Project Title:	Pheromone technology for management of capsid pests to reduce pesticide use in horticultural crops
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CONTENTS

GROWER SUMMARY

Headline

Male European Tarnished Plant Bugs have been attracted into cross vane funnel traps baited with the female sex pheromone.

Background and expected deliverables

In the past, lack of an effective monitoring tool has prevented effective control of capsids in horticultural crops and has led to unnecessary use of pesticides. Building on previous research at East Malling Research (EMR) and Natural Resources Institute (NRI), this project aims to develop effective and practical pheromone lures and traps for monitoring three species of capsids. Effective pheromone traps will be useful for improving the timing of spray applications and lead to a reduction in the use of broad-spectrum insecticides to control capsid pests on a range of horticultural crops. This will help to maintain or improve the level of control in both conventional and organic produce.

A major objective is to identify and confirm the components of the female sex pheromones of the capsid species European Tarnished Plant Bug, Common Green Capsid and the Nettle Capsid. Laboratory work and field bioassays will be undertaken to produce artificial lures. The use of such lures will directly or indirectly help to:

- reduce the incidence of residues of broad-spectrum pesticides.
- substantially improve capsid control in organic crops.
- reduce the likelihood of resistance to insecticides developing.
- simplify implementation of biological control programmes for other pests.
- maintain and enhance biodiversity, including natural horticultural pest enemies.

The project will investigate the effects of host-plant volatiles on capsid behaviour and control. It is possible that the incorporation of host-plant volatiles into lures with pheromones could

produce practical benefits in terms of enhancing the attractiveness and/or provide new attractants, particularly for female capsids.

Summary of the project and main conclusions

Volatile production

Volatiles were collected from individual females of European Tarnished Plant Bug and male and female Common Green Capsid and Nettle Capsid, at different times of day. This was conducted following field tests in 2007 which showed that female European Tarnished Plant Bug attracted males in the morning.

The collections of volatiles from **European Tarnished Plant Bug** in the morning resulted in a new ratio of the three main pheromone components (hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA)). When tested in field trials as a synthetic blend, released from microcapillary dispensers and a piezoelectric sprayer, it was apparent that the blend was attractive to males of the same species.

Volatiles collected from the **Common Green Capsid** were in the same order of magnitude and a similar ratio as that of the European Tarnished Plant Bug. The common Green Capsid appeared to produce the chemicals during the afternoon and evening period.

Both male and female **Nettle Capsids** produced HB, E2HB and KA but in very variable amounts. Attempts to determine the time of day of production of the potential pheromone components were rather inconclusive, although indications are that they are produced during the period 0000-0800 hrs.

Field trials

In field trials lasting several months, small numbers of female European Tarnished Plant Bug were attracted to lures containing phenyl acetaldehyde (PAA), but the plant volatile did not enhance the attraction of males to female sex pheromones. In shorter term field tests, females were not significantly attracted to lures containing the plant volatiles PAA, hexyl acetate, ocimene, methyl salicylate or (*E*)-β-caryophyllene.

No attraction of the Common Green Capsid to pheromone dispensers containing a 3 way mix of hexyl butyrate ('HB') : (*E*)-2-hexenyl butyrate ('E2HB') : (*E*)-4-oxo-2-hexenal ('KA') (1.0:0.7:1.0) was demonstrated in field trials. However, significant numbers of male *Lygus pratensis* (a closely related species to European Tarnished Plant Bug) along with a species damaging to apple in North America and a predatory species in the UK, (*Atractotomus mali*), were attracted to the 3 way mix. Sunflower oil helped to stabilise KA relative to HB in the dispensers.

Experiments exposing the antennae of male or female Nettle Capsid to the three compounds (HB, E2HB and KA) followed by reading the electrical signal were largely inconclusive. A small antennal response was obtained from male Nettle Capsid to volatiles from a female insect. Male European Tarnished Plant Bug antennae responded to all three of the potential pheromone components, but never to all three in the same analysis.

Using field and laboratory tests, it is still unclear whether male Nettle Capsids are attracted to females or vise versa. Indeed, still air (as opposed to wind-tunnel) laboratory bioassays failed to show attraction of Nettle Capsid or European Tarnished Plant Bug to the opposite sex or to the pheromone blend attractive to European Tarnished Plant Bug.

Trap designs

In tests comparing trap designs, green pre-moulded cross vane funnel traps (Agralan) captured more capsids than various designs of delta trap and sticky stake traps and, therefore, are the best choice for monitoring capsids. Water and a drop of detergent are used as a trapping agent.

Season of activity

Field collections of the three species by sweep netting and trap sampling showed that Nettle Capsid females were abundant from May, increasing in number through to July (males, females and nymphs) on nettles. Common Green Capsid was present in small numbers (mainly nymphs) from May onwards on blackcurrant. European Tarnished Plant Bug was abundant in July and populations continued to grow until October in fields sown with fat hen and scented mayweed. Laboratory culturing of European Tarnished Plant Bug and Nettle Capsid was successful through the growing season and provided many unmated males and females for testing.

Future work

Future work in this project will include confirming the attractiveness of the modified blend to European Tarnished Plant Bug and designing a dispenser which is user friendly to growers. In addition, we will explore the effects of release rate and blend ratio on attractiveness to male capsids. Nettle Capsid and Common Green Capsid will be further field tested, and volatiles collected in the laboratory, to determine the time of day females are releasing pheromone components, and reveal an accurate ratio and release rate of the 3 pheromone components.

Financial benefits

No direct financial benefits to growers resulted from the research this year, but the ability to monitor capsids in horticultural crops will result in better timing of pesticide applications in the future.

Action points for growers

No direct action points have arisen from this work to date.

SCIENCE SECTION

Pheromone technology for management of capsid pests to reduce pesticide use in horticultural crops

Scientific objectives

This project aims to reduce the use of broad-spectrum insecticides against capsid pests on a range of horticultural crops and maintain or improve the level of control in both conventional and organic produce. This is being achieved by building on previous research at East Malling Research (EMR) and Natural Resources Institute (NRI) to develop effective and practical pheromone lures for monitoring three species of capsids. Achievement of this will provide means to reduce substantially the need for prophylactic applications of broad-spectrum pesticides in several horticultural crops and encourage rational use of more specific approaches to control capsid pests and increase productivity. As a result, this will:

- reduce the incidence of residues of broad-spectrum pesticides, especially of organophosphates, in horticultural produce
- substantially improve control in organic crops where capsids are highly damaging pests and where only short persistence insecticides (e.g. pyrethrum) are available for control
- reduce the likelihood of resistance to insecticides developing in the target pests
- make it easier to implement biological control programmes for other pests
- maintain and enhance biodiversity, including natural enemies of important horticultural pests

The identity of the female sex pheromone components of *L. rugulipennis* and *L. pabulinus* will be confirmed and the components of the sex pheromone of *L. tripustulatus* identified. Laboratory windtunnel bioassay methods for each species will be developed to permit testing under controlled conditions essentially throughout the year. Laboratory and field experiments will be done to investigate the effects of pheromone blend, dispenser design and pheromone release rate on attractiveness for each species. Based on these results, lures and traps for monitoring these pests in commercial crops will be developed. Importantly, this will include the development and refinement of cost-effective, practical dispensers for use with capsid pheromones. Lack of these has been a major barrier in capsid pheromone research in general.

This project will also investigate the effects of host-plant volatiles on capsid behaviour. This is an area that has been largely ignored so far, although some very recent reports suggest host plant volatiles may be important in mate-finding as well as host plant location and oviposition. Incorporation of host-plant volatiles into lures could produce practical benefits in terms of enhancing the attractiveness of pheromones and/or providing new attractants, particularly for females.

The objectives of the project area as follows;

1. To confirm identification of components of the female sex pheromones of *L. pabulinus*
2. To identify components of the female sex pheromone of *L. tripustulatus*
3. To determine factors affecting attraction of *L. rugulipennis, L. pabulinus* and *L. tripustulatus* to synthetic pheromone lures in laboratory and field
4. To investigate the possibility of synergising the attractiveness of the pheromones of the three species with host plant volatiles in laboratory and field
5. To develop practical pheromone lures and traps for *L. rugulipennis*, *L. pabulinus* and *L. tripustulatus*
6. To calibrate pheromone traps for monitoring capsid pests in at least two field crops and one protected crop

Objective 1. Confirm identification of components of the female sex pheromones of *L. pabulinus*

Task 1.1. Establish cultures of *L. rugulipennis*, *L. pabulinus* and *L. tripustulatus* at EMR (EMR, Yrs 1-3)

Materials and methods

In March, potatoes and seeds of nettle (*Urtica dioica*), mayweed (*Matricaria recutita*) and fat-hen (*Chenopodium album*) were sown and grown in a glasshouse as food for the capsid cultures. Mirid cultures were maintained by feeding dwarf beans (*Phaseolus vulgaris*)/potato shoots/nettle shoots (also a place to the females to lay eggs), dead blowfly larva on moistened tissue paper in a plastic cup (4 cm diameter 3 cm depth) and bee collected pollen added to the bottom of the cages. In an attempt to reduce the time needed to feed the cultures whole potted plants were also used in the main mirid cultures, however, this method was abandoned as predators (namely, spiders) were introduced and consumed the mirids.

Once per week, the plant material from the culture boxes was removed and placed on top of shredded paper in a ventilated box (28x15x8.5 cm) (Fig. 1.1.1). A cup of moistened tissue paper was added to provide humidity. These boxes were checked weekly for nymphs. Mouldy plant material was removed and fresh food supplied each week.

After approximately 7 days eggs hatched, and in contrast to year 1, the capsids were only separated once they had moulted to adults (boxes checked every 3 days, mirids not sexually mature until after 5 days). They were separated into smaller ventilated culture boxes (13x7.5x5 cm) with shredded paper and food (Fig. 1.1.1). These culture boxes were maintained weekly to ensure humidity and provide a clean source of food for the insects. Separately reared insects were unmated males and females that could be used for the subsequent bioassays, field trials and pheromone collections. Cultures were kept in environmentally controlled rooms at 20^{o}C, with a light:dark regime of 16:8 h.

On 22 April sweep netting of nettles at different sites in and around EMR captured 27 *L. tripustulatus* adults (8 females + 19 males). A culture was set up in a large plastic culture cage (76x51x50 cm). Five separate collections in May resulted in 105 and 17 female and male *L. tripustulatus*, respectively. On 18 July, 290 adults and 170 nymphs were caught from areas of nettles at EMR, a blackcurrant crop surrounded by nettles at Stonebridge, Kent and nettle around a pear orchard in West Farleigh, Kent. A further 100 adults and nymphs were collected on 6 August.

L pabulinus was collected from 6 unsprayed rows of blackcurrant (Ben Alder) at Stonebridge, Kent by kind agreement of Tom Maynard, by tap sampling. Plants were tap sampled by knocking the branches with the arm onto a white beating tray. Collections of small numbers of *L. pabulinus* were made from May onwards. The samples collected on 20 May consisted mainly of nymphs. A few of the mirids were sent to Bernard Nau, a leading mirid entomologist, for confirmation of identification.

On 17 July, 119 *L. rugulipennis* (75 males, 44 females) were collected from the weed field ('Palmers Rough') at EMR and a culture set up (8 nymphs were also found at this time). From this time hundreds of *L. rugulipennis* were collected every two weeks to maintain the cultures and supply nymphs for the following experiments. On 1 October the few remaining capsid cultures were released.

Figure 1.1.1. Photographs of capsid culture apparatus a) large Perspex box containing egg laying adult mirids, b) food (blowfly larvae, pollen and dwarf beans), c) beans containing eggs in culture box, d) single adult capsid culture boxes.

Results

All 3 species of mirid fed on dwarf beans, pollen and blowfly larvae. *L. rugulipennis* and *L. tripustulatus* were cultured successfully throughout the year until August when the number of eggs laid by females declined. A combination of laboratory rearing and frequent field collections made it possible to maintain cultures for the lab and field experiments up to October. In culture the average lifespan of a female adult *L. rugulipennis* was 23 days, male and female *L. tripustulatus* 11 and 19 days, respectively, and *L. pabulinus* females 12 days at 20^{o}C. Separating only newly emerged adults drastically reduced the time needed to maintain the cultures, as time was not wasted singly rearing 4-5th instars that failed to emerge as adults (~50%). No attempts were made to over-winter the remaining few capsids this year, as new individuals will be collected in early 2009.

L. pabulinus cultures were, again, less successful. The main reason for this being the low numbers collected. Collecting this species is labour intensive and so it was more difficult to get the high numbers needed for the cultures. Eggs hatched successfully and small numbers were maintained from the end of May to the end of October.

Conclusions

- *L. tripustulatus* females were abundant from May and very abundant in July (males, females and nymphs). Populations had decreased by August.
- *L. pabulinus* was available in small numbers (mainly nymphs) from May onwards on blackcurrant.
- *L. rugulipennis* began to become abundant in July and populations continued to grow until October.
- Culturing of *L. rugulipennis* and *L. tripustulatus* was successful through the growing season and provided many unmated males and females for testing.
- Improvements on time spent culturing the insects were achieved this year by only individually rearing capsids which had successfully moulted to adult (instead of separating at 4-5th instar).
- Better sites and more collecting time are needed to obtain specimens of *L. pabulinus* to establish more permanent cultures.
- As healthy adult capsids can be obtained early in the year for cultures, overwintering was not attempted this year.
- Potted plants, although reducing labour by surviving longer in the cultures, harboured predators and are not recommended for future cultures. Potato shoots and supermarket purchased dwarf beans are adequate food sources for all 3 species.

Task 1.2. Confirm identity of chemicals produced by virgin female *L. pabulinus* (NRI, EMR Yr 1)

Materials and methods

1. Entrainment

Eight female and 8 male unmated adult *L. pabulinus* (field collected nymphs) were entrained for between 4 and 12 days (using the same filter for the whole period). In addition, 3 females were entrained at 3 times of day. Mirids were more than 6 days old (Table 1.2.1). Individuals were placed in entrainment chambers with a potato shoot or bean (as food and as a host plant to initiate pheromone production). The potato shoot/bean was placed in wet cotton wool and covered with aluminum foil to keep it fresh. Each chamber was connected to a pump by tubing with an airflow control valve. The air passed through a chamber of charcoal to 'scrub' the air before it passed over the mirid. Volatiles were trapped in Porapak Q filters (200 mg in Pasteur pipette 4 mm i.d.).

For the whole day (IV) entrainments six sets of entrainment apparatus were used simultaneously, each fitted with its own charcoal filter and Porapak Q entrapment filter, but sharing a common air pump (Fig. 1.2.1). The apparatus was operated in a controlled temperature (CT) room (number 2) at EMR. The apparatus was cleaned by passing a continuous air flow through a charcoal filter for 24 h before the collections began. The lighting in the CT room was on between 0900 and 0130 h. The room was in darkness between 0130 and 0900 h. The filters were connected and the pump was switched on for 30 minutes after placing the capsid in the chamber to give the insect time to settle. This was to reduce the likelihood of collection of any potential alarm compounds. The air speed in the entrainment apparatus was set at 450 ml/m (±50 ml). The 6 glass entrainment chambers contained either a male, female or plant only (Table 1.2.1).

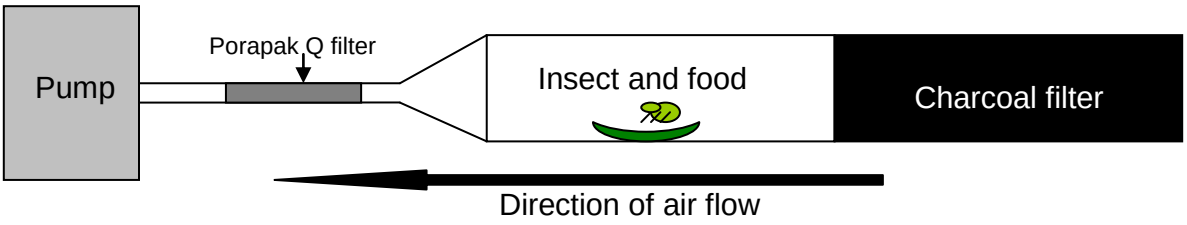

Figure 1.2.1. Schematic diagram of single entrainment apparatus used for collecting volatiles of capsids. Six entrainments were operating simultaneously.

For the 'time of day' analyses the day was divided into three periods;

Night 2400-0800 (light for 1.5 h, dark for 6.5 h) (period I)
Day 0800-1600 (dark for 1 h, light for 7 h) (period II)
Evening 1600-2400 (light for 8 h) (period III)

Each glass entrainment chamber was fitted with 3 parallel Porapak Q collection filters, one for each of the three periods of collection. Three pumps were used, one for each time period. The pumps were connected to timers and were turned on and off at the set periods of sampling (Fig. 1.2.2). Only one pump was in operation at any one time. The first collected volatiles during period I, the next period II and the last period III (Fig. 1.2.2). The apparatus was cleaned before entrainment began by allowing a continuous air flow through the system for 24 h. The room was set up for the lighting to be off between 0130-0900 h and on between 0900-0130 h. All filters were changed at the same time. Collections were made for different lengths of time (one or several days). The times included 1 h of dark in period II. At the end of the tests, filters were wrapped in aluminum foil and transported to NRI for analysis. Capsids were checked at the end of the entrainment to ensure they had survived. The details of the capsids used in the entrainment are given in Table 1.2.1.

At NRI the Porapak Q collection filters were extracted with dichloromethane (3 x 0.5 ml) and analysed by gas chromatography coupled to mass spectrometry (GC-MS) using a Varian CP-3800 GC and Saturn 2200 MS fitted with a fused silica capillary column coated with polar SupelcoWax (30 m x 0.25 mm i.d.) with helium carrier gas (1 ml/min) and oven temperature programmed from 50ºC for 2 min then at 6ºC/min to 250ºC. The identities of components were confirmed from the GC retention times and mass spectra and the three components hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal were quantified by reference to external standards.

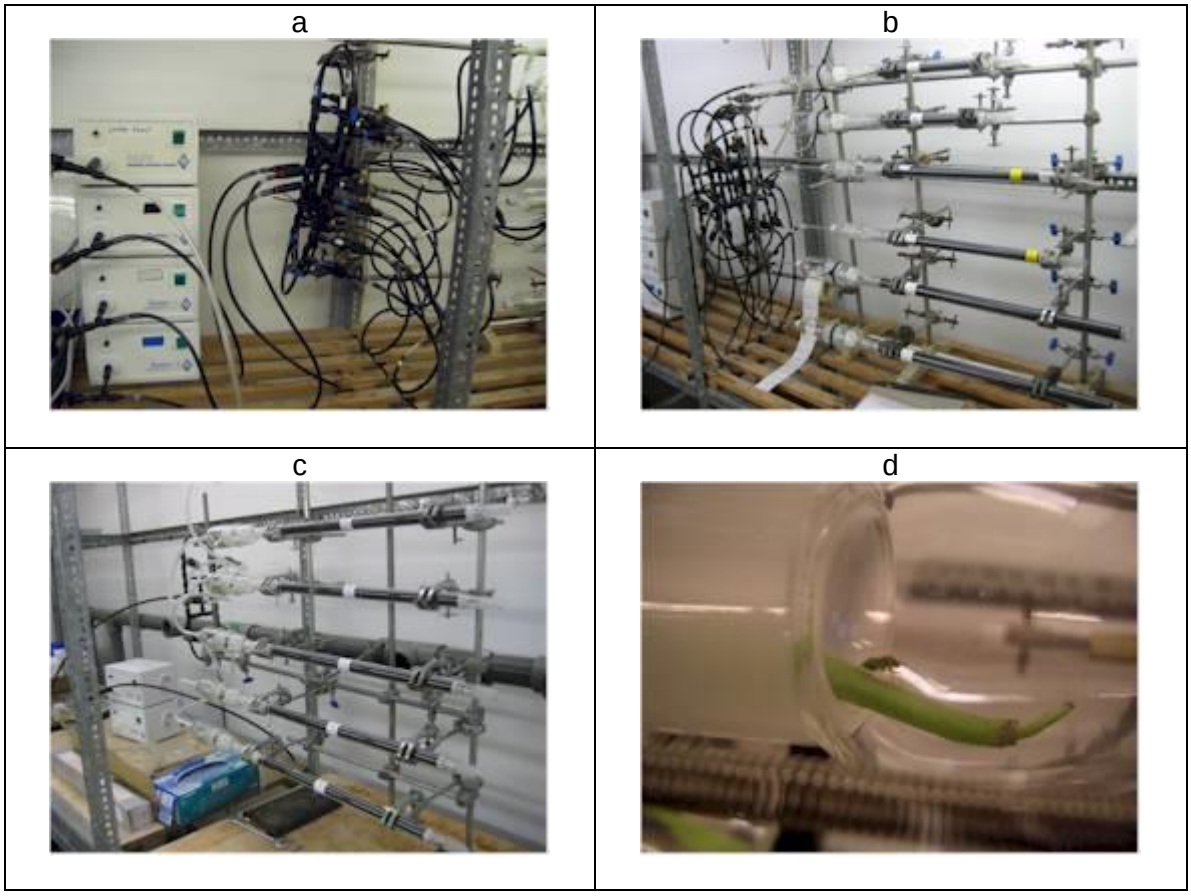

Figure 1.2.2. Entrainment apparatus used for sampling pheromones released by mirids at three time periods in the day. (a) pump and piping from glass chambers (b) entrainment apparatus for whole day entrainments (c) entrainment apparatus for different time of day entrainments (d) capsid feeding on bean in glass chamber.

Table 1.2.1. Details of *L. pabulinus* individuals used in the entrainment. I = treatment dark, II = treatment morning, III = treatment afternoon, IV = all day and night. m = male, f = female, na = not applicable.

NRI code 2008.036/	Capsid i.d.	Sex	Date adult emerged	Date in	Time in	Chamber No.	Date ended	Time ended	Treat.	Dead/Alive /Missing at end of test
1	501	m	29 May	02 Jun	14:00	1	04 Jun	15:30	IV	a
2	1	f	27 May	02 Jun	14:00	2	04 Jun	15:30	IV	a
3	control	-	-	02 Jun	14:00	3	04 Jun	15:30	IV	na
4	2	f	27 May	02 Jun	14:00	4	04 Jun	15:30	IV	a
5	502	m	29 May	02 Jun	14:00	5	04 Jun	15:30	IV	a
6	3	f	27 May	02 Jun	14:00	6	04 Jun	15:30	IV	a
7	503	m	27 May	05 Jun	13:30	1	07 Jun	13:00	IV	a
8	4	m	29 May	05 Jun	13:30	2	07 Jun	13:00	IV	a
9	504	m	29 May	05 Jun	13:30	3	07 Jun	13:00	IV	a
10	5	f	29 May	05 Jun	13:30	4	07 Jun	13:00	IV	a
11	control	-	-	05 Jun	13:30	5	07 Jun	13:00	IV	na
12	6	f	29 May	05 Jun	13:30	6	07 Jun	13:00	IV	a
13	7	f	02 May	09 Jun	17:00	1	11 Jun	19:00	IV	a
14	8	f	02 Jun	09 Jun	17:00	2	11 Jun	19:00	IV	d
15	9	f	02 Jun	09 Jun	17:00	3	11 Jun	19:00	IV	a
16	505	m	02 Jun	09 Jun	17:00	4	11 Jun	19:00	IV	d
17	506	m	02 Jun	09 Jun	17:00	5	11 Jun	19:00	IV	a
18	507	m	02 Jun	09 Jun	17:00	6	11/ Jun	19:00	IV	a
19	10	f	11 Aug	18 Aug	17:15	1	20 Aug	16:15	I	a
20	10	f	11 Aug	18 Aug	17:15	1	20 Aug	16:15	II	a
21	10	f	11 Aug	18 Aug	17:15	1	20 Aug	16:15	III	a
22	11	f	11 Aug	18 Aug	17:00	2	20 Aug	16:15	I	a
23	11	f	11 Aug	18 Aug	17:00	2	20 Aug	16:15	II	a
24	11	f	11 Aug	18 Aug	17:00	2	20 Aug	16:15	III	a
25	12	f	01 Sep	19 Sep	15:00	1	22 Sep	18:00	I	a
26	12	f	01 Sep	19 Sep	15:00	1	22 Sep	18:00	II	a
27	12	f	01 Sep	19 Sep	15:00	1	22 Sep	18:00	III	a
28	13	plant	na	19 Sep	15:00	2	22 Sep	18:00	I	a
29	13	plant	na	19 Sep	15:00	2	22 Sep	18:00	II	a
30	13	plant	na	19 Sep	15:00	2	22 Sep	18:00	III	a

2. Attraction in field

A small scale trial using caged females and males in delta traps with sticky bases was used to confirm attraction of male to female *L. pabulinus*. We also aimed to identify what time of day the female mirids were 'calling' (releasing pheromone).

The trial was set up at the Ben Alder blackcurrant plantation at Stonebridge, Kent (Table 3.3.2). The treatments were either male or female caged laboratory reared (Fig. 1.2.3) *L. pabulinus* (with food – potato shoot). Mirids were contained in a cage which consisted of a hair roller with gauze around the outside and a lid at either end, holding the gauze in place. The cage was placed vertically through a hole made in the top of the delta trap, so that it did not touch the sticky base. Green, 20 x 20 cm, delta traps with a brown (or white) sticky base were used for the first two tests (extra glue (ecotac) was added to the bases as capsids have been observed to walk off ordinary sticky bases). The traps were suspended at crop height, in contact with the blackcurrant bushes. The traps were checked every few days and mirids replaced with new virgin individuals. For the third observation the traps were changed for clear delta traps. Each treatment was replicated 5 times. The traps were arranged in row 3 of the blackcurrant field, 10 m apart. Counts of the number, sex and species of mirids caught in the traps were made. In total 18 female and 12 male baited traps were set up.

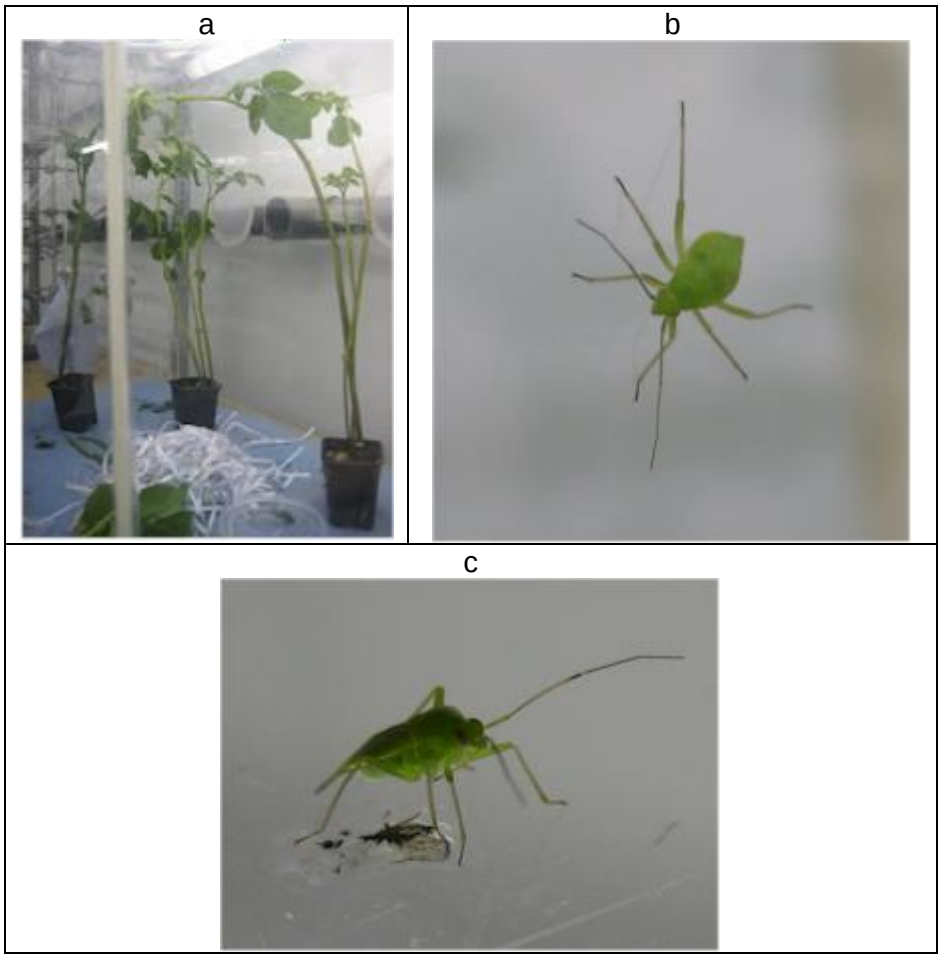

Figure 1.2.3. a) *L. pabulinus* culture with whole potato plants b) nymph c) adult

Results

1. Entrainment

Both female and male *L. pabulinus* produced hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA), and rates of production of hexyl butyrate over 48-hr periods are shown in Fig. 1.2.4. The mean rate for the 8 females used was 35.8 ng/hr (range 0-171 ng/hr; 5 out of 8 produced detectable amounts) and for males was 15.6 ng/hr (range 0-56 ng/hr; 4 out of 8 produced detectable amounts). The relative amounts of E2HB to HB were 0.04-0.08 with no obvious difference between males and females. Amounts of KA were very low and are still being quantified.

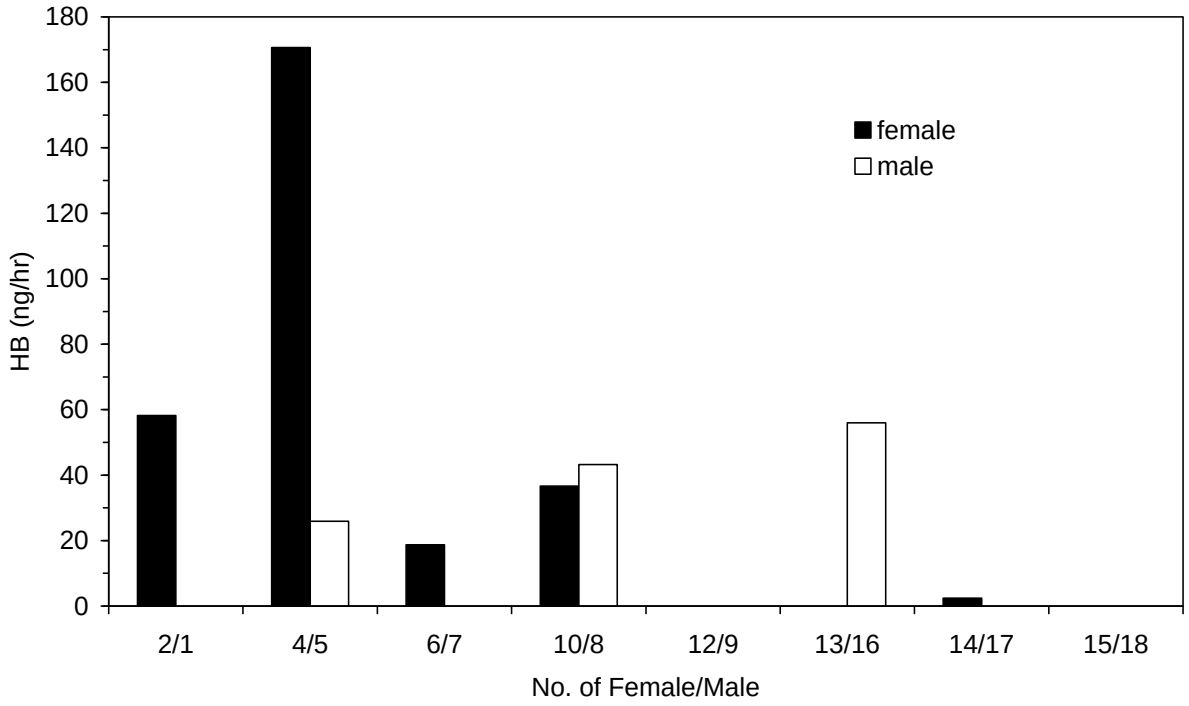

Figure 1.2.4. Amounts of hexyl butyrate collected from single male or female *L. pabulinus* over approx. 48 hr periods (No. of insect refers to NRI code in Table 1.2.1)

Collections of volatiles from two female *L. pabulinus* were made at different times of day and results are shown in Fig. 1.2.5. Significant amounts of HB were produced by both specimens in the afternoon and evening period, 16.00-24.00 hrs, and much smaller amounts in the other periods.

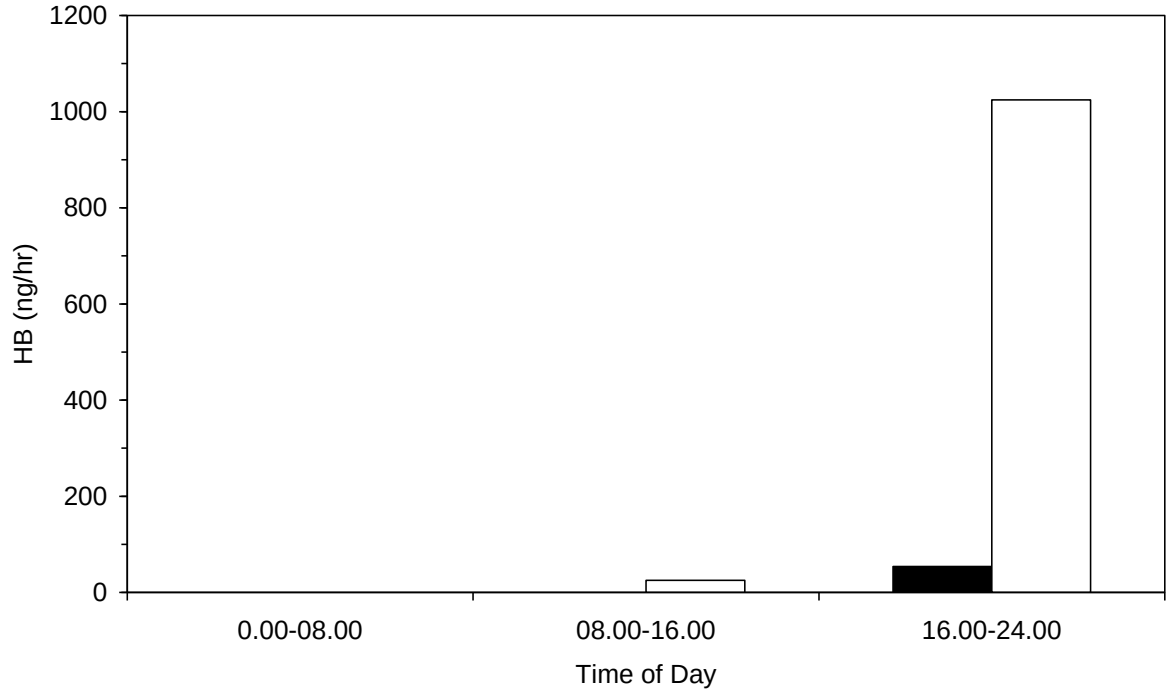

Figure 1.2.5. Amounts of hexyl butyrate collected from two separate female *L. pabulinus* over approx. 48 hr periods at different times of day

2. Attraction in field

From 9-30 June, 3 males were captured on three female baited traps and 1 male was captured on 1 male baited trap. Three of the 4 males were captured in the clear plastic delta traps. The attraction of males to females was not very successful. This was probably due to trap design.

Conclusions

- Both female and male *L. pabulinus* produce the three chemicals produced by *L. rugulipennis*, HB, E2HB and KA, although in the latter case only females produced these chemicals. This result is consistent with that of Drijfhout *et al.* (2002) who found that both male and female *L. pabulinus* produced the three compounds although only the antennae from males were responsive to these and those from females were not.
- The rate of production of HB is in the same order of magnitude as that from *L. rugulipennis*, and the ratio of E2HB:HB is also similar.
- *L. rugulipennis* females produced across morning, afternoon and evening. *L. pabulinus* appears to produce the chemicals during the afternoon and evening period. In both species, rates of production were very low during the night.
- More entrainments at different times of day are needed to confirm the ratio of the three compounds in *L. pabulinus.*
- Improvements need to be made on the trap design for *L. pabulinus*.

Task 1.3. Synthesise pheromone components of *L. rugulipennis* and *L. pabulinus* (NRI, Yr 1).

See year 1 report.

Objective 2. Identify components of the female sex pheromone of *L. tripustulatus*

Task 2.1. Identify chemicals produced by virgin female *L. tripustulatus* using air entrainment and analysis by GC-MS and GC-EAG, as above (NRI, EMR, Yr 2).

Materials and methods

1. Entrainment

For details of the entrainment methodology see Task 1.2. Bean and/or nettle were used as a food source for *L. tripustulatus* (Table 2.1.1). In total, 20 females and 23 males were entrained for whole days. Also, 10 females and 6 males were entrained at 3 times of day (see section 1.2 and Table 2.1.1).

Table 2.1.1. Details of *L. tripustulatus* individuals used in the entrainment. I = treatment dark, II = treatment morning, III = treatment afternoon, IV = all day and night. m = male, f = female, na = not applicable, * = field collected adult.

NRI code 2008.029/	Capsid i.d.	Sex	Date adult emerged	Date in	Time in	Chamber No.	Date ended	Time ended	Treat.	Dead/Alive /Missing at end of test
1	1	f	*	07-May	16:00	1	12-May	09:00	IV	d
2	2	f	*	07-May	16:00	2	12-May	09:00	IV	d
3	501	m	*	07-May	16:00	3	12-May	09:00	IV	m
4	3	f	*	07-May	16:00	4	09-May	14:30	IV	d
5	502	m	*	07-May	16:00	5	12-May	09:00	IV	d
6	503	m	*	07-May	16:00	6	09-May	10:00	IV	d
7	4	f	*	09-May	17:00	4	12-May	09:00	IV	a
8	504	m	*	09-May	17:00	6	12-May	09:00	IV	d
9	control		*	13-May	10:00	1	15-May	11:00	IV	-
10	505	m	*	13-May	10:00	2	15-May	11:00	IV	a
11	506	m	*	13-May	10:00	3	15-May	11:00	IV	a
12	5	f	*	13-May	10:00	4	15-May	11:00	IV	a
13	6	f	*	13-May	10:00	5	15-May	11:00	IV	a
14	507	m	*	13-May	10:00	6	15-May	11:00	IV	a
15	7	f	*	16-May	12:30	1	19-May	09:00	IV	a
16	8	f	*	16-May	12:30	2	19-May	09:00	IV	a
17	508	m	*	16-May	12:30	3	19-May	09:00	IV	a
18	509	m	*	16-May	12:30	4	19-May	09:00	IV	a
19	9	f	*	16-May	12:30	5	19-May	09:00	IV	a
20	10	f	*	16-May	12:30	6	19-May	09:00	IV	a
21	511	m	*	20-May	16:00	1	22-May	11:00	IV	a
22	17	f	*	20-May	16:00	2	22-May	11:00	IV	a
23	18	f	*	20-May	16:00	3	22-May	11:00	IV	a
24	512	m	*	20-May	16:00	4	22-May	11:00	IV	a
25	19	f	*	20-May	16:00	5	22-May	11:00	IV	a
26	20	f	*	20-May	16:00	6	22-May	11:00	IV	a
27	529	m	04-Jun	12-Jun	13:00	1	13-Jun	17:00	IV	a
28	41	f	04-Jun	12-Jun	13:00	2	13-Jun	17:00	IV	a

Table 2.1.1 continued

NRI code 2008.029/	Capsid i.d.	Sex	Date adult emerged	Date in	Time in	Chamber No.	Date ended	Time ended	Treat.	Dead/Alive /Missing at end of test
29	42	f	04-Jun	12-Jun	13:00	3	13-Jun	17:00	IV	a
30	530	m	07-Jun	12-Jun	13:00	4	13-Jun	17:00	IV	a
31	531	m	02-Jun	12-Jun	13:00	5	13-Jun	17:00	IV	a
32	43	f	09-Jun	12-Jun	13:00	6	13-Jun	17:00	IV	a
33	548	m	11-Jun	18-Jun	17:00	1	20-Jun	12:00	IV	a
34	549	m	09-Jun	18-Jun	17:00	2	20-Jun	12:00	IV	a
35	540	m	09-Jun	18-Jun	17:00	3	20-Jun	12:00	IV	a
36	550	m	11-Jun	18-Jun	17:00	4	20-Jun	12:00	IV	a
37	539	m	09-Jun	18-Jun	17:00	5	20-Jun	12:00	IV	a
38	551	m	09-Jun	18-Jun	17:00	6	20-Jun	12:00	IV	a
39	48	f	16-Jun	23-Jun	12:30	1	26-Jun	08:30	IV	a
40	552	m	13-Jun	23-Jun	12:30	2	26-Jun	08:30	IV	a
41	553	m	13-Jun	23-Jun	12:30	3	26-Jun	08:30	IV	a
42	49	f	16-Jun	23-Jun	12:30	4	26-Jun	08:30	IV	a
43	554	m	13-Jun	23-Jun	12:30	5	26-Jun	08:30	IV	a
44	50	f	16-Jun	23-Jun	12:30	6	26-Jun	08:30	IV	a
45	555	m	*	11-Aug	16:00	1	14-Aug	08:45	I	a
46	555	m	*	11-Aug	16:00	1	14-Aug	08:45	II	a
47	555	m	*	11-Aug	16:00	1	14-Aug	08:45	III	a
48	51	f	*	11-Aug	16:00	2	14-Aug	08:45	I	a
49	51	f	*	11-Aug	16:00	2	14-Aug	08:45	II	a
50	51	f	*	11-Aug	16:00	2	14-Aug	08:45	III	a
51	556	m	*	11-Aug	16:00	3	14-Aug	08:45	I	a
52	556	m	*	11-Aug	16:00	3	14-Aug	08:45	II	a
53	556	m	*	11-Aug	16:00	3	14-Aug	08:45	III	a
54	52	f	*	11-Aug	16:00	4	14-Aug	08:45	I	a
55	52	f	*	11-Aug	16:00	4	14-Aug	08:45	II	a
56	52	f	*	11-Aug	16:00	4	14-Aug	08:45	III	a
57	557	m	*	11-Aug	16:00	5	14-Aug	08:45	I	a
58	557	m	*	11-Aug	16:00	5	14-Aug	08:45	II	a
59	557	m	*	11-Aug	16:00	5	14-Aug	08:45	III	a
60	53	f	*	11-Aug	16:00	6	14-Aug	08:45	I	a
61	53	f	*	11-Aug	16:00	6	14-Aug	08:45	II	a

Table 2.1.1 continued

NRI code 2008.029/	Capsid i.d.	Sex	Date adult emerged	Date in	Time in	Chamber No.	Date ended	Time ended	Treat.	Dead/Alive /Missing at end of test
62	53	f	*	11-Aug	16:00	6	14-Aug	08:45	III	a
63	558	m	*	19-Sep	15:00	1	22-Sep	18:00	I	m
64	558	m	*	19-Sep	15:00	1	22-Sep	18:00	II	m
65	558	m	*	19-Sep	15:00	1	22-Sep	18:00	III	m
66	559	m	*	19-Sep	15:00	2	22-Sep	18:00	I	a
67	559	m	*	19-Sep	15:00	2	22-Sep	18:00	II	a
68	559	m	*	19-Sep	15:00	2	22-Sep	18:00	III	a
69	54	f	*	19-Sep	15:00	3	22-Sep	18:00	I	m
70	54	f	*	19-Sep	15:00	3	22-Sep	18:00	II	m
71	54	f	*	19-Sep	15:00	3	22-Sep	18:00	III	m
72	55	f	*	19-Sep	15:00	4	22-Sep	18:00	I	a
73	55	f	*	19-Sep	15:00	4	22-Sep	18:00	II	a
74	55	f	*	19-Sep	15:00	4	22-Sep	18:00	III	a
75	56	f	*	23-Sep	16:30	1	26-Sep	09:40	I	a
76	56	f	*	23-Sep	16:30	1	26-Sep	09:40	II	a
77	56	f	*	23-Sep	16:30	1	26-Sep	09:40	III	a
78	560	m	15-Sep	23-Sep	16:30	2	26-Sep	09:40	I	a
79	560	m	15-Sep	23-Sep	16:30	2	26-Sep	09:40	II	a
80	560	m	15-Sep	23-Sep	16:30	2	26-Sep	09:40	III	a
81	57	f	*	23-Sep	16:30	3	26-Sep	09:40	I	d
82	57	f	*	23-Sep	16:30	3	26-Sep	09:40	II	d
83	57	f	*	23-Sep	16:30	3	26-Sep	09:40	III	d
84	58	f	18-Sep	23-Sep	16:30	4	26-Sep	09:40	I	a
85	58	f	18-Sep	23-Sep	16:30	4	26-Sep	09:40	II	a
86	58	f	18-Sep	23-Sep	16:30	4	26-Sep	09:40	III	a
87	59	f	18-Sep	23-Sep	16:30	5	26-Sep	09:40	I	a
88	59	f	18-Sep	23-Sep	16:30	5	26-Sep	09:40	II	a
89	59	f	18-Sep	23-Sep	16:30	5	26-Sep	09:40	III	a
90	60	f	18-Sep	23-Sep	16:30	6	26-Sep	09:40	I	a
91	60	f	18-Sep	23-Sep	16:30	6	26-Sep	09:40	II	a
92	60	f	18-Sep	23-Sep	16:30	6	26-Sep	09:40	III	a

2. Attraction in field

Nine small scale field trials using caged females and males in delta traps with sticky bases (Fig. 2.1.1) were used to determine the attractiveness of female and male *L. tripustulatus* to the opposite sex. This is vital information for the future identification of sex pheromones. We also aimed to establish the time of day of 'calling', mirid releasing pheromone. Nettles in the border of an organic apple field at EMR were used for the tests (location NSRQ705 570). In addition, a pear orchard, with a nettle border to the north, in West Farleigh, Kent (OS ref: 532 734) was used (trial 3, Fig. 2.1.1). Clear delta traps were from Pherobank, Plant Research International, Wageningen.

The treatments were:
1) female + food
2) male +food
3) control (food only)

The methods were generally the same as for 'Task 1.2, *L. pabulinus*, *2. Attraction in field'.* Green or clear delta traps were used (Table 2.1.2). A fourth trial was set up to examine the attractiveness of female cohorts to other females after they were observed feeding in groups on potato stems in culture (Fig. 2.1.1). The traps were arranged in 1 row, 10 m apart. Counts of the number, sex and species of mirids caught in the traps were made.

Table 2.1.2 Details of nine trials set up to test the attractiveness of *L. tripustulatus* to the opposite sex.

Trial No	Treatments	Trap design	Cage design	Food	Date
1	6 x ♀, ♂ & control	Green delta trap - extra ecotac	Hair roller	Nettle	19-21 May
2	6 x ♀, ♂ & control	Green delta trap - extra ecotac	Hair roller	Nettle	22-27 May
3	7 x ♀, ♂ & 4 x control	Green delta trap - extra ecotac	Hair roller	Nettle	29 May-2 Jun
4	cohorts of ♀'s (4, 5, 6, 6, 7, 8 and 9)	Green delta trap - extra ecotac	round plastic container (4 holes in side) covered with netting - secured with twist tie	Nettle	3-6 Jun
5	5 x ♀ & ♂	Green delta trap - extra ecotac	Hair roller	Nettle	9-12 Jun
6	5 x ♀ & ♂	Green delta trap - extra ecotac	Hair roller	Nettle	12-16 Jun
7	5 x ♀ & ♂	Clear delta trap - extra ecotac	Hair roller	Nettle & bean	25-30 Jun
8	10 x ♀ 2 x ♂	Clear delta trap - extra ecotac	Hair roller	Nettle & bean	21-28 Jul
9	10 ♀, ♂ & control	Clear delta trap - extra ecotac	Hair roller	Nettle & bean	8-14 Aug

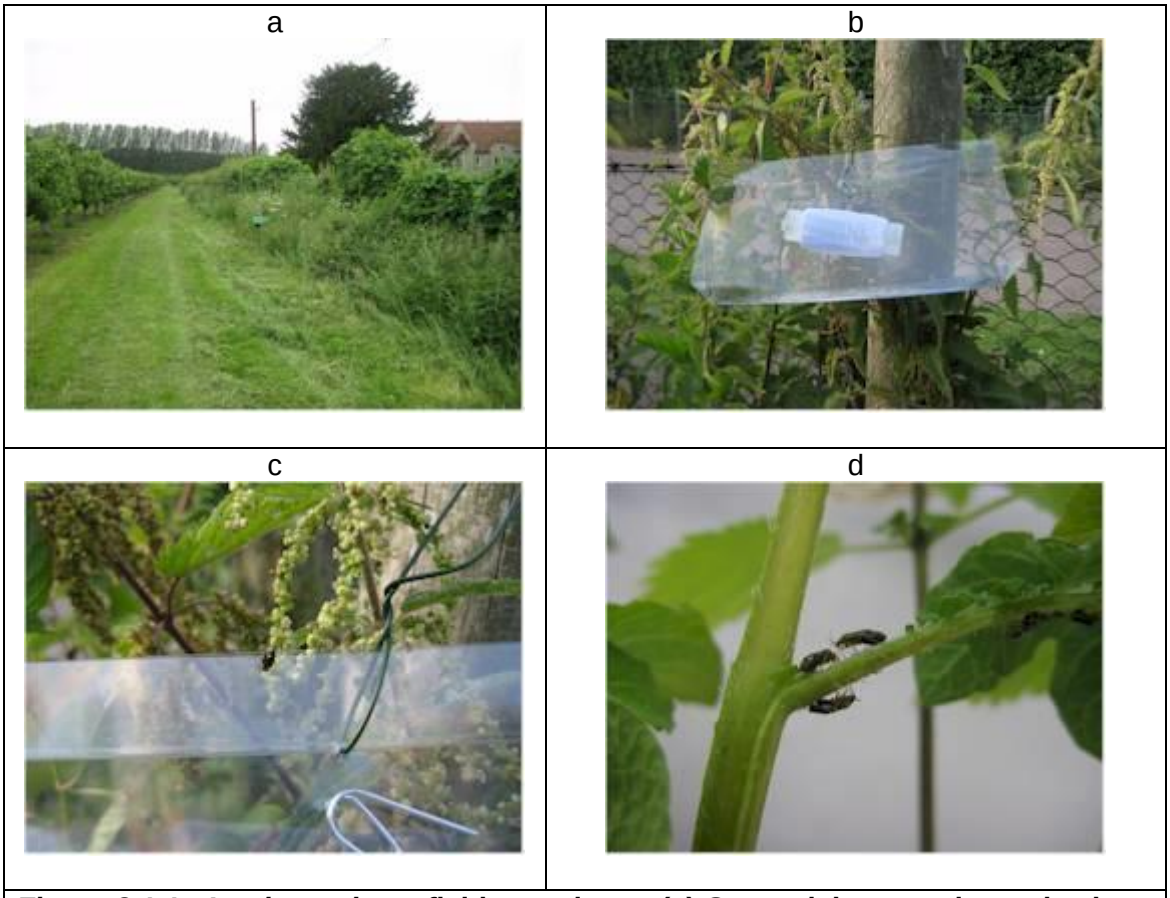

Figure 2.1.1. *L. tripustulatus* field experiment (a) Green delta traps in nettle alone edge of pear orchard (b) Clear delta trap with hair roller cage and sticky base (c) *L. tripustulatus* on top of trap (d) mirids feeing in group.

3. GC-EAG analyses

On 15 May, 27 May and 3 June, 4 (2 x male, 2 x female), 8 (4 x male, 4 x female) and 8 (4 x male, 4 x female), respectively, were transported to NRI for GC-EAD analysis. For coupled GC-EAG analyses the GC (HP6890; Agilent) was fitted with fused silica capillary columns coated with polar (Wax10, Supelco) and non-polar (SPB1, Supelco) phases. The column effluents were combined and split equally between the flame ionisation detector (FID) and a silanised glass T-piece. The contents of the latter were blown over the EAG preparation for 3 sec at 17-sec intervals with nitrogen (200 ml/min) as described by Cork *et al.* (1990). EAG preparations were set up using the portable device developed by Syntech (INR-02). The head of the insect was removed and the ground electrode was inserted into the back of the head. The tip of one antenna was removed and inserted into the recording electrode. Both FID and EAG signals were collected and analyzed with EZChrom software (Elite v3.0; Scientific Software Inc).

Results

1. Entrainment

Analysis of samples of volatiles collected from *L. tripustulatus* continuously (Table 2.1.1.) showed significant amounts of chemicals only in the first 6 samples and in these the insect was either dead or missing at the end of the experiment. In these, large amounts of HB were observed (3 females 84-1202 ng/hr; 3 males 68-562 ng/hr) with 5-10% of E2HB and significant amounts of KA. The only other samples where HB was detected were 7 (female) 83.5 ng/hr; 11 (male) 20.3 ng/hr; 17 (male) 10.0 ng/hr; 18 (male) 86.0 ng/hr; 20 (female) 11.3 ng/hr; 36 (male) 3.3 ng/hr; 43 (male) 92.4 ng/hr.

The relative amounts of HB, E2HB and KA were determined by GC-FID analysis. A sample from a female found to be dead at the end of the collection showed the three components in 1: 0.06 : 0.20 ratio and in a sample from a live female 1 : 0.05 : 0.12. Mean ratios from males dead at the end of the collection were 1 : 0.05 : 0.30 (n = 2) and from live males 1 : 0.04 : 0.04 (n = 4).

In analyses of collections made at different times of day (NRI codes 45-92) the only samples containing detectable amounts of the three potential components were 47 (male, 1600-2400 hr) 19.1 ng HB/hr; 63 (male, 0000-0800 hr) 683 ng/hr; 66 (male, 0000-0800 hr) 933 ng/hr; 87 (female, 000-0800 hr) 1,603 ng/hr.

2. Attraction in field

The field trails were inconclusive. Very few *L. tripustulatus* were captured in the green delta traps, however, clear delta traps were more effective (Table 2.1.3). It is possible that more males were captured because males were more active in seeking mates, but this is speculation and needs to be investigated further. The trap design should be improved.

Table 2.1.3. Number of *L. tripustulatus* captured in delta traps. See Table 2.1.2 for details of treatments.

Trial No	♂ in ♀ traps	♂ in ♂ traps	♀ in ♂ traps	♀ in ♀ traps
1	0	0	0	0
2	0	0	0	0
3	0	0	0	1
4	2	0	0	0
5	1	1	0	0
6	0	0	0	0
7	1	0	1	0
8	5	0	0	0
9	1	4	0	0
Total/traps	10/61	5/46	1/46	1/61
Ratio	0.2	0.1	0.02	0.02

3. GC-EAG analyses

GC-EAG analyses were carried out with a collection of volatiles from a female bug that contained the three potential pheromone components, HB, E2HB and KA, a collection from a female that had very little or none of these compounds and a collection from a male bug that contained the three potential pheromone components. Analyses were carried out with EAG preparations from both male (37 analyses) and female (29 analyses) bugs to all three collections.

Initial analyses were carried out with the polar GC column which separated the three potential pheromone components. No consistent and significant responses were observed from male (30 analyses) or female (29 analyses) antennae (e.g. Fig. 2.1.2).

Subsequently, 10 analyses were carried out using the non-polar GC column which did not separate the HB and E2HB and antennae from male *L. tripustulatus*. A small EAG response was obtained to the peak containing HB and E2HB in 4 analyses (Fig. 2.1.3).

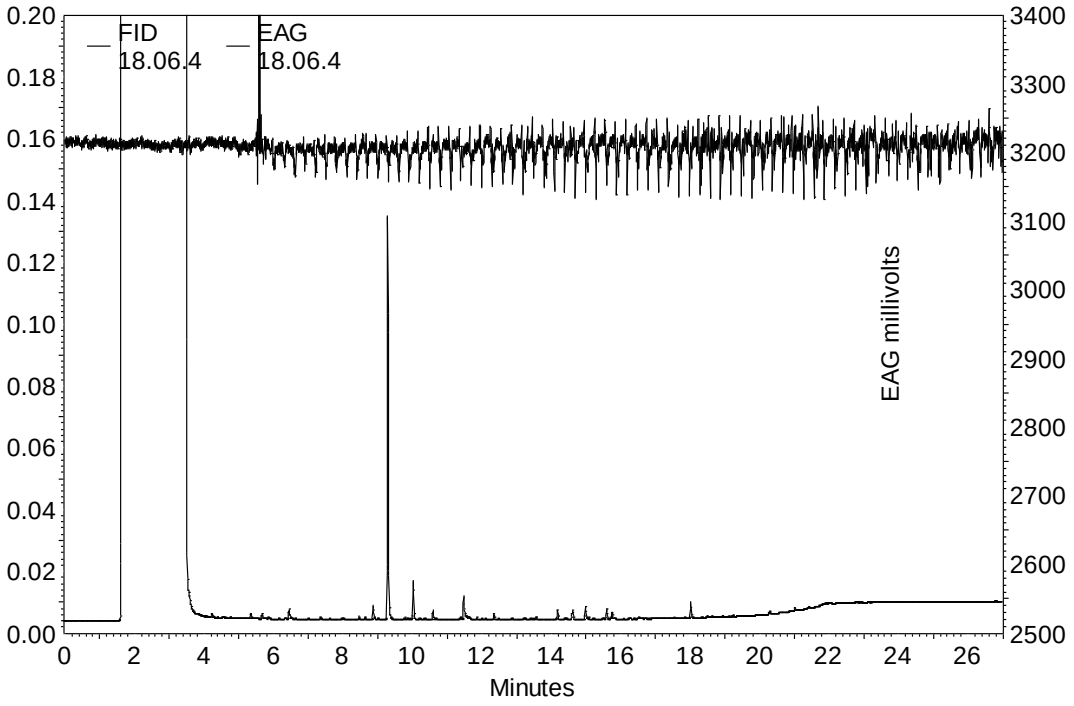

Figure 1.1.2. GC-EAG analysis with polar GC column of collection of volatiles from female *L. tripustulatus* and male EAG preparation (EAG upper trace, FID lower; EAGHB at 9.34 min, E2HB at 10.07 min, KA at 11.53 min).

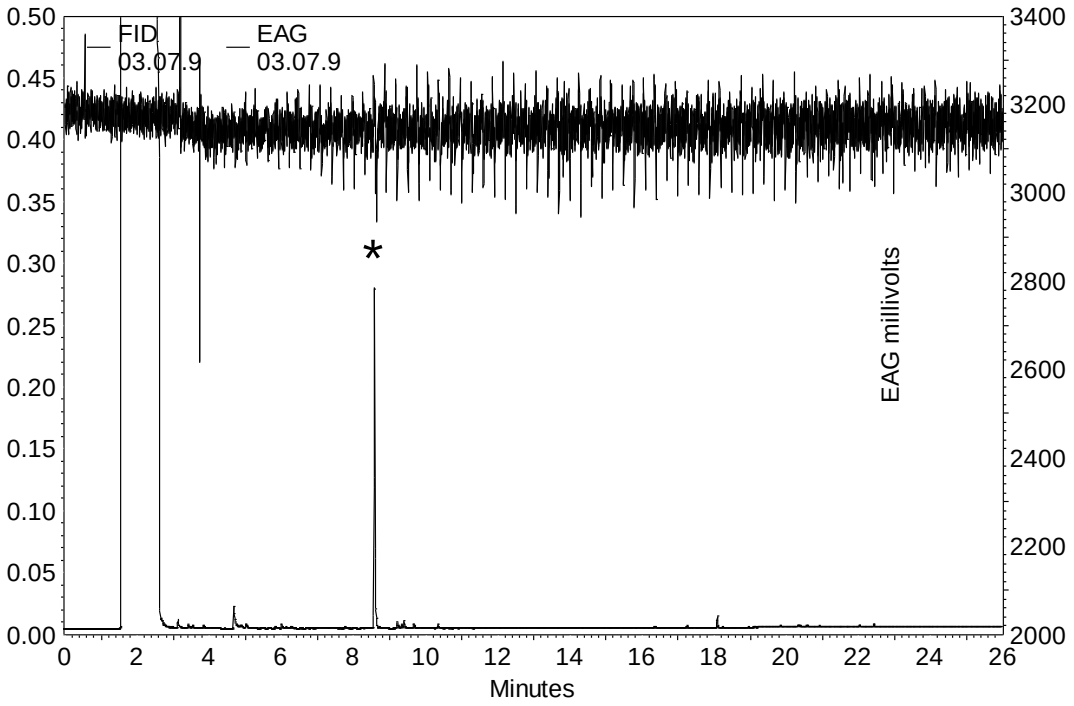

Figure 1.1.3. GC-EAG analysis with non-polar GC column of collection of volatiles from female *L. tripustulatus* and male EAG preparation showing EAG response (*) to HB+E2HB peak (EAG upper trace, FID lower; HB and E2HB at 8.63 min, KA at 4.71 min).

Conclusions

- Both male and female *L. tripustulatus* produced HB, E2HB and KA but in very variable amounts.
- The ratio of E2HB/HB was approximately 0.05 : 1 from both males and females, dead or alive.
- The proportion of KA/HB was higher from insects found to be dead at the end of the collection and from live insects the ratio was 0.12 : 1 from a female and 0.04 : 1 from males. These latter ratios are lower than those found with *L. rugulipennis* females (0.26 : 1 early in the season, 0.16 : 1 later).
- Attempts to determine the time of day of production of the potential pheromone components were rather inconclusive, although these were produced during the period 0000-0800 hrs for 3 out of the four collections containing significant amounts of material.
- In GC-EAG analyses, neither *L. tripustulatus* males nor females showed EAG responses to components in collections of volatiles from male or female insects when analysed on a polar GC column. Under the same conditions, males of *L. rugulipennis* showed responses to the three potential pheromone components, HB, E2HB, KA.
- In GC-EAG analyses using a non-polar column which did not separate HB and E2HB, a small EAG response was obtained from male *L. tripustulatus* to the peak containing the two compounds in volatiles from a female insect. No response to the KA or any other consistent responses were observed.
- Initial indications are that males are attracted to females, but improvements need to be made on the trap design for *L. tripustulatus*.

Task 2.2. Synthesise pheromone components of *L. tripustulatus* as above (NRI, Yr 2).

Hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal were produced by both male and female *L. tripustulatus*, but further work is required to determine whether these are pheromone components. Synthesis of these three compounds was carried out as described under Task 1.3.

Objective 3. Determine factors affecting attraction of *L. rugulipennis, L. pabulinus* and *L. tripustulatus* to synthetic pheromone lures in laboratory and field

Task 3.1. Develop laboratory bioassay to evaluate attraction of natural and synthetic pheromones and host plant volatiles to capsid bugs (EMR, Yr 1)

Materials and methods

Following work in year 1 a decision was made not to pursue laboratory bioassays using windtunnels. Windtunnel experiments were unsuccessful at demonstrating male attraction of *L. rugulipennis* to lures or unmated females. However, still air bioassays using large Perspex cages did show some (although variable) promise. This work was repeated with reproductively active capsids (main reproductive season).

1. L. rugulipennis

Single unmated females were placed, with food (dwarf bean), and a piece of moist tissue paper to maintain the humidity in small cages (hair roller covered with gauze and capped at both end). The hair roller was either placed inside a small clear plastic cage (12 cm deep, 9 cm diameter) with 4 small holes (1.5 cm diameter) or a sticky base with additional Ecotac added (Fig. 3.1.1). The small cages were placed within large Perspex cages (76x51x50 cm or 46x50x45 cm) (Fig. 3.1.1). The tests were run in a CT room (number 1) at 20^{o}C in 16:8 h L:D. The Perspex cages also contained a control cage (hair roller) with a bean, but no female. Twenty replicate cages were set up for each experiment. A cup with moistened paper and a bean was also included as humidity and food for the male capsids (Fig. 3.1.1). Into each large cage 2-7 males (dependent on availability) were released. Males were mostly field collected and then isolated. Observations began more than an hour after setting up the experiment, to give the mirids time to settle down. The first observation was 30 min before the lights came on and then every hour until 1400 h. A record was taken of the number of males inside or stuck to the female traps.

The first experiments was begun on 1 July (20 previously isolated females) and 4 males were released into each large cage. Caged females and free males were placed in the Perspex boxes at 1200 and 1300, respectively. On 2 July, 1400 h, the 4 hole trap design was changed for a sticky trap (1/4 sticky base insert on the bottom and top of each hair roller cage, Fig. 3.1.1). Observations were made on 1 July at 1400, 1500, 1600, 1700, 1800 h, 2 July at 0800, 0900, 1000, 1045, 1330, 1515, 1800 h and 3 July at 0800 and 0900 h. This test ended on 3 July.

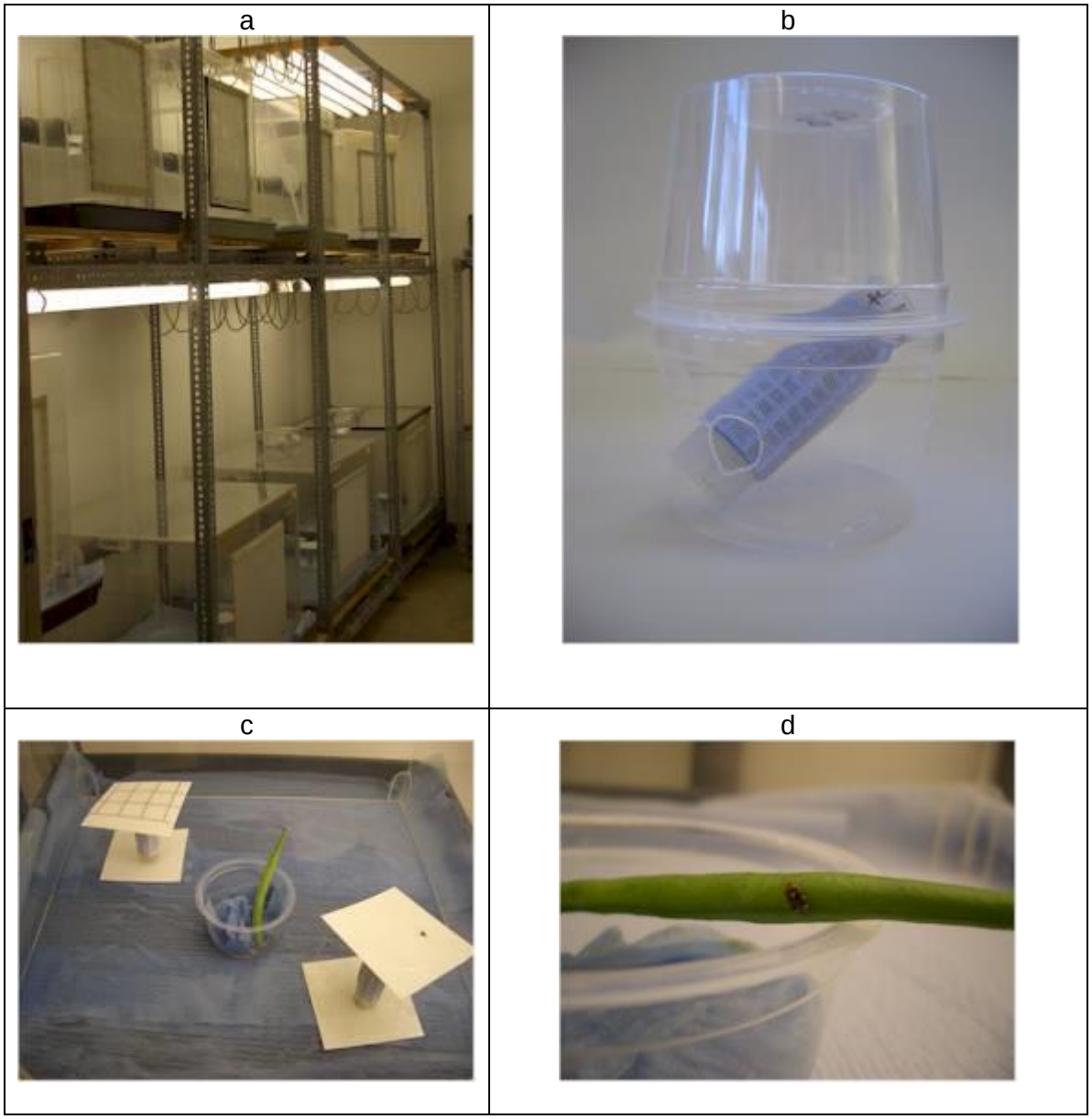

Figure 3.1.1. Apparatus used for the still air bioassay, (a) large bioassay cages (b) small clear plastic cage with 4 holes (c) sticky traps (d) male mirid feeding on bean.

The second test was set up on 1 September using reservoir lures with a 3 way combination of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal (ratio: 1:0.025:0.25, 10% in sunflower oil, release rate = 0.5 µg h^{-1}). The pheromone lures (odd numbered cages) were placed in 10 of the Perspex cages and a lure with sunflower oil only in the other 10 (even numbered cages). Initially each lure was placed in the centre of each cage on white 1/4 sticky insert (Fig. 3.1.2). At 0800 a single male was placed in each cage (the lights came on at 0900). On 2 September a second male was added to each cage at 1400 h. At this time the sticky trap was changed for a Petri dish water trap with a drop of detergent (as it was observed that some males could escape from the glue, Fig. 3.1.2). Observations were made on 1 September at 0930, 1000, 1030, 1130, 1300, 1400, 1500, 1600, 1700,

on 2 September at 0900, 1050, 1220, 1330, 1400, 1600, 1730 h, on 3 September at 0900, 1045, 1230 h, and 4 September 0830 and 0930 h.

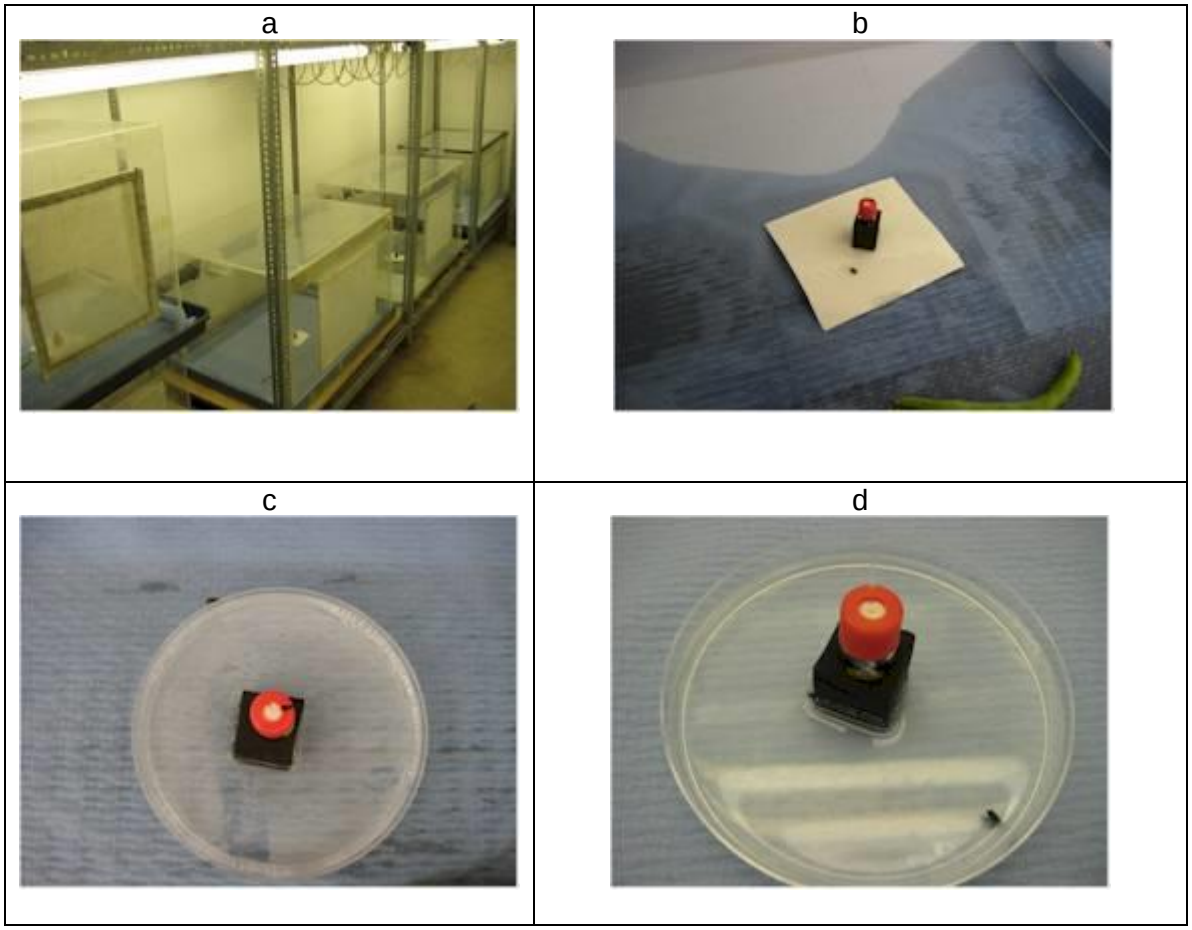

Figure 3.1.2. Apparatus used for the still air bioassay, (a) large bioassay cages (b) male mirid captured lure and sticky base trap (c) lure in Petri dish with water and detergent trap (d) male mirid captured in Petri dish trap.

2. L. tripustulatus

Three bioassays were done. In the first, 10 replicate caged single unmated males (with food (nettle), as above) were placed into large Perspex cages. A control cage was also included in each cage (hair roller with food only). Experiments were set up in CT room 1 with L:D 16:8 h, at 20°C. Three or 4 females were added to each Perspex cage on 16 June at 1200. A cup with wetted tissue paper and a bean was included as food and humidity for the test mirids. It was not known what time of day mirids were releasing pheromones and so sticky bases (10 x 10 cm sticky card) were used. A record was taken of the number of females stuck to the male traps. Observations were made on 16 June at 1400, 1500, 1600, 1700, 1800, on 17 June at 0800, 1600, 1700, 1800, and on 18 June at 0800, 0900, 1030, 1200, 1400. By the end of the test 6 caged males had died.

The second bioassay began on 24 June (1100) with females in cages (as bait) and a control (food only). Observations took place on 24 June at 1130, 1200, 1800, 2000 h and on 25 June at 0800, 0900, 1000, 1100 h. Three or 4 males were placed in each of the 10 Perspex cages. Only one female was dead by the end of the test.

The third bioassay, begun on 14 July (1015) also used females as bait and observations look place on 14 July at 1100, 1200, 1400, 1700. 1800, on 15 July at 0800, 1630, and on 16 July at 0900 h. Four males were placed into each cage.

3. GC-EAG analyses on L. rugulipennis

See Task 2.1 Materials and methods section for *L. tripustulatus*

Results

1. L. rugulipennis
At the first test a total of 7 and 10 males were observed/trapped on the female and control cages/sticky bases, respectively. Hence, no attraction of males to females could be confirmed using this bioassay.

The pheromone lures were then used because it was proposed that females, used as bait, under unnatural light conditions may not be 'calling', releasing pheromone. This might have explained why males were not responding as hoped. However, after 48 hours, 3 and 2 males were trapped in the pheromone and control traps, respectively.

2. L. tripustulatus
At the end of the first test ('are females attracted to males?'), 7 females were trapped on the male baited cages and 6 on the control cages, showing no sign that females are attracted to males (Fig. 3.1.3). The number of males trapped on female baited and control traps was between 9-10 and 2-8, respectively. Hence, the results were inconsistent and did not reveal whether it was males or females releasing sex pheromone.

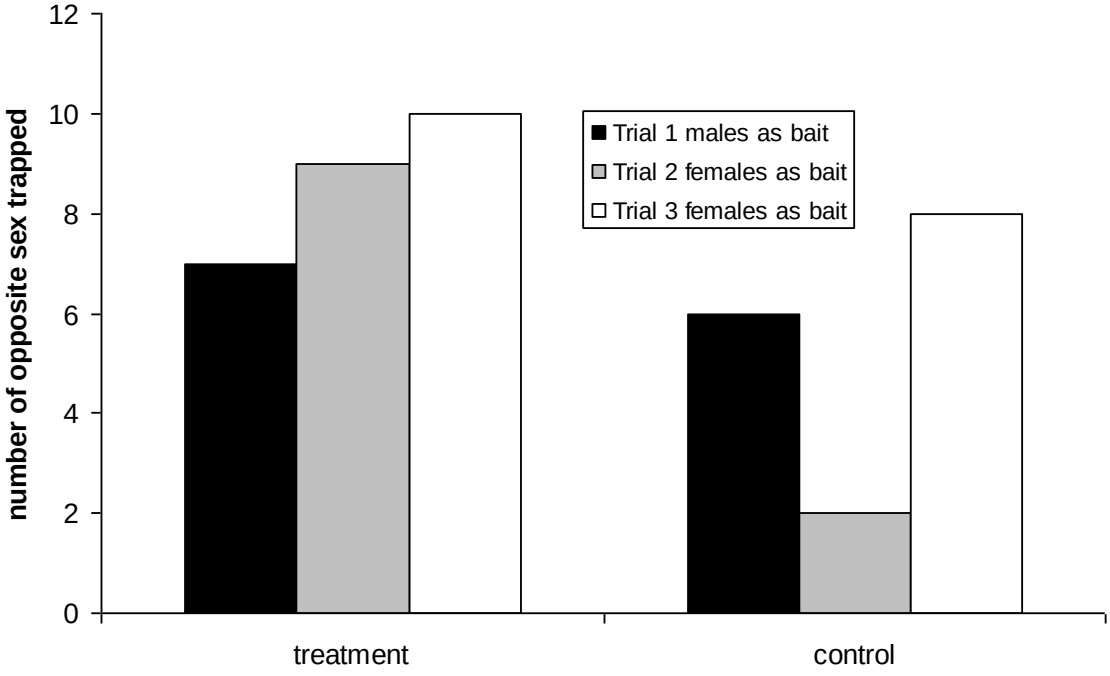

Figure 3.1.3. The number of mirids observed on the opposite sex and control sticky traps (10 replicates) on three test dates. 3-4 individuals of the opposite sex were added to each replicate.

4. GC-EAG analyses on L. rugulipennis

GC-EAG analyses (12) were carried out with EAG preparations from male *L. rugulipennis* and collections from female insects (Nos. 4, 70, 74 and 91 in Table 3.2.1). EAG responses were observed to all three of the potential pheromone components, HB, E2HB and KA, but never to all three in the same analysis (Fig. 3.1.4).

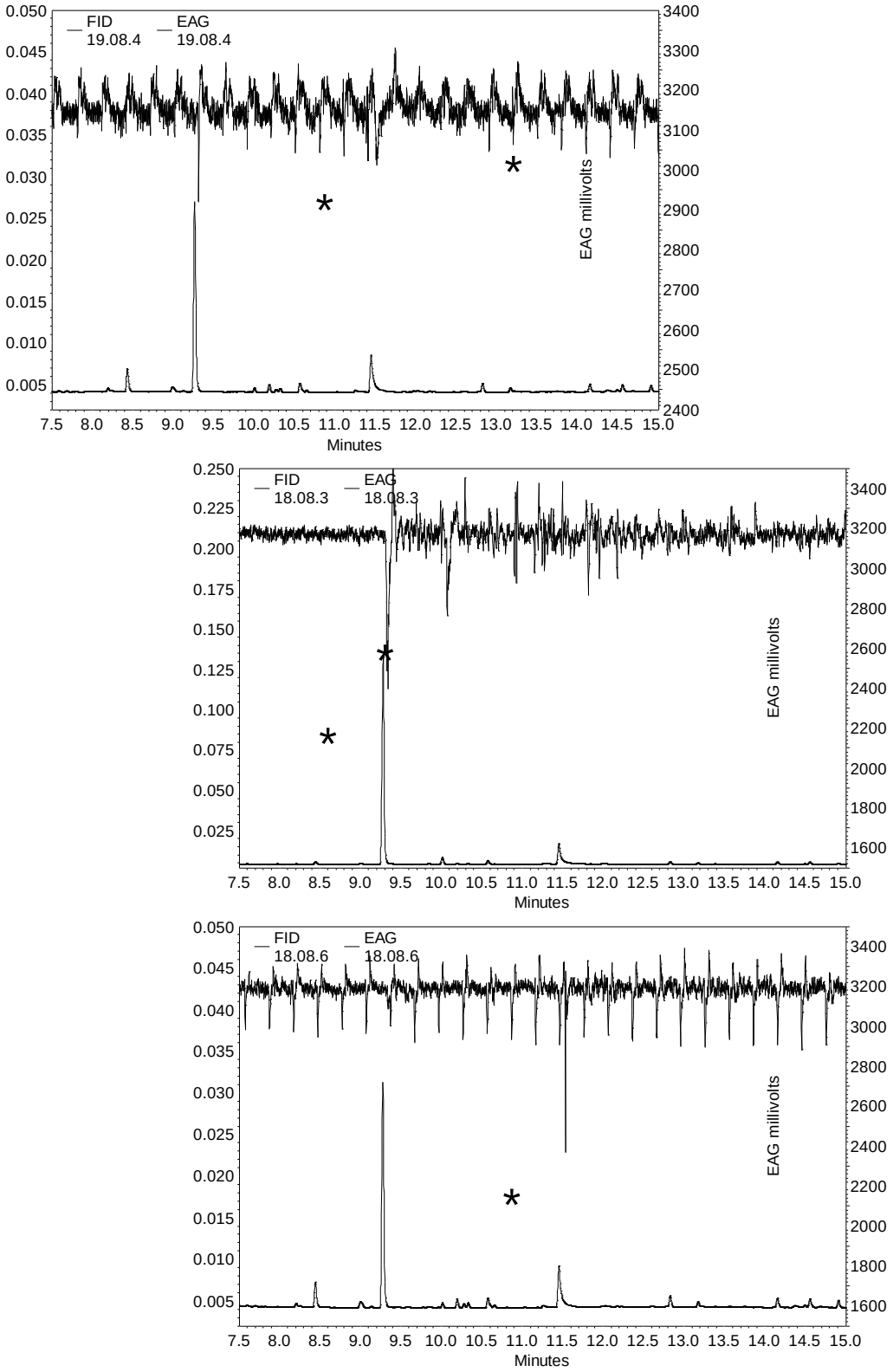

Figure 3.1.4. GC-EAG analyses of volatiles from female *L. rugulipennis* with male EAG preparation (* EAG response; HB at 9.32 min, E2HB at 10.08 min, KA at 11.52 min)

Conclusions

- Still air bioassays conducted this year failed to clarify results from year 1. Bioassays with both species failed to show attraction to the opposite sex.
- Male *L. rugulipennis* also failed to respond positively to the pheromone lures in the bioassay.
- Using mirids captured at the most reproductively active time of year failed demonstrate attraction.
- It is proposed that efforts at determining attraction of male and females should be concentrated in field trials in 2009.
- EAG responses were observed to all three of the potential pheromone components, HB, E2HB and KA, but never to all three in the same analysis.

Task 3.2. Use bioassay to evaluate attraction of *L. rugulipennis*, *L. pabulinus* and *L. tripustulatus* to blends of identified pheromone components released from different dispensing systems.

See section 3.1 for bioassay using pheromone reservoir lures in the laboratory with *L. rugulipennis*. Further studies were performed to identify the ratio of the 3 pheromone components released from *L. rugulipennis* (this section), *L. pabulinus* (see task 1.2) and *L. tripustulatus* (see task 2.1).

Materials and methods
Pheromone collections from *L. rugulipennis* were done using the standard air entrainment method in a controlled environment room at EMR (see task 1.2 for details of methodology) and GC analysis was performed at NRI.

37 females were entrained between 1 July and 20 August at 3 different times of day (Table 3.2.1);

Night 2400-0800 (light for 1.5 h, dark for 6.5 h) (period I)
Day 0800-1600 (dark for 1 h, light for 7 h) (period II)
Evening 1600-2400 (light for 8 h) (period III)

After chemical analyses of the filters, females that died before the end of the entrainment and females that emitted no compounds were omitted from the final calculation of the average amount of each of the 3 compounds released from females.

Table 3.2.1. Details of *L. rugulipennis* females used in the entrainment. I = treatment dark, II = treatment morning, III = treatment afternoon, * = field collected adult.

NRI code 2008.029/	Capsid i.d.	Date adult emerged	Date in	Time in	Filter No.	Date ended	Time ended	Treat.	Dead/Alive /Missing at end of test
1	49	23-Jun	01-Jul	16:00	1	04-Jul	09:30	I	d
2	49	23-Jun	01-Jul	16:00	1	04-Jul	09:30	II	d
3	49	23-Jun	01-Jul	16:00	1	04-Jul	09:30	III	d
4	50	23-Jun	01-Jul	16:00	2	04-Jul	09:30	I	a
5	50	23-Jun	01-Jul	16:00	2	04-Jul	09:30	II	a
6	50	23-Jun	01-Jul	16:00	2	04-Jul	09:30	III	a
7	51	27-Jun	01-Jul	16:00	5	04-Jul	09:30	I	a
8	51	27-Jun	01-Jul	16:00	5	04-Jul	09:30	II	a
9	51	27-Jun	01-Jul	16:00	5	04-Jul	09:30	III	a
10	52	27-Jun	04-Jul	18:00	1	07-Jul	09:00	I	a
11	52	27-Jun	04-Jul	18:00	1	07-Jul	09:00	II	a
12	52	27-Jun	04-Jul	18:00	1	07-Jul	09:00	III	a
13	53	27-Jun	04-Jul	18:00	2	07-Jul	09:00	I	a
14	53	27-Jun	04-Jul	18:00	2	07-Jul	09:00	II	a
15	53	27-Jun	04-Jul	18:00	2	07-Jul	09:00	III	a
16	54	27-Jun	04-Jul	18:00	3	07-Jul	09:00	I	a
17	54	27-Jun	04-Jul	18:00	3	07-Jul	09:00	II	a
18	54	27-Jun	04-Jul	18:00	3	07-Jul	09:00	III	a
19	55	27-Jun	04-Jul	18:00	4	07-Jul	09:00	I	a
20	55	27-Jun	04-Jul	18:00	4	07-Jul	09:00	II	a
21	55	27-Jun	04-Jul	18:00	4	07-Jul	09:00	III	a
22	56	27-Jun	04-Jul	18:00	5	07-Jul	09:00	I	a
23	56	27-Jun	04-Jul	18:00	5	07-Jul	09:00	II	a
24	56	27-Jun	04-Jul	18:00	5	07-Jul	09:00	III	a
25	57	27-Jun	04-Jul	18:00	6	07-Jul	09:00	I	a
26	57	27-Jun	04-Jul	18:00	6	07-Jul	09:00	II	a
27	57	27-Jun	04-Jul	18:00	6	07-Jul	09:00	III	a
28	58	01-Jul	07-Jul	18:00	1	10-Jul	08:30	I	a
29	58	01-Jul	07-Jul	18:00	1	10-Jul	08:30	II	a
30	58	01-Jul	07-Jul	18:00	1	10-Jul	08:30	III	a
31	59	01-Jul	07-Jul	18:00	2	10-Jul	08:30	I	a
32	59	01-Jul	07-Jul	18:00	2	10-Jul	08:30	II	a
33	59	01-Jul	07-Jul	18:00	2	10-Jul	08:30	III	a
34	60	01-Jul	07-Jul	18:00	3	10-Jul	08:30	I	a
35	60	01-Jul	07-Jul	18:00	3	10-Jul	08:30	II	a
36	60	01-Jul	07-Jul	18:00	3	10-Jul	08:30	III	a
37	61	01-Jul	07-Jul	18:00	4	10-Jul	08:30	I	a
38	61	01-Jul	07-Jul	18:00	4	10-Jul	08:30	II	a
39	61	01-Jul	07-Jul	18:00	4	10-Jul	08:30	III	a

Table 3.2.1. continued.

NRI code 2008.029/	Capsid i.d.	Date adult emerged	Date in	Time in	Filter No.	Date ended	Time ended	Treat.	Dead/Alive /Missing at end of test
40	62	*	07-Jul	18:00	5	10-Jul	08:30	I	a
41	62	*	07-Jul	18:00	5	10-Jul	08:30	II	a
42	62	*	07-Jul	18:00	5	10-Jul	08:30	III	a
43	63	*	07-Jul	18:00	6	10-Jul	08:30	I	a
44	63	*	07-Jul	18:00	6	10-Jul	08:30	II	a
45	63	*	07-Jul	18:00	6	10-Jul	08:30	III	a
46	64	03-Jul	10-Jul	17:30	1	14-Jul	14:00	I	a
47	64	03-Jul	10-Jul	17:30	1	14-Jul	14:00	II	a
48	64	03-Jul	10-Jul	17:30	1	14-Jul	14:00	III	a
49	65	*	10-Jul	17:30	2	14-Jul	14:00	I	a
50	65	*	10-Jul	17:30	2	14-Jul	14:00	II	a
51	65	*	10-Jul	17:30	2	14-Jul	14:00	III	a
52	66	*	10-Jul	17:30	3	14-Jul	14:00	I	d
53	66	*	10-Jul	17:30	3	14-Jul	14:00	II	d
54	66	*	10-Jul	17:30	3	14-Jul	14:00	III	d
55	67	*	10-Jul	17:30	4	14-Jul	14:00	I	a
56	67	*	10-Jul	17:30	4	14-Jul	14:00	II	a
57	67	*	10-Jul	17:30	4	14-Jul	14:00	III	a
58	68	*	10-Jul	17:30	5	14-Jul	14:00	I	a
59	68	*	10-Jul	17:30	5	14-Jul	14:00	II	a
60	68	*	10-Jul	17:30	5	14-Jul	14:00	III	a
61	69	*	10-Jul	17:30	6	14-Jul	14:00	I	a
62	69	*	10-Jul	17:30	6	14-Jul	14:00	II	a
63	69	*	10-Jul	17:30	6	14-Jul	14:00	III	a
64	80	07-Jul	15-Jul	08:30	1	17-Jul	09:00	I	m
65	80	07-Jul	15-Jul	08:30	1	17-Jul	09:00	II	m
66	80	07-Jul	15-Jul	08:30	1	17-Jul	09:00	III	m
67	81	07-Jul	15-Jul	08:30	2	17-Jul	09:00	I	a
68	81	07-Jul	15-Jul	08:30	2	17-Jul	09:00	II	a
69	81	07-Jul	15-Jul	08:30	2	17-Jul	09:00	III	a
70	82	07-Jul	15-Jul	08:30	3	17-Jul	09:00	I	a
71	82	07-Jul	15-Jul	08:30	3	17-Jul	09:00	II	a
72	82	07-Jul	15-Jul	08:30	3	17-Jul	09:00	III	a
73	83	07-Jul	15-Jul	08:30	4	16-Jul	10:00	I	d
74	83	07-Jul	15-Jul	08:30	4	16-Jul	10:00	II	d
75	83	07-Jul	15-Jul	08:30	4	16-Jul	10:00	III	d
76	84	07-Jul	15-Jul	08:30	5	17-Jul	09:00	I	d
77	84	07-Jul	15-Jul	08:30	5	17-Jul	09:00	II	d
78	84	07-Jul	15-Jul	08:30	5	17-Jul	09:00	III	d
79	85	07-Jul	15-Jul	08:30	6	17-Jul	09:00	I	a
80	85	07-Jul	15-Jul	08:30	6	17-Jul	09:00	II	a
81	85	07-Jul	15-Jul	08:30	6	17-Jul	09:00	III	a

Table 3.2.1. continued.

NRI code 2008.029/	Capsid i.d.	Date adult emerged	Date in	Time in	Filter No.	Date ended	Time ended	Treat.	Dead/Alive /Missing at end of test
82	86	07-Jul	18-Jul	16:00	1	21-Jul	08:30	I	a
83	86	07-Jul	18-Jul	16:00	1	21-Jul	08:30	II	a
84	86	07-Jul	18-Jul	16:00	1	21-Jul	08:30	III	a
85	87	07-Jul	18-Jul	16:00	2	21-Jul	08:30	I	a
86	87	07-Jul	18-Jul	16:00	2	21-Jul	08:30	II	a
87	87	07-Jul	18-Jul	16:00	2	21-Jul	08:30	III	a
88	88	07-Jul	18-Jul	16:00	3	21-Jul	08:30	I	a
89	88	07-Jul	18-Jul	16:00	3	21-Jul	08:30	II	a
90	88	07-Jul	18-Jul	16:00	3	21-Jul	08:30	III	a
91	89	07-Jul	18-Jul	16:00	4	21-Jul	08:30	I	a
92	89	07-Jul	18-Jul	16:00	4	21-Jul	08:30	II	a
93	89	07-Jul	18-Jul	16:00	4	21-Jul	08:30	III	a
94	90	07-Jul	18-Jul	16:00	5	21-Jul	08:30	I	a
95	90	07-Jul	18-Jul	16:00	5	21-Jul	08:30	II	a
96	90	07-Jul	18-Jul	16:00	5	21-Jul	08:30	III	a
97	91	07-Jul	18-Jul	16:00	6	21-Jul	08:30	I	a
98	91	07-Jul	18-Jul	16:00	6	21-Jul	08:30	II	a
99	91	07-Jul	18-Jul	16:00	6	21-Jul	08:30	III	a
100	92	11-Aug	18-Aug	17:15	3	20-Aug	16:15	I	a
101	92	11-Aug	18-Aug	17:15	3	20-Aug	16:15	II	a
102	92	11-Aug	18-Aug	17:15	3	20-Aug	16:15	III	a
103	93	11-Aug	18-Aug	17:15	4	20-Aug	16:15	I	a
104	93	11-Aug	18-Aug	17:15	4	20-Aug	16:15	II	a
105	93	11-Aug	18-Aug	17:15	4	20-Aug	16:15	III	a
106	94	11-Aug	18-Aug	17:15	5	20-Aug	16:15	I	d
107	94	11-Aug	18-Aug	17:15	5	20-Aug	16:15	II	d
108	94	11-Aug	18-Aug	17:15	5	20-Aug	16:15	III	d
109	95	11-Aug	18-Aug	17:15	6	20-Aug	16:15	I	a
110	95	11-Aug	18-Aug	17:15	6	20-Aug	16:15	II	a
111	95	11-Aug	18-Aug	17:15	6	20-Aug	16:15	III	a

Results

The majority of the hexyl butyrate (HB) was released in the daytime (0800-1600) or evening (1600-2400). Individuals that had died by the end of the entrainment period tended to release more HB and so were deleted from the final calculation of the mean amount of compounds released (individuals omitted from calculation = 49,66,83,84 dead; 80 missing). In general, more females released HB in the day (19) compared to the evening (9) or nighttime (3).

The quantity of each pheromone compound released varied from female to female. Some females did not release any compounds at all (e.g., female No 58). Hence, the ratio of the 3 compounds and time of day released also differed depending on the individual.

Between 2400-0800 (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA) were not detectable. It could also be seen that some individuals 'called' in the second part (e.g. female No 51) of the day instead of the morning (e.g. female No 89, Fig. 3.2.2). Equally, it can be seen (Fig. 3.2.3) that dieing females tended to produce far more HB than females alive by the end of the test. A comparison of the 3 compound ratios released by the 3 species under study in this project are given in Fig. 3.2.4.

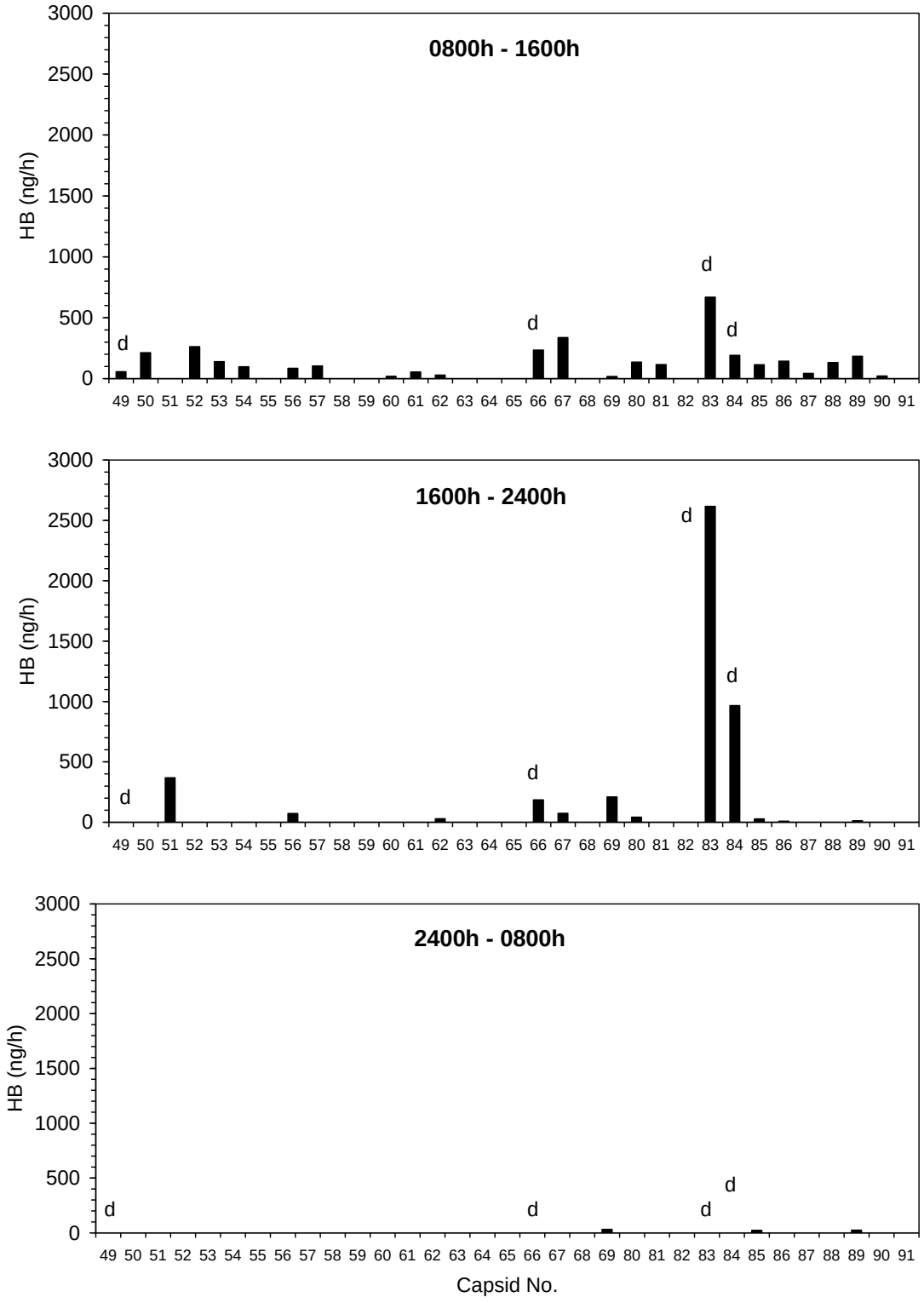

Figure 3.2.1. Quantity of hexyl butyrate (HB) released by single female *L. rugulipennis* (10-21 July) over 2-4 d separated into the 3 times of the day. Night 2400-0800, day 0800-1600 and evening 1600-2400.

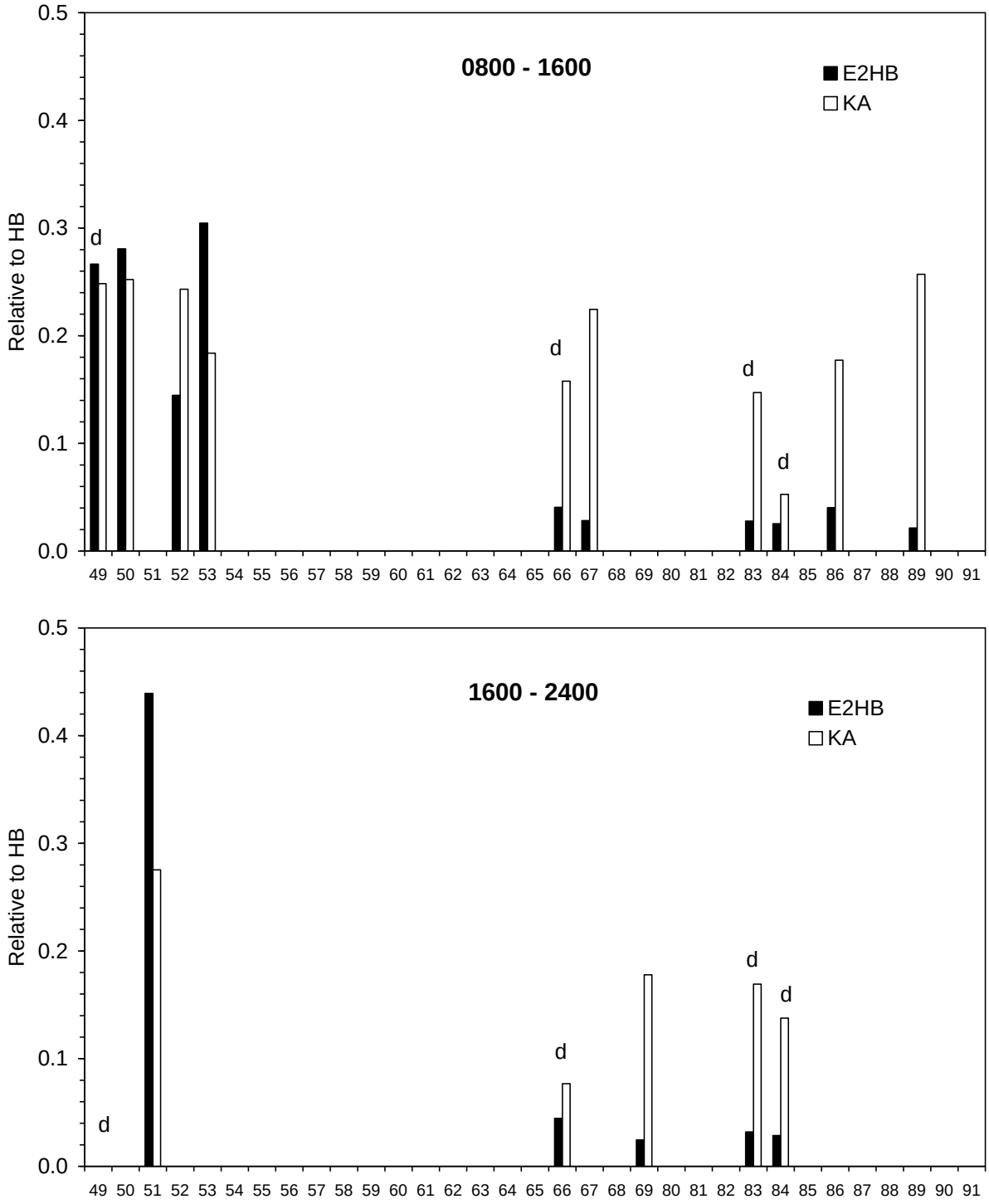

Figure 3.2.2. Quantity of (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA) relative to hexyl butyrate (HB) released by single female *L. rugulipennis* (10-21 July) over 2-4 d separated into the 2 times of the day. Day 0800-1600 and evening 1600-2400.

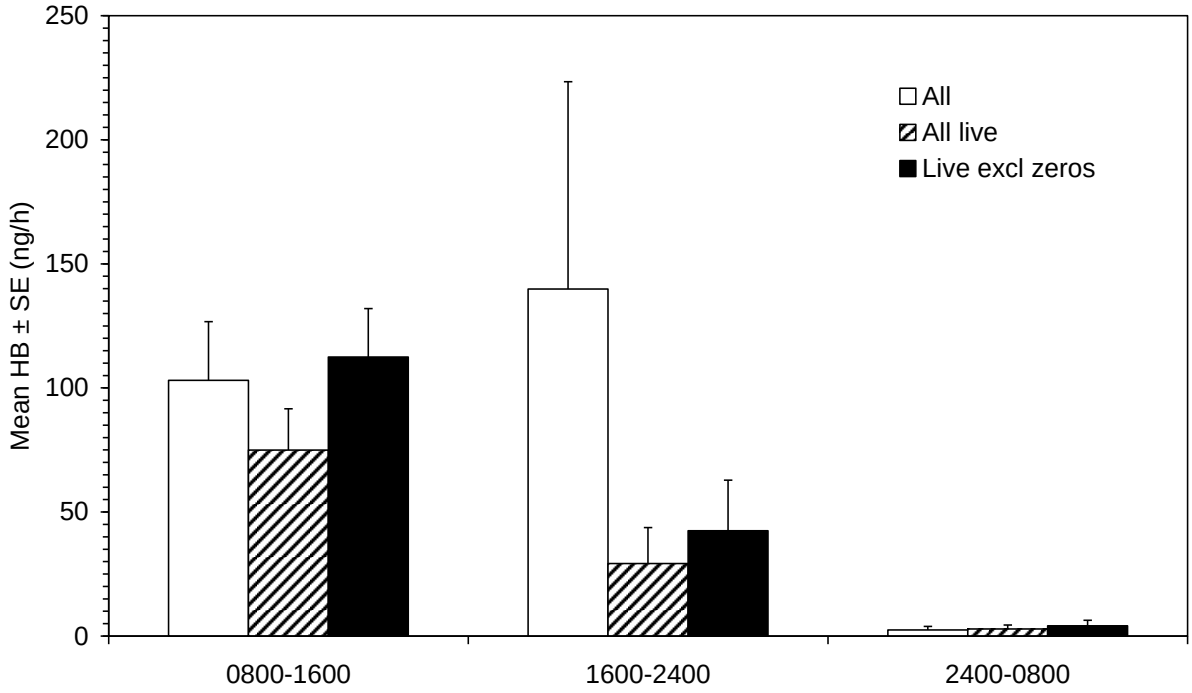

Figure 3.2.3. Average amount of hexyl butyrate (HB) released by all female *L. rugulipennis* (open bars), only live females (hatched bars) and all live females with those which produced no volatiles omitted (black bars). Quantity released over 2-4 days separated into the 3 times of the day (10-21 July).

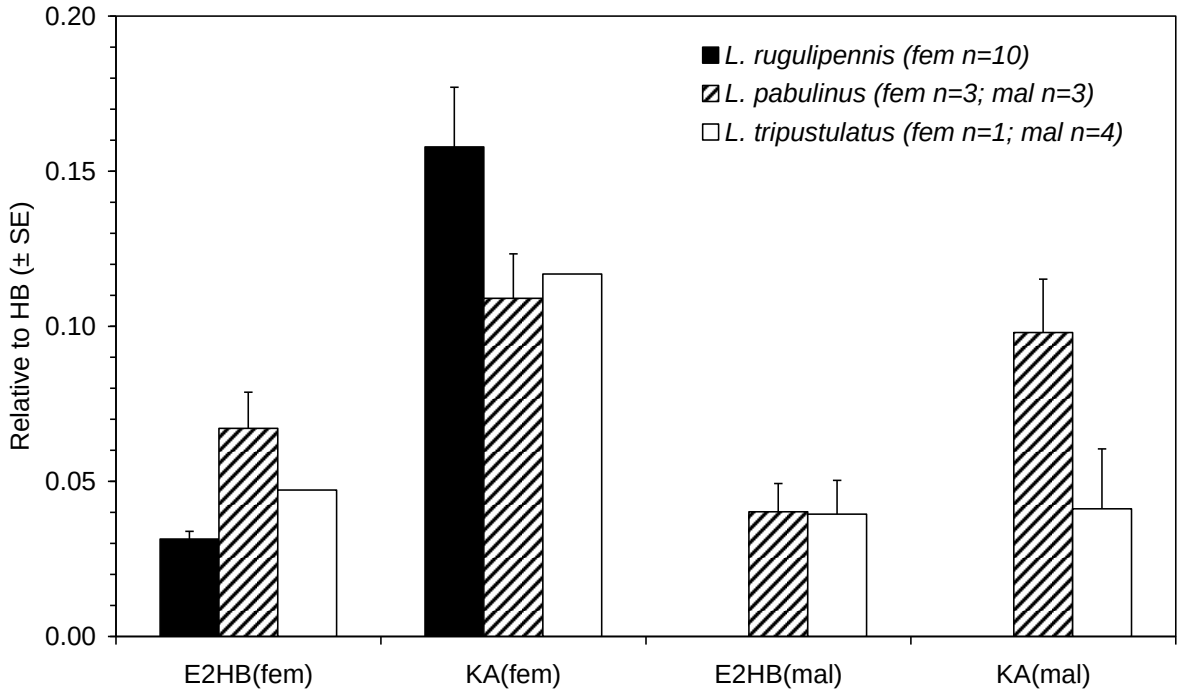

Figure 3.2.4. Amounts of E2HB and KA released by female *L. rugulipennis* and male and female *L. tripustulatus* and *L. pabulinus* in relation to HB.

Conclusions

- The amounts of HB, E2HB and KA released by female *L. rugulipennis* were very variable from individual to individual. Most material seemed to be released during the day (0800-1600 hrs) and evening (1600-2400 hrs) periods with little material released during the night (0000-0800 hrs) period. The mean amount of hexyl butyrate released was of the order of 100 ng/hr during the day period, but live individuals released at up to 500 ng/hr and up to 2,500 ng/hr was obtained from females which were dead at the end of the collection.
- The ratios of HB:E2HB:KA in the earlier collections made in late June were 1.00 : 0.29 (± 0.05 SE) : 0.24 (± 0.02) (n = 5). The ratios in collections made in July were 1.00 : 0.03 (± 0.002 SE) : 0.16 (± 0.02 SE) (n = 10).

Task 3.3. Carry out field trapping trials to relate laboratory results on pheromone blends and dispensers to attractiveness under field conditions (EMR, Yrs 1-2)

No attraction to micro-capillary reservoir dispensers was demonstrated in the still air bioassay. This trial aimed to test the attractiveness of blends of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal dispensed from glass micro-capillaries with a reservoir to *L. rugulipennis* and *L. pabulinus* in the field.

Materials and Methods

1. Field tests with micro-capillary reservoir lures - 3 way blends with PAA

Large scale randomised block experiments comparing catches of mirids in green delta traps or white cross vane funneled bucket traps bated with different blends of the 3 pheromone components with or without the phenyl acetaldehyde lures (PAA, 100 mg, Table 3.3.1) were tested in plots of apple, blackcurrant, cucumber, strawberry and weeds (Table 3.3.2).

The previously estimated ratio of HB:E2HB:KA released by a single *L. rugulipennis* female was 1.5:1.0:0.08. *L. rugulipennis* males were attracted to a blend of 1:1 HB:KA and *L. pratensis* was attracted to a blend of 3:2:3 HB:E2HB:KA (Innocenzi *et al.*, 2005). Two way and 3 way blends were made by combining components in the same ratio. In contrast to year 1, sunflower oil was used as a carrying agent. In total, nine treatments (A-I) comprising microcapillary lures containing different blends of hexyl butyrate ('HB'), (*E*)-2-hexenyl butyrate ('E2HB') and (*E*)-4-oxo-2-hexenal ('KA') with and without the plant volatile, phenyl acetaldehyde (PAA) were tested against an untreated (treatment J) control (Table 3.3.1). All possible two-way and three-way blends were tested in the same ratios. PAA was dispensed from a polyethylene sachet dispenser as described by Toth *et al.*, (pers. comm. 2008).

The lures were suspended vertically inside 20 x 20 cm green delta trap from a wire twist tie. The traps had a modified white sticky base (additional Ecotac applied, Fig. 3.3.1). Later in the season, a white cross vane funneled bucket trap containing water and detergent (Fig. 3.3.1) was used. Traps were suspended at mid crop height. Two replicates were placed on each site and plots were individual traps each containing lures (no lure in untreated) (Fig. 3.3.1).

The traps were deployed at site 1 on 11 June, at site 2 on 12 June and at site 3 on 17 June. Traps were moved from site 1 to site 5 on 27 June because of the low numbers of mirids caught (Table 3.3.2).
On 9 July the traps at site 5 were replaced with white cross vane bucket traps. The pheromone and PAA lures were also renewed at this time. The replicate 1 traps were also changed at Site 1 on 14

July. The same pheromone lures were used and the PAA lures replaced. The PAA lure and trap design was also changed at site 3 on 15 July.

The trials were ended on 6 August (site 2), 18 September (site 3) and 1 October (site 5). By 12 September the crop at site 4 was in full production. Bucket traps and delta traps were in place for 3 and 9 weeks, respectively, from 24 June at Cavgate Nursery and 28 June at Poolbank Nursery. Trap positions were changed periodically throughout the growing season at this site. This trial was ended on 23 September.

Weekly counts (where possible) of the numbers, sex and species of mirid trapped were determined. For green delta traps, sticky bases were stored in pizza boxes and capsid identification confirmed using a microscope in the laboratory. Funnel traps were sieved through gauze and the contents frozen until it could be identified in the laboratory.

Figure 3.3.1. (a) microcapillary reservoir lure (b) green delta trap with lure (c) PAA sachet in white cross vane funneled bucket trap (d) pheromone lure from second experiment in cross vane funneled bucket trap.

Table 3.1.1. Treatments used at the 5 sites (Table 3.1.2).

	Amounts of components in lures			
Treatment	HB (mg)	E2HB (mg)	KA (mg)	PAA (mg)
A	10	7	0	0
B	10	0	10	0
C	0	7	10	0
D	10	7	10	0
E (with A)	10	7	0	100
F (with B)	10	0	10	100
G (with C)	0	7	10	100
H (with D)	10	7	10	100
I	0	0	0	100
J	0	0	0	0

Notes: All + sunflower oil as a carrier.

Table 3.3.2. Sites used for testing the efficacy of the microcapillary lure with a reservoir.

Site No.	Crop	Site description	Target pest	Location	Person responsible
1	Apple (organic)	Various apple varieties. Traps in long line along perimeter of orchard next to hedgerow 12 m apart.	*L. pabulinus*	Nichol Farm, Deerton Street, Teynham ME9 9LJ	John Moor
2	Blackcurrant	23 rows of blackcurrant. Used 1st and 3rd row from edge (next to river), 12 m apart in 2 rows. Mown grass alleyways. Mixed hedgerow on 2 sides, road on one side and blackcurrant plot on another.	*L. pabulinus*	Stonebridge Ben Alder field rented from W Calcutt by T Maynard at NGR TQ 718 398	Rob Saunders & Bill Calcutt
3	Strawberry	Protected beds (5 per tunnel). Used row 50, 55 for rep 1 and 70 and 75 for rep 2. Traps 12 m apart.	*L. rugulipennis*	Tueslay Farm, Milford nr Godalming	Michelle Fountain and Gunnhild Jastaad
4	Cucumber	5 separate glasshouses – each with one replicate of each trap separated as far as possible. Traps orientated parallel to one another in line with the crop rows.	*L. rugulipennis*	Cavegate Nurseries, South Cave; Poolbank VHB, Welton; and Halsham Growers, Cottingham, E Yorks	Rob Jacobson
5	Weed field (organic)	Sown with fathen and mayweed. Traps spaced 10 m.	*L. rugulipennis*	Palmers Rough at East Malling Research, New Road, East Malling, Kent ME19 6BJ	Michelle Fountain and Gunnhild Jastaad

2. Micro-capillary reservoir lure with new combination of components from Task 3.2

A small scale randomised block experiment comparing catches of capsids in white cross vane funneled bucket traps baited with a lure was begun on 26 August in the weed field ('Palmers Rough') at EMR. The two treatments were microcapillaries containing;

1. 3-component mix 10% in sunflower oil giving 1:0.025:0.25 hexyl butyrate ('HB') : (*E*)-2-hexenyl butyrate ('E2HB') : (*E*)-4-oxo-2-hexenal ('KA') with a release rate of 0.5ug/h
2. control - oil only

The ratio of the blend was based on entrainment extractions from *L. rugulipennis* females in July 2008 (see Task 3.2). Dispensers for the pheromone components were sample vials fitted with a 5 µl glass micro-capillary (see above). The lure was suspended vertically inside a white cross vane funneled bucket trap and the trap was dug 5 cm into the ground. Water (250 ml) with a drop of detergent was poured into the bucket so that insects were unable to escape. The plots were individual traps each containing a lure. There were 10 replicates of each treatment positioned alternately and spaced 10 m apart.

Weekly counts (where possible) of the numbers, sex and species of mirid trapped were determined. Funnel traps were sieved through gauze and the contents frozen until it could be identified in the laboratory.

3. Piezoelectric sprayer

The piezoelectric sprayer was used to test the attractiveness of blends of female *L. rugulipennis* pheromone compounds to male capsids in the field by dispensing from a needle. The dispensing needle is attached to a piezoelectric disc which oscillates at a high frequency, volatilizing the liquid which passes through the needle and creates a point source of the material being dispensed (Fig. 3.3.2). Pheromone blends and entrainment extractions were released from the sprayer and mirids attracted to the plume sexed and identified. The sprayer was used in accordance with EMR Standard Operating Procedure 821 *Use of piezoelectric sprayer v1.* The sprayer was used in the weed field (1.2 ha, 'Palmers Rough'), at EMR (Fig. 3.3.2).

Using microcapillaries the most attractive blend to male *L. rugulipennis* males was found to be hexyl butyrate and *(E)*-4-oxo-2-hexenal (Innocenzi et al. 2005), whereas, all three components captured less *L. rugulipennis*, but more *L. pratensis.* The release rates of HB and KA would have been approximately 1 µg/h from these dispensers, although the ratio in volatiles from female *L. rugulipennis* was 19:1, HB:KA.

The tests were done on sunny still days (*L. rugulipennis* is less active in cold windy weather). The compounds were tested in the morning (when it has been shown that males are attracted to females), and at different ratios (30:1, 10:1, 1:1; HB:KA), maintaining the release rate of HB at 1 or 10 µg/h. The release from the syringe pump was set at a flow rate of 10 or 20 µl/min. Based on results from Task 3.2, a second set of solutions were tested;

1. 3-component mix 10% in sunflower oil giving 1:0.025:0.25 hexyl butyrate ('HB') : (*E*)-2-hexenyl butyrate ('E2HB') : (*E*)-4-oxo-2-hexenal ('KA') with a release rate of 0.5 µg/h
2. control - oil only

The 3-component mixture was 10% in solvent at a ratio of 1:0.025:0.25 at 2 µg/ml. In addition, natural collections (from entrainment) at similar concentrations in dichloromethane with and without GC peaks were tested. Different solvents were also tried, including hexane, dichloromethane and ethanol. The piezoelectric spray kit was placed at least 10 m in from the edge of the field and in a different location each day. The number of capsids in the field was high, as demonstrated by sweep netting and other field trials at the time. The species and sex of mirids attracted to the sprayer were recorded.

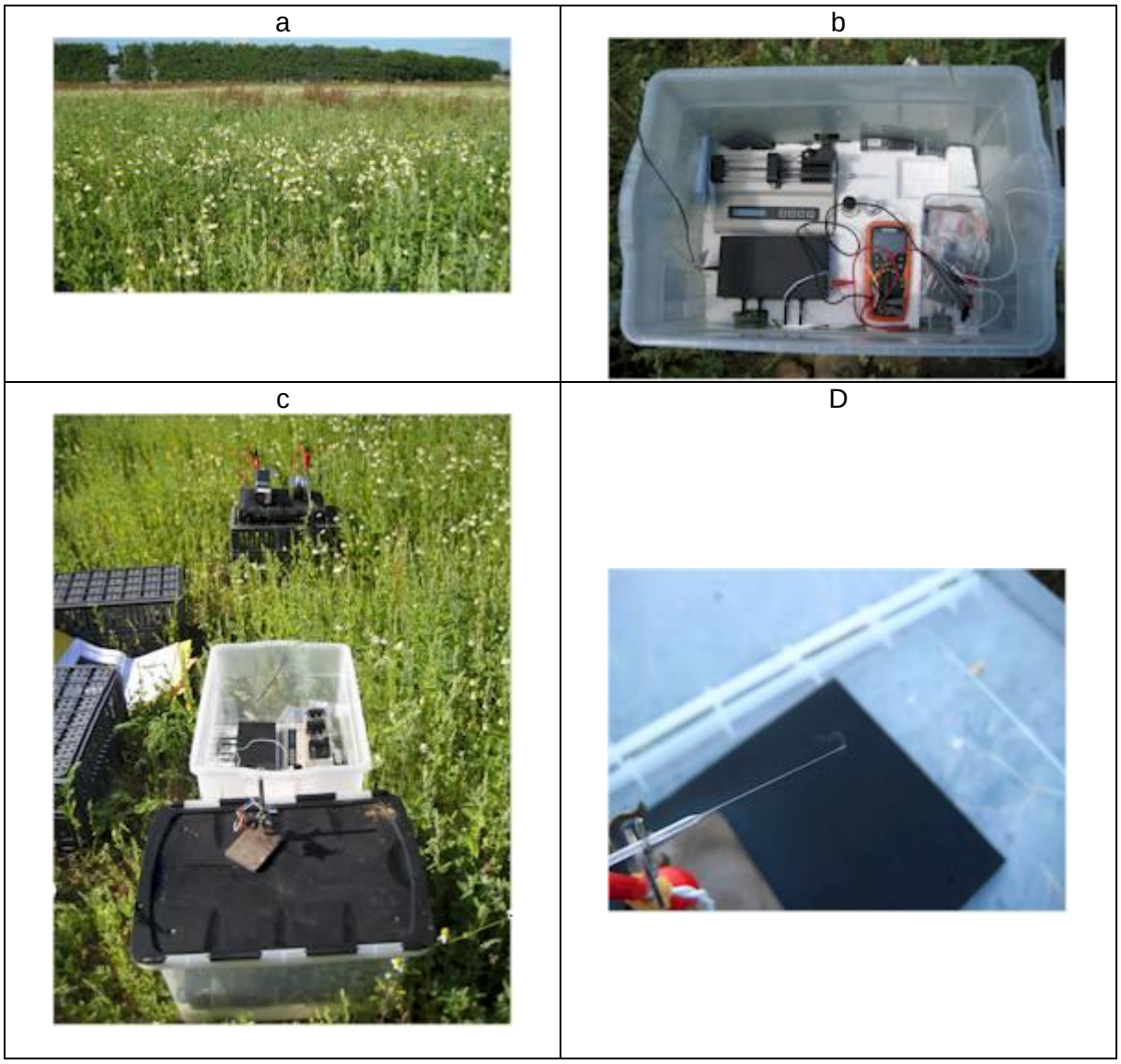

Figure 3.3.2. (a) Palmers Rough, weed field (b) piezoelectric spray equipment in box (c) Piezoelectric equipment set up in field (d) plume from end of needle.

Results

1. Field tests with micro-capillary reservoir lures - 3 way blends with PAA

At site 1 (apple) only one male *L. pabulinus* was trapped between 11 - 27 June in the E2HB 7 mg, KA 10mg, PAA 100mg treatment.

At site 2 (blackcurrant) very few *L. pabulinus* were trapped and those that were caught were not consistently in one treatment (Fig. 3.3.3). Other mirids trapped in low numbers at the blackcurrant site included *Heterogaster urticae*, *Calocoris norvegicus*, *Dichrooscytus rufipennis* and *Deraeocoris ruber.*

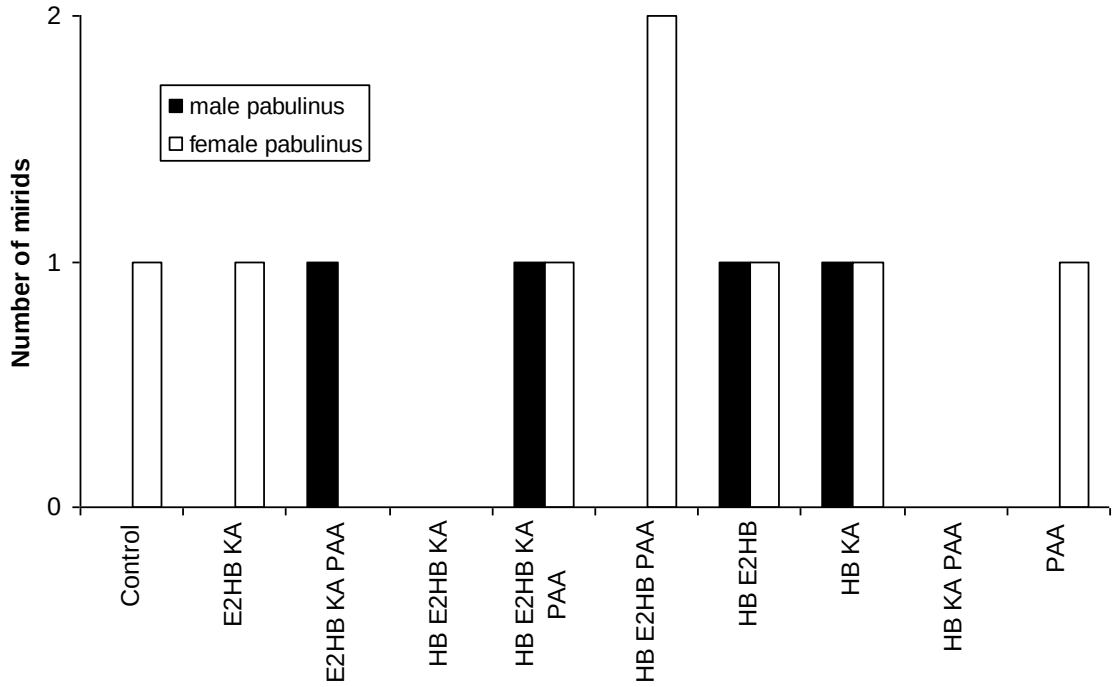

Figure 3.3.3. Numbers of male and female *Lygocoris pabulinus* trapped on the blackcurrant site between 12 June - 6 August

At site 3 (strawberry), between 17 June – 18 September, there was no significant attraction shown for male or female *L. rugulipennis* to the lures (Log_{10} transformed data; P=0.616, sed=0.3145, lsd=0.7114; P=0.213, sed=0.2617, lsd=0.5919, respectively). However, *Atractotomus mali* males were found in traps containing HB E2HB and KA for the first two weeks (Log_{10} transformed data; P=0.026, sed=0.1346, lsd=0.3045, Fig. 3.3.4)

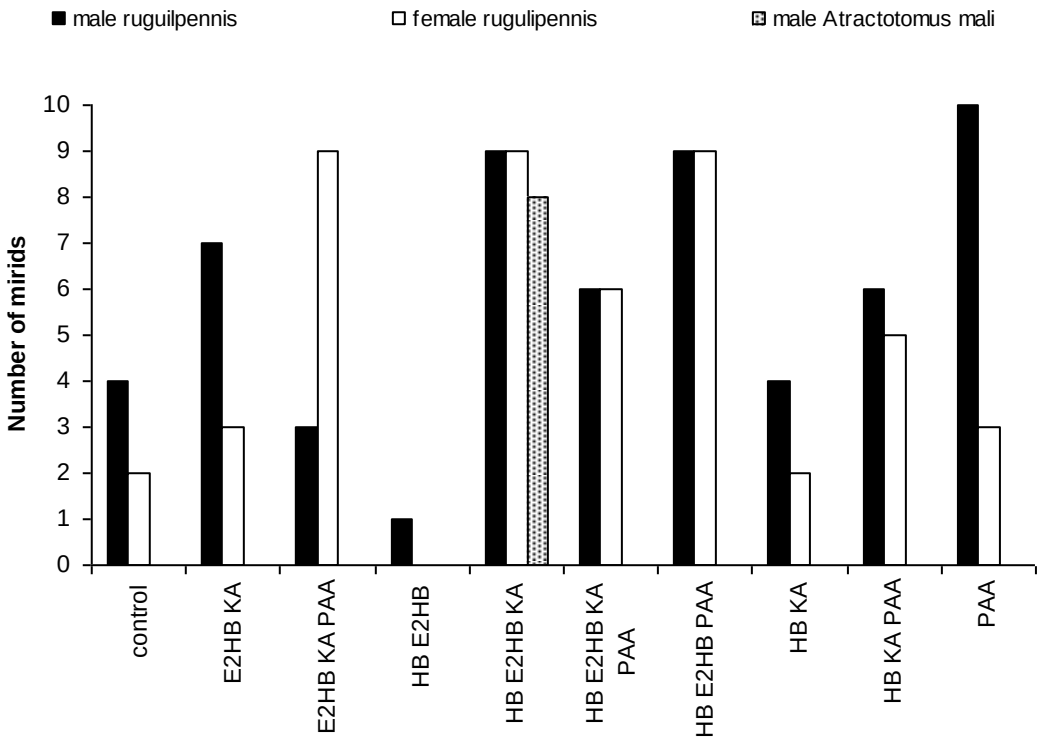

Figure 3.3.4. Numbers of male and female *Lygus rugulipennis and Atractotomus mali* trapped on the strawberry site between 17 June – 18 September.

Results from site 4 (cucumber) were also highly variable. There was some suggestion that female *L. rugulipennis* might be attracted to PAA. No males were trapped on this site (Fig. 3.3.5).

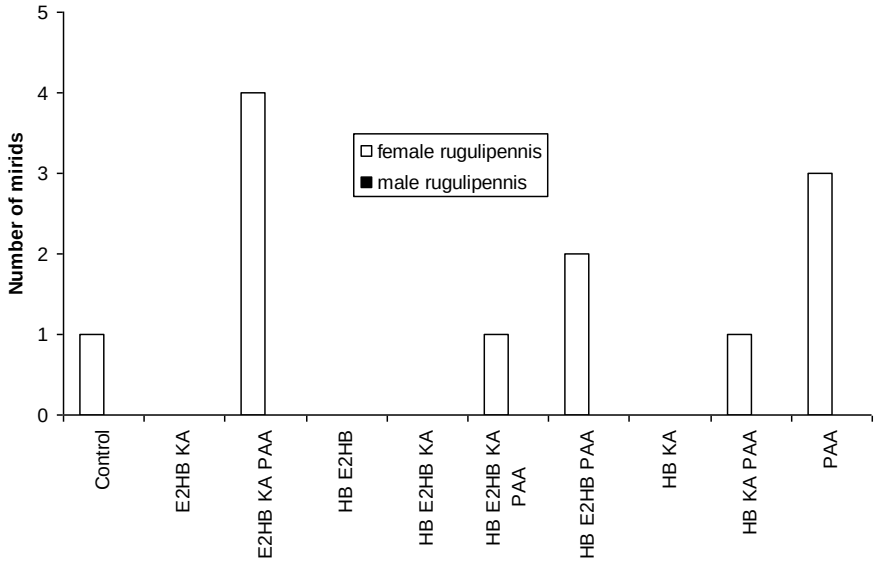

Figure 3.3.5. Numbers of male and female *Lygus rugulipennis* trapped on the cucumber sites between 17 June – 23 September.

In the weed field significantly more *L. rugulipennis* females were found in the PAA, compared to the control, E2HB KA, HB E2HB, HB E2HB KA and HB KA baited traps (Log_{10} transformed count data: P=0.02, sed=0.1635, lsd=0.3699, Fig. 3.3.6). The number of females was not significantly different from any of the traps which contained PAA plus pheromone. This may suggest that female *L. rugulipennis* were attracted to PAA. However, female *L. pratensis* were not significantly attracted into any of the traps compared to the control (Log_{10} transformed count data: P=0.175, sed=0.2143, lsd=0.6854, Fig. 3.3.7).

Male *L. pratensis* were more abundant in the HB E2HB KA treatment than the control, E2HB KA, HB E2HB, HB KA PAA and PAA baited traps. Hence, the males of this species were attracted to combinations with HB and E2HB, but only if they were combined with KA or PAA. PAA or HB:E2HB alone were not significantly attractive (Log_{10} transformed count data: P=0.029, sed=0.3845, lsd=0.8697, Fig. 3.3.7). Male *L. rugulipennis* were not significantly attracted into any of the traps compared to the control (Log_{10} transformed count data: P=0.222, sed=0.3352, lsd=0.7584, Fig. 3.3.6).

The numbers of other main invertebrate groups (bees, Heteroptera, grasshoppers, Diptera, Hymenoptera, spiders/harvestmen, molluscs and ants) trapped in the bucket traps in the weed field between 21 July- 5 August were not significantly different. Therefore, no other major groupings of invertebrates appeared to be attracted to the lures. Several small rodents did become trapped in the bucket traps.

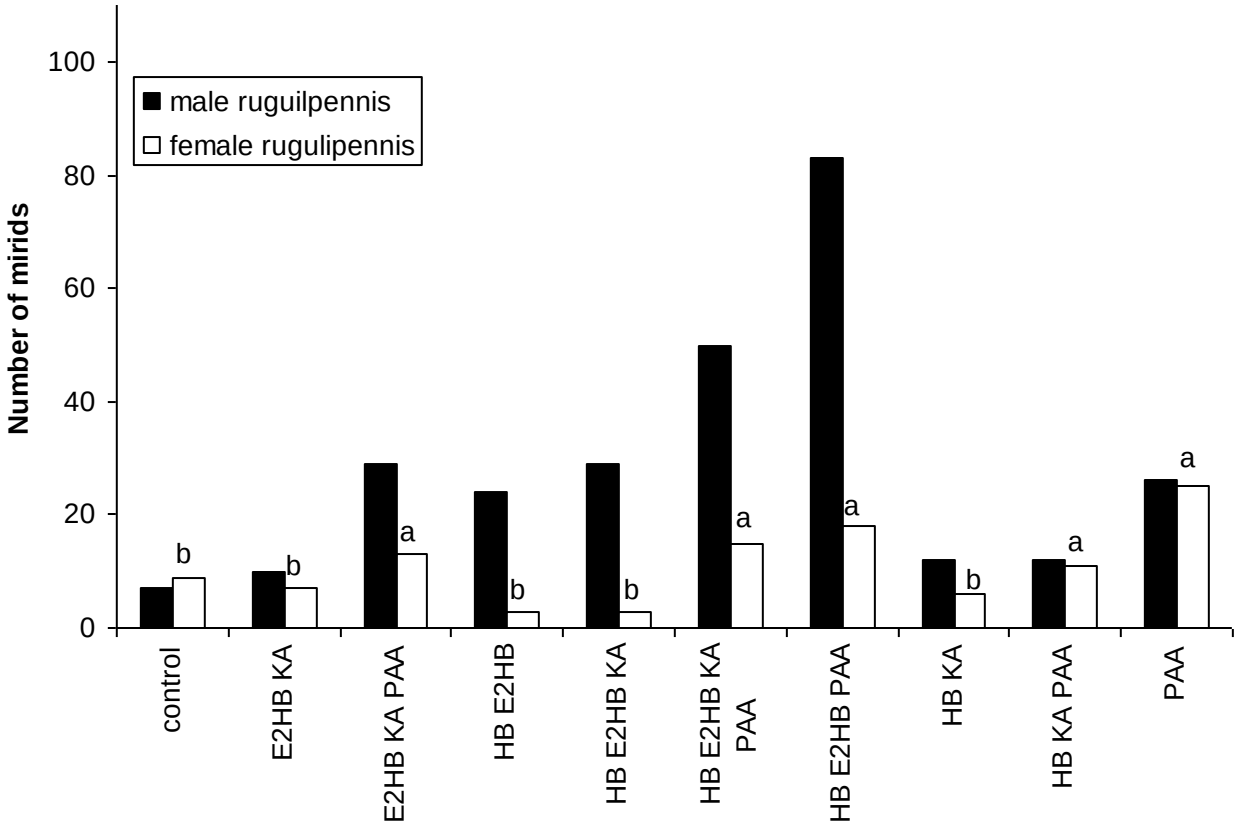

Figure 3.3.6. Numbers of male and female *Lygus rugulipennis* trapped on the weed field site between 27 June – 1 October. Different letter denote significant differences between the numbers of females in the treatments.

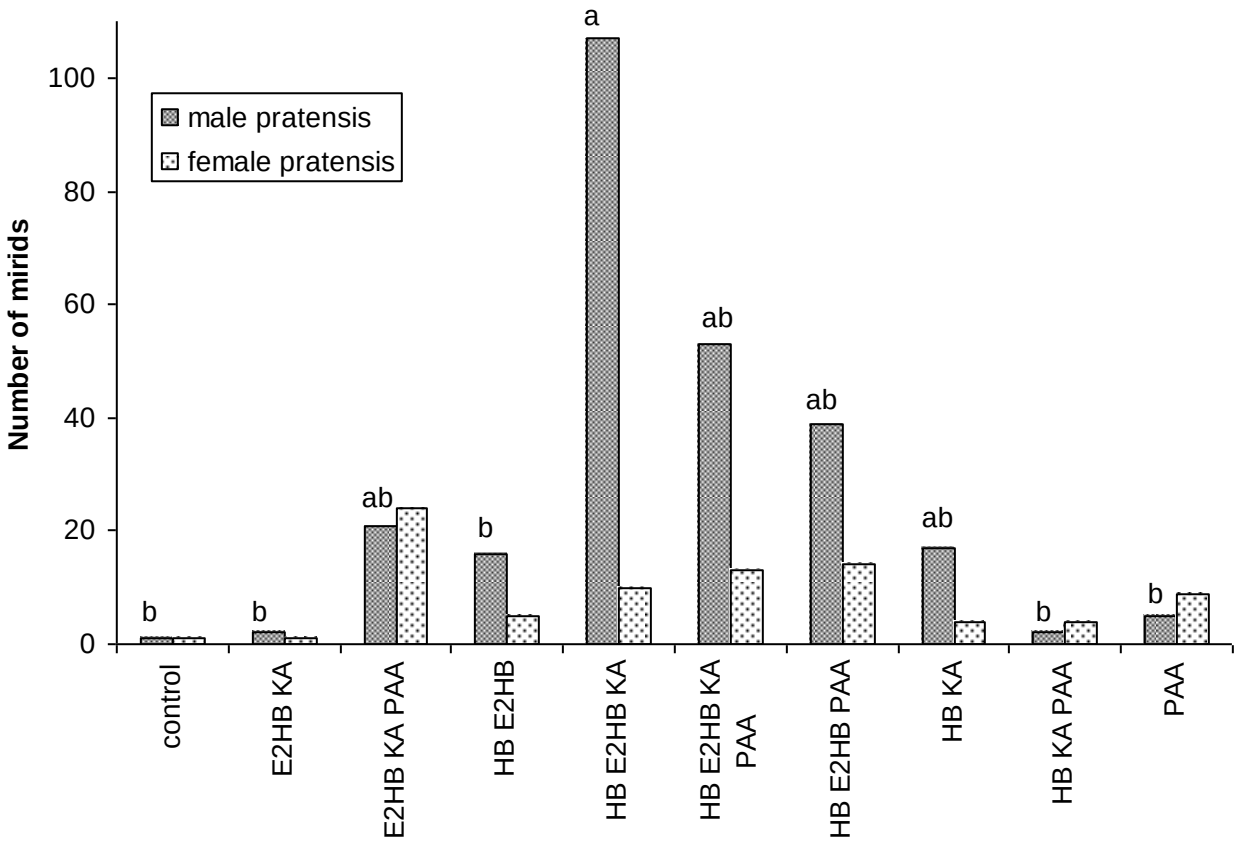

Figure 3.3.7. Numbers of male and female *Lygus pratensis* trapped on the weed field site between 27 June – 1 October. Different letter denote significant differences between the numbers of males in the treatments.

2. Micro-capillary reservoir lure with new combination of components from Task 3.2

Between 27 August – 1 October significantly more male *L. rugulipennis* males were trapped in the pheromone lures compared to the control lures (Log_{10} transformed count data: P=0.018, sed=0.0956, lsd=0.2009, Fig. 3.3.8). Numbers of female *L. rugulipennis* were not significantly higher in the pheromone treated baited traps (Log_{10} transformed count data: P=0.502, sed=0.0697, lsd=0.1465, Fig. 3.3.8), indicating that the ratio of compounds were functioning as a female sex pheromone. However, numbers of capsids had begun to decline in the field by this time so further work is needed to confirm these results.

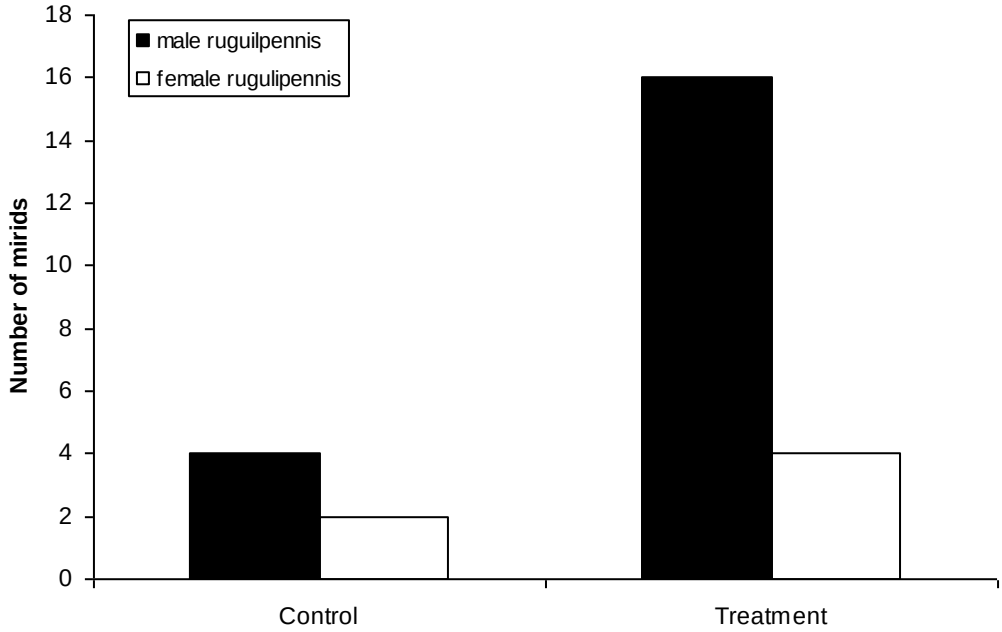

Figure 3.3.8. Numbers of male and female *Lygus rugulipennis* (n=5) trapped on the weed field site between 27 August – 1 October.

3. Piezoelectric sprayer

Over 23 hours in the field with 5 ratios of the 3 pheromone compounds and 3 different solvents attracted no mirids (Table 3.3.3). However, one treatment (18 Sep, 0825-0855) did attract 5 male *L. rugulipennis*, namely, 1:0.025:0.25 of hexyl butyrate ('HB') : (*E*)-2-hexenyl butyrate ('E2HB') : (*E*)-4-oxo-2-hexenal ('KA') with a release rate of 0.5 µg/h in hexane (Fig. 3.3.9). However, when this was repeated the next morning, no mirids were attracted. This may have been because it was an overcast day.

Ethanol had a tendency to expand the plastic connectors and cause the system to leak, whereas, DCM often froze and blocked the needle upon exiting the needle tip (Fig. 3.3.9).

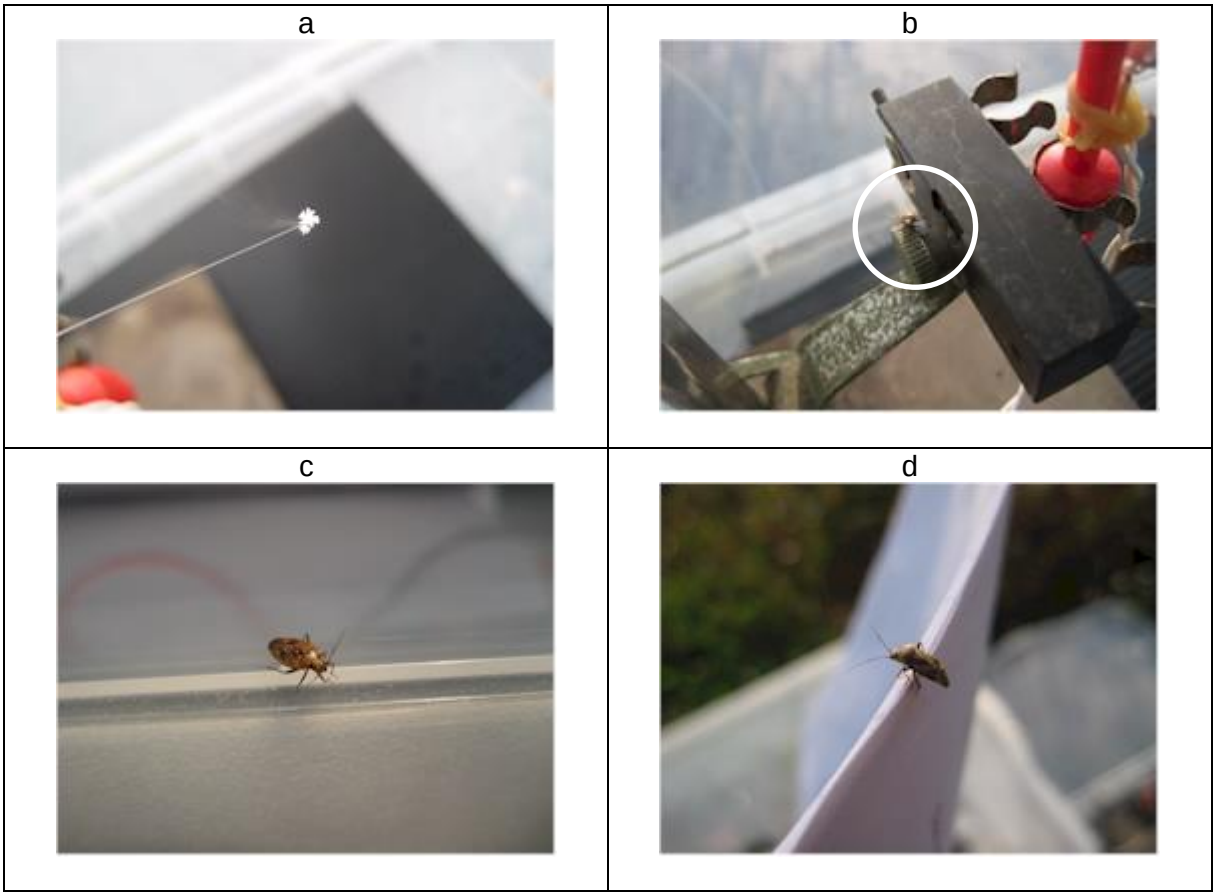

Figure 3.3.9. Photographs of the piezoelectric tests (a) DCM freezing at end of needle, (b) male *L. rugulipennis* on needle stand (c) male *L. rugulipennis* on box next to needle (d) male *L. rugulipennis* on notes. Photos b, c and d taken when ratio (1:0.025:0.25) of hexyl butyrate ('HB') : (*E*)-2-hexenyl butyrate ('E2HB') : (*E*)-4-oxo-2-hexenal ('KA') with a release rate of 0.5 µg/h in hexane was being used.

Table 3.3.3. Treatments tested with the piezoelectric sprayer. Amount and ratio of component in 1 ml solvent (hexane, ethanol or DCM) dispensed at 10 or 20 µl/min.

Date	Treatment and solvent	Time	Ratio HB:KA	No mirid observed
26 Jun	1 µg/h 023/1 hexane	0830-0930	30:1	0
26 Jun	1 µg/h 023/2 hexane	0945-1045	10:1	0
26 Jun	1 µg/h 023/3 hexane	1045-1145	1:1	0
26 Jun	10 µg/h 023/4 hexane	1150-1250	30:1	0
26 Jun	10 µg/h 023/5 hexane	1253-1353	10:1	0
26 Jun	10 µg/h 023/6 hexane	1356-1456	1:1	0
2 Jul	1 µg/h 023/3 hexane	0454-0530	1:1	0
2 Jul	1 µg/h 023/1 hexane	0531-0610	30:1	0
2 Jul	10 µg/h 023/6 hexane	0610-0655	1:1	0
2 Jul	10 µg/h 023/5 hexane	0656-0740	10:1	0
2 Jul	10 µg/h 023/4 hexane	0741-0827	30:1	0
2 Jul	1 µg/h 023/2 hexane	0828-0916	10:1	0
15 Jul	1 µg/h, 025/9 EtOH	0440-0455	0:1	0
15 Jul	10 µg/h, 025/16 EtOH	0455-0510	1:1	0
15 Jul	1 µg/h, 025/11 EtOH	0510-0525	10:1	0
15 Jul	1 µg/h, 025/12 EtOH	0525-0540	1:1	0
15 Jul	10 µg/h, 025/15 EtOH	0541-0556	10:1	0
15 Jul	10 µg/h, 025/13 EtOH	0557-0613	0:1	0
15 Jul	1 µg/h, 025/10 EtOH	0613-1629	30:1	0
15 Jul	10 µg/h, 025/14 EtOH	0629-0643	30:1	0
15 Jul	1 µg/h, 025/2 hexane	0644-0700	30:1	0
15 Jul	10 µg/h, 025/6 hexane	0701-0716	30:1	0
15 Jul	10 µg/h, 025/5 hexane	0717-0731	0:1	0
15 Jul	1 µg/h, 025/4 hexane	0732-0741	1:1	0
15 Jul	1 µg/h, 025/1 hexane	0747-0803	0:1	0
15 Jul	10 µg/h, 025/7 hexane	0803-0817	10:1	0
15 Jul	1 µg/h, 025/3 hexane	0818-0833	10:1	0
15 Jul	10 µg/h, 025/8 hexane	0833-0845	1:1	0

Table 3.3.3. continued

Date	Treatment and solvent	Time	Ratio HB:KA	No mirid observed
22 Jul	10 µg/h, 025/15 EtOH	0900-0911	10:1	0
22 Jul	10 µg/h, 025/15 EtOH	0912-0922	10:1	0
22 Jul	10 µg/h, 025/13 EtOH	0922-0933	0:1	0
22 Jul	10 µg/h, 025/13 EtOH	0933-0943	0:1	0
22 Jul	10 µg/h, 025/16 EtOH	0945-0955	1:1	0
22 Jul	10 µg/h, 025/16 EtOH	0956-1006	1:1	0
22 Jul	1 µg/h, 025/11 EtOH	1010-1020	10:1	0
22 Jul	1 µg/h, 025/12 EtOH	1026-1036	10:1	0
22 Jul	1 µg/h, 025/9 EtOH	1037-1047	0:1	0
22 Jul	1 µg/h, 025/10 EtOH	1049-1059	30:1	0
22 Jul	10 µg/h, 025/7 hexane	1100-1110	10:1	0
22 Jul	10 µg/h, 025/7 hexane	1153-1203	10:1	0
22 Jul	10 µg/h, 025/8 hexane	1204-1214	1:1	0
22 Jul	10 µg/h, 025/8 hexane	1215-1225	1:1	0
22 Jul	10 µg/h, 025/5 hexane	1227-1237	0:1	0
22 Jul	10 µg/h, 025/5 hexane	1237-1245	0:1	0
22 Jul	10 µg/h, 025/6 hexane	1248-1258	30:1	0
22 Jul	10 µg/h, 025/6 hexane	1300-1310	30:1	0
22 Jul	1 µg/h, 025/2 hexane	1315-1325	30:1	0
22 Jul	1 µg/h, 025/4 hexane	1326-1336	1:1	0
22 Jul	1 µg/h, 025/1 hexane	1345-1352	0:1	0
22 Jul	1 µg/h, 025/1 hexane	1352-1402	10:1	0

Table 3.3.3. continued

These solutions included the 3-component mixture, 10% in solvent at a ratio of 100:2.5:25 at 2ug/ml in; 1. hexane (027/4), 2. dichloromethane (027/5), 3. ethanol (027/6), 4. a natural collection at similar concentration in dichloromethane with GC peaks (027/9), 5. no GC peaks (027/10).				
Date	**Treatment and solvent**	**Time**	**Ratio HB:KA**	**No mirid observed**
18 Sep	hexane only	0735-0750	-	0
18 Sep	natural peaks DCM (027/9)	0753-0823	-	0
18 Sep	hexane (027/4)	0825-0855	100:2.5:25	5 male L. rugulipennis within 40 cm of needle
18 Sep	ethanol (027/6)	0855-0924	100:2.5:25	2 males flying away
18 Sep	hexane (027/4)	0924-0954	100:2.5:25	0
18 Sep	natural DCM with peaks (027/9)	0954-1024	-	0
18 Sep	natural DCM with no peaks (027/10)	1024-1054	-	0
18 Sep	DCM (027/5)	1054-1124	100:2.5:25	0
18 Sep	hexane (027/4)	1129-1143	100:2.5:25	0
18 Sep	hexane (027/4)	1145-1200	100:2.5:25	0
19 Sep	Hexane only	0742-0749		0
19 Sep	Hexane (027/4)	0749-0829	100:2.5:25	0
19 Sep	Ethanol (27/6)	0829-0845	100:2.5:25	0
19 Sep	DCM (027/5)	0845-0900	100:2.5:25	0

Conclusions

- No attraction of *L. pabulinus* to the pheromone field dispensers in the first trial was achieved.
- Significant numbers of *L. pratensis* males were attracted to a 3 way mix of hexyl butyrate ('HB') : (*E*)-2-hexenyl butyrate ('E2HB') : (*E*)-4-oxo-2-hexenal ('KA') (1.0: 0.7:1.0).
- *Atractotomus mali* was attracted to the 3 way mix of HB E2HB KA (1.0: 0.7:1.0) in the strawberry plots.
- Small numbers of female *L. rugulipennis* females were attracted to the PAA lures.
- Male *L. rugulipennis* males were attracted by the 1.0:0.025:0.25 of HB E2HB KA in hexane (piezoelectric sprayer) and sunflower oil (reservoir lures).
- PAA did not enhance the attraction of males to the pheromones.
- Numbers of capsids had begun to decline in the field by the end of September so further work is needed to confirm these results.
- Other main invertebrate groups were not attracted to the lures.
- Several small rodents became trapped in the bucket traps and so an improved design needed for 2009.

- Hexane is better for dispensing the pheromone components from the piezoelectric sprayer than DCM or ethanol.

Task 3.4. Measure release characteristics of new dispensing systems in order to define physicochemical parameters of effective lures (NRI, Yrs 1-2)

In previous work, blends of the three compounds, HB, E2HB and KA dispensed from open microcapillary tubes were found to attract males of *L. rugulipennis* and also the related *L. pratensis* (Innocenzi *et al.*, 2005). It was thought that these dispensers were successful because they released the chemical from a very small point source – comparable to the insect pheromone gland, perhaps – and at a specific release rate. However, these dispensers were very short-lived and the blend of chemicals released varied with time because only a small amount of the material could be loaded into the tube. Experiments were thus carried out with the same microcapillary tubes inserted into a reservoir to hold larger quantities of the compounds to be dispensed in the anticipation that these would be much easier to handle and would give a longer release period with relatively stable blend composition.

In view of the above results on the blend of chemicals and release rate measured from female *L. rugulipennis*, the aim was to devise a dispenser releasing a blend of HB:E2HB:KA in the region 1.00 : 0.03 : 0.20 with hexyl butyrate at approximately 1 µg/hr. Release rates were also determined for sachet dispensers containing the host-plant volatiles evaluated in field trapping tests.

Materials and methods

Microcapillary dispensers were constructed from 5 µl microcapillary tubes inserted into the Teflon-lined septum of a screw-top, conical sample vial (1.1 ml; Chromacol, Fig. 3.1.1). Care was taken to ensure a good seal round the microcapilary by making an initial hole in the septum from the Teflon side with a ferrule reamer. Duplicate dispensers were held in a laboratory windtunnel maintained at 27°C and 8 km/hr windspeed. At intervals volatiles were collected from individual dispensers held in silanised glass vessels (6 cm x 3 cm) by drawing charcoal-filtered air over them and trapping the volatiles on a filter containing Porapak Q (200mg, 50-80 mesh; Waters Associates) held in a Pasteur pipette (4 mm i.d.) between plugs of silanised glass wool. Volatiles were collected for 2-3 hr in the same room as the windtunnel and then eluted from the Porapak with dichloromethane (3 x 0.5 ml; Pesticide Residue Grade). After addition of decyl acetate (2 µg) as an internal standard, the solution was analysed by GC with FID. The fused silica capillary column (30 m x 0.32 mm i.d.) was coated with SupelcoWax10 (0.25 µm film thickness), carrier gas was helium (2.4 ml/min), injection was splitless (200°C) and the oven temperature was programmed from 60°C for 2 min then at 10°C/min to 250°C. Quantification was by comparison of peak area with that of the decyl acetate. Injection of known standards indicated that essentially no correction factor was necessary.

In an initial experiment the microcapillary was loaded with equal amounts of HB, E2HB and KA (20 mg each) as the neat mixture or diluted in 100 µl sunflower oil. The KA is unstable because of dimerisation and oxidation, and the sunflower oil was intended to help stabilise the KA by dilution and the presence of naturally-occurring antioxidants. This experiment gave an estimation of the relative release rates of the three compounds and, based on this, dispensers were made up containing 10 mg HB, 0.25 mg E2HB and 1.25 mg KA in 100 µl sunflower oil. These were used in field tests and also evaluated in the laboratory windtunnel.

Results

Results from collection of volatiles from the microcapillary dispensers seemed to be very dependant upon the collection filter used and its age, and the reasons for this are still being investigated. Results from collections made from the dispensers containing a blend of equal quantities of HB, E2HB and KA are shown in Fig. 3.4.1.

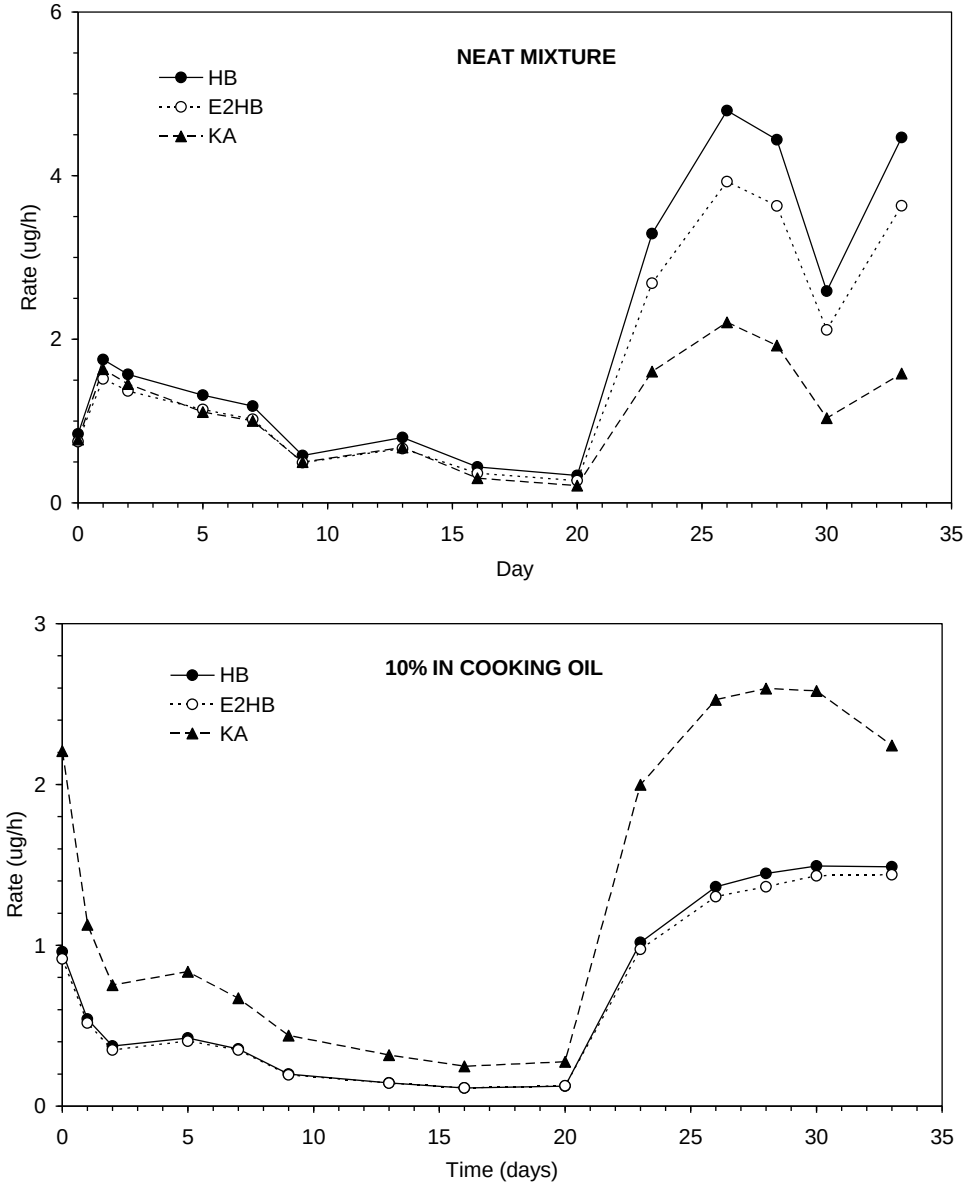

Figure 3.4.1. Release of HB, E2HB and KA from microcapillary dispensers containing a blend of equal quantities of the three components neat (upper) and 20% in sunflower oil (lower) maintained at 27°C and 8 km/hr windspeed. Results are mean from two dispensers.

Although rather inconsistent, the results suggest that release of HB from the dispensers containing the neat material were in the region of 2-4 µg/hr and from the dispensers containing a solution in sunflower oil were 1-2 µg/hr and release persisted for at least 33 days at 27°C.

Release rates of HB and E2HB were similar in each system, as expected for such similar chemical structures. However, whereas release of KA was lower than the HB from the neat mixture, it was significantly higher from the solution in sunflower oil. The ratio of KA/HB decreased from approximately 1.0 to 0.5 in 33 days for the neat material whereas the ratio dropped from approximately 2.0 to 1.5 from the sunflower oil solution (Fig. 3.4.2). This suggested that the sunflower oil did indeed provide some stabilisation of the unstable KA relative to the stable HB.

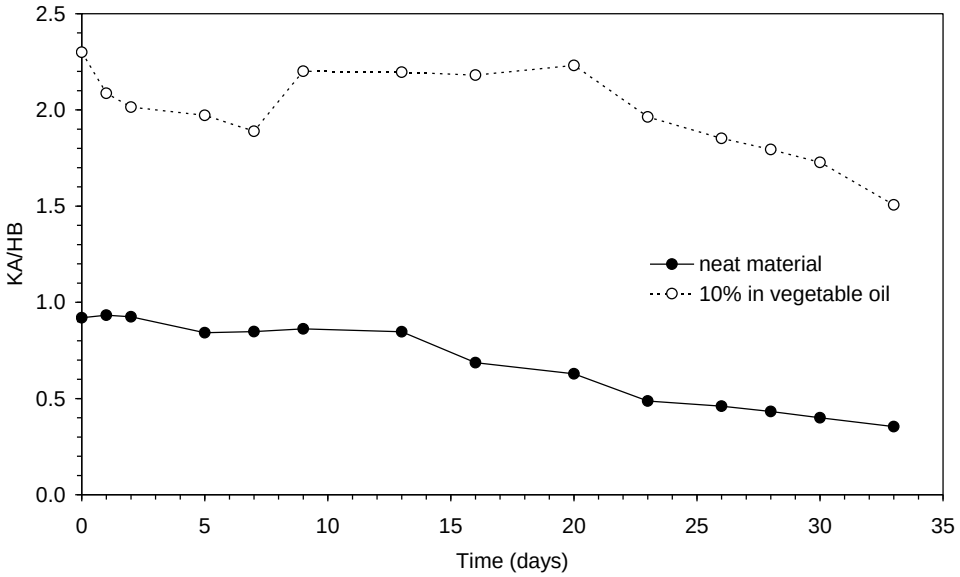

Figure 3.4.2. Ratio of KA/HB in volatiles released from microcapillary dispensers containing a blend of equal quantities of the three components neat (upper) and 20% in sunflower oil (lower) maintained at 27°C and 8 km/hr windspeed. Results are mean from two dispensers.

Dispensers for field use were made up with a blend of HB, E2HB and KA as a 10% solution in sunflower oil with the aim of releasing a 1.00 : 0.03 : 0.20 mixture mimicking that produced by females of *L. rugulipennis*. As the KA was released at approximately twice the rate of the HB for the solutions in sunflower oil, the blend put into the dispensers contained HB, E2HB and KA in a 1.00 : 0.025 : 0.125 ratio. Release rates of the three components from dispensers maintained in the laboratory windtunnel at 27°C and 8 km/hr windspeed are shown in Fig. 3.4.3. As above, results are very variable, but indicated release of HB was 1-2 µg/hr. The ratio of KA/HB decreased from approximately 0.25 to 0.1 over 33 days (Fig. 3.4.4). This may in part be due to some decomposition of the KA, but is also due to the faster release rate of KA distorting the blend remaining in the dispenser.

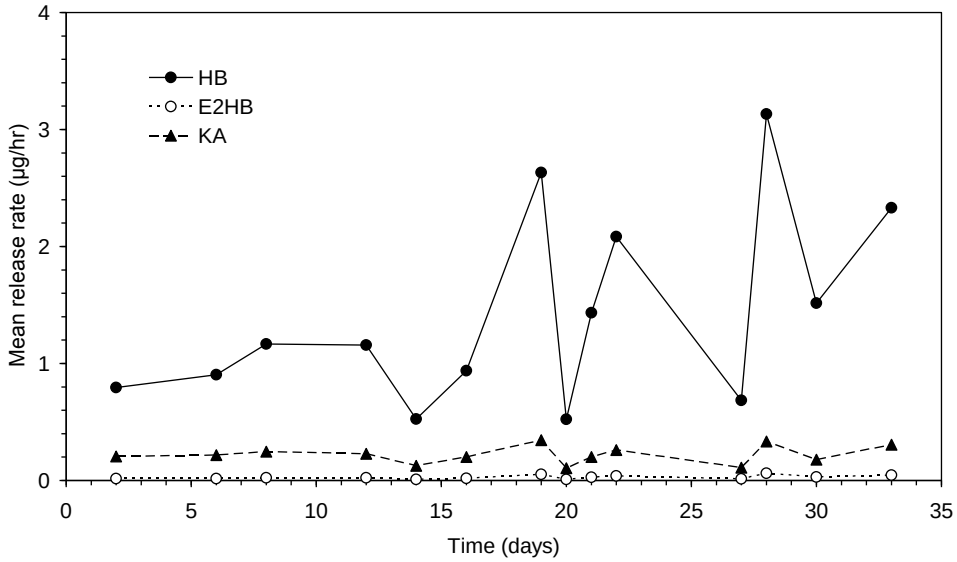

Figure 3.4.3. Release of HB, E2HB and KA from microcapillary dispensers containing a blend of the three components in 1.00 : 0.025 : 0.125 ratio 10% in sunflower oil maintained at 27°C and 8 km/hr windspeed. Results are mean from two dispensers.

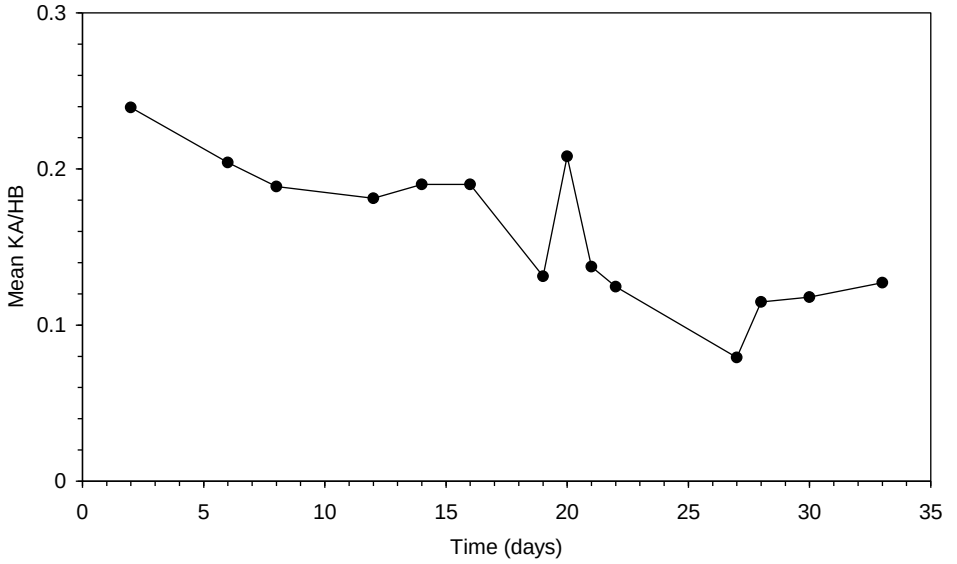

Figure 3.4.4. Ratio of KA/HB in volatiles released from microcapillary dispensers containing a blend of the three components in 1.00 : 0.025 : 0.125 ratio 10% in sunflower oil maintained at 27°C and 8 km/hr windspeed. Results are mean from two dispensers.

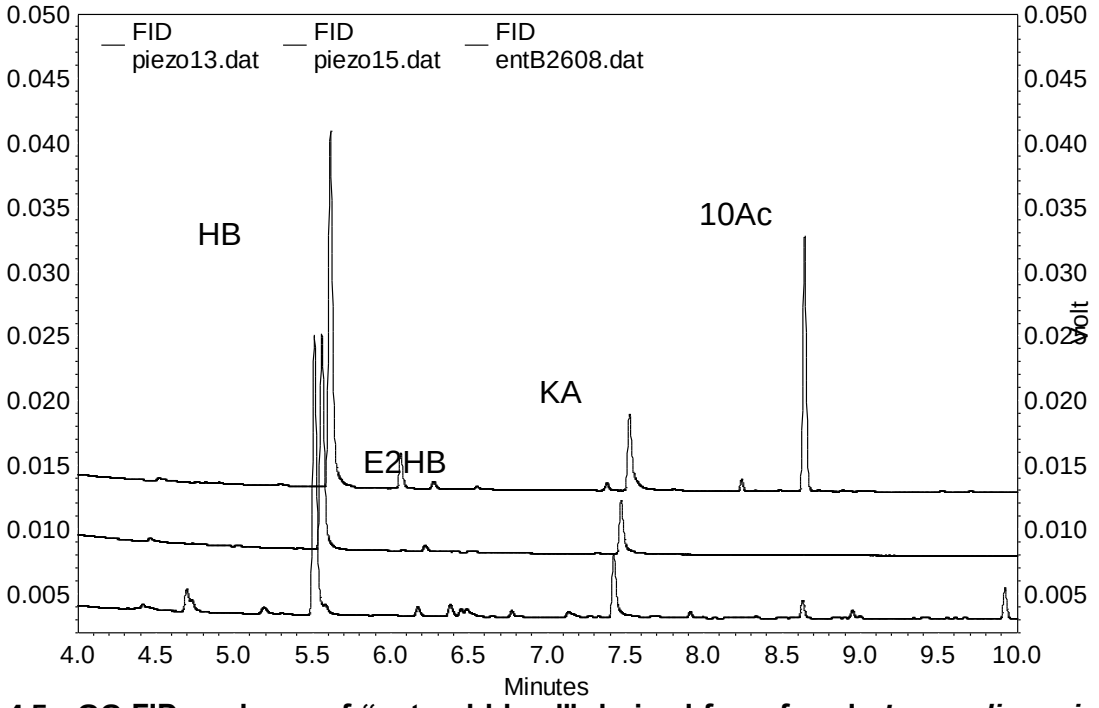

Figure 3.4.5. GC-FID analyses of "natural blend" derived from female *L. rugulipennis* used in piezoelectric experiments (lower), dichloromethane solution used in piezoelectric experiments (middle) and volatiles from microcapillary dispenser (upper) (HB hexyl butyrate; E2HB (*E*)-2-hexenyl butyrate; KA (*E*)-4-oxo-2-hexenal; 10Ac decyl acetate)

Release rates of methyl salicylate and phenylacetaldehyde (PAA) from white polyethylene sachets (2.5 cm x 2.5 cm x 120 µm thick) and clear polyethylene sachets (5 cm x 5 cm x 120 µm thick) were measured by recording weight loss of duplicate sachets maintained in a laboratory fume cupboard at 22°C. Release rate of PAA was also measured for the polyethylene sachet dispenser supplied by collaborators in Hungary.

As shown in Fig. 3.4.6, release of methyl salicylate persisted for only 10 days at 22°C at a mean rate of 11 mg/day. Release of PAA from the NRI dispensers lasted for at least 38 days at a mean rate of approximately 0.5 mg/day. Release of PAA was essentially complete within 10 days at a mean rate of 4 mg/day.

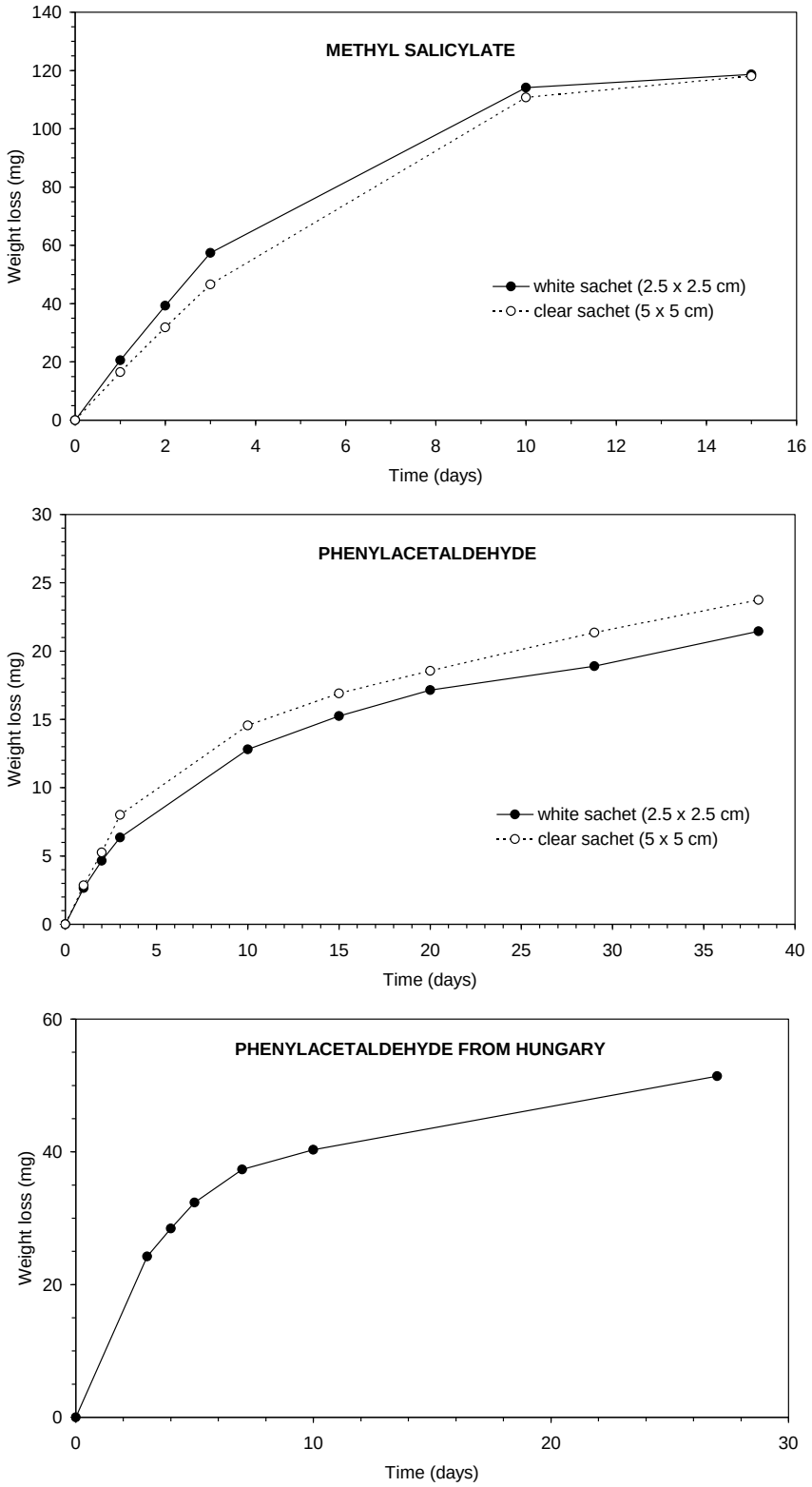

Figure 3.4.6. Release of methyl salicylate and phenylacetaldehyde from white and clear polyethylene sachet dispensers and of phenylacetaldehyde from sachet dispensers supplied by collaborators in Hungary at 22°C.

Conclusions

- Sunflower oil helped stabilise KA relative to the stable HB in the dispensers.
- Release of methyl salicylate persisted for only 10 days at 22°C at a mean rate of 11 mg/day.
- Release of PAA from the NRI dispensers lasted for at least 38 days at a mean rate of approximately 0.5 mg/day.

Objective 4. Investigate the possibility of synergising the attractiveness of the pheromones of the three species with host plant volatiles in laboratory and field

Task 4.1. Investigate possibility of synergising attractiveness of pheromone with known host plant volatiles using above laboratory and field bioassays (EMR, NRI, Yrs 1-2)

See Task 3.3, *1. Field tests with micro-capillary reservoir lures - 3 way blends with PAA,* for the results of pheromones in combination with phenylacetaldehyde (PAA) lures.

Materials and Methods

Two other studies were done to test the attractiveness of plant volatiles to *L. rugulipennis* males and females. Both were small scale randomised block field experiments comparing catches of capsids in white cross vane funneled bucket traps bated with different plant volatiles with and without mature female *L. rugulipennis*. The sites used were a weed field (1.2 ha, 'Palmers Rough'), at EMR, sown with Fathen and Scentless mayweed and a small area of set-aside, 'Churchfields East' (EMR), also dominated by Fathen and Scentless mayweed. The treatments tested were hexyl acetate, ocimene, ocimene, methyl salicylate, (*E*)-β-caryophyllene (Conti et al. 2006) and phenyl acetaldehyde (PAA, pers. comm. Miklos Toth) (Table 4.1.1).

Traps were modified AgriSense funnel/bucket traps with white Correx cross vanes. The traps contained 250 ml of water with a drop of detergent and were secured by digging into the ground, about 5 cm, so that the cross-vane was at weed flower height. Plant volatiles were dispensed from white polyethylene sachets (2.5 cm x 2.5 cm x 120 µ thick) containing 100 µg of the chemical (Table 4.1.1). In trial A, a direct comparison between 5 volatiles was made. In trial B, PAA was tested alone and in combination with a virgin (or field collected and then isolated) female *L. rugulipennis*. Control treatments consisted of a female with no plant volatiles and a trap with no female or plant volatile lure.

Table 4.1.1. Treatments of plant volatiles tested for attractiveness to *L. rugulipennis.*

Trial A, 9 July – 12 August			
Code	Treatment	Plant volatile concentration (mg)	No of female *L. rugulipennis*
A	hexyl acetate (2008/050/A)	100	0
B	ocimene (2008/050/B)	100	0
C	methyl salicylate (2008/050/C)	100	0
D	(*E*)-β-caryophyllene (2008/050/D)	100	0
E	phenyl acetaldehyde (2008/050/E)	100	0
F	control	0	0
Trial B, 23 August – 3 October			
G	phenyl acetaldehyde + female	100	1
H	female only	0	1
I	phenyl acetaldehyde only	100	0
J	control	0	0

The experiments were randomised block designs with each trap as a plot. There were 4 replicates of each treatment for trial A and 5 replicates for trial B. Traps were spaced at least 10 m apart. The traps were emptied weekly by sieving through gauze and females changed every ~5 days. The number, species and sex of capsids, and main groups of other invertebrates trapped were recorded. Trial A was set up in 'Palmers Rough', EMR on 10 July and ended on 25 July. Trial B was set up in Churchfields East on 18 August and ended on 1 September. See Task 3.4 for sachet release rates.

Results

Trial A

Female *L. rugulipennis* and *L. pratensis* were not significantly attracted into any of the plant volatile traps compared to the control (Log_{10} transformed data: P= 0.614, sed=0.1802, lsd=0.3840; P=0.262, sed=0.1503, lsd=0.3203, respectively). The same was true for male *L. rugulipennis* and *L. pratensis* (Log_{10} transformed data: P=0.624, sed=0.2166, lsd=0.4617; P=0.724, sed=0.1333, lsd=0.2841, respectively) (Fig. 4.4.1).

In addition, no attraction to the plant volatiles was shown for most of the invertebrate groups tested (all bees, Heteroptera, Diptera, Hymenoptera, spiders/harvestmen, earwigs, Coleoptera, ladybirds, Lepidoptera, molluscs and ants). However, there were more grasshoppers in the (E)-beta-caryophyllene and phenyl acetaldehyde compared to hexyl acetate, methyl salicylate and ocimene traps (Log_{10} transformed data; P=0.051, sed=0.1303, lsd=0.2777). There were also more grasshoppers in the (E)-beta-caryophyllene compared to the control traps.

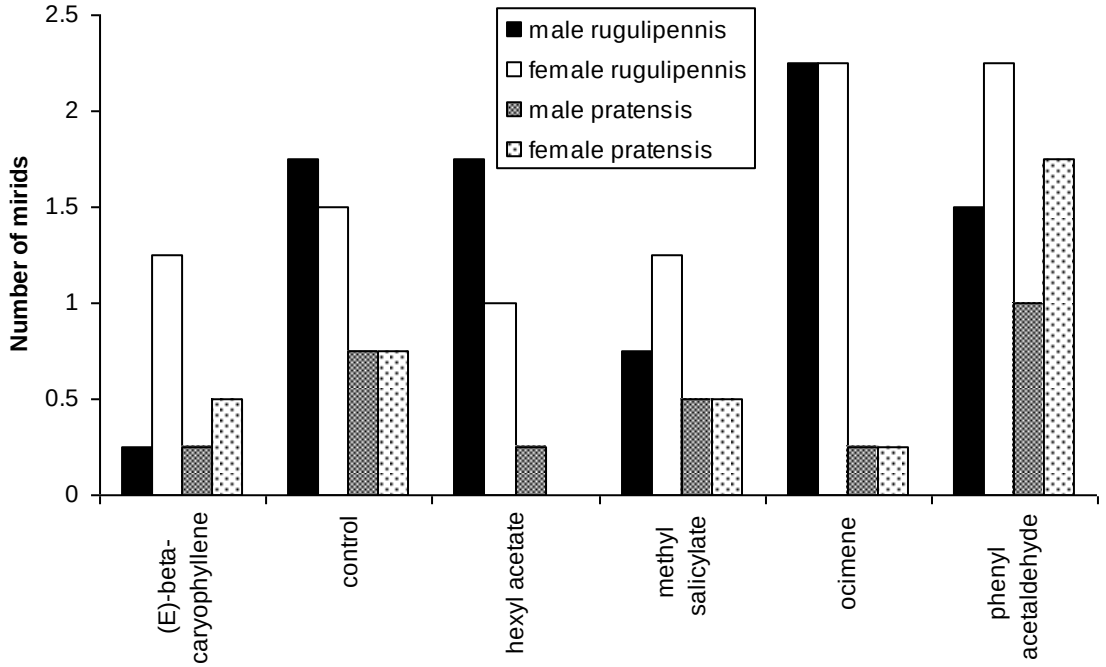

Figure 4.1.1. Numbers of *Lygus* attracted into plant volatile lure traps between 9 July – 12 August.

Trial B

Female *L. rugulipennis* and *L. pratensis* were not significantly increased in the PAA baited traps (Log_{10} transformed data: P=0.430, sed=0.1186, lsd=0.2584; P=0.085, sed=0.0959, lsd=0.2090, respectively). Equally, male *L. rugulipennis* and *L. pratensis* were not significantly attracted into the female or plant volatile baited traps (Log_{10} transformed data: P=0.318, sed=0.290, lsd=0.632; P=0.873, sed=0.1269, lsd=0.2765, respectively). The reason that numbers of male *L. rugulipennis* were not higher in the female baited traps was because the data was highly variable. For example, some females only lured 1 male, whereas others attracted up to 32 males, indicating variation in female calling. However, it can be seen that the males were more frequently found in traps that contained a female *L. rugulipennis* (Fig. 4.1.2). PAA did not increase the attraction of males to the traps in this test.

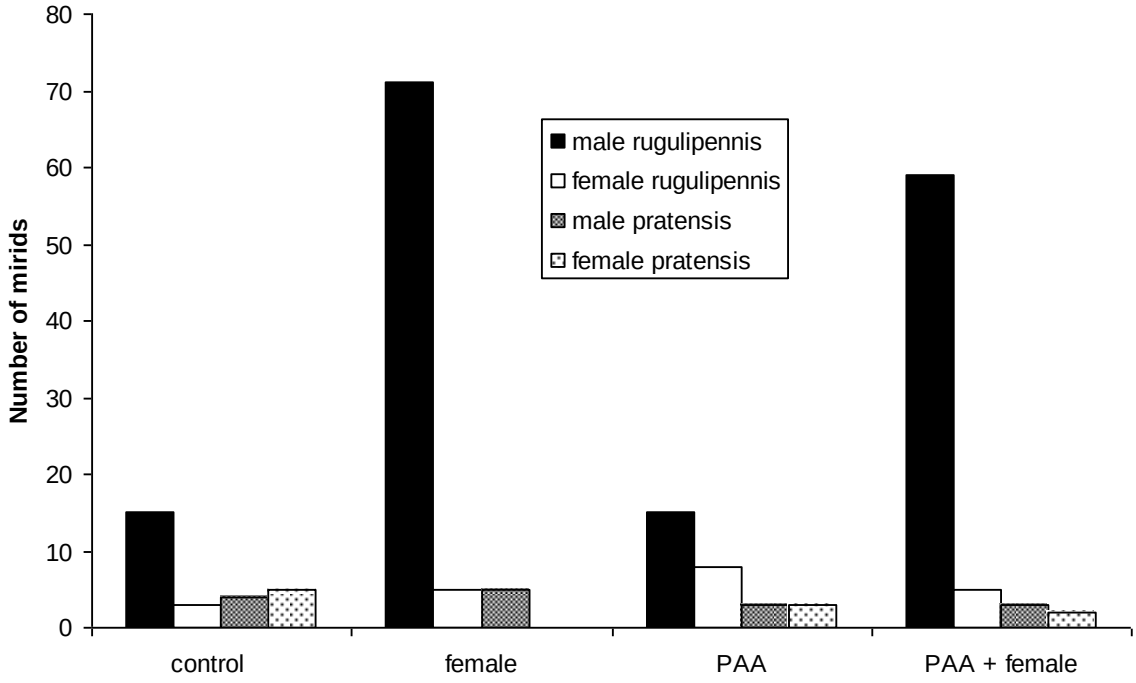

Figure 4.1.2. Numbers of *Lygus* attracted into PAA baited traps between 23 August – 3 October.

CONCLUSIONS

- In Task 3.3, conducted over several months *L. rugulipennis* females were attracted to the PAA lures. However, in this trial conducted over a couple of weeks, females were not significantly attracted into the traps.
- Both males and females of the two *Lygus* species were not attracted to hexyl acetate, ocimene, methyl salicylate, (*E*)-β-caryophyllene (Conti et al. 2006) or phenyl acetaldehyde.

Objective 5. Develop practical pheromone lures and traps for *L. rugulipennis*, *L. pabulinus* and *L. tripustulatus*

Task 5.1. Carry out field trials to develop robust, long-lived and practical dispensers for field use (EMR, Agrisense, KG Fruits, CGA, BGA, DJM Yrs 1-3)

Methods and materials

To test the efficacy of different trap designs for capturing mirids, small scale randomised block field experiments comparing trap designs were set up in the weed field (1.2 ha, Palmers Rough), at EMR.

Experiment 1
Green, 20 x 20 cm, delta traps with a brown (or white) sticky base were used for treatments A-D. Bucket traps (E-F) were had 250 ml water and a drop of detergent added. The traps were suspended at weed flower height on two canes (delta traps) or dug into the ground (bucket traps). In all traps mature virgin females (or females collected from the field and isolated for 2 days) were contained in a cage which consisted of a hair roller with gauze around the outside and a lid at either end, holding the gauze in place. The cage also contained a piece of damp paper to maintain the humidity and a section of bean as food (food needs to be added in order to stimulate the female to 'call'). The cage was placed vertically through a hole made in the top of the delta trap, so that the cage did not touch the sticky base or secured horizontally in the top of the bucket trap. Cypermethrin was sprayed onto the surface of the sticky base (20x20 cm) in a solvent at the recommended rate per ha (0.35 L/ha) (0.0014 ml/sticky base). The clear delta trap was made of clear vinyl sheets held together at the top with a paper binder (Fig. 5.1.1 and Table 5.1.1). There were 4 replicates of each trap which were spaced more than 10 m apart. Traps were checked and females changed every week. The number, species and sex of capsids trapped were recorded.

Table 5.1.1. Trap designs used in experiment 1, 27 June – 11 July (see Fig 5.1.1 for pictures).

Code	Trap	Capture device
A	Green delta	original sticky base
B	Green delta	'new' base
C	Green delta	original base with cypermethrin sprayed on
D	Green delta	original sticky base + ecotac
E	Cross vane funnel bucket trap	water with detergent
F	Cross vane funnel bucket trap	lambda-cyhalothrin cross veins + water with detergent
G	Clear delta trap	sticky base

A, C, D

E

B

F

G

Figure 5.1.1. Treatments with; A,C,D green delta traps, B green delta trap with 'new' sticky base, E white cross vane funneled bucket trap, E lambda-cyhalothrin cross vane funneled bucket trap, G clear delta trap. See Table 5.1.1 for corresponding description of traps.

Experiment 2

A randomised block design compared green delta traps, clear delta traps, sticky stake traps and white cross vane funneled bucket traps with commercially available pre-mounded cross vane bucket traps with different coloured cross vanes (Table 5.1.2, Fig. 5.1.2). There were 4 replicates of each trap which were spaced more than 10 m apart. Traps were checked and females changed every week. The number, species and sex of capsids trapped were recorded.

Table 5.1.2. Trap designs used in experiment 2, 27 August – 1 September. See Fig 5.1.2 for corresponding photographs.

Code	Trap	Capture device
H	Green delta	original sticky base with Ecotac
I	Clear delta trap	sticky base with Ecotac
J	White cross vane funneled bucket (Agrisense)	water with detergent
K	Premoulded green cross vane funnel bucket trap (Agralan)	water with detergent
L	Premoulded white cross vane funnel bucket trap (Agralan)	water with detergent
M	Premoulded yellow cross vane funnel bucket trap (Agralan)	water with detergent
N	Sticky stake trap	Ecotac

Figure 5.1.2. Treatments with; H green delta traps, I clear delta trap, J white cross vane funneled bucket trap, K pre-moulded green cross vane trap, L pre-moulded white cross vane trap, M pre-moulded yellow cross vane trap, N sticky stake trap. See Table 5.1.2 for corresponding traps.

Figure 5.1.2. continued. Treatments with; H green delta traps, I clear delta trap, J white cross vane funneled bucket trap, K pre-moulded green cross vane trap, L pre-moulded white cross vane trap, M pre-moulded yellow cross vane trap, N sticky stake trap. See Table 5.1.2 for corresponding traps.

Experiment 3

Insects have a thin covering of wax on their bodies that helps prevent water loss. Silica aerogels and diatomaceous earth are tiny, sharp particles that scratch through this protective layer and absorb the protective oils, leaving the insect's body vulnerable to water loss. Diatomaceous earth is a product of fossilised diatoms. A test was carried out to assess the feasibility of using it in the buckets traps in comparison to water and detergent.

Five replicates of each treatment were set up (Table 5.1.3). The traps were placed (alternating between the two treatments) in the weed field spaced 10 m apart. As with other trap design experiments the traps were baited with virgin/unmated female *L. rugulipennis*. Traps were checked and females changed every week and the number, species and sex of mirids trapped were recorded.

Table 5.1.3. Treatments used in experiment 3, 15-23 September.

Code	Trap	Capture device
O	Premoulded green cross vane funnel trap	diatomaceous earth
P	Premoulded green cross vane funnel trap	water with detergent

Results

Experiment 1

Although no significant differences were shown to exist between the trap types (Log_{10} transformed data: P=0.360, sed=0.0920, lsd=0.1934), the funnel traps using either white cross vanes or lambda-cyhalothrin cross vanes captured more male *L. rugulipennis* than the green delta sticky trap. The capsids in the delta traps are often captured on the edge of the sticky bases. It should also be noted that the lambda-cyhalothrin cross vane trap only began to catch mirids in the 4th (final) week of the experiment.

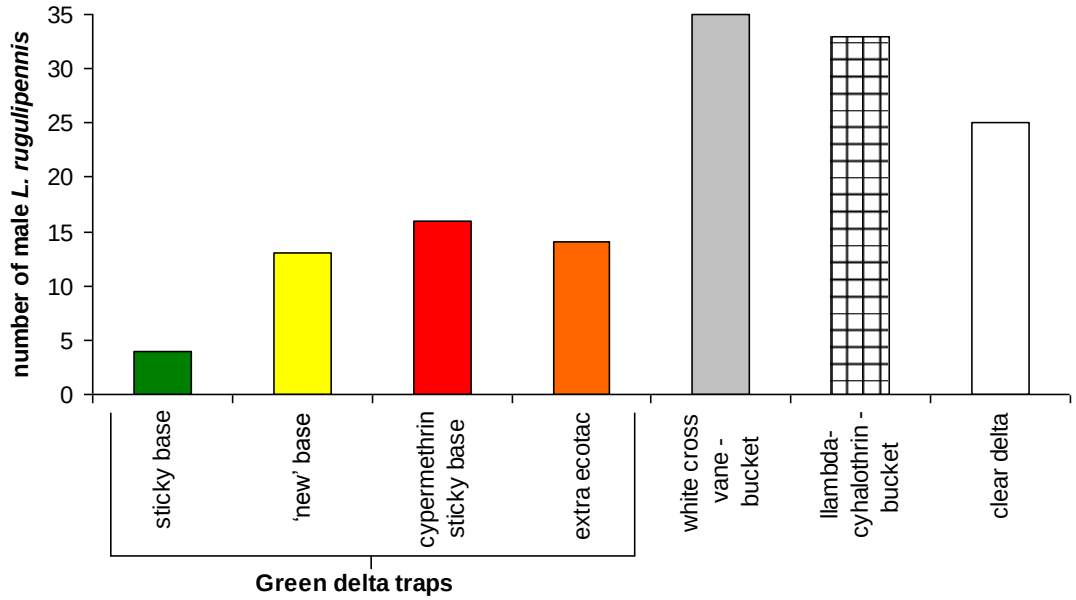

Figure 5.1.3. Comparison of the number of male *L. rugulipennis* in the different trap types in experiment 1.

Experiment 2

There were significantly more mirids captured in the green cross vane trap compared to the green or clear delta traps (Log_{10} transformed data: P=0.121, sed=0.3589, lsd=0.7541). The cross vane bucket traps and sticky stake trap captured the most male mirids. Sticky stake traps are impractical to use because of the contamination with glue. In addition, they can become covered in leaves and seeds reducing the area to contact for insects to become trapped (Fig. 5.2.1 – J).

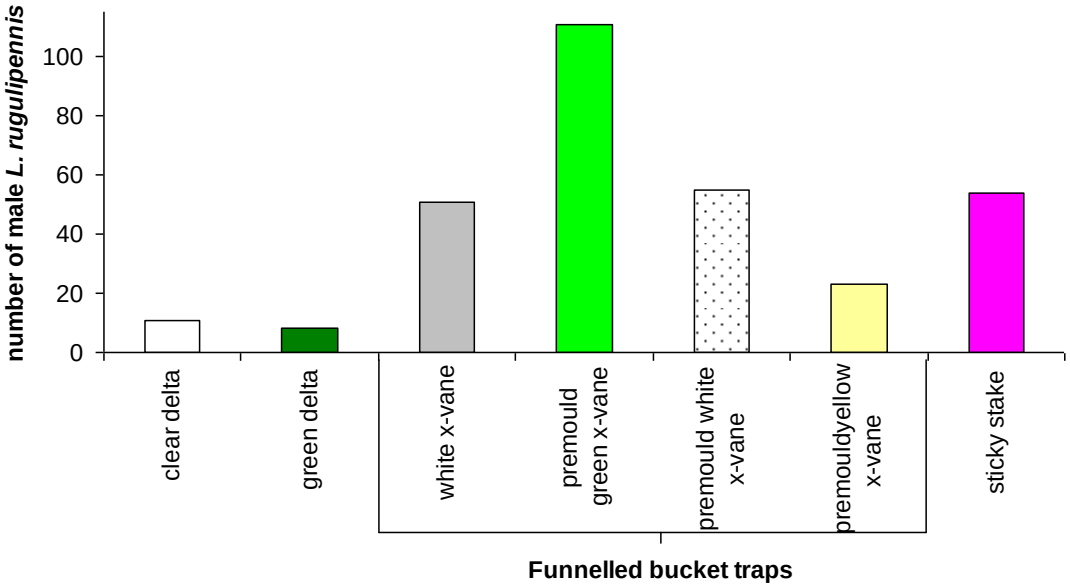

Figure 5.1.3. Comparison of the number of male *L. rugulipennis* in the different trap types in experiment 2.

Experiment 3

Numbers of mirids captured had begun to decline by this time and with 3 weeks of data only 4 and 11 male *L. rugulipennis* were captured in the diatomaceous earth and water traps, respectively. It was difficult to identify the species of mirid in the diatomaceous earth traps as they became encrusted in the earth. In addition, rainwater entered the bucket traps resulting in a paste, making identification even more difficult. It was noted that there were less invertebrates in the diatomaceous traps compared to the water traps, although these were not counted and analysed.

Conclusions

- Pre-moulded green cross vane funneled bucket traps captured the most mirids and are recommended for monitoring.
- Water and a drop of detergent are recommended as a trapping agent.

Task 5.2. Gather feedback from growers on use of lures and traps to guide development of commercial products (EMR, Agrisense, KG Fruits, CGA, BGA, DJM, Yrs 1-3)

In 2007 the green delta trap appeared to be effective at trapping *L. rugulipennis*, however, when this design was compared to cross vane funneled bucket traps it was not so efficient. Some modification is needed to prevent larger animals, such as, rodents falling into the funneled traps.

Objective 6. Calibrate pheromone traps for monitoring capsid pests in at least two field crops and one protected crop

Task 6.1. Carry out replicated trials in at least two field crops and one protected crop with best available traps and lures to investigate correlations between catches of male capsids in pheromone traps, capsid populations, as determined by independent means such as sweep-netting, and capsid damage (EMR, Agrisense, KG Fruits, CGA, BGA, DJM, Yrs 2-3)

From the traps baited with the 3 way combination of the pheromone components with the PAA lures (Task 3.3) it was possible to monitor *L. pratensis* and *L. rugulipennis* males and females throughout the latter part of the season in the weed field ('Palmers Rough') at EMR. This data will be further improved in 2009 with the use of the new 3 way mix attractive to *L. rugulipennis* males.

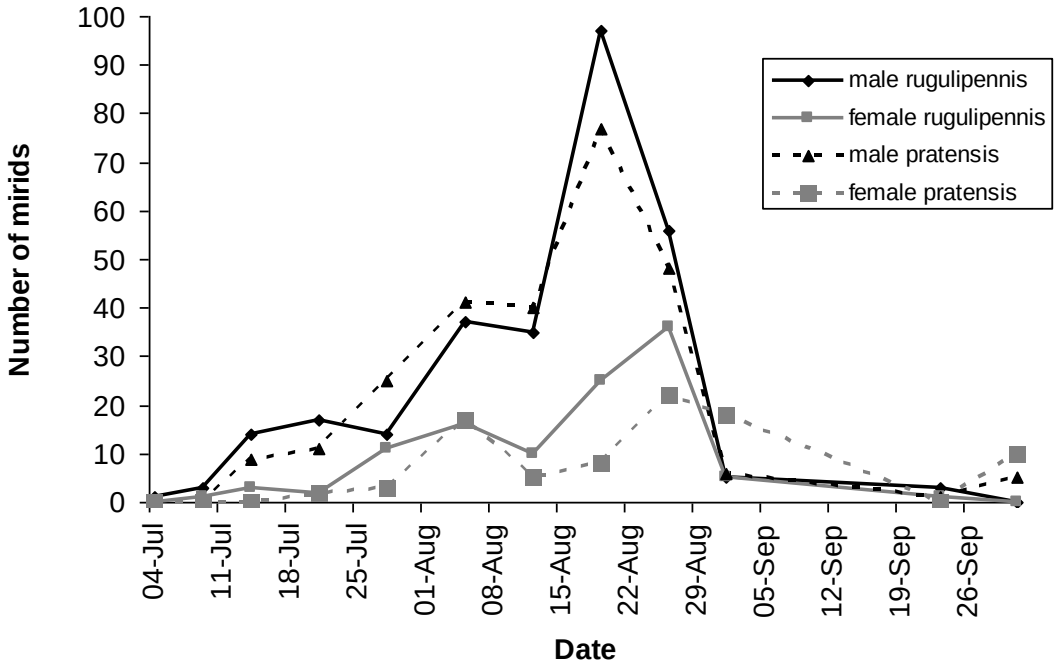

Figure 6.1.1. Phenology of *L. pratensis* and *L. rugulipennis* males and females in the weed field at EMR. Numbers are totals of each week in all traps used from July to September .

Task 6.2. Prepare protocol for using traps for monitoring capsids in different crops (EMR, Agrisense, KG Fruits, CGA, BGA, DJM; Yr 3)

Task 6.3 Prepare factsheet for HDC on use of traps, monitoring and spray programmes (EMR, KG Fruits, CGA, BGA, DJM; Yr 3).

Pheromone technology for management of capsid pests to reduce pesticide use in horticultural crops

Workplan for 2009

A brief discussion of research priorities for the final year of the project were discussed at the meeting on 11 December 2008, prior to a science partners meeting on 27 January 2009. The following priorities were proposed:

- Continue with laboratory rearing of mirids captured in the spring and summer.
- Abandon laboratory bioassays.
- Investigate dispensers for used by growers.

Lygus rugulipennis

- Confirm the attractiveness of the modified blend and to explore the effects of release rate using a range of different dispensers, including dispensers that could be practical for use by growers. Different sizes of source (micro-capillary point sources vs larger sources) will be included.
- This will require preliminary investigation of suitable dispensers and measurement of their release rates by NRI in January –April 2009. Female baited traps can be used as controls.
- The Agralan green cross vane funnelled trap will be modified to prevent rodents and bees entering, whilst ensuring maximum capture of mirids.
- Explore perturbation of the blend ratio to determine how this affects attractiveness as it is important to test the hypothesis that blend ratio is critical. This work could be done with the piezoelectric sprayer and/or other dispensers.

Lygocoris pabulinus

- More time of day entrainment data is needed to confirm the optimum blend.
- Nettles are a possible source of *Lygus pabulinus*. Field trials (on growers' holdings) in apple orchards adjacent to nettles testing dispensers with best known blend.

Liocoris tripustulatus

- Time of day entrainments are needed to determine the optimum blend.
- Field trials in areas dominated by nettles will be done.

Milestone status at end of Year 2 (2008)

Primary milestones					
Year 1	**Milestone**	**Target Date**	**In full**	**On time**	**Explanation**
1.1	Cultures of *L. rugulipennis* and *L. pabulinus* established.	31 Mar 08	Yes	Yes	*L. rugulipennis* and *L. tripustulatus* cultures established
1.2	Identification of components of sex pheromone of *L. pabulinus* completed	31 Mar 08	No	No	Good progress has been made. More work needed on time of day entrainments
1.3	Pheromone components for *L. rugulipennis* and *L. pabulinus* available in multi-gram quantities	31 Mar 08	Yes	Yes	This has been achieved by NRI
3.1	Laboratory bioassay(s) developed for measuring behaviour of *L. rugulipennis* and *L. pabulinus* in response to pheromone	31 Mar 08	No	No	Windtunnel and still air bioassays have proved to be unreliable and no comparison to the field tests.
Year 2					
2.1	Identification of components of pheromone of *L. tripustulatus* completed	31 Mar 09	No	No	Progress has been made. More work needed on time of day entrainments
3.2	Pheromone lures attractive to *L. rugulipennis* and *L. pabulinus* in laboratory and field developed	31 Mar 09	Yes	Yes	In progress. Good progress made in 2008 on *L. rugulipennis*. More work needed on *L. pabulinus*
4.1	Effect of adding selected host plant volatiles to pheromone lures for *L. rugulipennis* and *L. pabulinus* determined in laboratory and field	31 Mar 09	Yes	Yes	6 volatiles tested in 2008 – low attractiveness demonstrated with *L. rugulipennis* females.
~~5.1~~	Attractive lures and traps developed for L. rugulipennis and L. pabulinus which are adequate for evaluation as monitoring tools for capsid populations in the field	31 Mar 09	-	-	It is unrealistic to reach this milestone by Mar 2009, hence this milestone has been moved to year 3.
Year 3					
2.1	Pheromone lures attractive to *L. tripustulatus* in laboratory and field	31 Mar 10	-	-	-

	developed				
5.1	Effective lures and traps developed for *L. rugulipennis*, *L. pabulinus* and *L. tripustulatus* which are suitable for commercial production and distribution	31 Mar 10	-	-	-
6.1	Traps and lures validated for monitoring *L. rugulipennis*, *L. pabulinus* and/or *L. tripustulatus* in at least two field crops and one protected crop	31 Mar 10	-	-	-
6.2	Protocols prepared for using lures and traps to monitor capsid populations	31 Mar 10	-	-	-
5.1 (was Yr 2)	Attractive lures and traps developed for *L. rugulipennis* and *L. pabulinus* which are adequate for evaluation as monitoring tools for capsid populations in the field	31 Mar 10 (new target)	-	-	Agralan, green cross vane funneled traps are considered the most effective and practical for growers to use
Secondary milestones					
Year 1					
1.1	Methods for collecting pheromone from capsids optimised	31 Mar 08	Yes	Yes	Done
1.2	Methods for carrying out GC-EAG analyses on capsids further improved	31 Mar 08	Yes	No	Ongoing
3.1	Fields of weeds prepared at EMR for field trapping trials	31 Mar 08	Yes	Yes	Done, ‘Palmers Rough’, EMR
3.2	Release rates of pheromone components from dispensers used in bioassays determined	31 Mar 08	Yes	No	Ongoing
4.1	List of potential host-plant volatiles prepared in collaboration with Italian workers and synthetic compounds available	31 Mar 08	Yes	Yes	These have been tested. Also a volatile suggested by Hungarian scientist.
5.1	Trials initiated in growers’ fields	31 Mar 08	Yes	Yes	Trials done in both years. Newly discovered pheromone ratio will be tested in 2009.
Year 2					

2.1	Volatiles collected from *L. tripustulatus* males and females	31 Mar 09	Yes	Yes	Collections made. Ongoing
2.2	EAG recordings made from *L. tripustulatus* males and females	31 Mar 09	Yes	Yes	Ongoing
3.1	Fields of weeds prepared at EMR for field trapping trials	31 Mar 09	Yes	Yes	Done, 'Palmer's Rough' at EMR
3.2	Release rates of pheromone components from dispensers used in bioassays determined	31 Mar 09	Yes	Yes	Yes
5.1	Feedback from growers on use of lures and traps gathered and evaluated	31 Mar 09	No	No	Still working on effective lure development.
Year 3					
5.1	Feedback from growers on use of lures and traps gathered and evaluated	31 Mar 10	-	-	-