Project title:	Exploiting semiochemicals, conservation biocontrol and selective physical controls in integrated management of pear sucker
Project number:	TF 181 (HL0194)
Project co-ordinator:	Oliver Doubleday
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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CONTENTS

GROWER SUMMARY

Headline

Nettles, willow and hazel should be provided round pear orchards as the best sources of anthocorid predators

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. The pear sucker sex pheromone is known to exist and could be identified. This would provide 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 overwinter 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. The 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 project and main conclusions

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. During 2010 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.

Analytical results were similar to those obtained during 2009 but are more definitive with more replicates and the replicates done under very standard conditions and analysed soon after preparation. As previously, analyses of washes from males and females showed that no compounds existed in the males that were not present in the females and vice versa. In addition, there were no significant differences in the relative amounts of each compound between males and females. 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. pyricola*.

No attraction of the opposite sex of *C. pyri* has been demonstrated in the field using 13-Methylheptadecane 1 mg mL^{-1} or unmated males or females. Hexane washes of females also failed to attract male *C. pyri* males.

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

Sampling of the replicate tree species hedgerow plots planted in spring 2008 was started. A data base of 5753 arthropods sampled was constructed. However, numbers of anthocorids collected were rather small and erratic. Identification of the dominant psyllids and aphids from shoot samples collected from the established existing hedgerow plots in 2009 was completed. 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 started to establish. Nettles had established strongly at all 3 sites and were tall and the abundant arthropod fauna of nettles was present on many subjects. Further sampling is planned for 2011, in the final year of the project.

An experiment using protein (milk and egg white) markers and monoclonal antibody detection methods demonstrated low levels of migration 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. Migration occurred for distances > 50 m.

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

Work is ongoing to characterise the chemical signature of pear sucker infested pear foliage and to try to emulate the attractive signal with synthetic lures. 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

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).

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 orchards are so badly attacked by the pest that they have become unviable and have to be grubbed. Loss/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.

Action points for growers

- Growers who would like a copy of the pear sucker identification guide or who would like the species of pear sucker present in their orchards checked, should contact Jerry Cross or Michelle Fountain at East Malling Research (Email: jerry.cross@emr.ac.uk; michelle.fountain@emr.ac.uk, Office: 01732 523748).

- Growers should conserve nettles, willow and hazel trees in the vicinity of pear orchards to act as early season sources of Anthocorids and consider planting these if they are not present.

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

SCIENCE SECTION

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.

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)

Ongoing

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)

Materials and Methods

Collections

Winterform pear sucker were collected from various farms sites (Broadwater Fm (St. Leonard's), Westerhill Fm (Coxheath), “Churchfield” (West Farleigh), Foxbury Fm (Ivy Hatch), Marsh Gate Fm (Cooling)). Summerform pear sucker were sampled from a culture kept on conference pear in a glasshouse at EMR (Fig. 1.1.1). Over 270 female and 300 male *C. pyri* were hexane washed.

Figure 1.1.1. Nymphs, eggs and newly emerged adults on leaves in glasshouse culture. Honeydew is also present from the feeding nymphs.

Body washes

Body washes were obtained by immersing 1-10 individuals in hexane (0.5 ml) for 5 min and then transferring the hexane into a sample vial with a glass pipette (Fig. 1.1.2). Between 11 February and 11 March 2010, 38 samples were prepared and these were designated “winterform”. During 4-5 May 2010, 46 samples were prepared and these were designated “summerform”. Females were dissected after washing to note the development of ova and look for signs of mating.

Figure 1.1.2. Vials containing hexane washed *C. pyri*.

Samples were analysed by GC-MS under conditions for examination of relatively involatile, high-molecular weight hydrocarbons (up to 34C) using a HP6890N GC coupled to a HP5973 MS (Agilent) with GC column coated with non-polar DB5 (Supelco). The carrier gas was helium (1 ml/min), injection splitless (270°C) and oven temperature programmed from 60°C for 2 min, then at 10°C/min to 300°C and held for 15 min. The transfer line was turned up from 250°C to 280°C. The samples were also analysed by GC with flame ionisation detection (FID) under similar conditions with the detector at 300°C.

Compounds were identified from their Retention Indices relative to the retention times of *n*-alkanes and their mass spectra. GC-FID was used for quantification.

Results

Body washes

Comparison of the GC profiles of the high-molecular weight hydrocarbons (23C – 35C) in hexane body washes of male and female *C. pyri* showed that no compounds existed in the males that were not present in the females and vice versa. In addition, there were no significant differences in the relative amounts of each compound between males and females. This was true for both winterform and summerform insects (Fig. 1.1.3).

Most of the compounds were identified from their GC Retention Indices and mass spectra (Table 1.1.1). The majority were *n*-alkanes, 2- and 3-methylalkanes and long chain aldehydes.

There were, however, significant quantitative differences between the profiles from winterform and summerform insects (Fig. 1.1.4). In the latter the relative amounts of the *n*-alkanes and aldehydes were higher.

Guedot et al. (2010) proposed 13Me27:H to be the female sex pheromone of *C. pyricola.* This compound was detected as a minor component at RI 2733 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 (Fig. 1.1.3).

These results are very similar to those obtained during 2009 but are more definitive with more replicates and the replicates done under very standard conditions and analysed soon after preparation.

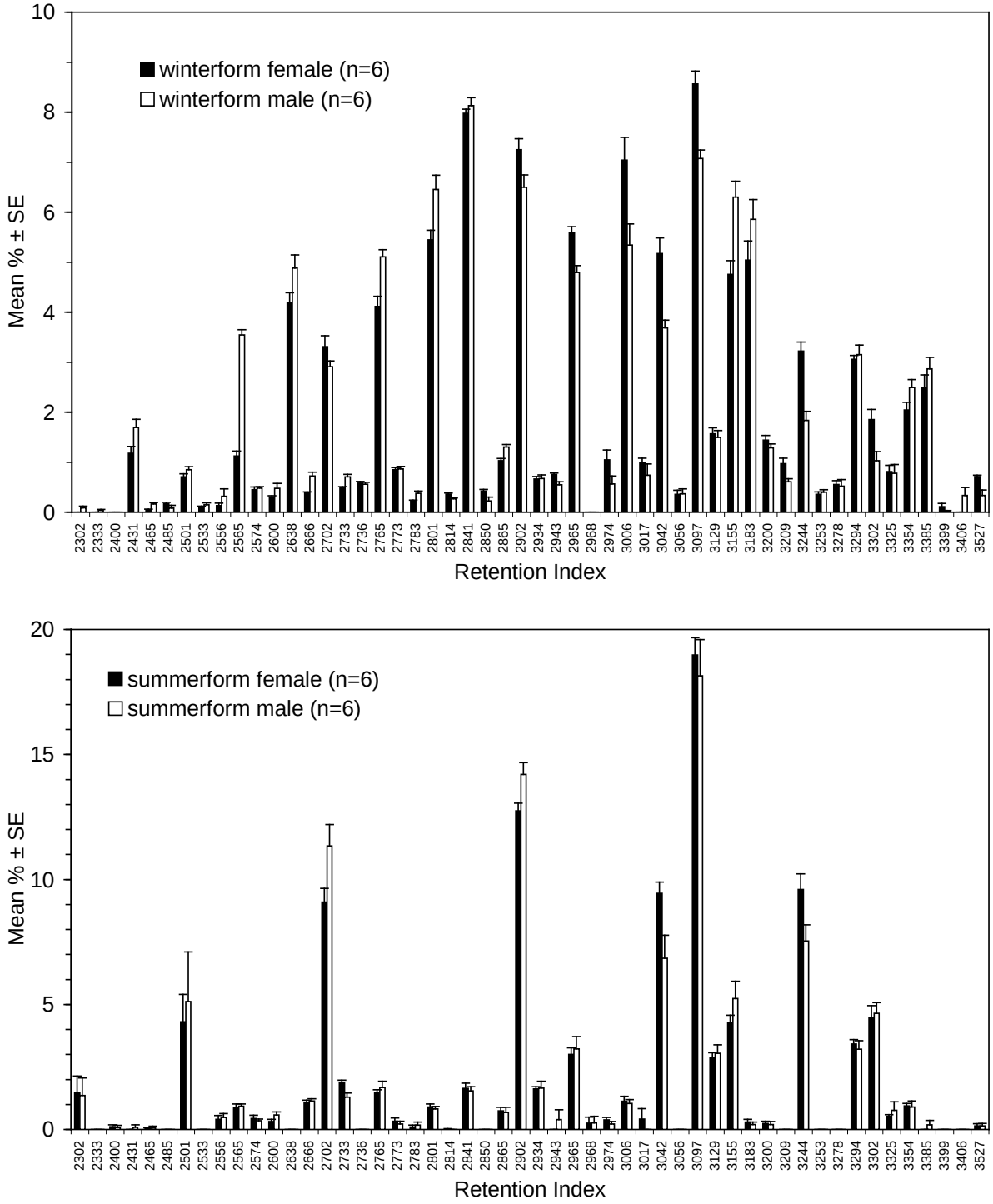


Figure 1.1.3. Comparison of hexane body washes from female and male winterform (upper) and summerform (lower) *C. pyri*.

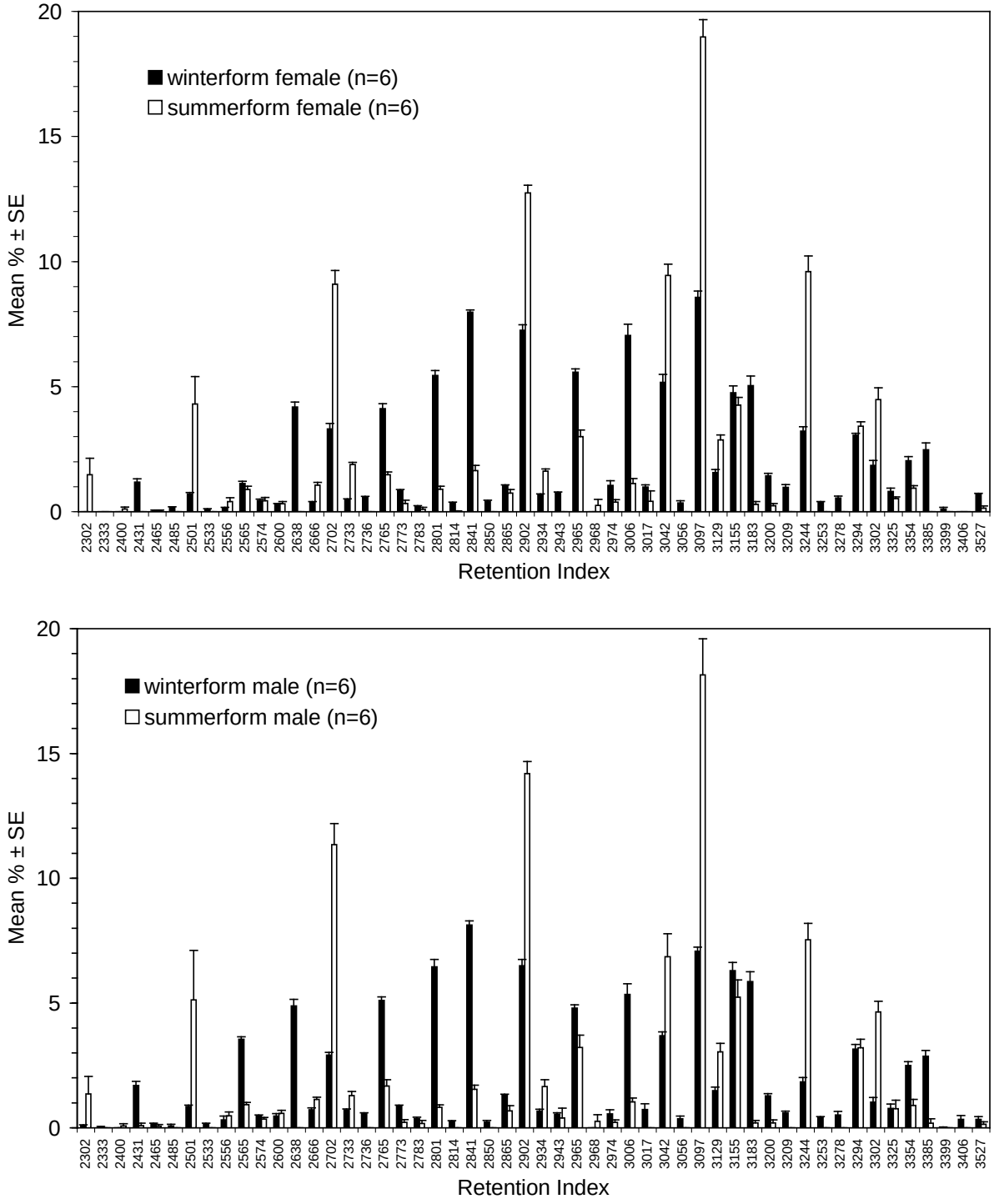


Figure 1.1.4. Comparison of hexane body washes from female (upper) and male (lower) winterform and summerform *C. pyri*.

Table 1.1.1. Compounds present in hexane washes from *C. pyri* identified from GC Retention Indices (RI) and mass spectra

RI	Compound		RI	Compound	
2302	tricosane		2934	11-, 13-, 15-methyl-nonacosane	11,13,15Me29:H
2333			2943	heptacosanal	27:Ald
2400	tetracosane		2965	2-methyl-nonacosane	2Me29H
2431	docosanal	22:Ald	2974	3-methyl-nonacosane	3Me29:H
2465			3006	triacontane	
2485			3017	unidentified	???
2501	pentacosane	25:H	3042	octacosanal	28:Ald
2533			3056		
2556			3097	hentriacontane	31:H
2565	2-methylpentacosane	2Me25:H	3129	11-, 13-, 15-methyl-hentriacontane	11,13,15Me31:H
2574	3-methylpentacosane	3Me25:H	3155	11,15-,13,17-diMe- hentriacontane	13,17-diMe31H
2600	hexacosane	26:H	3183	unidentified	
2638	tetracosanal	24:Ald	3200	dotriacontane	32:H
2666	2-methylhexacosane	2Me26:H	3209		
2702	heptacosane	27:H	3244	triacontanal	30:Ald
2733	13-methylheptacosane	13Me27:H	3253		
2736	pentacosanal	25:Ald	3278		
2765	2-methylheptacosane	2Me27:H	3294		
2773	3-methylheptacosane	3Me27:H	3302	tritriacontane	33:H
2783			3325	11-,13-,15-methyl-tritriacontane	11,13,15Me31H
2801	octacosane	28:H	3354	unidentified	
2814	2804 unidentified		3385		
2841	hexacosanal	26:Ald	3399		
2850			3406	tetratriacontane	34:H
2865	2-methyloctacosane	2Me28:H		pentatriacontane	35:H
2902	nonacosane	29:H	3527		

Summary

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. During 2010 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.

Analytical results were similar to those obtained during 2009 but are more definitive with more replicates and the replicates done under very standard conditions and analysed soon after preparation. As previously, analyses of washes from males and females showed that no compounds existed in the males that were not present in the females and vice versa. In addition, there were no significant differences in the relative amounts of each compound between males and females. 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*.

References

Brown, R.L., Landolt, P.J., Horton, D.R. and Zack, R.S. 2009. Attraction of *Cacopsylla pyricola* (Hemiptera: Psyllidae) to female psylla in pear orchards. *Environmental Entomology*, 38:815-822.

Guedot, C., Horton, D.R. and Landolt, P.J. 2009. Attraction of male winterform pear psylla to female-produced volatiles and to female extracts and evidence of male-male repellency. *Entomologia Experimentalis et Applicata* 130:191-197.

Guedot, C., Millar, J.G., Horton, D.R. and Landolt, P.J. (2010) Identification of a sex attractant pheromone for male winterform pear psylla, *Cacopsylla pyricola. journal of Chemical Ecology,* accepted.

Horton, D. R. & Landolt, P. J. 2007. Attraction of male pear psylla, *Cacopsylla pyricola*, to female-infested pear shoots. *Entomologia Experimentalis et Applicata* 123, 177–183.

Horton, D. R., Guédot, C. & Landolt, P. J. 2007. Diapause status of females affects attraction of male pear psylla, *Cacopsylla pyricola*, to volatiles from female-infested pear shoots. *Entomologia*

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 1.2. 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)

Methods and Materials

Synthesis

13-Methylheptadecane was synthesised by reaction of tetradecyl triphenylphosphonium bromide with 2-tetradecanone in the presence of potassium t-butoxide in THF. The resulting mixture of alkenes was hydrogenated at atmospheric pressure over 10% palladium on charcoal as catalyst

For field tests, rubber septa (20 mm x 10 mm; International Pheromone Systems Ltd.) or polythene vials, were impregnated with 1 mg as a hexane solution and the solvent allowed to evaporate.

Field tests

7 field tests were done at Clive Baxter's Farm (J L Baxter & Son, Westerhill Farm, Westerhill Lane, Linton, Maidstone, Kent ME17 4BS) cv. Conference pear orchard, and Churchfield, West Farleigh (OS ref:532 734). This site had abundant populations of *C. pyri* and a smaller proportion of *C. pyricola.*

Potential sex pheromone lures (produced at NRI) were tested in the field inside 30 x 20 cm, 1 mm, insect mesh bags or in the centre of white sticky bases. The lures (rubber septa or polythene vial, Fig. 1.2.2) were suspended on a wire with a 3 x 3 cm square of black Correx above to prevent the lure sticking to the bag. The bags were coated with Ecotac by pressing the mesh onto a white tray coated with the glue. The wire holding the lure and black Correx square was secured at the top of the bag with a twist tie (Fig. 1.2.2). Experiments comparing lures to live virgin pear sucker were comprised of caged laboratory reared *C. pyri* inside a capped hair roller.

Bags or sticky bases were hung in the canopy of the trees and inspected regularly for pear sucker (1.2.3). At 'Churchfield' the bags were hung in row 4 and 8 just inside the gate of the orchard on every 4th tree in the row (~12 m). At Westerhill the traps were hung on every 6th tree (~12 m, row spacing 4 m). Randomised block designs were done for all tests. The numbers, sex and species of pear sucker was recorded and entered onto Excel spread sheets.

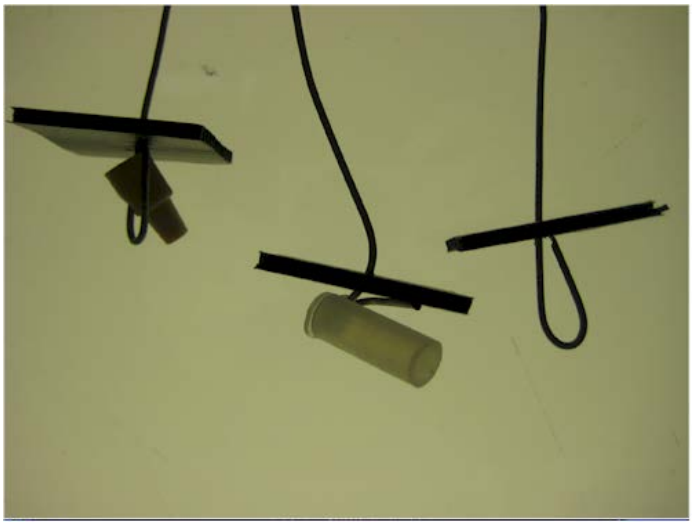


Figure 1.2.2. **I**eft; rubber septa, middle; polythene vial, right; control (no vial); lure inside mesh bag

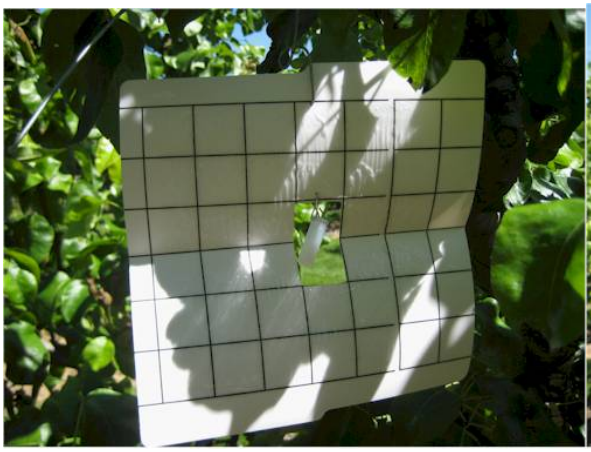


Figure 1.2.3. top left; polythene vial, top right; septa, bottom left; control (no lure), bottom right; caged male or female pear sucker

Laboratory tests

The first laboratory test (2 July 2010) used a filter paper in the bottom of a Petri dish onto which a line (trail) of the 13-Methylheptadecane 1 mg mL^{-1} in hexane was painted across the centre with a paint brush. A single male was added to each Petri dish and behaviour observed (Fig 1.2.4). There were 5 replicates of the treatment and 5 replicates of a control (hexane only).

In the second experiment (22 July 2010) the trail was added in a line with a micropipette (D Farman). This time males and females were tested (5 replicates of each) and a new individual was added every 10 minutes. 6 different solutions were used; a blank, the pheromone and hexane washes from female *C. pyri* (13-Methylheptadecane 1 mg mL^{-1}, H62, H103, H100, H60, blank).

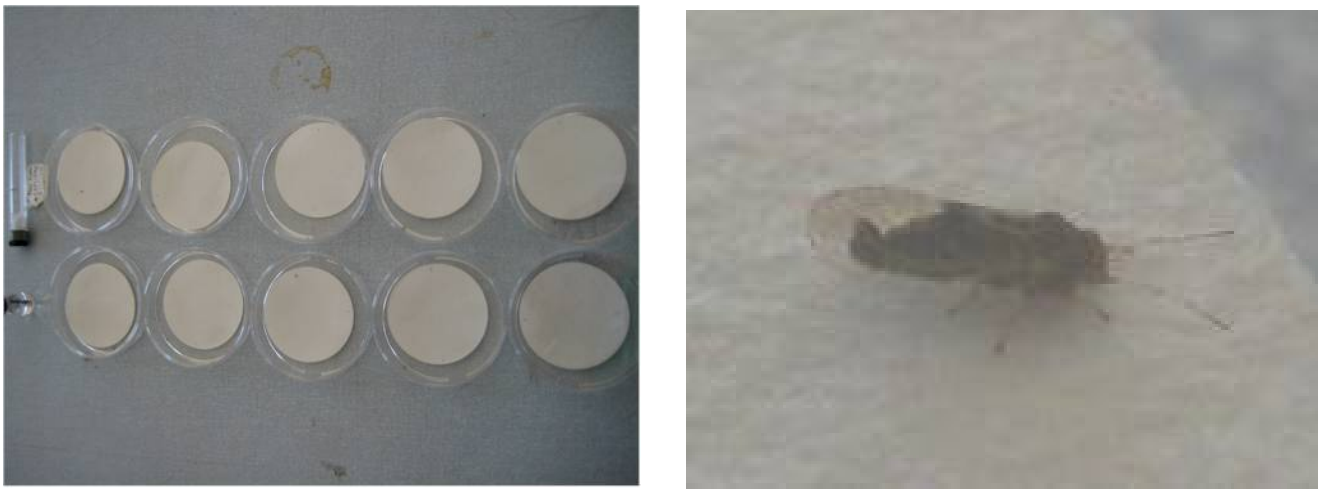


Figure 1.2.4. left; Petri dishes containing filter paper with trail, right; single male on filter paper

Results

Field tests

Of the seven field tests carried out there was no clear difference between traps baited with either virgin male or female or 'pheromone' baited septa or vials (ANOVA on LOG_{10} (+1) transformed data) (Table 1.2.1). Average numbers were remarkably similar across treatments indicating that the insects were not attracted to pear sucker in cages or the postulated pheromone.

Table 1.2.1. Numbers, sex and species of pear sucker found on baited ('pheromone', female or male) and unbaited traps

Date	Dose	Trap	Treatment	pyri male	pyri female	pyricola male	pyricola female
			control	60	8	2	1
17 Feb - 1 Mar	1 mg	Sticky mesh bag	septa	28	16	1	4
			vial	29	12	4	1
			control	3	2	0	0
8 - 11 Mar	1 mg	Sticky mesh bag	septa	2	2	0	0
			vial	0	0	0	0
			control	6	3	3	3
12-19 Mar	1 mg	White sticky base	septa	10	7	8	4
			vial	6	3	2	2
			control	11	5	5	2
19 Mar-19 Apr	1 mg	White sticky base	female	23	6	1	2
			male	18	11	5	4
19 Apr-4 May	1 mg	White sticky base	control	1	0	0	0
			septa	0	4	0	0
			control	7.2	8	0	0
			female	5	6	0	0
10 May - 17 May	1 mg	White sticky base	male	6.2	5.6	0	0
			septa	4.4	3.2	0	0
			vial	10.6	7.4	0	0
			control	5	13	2	2
			female	10	5	0	1
24 Jun- 16 Jul	1 mg	White sticky base	male	11	9	0	1
			septa	8	9	3	6
			vial	3	9	1	3
			control	**13**	**6**	**2**	**1**
			female	**13**	**6**	**0**	**1**
		AVERAGE	**male**	**12**	**9**	**2**	**2**
			septa	**9**	**7**	**2**	**2**
			vial	**10**	**6**	**1**	**1**

Laboratory tests

No attraction was observed of either male or female *C. pyri* to the synthetically produced pheromone or female hexane washes when applied to a filter paper as a trail (Tables 1.2.2 and 1.2.3). NB: all pear sucker were attracted towards the window (edges of Petri dishes).

Table 1.2.2. First trail finding test (2 July 2010)

Time	Action
10:50	Filter paper painted
11:00	males added in (1x male/dish)
11:09	no attraction
11:16	2 starting to walk around (not trail)
11:20	2 males crossed over 'pheromone' trail - did not show interest in trail
11:23	no attraction
11:32	no attraction
11:40	no attraction
11:50	no attraction
12:00	no attraction

Table 1.2.3. Second trail following test (22 July 2010)

	Time (m)	Action
male rep.		
1	10	no attraction
2	20	H103, 2 males tried to mate
3	30	no attraction
4	40	no attraction
5	50	no attraction
female rep.		
1	10	no attraction
2	20	no attraction
3	30	no attraction
4	40	no attraction
5	50	no attraction

Summary

No attraction of the opposite sex of has *C. pyri* been demonstrated in the field using 13-Methylheptadecane 1 mg mL^{-1} or unmated males or females. Hexane washes of females also failed to attract male *C. pyri* males.

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, 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 were done at 2-3 week intervals from 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 planted in early spring 2008 at the following 3 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 for evaluation (Treatments)

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

Table 3.1.3.1. 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
Common alder†	*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
Hawthorne	*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

Sampling of hedgerows/windbreaks and adjacent pear crops for spring and summer predator and prey communities

Each plot was separately sampled at 2-3 week intervals from late March to September 2010 to characterise predator communities, especially anthocorids. Populations of key prey species including the main aphids and psyllids that are present on the woody hosts were quantified. As much as possible of the counting and identification was done in the field on the day of sampling.

Sampling woody species for predators

A 0.25 m^2 beating tray was used for beat sampling the plots of woody species and the adjacent pear orchard at each site.

It is important that the sampling effort/method is kept as uniform as possible, both between plots on a site, sampling dates and between sites.

No: of beats per plot: 10 = 1 per meter

After each five beats, the numbers of each target insect in the beating tray are to be counted and recorded, as shown in Table 4

Table 2.1.3.3. Predators recorded by beat sampling

Taxa	species	Life stage	Notes
Heteroptera	*Anthocoris nemoralis*	Adult	
		N1-3	
		N3-5	
	Anthocoris nemorum	Adult	
		N1-3	
		N3-5	
	Other predatory sp		Pooter and bring back for ID
Coccinellidae	*Propylea 14-punctata*	Adult	
		Larvae	
	Coccinella 7 punctata	Adults	
		Larvae	
	Harmonia axyridis	Adults	
		Larvae	
Dermaptera	*Forficula auricularia*	Adults	
		L1	
		L2	
		L3	
		L4	
Neuroptera	*Heamerobidae*	Adults	
		Larvae	
	Chrysopidae	Adults	
		Larvae	
Araneae			Total numbers. ID dominant sp if possible

Insects for identification in the lab were collected with a pooter or paint brush and transferred to a glass tube with 70% alcohol a cardboard label inscribed in pencil with the site, plot number and date was then placed in the tube which was sealed with a stopper. All the samples from 1 sampling date were held together in one larger bag.

Sampling woody species for prey

A random sample of ten 10 cm long shoots (latest growth) was inspected *in situ* and an approximate count made of numbers of the dominant aphids and psyllid eggs and nymphs for the particular tree species.

A pictorial guide to identification of the dominant aphid and psyllid species on each woody species provided below:

List of key prey

Dominant species are in bold

Field maple *(Acer campestre*)

Aphids:
- *Drepanosiphum acerinum*
- *Drepanosiphum dixoni*
- ***Drepanosiphum platanoidis***
- *Mimeuria ulmiphila*
- *Periphyllus aceris*
- *Periphyllus californiensis*
- *Periphyllus hirticornis*
- *Periphyllus obscurus*
- ***Periphyllus testudinaceus***

Psyllids:
- ***Rhinocola aceris* (dominant)**

Norway maple *(Acer platanoides*)

Aphids:
- ***Drepanosiphum platanoidis***
- *Periphyllus aceris*
- *Periphyllus lyropictus*
- ***Periphyllus testudinaceus***

Psyllids:
- ***Rhinocola aceris* (dominant)**

Grey Alder *(Alnus glutinosa*)

Aphids:
- *Clethrobius comes*
- *Glyphina betulae*
- *Pterocallis alni*
- *Stomaphis quercus*

Psyllids:
- *Baeopelma foersteri*
- ***Psylla alni* (dominant)**

Birch *(Betula pendula*)

Aphids:
- *Betulaphis quadrituberculata*
- *Calaphis betulicola*
- *Calaphis flava*
- *Calliopterinella calliptera*
- *Callipterinella minutissima*
- *Callipterinella tuberculata*
- *Clethrobius comes*
- ***Euceraphis betulae***
- *Glyphina betulae*
- *Hamamelistes betulinus*
- *Monaphis antennata*
- *Stomaphis quercus*
- *Symydobius oblongus*

Psyllids:
- ***Chamaepsylla hartigii* (dominant)**
- *Psylla betulae*

Hazel *(Corylus avellana*)

Aphids:
- *Corylobium avellanae*
- *Myzocallis coryli*

Hawthorn (*Crataegus monogyna*)

Aphids:

Aphis pomi
Dysaphis angelicae
Dysaphis apiifolia ssp. petroselini
Dysaphis crataegi
Dysaphis lauberti
Dysaphis ranunculi
Nearctaphis bakeri
Ovatus crataegarius
Prociphilus pini
Rhopalosiphum insertum

Psyllids:

Cacopsylla affinis
Cacopsylla crataegi
Cacopsylla melanoneura (dominant)
Cacopsylla peregrine **(dominant)**

Ash (*Fraxinus excelsior*)

Aphids:

Prociphilus bumeliae
Prociphilus fraxini

Psyllids:

Psyllopsis discrepans
Psyllopsis distinguenda
Psyllopsis fraxini (dominant)
Psyllopsis fraxinicola **(dominant)**

Blackthorn *(Prunus spinosa*)

Aphids:

Brachycaudus cardui
Brachycaudus helichrysi
Brachycaudus prunicola
Hyalopterus pruni
Phorodon humuli
Rhopalosiphum nymphaeae
Rhopalosiphum padi

Psyllids:

Cacopsylla pruni **(dominant)**

Elder (*Sambucus nigra*)

Aphids:

Aphis sambuci

Goat willow *(Salix caprea*)
Aphids:

Aphis farinosa
Cavariella aegopodi
Cavariella archangelicae
Cavariella pastinacea
Cavariella theobaldi
Chaitophorus capreae
Chaitophorus horii
Chaitophorus niger
Chaitophorus salicti
Chaitophorus salijaponicus
Chaitophorus vitellinae
Plocamaphis flocculosa ssp. *brachysiphon*
Plocamaphis flocculosa ssp. *goernitzi*
Pterocomma pilosum
Pterocomma rufipes
Pterocomma salicis

Psyllids:

Cacopsylla ambigua **(dominant)**
Cacopsylla brunneipennis **(dominant)**
Cacopsylla moscovita
Cacopsylla pulchra
Cacopsylla saliceti
Bactericera curvatinervis
Bactericera salicivora

Grey willow (*Salix cinerea*)
Aphids:

Aphis farinosa
Cavariella aegopodi
Cavariella theobaldi
Chaitophorus capreae
Chaitophorus niger
Chaitophorus salicti
Plocamaphis flocculosa ssp. *brachysiphon*
Plocamaphis flocculosa ssp. *goernitzi*
Pterocomma pilosum
Pterocomma rufipes
Pterocomma salicis

Psyllids:

Cacopsylla ambigua **(dominant)**
Cacopsylla brunneipennis **(dominant)**
Cacopsylla moscovita
Cacopsylla pulchra
Cacopsylla saliceti
Bactericera curvatinervis
Bactericera salicivora

Lime *(Tilia cordata*)
Aphids:

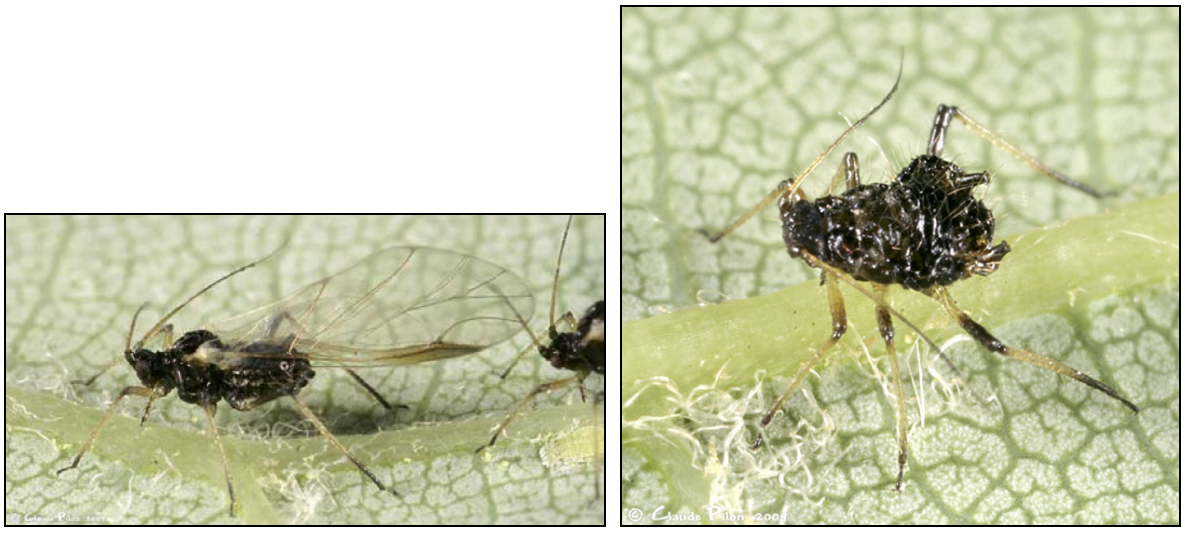
Eucallipterus tiliae
Patchiella reaumuri

Pear (*Pyrus communis*)
Aphids**:**

Anuraphis catonii
Anuraphis farfarae
Anuraphis pyrilaseri
Anuraphis subterranea
Aphanostigma pyri
Aphis craccivora
Aphis gossypii

Aphis pomi
Aphis spiraecola
Brachycaudus cardui
Brachycaudus helichrysi
Brachycaudus persicae
Dysaphis plantaginea
Dysaphis pyri
Dysaphis reaumuri
Eriosoma lanigerum
Eriosoma lanuginosum
Eriosoma pyricola
Longistigma caryae
Melanaphis pyraria
Myzus persicae
Nearctaphis bakeri
Ovatus crataegarius
Ovatus insitus
Pterochloroides persicae
Rhopalosiphum insertum
Schizaphis pyri
Toxoptera aurantii
Toxoptera citricida

Drepanosiphum platanoidis



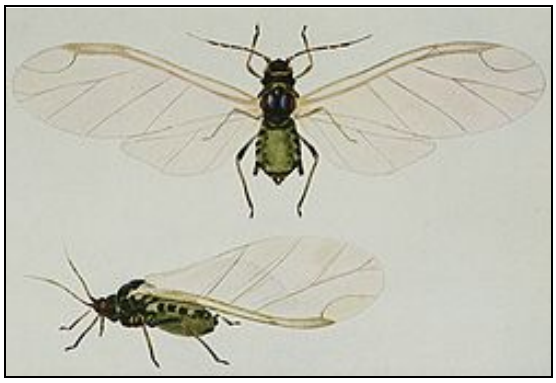
Periphyllus testudinaceus

Rhinocola aceris

Psylla alni

Euceraphis betulae

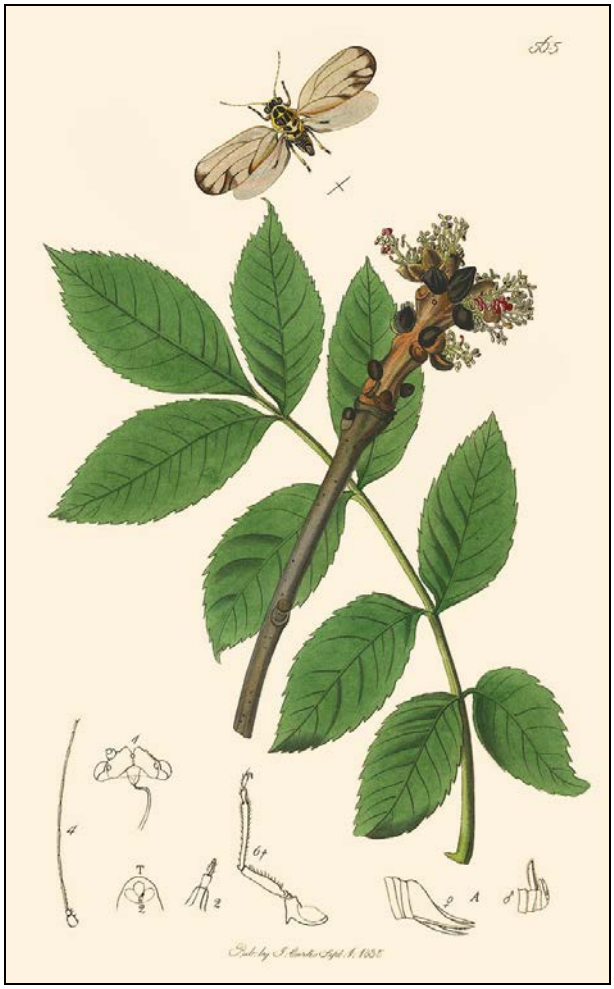
Corylobium avellanae



Rhopalosiphum insertum

Myzocallis coryli

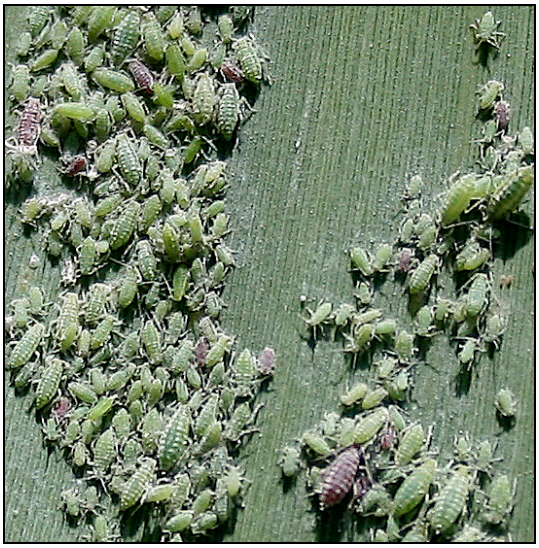
Cacopsylla melanoneura



Psyllopsis fraxini

Psyllopsis fraxinicola

Brachycaudus helichrysi

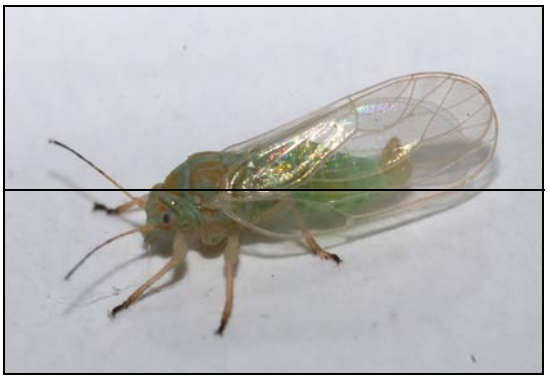


Hyalopterus pruni

Phorodon humuli

Cacopsylla pruni

Cavariella aegopodi



Cacopsylla ambigua

Eucallipterus tiliae

teneral female

winter male

female winter

Cacopsylla brunneipennis

Results

2010

A large data base of 5753 arthropods sampled was constructed (Table 2.1.3.4). However, numbers of anthocorids collected were rather small and erratic (Table 2.1.3.5).

Beat samples showed psyllid adults were most abundant on the pear trees in the adjacent orchard, with comparatively small numbers on the other subjects which were immature (Table 2.1.3.6). Aphids were very abundant on birch followed by *Acer campestre* and *Salix cinerea* (Table 2.1.3.7). Shoot sampling was more effective for aphid enumeration than beat sampling. It revealed the presence of quite high numbers of aphids on *Corylus avellana* (Table 2.1.3.8). Shoot samples revealed that psyllid nymphs were by far the most abundant in the established adjacent pear orchards (Table 2.1.3.9).

Detailed results are as follows:

-Anthocoridae:

The anthocorid numbers were much lower than in old hedges. In most cases *A. nemoralis* was more common on the plants, which is also evidence for nettle effect, because from the results of the previous years this species should be dominant only on plants with high numbers of psyllids (*Pyrus*, *Salix*, *Crataegus*, *Urtica*). On all the other, aphid-dominated plants, the mostly aphid-feeder *A. nemorum* should be the dominant species.

Also, there were more *Orius* sp. specimens found on some plants, where their dominance should not be important. From the results of previous years, their number should be important only on *Salix* and *Urtica*.

-Miridae:

The total numbers of Miridae were generally low on the sites of Rodmersham and Broadwater, probably because of the young ages of the plants. The most common predatory species was *Heterotoma planicornis*, but because this species is very common on nettle too, the data should be interpreted carefully. The Miridae numbers were higher on the site Ballingham - the specimens were collected but they need to be identified before interpretation.

-Psyllidae:

The most typical species were already present on the plants at the Rodmersham and Broadwater sites, but in low numbers compared to the old hedges.

Acer campestre and *A. platanoides*:
The associated *Rhinocola aceris* was not found - only other species were found, which came from the other plants.

Alnus glutinosa:
Both of the associated psyllid species (*Psylla alni*, *Baeopelma foersteri*) were found on the Rodmersham site.

Betula pendula:
Only one specimen of *Chamaepsylla hartigii* was found, on the Rodmersham site. Anyway, maybe the other unidentified specimens can belong to this species too.

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 too, but in a much lower number compared with old hedges.

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

Prunus spinosa:
The associated *Cacopsylla pruni* was not 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 dominant species on both the Rodmersham and Broadwater sites, the psyllids of Ballingham site are not identified yet. *C. pyricola* was also present on the Rodmersham and Broadwater sites, but in lower numbers.

Salix caprea and *S. cinerea*:
The associated *Cacopsylla ambigua* adults were found on the Rodmersham and Broadwater sites too, but in low numbers. Probably the few specimens at the Ballingham site could belong to this species too, but identification is yet to be done. The other common willow feeding species (*Cacopsylla brunneipennis*) was not found so far, but some of the unidentified specimens could belong to this species too.

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

Sambucus nigra:
There are no associated species and only guest specimens might be present.

Tilia cordata:

Psyllids were not found at all on *Tilia*.

General comment:

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

Aphids:

The most typical species were already present, and in some cases they could reach quite a high number.

Acer campestre:

Two important aphid species/genera were dominant on field maple trees. In the early season, *Periphyllus testudinaceus* was the dominant one and later on *Drepanosiphum* sp. became dominant. *P. testudinaceus* had large colonies in the early spring, which were visited by ants many times. In May and June they produced winged forms in high numbers and so their numbers are overrepresented in the beating samples compared with the shoot checking samples. *Drepanosiphum* sp. has only winged asexual females, so it can colonise the trees easily at any time. This is a large bodied species. The Rodmersham site was much more infested than the Broadwater site.

Acer platanoides:

Two important aphid species/genera were dominant on field maple trees. In the early part of the season *Periphyllus testudinaceus* was the dominant one and later on *Drepanosiphum* sp. became dominant. The Ballingham site was more infested than the Broadwater site.

Alnus glutinosa:

Pterocallis alni was the dominant species at each sampling time. This is a small bodied species sometimes reaching high numbers on the underside of alder leaves. The Rodmersham site was the most infested and the Ballingham site was the least infested.

Betula pendula:

Euceraphis betulae is the most common species on *Betula pendula*. This is a large bodied species that has only winged asexual females. It can colonise trees at any time and also move if necessary. The nymphs are able to move fast and they do not aggregate in high numbers. Because of their fast moving ability they are overrepresented in beating samples compared with other, smaller bodied and aggregating species. Nymphs are effectively collectable with the beating method. The Ballingham site

was much more infested than the Rodmersham site.

Corylus avellana:

Myzocallis coryli was the dominant species on hazel. This is a small bodied aphid. It has only winged asexual females and so it can easily colonise plants at any time. They are distributed randomly on the leaves and are not visited by ants. Because of their small size and good attaching ability, their numbers are really underrepresented by the beating method compared with shoot checking. The results of 2008 and 2009 need to be corrected taking this into account. The Rodmersham site was much more infested than the Broadwater site.

Crataegus monogyna:

Aphis pomi and one or more generalist species (mostly *Macrosiphum* sp.) were the dominant species on hawthorn. *A. pomi* is a highly ant visited species and can reach high numbers on hawthorn. It aggregates on the young shoots and can be underrepresented by using the beating method. *Macrosiphum* species are large bodied aphids with long legs and so are probably overrepresented in beating samples compared with *A. pomi*.

Fraxinus excelsior:

There were very low numbers of aphids on *Fraxinus* and identification needs to be done.

Prunus spinoza:

There were few numbers of aphids collected and identification needs to be done.

Pyrus communis:

Aphid infestation was generally low on pear. Two associated species were identified to date. *Rhopalosiphum insertum* was common in the early spring and *Aphis pomi* was common during the summer. None of them are easily collectable by the beating method and so their numbers are underrepresented. Winged asexual females of various species romf the hedgerow plants occurred in quite high numbers on pear (mostly *Periphyllus testudinaceus* and *Drepanosiphum* sp. from maples). Their numbers are highly overrepresented in beating samples compared with *A. pomi* and *R. insertum*.

Salix caprea and *S. cinerea*:

Two kinds of aphids were dominant on willows. The larger bodied *Aphis farinosa* is a highly ant visited species making large colonies on the young shoots of willows whose numbers depend on protection by ants. The other group/species is from the genus *Chaitophorus*, which can reach quite large numbers on the underside of the leaves. Their size is smaller and ants normally do not visit them. Their numbers are underrepresented by beating methods compared with shoot checking. The

Rodmersham site was the most infested.

Sambucus nigra:

Aphis sambuci is the only associated species and it was found only on the Rodmersham site. This species is highly visited by ants. There were winged asexual females of other species present on elder, which are overrepresented in beating samples.

Tilia cordata:

There were very few numbers of aphids collected on *Tilia*, mostly winged asexual females that came from the other plants.

-Other predators:

Coccinellidae:

There were numerous Coccinellidae species on the studied plants collected by beating methods, the most common ones were *Coccinella septempunctata*, *Harmonia axyridis* and *Propylea quatuordecimpunctata*, respectively. Four species were found on pear trees this year (*C. septempunctata*, *H. axyridis*, *Adalia bipunctata* and *P. quatuordecimpunctata*, respectively).

Cantharidae:

The most common species was *Rhagonycha fulva*. It was found on each plant species, the highest numbers were on *Salix cinerea* and *Pyrus communis*, respectively.

Forficulidae:

Forficula auricularia numbers were rather low this year. *Corylus avellana* had the highest numbers collected by the beating method.

2009

Identification of the dominant psyllids and aphids from shoot samples collected from the established existing hedgerow plots in 2009 was done. The seasonal dynamics of the key species are shown in Figures 2.1.3.1 – 2.1.3.16.

Discussion

Two important problems were encountered that make the results of the above work of limited, confirmatory value:

1. The trees planted in year 1 of the project were only in their third season of growth. Those at

Ballingham had grown well but at Broadwater and Rodmersham growth was comparatively poor. The characteristic aphid, psyllid and heteroptera fauna associated with each subject was only just starting to establish.

2. Nettles had established strongly at all 3 sites and were tall, swamping the smaller species at all 3 sites. The strong fauna of nettles was present throughout and furthermore, sampling was difficult for some subjects.

Final conclusions based mostly on the Anthocoris results of 2008 and 2009:

In the case of aphids, those plants that appear to be the most reliable sources for anthocorids are those which have one or more species of the kind of aphids which have:

- asmall body size
- liveon the underside of leaves
- are able to reach high numbers
- can colonise plants at any time
- aopulation dynamic that is less dependent on ants.

These plants (and their associated aphids) are:

Alnus glutinosa (*Pterocallis alni*)
Corylus avellana (*Myzocallis coryli*)
Salix caprea (*Chaitophorus* sp.)
Salix cinerea (*Chaitophorus* sp.)

Other potentially good sources are the plants with ant visited aphid species, but on them the situation depends strongly on the presence of ants. These aphids cannot reach large population sizes without ants. These plants are:

Acer campestre (*Periphyllus testudinaceus*)
Acer platanoides (*Periphyllus testudinaceus*)
Crataegus monogyna (*Aphis pomi*)
Prunus spinoza (*Brachicaudus helichrysi*)
Pyrus communis (*Aphis pomi*)
Salix caprea (*Aphis farinosa*)
Salix cinerea (*Aphis farinosa*)
Sambucus nigra (*Aphis sambuci*)

Table 2.1.3.4. Numbers of main arthropod groups on plants collected by the beating method. Minor groups have been deleted.

Tree sp.	Site	Aphidoidea *	Araneae	Auchenorr-hyncha	Coleoptera	Forficulidae	Syrphidae	Heteroptera	Hymenoptera	Chrys-opidae	Psyllidae	Totals
Acer campestre	S1	378	35		13	2	1	11	24	2	6	**490**
	S3	32	29	5	31	2		5	26		13	**158**
Acer platanoides	S1	63	6		6		1	4	15		5	**114**
	S2	28	21		6			7	1			**75**
Alnus glutinosa	S1	131	12		9			10	9		36	**223**
	S2	6	23	2	1			63	0		1	**108**
	S3	44	15	3	31	1		5	3		3	**119**
Betula pendula	S1	143	5		7			13	3		6	**193**
	S2	1306	12		6			9	0		3	**1349**
Corylus avellana	S1	47	23		11			37	15		2	**150**
	S3	5	16	1	39	9		3	15		5	**106**
Crataegus monogyna	S1	48	45		6			24	3	1	22	**166**
	S3	8	52		23			12	6	1	23	**139**
Fraxinus excelsior	S1	12	20		3		1	14	13	1	25	**106**
	S2	4	42		3			49	0		24	**134**
Prunus spinosa	S2	21	54		5			18	0		1	**112**
	S3	12	43	3	30	1		7	4	1	2	**117**
Pyrus communis	S1	61	2		13		1	8	9		129	**238**
	S2	4	31	1	6			14	0		20	**88**
	S3	5	4	1	35	3		7	59		118	**244**
Salix caprea	S1	24	15	1	12			9	33	1	5	**120**
	S2	2	18	5	1			53	0		9	**100**
	S3	7	33	3	33	2		4	12		14	**121**
Salix cinerea	S1	139	23		34	1		66	37	5	6	**328**
	S2	60	26	1	6		1	32	0		4	**142**
Sambucus nigra	S1	19	31	50	12			68	14	3	2	**216**
	S2	4	68	7	4			54	1		4	**158**
Tilia cordata	S2	9	24		13			14	0		4	**76**
	S3	3	19		9	3		1	4	1	1	**55**
Totals		**2625**	**749**	**83**	**408**	**24**	**5**	**621**	**307**	**15**	**493**	**5753**

Table 2.1.3.5. Numbers of identified heteroptera on plants collected by the beating method. Minor groups have been deleted. A=adult, N=nymph

Tree sp.	Site	*Anthocoris nemoralis*		*Anthocoris nemorum*		*Anthocoris sp.*		*Orius sp.*		*Heterotoma planicornis*		Grand total
		A	***N***	***A***	***N***	***A***	***N***	***A***	***N***	***A***	***N***	
Acer campestre	S1	1						1			2	**11**
	S3	3		1								**5**
Acer platanoides	S1											**4**
	S2	3		1								**7**
Alnus glutinosa	S1	2							1			**10**
	S2										5	**63**
	S3	1		1	1							**5**
Betula pendula	S1	1	10									**13**
	S2	2		5		1						**9**
Corylus avellana	S1	2	12	6	1			2	1	5	2	**37**
	S3	1		1								**3**
Crataegus monogyna	S1	5	2		1			5	3	4	1	**24**
	S3	5	1									**12**
Fraxinus excelsior	S1	2	3					3		3		**14**
	S2	4		1		1						**49**
Prunus spinosa	S2			1			1				10	**18**
	S3	1										**7**
Pyrus communis	S1	3	3									**8**
	S2	5		4			2					**14**
	S3	3	3					1				**7**
Salix caprea	S1		1					2	2	2		**9**
	S2	4		3		2	3	1				**53**
	S3	1	1									**4**
Salix cinerea	S1	4	11					13	12	1		**66**
	S2	3				1	1				1	**32**
Sambucus nigra	S1		4					8	4	7		**68**
	S2			3						2	7	**54**
Tilia cordata	S2	1				3	1	1				**14**
	S3											**1**
Total		**57**	**51**	**27**	**3**	**8**	**8**	**37**	**23**	**24**	**28**	**621**

Table 2.1.3.6. Numbers of identified psyllids on plants collected by the beating method. Minor groups and nymph stages have been deleted.

Tree sp.	Site	*Cacopsylla ambigua*	*Cacopsylla melanoneura*	*Cacopsylla peregrina*	*Cacopsylla pyri*	*Cacopsylla pyricola*	*Cacopsylla sp.*	*Psylla foersteri*	*Psyllopsis fraxini*	*Psyllopsis fraxinicola*	*Trioza urticae*	Grand total
Acer campestre	S1											**6**
	S3		1	1							3	**13**
Acer platanoides	S1		2		1					2		**5**
	S2											
Alnus glutinosa	S1							27				**36**
	S2							1				**1**
	S3		1									**3**
Betula pendula	S1				2							**6**
	S2											**3**
Corylus avellana	S1				1							**2**
	S3	1									3	**5**
Crataegus monogyna	S1		11	1	1							**22**
	S3		7	10	1						2	**23**
Fraxinus excelsior	S1				1				1	15		**25**
	S2								13	2		**24**
Prunus spinosa	S2											**1**
	S3		1									**2**
Pyrus communis	S1		2		75	7						**129**
	S2						18					**20**
	S3				56	36						**118**
Salix caprea	S1	1										**5**
	S2											**9**
	S3	10										**14**
Salix cinerea	S1	3			2							**6**
	S2											**4**
Sambucus nigra	S1											**2**
	S2											**4**
Tilia cordata	S2											**4**
	S3		1									**1**
Total		**15**	**26**	**12**	**140**	**43**	**18**	**28**	**14**	**19**	**8**	**493**

Unsurprisingly there were more psyllids on pear than on other tree sp. The majority of the psyllids on pear were *C. pyri*. There was a third species at site 2 that is yet to be identified.

Table 2.1.3.7. Numbers of identified aphids on plants collected by the beating method. Minor groups have been deleted.

Tree sp.	Site	*Drepanosiphum platanoidis*	*Euceraphis betulae*	*Myzocallis coryli*	*Periphyllus testudinaceus*	*Pterocallis alni*	Grand total
Acer campestre	S1	32			139		**378**
	S3	1			29		**32**
Acer platanoides	S1				46		**63**
	S2	2					**28**
Alnus glutinosa	S1					96	**131**
	S2						**6**
	S3				1	12	**44**
Betula pendula	S1		135				**143**
	S2		1000				**1306**
Corylus avellana	S1			42			**47**
	S3			2	3		**5**
Crataegus monogyna	S1						**48**
	S3						**8**
Fraxinus excelsior	S1				3		**12**
	S2						**4**
Prunus spinosa	S2						**21**
	S3						**12**
Pyrus communis	S1	42		3	1		**61**
	S2						**4**
	S3						**5**
Salix caprea	S1				2		**24**
	S2						**2**
	S3				1		**7**
Salix cinerea	S1				2		**139**
	S2						**60**
Sambucus nigra	S1				3		**19**
	S2						**4**
Tilia cordata	S2						**9**
	S3						**3**
Total		**77**	**1135**	**47**	**230**	**108**	**2625**

Table 2.1.3.8. Numbers of identified aphids on plants collected by examining shoot in the field 2010. Minor groups have been deleted

Tree sp.	Site	*Aphis farinosa*	*Aphis pomi*	*Aphis sambuci*	*Drepanosiphum platanoidis*	*Euceraphis betulae*	*Myzocallis coryli*	*Periphyllus testudinaceus*	Grand total
Acer campestre	S1				52		101	5	**1028**
	S3							24	**112**
Acer platanoides	S1				6			83	**309**
	S2								**81**
Alnus glutinosa	S1				11				**210**
	S2								**3**
	S3								**132**
Betula pendula	S1					29			**33**
	S2					81			**160**
Corylus avellana	S1				21		2303		**2343**
	S3						303	1	**314**
Crataegus monogyna	S1		28						**139**
	S3		27						**51**
Fraxinus excelsior	S1								**4**
	S2								**6**
Prunus spinosa	S2								**1**
	S3								**21**
Pyrus communis	S1								**48**
	S2								**2**
	S3		19						**68**
Salix caprea	S1	121							**339**
	S2								**2**
	S3								**43**
Salix cinerea	S1	57							**1707**
	S2								**11**
Sambucus nigra	S1			222					**222**
	S2								**2**
Tilia cordata	S2								**11**
	S3								*****
Total		**178**	**74**	**222**	**90**	**110**	**2717**	**113**	**7402**

Table 2.1.3.9. Numbers of psyllids on plants collected by examining shoots in the field 2010. Species still to be identified

Tree sp.	Site	Grand total
Acer campestre	S1	1
	S3	0
Acer platanoides	S1	6
	S2	0
Alnus glutinosa	S1	0
	S2	0
	S3	0
Betula pendula	S1	0
	S2	0
Corylus avellana	S1	0
	S3	0
Crataegus monogyna	S1	20
	S3	11
Fraxinus excelsior	S1	6
	S2	0
Prunus spinosa	S2	0
	S3	0
Pyrus communis	S1	3063
	S2	13
	S3	1616
Salix caprea	S1	2
	S2	1
	S3	1
Salix cinerea	S1	2
	S2	1
Sambucus nigra	S1	0
	S2	0
Tilia cordata	S2	0
	S3	0
Totals		**4743**

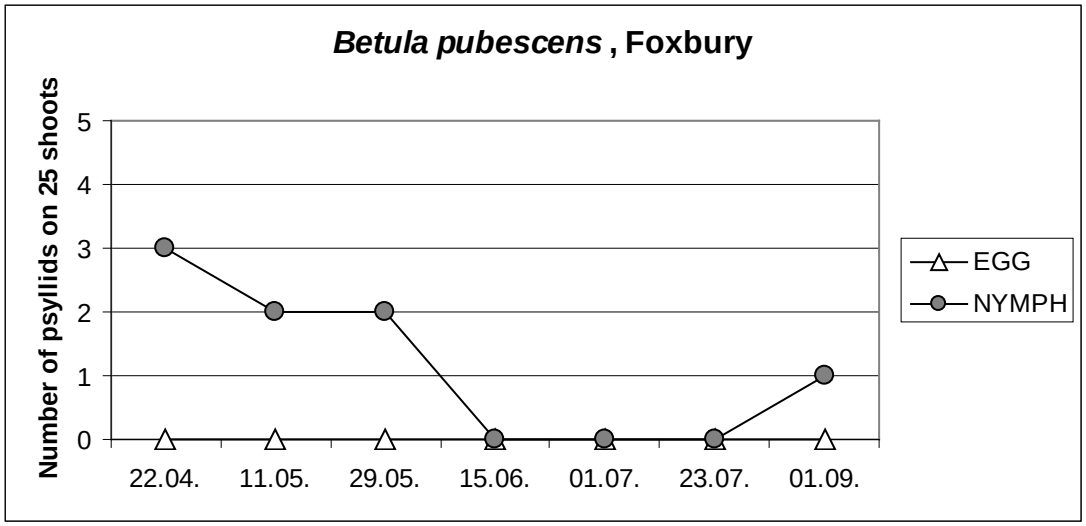
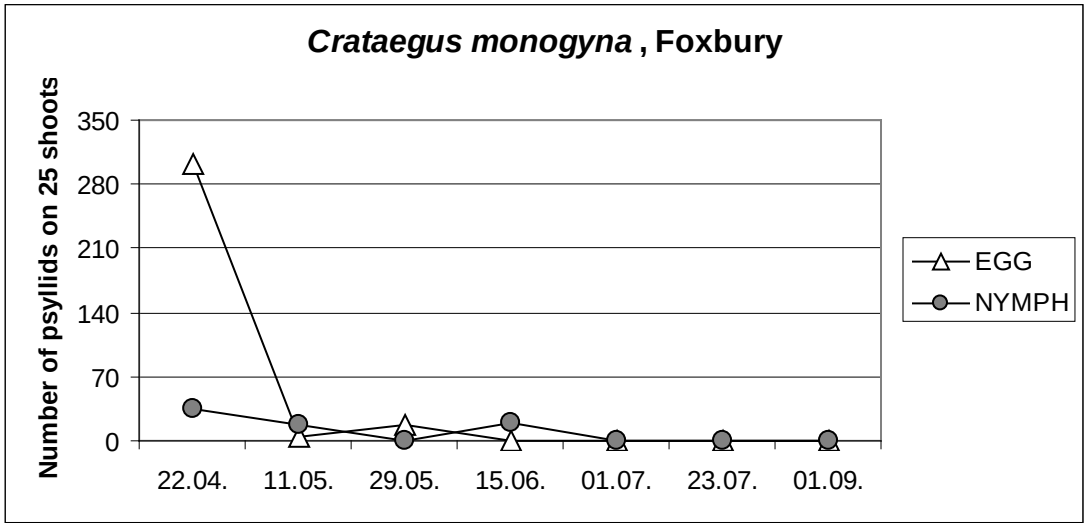


Figure 2.1.3.1. Seasonal dynamics of psyllids on *Betula pubescens* in 2009



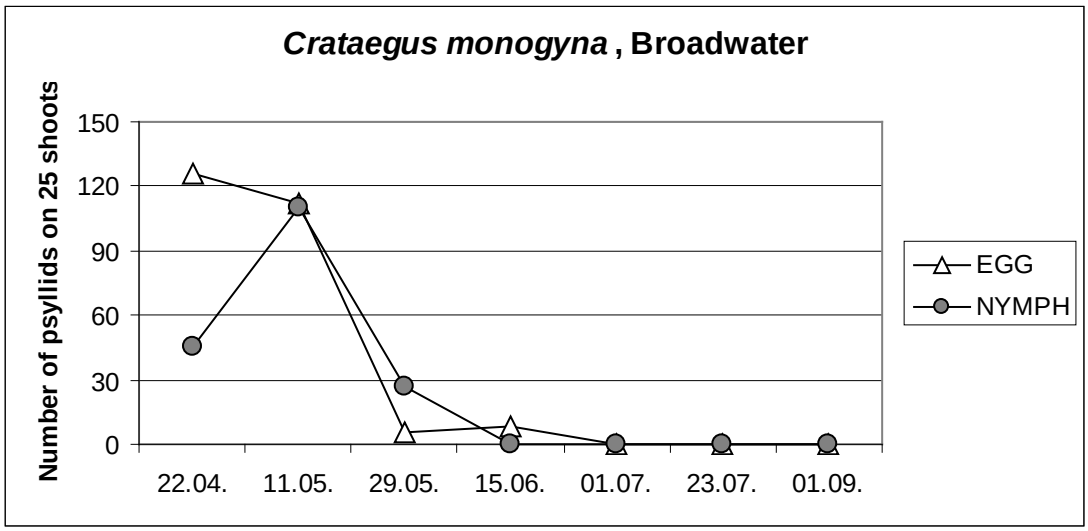


Figure 2.1.3.2. Seasonal dynamics of psyllids on *Crataegus monogyna* in 2009

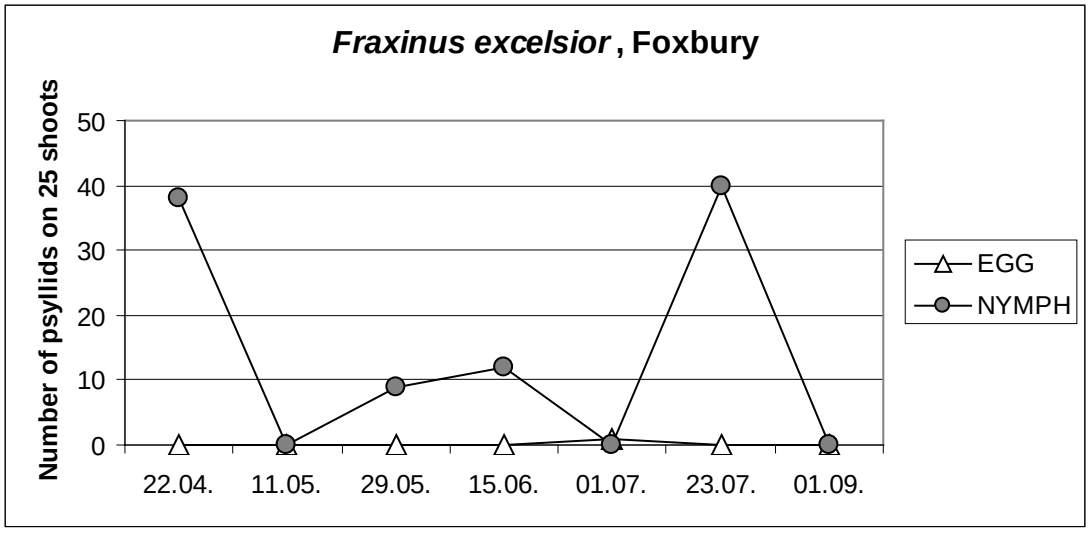
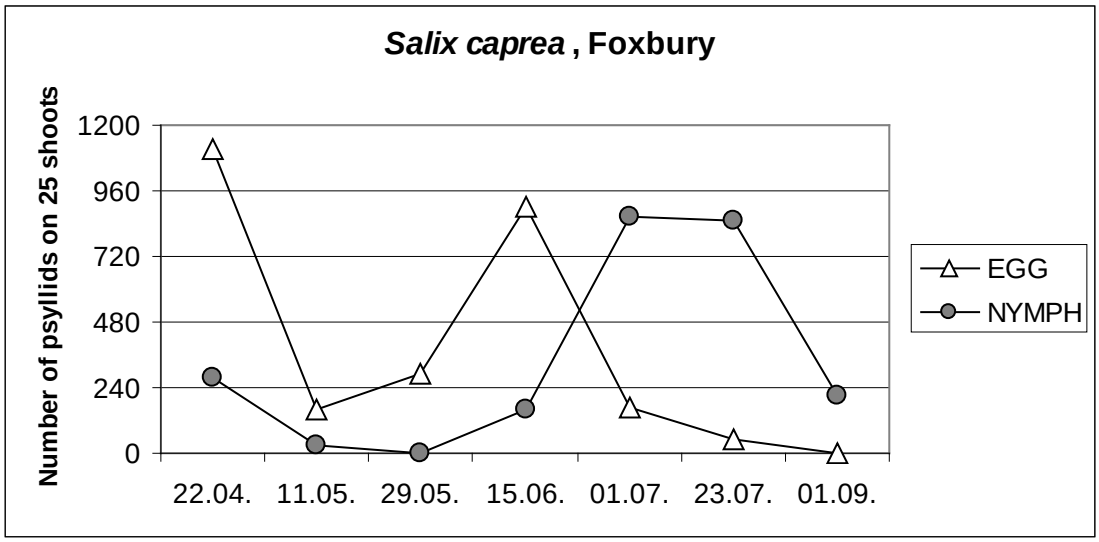


Figure 2.1.3.3. Seasonal dynamics of psyllids on *Fraxinus excelsior* in 2009



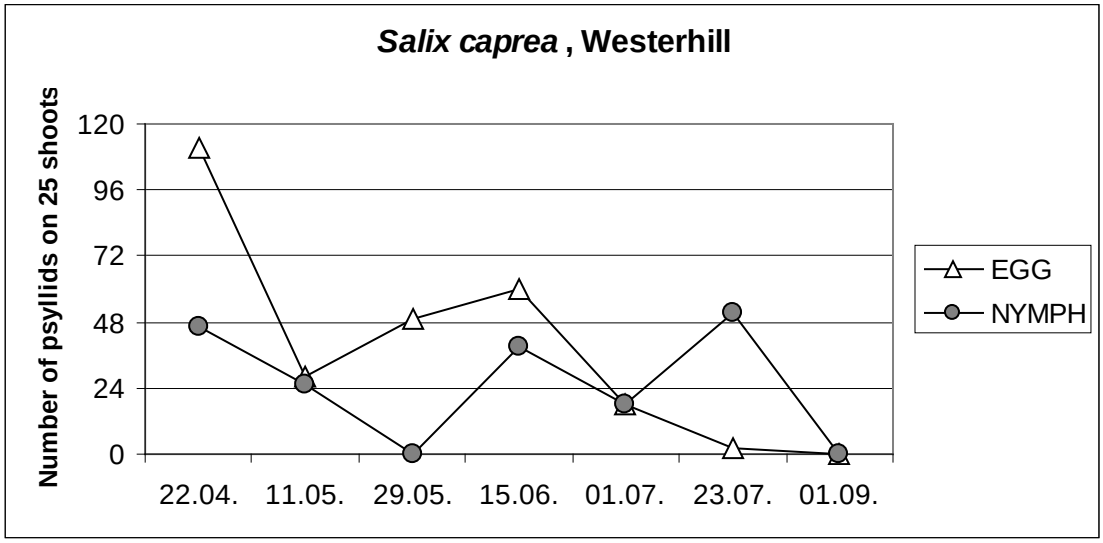


Figure 2.1.3.4. Seasonal dynamics of psyllids on *Salix caprea* in 2009

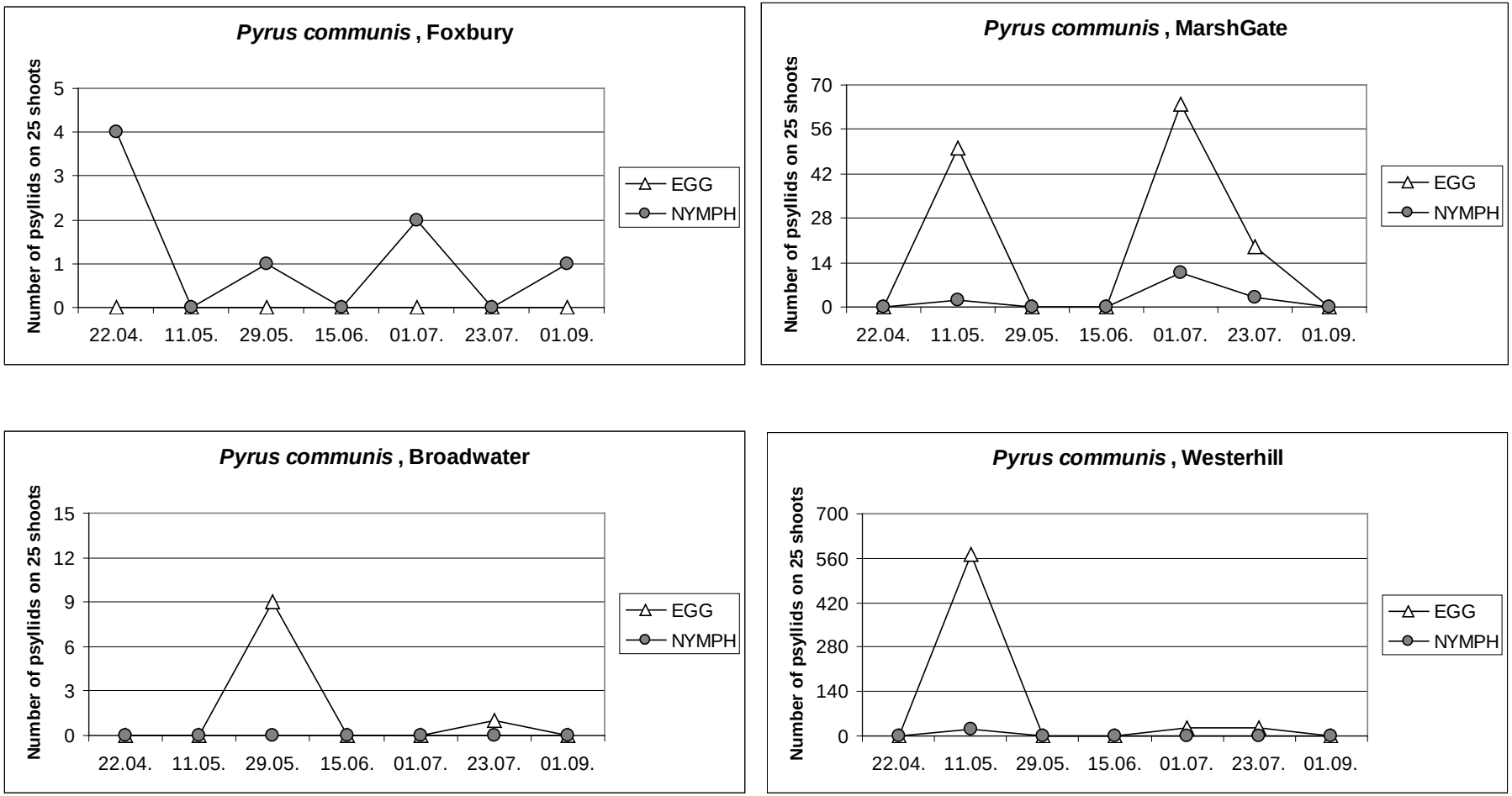
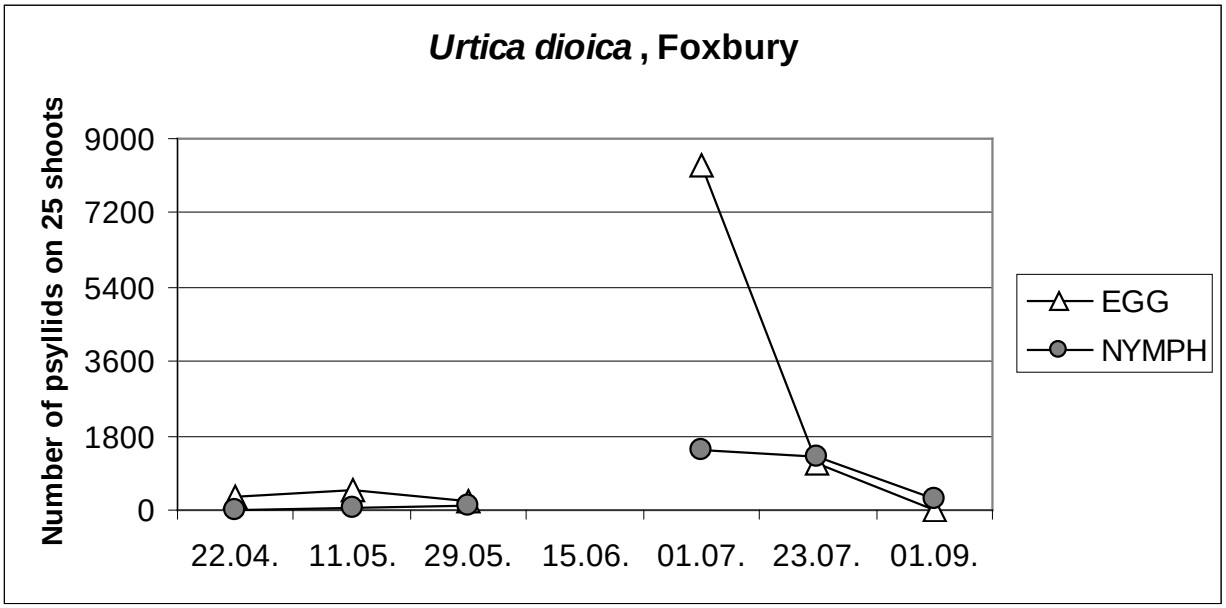


Figure 2.3.1.5 Seasonal dynamics of psyllids on *Betula pubescens* in 2009



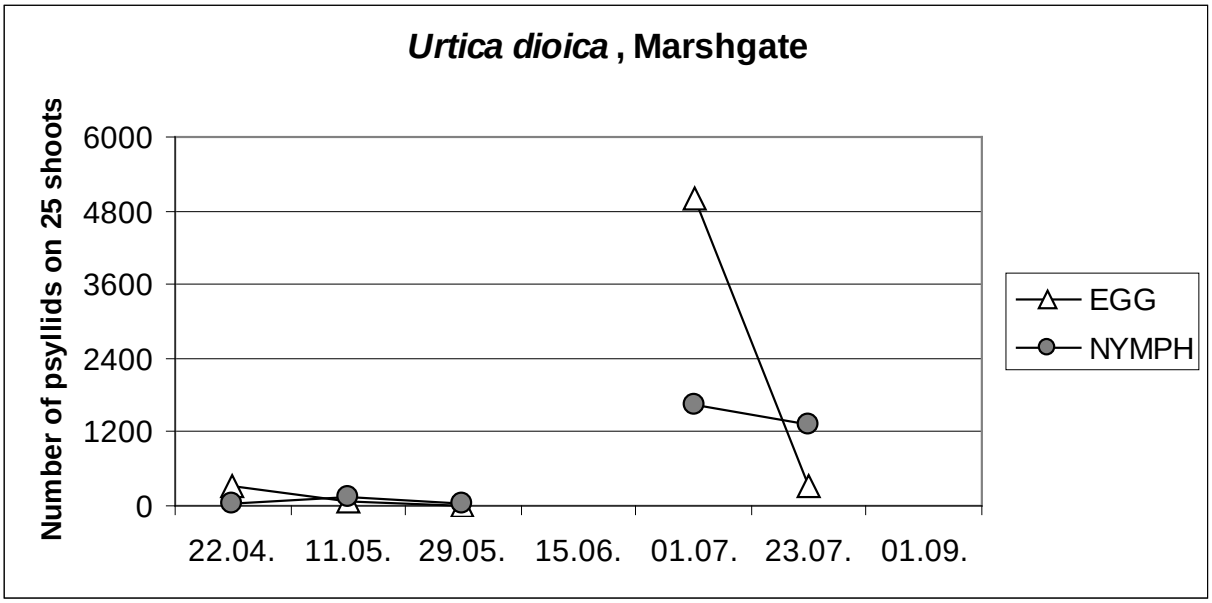
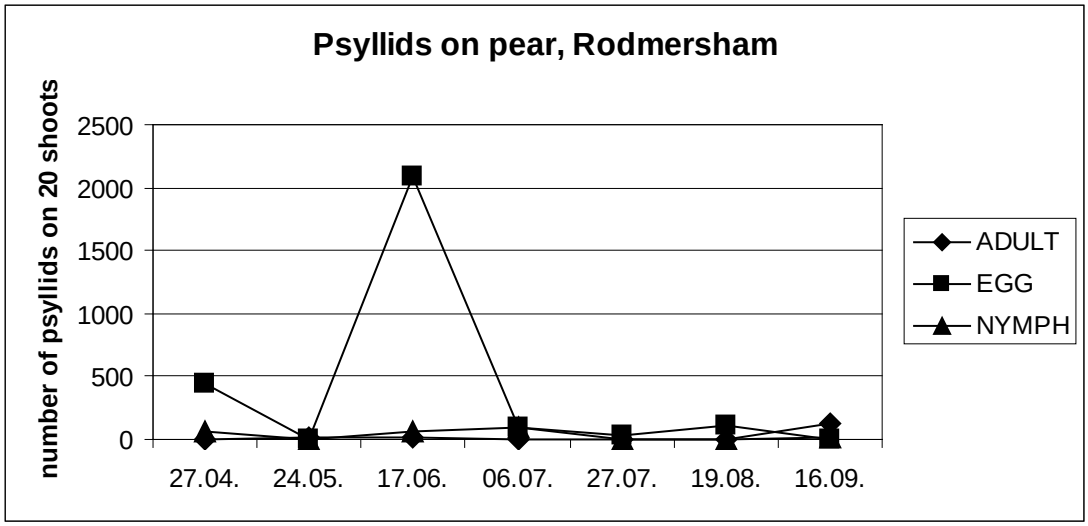
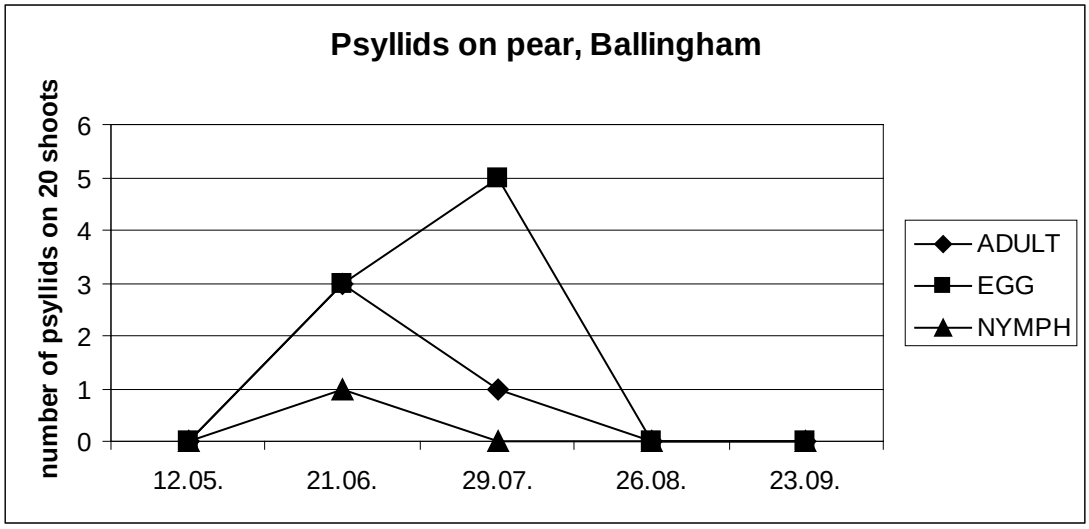


Figure 2.1.3.6 Seasonal dynamics of psyllids on *Urtica dioica* in 2009





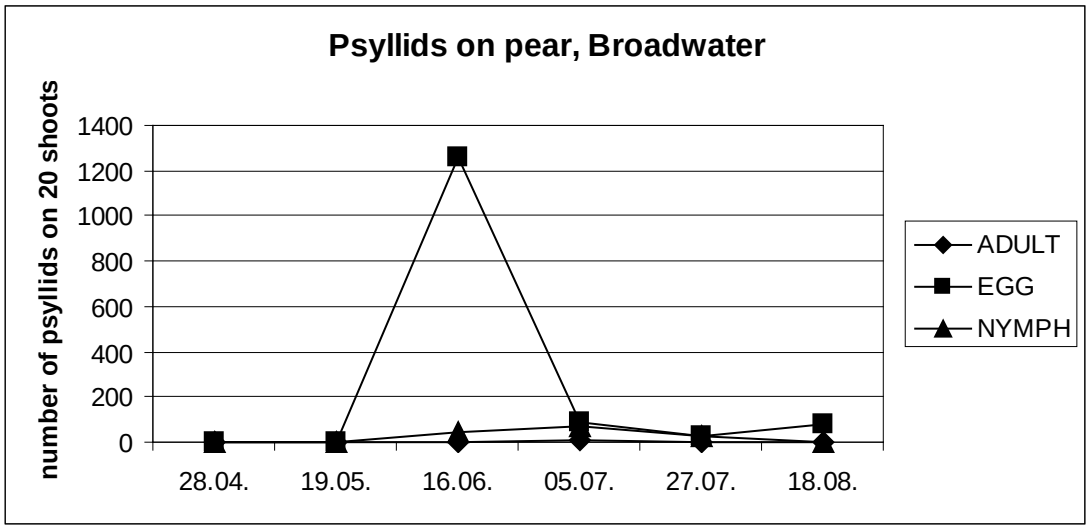
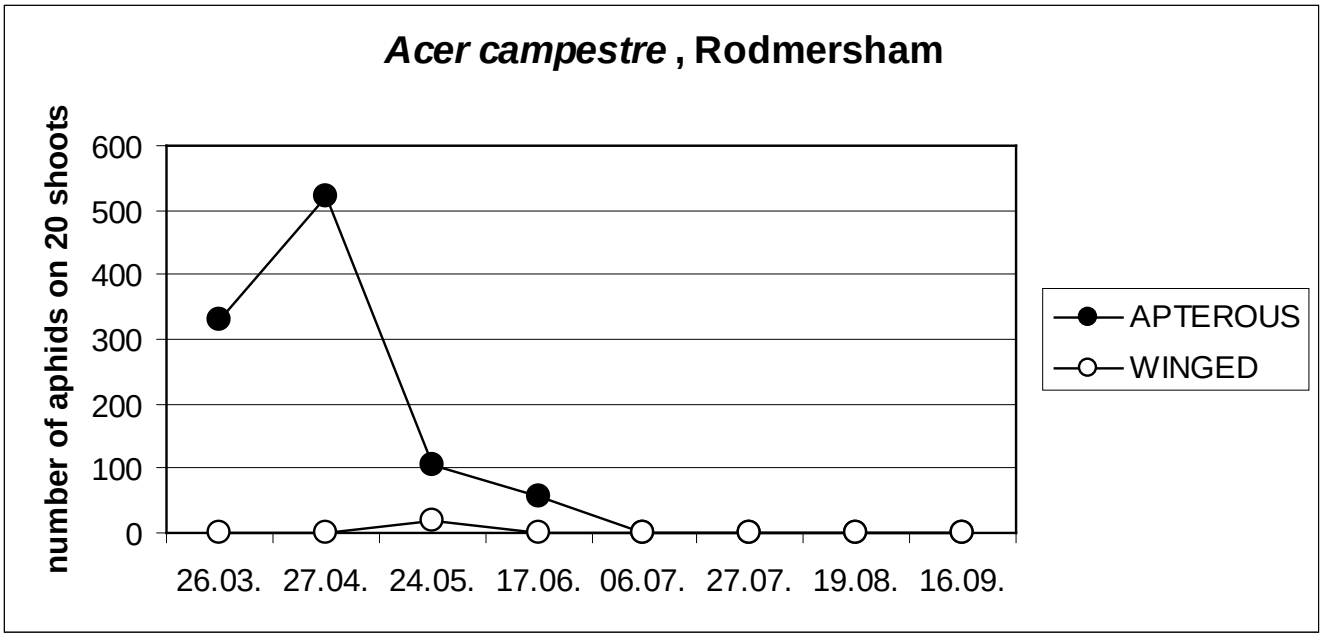


Figure 2.1.3.7. Seasonal dynamics of psyllids on pear in 2010



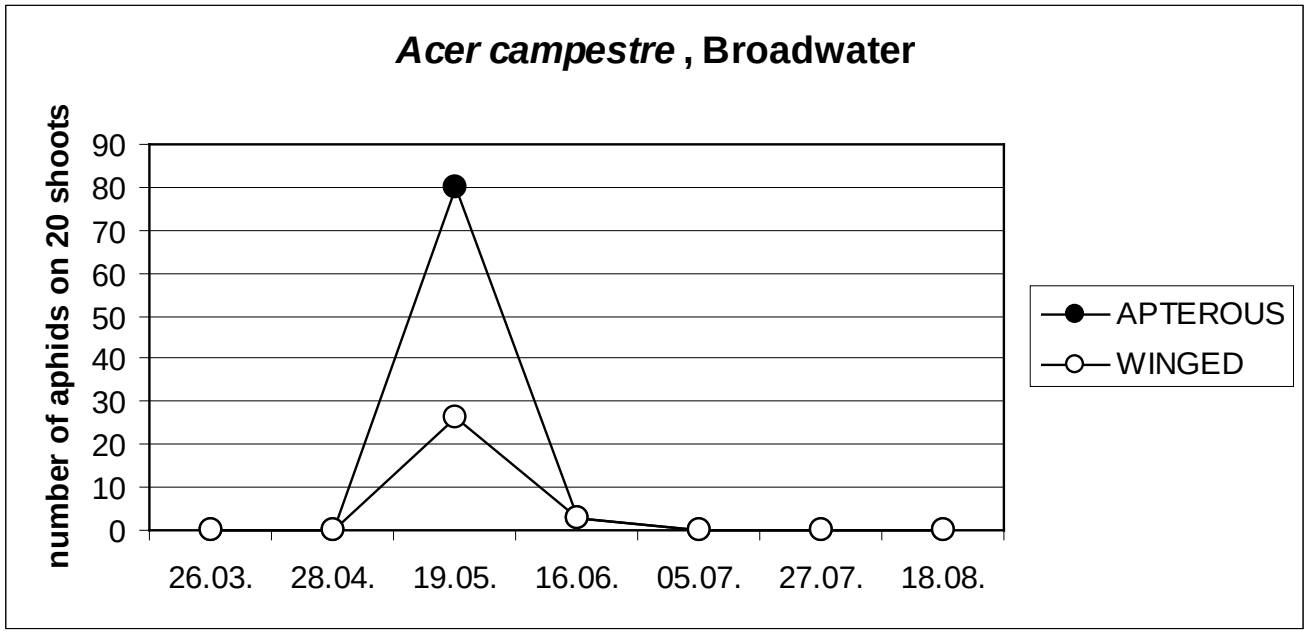
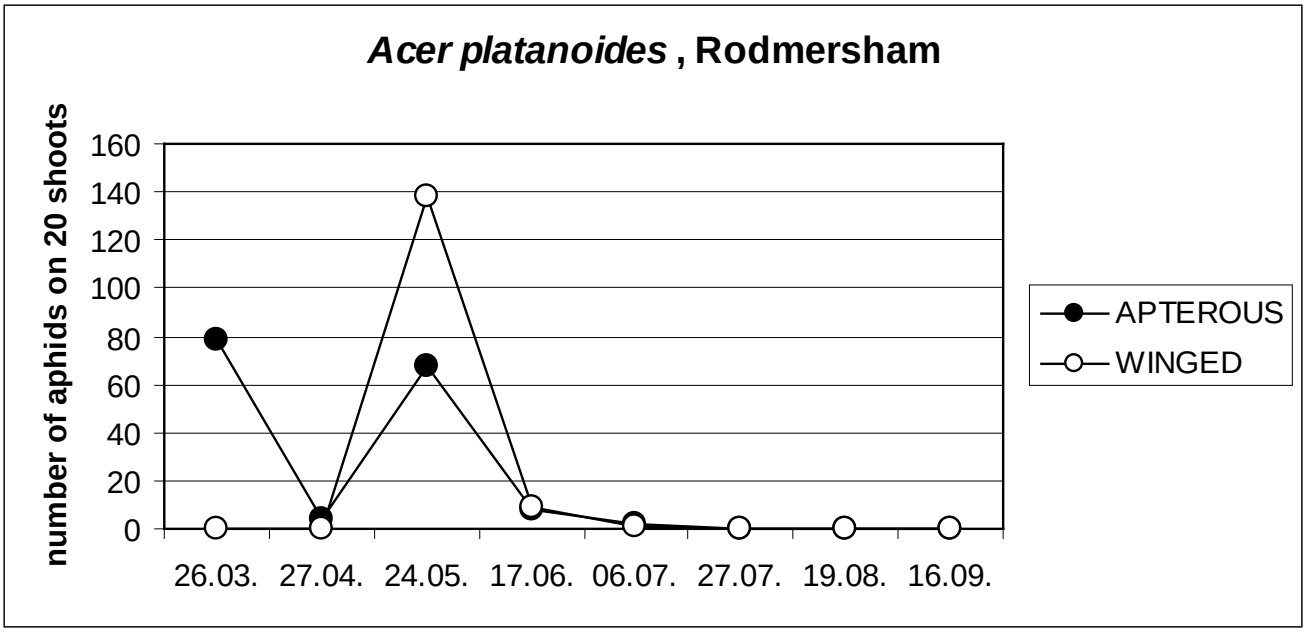


Figure 2.1.3.8. Seasonal dynamics of aphids on *Acer campestre* in 2010



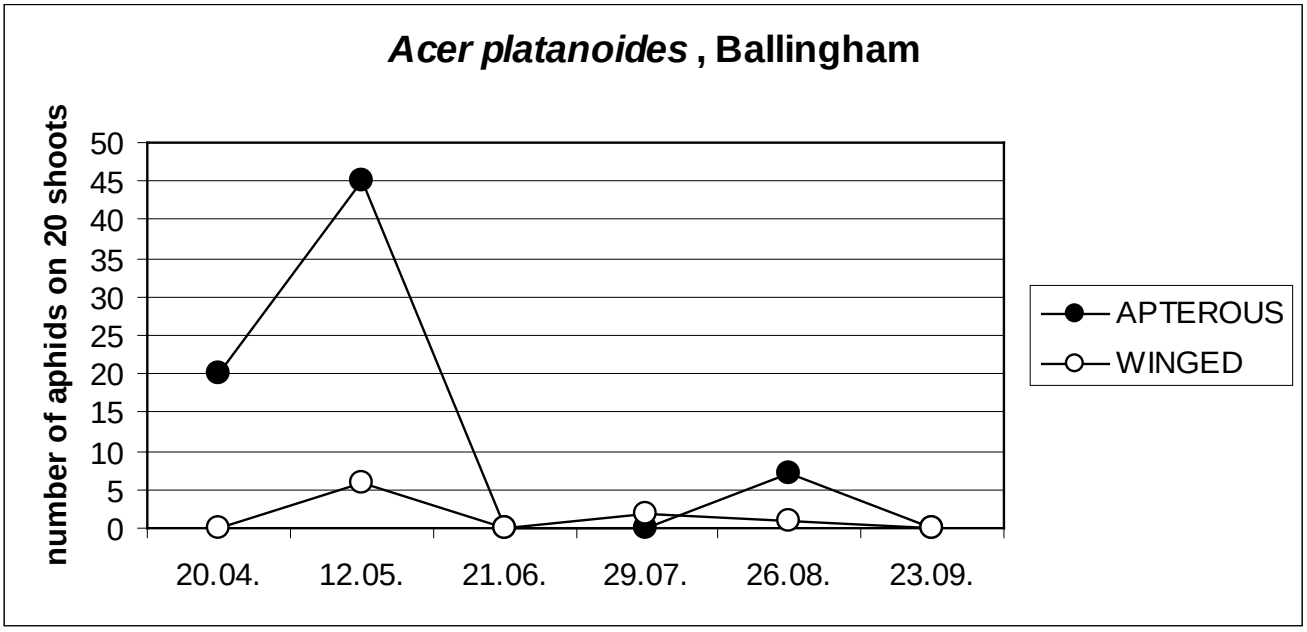
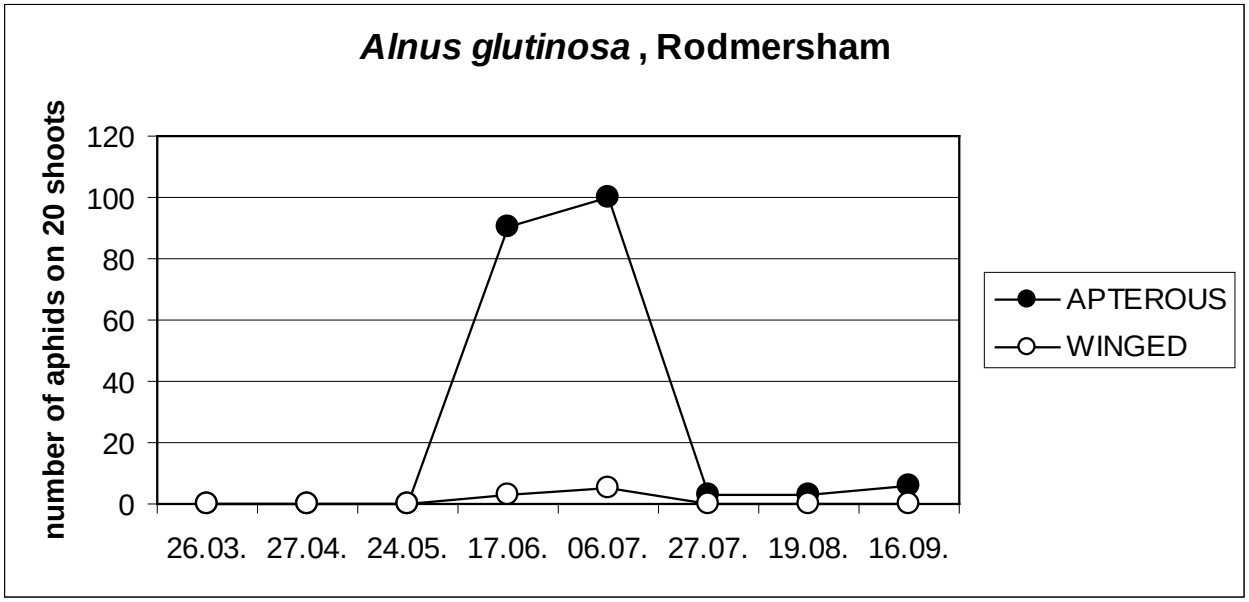


Figure 2.1.3.9. Seasonal dynamics of aphids on *Acer platanoides* in 2010



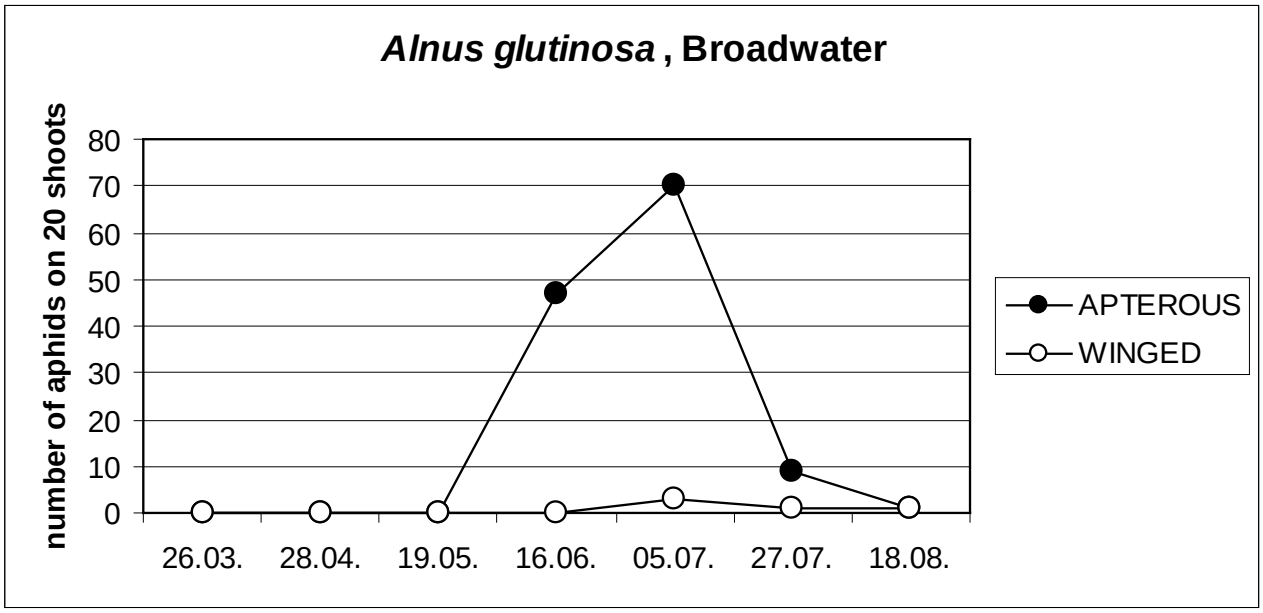
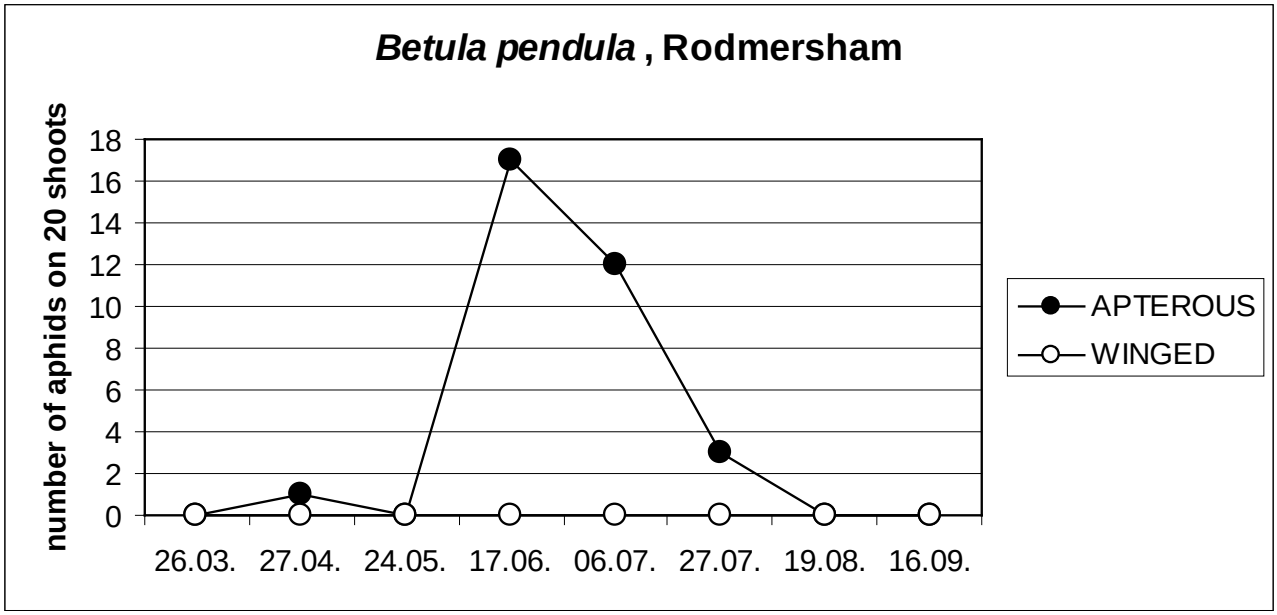


Figure 2.1.3.10. Seasonal dynamics of aphids on *Alnus glutinosa* in 2010



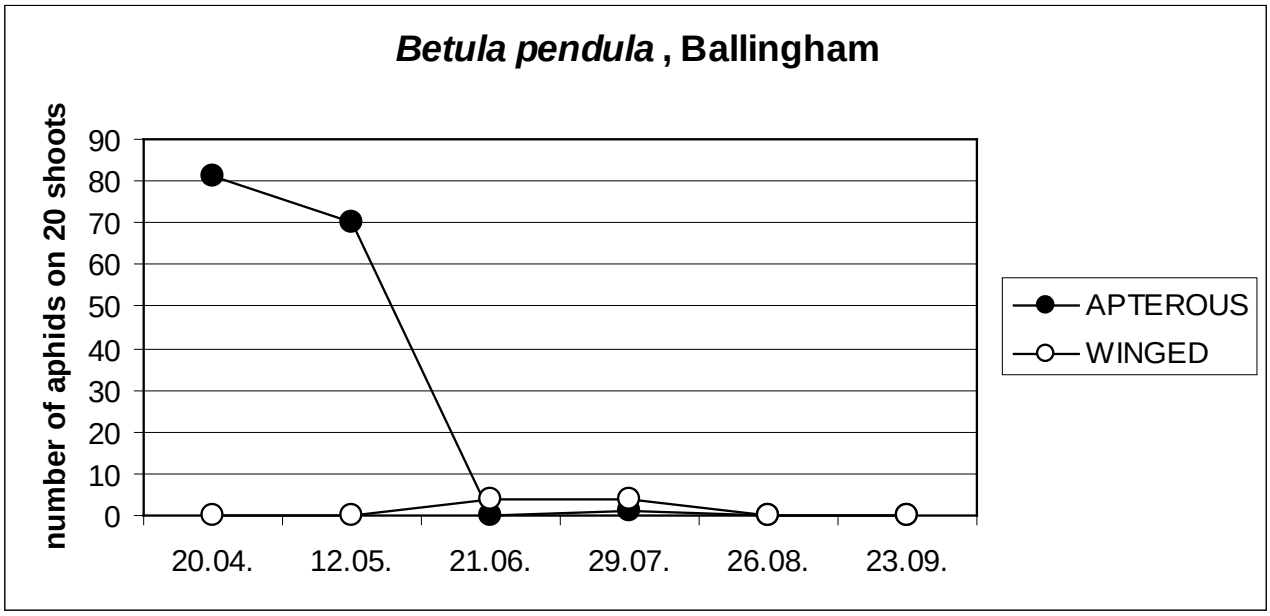
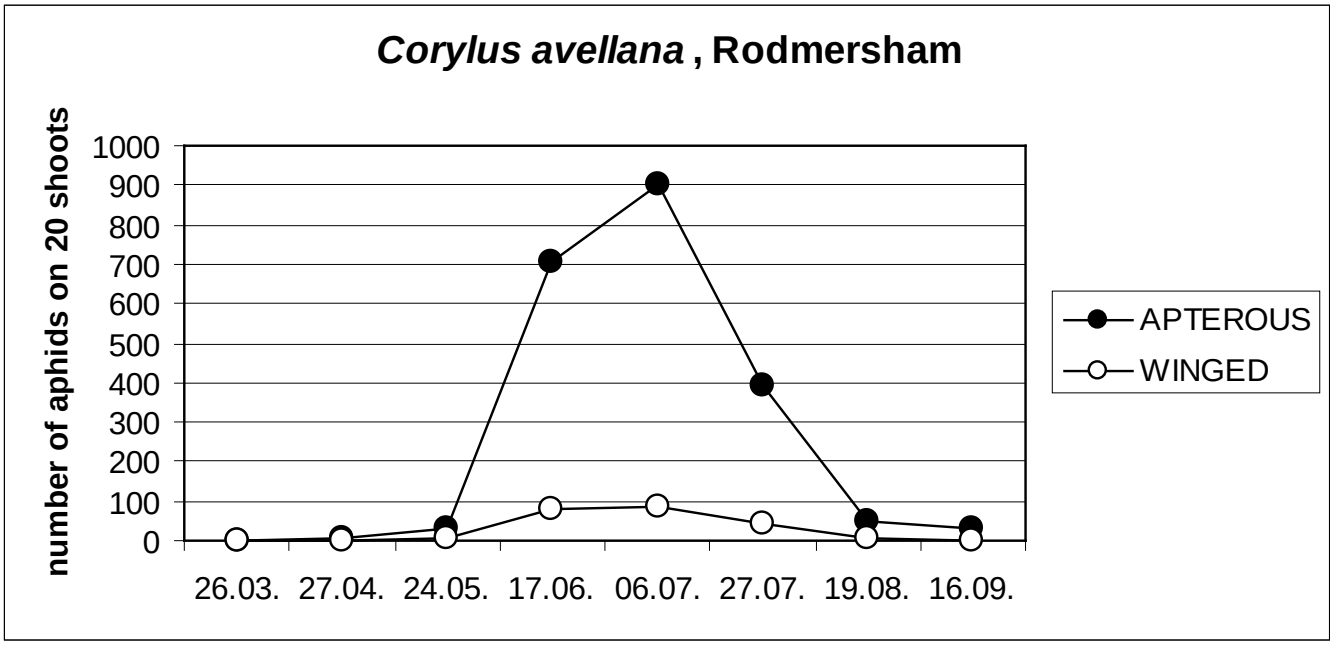


Figure 2.1.3.11. Seasonal dynamics of aphids on *Betula pendula* in 2010



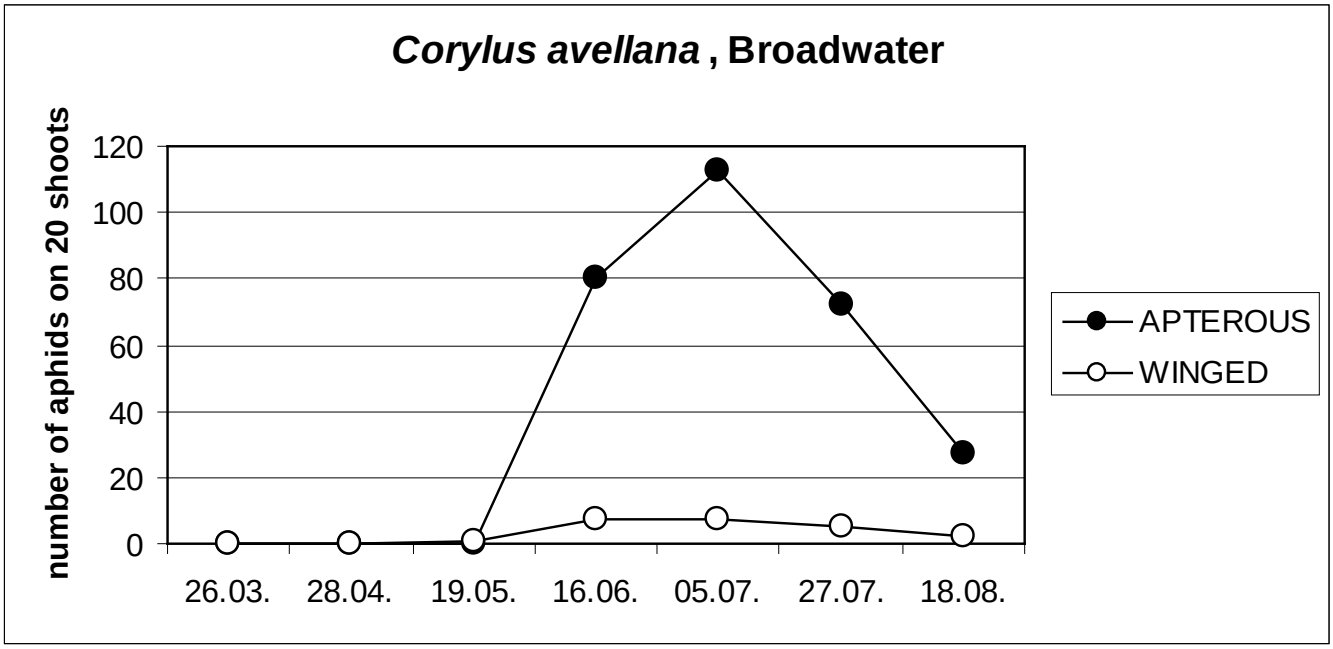
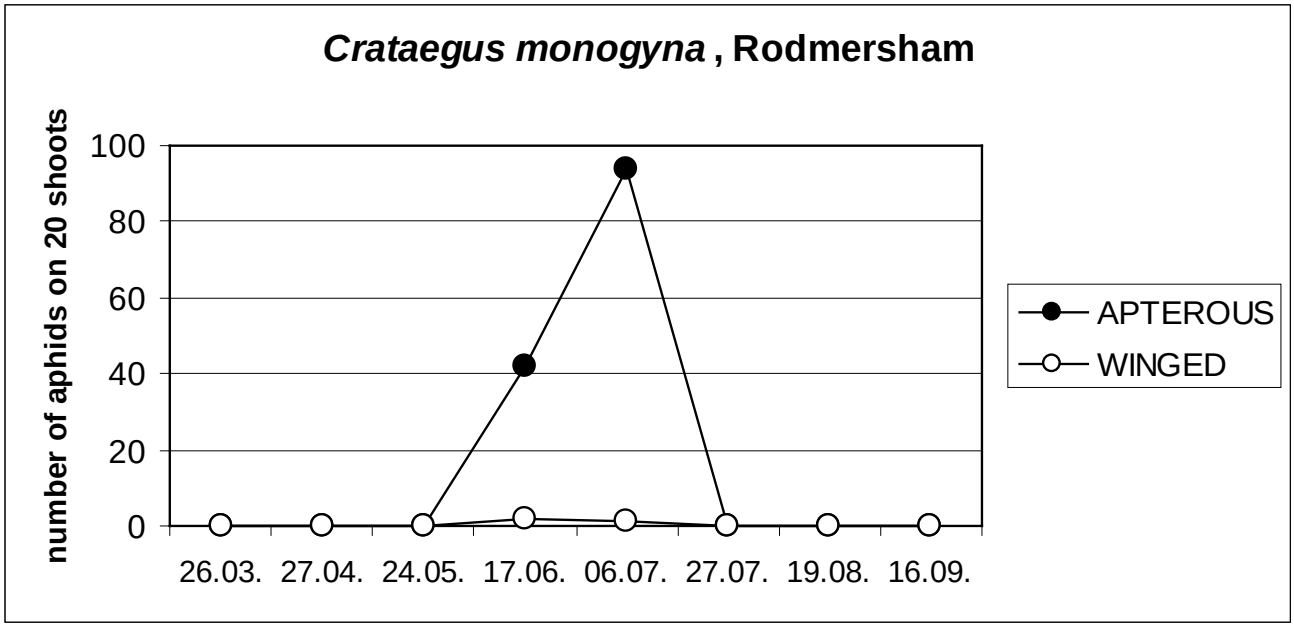


Figure 2.3.1.12. Seasonal dynamics of aphids on *Corylus avellana* in 2010



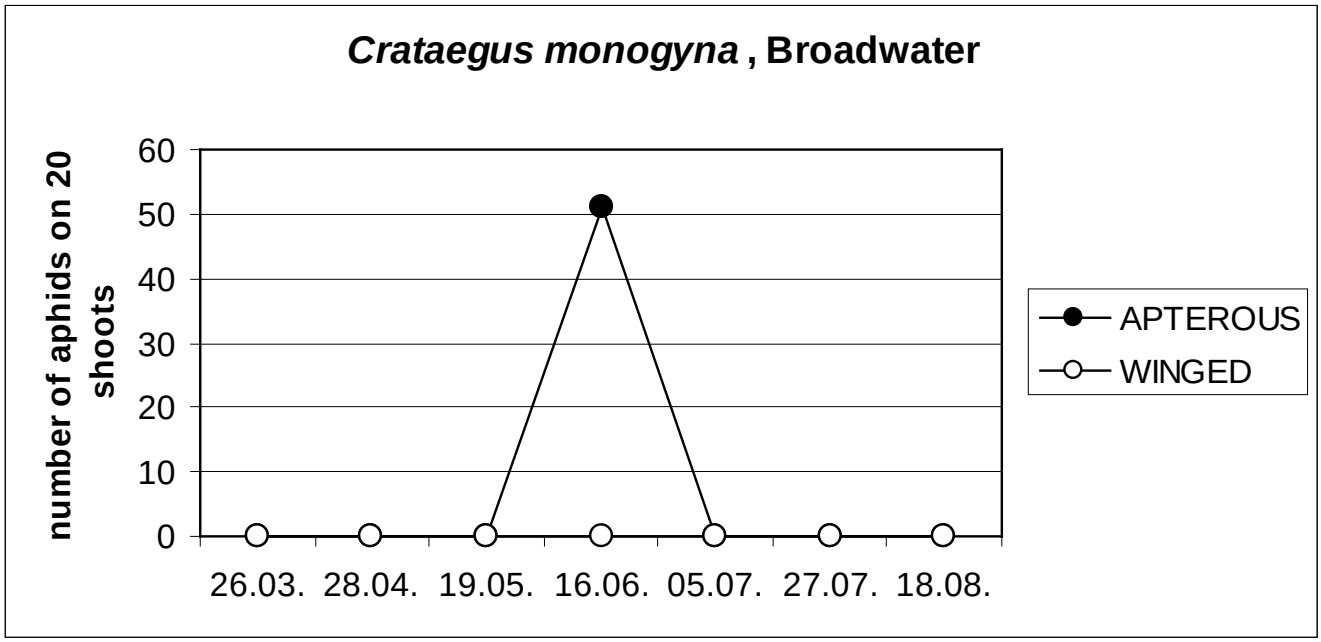
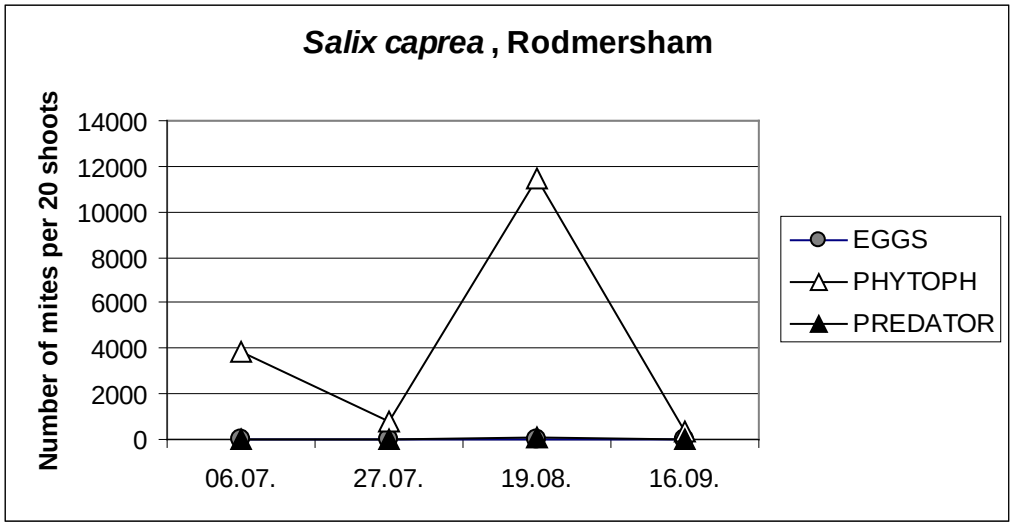
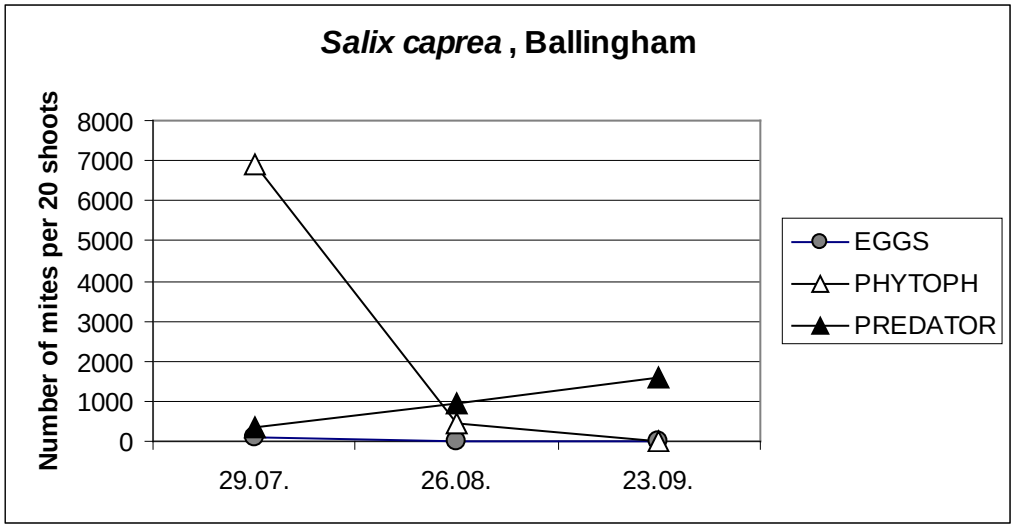


Figure 2.1.3.13. Seasonal dynamics of aphids on *Cratagus monogyna* 2010





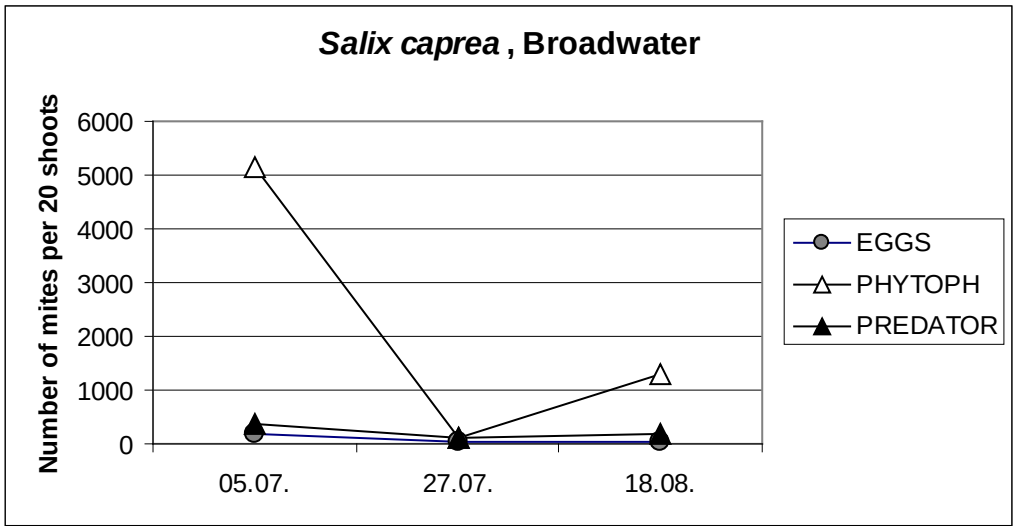
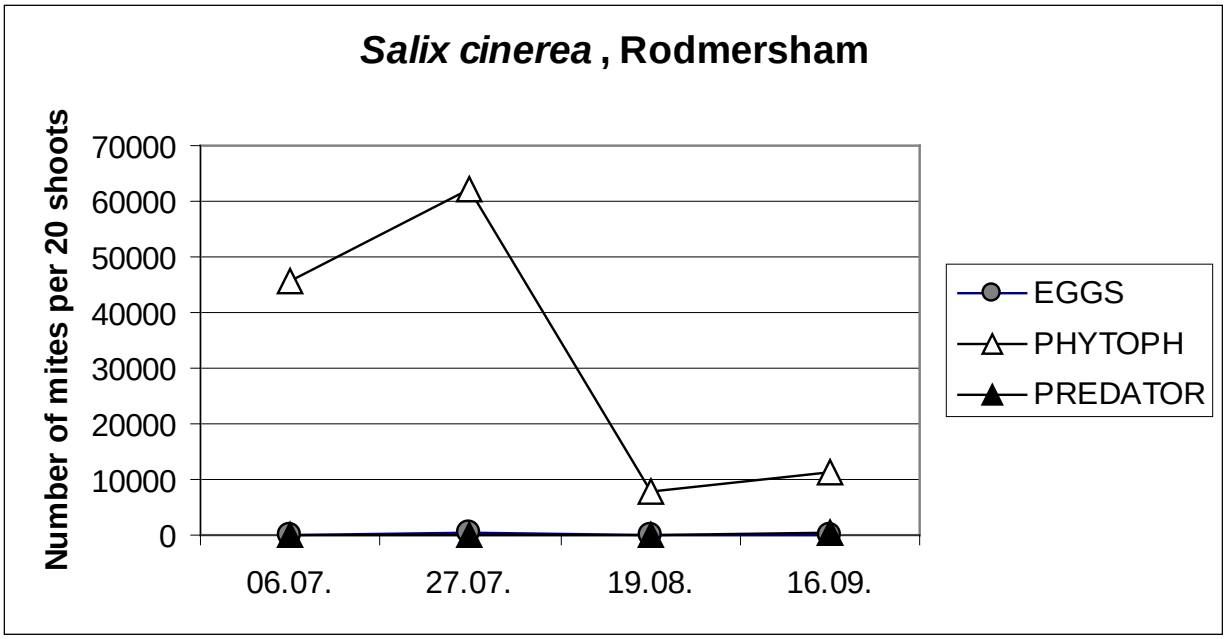


Figure 2.1.3.14. Seasonal dynamics of mites on *Salix caprea* in 2010



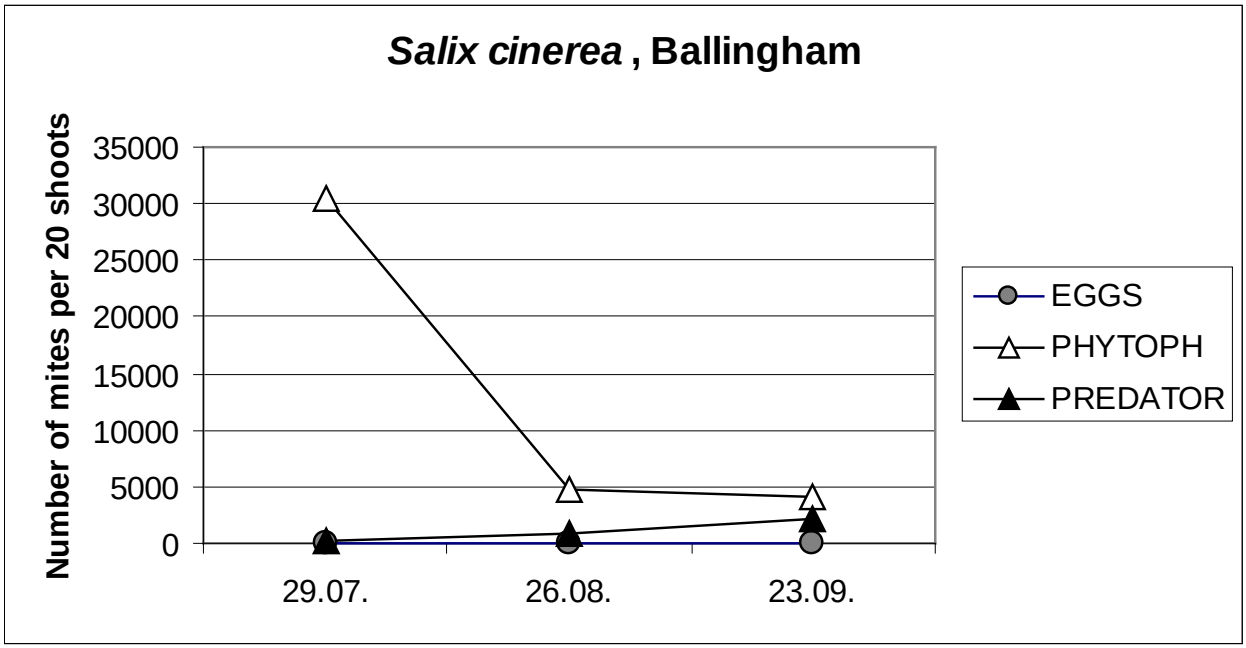
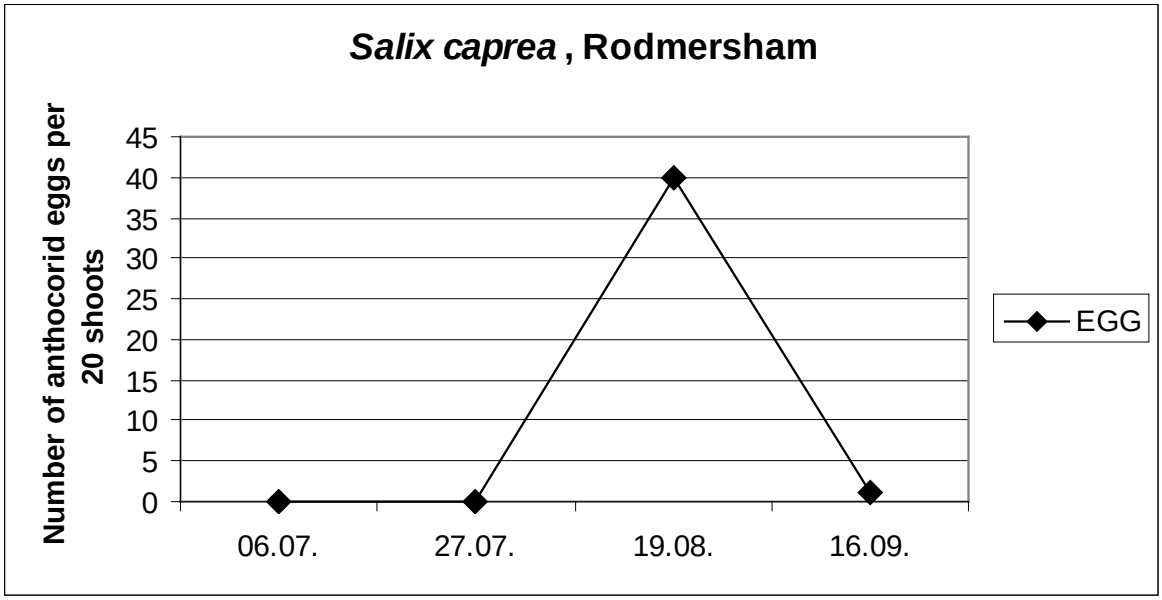


Figure 2.1.3.15. Seasonal dynamics of mites on *Salix cinerea* in 2010



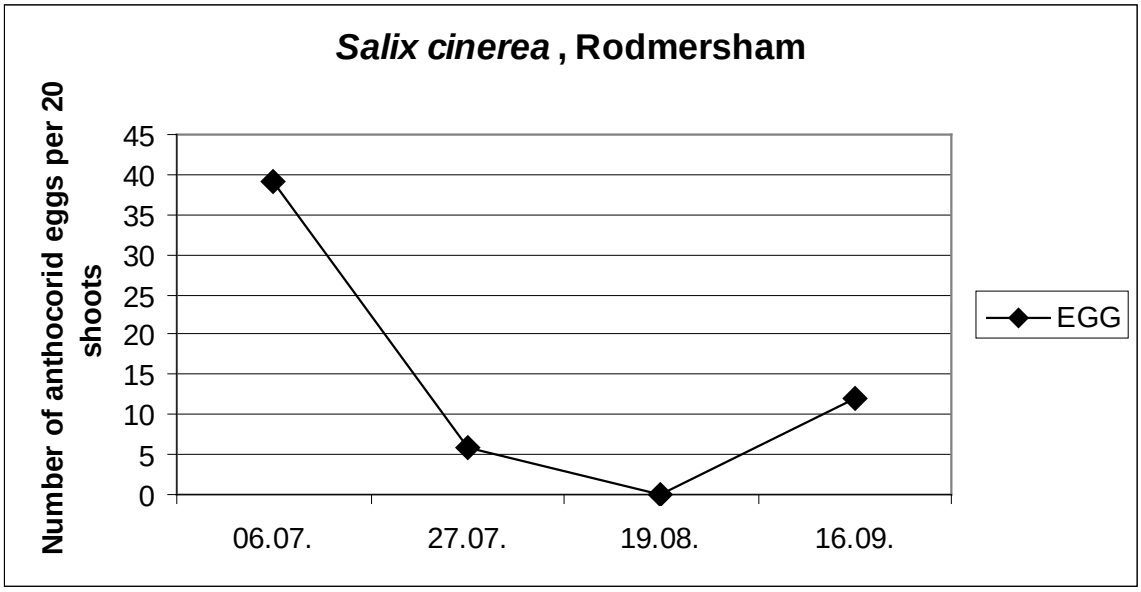


Figure 2.1.3.16. Seasonal dynamics of anthocorid eggs on *Salix caprea* and *Salix cinerea* in 2010

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

Field experiments were conducted in 2010 to quantify the spatial and temporal pattern of the migration of anthocorids into pear sucker infested orchards from adjacent nettle strips and whether the influx into pear could be enhanced by cutting down the nettles. Protein (milk, albumin etc) monoclonal antibody based 'mark and recapture' methods developed in the USA were used (Hagler & Naranjo, 2004; Hagler, 2006). Nettle plots alongside a pear orchard were sprayed with dilute solutions of different protein markers and the subsequent dispersal of the anthocorids from nettle to pear determined by sampling in the orchard and testing anthocorids collected using ELISA. A comparison was made between nettle plots which were trimmed shortly after marker application and untrimmed plots.

Background and outline

Anthocorid predatory bugs are the key natural enemies of pear sucker. Nettles, which are host to large populations of nettle aphid and nettle psyllid, harbour high populations of anthocorids. A protein based monoclonal antibody mark and recapture method was used to study the migration of adult anthocorids from nettles, not cut down versus after they have been cut, into adjacent pear orchards. Milk and albumin were used as the marker proteins. When adult anthocorid numbers were at their peak in 2010, a 60 m plot of nettles adjacent to a pear orchard was sprayed with a milk solution and a 60 m plot was sprayed with an albumin solution. Once the spray deposits had dried, the nettles sprayed with milk were cut to the ground using a strimmer. The subsequent dispersal of the anthocorids to the adjacent pear orchards was studied over a period of 7 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.

Methods

Date and duration of study

The study was done over a period of one week in late August to early September 2010. A period of stable dry weather was needed.

Site

The site for the work is owned by Robert Mitchell Partnership, Foxbury Farm, Stone Street, Sevenoaks TN15 0LW Contact: Robert Mitchell

The experimental orchard chosen for the study was Redshed conference pears at Sheet Hill Farm,

Plaxtol TN15 0LZ. Redshed conference pears (2.64 ha) is marked in red surround in Figure 2.1.4.1 below. The orchard is at National Grid Reference TQ 603 551. Access to the field was from the south from Winfield Lane.

Control nettle plots were provided on the north eastern edge of Crowhurst Orchard, to the north of Redshed, as far away as practical from the experimental orchard (Fig. 2.1.4.1).

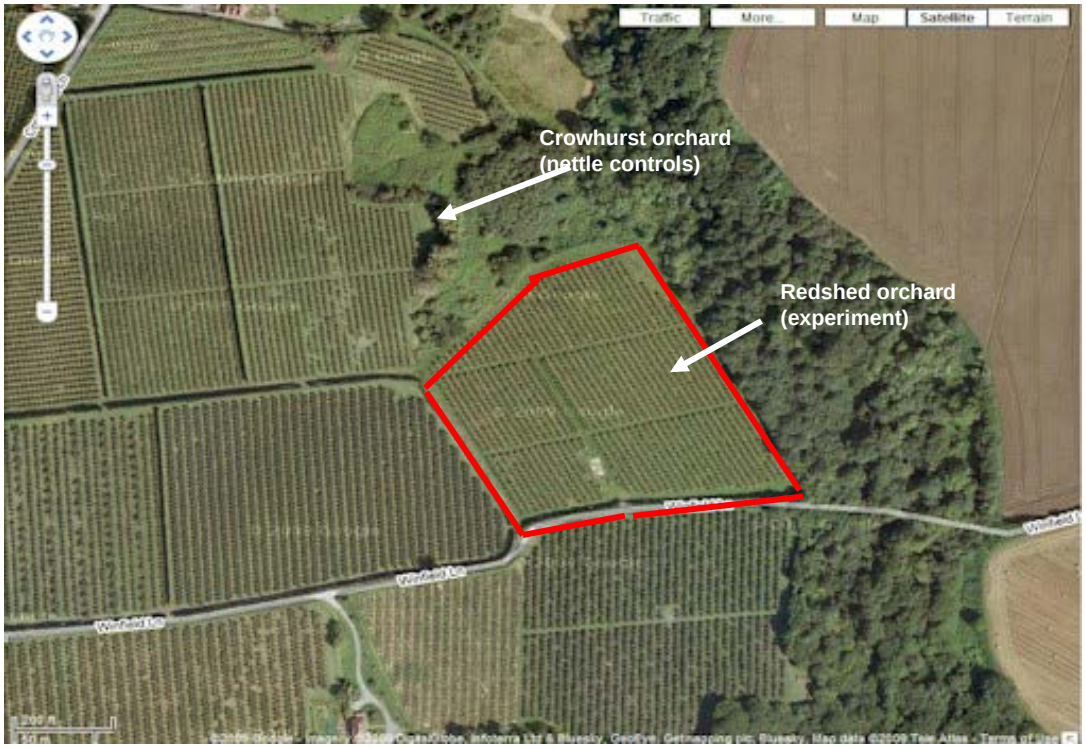


Figure 2.1.4.1. Redshed Conference pear orchard at Sheet Hill Farm Plaxtol (red surround)

Treatments

A 120 m x ~ 2 m wide strip of nettles on a bank along the south western border of Redshed Orchard was divided into four 30 m long plots, labelled N1-N4 (Fig. 2.1.4.2).

Three ~ 30 m long control nettle plots were marked out end to end on the north eastern edge of Crowhurst Orchard (Fig. 3). They were: C1 = untreated control (green line); C2 = uncut milk and egg control (blue line). Note that none of these plots were to be cut after spraying.

Figure 2.1.4.2. Location of the experimental plots: N1, N3 = sprayed with cows milk powder (white lines); N2, N4 = sprayed with egg white powder (yellow lines) and cut

Figure 2.1.4.3. Approximate locations of the nettle control plots: C1 = untreated control (green line); C2 = uncut egg and milk (blue line) in Crowhurst Orchard to the north of the experimental orchard

On day 0 of the study, the white nettle plots N1, N3 were sprayed to run off with a 20% (=200 ml/l) solution of fresh cow's milk in water containing 0.3 g/l EDTA using a motorised air-assisted back pack sprayer. The yellow nettle plots were similarly sprayed with egg white powder at a concentration of 25 g/l plus 0.3 g/l EDTA. The blue plot (C2) was sprayed with 20% fresh cow's milk plus 25 g/l egg white powder plus 0.3 g/l EDTA. The sprays were applied with different sprayers to avoid cross contamination.

As soon as possible after the sprays had dried, the white nettle plots sprayed with milk N1 and N3 were cut to the ground using a petrol strimmer, minimising the amount of pulverisation of the nettles. Note the blue plot C2 was not cut. Treatments are summarised in Table 2.1.4.1.

Table 2.1.4.1. Treatments

Treatment number/name	Colour of lines in Figures 2 & 3	Plot numbers	Sprayed	Cut after spraying
1. Cut	White	N1, N3	With milk	Yes
2. Not-cut	Yellow	N2, N4	With egg white	No
3. Control	Green	C1	Not sprayed	No
4. Uncut milk and Egg control	Blue	C2	With egg white powder + milk powder	No

Sampling

Pear trees: Adult anthocorids and other adult predatory heteroptera were sampled by beat sampling over a large paper sheet on the ground. For each sampled pear tree, 10 sharp taps were done over a pair of large clean white paper sheets, each 1 m x 2m and laid on the ground under the tree to form a 2 m x 2 m square centred under the tree. The numbers of anthocorid adults and nymphs recovered from each tree were recorded. Each adult individual was transferred with forceps to an individual Eppendorf tube. The forceps were carefully wiped clean with a fresh damp paper towel each time that the forceps were used. The batches of tubes for each tree on each sampling date were held together in a bag labelled with the date and row and tree number. A list of the samples collected was made.

Nettles: Adult anthocorids and other adult predatory heteroptera were sampled by beat sampling over an A2 sized (60 cm x 42 cm) board covered with an A2 sheet of white paper, which was refreshed for sampling each plot. The paper was secured to the board with bulldog clips. For each plot, 10 tap samples were done over the board, the numbers of anthocorid adults and nymphs being recorded for each tap. Each adult individual was transferred using forceps to an individual Eppendorf tube. The

forceps were carefully wiped clean with a fresh damp paper towel each time the forceps were used. The batches of tubes for each tree on each sampling date were to be held together in a bag labelled with the date and plot number. A list of the samples collected was made.

Avoiding cross-contamination

Avoiding cross contamination of individuals was critical. In general, the pear trees were sampled first starting at the furthest point from the sprayed area. The unsprayed nettles were sampled next and the sprayed nettles were sampled last.

Pre- and post treatment sampling

Immediately before spraying, three samples, each of 10 adult anthocorids, were collected by beat sampling, one bulk sample taken overall from the four experimental nettle plots N1-N4, one overall from the two control nettle plots (C1-C2) and one from Redshed Orchard pears. Each adult anthocorid was held in an individual Eppendorf tube to avoid cross contamination. The batches of 10 tubes comprising each sample were held together for each plot and labelled ‘pre-nettle N1-N4’, ‘pre-nettle C1-C2’ and ‘pre-pear Redshed’, respectively. See Table 2 for list of samples.

As soon as the sprays had dried and before the milk sprayed plots of nettles were cutfive further samples, each of 10 adult anthocorids, were collected by beat sampling in the same way. One sample was taken from each of the plots C1 and C2, one sample one from the pear trees in row 1 alongside plots N1-N4 in Redshed Orchard and one from the pear trees on the northern boundary of Redshed Orchard. The samples were labelled Day0 C1, Day0 C2, Day0 pear W, Day0 pear N, respectively. See Table 2 for list of samples.

Post treatment sampling from plots C1 and C2 was repeated on each of the post applications sampling dates. See Table 2 for list of samples.

Anthocorid migration monitoring samples

Full sampling was done 1, 2, 4 and 7 days after spraying

Four sampling trees in each of five pear rows (rows 1, 2, 4, 8 and 16) from the west edge of the orchard) were designated for sampling along four transects perpendicular to the nettle plot N1-N4 as marked by red lines in Figure 4. The fruit from these 20 trees was picked/removed before the experiment commenced and the trees were labelled with the transect and row number.

Figure 2.1.4.4. Sampling transects (red line), two perpendicular to each sprayed nettle plot

On each of the four sampling occasions, each of the 20 trees in the sampling grid was sampled over a 2 x 2 m paper square, the paper was renewed for each replicate and row.

Each anthocorid was transferred with forceps to an individual Eppendorf tube. The samples from each plot were held together in a bag labelled with the experimental day, row and transect number e.g. ‘Day1 R16 T4’. See Table 2.1.4.2 for list of samples.

Table 2.1.4.2. List of samples

Day	Plot	Descriptor	Label
Day 0, Pre-spraying	Overall from nettles N1-N4	10 adult anthocorids	Pre-nettle N1-N4
	Overall from nettle C1-C2	10 adult anthocorids	Pre-nettle C1-C2
	Overall from Redshed pears	10 adult anthocorids	Pre-pear Redshed
Day 0, Post-spraying	Nettles C1	10 adult anthocorids	Day0 C1
	Nettles C2	10 adult anthocorids	Day0 C2
	Pears Redshed row 1	10 adult anthocorids	Day0 pear W
	Pears Redshed north edge	10 adult anthocorids	Day0 pear N
Day1	Nettles C1	10 adult anthocorids	Day1 C1
	Nettles C2	10 adult anthocorids	Day1 C2
	Each of 20 pear trees in sampling grid	All adult anthocorids & adult heteroptera	Day1 R1 T1 – Day1 R16 T4
Day 2	Nettles C1	10 adult anthocorids	Day2 C1
	Nettles C2	10 adult anthocorids	Day2 C2
	Each of 20 pear trees in sampling grid	All adult anthocorids & adult heteroptera	Day2 R1 T1 – Day2 R16 T4
Day 4	Nettles C1	10 adult anthocorids	Day4 C1
	Nettles C2	10 adult anthocorids	Day4 C2
	Each of 20 pear trees in sampling grid	All adult anthocorids & adult heteroptera	Day4 R1 T1 – Day4 R16 T4
Day 7	Nettles C1	10 adult anthocorids	Day7 C1
	Nettles C2	10 adult anthocorids	Day7 C2
	Each of 20 pear trees in sampling grid	All adult anthocorids & adult heteroptera	Day7 R1 T1 – Day7 R16 T4

Sample storage

Samples were held overnight in a fridge before ELISA testing the following day. If necessary, samples were stored in a -20 ˚C freezer until processing at a later date.

Sample analysis

MAB ELISA testing was done to determine the presence of the milk and/or albumin tracer on each individual 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 and PBS + 20% bovine serum + 1300 ppm Silwet for the egg 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. The primary antibody for the egg was a mouse monoclonal antibody to ovalbumin (ab17291-100 lot 808018, Abcam, Cambridge, UK), which was diluted in PBS + 1300 ppm Silwet + 30% Bovine serum at 1:2000. These were 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 for the egg was a rabbit polyclonal anti mouse with a horse radish peroxidase conjugation (ab6728, lot 883197, Abcam, Cambridge, UK). This was diluted using an antibody diluent at a rate of 1:2000. 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 of the negative 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 last year, 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 this experiment wells were deemed to be positive if they were 1.84 x greater than the mean of the negative wells for the milk or 1.66 x the mean of the negative controls for the egg. Whilst this may give a conservative estimate compared to using 3 x SD, it protects against false positives which would overestimate movement.

Results

The results for the egg marker are shown in Tables 3 a - e and the results for the milk marker are shown in Tables 4 a – e.

The numbers of anthocorids marked with milk from the sprayed nettle plots were low, with between 0 and three marked insects out of 10 sampled on each sample date, whereas the numbers of anthocorids marked with egg from the sprayed nettle plots was between 4 and 8 out of 10 on each sample date.

Anthocorids marked with egg were found on days 1, 2 and 4 but not on day 7 of sampling (four were found in total). Although the nettles sprayed with egg were not cut, the anthocorids were found throughout the orchard and as far as row 16.

Anthocorids marked with milk were not found on day 0 in the pears, but were found in on days 2, 4 and 7. As previously, they were found throughout the orchard, with one in row 1, two in row 8 and one in row 16.

It is interesting to note that the number of anthocorids caught in row 1 was similar across all dates, but was lower at day 7 compared to day 1 for some of the rows farther away, which may indicate an influx of unmarked anthocorids from the nettle bank.

The majority of anthocorids that were caught were *Anthocoris nemorum* (279) although *A. nemoralis*

were also caught (75).

Tables 2.1.4.3 a - e. The number of anthocorids found marked with an egg marker at day 0, 1, 2, 4 and 7 after treatment.

a) Day 0, EGG

Nettle plots

Plot	No. negative	No. positive
C1	10	0
C2	2	8

Pre-spraying sample

Plot	No. negative	No. positive
Pre-Nettle C	10	0
Pre-Nettle N	9	0
Pre-pear	10	0
Total Pre	***29***	***0***

Post-spray, pre-cutting sample

Plot	No. negative	No. positive
Pear North	7	3
Pear West	8	2
Total Post	***15***	***5***

b) Day 1, EGG

Nettle plots

Plot	No. negative	No. positive
C1	10	0
C2	5	5

Sampled pear trees

	Row1		Row2		Row4		Row8		Row16	
	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive
Tree1	4	0	3	0	0	0	3	0	3	0
Tree2	2	0	2	0	5	0	5	0	5	0
Tree3	7	1	6	0	4	0	1	0	1	0
Tree4	4	0	10	0	4	0	7	0	10	0
Total	***17***	***1***	***21***	***0***	***13***	***0***	***16***	***0***	***19***	***0***

Total no. negative = 86 **Total no. positive =1**

c) Day 2, EGG

Nettle plots

Plot	No. negative	No. positive
C1	10	0
C2	3	7

Sampled pear trees

	Row1		Row2		Row4		Row8		Row16	
	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive
Tree1	6	0	3	0	1	0	4	0	1	0
Tree2	2	0	4	0	4	0	3	0	2	0
Tree3	7	0	0	1	2	0	0	0	0	0
Tree4	3	0	3	0	5	0	3	1	0	0
Total	***18***	***0***	***10***	***1***	***12***	***0***	***10***	***1***	***3***	***0***

Total no. negative = 53 **Total no. positive =2**

d) Day 4, EGG

Nettle plots

Plot	No. negative	No. positive
C1	10	0
C2	6	4

Sampled pear trees

	Row1		Row2		Row4		Row8		Row16	
	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive
Tree1	3	0	5	0	1	0	1	0	0	0
Tree2	4	0	4	0	0	0	1	0	0	0
Tree3	10	0	3	0	2	0	2	0	0	0
Tree4	7	0	8	0	2	0	3	0	1	0
Total	***24***	***0***	***20***	***0***	***5***	***0***	***7***	***0***	***1***	***0***

Total no. negative = 57 **Total no. positive =0**

e) Day 7, EGG

Nettle plots

Plot	No. negative	No. positive
C1	11	0
C2	5	5

Sampled pear trees

	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive
	Row1		**Row2**		**Row4**		**Row8**		**Row16**	
Tree1	5	0	2	0	2	0	1	0	2	0
Tree2	0	0	0	0	1	0	2	0	2	0
Tree3	8	0	1	0	1	0	0	0	1	0
Tree4	4	0	4	0	0	0	0	0	1	0
Total	***17***	***0***	***7***	***0***	***4***	***0***	***3***	***0***	***6***	***0***

Total no. negative = 37 Total no. positive =0

Tables 2.1.4.4 a - e. The number of anthocorids found marked with a milk marker at day 0, 1, 2, 4 and 7 after treatment.

a) Day 0, MILK

Nettle plots

Plot	No. negative	No. positive
C1	10	0
C2	8	2

Pre-spraying sample

Plot	No. negative	No. positive
Pre-Nettle C	10	0
Pre-Nettle N	10	0
Pre-pear	10	0
Total Pre	***30***	***0***

Post-spray, pre-cutting sample

Plot	No. negative	No. positive
Pear North	10	0
Pear West	10	0
Total Post	***20***	***0***

b) Day 1, MILK

Nettle plots

Plot	No. negative	No. positive
C1	10	0
C2	8	2

Sampled pear trees

	Row1		Row2		Row4		Row8		Row16	
	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive
Tree1	4	0	3	0	0	0	3	0	3	0
Tree2	2	0	2	0	5	0	5	0	5	0
Tree3	8	0	6	0	4	0	1	0	1	0
Tree4	4	0	10	0	4	0	7	0	10	0
Total	***18***	***0***	***21***	***0***	***13***	***0***	***16***	***0***	***19***	***0***

Total no. negative = 87 Total no. positive =0

c) Day 2, MILK

Nettle plots

Plot	No. negatives	No. positives
C1	10	0
C2	10	0

Sampled pear trees

	Row1		Row2		Row4		Row8		Row16	
	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive
Tree1	6	0	3	0	1	0	4	0	1	0
Tree2	2	0	4	0	4	0	3	0	2	0
Tree3	7	0	1	0	2	0	0	0	0	0
Tree4	3	0	3	0	5	0	3	1	0	0
Total	***18***	***0***	***11***	***0***	***12***	***0***	***10***	***1***	***3***	***0***

Total no. negative = 54 Total no. positive =1

d) Day 4, MILK

Nettle plots

Plot	No. negatives	No. positives
C1	10	0
C2	7	3

Sampled pear trees

	Row1		Row2		Row4		Row8		Row16	
	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive
Tree1	3	0	5	0	1	0	1	0	0	0
Tree2	4	0	4	0	0	0	1	0	0	0
Tree3	9	1	3	0	2	0	2	0	0	0
Tree4	7	0	8	0	2	0	3	0	1	0
Total	***23***	***1***	***20***	***0***	***5***	***0***	***7***	***0***	***1***	***0***

Total no. negative = 56, Total no. positive =1

e) Day 7, MILK

Nettle plots

Plot	No. negatives	No. positives
C1	10	0
C2	7	3

Sampled pear trees

	Row1		Row2		Row4		Row8		Row16	
	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive	No. negative	No. positive
Tree1	5	0	2	0	2	0	0	1	2	0
Tree2	0	0	0	0	1	0	2	0	2	0
Tree3	8	0	1	0	1	0	0	0	1	0
Tree4	4	0	4	0	0	0	0	0	0	1
Total	***17***	***0***	***7***	***0***	***4***	***0***	***2***	***1***	***5***	***1***

Total no. negative = 35 Total no. positive =2

Table 5. The total number of anthocorids in caught in each row at each sampling date.

	Row 1	Row 2	Row 3	Row 4	Row 5
Day 1	17	21	13	16	19
Day 2	18	11	12	11	3
Day 4	24	20	5	7	1
Day 7	17	7	4	3	6

Discussion

This experiment has shown that anthocorids will move from banks of nettles throughout an adjoining orchard, although it has not been able to prove conclusively that cutting the nettles is of benefit.

It was disappointing that the number of anthocorids marked with milk was low, especially as the cut nettles were sprayed with milk so we would have expected to retrieve more milk-marked anthocorids. Optical density readings from the plate reader for the standard control samples on the ELISA plates were similar to those obtained last year, so it is unlikely to be a molecular problem. Jones *et al*. 2006 have found that insect numbers acquiring the marker by walking across the residues was lower for milk than for egg, with the percentage of psylla scoring positive for milk being 3- to 4- fold lower than with egg. However the advantage of using the milk marker is that it has a greater rain-fastness than the egg marker.

References

Jones, V.P., Haggler, J.R., Brunner, J.F., Baker, C.C. and Wilburn, T.D. 2006. An inexpensive immunomarking technique for studying movement patterns of naturally occurring insect populations *Environ. Entomol*. **35 (4):**827-836

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 anothocorid 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 & 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 & 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)

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 *Anthocoris 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 we collected volatiles 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 2-phenylethanol as a good candidate for involvement in attraction of anthocorid predators to pear trees infested with pear sucker.

During 2010 efforts were focussed on:

- confirming production of 2-phenylethanol by potted pear trees infested with pear sucker nymphs in the laboratory;
- investigating production of 2-phenylethanol and other volatile compounds by potted pear trees

infested with pear sucker nymphs in the field;

- investigating production of 2-phenylethanol and other volatile compounds by pear trees naturally infested with pear sucker in an orchard;
- 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.

Materials and Methods

Plant volatile collections from potted plants

In the laboratory volatile collections were made from potted cv. Conference pear trees indoors (5-21 May). The leaves of the trees were either punctured with a hypodermic needle or had nymphs of pear sucker, *C. pyricola,* added (Table 3.1.1)

Volatiles were also collected from potted cv. Conference pear trees in the field (16 June and 6 July). In this experiment leaves with nymphs of pear sucker, *C. pyri,* introduced were compared to leaves with no pear sucker (Table 3.1.2)

The upper parts of a potted pear plant were contained in a transparent bag (Sainsbury's Oven Bags) loosely attached round the stem at EMR. These clear oven bags enclosed the shoot and the psyllid nymphs (from cultures) and were anchored in place with wire ties on a clamp stand. The inlet tube was held in place at the bottom (open end) of the bag and the outlet tube at a cut top corner, both with a wire tie. The tubing consisted of Teflon and glass. Insects were placed in the bag and charcoal filtered air introduced into the base of the bag (1 litre/min) while volatiles were collected onto two Porapak filters at the top of the bag (450 ml/min each). After various time intervals filters were removed and, stored at -20^{o}C and then transported to NRI for analyses. Trapped volatiles were removed and analysed by GC-MS at NRI.

Collection and analysis of volatiles in pear orchards

Collections were made at Marshgate Farm, Cooling, Rochester, ME3 8DP on 6 August and 26 October 2010. Volatiles were collected by drawing the air through a Tenax adsorbent trap (89 mm x 6.4 mm o.d.; 200mg of Tenax TA 35/60) using a portable pump (100 ml/min).

Collections were analysed by thermally desorbing the collection filter (Markes Unity Thermal Desorber; 200°C) followed by analysis by gas chromatography linked to mass spectrometry (Agilent HP6890 GC and HP5973 MS) using a non-polar GC column (DB5, Supelco) temperature programmed from 50°C for 2 min then at 6°C/min to 250°C with helium carrier gas (1 ml/min).

Table 3.1.1. Artificial damaged vs. pear sucker nymph damaged leaf entrainments

Date Started	Time filter in	Date ended	Time ended	NRI code	flow rate (ml/min)	EMR code	5th instar	Plant stage
06/05/2010	11:10	07/05/2010	15:00	2010-015CT-001	450	CT49	~10	leaf
06/05/2010	11:10	07/05/2010	15:00	2010-015CT-002	450	CT50	~10	leaf
06/05/2010	11:10	07/05/2010	15:00	2010-015CT-003	450	CT51	punctured with needle (leaves, green and woody stems)	leaf
06/05/2010	11:10	07/05/2010	15:00	2010-015CT-004	450	CT52	punctured with needle (leaves, green and woody stems)	leaf
10/05/2010	09:30	11/05/2010	14:30	2010-015CT-005	450	CT53	~10	leaf
10/05/2010	09:30	11/05/2010	14:30	2010-015CT-006	450	CT54	~10	leaf
10/05/2010	09:30	11/05/2010	14:30	2010-015CT-007	450	CT55	punctured with needle (leaves, green and woody stems)	leaf
10/05/2010	09:30	11/05/2010	14:30	2010-015CT-008	450	CT56	punctured with needle (leaves, green and woody stems)	leaf
20/05/2010	09:00	21/05/2010	14:30	2010-015CT-009	450	CT57	~10	leaf
20/05/2010	09:00	21/05/2010	14:30	2010-015CT-010	450	CT58	~10	leaf
20/05/2010	09:00	21/05/2010	14:30	2010-015CT-011	450	CT59	punctured with needle (leaves, green and woody stems)	leaf
20/05/2010	09:00	21/05/2010	14:30	2010-015CT-012	450	CT60	punctured with needle (leaves, green and woody stems)	leaf
25/05/2010	12:00	28/05/2010	14:00	2010-015CT-013	450	CT61	~10	leaf
25/05/2010	12:00	28/05/2010	14:00	2010-015CT-014	450	CT62	~10	leaf
25/05/2010	12:00	28/05/2010	14:00	2010-015CT-015	450	CT63	punctured with needle (leaves, green and woody stems)	leaf
25/05/2010	12:00	28/05/2010	14:00	2010-015CT-016	450	CT64	punctured with needle (leaves, green and woody stems)	leaf

Table 3.1.2. No damage vs. pear sucker nymph damaged leaf entrainments

Date Started	Time filter in	Date ended	Time ended	NRI code	flow rate (ml/min)	EMR code	5th instar	Plant stage
23/06/2010	14:00	28/06/2010	15:00	2010-015SB-001	390	SB01	~10	new shoot
23/06/2010	14:00	28/06/2010	15:00	2010-015SB-002	390	SB02	~10	new shoot
23/06/2010	14:00	28/06/2010	15:00	2010-015SB-003	390	SB03		new shoot
23/06/2010	14:00	28/06/2010	15:00	2010-015SB-004	390	SB04		new shoot
28/06/2010	14:00	01/07/2010	16:00	2010-015SB-005	390	SB05	~20	new shoot
28/06/2010	14:00	01/07/2010	16:00	2010-015SB-006	390	SB06	~20	new shoot
28/06/2010	14:00	01/07/2010	16:00	2010-015SB-007	390	SB07		new shoot
28/06/2010	14:00	01/07/2010	16:00	2010-015SB-008	390	SB08		new shoot
06/07/2010	12:30	09/07/2010	12:00	2010-015SB-009	390	SB09	~20	new shoot
06/07/2010	12:30	09/07/2010	12:00	2010-015SB-010	390	SB10	~20	terminated shoot
06/07/2010	12:30	09/07/2010	12:00	2010-015SB-011	390	SB11		terminated shoot
06/07/2010	12:30	09/07/2010	12:00	2010-015SB-012	390	SB12		terminated shoot
12/07/2010	11:00	15/07/2010	15:30	2010-015SB-013	390	SB13	~20	terminated shoot
12/07/2010	11:00	15/07/2010	15:30	2010-015SB-014	390	SB14	~20	terminated shoot
12/07/2010	11:00	15/07/2010	15:30	2010-015SB-015	390	SB15		terminated shoot
12/07/2010	11:00	15/07/2010	15:30	2010-015SB-016	390	SB16		terminated shoot

Gas chromatography coupled to electroantennographic recording (GC-EAG)

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. The end was removed from one antenna and inserted into the recording electrode. In other approaches both antennae were inserted into the recording electrode or a single, excised antenna was used. Whole body preparations were also used: the wings and legs were removed from the insect and the entire body was inserted into the saline filled glass electrode, the end was then removed from one antenna and inserted into the recording electrode. In all cases, insects were held in sample tubes on ice before preparation. Commercially available *Orius laevigatus* were used to develop techniques as well as *Anthocoris nemoralis* collected from pear orchards in June and July.

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.

Two methods of linking the GC to the EAG were evaluated. In one the column effluent was split (1:1) with equal lengths of deactivated fused silica capillary leading to the flame ionisation detector and to a glass T-piece in the column oven. At intervals (17 sec) the contents of the T-piece were blown out (3 sec) over the insect preparation with air (300 ml/min) as described by Cork et al., 1990.

Alternatively the column effluent was split (1:1) as above except that the deactivated fused silica capillary leading to the EAG preparation was passed out of the GC oven, through a heated jacket (250°C) and into a continuous stream of humidified air (1000 ml/min) passing through a glass tube (6 mm i.d.) and over the EAG preparation (c.f. Struble and Arn, 1984).

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

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

Plant volatile collections from potted pear trees

Volatiles were collected in the laboratory from potted pear trees which either had pear sucker nymphs on them or the leaves were punctured with a dissecting needle. In analyses of the collections by GC-MS, only (*Z*)-3-hexenyl acetate and methyl salicylate were detected in significant amounts. 2-Phenylethanol was not detected, in contrast to results in 2009 where this compound seemed to be associated with feeding of the nymphs.

Furthermore, there were no clear qualitative differences between collections from trees with nymphs and collections from artificially wounded trees in terms of presence and absence of compounds, as shown in Table 3.1.3. Inspection of the data indicated that there seemed to be larger amounts of (*Z*)-3-hexenyl acetate in collections from the artificially wounded plants, and this was supported by the simple means. Representative GC-MS traces are shown in Figure 3.1.1.

Table 3.1.3. GC-MS analyses of volatiles collected from potted pear trees in the laboratory with either pear sucker nymphs or artificial punctures (TIC is Total Ion Current from GC-MS analysis; Z3HexAc is (Z)-3-hexenyl acetate; Z3HexOl is (Z)-3-hexenol; MeSal is methyl salicylate).

			GC-MS TIC		
NRI code	**Treatment**	**Time (hr)**	**Z3HexAc**	**Z3HexOl**	**MeSal**
2010-015CT-001	10 nymphs	28.0	0		2,945
2010-015CT-002	10 nymphs	28.0	12,039		82,076
2010-015CT-003	puncture	28.0	23,529		769
2010-015CT-004	puncture	28.0	426,928	40,294	58,791
2010-015CT-005	10 nymphs	29.0	0		0
2010-015CT-006	10 nymphs	29.0	0		13,313
2010-015CT-007	puncture	29.0	0		0
2010-015CT-008	puncture	29.0	55,532	5,591	5,628
2010-015CT-009	10 nymphs	29.5	95,659		560
2010-015CT-010	10 nymphs	29.5	21,807	2,950	10,454
2010-015CT-011	puncture	29.5	8,469	1,433	1,907
2010-015CT-012	puncture	29.5	112,602	13,995	32,374
2010-015CT-013	10 nymphs	74.0	919		1,222
2010-015CT-014	10 nymphs	74.0	6,480		48,773
2010-015CT-015	puncture	74.0	10,360		552
2010-015CT-016	puncture	74.0	42,980		6,341
mean with nymphs			15,212	1,475	17,705
mean with puncture			85,050	15,328	13,295

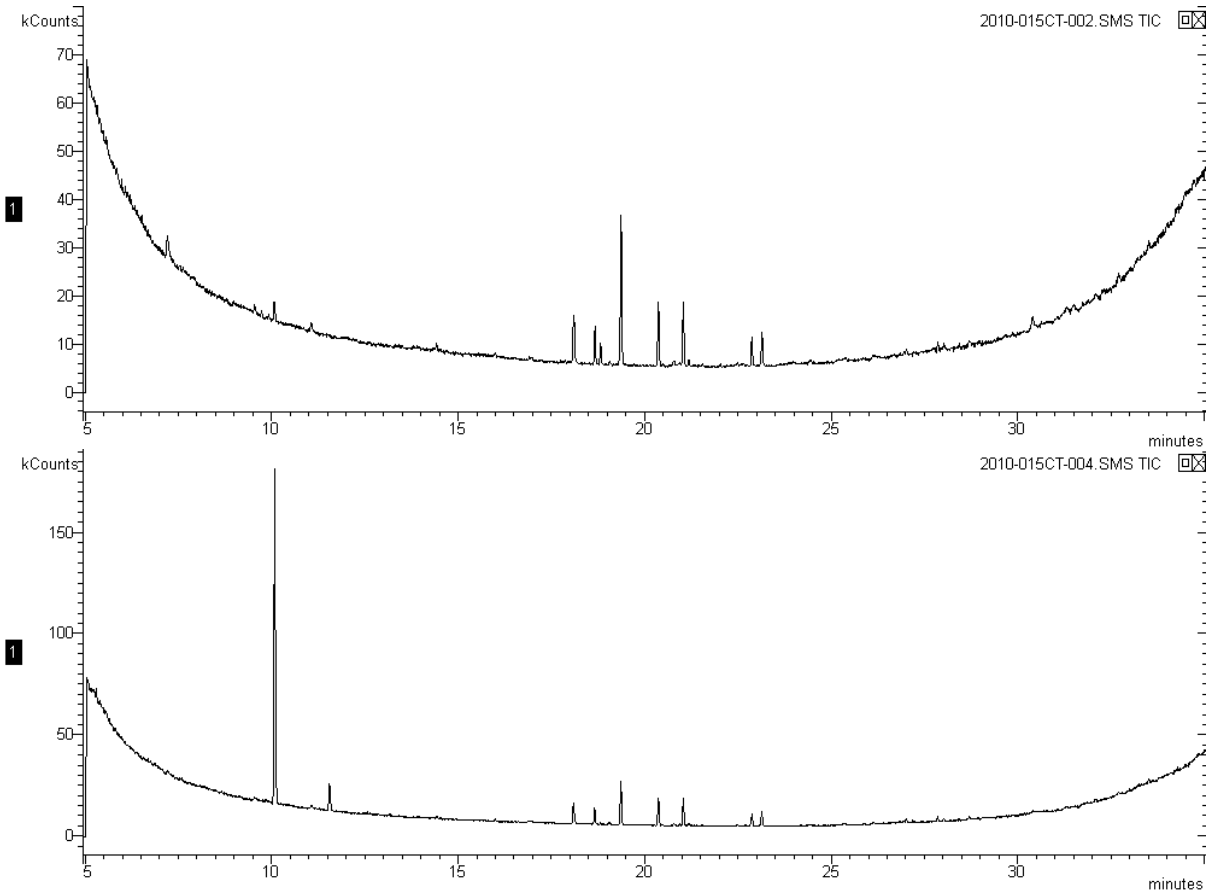


Figure 3.1.1. Representative GC-MS analyses of volatiles from pear trees with pear sucker nymphs (upper) and artificially punctured leaves (lower) ((*Z*)-3-hexenyl acetate at 10.07 min, (*Z*)-3-hexenol at 11.55 min, methyl salicylate at 19.37 min; 2-phenylethanol would be at 21.93 min; peaks at 18.10, 18.70, 20.37, 21.04, 22.88 and 23.15 min are impurities from Porapak).

Volatiles were also collected from potted pear trees under field conditions. These were either clean or artificially infested with 5th instar pear sucker nymphs. Analyses of the collections by GC-MS showed at least eight significant compounds (Table 3.1.4).

(*Z*)-3-Hexenyl acetate and methyl salicylate were detected in all the samples as above. With the other compounds there were no consistencies in terms of presence or absence associated with presence or absence of pear sucker nymphs and even simple mean amounts showed no clear differences (Table 3.1.4).

Representative GC-MS chromatograms are shown in Figure 3.1.2.

Table 3.1.3. GC-MS analyses of volatiles collected from potted pear trees in the field with and without pear sucker nymphs (TIC is Total Ion Current from GC-MS analysis; Z3HexAc is (*Z*)-3-hexenyl acetate; lin oxide is linalool oxide; caryoph is caryophyllene; germD is germacrene-D; MeSal is methyl salicylate; sample 1 was too heavily contaminated for analysis).

Sample No.	No. nymphs	Time (hr)	GC-MS TIC								
			ocimene	Z3HexAc	lin oxide	copaene	linalool	caryoph	germD	MeSal	eugenol
1	10	121									
2	10	121		138,632	19,542	93,037				111,859	
3		121		57,183	16,046					252,103	20,999
4		121		389,529	50,100					1,010,000	
5	20	74	83,292	86,977	46,311	72,389	43,992	88,980	60,573	546,702	
6	20	74		552,920	13,175	49,667				622,543	
7		74	41,396	51,954	27,482		59,906	22,332		187,295	19,354
8		74	76,960	64,309	23,558	18,538	65,151	33,423	28,306	306,605	31,636
9	20	71.5		83,039		64,440				43,976	
10	20	71.5	25,181	286,503		41,092			27,766	425,065	25,451
11		71.5		28,266	33,459	24,602				128,067	
12		71.5	140,324	294,932	50,052	134,334	73,499	73,499	127,170	526,176	181,992
13	20	76.5	25,231	162,807						224,129	
14	20	76.5		77,945						254,444	
15		76.5	13,946	54,230	19,207	48,799				96,391	55,024
16		76.5		74,806	16,291	44,945				64,422	21,891
mean + nymphs			44,568	198,403	26,343	19,542	43,992	88,980	44,170	318,388	25,451
mean - nymphs			68,157	126,901	29,524	54,244	66,185	43,085	77,738	321,382	55,149

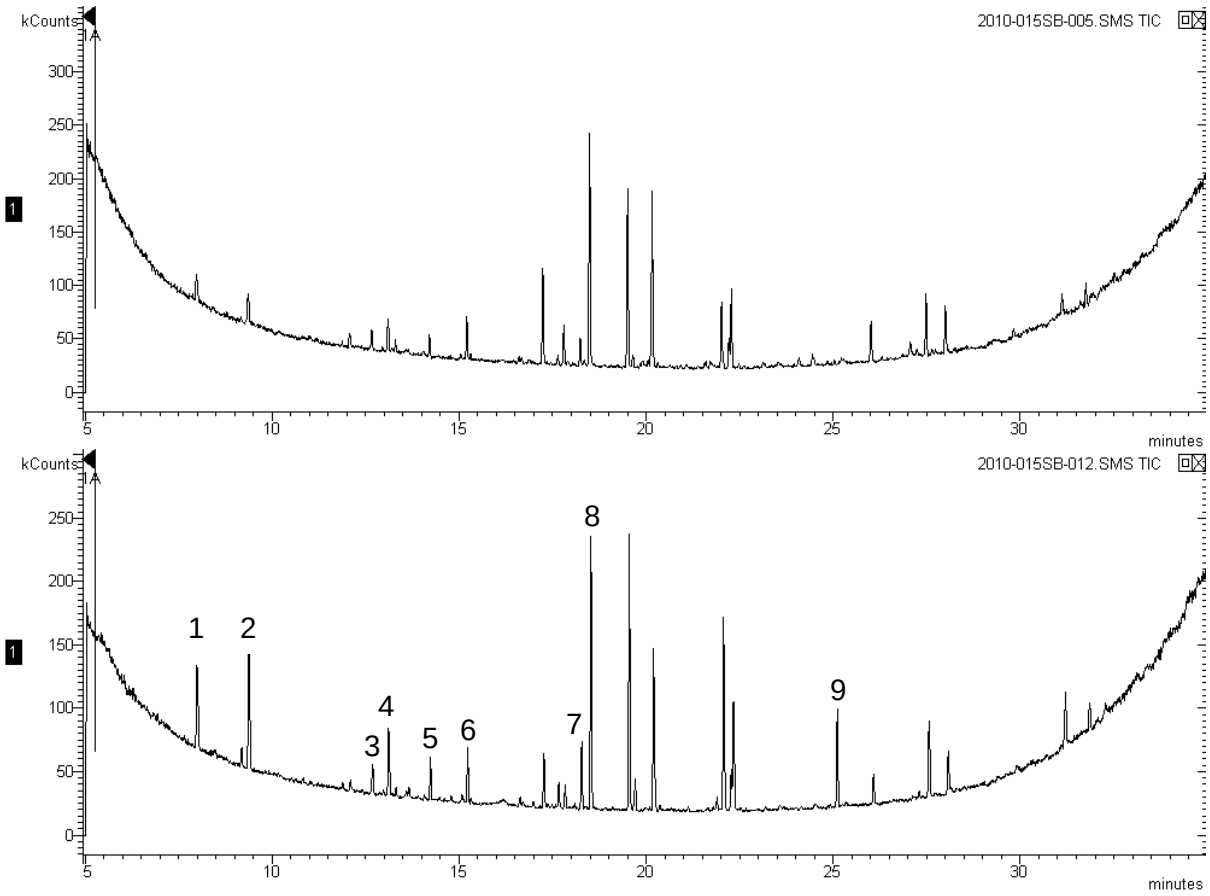


Figure 3.1.2. Representative GC-MS analyses of volatiles from pear trees with (upper) and without (lower) pear sucker nymphs (1 ocimene; 2 (*Z*)-3-hexenyl acetate; 3 linalool oxide; 4 copaene; 5 linalool; 6 caryophyllene; 7 germacrene-D; 8 methyl salicylate; 9 eugenol; other peaks are impurities from Porapak; 2-phenylethanol would be at 20.87 min).

Volatile collections in pear orchards

The only compound detected in analyses of volatiles from a pear orchard was methyl salicylate. In the collection made on 6 August 2010, when it was dry and there were hardly any insects, 0.463 µg/l of methyl salicylate was detected in a 15-hour collection. On 26 October 2010, when it was wet and the orchard was heavily infested with pear sucker, 0.093 µg/l of methyl salicylate was detected in a 24-hour collection.

These results suggest that, even using the most sensitive equipment, it is difficult to collect and analyse the volatiles present in a pear orchard. The fact that less methyl salicylate was detected in the later collection, even though the trees were more heavily damaged, may have been due to the lower temperature.

EAG Studies

Coupled GC-EAG analyses were carried out with 6 female and 11 male *O. laevigatus* EAG preparations and 10 female and 9 male *A. nemoralis* preparations.

Great difficulty was experienced in obtaining stable EAG preparations. Some of the best results are shown below using the method of Cork et al. (1990) to couple the GC to the EAG and an insect head with recording from a single antenna. In Fig. 3.1.3 there were possible responses to methyl salicylate and 2-phenylethanol (10 ng injected), but these were not apparent in Figs. 3.1.4 and 3.1.5.

These studies will be repeated with improved EAG equipment.

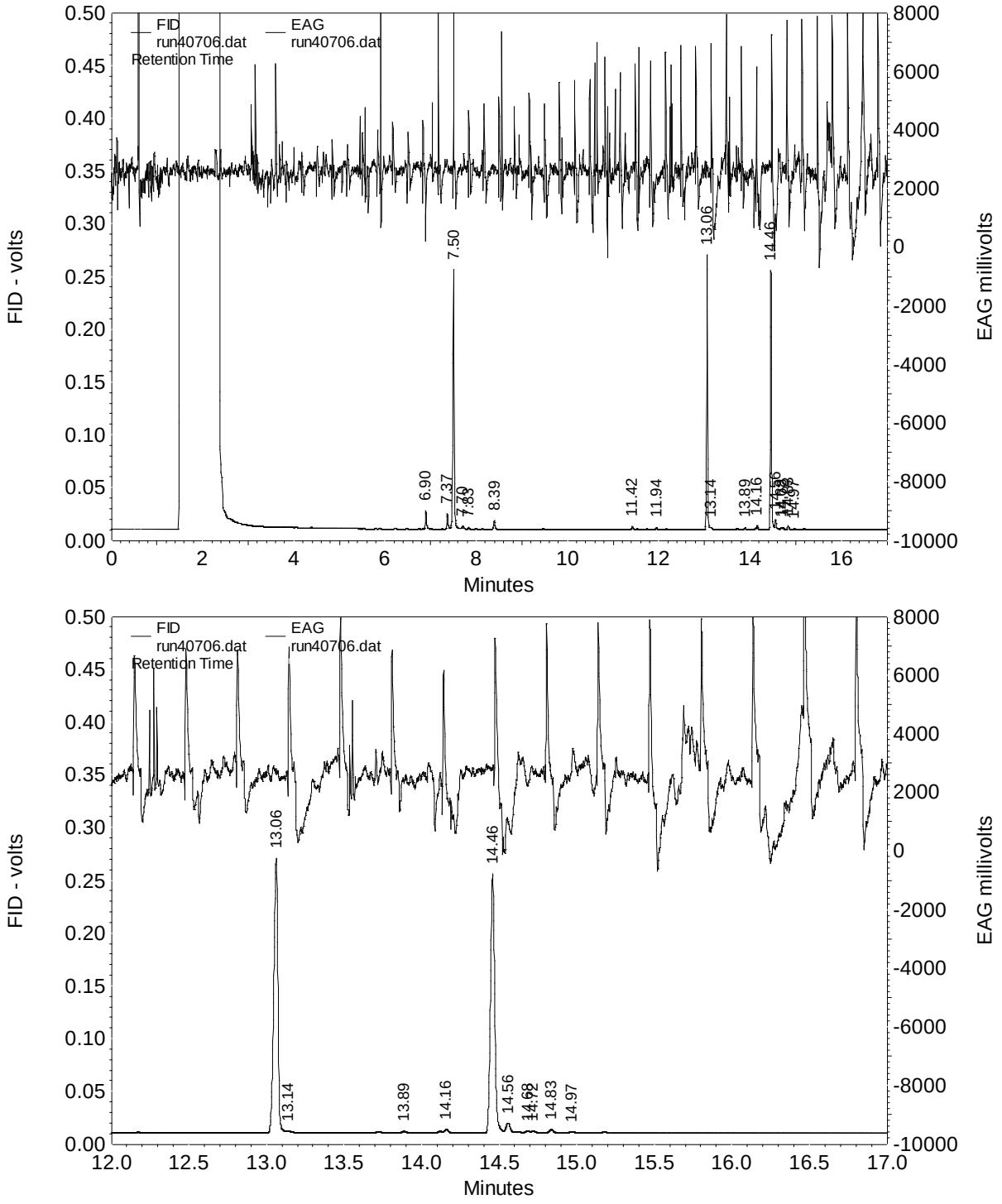


Figure 3.1.3. Male *Orius*, EAG without continuous by-pass, (Z)-3-hexenyl acetate at 7.50 min, methyl salicylate at 13.06 min, 2-phenylethanol at 14.46 min. Lower is expansion of upper showing possible responses to methyl salicylate and phenylethanol.

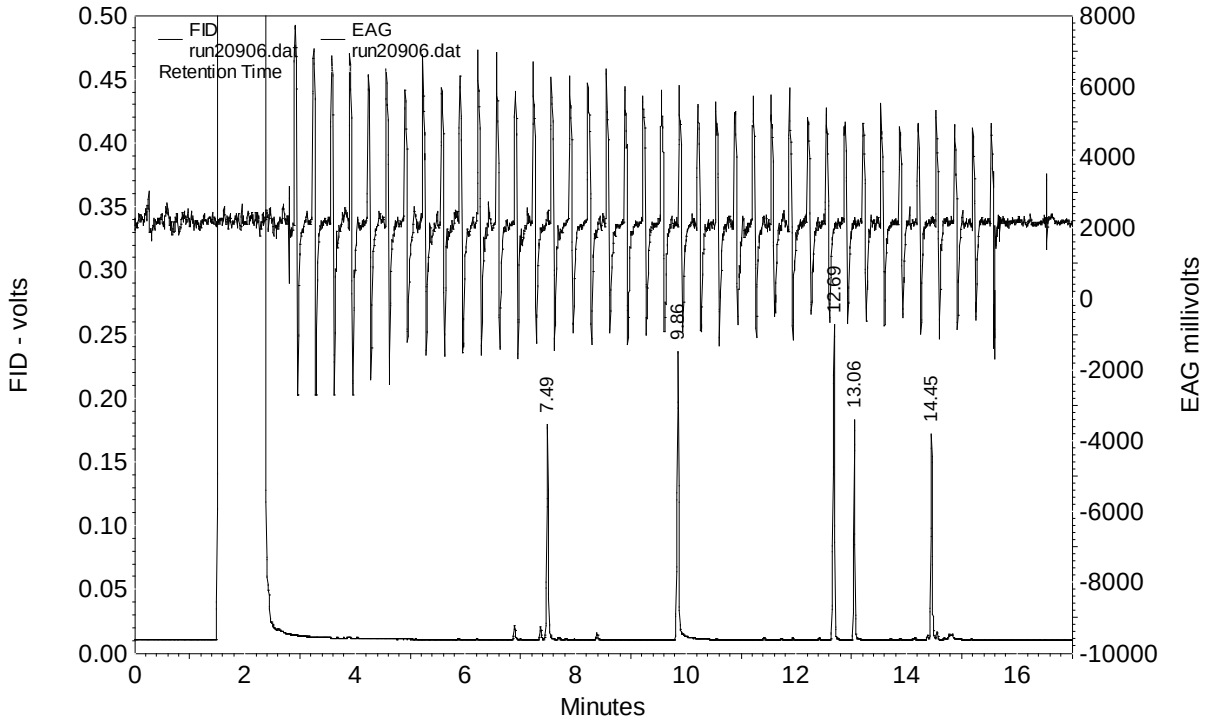


Fig. 3.1.4. Male *Orius*, EAG without continuous by-pass, (Z)-3-hexenyl acetate at 7.49 min, decanal at 9.86 min, (*E,E*)-α-farnesene at 12.69 min, methyl salicylate at 13.06 min, 2-phenylethanol at 14.45 min.

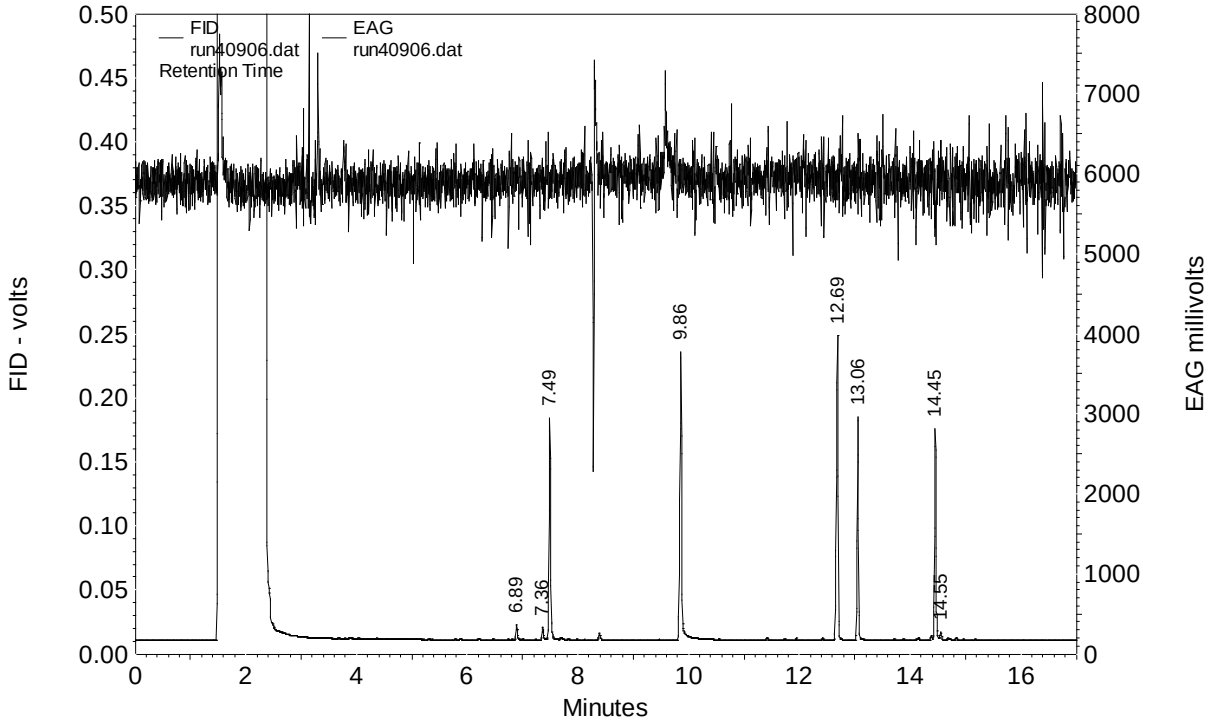


Figure 3.1.5. Male *Orius*, EAG with continuous by-pass, (Z)-3-hexenyl acetate at 7.49 min, decanal at 9.86 min, (*E,E*)-α-farnesene at 12.69 min, methyl salicylate at 13.06 min, 2-phenylethanol at 14.45 min.

Summary and Conclusions

Volatiles were collected in the laboratory during May from potted pear trees which either were artificially infested with pear sucker nymphs or had leaves artificially wounded with a dissecting needle. Analysis of the volatiles by GC-MS failed to confirm previous results which suggested 2-phenylethanol was associated with feeding of the nymphs. (*Z*)-3-Hexenyl acetate and methyl salicylate were the only significant compounds detected, with the former present in relatively greater amounts in volatiles from the artificially-wounded trees.

Volatiles were also collected in the field during June-July from potted plants with and without pear sucker nymphs. Analyses of these by GC-MS showed no obvious differences between collections from trees with nymphs and those without. As above, (*Z*)-3-hexenyl acetate and methyl salicylate were observed in all collections. Seven other compounds were also present in significant amounts.

Volatiles were collected by air sampling in a pear orchard on two occasions. Methyl salicylate was the only compound identified at the limits of detection using the most sensitive thermal desorption technique.

EAG studies were carried out using commercially-available *Orius laevigatus* as a model anthocorid and *Anthocoris nemoralis* collected from the field. Different approaches to linking the GC to the EAG and methods for making the EAG preparations were evaluated. Attempts were made to record EAG responses from both synthetic compounds and collections of volatiles from pear trees infested with psyllids. Great difficulty was experienced in obtaining stable EAG preparations. Possible responses to methyl salicylate and 2-phenylethanol were recorded, but these studies need repeating with improved EAG equipment.

Studies to date have demonstrated methyl salicylate and (*Z*)-3-hexenyl acetate as the only compounds produced consistently in significant amounts by pear trees infested with pear sucker. While the latter is a general “green leaf volatile”, the former is a well-known compound produced by stressed plants. Furthermore, there are numerous reports where it has been used to attract natural enemies into crops (e.g. Simpson et al., 2010; James, 2005) and it is now commercially available for this use (“Predalure”).

References

Cork, A., Beevor, P.S., Gough, A.J.E. and Hall, D.R. 1990. Gas chromatography linked to electroantennography: a versatile technique for identifying insect semiochemicals. in "Chromatography and Isolation of Insect Hormones and Pheromones". eds. A.R. McCaffery and I.D.

Wilson. Plenum press, New York and London. pp. 271-279.

James, D.G. 2005. Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *Journal of Chemical Ecology*, 31:481-495.

Simpson, M., Gurr, G., Simmons, A.T., Wratten, S.D., James, D.G., Leeson, G. and Nicol, H.I. 2010. Insect attraction to synthetic herbivore-induced plant volatile-treated crops. *Agricultural and Forest Entomology*, doi: 10.1111/j.1461-9563.2010.00496.xx

Struble, D.L. and Arn, H. 1984. Combined gas chromatography and electroantennogram recording of insect olfactory responses. in "Techniques in Pheromone Research", eds. H.E. Hummel and T.A. Miller. Springer-Verlag, New York, pp. 161-178.

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.

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 hung in white delta traps with sticky bases which were placed in the pear orchard on 18 May 2010. The three treatments, methyl salicylate, phenyl ethanol or the 'no-lure' control, were

arranged in a randomised block design with five replicates of each treatment. Lures were replaced on 24 June.

Assessments

Traps were checked and sticky bases changed on 2 June, 24 June, 13 July and 18 August 2010 and predators were identified and counted. Tap samples were done on the tree on which the traps were hung. Five areas were tapped with each tapping area having five beats. An initial tap sample was done on 18 May before the traps were set up and further tap samples were done on 6 June, 24 June and 13 July. The numbers of pests and beneficials were recorded. Pre-treatment leaf samples were taken immediately prior to experimental set-up. For the pre-treatment assessment 20 leaves from the tree on which the trap was to be hung were sampled into a plastic bag and in the laboratory the numbers of psyllid eggs and nymphal instars were counted under a stereomicroscope and recorded in three categories: 1st and 2nd combined, 3rd, 4th and 5th combined. Leaves were held at 4°C until assessment. Further leaf samples were taken on 6 June, 24 June and 19 July. 25 leaves per tree were assessed.

Statistical analysis

The results were analysed using ANOVA (actual number and log 10 (n+1) transformation) where possible.

Results

The psyllids and beneficial insects caught on the sticky bases can be seen in Table 3.2.1.3. The numbers caught were too low for statistical analysis in the majority of cases. There was no statistical difference between the treatments on the numbers of psyllid adults on either 2nd or 24th June. There was a significant increase in the numbers of hoverflies caught in the traps with both methyl salicylate and phenyl ethanol lures on the 18th August, where the log transformed values were 0.626 for the control, 1.298 for methyl salicylate and 1.201 for phenyl ethanol (p=0.007, sed=0.1633, lsd=0.3766, 8 d.f.). Although it was not possible to analyse the data where there were few catches, it is interesting to note that higher numbers of parasitoids were caught in the methyl salicylate and phenyl ethanol treatments. There were also higher numbers of silver Y moths *Autographa gamma* (Lepidoptera: Noctuidae) caught in the traps with methyl salicylate lures.

Table 3.2.1.3. Total numbers of psyllids and beneficials caught in delta traps with methyl salicylate (m) or phenyl ethanol (p) lures or no lure (c), 5 traps per treatment.

	02-Jun			24-Jun			13-Jul			18-Aug		
	M	**P**	**C**	**M**	**P**	**C**	**M**	**P**	**C**	**M**	**P**	**C**
psyllid a	30	10	12	32	30	38	6	3	9	1	1	1
psyllid n	0	0	0	5	3	2	0	0	0	0	0	0
anthocorid a	0	11	4	1	2	7	0	2	2	0	7	0
anthocorid n	0	1	1	0	0	0	0	0	0	1	0	0
parasitoid	1	0	0	4	4	1	0	0	2	2	3	2
spiders	1	2	0	1	1	1	0	2	0	1	0	1
lacewing	1	1	0	0	0	0	0	0	2	2	0	0
hoverfly	1	1	0	4	1	2	7	9	1	100	75	25
coccinelid a	1	2	0	0	1	1	1	0	0	1	0	0
coccinelid lv	0	0	0	0	3	0	1	2	0	0	0	1
silver y moths	0	0	0	9	1	0	2	0	0	0	0	0
other moths	0	0	0	0	1	0	0	0	0	3	1	0

a = adult, n = nymph, lv = larva

The numbers of psyllids and beneficial insects caught in tap samples are shown in Table 3.2.1.4. For beneficials numbers were too low to be analysed statistically. There was no statistical difference in the numbers of psyllid adults between treatments.

Table 3.2.1.4. Total numbers of psyllids and beneficials caught in tap samples from pear trees containing delta traps with methyl salicylate (m) or phenyl ethanol (p) lures or no lure (c), 5 traps per treatment.

	06-Jun			24-Jun			13-Jul		
	M	**P**	**C**	**M**	**P**	**C**	**M**	**P**	**C**
psyllid a	31	51	41	8	21	15	22	16	17
anthocorid a	0	1	0	9	6	4	7	4	4
anthocorid n	0	0	0	0	0	0	8	9	1
parasitoid	0	0	0	0	0	0	1	2	0
spider	5	1	2	3	4	1	1	3	3
coccinelid a	3	4	1	2	0	1	2	0	0
coccinelid lv	1	2	0	1	2	2	1	3	0

a = adult, n = nymph, lv = larva

There was no effect of treatment on the numbers of psyllid nymphs or eggs in the leaf samples on any of the dates (Table 3.2.1.5.). The analysis was done on the log 10 n+1 numbers, but actual numbers

are presented in the table.

Table 3.2.1.5. The mean number of pear psyllid eggs and nymphs, counted in leaf samples (25 leaves), taken from pear trees containing delta traps with methyl salicylate (m) or phenyl ethanol (p) lures or no lure (c).

Date	Pear psyllid stage		M	P	C
06-Jun-10	Nymphs	1st and 2nd	46.6	53.6	46.6
		3rd	1.4	1.6	0.8
		4th and 5th	2	1.2	3.4
	Eggs		359	278	272
24-Jun-10	Nymphs	1st and 2nd	32	18	18.2
		3rd	4.6	5.0	5.0
		4th and 5th	4.8	1.8	1.6
	Eggs		181	200	190
17-Jul-10	Nymphs	1st and 2nd	0	0	0.2
		3rd	0	0	0
		4th and 5th	0.2	0	0
	Eggs		8.8	9.4	5.2

Discussion

As in previous experiments there was a significant increase in the numbers of hoverflies caught in the traps with the methyl salicylate and phenyl ethanol lures in August. This catch was after the major sampling had taken place for this experiment, which finished in July, and the sticky bases were simply changed to see if there was an extended effect of the phenyl ethanol lures, which were already out. At this time the methyl salicylate lures should not have been effective. At a release rate of 17 mg/day, the methyl salicylate lures would be most effective for approximately 2 weeks i.e. between 18 May to 8 June and 24 June to 9 July. Therefore for the sampling dates of 24 June and 18 August it would not be expected that any effect due to the methyl salicylate lure would be seen. The phenyl ethanol sachets with a release of 1.8 mg/day would be effective for 20 weeks, i.e. for the duration of the experiment (although they were also changed on 24 June). However, as the trap catches were still significantly different to the control, the release rate of methyl salicylate may have been lower in the field. Release rate experiments in the laboratory were done at 22°C and previous work (NRI)

suggested a doubling of the release rate for a 6°C rise in temperature. Temperatures in the field at the East Malling Research Met Station show that between the 18 May and 23 June the mean daily temperature was 14.7, 7°C lower than the laboratory standard temperature. This would suggest that the lures would last for twice as last long. Lures were loaded with 250 µl of volatile (which is approximately 200 mg). If the average release was lower at 5 mg per day then the lures would last approximately 40 days.

There were also higher catches of silver Y moths caught in the traps with methyl salicylate lures, especially on the outer edge of the experiment.

There was no effect of the lures on the numbers of anthocorids and thus on pear psyllid populations. In this experiment the psyllids were monitored to determine if the lures were attracting beneficials which were not caught in the traps but which may have had an effect on pest populations.

In this experiment the lures were set out as point sources. An alternative strategy may be to set up higher numbers of lures in large 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.

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)

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

Anthocorids for bioassays have been sourced and ordered from Syngenta Bioline. These will be used to complement the experiments conducted on *Orius laevigatus* in year 2, using the direct application bioassay. Although there has been an anthocorid supply problem it is envisaged that all bioassays will be completed ahead of the field experiment.

Task 4.3. Evaluate best treatment (from tasks 4.1 and 4.2) in the field (EMR, Yr 4)

Introduction

Many UK pear growers apply a programme of 6 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. However, the ways in which the materials act is unclear. If they act by direct toxicity, 1st and 2nd instar nymphs are most likely to be affected, as these are generally most susceptible. However, the compounds may act by deterring oviposition, or they may adversely affect foliage quality and the suitability of the host plant for pear sucker. No research has been done to optimise dose rates or the degree of cover required for optimum control, or any possible adverse effects on anthocorids with these materials.

Insecticidal activity of sulphur, magnesium sulphate, non-ionic wetter and mixtures have been studied in lab bioassays in years 1 and 2. This has found that even at eight times the rates commonly used 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 by the sulphur treatment. Activator 90 at two to eight times the standard field rate significantly reduced psyllid numbers. Laboratory bioassays were also conducted to look at the effects on a predator. *Orius laevigatus* nymphs were exposed to different concentrations of non-ionic wetter, micronised sulphur and magnesium sulphate either in isolation or combination. There was a low mortality of nymphs (≤15%) with wetter alone, or in combination with sulphur and magnesium sulphate up to four times field rate.

Although this objective is not due to be completed until year 4, a preliminary field experiment was conducted in year 3. The aims of this experiment were to evaluate spray treatments for control of pear sucker identifying the relative efficacy of the different treatments for control of pear sucker eggs and nymphs and possible effects on natural enemies. Treatments tested were a foliar spray of sulphur, wetter or Agri 50E in comparison with wetter only and untreated controls. Agri 50E has been shown to give control of the nymphal stages of other sucking pests (Cuthbertson *et a*l., 2009). As the higher rates of Activator 90 and sulphur were shown to be effective in laboratory studies in years 1 and 2, the standard field rate of sulphur (3 l/ha) was compared with the maximum field rate (11 l/ha). The standard concentration of Activator 90 (0.1% solution) was used, as well as a high rate, which was a 0.4% solution when applied in 500 l/ha; however if the crop was sprayed at 2000 l/ha the same amount of product could be applied as a 0.1% solution. These treatments were used singly and in combination.

Methods

Dates and duration of study

5-25 August 2010

Site

The study was done in a Conference pear orchard (the Bank) at Marsh Gate Farm, Cooling, Kent, which 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 3.6 x 3.7 m, tree density = 751 trees/ha.

Treatments

Six treatments were included as shown in Tables 4.3.1. and 4.3.2.

Table 4.3.1. Treatments

Trt	Colour code	Product	Product Dose /ha
1	Yellow	Sulphur SC	11.0 l
2	Green	Activator 90	2 l
3	Yellow and Green	Sulphur + Activator 90	11.0 l + 2 l
4	Grey	Sulphur + Activator 90	3.0 l + 500 ml
5	Red	Agri-50E	1.5 l
6	Blue	Water	-
7	White	Untreated	-

Table 4.3.2. Products and their formulation details

Product	Parent Company	a.i. & formulation	Product dose rate/ha	Approval status on pear#
United Phosphorus Sulphur SC	Headland	sulphur 800 g/l SC	Maximum of 11 litres	Approved
Activator 90	De Sangosse	alkylphenyl hydroxypolyoxyethylene 750 g/l + natural fatty acids 150 g/l	0.1 % conc	Approved
Agri 50E	Fargro Ltd	seaweed gum (propylene glycol alginate)	3ml/l water volume 1000 l/ha	Exempt from registration

Timing of sprays

The treatments were applied when pear sucker populations were present in the crop. As this was an experiment for scientific purposes to complement laboratory bioassays, rather than to achieve adequate control in the field throughout the season, the first treatment applications were made when nymphs were already present, and allowed an appropriate harvest interval. Sprays were applied on 5 and 12 August 2010.

Spray application

Two sprays were applied at a volume of 500 l/ha with a Birchmeier motorised air-assisted knapsack sprayer with a red micron restrictor. Each tree was sprayed to deliver a volume of 660 ml of spray solution. The amounts of sprayate remaining were measured to determine the accuracy of spray applications (Table 4.3.3.).

Table 4.3.3. Accuracy of spray applications (%)

Treatment	5/08/10	12/08/10
1	93	93
2	96	98
3	94	101
4	97	92
5	94	96
6	75	96

Experimental design and layout

A randomised complete block experimental design with four replicate plots of each treatment was used (see table 4.3.4). Each plot consisted of two pear trees plus one guard tree at either side in the row. Plots in each block were arranged end to end in the row. Guard rows between adjacent rows of plots were included to minimise interplot contamination by spray drift.

Table 4.3.4. The experimental design of the pear sucker spray trial

Plot No.	Colour code	Trt	**Plot No.**	Colour code	Trt	**Plot No.**	Colour code	Trt	**Plot No.**	Colour code	Trt
101	White	-	**201**	Green	A90	**301**	Grey	S+A90 low	**401**	Yellow	S
102	Red	Agri	**202**	Blue	Water	**302**	Ye Gr	S+A90 high	**402**	Blue	Water
103	Green	A90	**203**	Yellow	S	**303**	Yellow	S	**403**	Ye Gr	S+A90 high
104	Grey	S+A90 low	**204**	Grey	S+A90 low	**304**	White	-	**404**	Red	Agri
105	Blue	Water	**205**	Ye Gr	S+A90 high	**305**	Green	A90	**405**	Grey	S+A90 low
106	Ye Gr	S+A90 high	**206**	Red	Agri	**306**	Blue	Water	**406**	White	-
107	Yellow	S	**207**	White	-	**307**	Red	Agri	**407**	Green	A90

Maintenance sprays

A full maintenance programme of fungicide (and PGR programme) was applied as for the rest of the orchard. No other insecticides were sprayed.

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 and approximately 5-7 days after each spray treatment.

Pear sucker: Assessments concentrated on determining the effects of treatments on eggs and nymphs. Counts of pear sucker eggs (all ages counted together), and nymphs of each life stage were

made on a randomly selected sample of 20 leaves from each plot for the pre-treatment and the first post-treatment assessment. Ten leaves were sampled per plot for the second post-treatment assessment.

Natural enemies: Anthocorids and other predators were assessed by tap sampling the trees, with five taps per tree (two trees per plot were sampled). Numbers of adult psyllids were also recorded.

Statistical analysis

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

Results

There were no significant differences between plots at the pre-treatment assessment (Table 4.3.5.). At the first assessment, seven days after the first spray and prior to the second spray, none of the products showed a significant difference between the control (Table 4.3.6.). The water spray alone was shown to significantly reduce the number of fourth and fifth instar nymphs. However, as this amount of water was also applied with the other treatments it is unclear why this happened. Data were analysed for this date both with and without the pre-treatment data as a covariate. However, results for both analyses were similar and so the counts of the 18 August were analysed without using a covariate. On the 18 August, after two sprays, significant differences were seen between treatments (Table 4.3.7.). The numbers of first and second instar nymphs were reduced in the sulphur plus wetter combined treatments (both low and high rates), sulphur alone and Agri 50E. Third instar nymphs were significantly reduced in the sulphur plus wetter combined treatments (both low and high rates), sulphur alone and wetter alone. There was an indication of a reduction in the Agri 50E treatment, but this was not significant. There were no significant reductions for the fourth and fifth instar nymphs in any of the treatments or for the eggs.

Table 4.3.5. Log values for the pre-treatment assessment on the 5 August 2010

Stage	Control	Water	Sulphur and wetter low rate	Sulphur and wetter high rate	Sulphur high rate	Wetter high rate	Agri-50E	p (18 df)	s.e.d.	l.s.d. (p=0.05)
Eggs	0.632	0.639	0.575	0.615	0.620	0.746	0.611	0.551	0.082	0.172
1&2	0.290	0.246	0.308	0.257	0.266	0.324	0.227	0.319	0.044	0.092
3	0.0750	0.0750	0.0662	0.0549	0.0458	0.0768	0.0749	0.772	0.024	0.049
4&5	0.0552	0.0195	0.0574	0.0323	0.0511	0.0549	0.0263	0.480	0.023	0.048

Table 4.3.6. Log values for the first post-treatment assessment on 12 August 2010. Figures in bold are significantly different to the control.

Stage	Control	Water	Sulphur and wetter low rate	Sulphur and wetter high rate	Sulphur high rate	Wetter high rate	Agri-50E	p (18 df)	sed	lsd (p=0.05)
Eggs	0.869	1.046	0.798	0.791	0.933	0.815	0.956	0.104	0.0934	0.1963
1&2	0.331	0.471	0.263	0.182	0.308	0.310	0.387	0.097	0.0878	0.1845
3	0.1634	0.1759	0.1449	0.1191	0.1274	0.1601	0.1711	0.458	0.0310	0.0652
4&5	0.1705	**0.0816**	0.1452	0.1247	0.1060	0.1608	0.2069	0.036	0.0349	0.0732

Table 4.3.7. Log values for the second post-treatment assessment on 18 August 2010. Figures in bold are significantly different to the control.

Stage	Control	Water	Sulphur and wetter low rate	Sulphur and wetter high rate	Sulphur high rate	Wetter high rate	Agri-50E	p (18 df)	sed	lsd (p=0.05)
Eggs	1.037	1.168	1.034	0.899	1.245	1.132	1.081	0.071	0.1016	0.2136
1&2	0.454	0.416	**0.148**	**0.115**	**0.194**	0.314	**0.218**	0.014	0.0968	0.2034
3	0.213	0.228	**0.065**	**0.023**	**0.045**	**0.077**	0.130	0.014	0.06	0.126
4&5	0.205	0.187	0.138	0.072	0.135	0.166	0.095	0.18	0.0521	0.1094

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, soldier beetles, lacewings and ants. These were analysed when there was sufficient data, i.e. individuals were found in more than four plots, using ANOVA with and without log (N+1) transformation. There was no significant difference in the numbers of pear sucker adults (Table 4.3.8.), anthocorid adults or parasitoids on 5 August before treatments were applied. On 12 August there was also no difference between the treatments in the numbers of pear sucker adults (Table 4.3.8.), anthocorid adults or nymphs, ladybird adults or spiders present. On 18 August there was no reduction of the numbers of anthocorid nymphs, ladybird adults or spiders between any of the treatments compared to the control. The numbers of pear sucker adults were not analysed at this date as the numbers were estimated, with approximately 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 efficacy in small plot experiments.

Table 4.3.8. The mean numbers of pear sucker adults per plot

Date		Control	Water	Sulphur and wetter low rate	Sulphur and wetter high rate	Sulphur high rate	Wetter high rate	Agri-50E
5 Aug	Pre-treat	98	91	80	91	99	108	119
12 Aug	7D post-treat	121	122	106	173	152	141	138

Table 4.3.9. The mean numbers of beneficials per plot

Date		Control	Water	Sulphur and wetter low rate	Sulphur and wetter high rate	Sulphur high rate	Wetter high rate	Agri-50E
5 Aug	Anthocorid adults	1	1.5	1	0.75	0	0.75	0
	Parasitoids	0.75	0	0.25	0	0.5	0.5	0.25
12 Aug	Anthocorid adults	0.75	1	1	0.5	0.5	0	0.75
	Anthocorid nymphs	1	0.75	1.25	0.25	0.25	0.75	0.5
	Ladybirds	0.75	0.75	0.5	0.75	0.5	0.5	0.75
	Spiders	0	0.5	0.75	0	0	0	0.25
18 Aug	Anthocorid adults	1	0.75	2	0.75	1.75	0.75	0
	Anthocorid nymphs	1	0.75	0.75	0.5	1	1.25	0.5
	Ladybirds	0.75	0.25	2	0.25	1.25	0.5	0.5
	Spiders	1.25	0.25	0.25	0	0.25	0.5	0.75

Discussion

These results showed that by the second assessment date, after two sprays had been applied, all products significantly reduced either first and second instar or third instar nymphs when compared to the untreated control. The water alone treatment was only effective on fourth and fifth instar nymphs on the first assessment. The maximum rate of sulphur left a visible deposit when applied and this should be taken into consideration when advising on spray rates. A further field experiment should be

conducted to look at further combinations of sulphur and Activator 90, and to further explore the effects of Agri 50E with repeated sprays early in the season when populations are low.

References

Leaf dipping as an environmental screening measure to test chemical efficacy against *Bemisia tabaci* on poinsettia plants, A. G. S. Cuthbertson; L. F. Blackburn; P. Northing; W. Luo; R. J. C. Cannon; K. F. A. Walters, Int. J. Environ. Sci. Tech., 6 (3), 347-352, Summer 2009, ISSN: 1735-1472

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.

A large scale experiment using replicated plots was conducted to evaluate the efficacy of dormant period sprays of kaolin (Surround) for control of pear sucker adults. Large plots were needed for this work because of the dispersive nature of pear sucker adults.

Methods and Materials

Yearly experiments were conducted in pear sucker infested orchards (Table 4.4.1). Different numbers of sprays were applied each year (Table 4.4.1). Orchards were divided into equal large plots. Two plots were sprayed (one in each half, allocated at random) and two left unsprayed. Surround WP application rate: the recommended rate for application on the label is 50 lb in 100 gallons water per acre = 56 kg in 1100 litres water per ha. The sprays were applied with the grower's air-assisted orchard sprayer.

Table 4.4.1. Dates of Kaolin spray applications and assessments

Year	Spray dates	No. sprays	Assessment dates	Farm
2008	5-14 March	1	18, 31 March, 11 April	J L Baxter & Son, Westerhill Farm, Westerhill Lane, Linton, Maidstone, Kent ME17 4BS
2009	21 February, 7 March and 1 April	3	27 February, 3 March, 4 April	G H Dean & Co. Hempstead Farm, Bapchild, Sittingbourne, Kent ME9 9BH
2010	11, 20 March	2	17, 24 March, 12, 28 April	G H Dean & Co. Hempstead Farm, Bapchild, Sittingbourne, Kent ME9 9BH

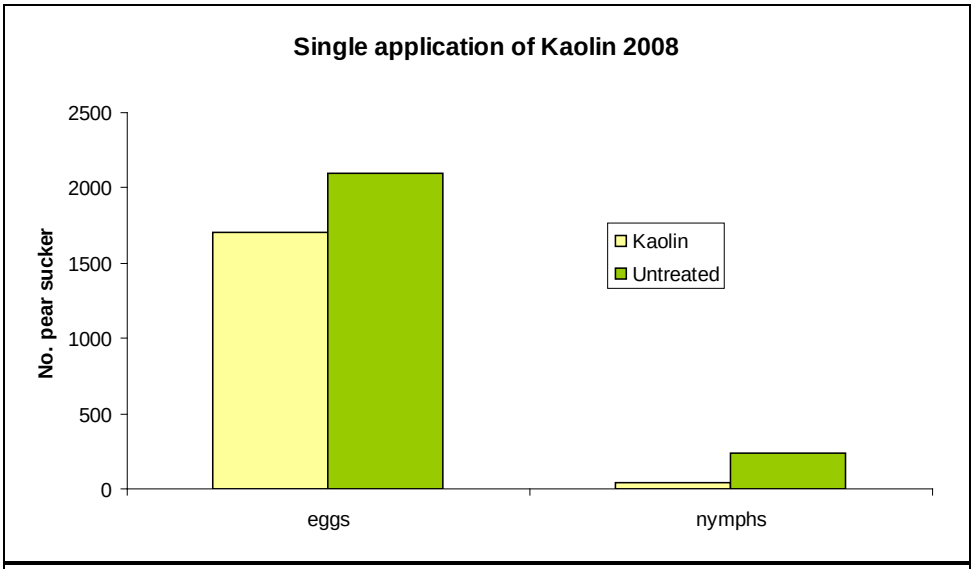
Twenty trees in each plot were tap sampled over a white tray after the spray applications. The numbers of adult pear sucker and anthocorids were recorded. The numbers of eggs and nymphs were also recorded around the base of 40 fruiting buds/clusters, two from each of 20 trees were counted under a microscope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

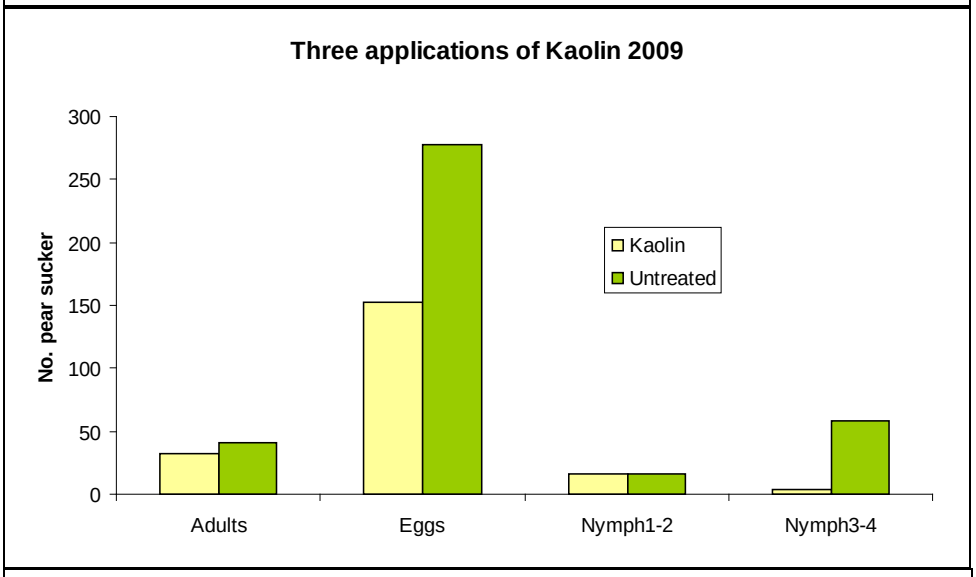
Results

Dormant season sprays of kaolin gave good control of pear sucker, reducing numbers of nymphs by over 75% in many cases (Fig. 4.4.1). This is important for reducing the numbers of subsequent egg laying females in the first generation. The cost of a single spray of kaolin is around £35/ha.

Summary

These trials show good promise for the use of spray applications of kaolin for the control pear sucker early on in the season (pre bud burst).





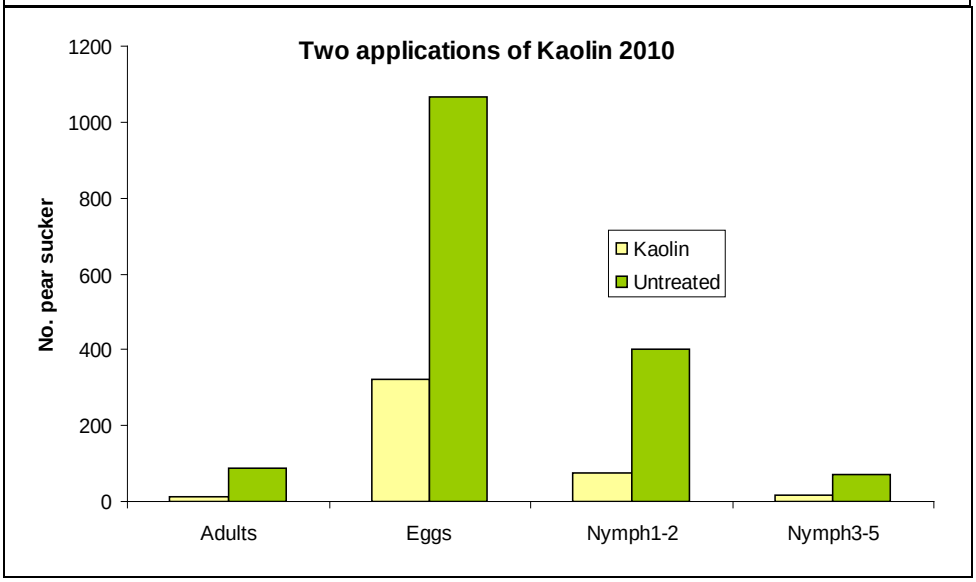


Figure 4.4.1. Numbers of pear sucker adults, nymphs and eggs in plots treated with Surround compared to untreated plots

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 three half to one day workshops on pear production (English Apples & 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

SIX MONTHLY REPORT TO HORTICULTURE LINK PROGRAMME MANAGEMENT COMMITTEE

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 & pears Ltd; J Sainsbury's plc
Report Written by:	J Cross
Project Start/Completion Dates:	1 April 2008 – 31 March 2012
Reporting Period:	30 September 2010 – 31 March 2011
Number of Months Since Commencement:	31-36
Date of Last Management Meeting:	3 February 2011
Dates of Next Meetings	14 July 2011, 7 March 2012

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	
P2.1.1	6	Experimental hedgerows planted	Y
P2.1.2	3	5 existing hedgerows identified and characterised for future study	Y
P2.1.3	42	4 season data set characterising predator and prey communities in hedgerows complete	

P2.1.5	48	Recommendations on choice of hedgerow woody species and management practices formulated	
P2.2.2	45	4 season data set on the occurrence of over wintering predators in refuges complete	
P2.2.3	48	Recommendations for growers on the use of artificial refuges for anthocorid over wintering formulated	
P2.3.1	6	Strip plantings of nettles and flowering herbs on grower farms established	N[1]
P2.3.2	42	4 season data set characterising predator and prey communities in strips completed	
P2.3.4	48	Recommendations on benefits of nettle/flowering herb strips and management practices formulated	
P2.4.1	6	Tall flowering herb mix sown in alleys of 2 orchards	Y
P2.4.2.	45	4 season data set characterising predator and prey communities in alleyway herbage and attendant pear trees	
P2.4.4.	48	Benefits of alleyway flowering herbage determined and recommendations for growers formulated	
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	
P4.3	42	Field evaluating effects of numbers sprays and spray cover of physically acting spray treatment completed	
P4.4	48	Four field experiments evaluating winter spray treatments with kaolin completed and benefits of treatment and timing and number of sprays determined	
P5.1	12	First ½-1 day workshop focusing on UK pear growing held	Y
P5.2	36	Second ½-1 day workshop focusing on UK pear growing held	N

P5.3	48	Third ½-1 day workshop focusing on UK pear growing held	

[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 & winterform *C pyricola* established	Y
S1.1.2	18	Volatiles collected from winterform & 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	
S2.1.4	42	Two MAB experiments investigating hedgerow trimming completed	
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	
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

3. Milestones for the six month period:

(from project proposal, or other more recently approved planning document)

Primary milestones in this reporting period are highlighted in grey above. 'P1.2.2: Pheromone attractiveness demonstrated and lure and trap optimised' has not been met because despite great efforts, we have not been able to identify any sex specific compounds which might constitute a sex pheromone. The cuticular hydrocarbon proposed as the sex pheromone of *Cacopsylla pyricola* by USA workers was not sex specific and we could not detect any attraction of either *C. pyri* or *C. pyricola* to it. 'P5.2 Second ½-1 day workshop focusing on UK pear growing held' was not met because the first full one day workshop on pear growing had only just been held and the consortium felt that the holding of a second one day workshop at the end of the project in Spring 2012 was more suitable than 3 ½ days ones.

4. Research report (new developments since full end of year 2 report issued 10 February 2010) (concise account including comments on whether targets are being met)

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

Despite careful comparisons of many volatiles and cuticular chemicals collected from female and male *C. pyri* we have not yet shown any significant and consistent differences that might be attributed to components of a female sex pheromone. We have found the compound (13-methylheptacosane), recently reported as the female pheromone of *C. pyricola* by investigators in the USA, to be present in body washes from *C. pyri* but relative amounts present in washes from males and females were similar. We have synthesised and tested the compound for attraction in pear orchards containing a mixture of *C. pyri* and *C. pyricola* in Kent in Spring and Summer 2010, but have not yet demonstrated attraction so far. Similarly we have not observed responses from males in laboratory trail following experiments to either the synthetically produced compounds or insect extracts.

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

The main focus of field work in 2010 was the regular sampling of the replicated purpose planted experimental hedgerow plots at 3 sites. The trees are in their 3rd year of growth and the results indicate that their characteristic fauna is starting to develop, though is not fully established. A large data base of many thousands of arthropods is being generated. Additional work to examine arthropod communities on *Salix caprea* through the season was also done. Only very small numbers of anthocorids were found to migrate into pear in a large scale mark and capture field experiment examining the migration of anthocorid predators from uncut versus cut-down nettles in August-September 2010. Numbers were too small to indicate whether there was a difference between cut an duncut nettles, but individuals were found to migrate >60 m over the 6 days of the experiment. The

experimental technique used was successful and the experiment will be repeated in 2011.

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.

Work is ongoing to characterise the chemical signature of pear sucker infested pear foliage and to try to emulate the attractive signal with synthetic lures. To date, we have not been able to demonstrate attraction to anthocorids to the compound identified in previous Dutch work, either singly or in mixtures.

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

Programmes of sprays of Kaolin in the late dormant period have been shown to deter egg-laying. 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).

5. Project changes:

(proposed or agreed with the LINK programme, and including any changes to expected profile of grant claims)

At the consortium meeting on 3 February 2011, the following adjhustments to the work programme for year 4 of the project were agreed:

Objective 1

It was agreed that work in 2011 should focus on EAG of the numerous collections already acquired, to see whether any active substances could be identified.

Objective 2

No adjustment to the work programme for this objective is needed. Completion of the identification of the material from sampling the established hedgerows in 2009 would also be a priority in year 4 of the project.

Objective 3

The need to evaluate grids of dispensers of methyl salicylate and other volatiles in larger plots and to do further EAG on anthocorids in year 4 of the project was identified. The sex pheromone of the vine mealy bug (*Plannococcus ficus*) has been shown to be attractive to a wide range of natural enemies in vineyards and it will be tested for attracting pear sucker natural enemies in pear orchards in year 4 of the project.

Objective 4

It was agreed that work in year 4 of the project would focus on further quantifying the effects of early season sprays of kaolin as this was yielding promising results

6. Publications and technology transfer outputs:

(including public presentations/talks given. Indicate additions since last report by use of bold type)

<u>Publications</u>

Anon. 2010. Conference takes pears into the next decade. HDC News April 2010. 18-19.

Cross, J.V. 2010. Improving biocontrol and management of pear sucker. HDC project profile TF 181.

Cross, J.V. 2010. Pear sucker research. Proceeding of the Sainsbury/EAP/EMRA conference

Cross, J. V., Fountain, M.T. 2010. Pear Suckered? National Fruit Show Handbook 2010

Cross, J V. Nagy, C., Batki, M., Linka, J. 2010. Conservation biocontrol of pear sucker. Proceeding of the 150th year Anniversary of the Austrian Federal College and Institute of Pomology and Viticulture. International Symposium on the maintenance of biodiversity in Pomology. 23 October 2010, Vienna, Austria, 8 pp.

Article in the HDC Top Fruit Review (2010) Biocontrol and management of pear sucker.

Technology transfer

'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.

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7. Exploitation plans:

(give an update on perceived exploitation opportunities and future plans.)

These have yet to be agreed by the project consortium

