3.2.1.2.) have shown that the methyl salicylate sachets have a release rate of 17 mg/day. The phenyl ethanol sachets last longer with a release rate of 1.8 mg/day.

Table 3.2.1.1. Chemicals and their rates of application

Treatment	Active substance	Loading	Dispenser	Release rate
1	Methyl salicylate	250 µl	Sachet	High
2	Phenyl ethanol	250 µl	Sachet	High
untreated	none			-

Table 3.2.1.2. Release rates in laboratory conditions (NRI).

Dispenser	Size	Amount	Temp	mg/day	Ref
Methyl salicylate					
clear sachet	5x5	100ul	22°C	17	2008/39
Phenyl ethanol					
clear sachet	5x5	100ul	22°C	1.8	2009/066

Experimental design

Lures were arranged in a 3 x 3 grid in each plot which spanned seven trees in a row (with a lure on trees 1, 4 and 7) across five rows (with lures in rows 1, 3 and 5). The central lure in each plot was hung in a white delta trap with a sticky base. The three treatments: methyl salicylate, phenyl ethanol or the 'no-lure' control were arranged in a randomised block design with three replicates of each treatment. One yellow and one blue sticky trap (10 cm x 24 cm) were hung in different rows adjacent to the central lure. The lures and traps were put out on 21 April 2011.

Assessments

On 21 April pre-treatment tap samples were done on the central tree per plot. Five areas were tapped with each tapping area having five beats. Traps were checked and sticky bases changed on 28 April and 9 May. Tap samples were also done on these dates, with the tapping area increased to three trees per plot on the last occasion. The numbers of pests and beneficials were recorded.

Results

The total numbers of psyllids and beneficials in tap samples is shown in Table 3.2.1.3. There was no difference between the treatments.

Table 3.2.1.3. Numbers of psyllids and beneficials in tap samples of the central trees in a grid of lures with methyl salicylate (MeSa) or phenyl ethanol (PhEth) or no lure (Control); total numbers in 3 plots per treatment.

		Pear psyllids		Anthocorids				
		Adults	**Nymphs**	**Adults**	**Nymphs**	**Coccinelids**	**Spiders**	**Lacewings**
21.4.11	**MeSa**	0	0	0	0	1	4	0
1 tree per	**PhEth**	3	0	0	0	2	5	0
plot	**Control**	5	1	1	0	2	5	0
28.4.11	**MeSa**	44	3	0	0	0	4	0
1 tree per	**PhEth**	55	3	0	2	1	7	0
plot	**Control**	125	1	1	1	0	2	2
9.5.11	**MeSa**	151	0	0	5	6	3	0
3 trees	**PhEth**	194	0	3	3	1	15	0
per plot	**Control**	174	0	2	9	2	9	0

The psyllids and beneficial insects caught on the sticky traps included coccinelids, hoverflies and anthocorids (Table 3.2.1.4.) but numbers caught were too low for statistical analysis. Psyllids were most attracted to yellow traps and on the second sampling date were not assessed on the blue and white traps as numbers were low. The coccinelids caught were *Calvia 14-guttata, Harmonia axyridis, Propylea 14-punctata* and *Psyllobora 22-punctata*.

Table 3.2.1.4. Numbers of psyllids and beneficials caught on different colour sticky traps placed within a grid of lures with methyl salicylate (MeSa) or phenyl ethanol (PhEth) or no lure (Control); total numbers in 3 plots per treatment.

		Psyllids	Anthocorids	Coccinelids	Spiders	Lacewings	Hoverflies
28 April 2011							
MeSa	**Yellow**	36	2	3	1	0	0
	Blue	4	0	0	1	0	0
	White	0	0	0	0	0	0
PhEth	**Yellow**	26	0	8	2	0	0
	Blue	5	0	0	1	0	2
	White	0	0	0	0	1	0
Control	**Yellow**	25	0	2	1	0	0
	Blue	10	1	0	2	0	0
	White	0	0	0	0	0	0
9 May 2011							
MeSa	**Yellow**	181	0	1	2	0	0
	Blue	NA	0	0	0	0	1
	White	NA	0	0	0	0	6
PhEth	**Yellow**	105	0	1	2	1	0
	Blue	NA	0	0	1	0	4
	White	NA	0	0	0	4	7
Control	**Yellow**	176	0	5	1	0	0
	Blue	NA	0	0	0	0	4
	White	NA	0	1	0	0	3

NA = Not Assessed

Experiment 3.2.1.2. - Field experiment to determine the effect of two volatiles on anthocorid populations in pear orchards in late summer/autumn

Methods

Pear orchard site

The study was done in a conference pear orchard 'Stony Rocks' at Marsh Gate Farm, Cooling, Kent, by kind permission of David Long. This orchard had been identified as being infested with pear sucker. The orchard was situated at NGR 763 761 (Landranger sheet 178 Thames Estuary).

Treatments

Treatments were high release rates of methyl salicylate, phenyl ethanol, farnesene (all supplied by NRI) and lavandulyl senecioate 8.61% (Checkmate^{R} UMB-XL Suterra Oregon USA) plus a 'no-lure' control.

Experimental design

Lures were arranged in a 3 x 3 grid in each plot which spanned five trees in a row (with a lure on trees 1, 3 and 5) across three rows (with lures in rows 1, 2 and 3). The five treatments were arranged in a randomised block design with four replicates of each treatment. One yellow and one blue sticky trap (10 cm x 24 cm) were hung at least 1m from the central lure. The lures and traps were put out on 1 September 2011

Assessments

On 1 September pre-treatment tap samples were done on five trees in the orchard to determine the numbers of beneficials present in the orchard. On 8th September three trees were tapped per plot; each tree had five tapping areas with each of those being tapped five times. Traps were collected and the numbers of pests and beneficials were recorded in the laboratory.

For the trap results each insect count was analysed as a split plot with the two trap types as the subplot treatment. A log transformation was used throughout. All insect counts were included, i.e. psyllids, anthocorids, orius, coccinelids, lacewings, hoverflies, spiders, earwigs and opiliones.

Results

Both trap catches and tap catches were assessed. The number of lacewings and earwigs on the trap catches were too low to be assessed. For spiders there was no evidence of any treatment type or trap colour differences.

Insects for which there were significant differences in catch for trap type but no evidence of treatment differences were:

(i) **Anthocorid adults**: yellow > blue ($p<0.001$)
(ii) **Pysllids:** yellow > blue ($p<0.001$)
(iii) **Coccinelids:** yellow > blue ($p<0.001$)
(iv) **Hover flies:** blue > yellow ($p=0.002$)

Insects for which there were significant differences between treatments but not trap type were:

(i) **Orius**: treatment $p=0.007$ (12 d.f., s.e.d. 0.3681), with volatile lavandulyl seneciaote significantly greater than the rest (control, farnesene, methyl salicylate and phenyl ethanol),

with the mean log value being 1.843, 0.484, 0.433, 0.865 and 0.260 for each of those treatments respectively.

The total numbers of psyllids and beneficials in tap samples is shown in Table 3.2.1.5.

Table 3.2.1.5. Numbers of psyllids and beneficials in tap samples of the central trees in a grid of lures with methyl salicylate (MeSa), phenyl ethanol (PhEth), farnesene (Far), lavandulyl senecioate (LaSe) or no lure (Control); total numbers in four plots per treatment on 8 September 2011.

	MeSa	**PhEth**	**Far**	**LaSe**	**Control**
Pysllids	659	782	963	719	563
Anthocorid nymphs	3	5	4	3	0
Anthocorid adults	24	50	38	40	28
Orius	2	0	0	0	0
Coccinellid adults	35	22	21	32	26
Coccinelid larvae	0	2	4	1	2
Lacewing adults	8	16	28	19	8
Lacewing larvae	0	0	1	1	1
Hoverflies	0	0	0	0	2
Spiders	15	17	25	8	22
Earwigs	7	8	0	4	3
Opiliones	6	21	10	15	23

The data was $\log_{10}(n+1)$ transformed and analysed using ANOVAR. The only category that showed a significant treatment effect at $P < 0.05$ was for spiders, where there was a reduction in the number of spiders in the lavandulyl senecioate treatment compared to the control (mean log value for MeSa, PhEth, Far, LaSe and Control was 0.64, 0.68, 0.82, 0.45 and 0.78 respectively, least significant difference 0.247, 12 d.f.).

Conclusions

Results showed that different predators were attracted to different coloured sticky traps. However, there was only a significant effect of volatile treatment on *Orius* numbers, with higher numbers caught in the lavandulyl senecioate treatment than in any of the other treatments. These experiments used one release rate per volatile and there is potential to explore different release rates further, both in the field and in the laboratory.

References

James, D.G., Castle, S.C., Grasswitz, T. and Reyna, V. 2005. Using Synthetic Herbivor-Induced Plant Volatiles to Enhance Conservation Biological Control: Field Experiments in Hops and Grapes from Second International Symposium on Biological Control of Arthropods Volume I, Davos, Switzerland - September 12-16, 2005

Objective 4. Efficacious, physically acting spray treatment that is safe to anthocorid predators

Task 4.1. Determine insecticidal activity of sulphur, magnesium sulphate, non-ionic wetter and mixtures in lab bioassays (EMR, Yrs 1,2)

Work completed

Task 4.2. Determine effects of best treatment (from task 4.1) on anthocorids in lab bioassays (EMR, Yrs 2, 3).

In 2009 different bioassay techniques were assessed using *Orius laevigatus* as a model predator. Dipping pots proved to give too high a mortality of first and second instar nymphs and topical application was effective and reduced control mortality. In the 2009 bioassay using a paintbrush to apply the compounds there was no mortality in the water control treatment and between 10 and 15% mortality in all other treatments with Activator 90, Sulphur and magnesium sulphate either alone or in combination. As the compounds to be tested are reported to have a physical mode of action it is important to test them by immersing the insects in the test solutions.

In 2010 and 2011 anthocorids were sourced and ordered from Syngenta Bioline. These were used to complement the experiments conducted on *Orius laevigatus* in year 2, using dip techniques and a direct application bioassay. Bioassays were undertaken to determine the effects of the compounds on *O. laevigatus* and *A. nemoralis* nymphs and on O. laevigatus egg hatch.

Experiment 4.2.1. To determine the effect of sulphur, magnesium sulphate and a wetter on A. nemoralis eggs.

This experiment aimed to determine if sulphur, magnesium sulphate and a wetter affect anthocorid egg hatch.

Anthocorid cultures

Adult *O. laevigatus* and *A. nemoralis* were obtained from Syngenta Bioline and held at either 10 or 20°C as required. Adult anthocorids were used to produce a supply of eggs for bioassay. Adults were placed into culture boxes with beans (as egg laying substrates) cut in half, and held in moist cotton wool at one end to provide moisture. Beans were replaced after two days and new beans were introduced to provide a supply of eggs of known age.

Bioassay techniques

Bioassays of eggs were done using the dip technique.

The compounds were tested separately and in combination, based on a field application rate of 500 l per ha and field concentrations of:

Sulphur at 3 l per ha

$MgSO_4$ at 7.5 kg per ha

Activator 90 at 0.1% (1 ml per l)

This gives the treatments as in Table 4.2.1.1 below:

Table 4.2.1.1 Treatments

Compound	Trt	Rate	Amount/l00ml
Activator 90	1	1x	0.1 ml
Sulphur	2	1x	0.6 ml
$MgSO_4$	3	1x	1.5 g
Activator 90 + Sulphur + $MgSO_4$	4	1x	Activator 90 0.1ml, Sulphur 0.6 ml, $MgSO_4$ 1.5 g
Water	5	-	-

Dip method

Beans, into which anthocorid eggs had been laid, were dipped until fully immersed into a solution of the test compound. Once dipped, the bean was placed upright in a plastic tube and allowed to air dry for two hours, before placing a plastic cap in the end of the tube to prevent emerging nymphs from escaping. Beans were held at 16°C after treatment.

The number of eggs hatched was assessed one day after treatment (DAT) and again at 3, 4, 7 and 12 DAT. A distilled water dip was used as a control.

Results

Results of the dip bioassay on *A. nemoralis* eggs are shown in Table 4.2.1.2.

Table 4.2.1.2. Effect of treatment with physically acting compounds on % of *A. nemoralis* eggs that hatched successfully in laboratory dip bioassays

Treatment	Initial number of eggs	Days after treatment				
		1	3	5	7	12
Activator 90	93	0	0	14	33	73
Sulphur	101	0	8	8	53	81
$MgSO_4$	66	0	0	21	64	70
Mixture	72	0	0	4	39	81
Water control	72	0	3	11	63	82

Conclusion

There was no significant effect of any treatment on egg hatch of *A. nemoralis* in this bioassay. Therefore, the use of these compounds at these rates in the field is unlikely to have a short-term effect on predator numbers.

Experiment 4.2.2. To determine the effect of different rates of sulphur on O. laevigatus egg hatch

Initial laboratory bioassays had suggested that there was a slight effect of sulphur application at high rates on *O. laevigatus* egg hatch. This experiment was done to determine if this was a true effect.

Methods

Methods were as for the dip experiment with *A. nemoralis* (Expt 4.2.1.). Four rates of sulphur were compared with a water control. Sulphur treatments were 1, 2, 4 and 8 times field recommended rates for this compound.

Results

Results are shown in Table 4.2.1.3.

Table 4.2.1.3. Effect of treatment with different rates of sulphur on *O. laevigatus* egg hatch and subsequent survival of larvae

Treatment	Initial number of eggs	3 days after treatment		5 days after treatment	
		Mean % hatched	Mean % dead or moribund	Mean % hatched	Mean % dead or moribund
Control	134	35	4	48	48
Sulphur 1	178	23	35	41	61
Sulphur 2	128	21	37	26	46
Sulphur 4	155	34	53	42	57
Sulphur 8	141	30	15	38	35

Conclusion

There was no significant effect of any treatment on egg hatch of *O. laevigatus* in this bioassay. Therefore the use of these compounds at these rates in the field is unlikely to have a short-term effect on predator numbers.

Experiment 4.2.3. To determine the effect of sulphur, magnesium sulphate and a wetter on A. nemoralis nymphs.

Methods

Laboratory bioassays were done to determine the mortality of first-second instar anthocorid nymphs exposed to a combination of a non-ionic wetter, micronised sulphur and magnesium sulphate.

Anthocorid cultures

These were obtained from Syngenta Bioline. Eggs laid into a runner bean were supplied and held at either 10 or 20°C as required to produce a supply of nymphs. These were monitored

daily until hatching. Once anthocorids had hatched the larvae were provided with sliced fine beans and *Ephestia* eggs.

Bioassay techniques

Bioassays were done using a topical dosing bioassay. Chemicals tested were sulphur, magnesium sulphate and Activator 90 with appropriate standards.

Dosing method

Nymphs were topically dosed with 0.5 µl of treatment solution and then immediately transferred using a fine sable paintbrush (0 or less in brush size) to individual plastic dishes with a supply of *Ephestia* eggs and fine beans as food. The dish was sealed using Nescofilm™.

One/two day old first instar nymphs were used in the experiment. The compounds were tested in combination and compared to a water control or untreated control and a chlorpyrifos insecticide control. This was based on a field application rate of 500 l per ha and field concentrations of sulphur at 3 l per ha, magnesium sulphate at 7.5 kg per ha and Activator 90 at 0.1% (1 ml per l) (Table 4.2.3.1). The mortality of the nymphs was assessed 1 and two days after treatment (DAT).

Table 4.2.3.1 Treatments

Compound	Trt	Rate	Amount/l00ml
Activator 90 + Sulphur + $MgSO_4$	1	1x	Activator 0.1ml, Sulphur 0.6 ml, $MgSO_4$ 1.5 g
Water control	2	-	-
Untreated control	4	-	-
Chlorpyrifos insecticide control	4	1x	

Results

All nymphs tested with chlorpyrifos had died within 24 hours, showing that the bioassay technique was effective at dosing individuals (Table 4.2.3.2). There was no evidence that the sulphur, magnesium sulphate and Activator 90 combination increased mortality when compared with the water controls. In addition, nymphs that were still alive after one week were able to moult to second instar in both the water control and the combination treatment.

Table 4.2.3.2. The effect of sulphur, magnesium sulphate and a wetter, and an insecticide standard on 1-2 day old anthocorid nymphs

Compound	Number of nymphs treated	Number of nymphs alive	
		Day 1	Day 2
Activator 90 + Sulphur + $MgSO_4$	30	26 (86%)	22 (73%)
Water control	30	20 (66%)	17 (56%)
Untreated control	20	15 (75%)	12 (60%)
Chlorpyrifos insecticide control	10	0 (0%)	0 (0%)

Conclusion

This experiment showed no detrimental effects of using the combination of Activator 90 + sulphur + magnesium sulphate at the standard field rates on first instar anthocorid nymphs. This stage is deemed to be the most susceptible to insecticidal activity. Topically dosing was used, which provides more contact with the product than purely allowing the insect to walk over the surface of sprayed foliage, and therefore provides a 'worse-case scenario'. Thus it is unlikely that the use of these compounds in the field at the rates tested would have any short term effects on predator numbers. However effects of multiple applications should be tested in the field.

Task 4.3 - To identify the most effective physically-acting spray treatment of those used currently that is safe to anthocorid predators and to determine optimum concentration and spray cover requirements (EMR, Yr 4).

Introduction

Many UK pear growers apply a programme of six or more sprays of sulphur or sulphur + magnesium sulphate + non-ionic wetter per season to control pear sucker. The materials are applied at high doses and volumes. These programmes are widely considered by growers to give a useful degree of control of pear sucker. In 2010 a preliminary field experiment was done using Sulphur SC, Activator 90 and Agri-50E. This showed that after two sprays had been applied, all products significantly reduced first and second or third instar nymphs when compared to the untreated control. A water alone treatment was only effective on fourth and fifth instar nymphs on the first assessment. In 2011 two additional experiments were done in the field and in a gauzehouse to gain more information about the effect of these compounds and of different adjuvants.

Experiment 4.3.1. To evaluate the effect of adjuvants for the control of pear psyllid and effects on natural enemies.

The aims of this experiment were to evaluate the effect of different adjuvants for control of pear sucker and to identify the relative efficacy of the different treatments for control of pear sucker eggs and nymphs and possible effects on natural enemies. Treatments tested were foliar sprays of Activator 90 (0.1 solution %), Activator 90 (0.4 % solution), BreakThru (0.15 % solution), Silwet (0.15 % solution), Transcend (0.5 % solution), water alone at 1000 l per hectare and untreated controls. These treatments were used singly and five sprays were applied.

Methods

Dates and duration of study -26 April to 31 May 2011

Site

The study was done in a conference pear orchard at Marsh Gate Farm, Cooling, Kent, by kind permission of David Long. This orchard had been identified as being infested with pear sucker. The orchard was situated at NGR 763 761 (Landranger sheet 178 Thames Estuary). The plant spacing was 3 x 4.3 m, tree density = 775 trees/ha.

Treatments

Six treatments were included as shown in Table 4.3.1.1. below:

Table 4.3.1.1. Treatments

Trt	Product	Product Concentration
1	Activator 90	0.1 %
2	Activator 90	0.4 %
3	BreakThru	0.15 %
4	Silwet	0.15 %
5	Transcend	0.5 %
6	Water	-
7	Untreated	-

Timing of sprays

The treatments were applied when pear sucker eggs were present in the crop. Sprays were applied on 26 April, 4, 11, 19 and 25 May.

Spray application

Five sprays were applied at a volume of 1000 l/ha with a Birchmeier motorised air-assisted knapsack sprayer with a pink micron restrictor. Each tree was sprayed to deliver a volume of

1.29 l of spray solution. The amounts of sprayate remaining were measured to determine the accuracy of spray applications.

Experimental design and layout

A randomised complete block experimental design with four replicate plots of each treatment was used. Each plot consisted of a single pear tree plus one guard tree at either side in a single row. Plots in each block were arranged end to end in the row. Guard rows at either side of the sprayed row were included.

Maintenance sprays

A full maintenance programme of fungicide (and PGR programme) was applied as for the rest of the orchard. Due to the high pest pressure and warm weather, the grower continued to apply sulphur, magnesium sulphate and kaolin throughout the trial and also applied a detergent spray.

Meteorological records

Wet and dry bulb temperature, wind speed and direction were recorded before and after spraying. Full records for the trial duration were available from the EMR met station.

Assessments

Assessments of pear sucker and natural enemy populations were made before the treatment was applied on 26 April 2011 and approximately five-seven days after each spray treatment on 4, 11, 19, 25 and 31 May.

Pear psyllid: Assessments concentrated on determining the effects of treatments on eggs and nymphs. Counts of pear psyllid eggs (all ages counted together), and nymphs of each life stage were made on a randomly selected sample of leaves. Ten old and five young leaves were collected from each plot. All leaves from all plots were assessed on 26 April, 5 and 31 May, 10 old leaves were assessed on 19 May. On 11 and 25 May a sub-sample of leaves from the control plots only were assessed to gain an indication of the developmental stage.

Natural enemies: Anthocorids and other predators were assessed by tap sampling the trees, with five taps per tree. Numbers of adult psyllids were also recorded. A pre-treatment assessment was made on 26 April 2011 and post-treatment assessments were done on 4, 19 and 31 May.

Statistical analysis

ANOVA of counts and other variates with transformation were carried out as necessary.

Results

Results of the full plot assessments on 26 April, 4, 19 and 31 May are shown below in Tables 4.3.1.2 to 4.3.1.5.

Table 4.3.1.2. $Log_{10}(n+1)$ values for the pre-treatment assessment on the 26 April 2011

			Nymphs					
	Eggs		**First and second**		**Third**		**Fourth and fifth**	
Activator 90 low	1.81	89	0.508	3.3	0.619	3.3	1.299	22.8
Activator 90 high	1.03	68	0.807	9.3	0.639	4.0	1.378	32.3
BreakThru	1.87	69	0.590	3.0	0.846	8.7	1.559	42
Silwet	1.32	51	0.712	12	0.369	1.8	1.376	23.8
Transcend	1.61	44	0.467	18.3	0.445	2.5	1.257	17.8
Water	1.31	29	0.608	4.8	0.712	7.0	1.248	22
Untreated	0.99	33	0.345	1.5	0.175	1.0	1.100	15.5
P (17 d.f.)	ns		ns		ns		ns	
s.e.d.	0.499		0.353		0.199		0.204	
l.s.d.	1.053		0.744		0.420		0.431	

Table 4.3.1.3. $Log_{10}(n+1)$ values for the post-treatment assessment on 4 May 2011

			Nymphs					
	Eggs		**First and second**		**Third**		**Fourth and fifth**	
Activator 90 low	2.256	375	0.119	0.5	0.075	0.3	0.075	6.0
Activator 90 high	2.297	243	0.075	0.3	0.195	0.8	0.195	6.0
BreakThru	2.658	494	0.075	0.3	0.075	0.3	0.075	5.3
Silwet	2.068	119	0	0	0	0	0	2.5
Transcend	2.176	206	0.226	1.8	0	0	0	2.5
Water	2.361	305	0.151	0.5	0.119	0.5	0.119	3.8
Untreated	2.118	299	0.151	0.5	0	0	0	3.0
P (17 d.f.)	ns		ns		ns		ns	
s.e.d.	0.242		0.161		0.098		0.098	
l.s.d.	0.508		0.338		0.206		0.206	

Table 4.3.1.4. $Log_{10}(n+1)$ and backtransformed values for the post-treatment assessment on 19 May 2011

	Eggs		**Nymphs**					
			1 + 2		3		4 + 5	
	trans	backtrans	trans	backtrans	trans	backtrans	trans	backtrans
Activator 90 low	2.974	980	1.857	73	0.455	3.3	0.119	0.5
Activator 90 high	2.899	853	1.661	50	0	0	0	0
BreakThru	3.042	1268	1.845	83	0.151	0.5	0	0
Silwet	2.915	885	**1.405**	45	0.195	0.8	0	0
Transcend	2.917	1026	1.691	58	0.464	3	0.119	0.5
Water	3.049	1181	2.014	121	0.612	4.3	0.075	0.25
Untreated	3.090	1372	1.842	82	0.349	2	0.239	1

P (17 d.f.)	ns	0.034	ns	ns
s.e.d.	0.114	0.160	0.249	0.110
l.s.d.	0.240	0.337	0.523	0.230

Significant difference from the untreated shown in bold.

Table 4.3.1.5. $Log_{10}(n+1)$ values for the post-treatment assessment on 31 May 2011

	Eggs		**Nymphs**					
			1 + 2		3		4 + 5	
	trans	backtrans	trans	backtrans	trans	backtrans	trans	backtrans
Activator 90 low	**2.621**	424	**1.474**	30	**0.753**	5.5	**0.195**	0.75
Activator 90 high	**2.625**	432	**1.44**	29	**0.437**	2.3	**0.151**	0.5
BreakThru	**2.628**	433	**1.306**	27	**0.175**	1	**0**	0
Silwet	**2.354**	232	**1.276**	20	**0.075**	0.3	**0.151**	0.75
Transcend	**2.602**	412	1.745	58	**0.726**	6	**0.151**	0.5
Water	2.776	646	**1.615**	47	**0.929**	10.3	0.602	5.75
Untreated	2.894	876	2.016	106	1.563	37	0.709	7.75
P (17 d.f.)	<.001		<.001		<.001		0.05	
s.e.d.	0.082		0.1445		0.193		0.228	
l.s.d.	0.172		0.3036		0.405		0.479	

Significant difference: from the untreated shown in bold; and from the water shown as shaded.

A repeated measures ANOVA was also used on numbers per category per leaf with a square root transformation. This showed no strong evidence of differences between treatments for eggs, although at the later times numbers in the control are higher than the treated. There is also evidence that Silwet gave a significant reduction compared to the combined control. The treatment effects for the third instar nymphs are most apparent on 31 May. At this time there is a large decrease from the untreated to the water control. Activator 90, BreakThru and Silwet are significantly lower than the water control at this time. At the last assessment date the control plots had significantly greater numbers of fourth and fifth instar nymphs than the treated plots overall.

Numbers of pear sucker adults and natural enemies were assessed by tap sampling on each occasion. Categories of natural enemies recorded were ladybird, anthocorid and orius adults and nymphs, spiders, harvestmen, parasitoids and lacewings. These were not analysed due to the low numbers present. The numbers of pear sucker adults were not analysed since the numbers had been estimated, with 100+ per plot for most plots. As pear sucker adults are highly mobile, assessing the effects on the less mobile nymphal stages is more appropriate to determine pesticide efficacy in small plot experiments.

The total numbers of anthocorids across the trial area increased from three adults on 26 April to 34 adults and 23 nymphs on 31 May. These were all *A. nemoralis*. Only two spiders, five ladybirds and 11 parasitoids were found on 31 May.

Conclusion

Silwet (a drift retardant/spreader/wetter) and BreakThru (a wetter) were the most effective treatments at reducing the numbers of first and second instar nymphs. Both of these are 80-85% w/w polyalkylene oxide modified heptamethyl trisiloxane. Activator 90 (a non-ionic surfactant/wetter) also reduced nymph numbers; this contains alcohol ethoxylates and natural fatty acids.

Any applications of these products must be based on label recommendations, as adjuvants must be used with another control product and not alone.

Experiment 4.3.3. To evaluate the effect of sulphur, magnesium sulphate and non-ionic wetter both singly and in combination for the control of pear psyllid.

Methods

Dates and duration of study -17 August to 24 August

Site

The study was done on potted pear trees that had been previously infested with pear psyllids. Trees were held in a gauzehouse at EMR.

Treatments

Ten treatments were included as shown in Table 4.3.3.1. below:

Table 4.3.3.1. Treatments

Trt	Product	Product Dose/ha
1	Sulphur, Activator 90, $MgSO_4$	11 l, 7.5 l, 500 ml
2	Sulphur, $MgSO_4$	11 l, 7.5 l
3	Activator 90, $MgSO_4$	7.5 l, 500 ml
4	Sulphur, Activator 90,	11 l, 500 ml
5	Sulphur	11 l
6	$MgSO_4$	7.5 l
7	Activator 90	500 ml
8	Agrimec/Dynamec	750 ml
9	Water	-
10	Untreated	

Spray application

A single spray was applied at a volume of 500 l/ha with a Birchmeier motorised air-assisted knapsack sprayer. Each tree was sprayed to deliver a volume of 0.5 l of spray solution. The amounts of sprayate remaining were measured to determine the accuracy of spray applications. Trees were removed from the gauzehouse to be sprayed and were returned to the gauzehouse once sprays had dried.

Experimental design and layout

A randomised complete block experimental design with four replicates of each treatment was used. Each replicate consisted of a single potted pear tree.

Meteorological records

Wet and dry bulb temperature, wind speed and direction were recorded before and after spraying.

Assessments

Five leaves were collected from each tree on 17 August prior to spraying and following spraying on 20 August and 24 August.

Pear psyllid: Assessments concentrated on determining the effects of treatments on eggs and nymphs. Counts of pear psyllid eggs (all ages counted together), and nymphs of each life stage were made on a randomly selected sample of leaves.

Statistical analysis

ANOVA of counts and other variates with transformation were carried out as necessary. Initial pre-treatment counts were used as covariates. The treatment set was structured as a 2x2x2 factorial set for Sulphur, magnesium sulphate and Activator 90 plus the standard treatment of Dynamec and also the water control, with the untreated control as the 'null' treatment in the factorial set. Unstructured and structured ANOVAs were provided. All counts were divided by 5 (the number of leaves assessed, to put numbers on a per leaf basis) and then square root transformed before analysis. As there were two dates of recording post treatment this repeated measures ANOVA was a split plot analysis with time as the subplot 'treatment'.

Results

The statistical analysis structure gives a significant effect of Dynamec as expected. The interactions between the treatments are described below and full data are shown in Table 4.3.3.2.

(i) Eggs

There was no significant reduction in any treatment.

(ii) Nymphs 1 and 2

The pre-treatment count was not significant as a covariate, but it has been included for consistency. Here there is overall evidence of treatment differences present (p=0.003), with numbers in the Dynamec treatment significantly lower than all other treatment combinations (this is emphasised in the structured analysis where the comparison of Dynamec with the water control is highly significant (p<0.001)).

(iii) Nymphs 3

Here the covariate had only a slight effect (p=0.081). There are overall treatment differences present (p=0.001), due to numbers in the Dynamec treatment being significantly lower than the rest (p<0.001 in the structured analysis). However, there were no differences present between the other 9 treatment combinations.

(iv) Nymphs 4

The pre-treatment count was significant as a covariate (p=0.005) and overall treatment differences were present (p=0.027). Numbers in the Dynamec treatment were significantly lower than all other treatments except water (p=0.102). There was a fairly strong indication of an interaction between time and magnesium sulphate applications – numbers at time 1 + $MgSO_4$ > - $MgSO_4$, whereas at time 2 + $MgSO_4$ < - $MgSO_4$.

(v) Nymphs 5

The pre-treatment count was significant as a covariate (p=0.003) but there was no indication of any treatment differences present. From the structured ANOVA there was evidence of an overall difference between the factorial set and the other two treatments (water and Dynamec) (p=0.022) but there were no significant differences between any of the treatments and either of the controls.

Table 4.3.3.2. Transformed values per leaf (adjusted for covariate) for the post-treatment assessments on 20 and 24 August 2011

Treatment	Eggs		Nymphs							
			First + second		Third		Fourth		Fifth	
	20/8	24/8	20/8	24/8	20/8	24/8	20/8	24/8	20/8	24/8
Sulphur, Activator 90, $MgSO_4$	2.94	2.70	2.32	3.22	1.24	1.57	0.92	0.82	0.48	0.40
Sulphur, $MgSO_4$	2.11	0.50	3.13	2.06	1.50	1.56	0.99	0.59	0.43	0.59
Activator 90, $MgSO_4$	2.41	1.87	1.90	1.91	1.03	1.00	0.52	0.71	0.72	0.28
Sulphur, Activator 90,	2.99	1.49	2.13	1.97	1.22	1.27	0.49	0.88	0.60	0.67
Sulphur	1.94	1.61	2.21	1.82	0.95	0.81	0.38	0.69	0.22	0.64
$MgSO_4$	1.43	1.37	2.11	2.24	1.01	1.23	0.94	0.66	0.45	0.40
Activator 90	2.40	1.99	2.21	1.86	1.12	1.20	0.32	0.88	0.43	0.46
Agrimec/Dynamec	1.92	1.53	0.47	0.26	0.30	0.03	0.06	-0.05	0.27	-0.03

Water	2.42	1.55	2.23	2.14	1.30	0.97	0.35	0.49	0.44	0.19
Untreated	2.29	2.11	2.69	3.04	1.22	1.70	0.91	1.13	0.37	0.52

Conclusion

As a single spray none of the treatments could reduce the levels of pear psyllid nymphs to those achieved by the Dynamec application. However, a repeated programme of sprays may have a different effect.

Task 4.4. Evaluate late winter spray treatments with Kaolin (EMR, A Scripps, D Long, J Baxter, FAST, Yrs 1-4)

Introduction

Research in several other countries has demonstrated that late winter treatments with particulate films of kaolin (Surround) give good suppression of over wintering pear sucker adults. Sprays at this time are unlikely to affect subsequent photosynthesis or have harmful effects on anthocorid predators as these are not present in pear orchards in substantive numbers in the dormant period. The value of this approach in the UK, and the effects of timing and number of sprays, need to be investigated.

Methods and Materials

A large scale experiment using replicated eight orchard plots was done to evaluate the efficacy of dormant period sprays of kaolin (Surround) for control of pear sucker adults and nymphs. Large plots were needed for this work because of the dispersive nature of pear sucker adults. In the previous three years, one-three sprays of late season Kaolin have given good control of pear sucker nymphs at assessments in April. However, it is reported that pear sucker recovers by the summer. In 2011 the treatments were assessed in the summer, as well at the spring, to determine if Kaolin had effects well into the second generation.

Eight orchards were selected (Table 4.4.1) for Kaolin treatments to be applied. Growers aimed to apply four sprays of kaolin between February and April (Table 4.4.2).

There were two experimental treatments in each orchard, a programme of applications of Surround from mid- February to April, and an untreated control. Surround WP was applied at 12.5kg/ha in 250 litres water/ha. The lower water volume was used to give adequate coverage on the young trees (recommended rate on label; 50 lb in 100 gallons water per acre = 56 kg in 1100 litres water per ha). Approximately 10% of each orchard at one end was left unsprayed as an untreated control (see Fig. 4.4.1 as an example). The cost of a single spray of Kaolin is around £35/ha.

The sprays were applied with the grower's air-assisted orchard sprayer using hollow cone nozzles. The orchards were divided into sprayed and unsprayed. The sprayed area was about 90% of the orchard and the unsprayed area about 10%. This gave eight replicates of the treatment.

Two assessments were done at each site (11-13 April and 14 June). The first up to 2 weeks

after the final spray application and the second at peak population in June. Assessments were done by EMR staff. The centre of the control plots and the treated area furthest from the control plot was sampled.

20 trees in each plot were tap sampled over a white tray after the spray applications. The numbers of adult pear sucker and anthocorids were recorded. The numbers of eggs and nymphs were also recorded around the base of 40 fruiting buds/clusters: two of each from 20 trees were counted under a microscope in the laboratory at EMR.

Kaolin sprays were part of an overall IPM programme, so the growers applied other pear sucker treatments later in the season as and when required.

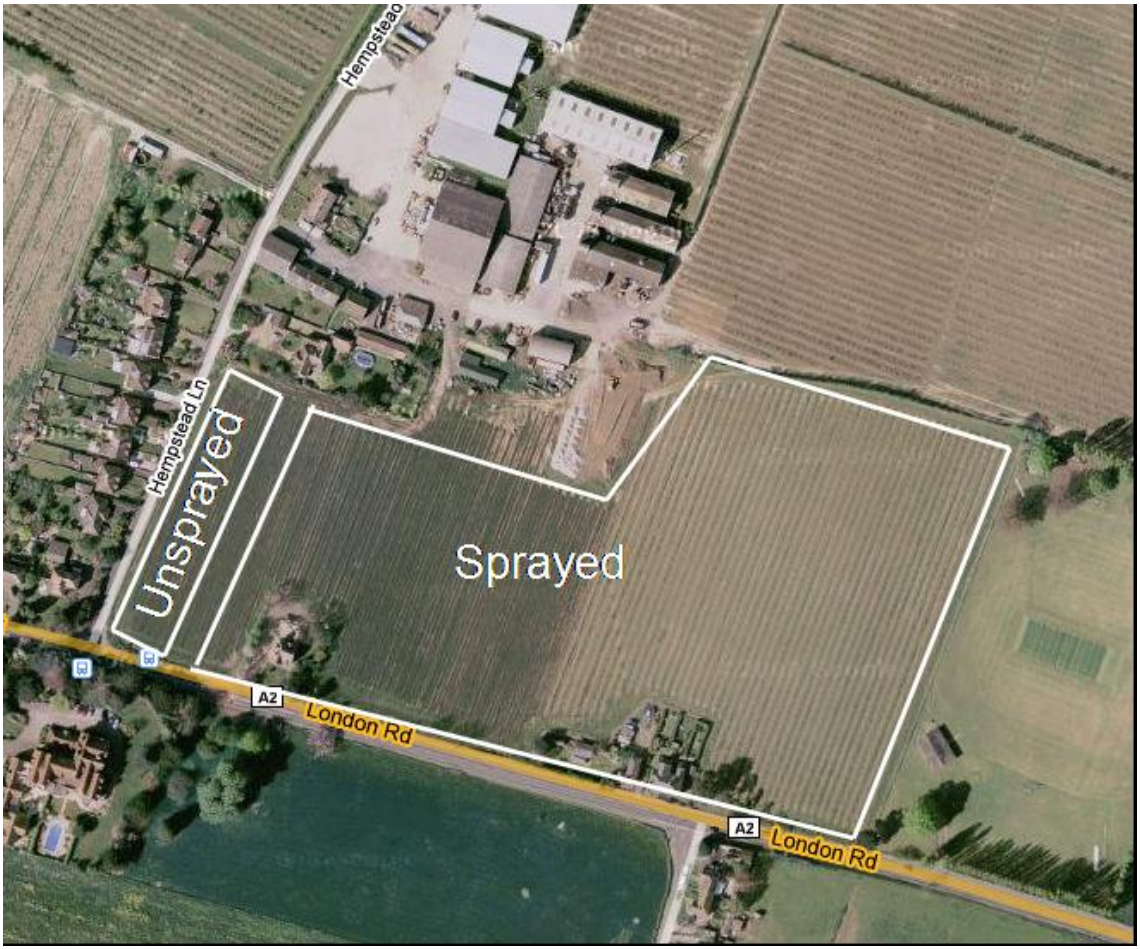


Figure 4.4.1. Example of spray location at Firs Orchard. The majority of the orchard was sprayed, leaving just 10% of the rows untreated as a control.

Table 4.4.2. Dates of spray applications of Surround (Kaolin)

Farm	Field	Spray date	Other
Batteries	Picklin's Pears	10, 22 March	Insegar on 24 March
G H Dean & Co	"All orchards"	18, 21 February and 7 March	
Sherenden Farm	Long Orchard	4, 28 March	
Elphicks Farm	Potato Ground	8, 16, 24, 31 March	

Table 4.4.1. Growers orchards sprayed with kaolin

Site	Contact	Grower	Farm	Contact details	Orchard	Variety	Planting date	Area (ha)	Plant spacing
1	David Butler	Oliver Doubleday	G H Dean & Co., Hempstead Farm, Bapchild, Sittingbourne, Kent ME9 9BH	07970 629896 david@ghdean.co.uk Oliver: 07747 773443 Email: oliver@ghdean.co.uk	Firs NGR TQ 935630	Conference	Jan 2005 and Jan 2008	2.25	3.75m x 1.5m and 1m apart in row, respectively
2					Newbury Stables NGR TQ 929600	Conference	~1981	3.5	
3					Banks NGR TQ 965651	Conference	~1981	3.15	
4					Cricket meadow NGR TQ 923618	Conference	~1999	2.3	
5					Prospect NGR TQ 914617	Conference	~1981	4.3	
6	James Shillitoe (FAST)	Adrian Scripts	Sherenden Farm, Sherenden Rd, Tudeley, Tonbridge, Kent TN11 0PE	07590 775160 james.shillitoe@fastltd.co.uk	Long Orchard NGR TQ 625461	Conference	2005.		4.0 m x 1.25 m
7		Clive Baxter	Elphicks Farm, Water Lane, Maidstone, ME15 0SG		Potato Ground NGR TQ 715488	Conference			
8	Mike Barnett	Melvyn Newman Newmafruit 07976 738173	Batteries Farm Claxfield Road Lynsted Sittingbourne Kent ME9 0JQ	Mob: 07889 768338 mike@newmafruit.co.uk	Picklin's Pears NGR 613937	Conference (Comice 1 and 9)	1948	3.0	5.4 m x 5.4 m

Results

Dormant season sprays of kaolin gave good control of pear sucker, reducing numbers of nymphs by over 75% in many cases in previous years (Fig. 4.4.2).

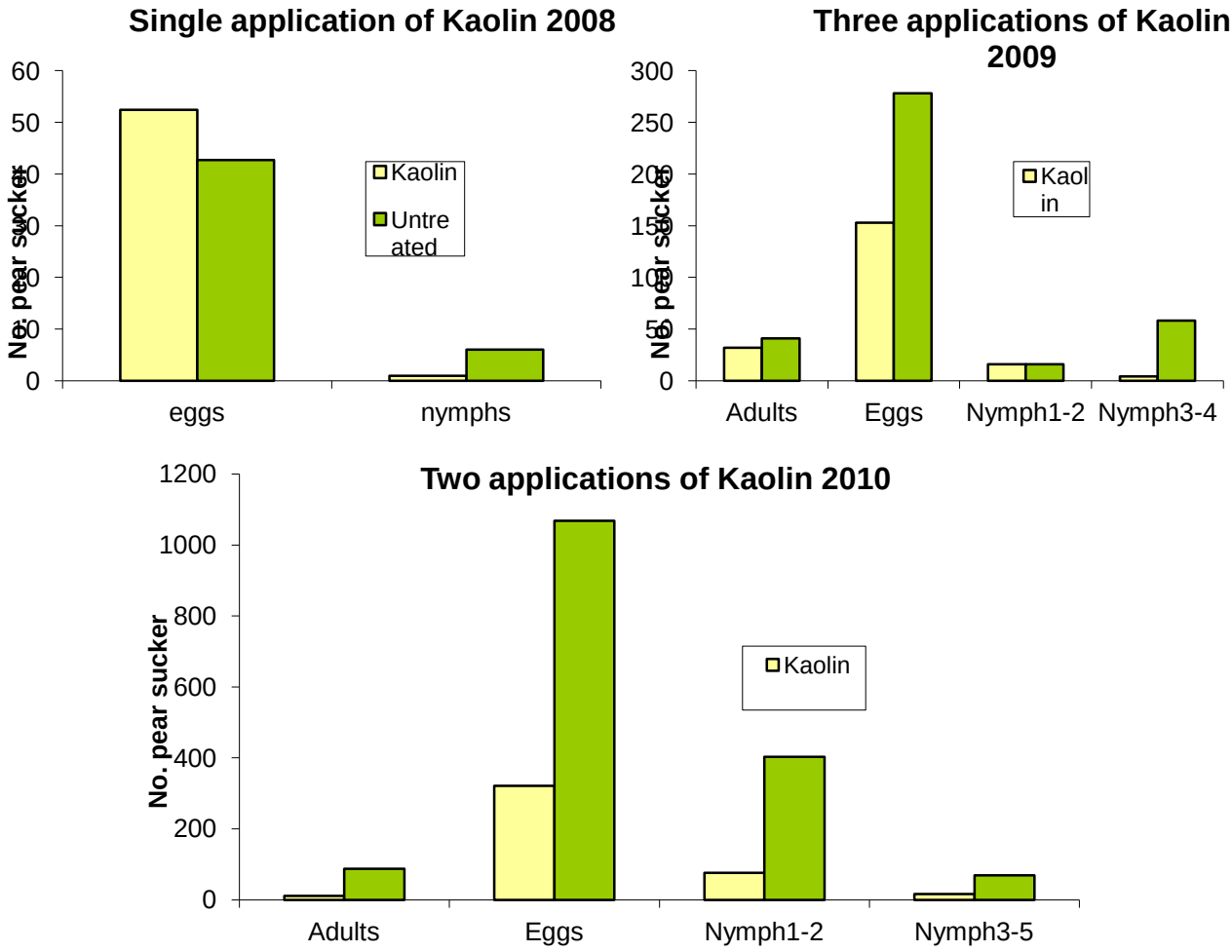


Figure 4.4.2. Numbers of pear sucker adults, nymphs and eggs from beat sampling and on shoot samples for 2008, 2009 and 2010 with one, three or two spray applications respectively.

Generally, at the 11-13 April 2011 assessment, across all eight sites there were fewer adult, nymph and eggs of pear sucker on the kaolin treated compared to untreated plots across all sites. However, the decrease was not statistically significant (Fig. 4.4.3). Six of the eight orchards had fewer eggs, nymphs and adult pear sucker with the exception of 'Prospect' and 'Long' orchards. There were significantly fewer adult anthocorids on the kaolin treated plots (ANOVA on Log_{10} transformed data, P=0.018, sed=0.1127, lsd=0.2665, Fig. 4.4.3). There were fewer anthocorid eggs on the treated plots, but this was not significant (Fig. 4.4.4). There were no significant effects on *Orius*, ladybird or spider numbers

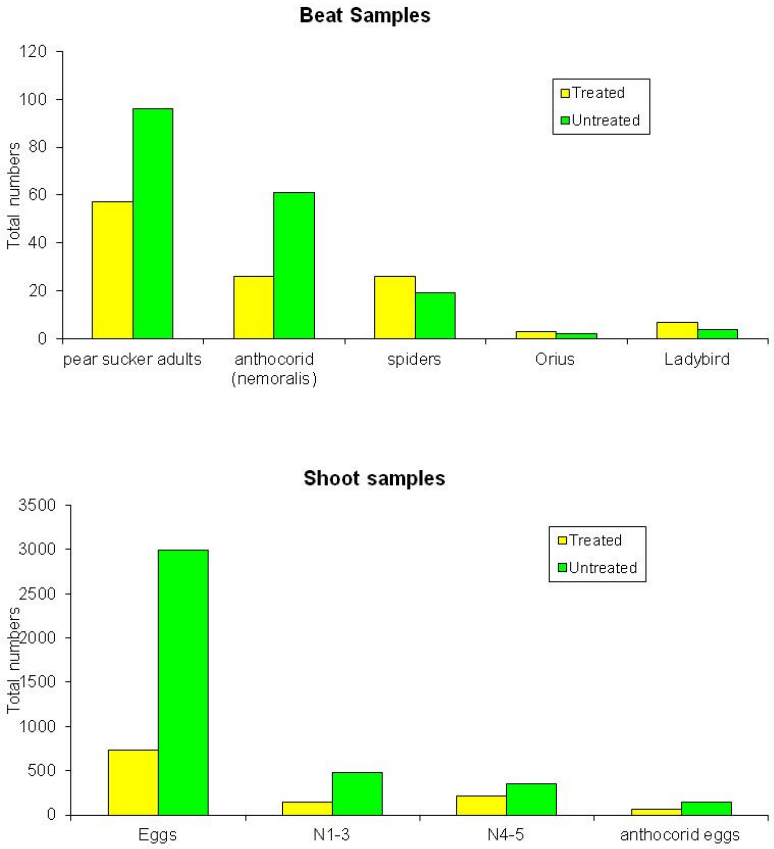


Figure 4.4.3. Numbers of arthropods from beat sampling (above) and on shoot samples (below)

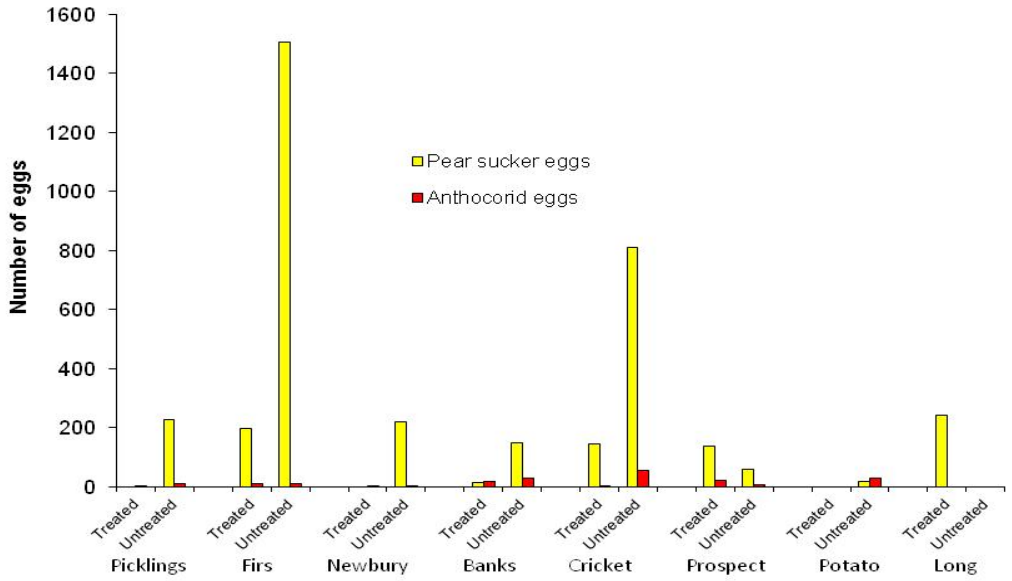


Figure 4.4.4. Numbers of pear sucker and anthocorid eggs in the April assessments on shoots in the kaolin treated and untreated plots

At the 14 June assessment there fewer, although not quite significantly, pear sucker eggs on the treated plots (ANOVA on Log_{10} transformed data, P=0.079, sed=0.1443., lsd=0.3413, Fig. 4.4.5), three months after the kaolin had been applied. The adult pear sucker numbers were significantly lower (ANOVA on Log_{10} transformed data, P=0.007, sed=0.0782., lsd=0.1849, Fig. 4.4.5), overall, across the eight sites on the kaolin treated compared to untreated plots. This demonstrates lasting effects into the second generation (Fig. 4.4.5). Anthocorid eggs and adults were unaffected indicating that the early sprays of kaolin had no lasting effects on their populations in the summer – probably because most had not migrated into the orchards at the time of spraying.

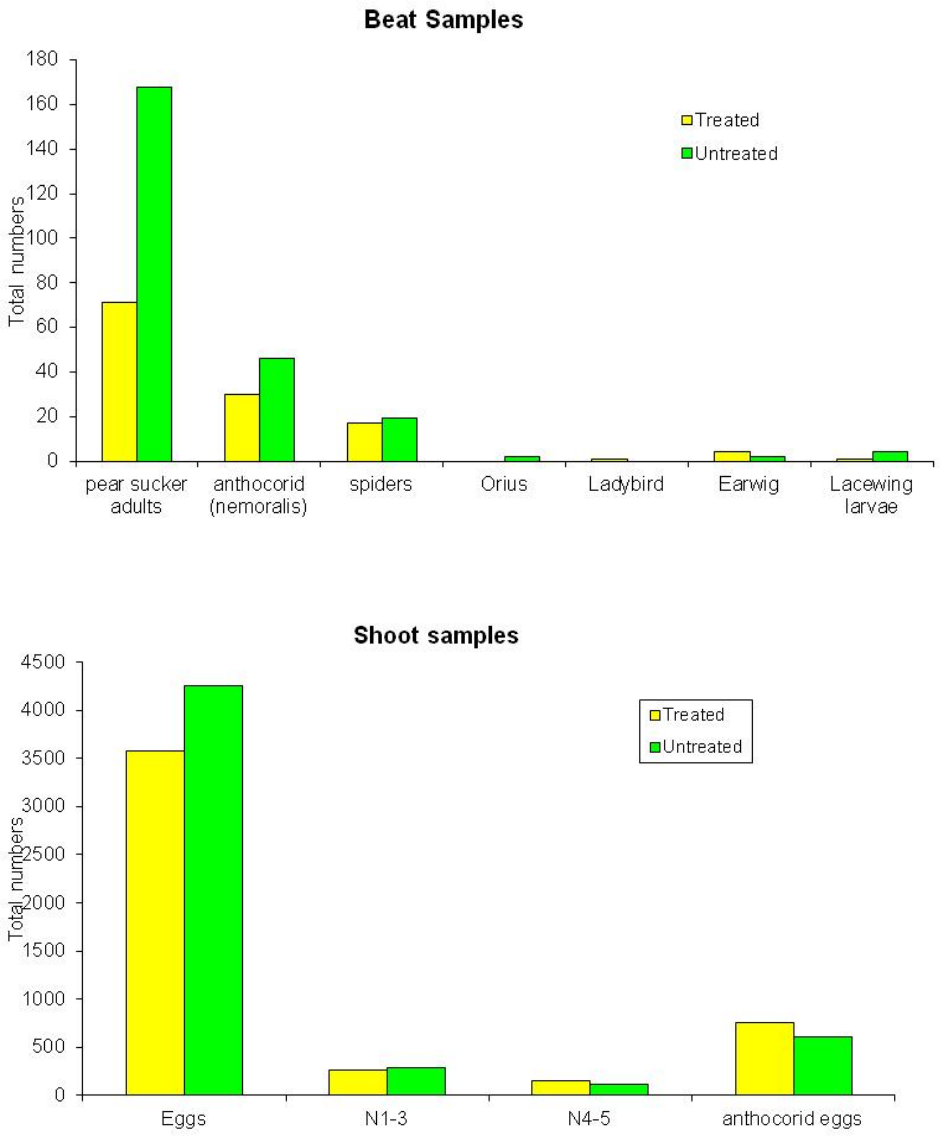


Figure 4.4.5. Numbers of arthropods from beat sampling (above) and on shoot samples (below)

Numbers of pear sucker eggs were lower in the treated compared to untreated orchards in five of the eight orchards (Fig 4.4.6) in June. However, although most orchards had low numbers of pear sucker, a noticeable difference was the dramatic increase in eggs in 'Long' orchard. Table 4.4.2 shows the spray records for the orchards. It can be seen that 'Long' orchard received multiple sprays of chemical insecticides, including chlorpyrifos, fenoxicarb, spirodiclofen, diflubenzuron and indoxacarb; nine spays in total. These applications were likely to have killed natural predators, including anthocorids and earwigs. From the data in Table 4.4.2 it appears that a small number of sprays of methoxyfenozide and fenoxycarb against moths are not detrimental to predators, but more research is needed to confirm this.

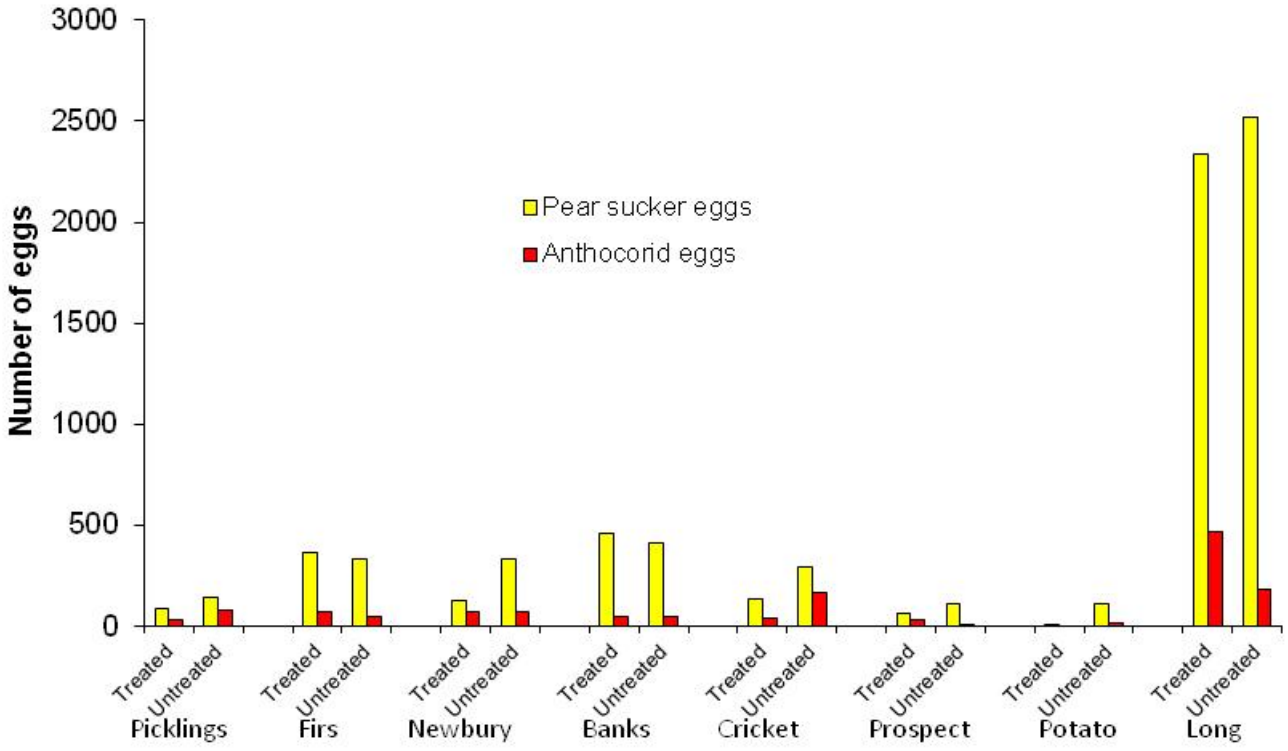


Figure 4.4.6. Numbers of pear sucker and anthocorid eggs in the April assessments on shoots in the kaolin treated and untreated plots

When the data across all eight sites was combined for the two dates there was a significant reduction in pear sucker eggs (ANOVA on Log_{10} transformed data, P=0.023, sed=0.1576., lsd=0.3726) and pear sucker adults (ANOVA on Log_{10} transformed data, P=0.0006, sed=0.0815., lsd=0.1927). There was almost a significant reduction in nymph stages 1-3 (P=0.059).

Orchard	Date	a.i.	Product	Rate/ha
Firs	03/03/11	adjuvant	Spraygard	0.25 l
	22/03/11	Magnesium sulphate	Bittersalz	5 Kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Fenoxycarb	Insegar	0.4 kg
	06/04/11	Magnesium sulphate	Bittersalz	5 Kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
	23/04/11	Spirodiclofen	Envidor	0.6 l
		Magnesium sulphate	Bittersalz	5 Kg
		Sulphur	Headland sulphur	3 kg
	04/05/11	Sulphur	Headland sulphur	4l
		Magnesium sulphate	Bittersalz	5 kg
	11/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	5 kg
	19/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	6 kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
	27/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	8 kg
	08/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	15/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	18/06/11	Runner	Methoxyfenozide	0.45 l
		Magnesium sulphate	Bittersalz	7.5 kg
	11/07/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	22/09/11	Sulphur	Headland sulphur	3 l
		Magnesium sulphate	Bittersalz	8 kg
Newbury Stable	19/03/11	adjuvant	Spraygard	0.25 l
	28/03/11	Magnesium sulphate	Bittersalz	5 Kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Fenoxycarb	Insegar	0.4 kg
	07/04/11	Magnesium sulphate	Bittersalz	5 Kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
	23/04/11	Spirodiclofen	Envidor	0.6 l
		Magnesium sulphate	Bittersalz	5Kg
		Sulphur	Headland sulphur	3 kg
	09/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	5 kg
	12/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	5 kg
	21/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	6 kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
	03/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	8 kg
	10/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	23/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	30/06/11	Methoxyfenozide	Runner	0.45 l
		Bittersaz	Magnesium sulphate	7.5 kg
	18/07/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	23/09/11	Sulphur	Headland sulphur	3 l

		Magnesium sulphate	Bittersalz	8 kg
Prospect	17/03/11	adjuvant	Spraygard	0.25 l
	23/03/11	Magnesium sulphate	Bittersalz	5 Kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Fenoxycarb	Insegar	0.4 kg
	06/04/11	Magnesium sulphate	Bittersalz	5 Kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
	08/04/11	Magnesium sulphate	Bittersalz	5 Kg
	20/04/11	Spirodiclofen	Envidor	0.6 l
		Magnesium sulphate	Bittersalz	5 Kg
		Sulphur	Headland sulphur	3 kg
	05/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	5 kg
	12/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	5 kg
	20/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	6 kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
	27/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	8 kg
	10/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	24/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	01/07/11	Runner	Methoxyfenozide	0.45 l
		Magnesium sulphate	Bittersalz	7.5 kg
	21/09/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	8 kg
Banks	04/03/11	adjuvant	Spraygard	0.25 l
	19/03/11	adjuvant	Spraygard	0.25 l
	24/03/11	Magnesium sulphate	Bittersalz	5 Kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Fenoxycarb	Insegar	0.4 kg
	06/04/11	Magnesium sulphate	Bittersalz	5 Kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
	08/04/11	Magnesium sulphate	Bittersalz	5 Kg
	20/04/11	Spirodiclofen	Envidor	0.6 l
		Magnesium sulphate	Bittersalz	5 Kg
	04/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	5 kg
	12/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	5 kg
	20/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	6 kg
	02/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	8 kg
	09/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	23/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	30/06/11	Methoxyfenozide	Runner	0.45 l
		Bittersaz	Magnesium sulphate	7.5 kg
	15/07/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	22/09/11	Sulphur	Headland sulphur	3 l
		Magnesium sulphate	Bittersalz	8 kg

Cricket meadow	03/03/11	adjuvant	Spraygard	0.25 l
	19/03/11	adjuvant	Spraygard	0.25 l
	24/03/11	Magnesium sulphate	Bittersalz	5 Kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Fenoxycarb	Insegar	0.4 kg
	04/04/11	Magnesium sulphate	Bittersalz	5 Kg
		Mancozeb	Karamate Dry Flo Neotec	2.0 kg
	23/04/11	Spirodiclofen	Envidor	0.6 l
		Magnesium sulphate	Bittersalz	5 Kg
		Sulphur	Headland sulphur	3 l
	30/04/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	5 kg
	14/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	5 kg
	20/05/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	6 kg
	01/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	8 kg
	11/06/11	Mancozeb	Karamate Dry Flo Neotec Headland	2 kg
		Sulphur	sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	15/06/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	30/06/11	Methoxyfenozide	Runner	0.45 l
		Bittersaz	Magnesium sulphate	7.5 kg
	15/07/11	Sulphur	Headland sulphur	4 l
		Magnesium sulphate	Bittersalz	7.5 kg
	22/09/11	Sulphur	Headland sulphur	3 l
		Magnesium sulphate	Bittersalz	8 kg
Pick Wood	31/03/11	Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Fenoxycarb	Insegar	0.2 kg
	13/04/11	Mancozeb	Karamate Dry Flo Neotec	2.0 kg
	05/05/11	Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Sulphur	Sulphur Flowable	2.0 l
	16/05/11	Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Sulphur	Sulphur Flowable	2.0 l
		Fenoxycarb	Insegar	0.15 kg
	23/05/11	Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Sulphur	Sulphur Flowable	2.0 l
		Fenoxycarb	Insegar	0.15 kg
	08/06/11	Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Sulphur	Sulphur Flowable	2.0 l
	17/06/11	Mancozeb	Karamate Dry Flo Neotec	2.0 kg
		Sulphur	Sulphur Flowable	2.0 l
		Fenoxycarb	Insegar	0.15 kg
	30/06/11	Sulphur	Sulphur Flowable	2.0 l
	13/07/11	Chlorpyrifos	Equity	1.0 l
		Sulphur	Sulphur Flowable	2.0 l
Long field	04/03/11	Kaolin	Surround	15.55 kg
	24/03/11	Sulphur	Sulphur Flowable	1.996 l
	31/03/11	Chlorpyrifos	Govern	0.596 kg
	20/04/11	Magnesium sulphate	Bittersalz	2.02 kg
	28/04/11	Fenoxycarb	Insegar	0.408 kg
		Sulphur	Headland sulphur	2.051 l
		Magnesium sulphate	Bittersalz	2.070 kg

05/05/11	Fenoxycarb	Insegar	0.453 kg
	Sulphur	Headland sulphur	2.070 l
	Magnesium sulphate	Bittersalz	2.278 kg
12/05/11	Mancozeb	Karamate Dry Flo Neotec	2.040 kg
	Fenoxycarb	Insegar	0.408 kg
	Sulphur	Headland sulphur	3.065 l
	Magnesium sulphate	Bittersalz	3.416 kg
19/05/11	Chloryrifos	Goven	1.203 kg
	Sulphur	Headland sulphur	3.065 l
	Magnesium sulphate	Bittersalz	3.416 kg
25/05/11	Spirodiclofen	Envidor	0.620 kg
	Sulphur	Headland sulphur	3.088 l
	Magnesium sulphate	Bittersalz	3.416 kg
02/06/11	Mancozeb	Karamate Dry Flo Neotec	2.040 kg
	Sulphur	Sulphur Flowable	3.065 l
	Magnesium sulphate	Bittersalz	3.416 kg
	Diflubenzuron	Dimilin flo	0.307 l
08/06/11	Mancozeb	Karamate Dry Flo Neotec	2.040 kg
	Sulphur	Sulphur Flowable	3.065 l
	Magnesium sulphate	Bittersalz	3.416 kg
15/06/11	Sulphur	Sulphur Flowable	2.043 l
	Magnesium sulphate	Bittersalz	3.416 kg
21/06/11	Sulphur	Sulphur Flowable	2.043 l
	Magnesium sulphate	Bittersalz	3.416 kg
29/06/11	Sulphur	Sulphur Flowable	2.043 l
	Magnesium sulphate	Bittersalz	3.416 kg
05/07/11	Sulphur	Sulphur Flowable	2.043 l
	Magnesium sulphate	Bittersalz	3.416 kg
	Diflubenzuron	Dimilin flo	0.317 l
12/07/11	Sulphur	Sulphur Flowable	2.043 l
	Magnesium sulphate	Bittersalz	3.416 kg
20/07/11	Magnesium sulphate	Bittersalz	3.416 kg
29/07/11	Sulphur	Sulphur Flowable	2.043 l
	Magnesium sulphate	Bittersalz	3.416 kg
	Spinosad	Tracer	0.258 l
05/08/11	Sulphur	Sulphur Flowable	2.043 l
	Magnesium sulphate	Bittersalz	3.410 kg
18/08/11	Indoxacarb	Steward	0.251 kg
20/09/11	Sulphur	Sulphur Flowable	2.042 l
28/09/11	Mancozeb	Diflubenzuron	2.032 kg
	Sulphur	Sulphur Flowable	2.042 l
	Magnesium sulphate	Bittersalz	5.079 kg

Objective 5. To transfer the results of the research to UK pear growers in a series of workshops as part of a wider focus on improving and increasing UK pear production.

Task 5.1. Hold a series of 3 half to one day workshops on pear production (English Apples and Pears, Sainsbury's, Grower partners, EMR, NRI (Yrs 1, 3, 4)

Pear growing for the future' - A one day conference focusing on UK pear growing took place on Thursday 25 February 2010, Conference Centre, East Malling Research. It was organized as part of the technology transfer activities of this project by EMR (J Cross), EAP (A Barlow) and Sainsbury's (T Huxley). It was attended by ~80 delegates mainly from the industry, including many UK pear growers.

16 Feb-2011 Sainsbury/Chingford UK Pear Grower Focus Group - EMR Concept Pear Orchard - Demonstration Morning

Profitable pear production in the UK - A one day conference focusing on UK pear growing took place on 27 February 2012, Conference Centre, East Malling Research. It was organized as part of the technology transfer activities of this project by EMR (J Cross), EAP (A Barlow) and Sainsbury's (T Huxley). It was attended by ~80 delegates mainly from the industry, including many UK pear growers.

SIX MONTHLY REPORT TO HORTICULTURE LINK PROGRAMME MANAGEMENT COMMITTEE
(48 month report due 31 March 2012)

Project Number:	HL0194
Project Title:	Exploiting semiochemicals, conservation biocontrol and selective physical controls in integrated management of pear sucker
Project Partners:	Horticultural Development Council; East Malling Trust; WorldWideFruit; FAST; H L Hutchinson; UAP Ltd; Agrisense BSG Ltd; G H Dean Ltd A Scripps Ltd; H Chapman Ltd; D G Long; J L Baxter & Son; H Rudge; Robert Mitchell; partnership; East Malling Ltd; English Apples and Pears Ltd; J Sainsbury's plc
Report Written by:	J Cross
Project Start/Completion Dates:	1 April 2008 – 31 March 2012
Reporting Period:	1 October 2011 – 31 March 2012
Number of Months Since Commencement:	48
Date of Last Management Meeting:	7 March 2012
Dates of Next Meetings	

1. Project objectives:

1. To identify the sex pheromone of the pear sucker, *Cacopsylla pyricola*, and exploit it for pest monitoring

   Sub-objective 1.1. To identify pear sucker, *Cacopsylla pyricola*, sex pheromone
   Sub-objective 1.2. Demonstrate pheromone activity, develop a lure and trapping system, and calibrate in the field.

2. To develop conservation biocontrol methods to maximise anthocorid populations and other natural enemies of pear sucker

   Sub-objective 2.1. Identify woody species and species mixes for hedgerows / windbreaks
   Sub-objective 2.2. Investigate anthocorid over wintering and the benefits of artificial refuges
   Sub-objective 2.3. Investigate use and management of strips of stinging nettle versus a purpose-sown flowering ground herbage mix adjacent to hedgerow/windbreak bordering pear orchards
   Sub-objective 2.4. Investigate the benefits of more diverse flowering ground herbage in pear orchard alleyways

3. To exploit synomones (of pear foliage fed on by pear sucker) to attract anthocorids into pear orchards in spring

   Sub-objective 3.1. Establishment of blends and release rates of synomones for attracting anthocorids.
   Sub-objective 3.2. Development of method for deployment of synomones for attracting anthocorids into pear.

4. To identify the most effective physically-acting spray treatment of those used currently that is safe to anthocorid predators and to determine optimum concentration and spray cover requirements.

To transfer the results of the research to UK pear growers in a series of workshops as part of a wider focus on improving and increasing UK pear production.

2.	**Table showing overview of progress against milestones for project as a whole** (from project proposal, or other more recently approved planning document)		
Milestone	Target month	Title	
P1.1.5	24	Chemical structures of *C. pyricola* pheromone components determined and synthesised	N
P1.2.2	36	Pheromone attractiveness demonstrated and lure and trap optimised	N
P1.2.4	48	Protocol for use of pear sucker sex pheromone trap prepared	N
P2.1.1	6	Experimental hedgerows planted	Y
P2.1.2	3	Five existing hedgerows identified and characterised for future study	Y
P2.1.3	42	Four season data set characterising predator and prey communities in hedgerows complete	Y
P2.1.5	48	Recommendations on choice of hedgerow woody species and management practices formulated	Y
P2.2.2	45	Four season data set on the occurrence of over wintering predators in refuges complete	Y
P2.2.3	48	Recommendations for growers on the use of artificial refuges for anthocorid over wintering formulated	N
P2.3.1	6	Strip plantings of nettles and flowering herbs on grower farms established	N[1]
P2.3.2	42	Four season data set characterising predator and prey communities in strips completed	N
P2.3.4	48	Recommendations on benefits of nettle/flowering herb strips and management practices formulated	Y
P2.4.1	6	Tall flowering herb mix sown in alleys of two orchards	Y
P2.4.2.	45	Four season data set characterising predator and prey communities in alleyway herbage and attendant pear trees	N
P2.4.4.	48	Benefits of alleyway flowering herbage determined and recommendations for growers formulated	Y
P3.1.3	24	Best blend/release rate of synomones for attracting anthocorids determined	N
P3.2.1	42	Two large scale experiments evaluating efficacy of synomone dispensers completed and benefits of treatment determined	Y
P4.3	42	Field evaluating effects of numbers sprays and spray cover of physically acting spray treatment completed	Y
P4.4	48	Four field experiments evaluating winter spray treatments with kaolin completed and benefits of treatment and timing and number of sprays determined	Y
P5.1	12	First half-one day workshop focusing on UK pear growing held	Y

P5.2	36	Second half-one day workshop focusing on UK pear growing held	N
P5.3	48	Third half-one day workshop focusing on UK pear growing held	Y
[1] The nettles were sown but they failed to establish. It was decided to use existing nettle patches for the work			
S1.1.1	12	Rearing methods for summerform and winterform *C pyricola* established	Y
S1.1.2	18	Volatiles collected from winterform and summerform adult *C pyricola*	Y
S1.1.3	24	Chemical analysis of volatile collections completed	Y
S1.1.4	24	GC-EAG of volatile collections completed	N[2]
S1.2.1	30	Dispensers for *C pyricola* pheromone components prepared	N
S1.2.3	42	Sex pheromone trap calibrated for monitoring	N
S2.1.4	42	Two MAB experiments investigating hedgerow trimming completed	Y
S2.2.1	28	Over wintering of anthocorids in natural habitats investigated	Y
S2.3.3	42	Two MAB experiments investigating nettle/flowering strip cutting completed	Y
S3.1.1	18	Attractive compounds from pear sucker infested pear seedlings investigated	Y
S3.1.2	18	Dispensers for synomones developed	Y
S4.1	24	Insecticidal activity of sulphur, magnesium sulphate and wetter determined	Y
S4.2	36	Effects of best treatment on anthocorids determined	Y
[2]Trail following experiments used to assess attractiveness of extracts and synthetics			
Note: The rest of this form has not been completed as the project has ended and a LINK completion form prepared			

GUIDANCE NOTES

- All sections should be written by the project research co-ordinator in consultation with the project partners.

- The report should normally be 2-4 pages long, excluding the list of publications.

- The approved project proposal document should be used as a reference when describing research progress in sections 2 and 4 and for reporting on how the exploitation opportunities have developed during the course of the research in section 7.

- Proposed and actual dates of completion for objectives and milestones should be given in sections 2 and 3.

- The research report in section 4 needs to contain enough detail to give a clear idea of the state of the project, highlights and any critical issues. Simply stating that the project is on schedule and meeting its objectives is not sufficient.

- When commenting in section 4 on any delay or non-achievement (or indeed early achievement) of project milestones an assessment should be given of the reasons for this, the likely impact on the project overall. This must be related to any proposals to change the project plan in section 5.

- Any changes in project staffing and their impacts should be included in section 5.

- Only publications and presentations arising as a direct result of the LINK project should be listed in section 6.

- The report, once approved by the consortium, should be submitted to the joint LINK programmes Secretariat who will pass to the relevant sponsor Project Monitoring Officer and the Programme Management Committee

- Please note that as well as referring to technical issues and objectives the sponsor and PMC will consider the performance of the consortium (is good collaboration evident? Are in-kind contributions from companies being received as planned? Are future plans realistic? etc).

- Submission of reports will be on a rolling six monthly basis, deadline dates to be agreed project by project.

Reports should be sent to: **hortlink@defra.gsi.gov.uk**
Joint LINK Programmes Secretariat
Area 8A LMB
Nobel House
17 Smith Square
London
SW1P 3JR

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