Grower summary

PC/SF 276

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

Year 1 Report 2007

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the inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

CONTENTS

Grower summary

Headline

- Progress is being made in the development of sex pheromone monitoring traps for capsids.

Background and expected deliverables

This project aims to reduce the use of broad-spectrum insecticides to control capsid pests on a range of horticultural crops whilst maintaining or improving the level of control in both conventional and organic produce. Building on previous research at East Malling Research (EMR) and Natural Resources Institute (NRI), progress is being made to develop effective and practical pheromone lures for monitoring three species of capsids.

A major objective is to identify and confirm the components of the female sex pheromones of the capsid species *Lygus rugulipennis*, *Lygocoris pabulinus* and *Liocoris tripustulatus.* Laboratory work and field bioassays will be undertaken to produce artificial lures. The use of such lures will;

- 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.

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. In the future, 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

Collections of volatiles were made from individual males and females of *L. rugulipennis* and *L. tripustulatus*. With *L. rugulipennis*, previous results showing that only females produce hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal were confirmed. Preliminary results with *L. tripustulatus*, have shown that the same 3 compounds were produced by both males and females. However, the amounts and ratios of the compounds produced were very erratic. In the field, good attraction of male *L. rugulipennis* to standard green delta traps baited with unmated laboratory reared females occurred primarily in the morning. However, there was no obvious pattern of production of the compounds at different times of day in the laboratory.

Analysis of volatile collections by gas chromatography linked electroantennagrams (GC-EAG) from *L. rugulipennis* and *L. tripustulatus* showed weak and erratic responses to the 3 identified compounds and no significant responses to any other compounds.

Laboratory wind tunnel bioassays proved unsuccessful partly because it was not possible to determine when a female was 'calling'; releasing sex pheromone. *L. rugulipennis* males did not respond to females or synthetic lures. Some positive responses were obtained for both *L. rugulipennis* and *L. tripustulatus* in large cage still air bioassays. These suggested attraction of male *L. rugulipennis* to females, and of female *L. tripustulatus* to males, but, in the latter species, numbers responding were small and results were not wholly consistent.

Field tests were carried out with various combinations of the 3 compounds shown to be produced by female *L. rugulipennis* using either open capillary tube dispensers or capillary dispensers with a reservoir. No attraction was found even though attraction had been demonstrated in previous field experiments prior to this project.

The 3 capsid species were cultured in the laboratory, with most effort being devoted to *L. rugulipennis* and *L. tripustulatus*. This proved labour intensive, somewhat unpredictable and productivity declined later in the growing season due to the insects entering diapause as winter approached. Attempts to collect *L. pabulinus* from the field were largely unsuccessful as populations in 2007 were very low.

Future work will concentrate on confirming sex pheromone components and estimating the time of day males are attracted to females of *L. pabulinus*. The wind tunnel bioassay will be abandoned and efforts concentrated on the still air, large cage bioassay which will then provide the basis for laboratory testing the development of synthetic lures.

New lures and pheromone dispensing technologies will be employed for field testing blends and release rates of the 3 known compounds. Collection and analysis of pheromones from *L. rugulipennis* and *L. tripustulatus* will be repeated when the insects

are highly reproductively active in the main season, including volatile release analyses at different times of day.

Financial benefits

No direct financial benefits to growers resulted from the trial this year, but the ability to monitor capsids in horticultural crops will result in reduced numbers and better timed pesticide applications in the future.

Action points for growers

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

Science Section

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

On 28 June 2007, a collection of adult *L. pabulinus* was made from IPM blackcurrant plots at Pixley Farm, Hereford. Plants were beat sampled by knocking the branches with the arm onto a white beating tray (total sampling time was approximately 4 h). Around 20 individuals were found and identification confirmed by leading mirid entomologists. The numbers collected were not as high as hoped and although some success was achieved at rearing eggs of the capsids, the culture died out within a few weeks. Apple orchards and hop gardens at EMR and other sites in Kent were searched for *L. pabulinus*, but high populations could not be found.

Over one hundred adult female and 30 male *L. rugulipennis* were collected by sweep netting from the mayweed (*Matricaria recutita*) and fat-hen (*Chenopodium album*) plants growing between the cultivated rows of a young vine crop at an organic farm in Marden, Kent (Peter Hall, Poultry Farm) on 17 July 2007.

Over 40 individuals of *L. tripustulatus* were collected from nettles on the organic farm at Marden on 17 July 2007.

All three capsid species were cultured in BugDorm boxes (29x29x29 cm) (Fig. 1.1.1). Approximately 20 individuals (10 male and 10 female) were added to each box. Food consisted of 10 whole dwarf beans (*Phaseolus vulgaris*), sections of bean and a dead blowfly larva on moistened tissue paper in a plastic cup (4 cm diameter 3 cm depth) (Fig. 1.1.1). Bee collected pollen was also added to the bottom of the cages. In addition, *L. tripustulatus* cultures were, initially, provided with whole nettle plants, as this was thought to be essential for their development. *L. pabulinus* were also provided with potato shoots. In addition to being food the whole beans and potato shoots provided egg laying sites for the females. Culture boxes were refreshed with new individuals of field collected adults as needed.

Once per week, the whole beans and potato shoots were 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 once the nymphs reached their 4^{th}-5^{th} instar, they were separated into smaller ventilated culture boxes (13x7.5x5 cm) with shredded paper (place to hide and moult) and food. These culture boxes were

attended twice per week to maintain humidity and a clean source of food for the insects. Separately reared insects were unmated males and females that could be used for the subsequent bioassays and pheromone collections. Cultures were maintained in environmentally controlled rooms at 20–24°C, with a light:dark regime of 16:8 h.

Figure 1.1.1. Photographs of capsid culture apparatus a) Bugdorm, b) feeding cup, c) beans containing eggs in culture box, d) single adult capsid culture box.

Results

All 3 species of mirid were observed feeding on dwarf beans, pollen and blowfly larvae. Approximately, 50% of nymphs survived the final moult from 5th instar to adult. *L. rugulipennis* was cultured successfully throughout the year until August when the number of eggs laid by females reduced. Although more individuals were collected from the field, the number of nymphs produced reduced significantly even when the temperature in the environmentally controlled room was raised from 20 to 24^{o}C. However, with a combination of laboratory rearing and frequent field collections it was possible to maintain cultures for the experiments up to December. Many of the *L. rugulipennis* individuals toward the end of the season were collected in weed fields at EMR which were dominated by mayweed (*Matricaria recutita*) and fat-hen (*Chenopodium album*). It was noted that towards the end of the season (October) there was a bias towards more individuals of *Lygus pratensis*. *L. tripustulatus* cultures

were relatively successful. As with *L. rugulipennis*, the number of resulting nymphs reduced toward the end of the season. Frequent collections from the field, maintained the cultures. No eggs were laid in beans after August. The average lifespan of an adult at 20-24°C was 28 and 25 days for *L. rugulipennis* and *L. tripustulatus*, respectively. Attempts were made to over-winter the remaining cultures in order to break their diapause. Insects were gradually cooled to 5°C and the light hours were reduced to 8 hours/day for 6-10 weeks before steadily increasing the temperature (20°C) and daylight hours again. The insects were provided with rolled corrugated card in which to hibernate. None of the insects survived the artificial over-wintering.

L. pabulinus cultures were less successful. Eggs were observed in the dwarf beans and potato shoots and hatched successfully. Cultures died out within 4 weeks even though eggs were hatching successfully. The difficulty of establishing the cultures was further exacerbated by the inability to find an abundant field population to renew the cultures.

Conclusions

- Culturing of *L. rugulipennis* and *L. tripustulatus* was particularly successful through the summer months and provided many unmated males and females for testing. However, culturing of these insects is labour intensive.
- Better sites are needed to obtain specimens of *L. pabulinus* to establish more permanent cultures.
- Improved over-wintering culture conditions are needed.

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

Because of a lack of unmated *L. pabulinus* individuals from culture no volatiles were collected this year. This task will be given priority in year 2.

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

Hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal were identified as major components in volatiles from females of *L. rugulipennis* and males and females of *L. tripustulatus.* Hexyl butyrate and (*E*)-2-hexenyl butyrate were synthesised

previously by reaction of butyryl chloride and pyridine with hexanol or (*E*)-2-hexenol, respectively, in dichloromethane.

(*E*)-4-Oxo-2-hexenal was synthesised previously by a multi-step route in low yield, and two new reactions applicable to synthesis of this compound and analogues were investigated. Reaction of (*Z*)-3-hexenol with pyridinium chlorochromate in dichloromethane (Fernandes and Kumar, 2003) gave (*E*)-4-oxo-2-hexenal among several other products. Workup to remove chromium salts followed by flash chromatography were tedious and the product was obtained in only 10% yield. In the second approach, reaction of commercially-available 2-ethylfuran with N-bromosuccinimide and pyridine (Moreira and Millar, 2005) gave (*E*)-4-oxo-2-hexenal contaminated only with succinimide and this was easily removed by flash chromatography to give the highly pure (*E*)-4-oxo-2-hexenal in 45% yield.

Thus the second route (Figure 1.3.1.) gives the pure (*E*)-4-oxo-2-hexenal in gram quantities, a single-step reaction from readily-available starting materials. Previous findings that the compound is rather unstable in neat form were confirmed, but the product of the above reaction could be stored for months without decomposition as a 10 mg/ml solution in diethyl ether at -20ºC.

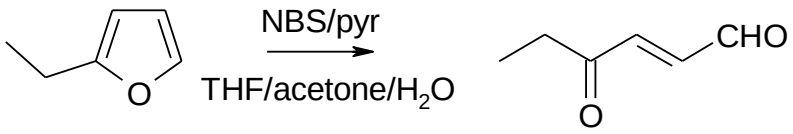


Figure 1.3.1. Synthesis of (E)-4-oxo-2-hexenal from 2-ethylfuran.

No entrainment was carried out on *L. pabulinus*, so no additional compounds were found.

References

Fernandes R.A. and Kumar P. (2003). PCC-mediated novel oxidation reactions of homobenzylic and homoallylic alcohols. *Tetrahedron Letters*, 44: 1275-1278.

Moreira J.A. and Millar J.G. (2005). Short and simple syntheses of 4-oxo-(*E*)-2-hexenal and homologs: Pheromone components and defensive compounds of Hemiptera. *Journal of Chemical Ecology*, 31: 965-968.

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

In order to determine if the species is producing sex pheromones and identify the pheromone components, volatiles were collected from single unmated *L. tripustulatus* females and males by the air entrainment method (Fig. 2.1.1) at EMR, followed by chemical analyses and GC-EAD at NRI.

Single unmated females and males were used in the entrainment and were more than 6 days old. Individuals were held in the entrainment apparatus with a bean and nettle (as food and host plant, respectively) to initiate pheromone production. In the entrainment set up on 9 November, only bean was used (no nettle was included). Where possible, males which had attracted females in the still air bioassay (Obj. 3) were used as it was more likely that they were releasing the pheromone. Volatiles were trapped in Porapak Q filters (200 mg in Pasteur pipette 4 mm i.d.).

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. The apparatus was operated in 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. Collections from each individual were made for 3-4 days (using the same filter for the whole period). 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).

Each sample was labeled according to the following scheme; species, individual number (females 1-499 and males >500), number of charcoal filter (1-6) and date of sampling, for example, Lt-42-3-11/09/07 or Lt-15-6-27/09/07. A record was made of the date and time the entrainment began and ended. Collections were carried out between the 9-12, 12-15, 15-18, 18-22, 22-26 October, 29 October-2 November, 9-13, 14-16 and 16-19 November (Table 2.1.1).

At NRI the 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.

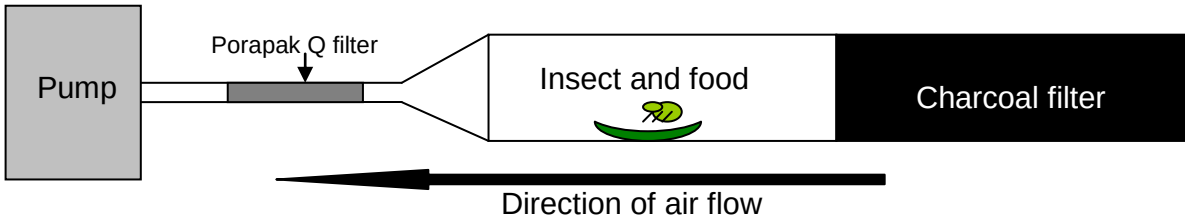


Figure 2.1.1. Schematic diagram of single entrainment apparatus used for collecting volatiles of capsids. 6 entrainments were operating simultaneously.

Table 2.1.1. Details of *L. tripustulatus* individuals used in the entrainment. ? = date not known.

Capsid i.d.	Sex	Date adult emerged	Date in	Time in	Filter No.	Date finished	Time finished	Dead/ alive end test
2	f	21/09/2007	09/10/2007	1035	1	12/10/2007	0930	a
501	m	24/09/2007	09/10/2007	1035	2	12/10/2007	0930	a
502	m	24/09/2007	09/10/2007	1035	3	12/10/2007	0930	a
503	m	24/09/2007	09/10/2007	1035	4	12/10/2007	0930	a
3	f	21/09/2007	09/10/2007	1035	5	12/10/2007	0930	a
4	f	24/09/2007	09/10/2007	1035	6	12/10/2007	0930	a
1	f	21/09/2007	12/10/2007	1400	1	15/10/2007	1100	a
504	m	21/09/2007	12/10/2007	1400	2	15/10/2007	1100	a
10	f	21/09/2007	12/10/2007	1400	3	15/10/2007	1100	a
11	f	28/09/2010	12/10/2007	1400	4	15/10/2007	1100	a
505	m	24/09/2007	12/10/2007	1400	5	15/10/2007	1100	a
506	m	24/09/2007	12/10/2007	1400	6	15/10/2007	1100	a
12	f	24/09/2007	15/10/200	1730	1	18/10/2007	1100	a

			7					
			15/10/200					
13	f	24/09/2007	7	1730	2	18/10/2007	1100	a
			15/10/200					
14	f	21/09/2007	7	1730	3	18/10/2007	1100	a
			15/10/200					
507	m	24/09/2007	7	1730	4	18/10/2007	1100	d
			15/10/200					
508	m	21-28/09/07	7	1730	5	18/10/2007	1100	a
			15/10/200					
509	m	21-28/09/07	7	1730	6	18/10/2007	1100	a
			18/10/200					
510	m	08/10/2007	7	1800	1	22/10/2007	0900	a
			18/10/200					
15	f	08/10/2007	7	1800	2	22/10/2007	0900	a
			18/10/200					
16	f	08/10/2007	7	1800	3	22/10/2007	0900	a
			18/10/200					
511	m	08/10/2007	7	1800	4	22/10/2007	0900	a
			18/10/200					
512	m	08/10/2007	7	1800	5	22/10/2007	0900	a
			18/10/200					
17	f	21/09/2007	7	1800	6	22/10/2007	0900	a
			22/10/200					
18	f	01/10/2007	7	1600	1	26/10/2007	1625	a
			22/10/200					
513	m	08/10/2007	7	1600	2	26/10/2007	1625	a
			22/10/200					
514	m	08/10/2007	7	1600	3	26/10/2007	1625	a
			22/10/200					
515	m	08/10/2007	7	1600	4	26/10/2007	1625	a
			22/10/200					
19	f	24/09/2007	7	1600	5	26/10/2007	1625	a
			22/10/200					
20	f	08/10/2007	7	1600	6	26/10/2007	1625	a
			29/10/200					
516	m	08/10/2007	7	0955	1	02/11/2007	0900	a
			29/10/200					
21	f	08/10/2007	7	0955	2	02/11/2007	0900	a
			29/10/200					
517	m	28/08/2007	7	0955	3	02/11/2007	0900	a

518	m	19/10/2007	29/10/2007	0955	4	02/11/2007	0900	a
22	f	22/10/2007	29/10/2007	0955	5	02/11/2007	0900	a
23	f	08/10/2007	29/10/2007	0955	6	02/11/2007	0900	a
522	m	01/11/2007	09/11/2007	1400	1	13/11/2007	0900	a
517	m	28/08/2007	09/11/2007	1400	2	13/11/2007	0900	a
26	f	24/09/2007	09/11/2007	1400	3	13/11/2007	0900	*
532	m	01/11/2007	09/11/2007	1400	4	13/11/2007	0900	a
525	m	01/11/2007	09/11/2007	1400	5	13/11/2007	0900	a

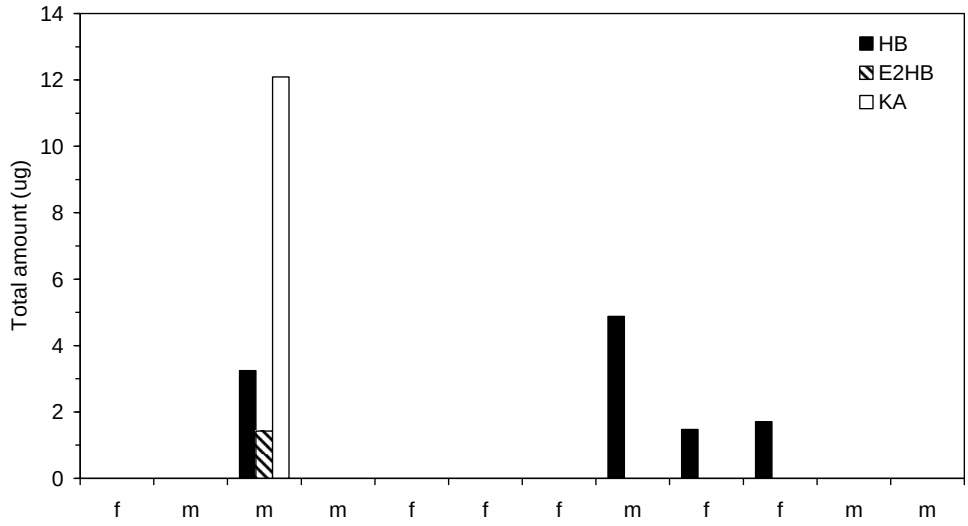
Table 2.1.1. Continued. Details of *L. tripustulatus* individuals used in the entrainment. ? = date not known.

Capsid i.d.	Sex	Date adult emerged	Date in	Time in	Filter No.	Date finished	Time finished	Dead/ alive end test
27	f	24/09/2007	09/11/2007	1400	6	13/11/2007	0900	a
538	m	?	14/11/2007	1820	1	16/11/2007	1045	a
540	m	?	14/11/2007	1820	2	16/11/2007	1045	a
546	m	08/10/2007	14/11/2007	1820	3	16/11/2007	1045	a
548	m	08/10/2007	14/11/2007	1820	4	16/11/2007	1045	a
535	m	08/10/2007	14/11/2007	1820	5	16/11/2007	1045	a
544	m	?	14/11/2007	1820	6	16/11/2007	1045	a
28	f	?	16/11/2007	1700	1	19/11/2007	0900	a
29	f	?	16/11/2007	1700	2	19/11/2007	0900	a

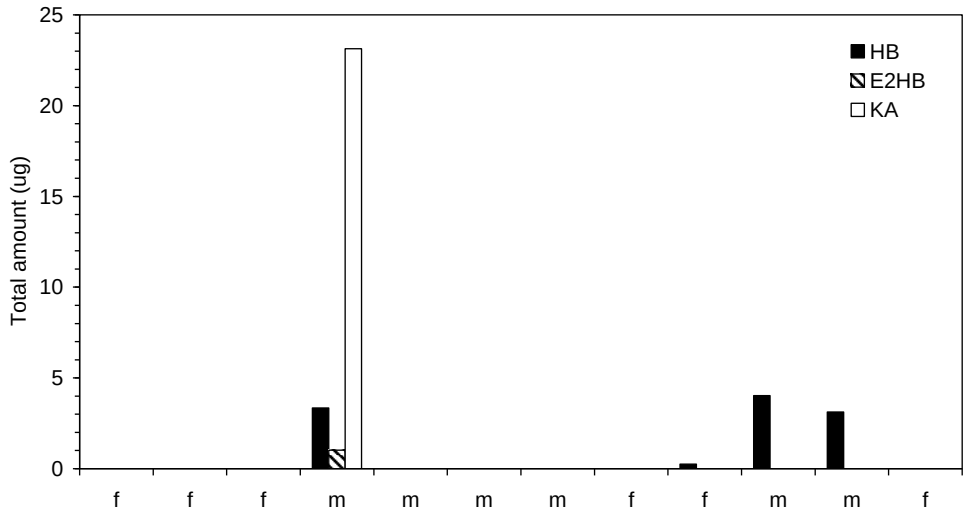
30	f	?	16/11/2007	1700	3	19/11/2007	0900	a
31	f	?	16/11/2007	1700	4	19/11/2007	0900	a
32	f	?	16/11/2007	1700	5	19/11/2007	0900	a
33	f	?	16/11/2007	1700	6	19/11/2007	0900	a

NB: * = capsid missing at end of test

a.



b.



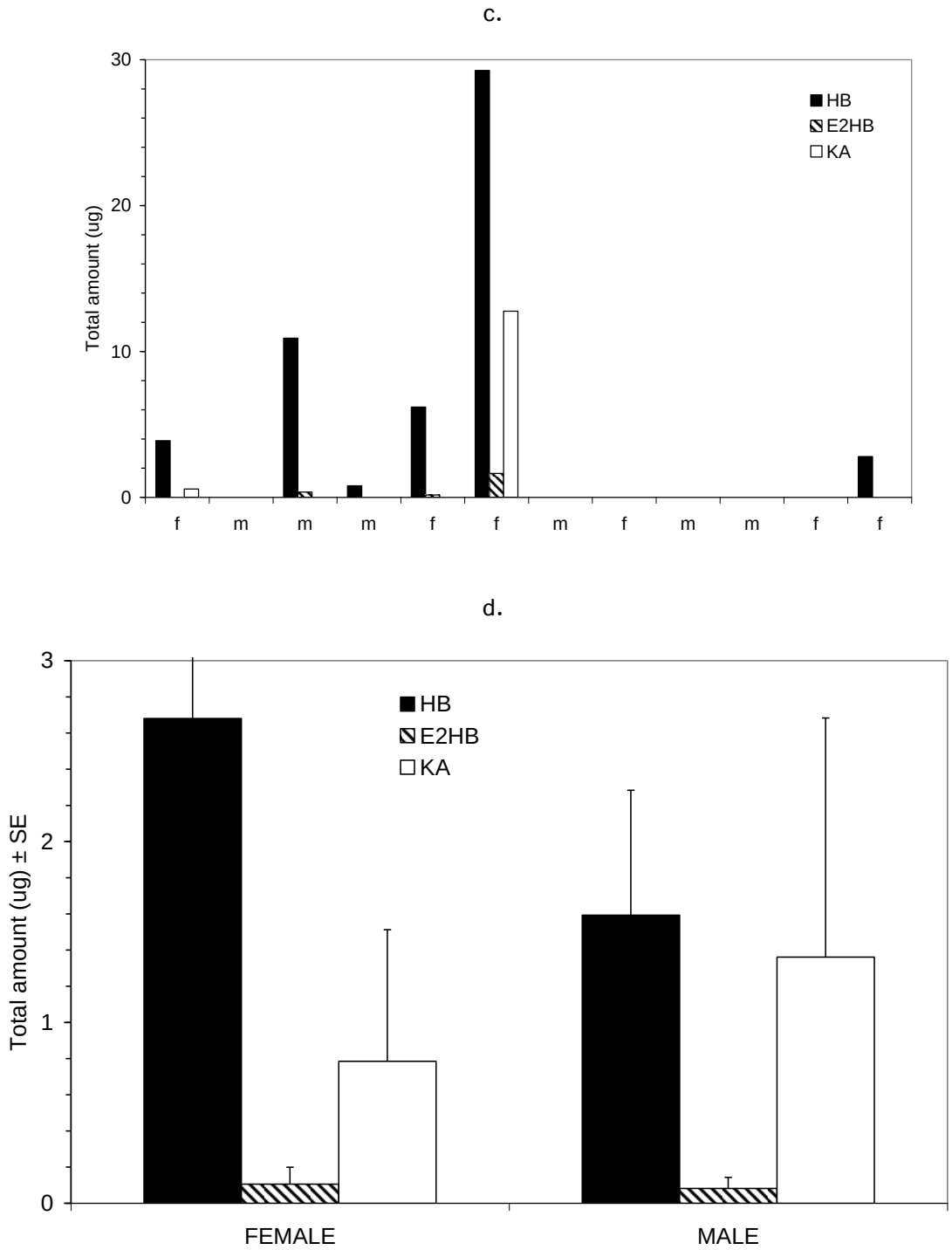


Figure 2.1.2. Compounds released by individuals (a–c) of *L. tripustulatus* and (d) total of compounds released by all males and females. Hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA).

Results

GC analyses were carried out on collections from 48 individual unmated *L. tripustulatus* (25 males and 23 females (18 November)) (Table 2.1.1). Both male and female *L. tripustulatus* were found to release hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal (Fig. 2.1.2). However, the variation between individuals was high and only 2 males and one female released significant amounts of all three compounds. Many of the capsids released hexyl butyrate. The grand mean amounts produced by males and females were remarkably similar and there was no evidence that any of these 3 compounds were sex specific. Filters from the last samples (12) have still to be analysed. GC-EAG analysis is in progress, using both male and female antennae, to try to identify electrophysiologically active compounds.

Conclusions

- Both males and females were found to release hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal, but the amounts produced by individuals were erratic. None of these compounds appeared to be sex specific and whether or not they are pheromone components is yet to be determined.
- It is possible that there are other components, as yet unidentified. GC-EAG is in progress currently.
- No consistent response has be found with GC-EAG at present.

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 for laboratory bioassays

Work began to develop a bioassay to to be used in the laboratory. Three tests were used; 1) Windtunnel, 2). Windtunnel and entrainment combined 3) Still air bioassay (*L. rugulipennis* and *L. tripustulatus*).

1. Windtunnel experiments

The windtunnel was set up in the culture room with fans drawing air in at one end and an extractor fan at the other end removing air (Fig. 3.1.1). Experiments were set up to test the synthetic compounds (hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA)) in microcapillary reservoir vials (see Task 3.3). The lures were made on 24 April and each contained 10 mg of synthetic pheromone and 1.0 mg BHT as an antioxidant (Table 3.1.1). Lures were stored at -20°C until used. A laboratory reared unmated female was used as a control. *L. rugulipennis* males (singly or in groups) were released downwind of either a single lure or an unmated female and observed for an hour with each. Experiments began on 24 July. Various methods were incorporated including observations under red light in darkness, observations at different times of day and repeated tests with unmated females to observe premating behaviour. The tests were run in a controlled temperature room at 16:8 h L:D and 20-24°C. The wind speed was varied between 0.01-0.65 m/s.

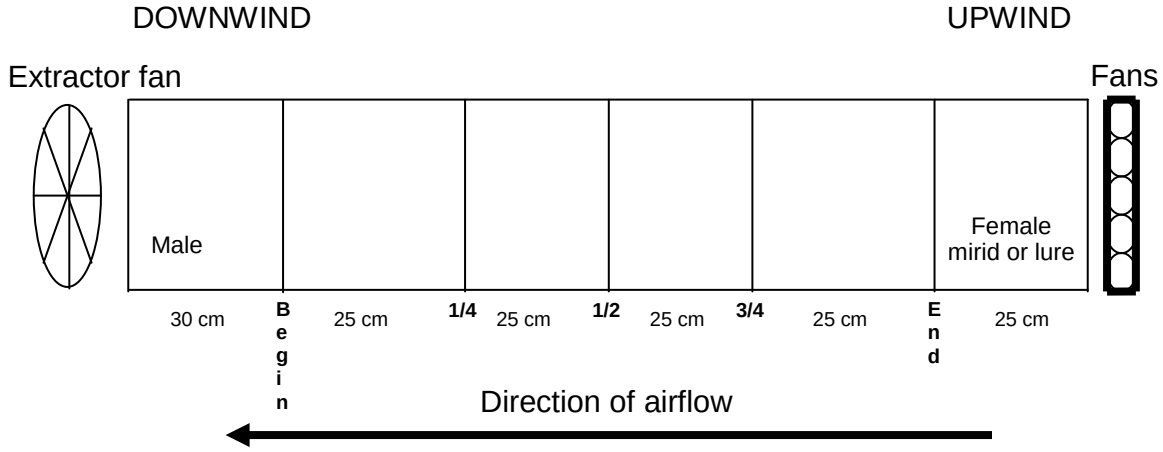


Figure 3.1.1. Schematic diagram of the windtunnel used for the capsid bioassays.

Table 3.1.1. Treatments and amount of each of the 3 synthesised compounds in each reservoir vial.

Code	HB (mg)	E2HB (mg)	KA (mg)
A	300	200	-
B	470	-	30
C	-	450	50
D	290	190	20
E	Laboratory reared unmated female		

2. Windtunnel and entrainment combined

On 5 October, a preliminary trial was set up to combine a windtunnel with an entrainment system so that it was possible to establish if the female was releasing pheromones whilst the male demonstrated an attraction to the female. The test with a caged female (i.d. 46) plus food and male was run for 3 days. The test was run in a controlled temperature room at 16:8 h L:D and 24°C. It was not possible to measure the air speed in this device directly, but the volume of air drawn through it was 500 ml/min.

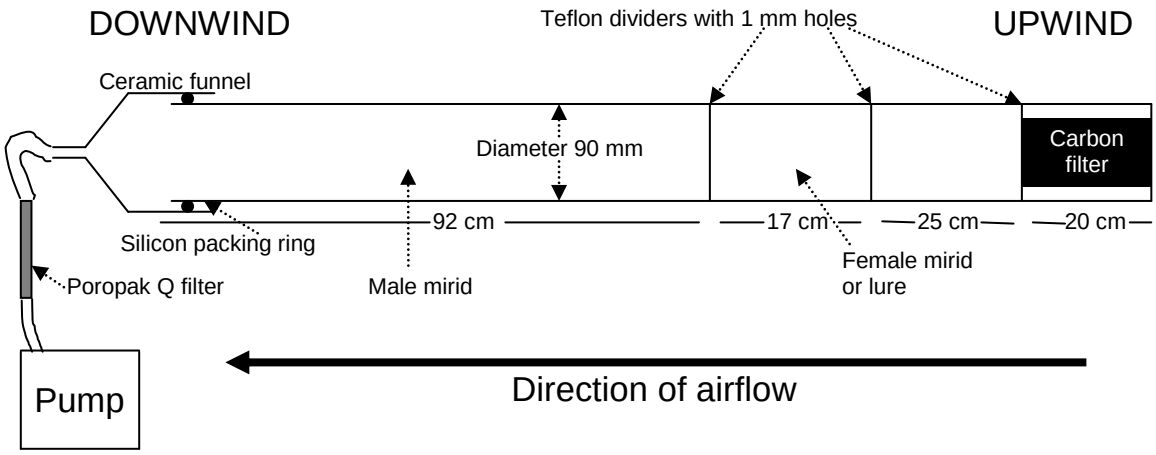


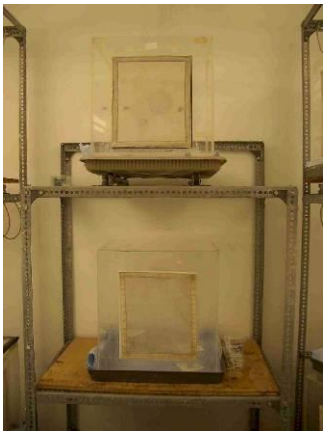
Figure 3.1.2. Schematic diagram of the windtunnel-entrainment device. The device was of circular cross section with a diameter of 90 mm.

3. Still air bioassay – 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 (Figures 3.1.3 and 3.1.4). These small cages were placed within large bioassay cages (76x51x50 cm or 46x50x45 cm) (Fig. 3.1.3). The tests were run in a controlled temperature room at 24°C in 16:8 h L:D. The bioassay cages also contained a control cage (hair roller) with a bean, but no female. 20 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. Into each large cage 2-7 males (dependent on availability) were released. Males were mostly field collected. Observations began the morning after setting up the experiment. The first observation was 30 min before the lights came on and then every hour until 1400 h for *L. rugulipennis*. A record was taken of the number of males inside or stuck to the female traps. One test was done with sticky traps and three with non-sticky plastic cup cages.

a

b



c

d

Figure 3.1.3. Apparatus used for the still air bioassay, a) large bioassay cages, b) front view of bioassay cages, c) caged capsid inside small plastic non-sticky trap, d) bioassay set up with sticky base traps.

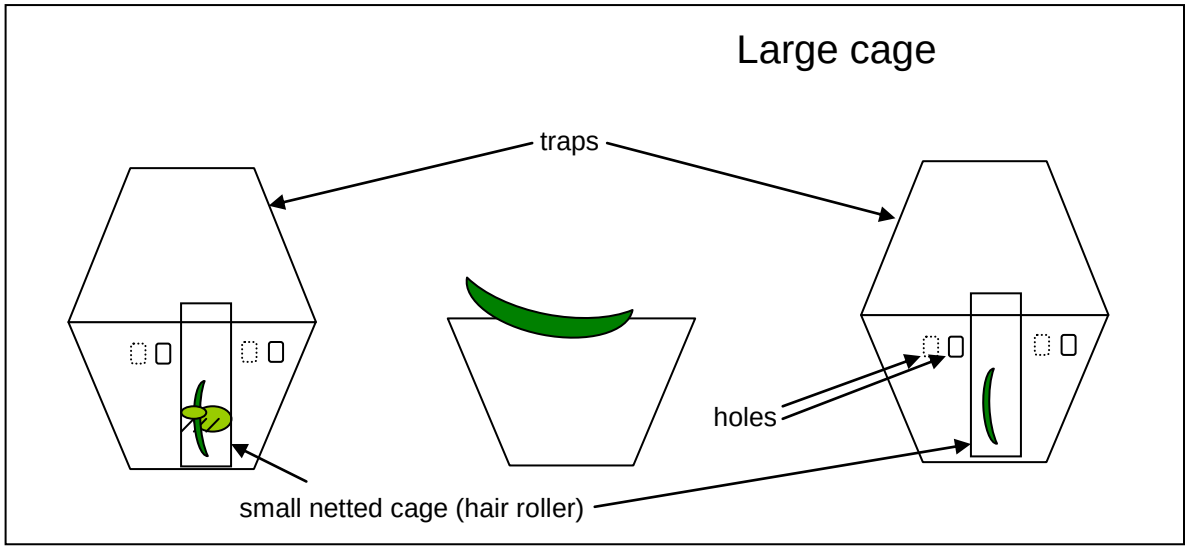


Figure 3.1.4. Design of small plastic cages (treatment and control), placed in large bioassay cage. 20 replicates were used for each test.

L. tripustulatus

For this species the same procedure was carried out as for *L. rugulipennis*, however, males are believed to be attracting females (see Obj. 2), hence, the sexes were reversed for this test. It is also not known what time of day the males were releasing pheromones and so the traps were set up using sticky bases to be able to get information about the number of females captured at night. A record was taken of the number of females stuck to the male traps. Observations began in the morning after setting up the experiment. The first observation was 30 min before the lights came on and then every hour until 1400 h. Each test was marked with species (L.t.), female/male number (1-), cage number (1-2), number of free males/females released and the date the test began and ended. Two trials were set up (6-8 and 13-14 november).

Results

1. Windtunnel experiments

In the first test on 24 July, a male and female *L. rugulipennis* were released into the tunnel and watched for 78 min. No contact or mating behaviour was observed and both insects were stationary at the downwind end of the tunnel by the end of the test. In the same test the male was observed taking short flights and short walks

towards lure A with a flight over the lure 37 min into the test. No attraction to lures B, C and D was demonstrated.

The test was repeated the next day, but no attraction was shown to the lures or the female. It was decided to use only unmated females to demonstrate that attraction could be achieved in the windtunnel before further lure testing was done.

On 1 August a female was placed in the tunnel with a food plant (fat-hen). Whilst the female remained on the plant feeding the male stayed downwind. After 1h, two more males were added, but there was no response to the female. After 2 h another female was added. This individual spent most of the time in short bursts of flight, but no attraction of males to either of the females was seen.

On 2 August the bioassay was performed under darkness using a red light. After 40 min a male approached the female from the side, touched her with his antennae and walked away. No further courtship or mating behaviour was observed. Both sexes were more active in darkness compared to light. Two more males were added, but no attraction was demonstrated.

On 3 October an unmated female was placed in a glass tube enclosure with gauze at each end, with a bean as food. A male was placed in the tunnel at the downwind end and observed for 30 min in darkness, at which point the lights were switched on. After an hour no attraction to the female was demonstrated. The next day this was repeated with 3 males but no attraction to the female was seen.

Following this, 6 tests using various traps (funnel and sticky) to catch males were used (Fig 3.1.5) so that continuous observations were not necessary. Instead the insects were checked every hour and position in the tunnel recorded. No males were found in funnel traps and there were no significant differences between the number of males trapped on sticky female and control cages.

a	b
	
c	d

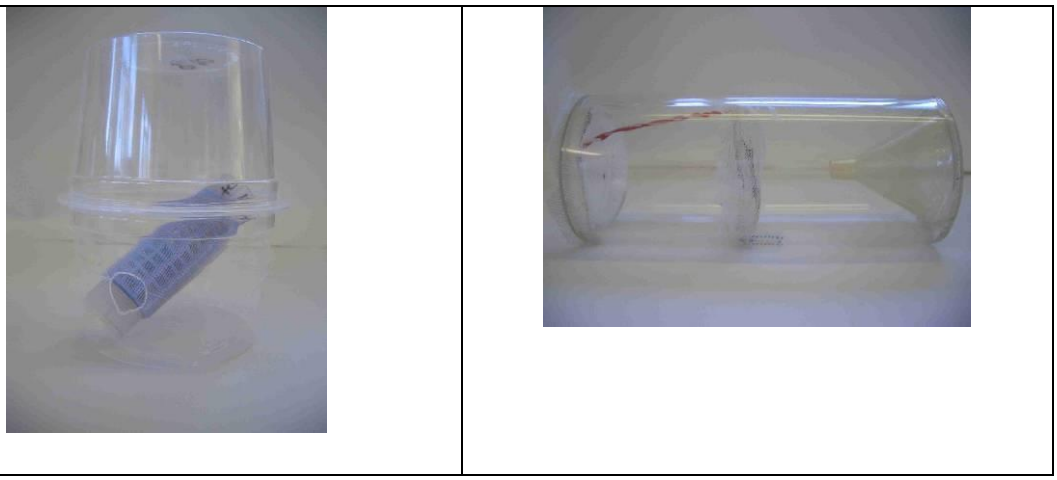


Figure 3.1.5. Various trap designs used in the windtunnel tests a) sticky traps with hair-roller cages, b) sticky plastic pot, c) non-sticky plastic pot trap, d) funnel trap.

2. Windtunnel and entrainment combined

The male was observed sitting on the mesh of the female cage, but it was unclear whether this was attraction to the female or the airflow. It was not possible to have a control in this device. Only hexyl butyrate was detected in the filter, indicating that the system was effective at collecting volatiles, but attraction was not demonstrated, presumably because the female on this occasion was not releasing pheromones.

3. Still air bioassay

L. rugulipennis

From the test done on 16 October using sticky bases with seven males added to each cage there was a total of 20 males (mean =1.0) and 30 males (mean = 1.5) on the female and control cages, respectively. After this date the non-sticky traps with holes were used. Evidence from field tests (Obj. 4) suggested that the females were attracting males in the morning, so observations were made then. On each of the sampling dates (18-23 October, 3 October and 31 October–2 November) there were totals of 84, 27 and 52 males in the female traps and 53, 39 and 59 in the control traps respectively (Fig. 3.1.6). On the first test dates the results were encouraging. More males were captured in the female cages compared to the male cages (Fig. 3.1.6. a). However, the subsequent tests were less clear.

This variation is partly due to the high variation in the number of males that females attract, or, indeed, the number of males attracted to females (Fig. 3.1.7). The mechanism for this difference is not known, but is likely to be due to the physiological state of the female – whether she is releasing pheromone (see also Obj. 4).

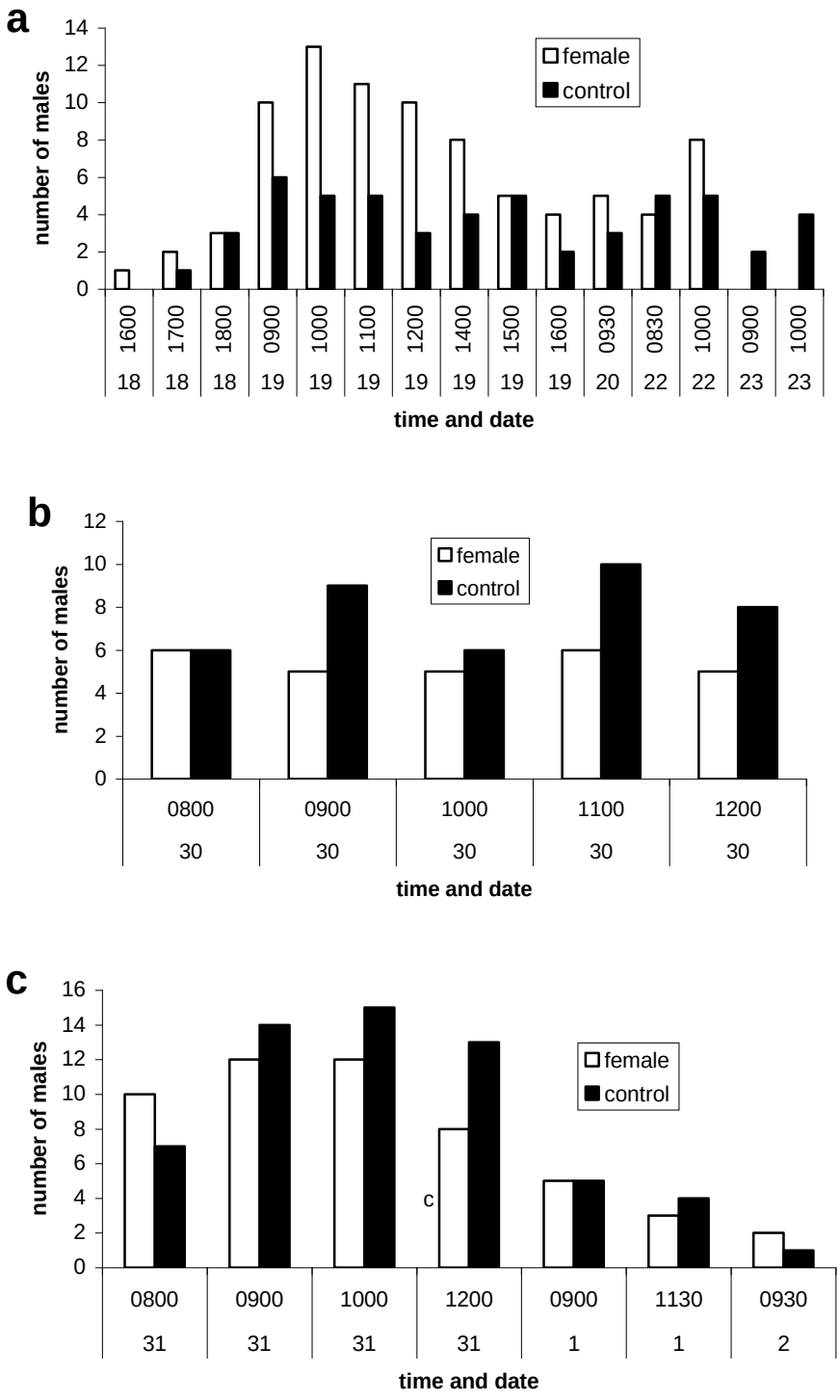


Figure 3.1.6. The number of male *L. rugulipennis* observed inside the female and control traps (20 replicates) over time at each test (a) 18–23 October, (b) 30 October and (c) 31 October-2 November. Bioassays were set up the previous day on each of the three occasions with 5 males added on the first, 4 on the second and 2 on the third date.

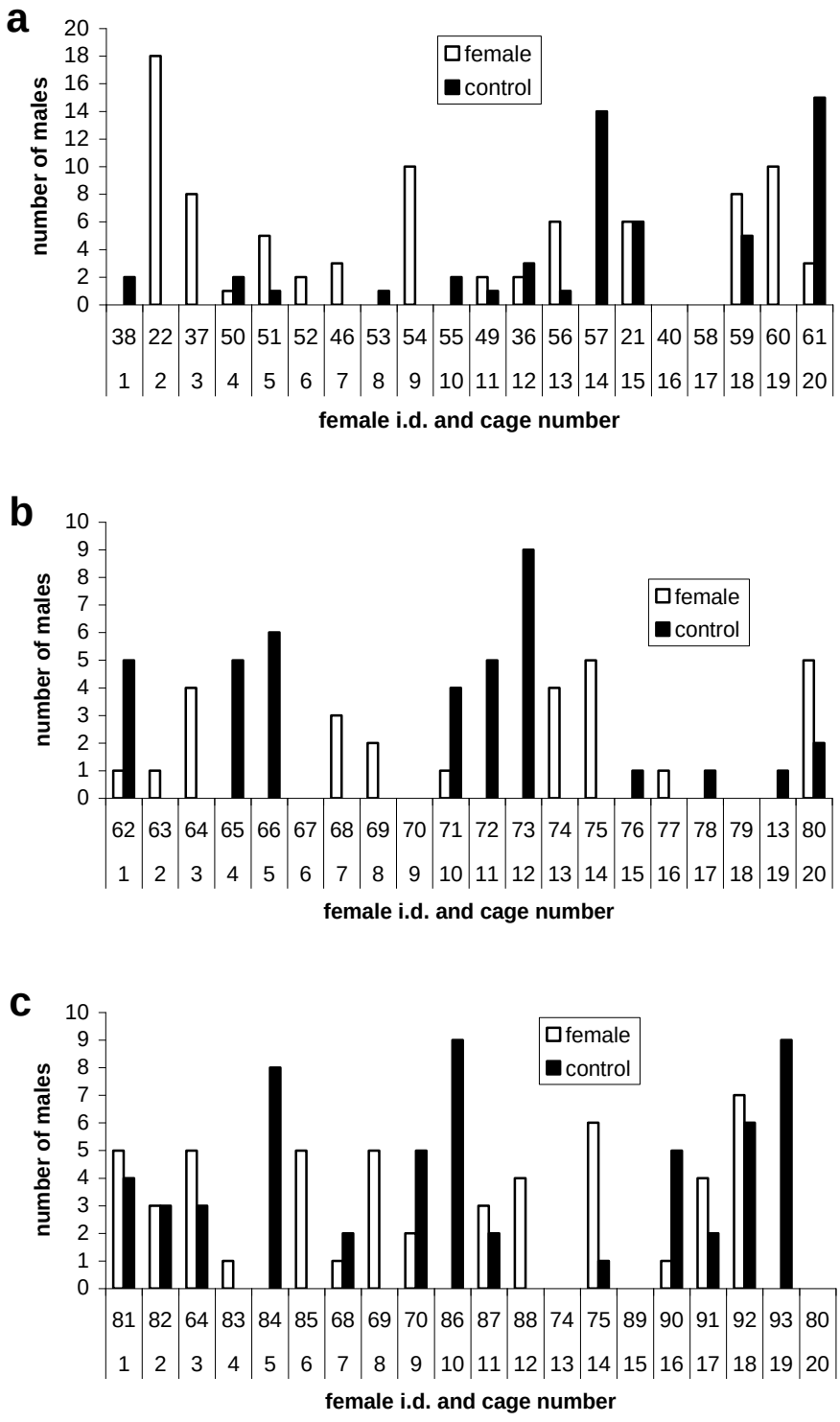
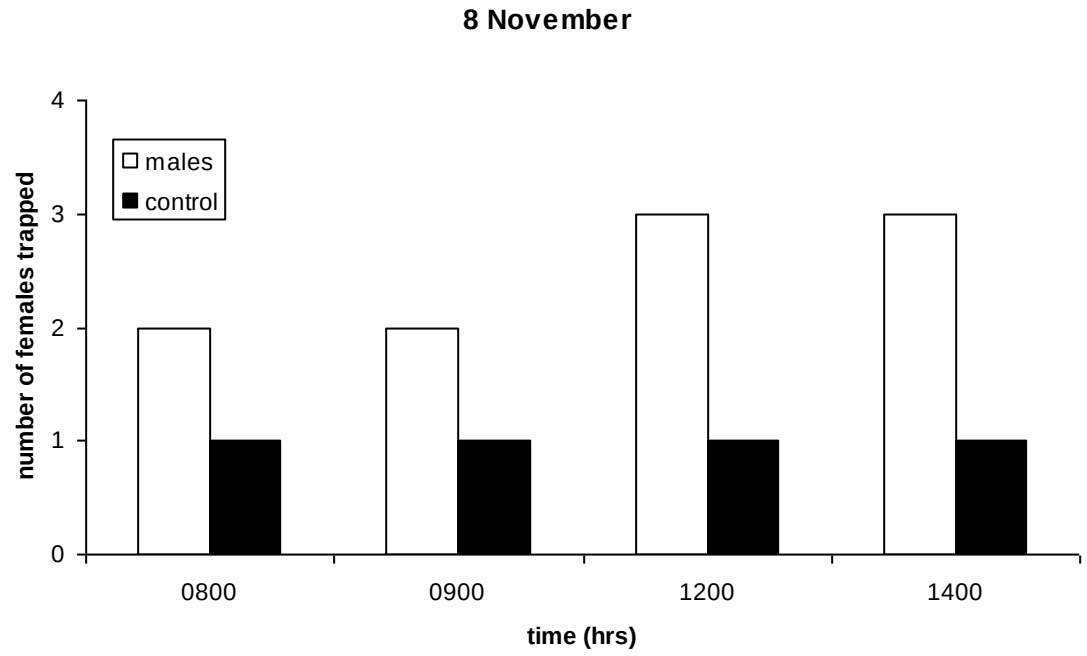


Figure 3.1.7. The cumulative number of male *L. rugulipennis* observed inside the individual female and control traps for replicates 1–20 on each test date (a) 18–23 October, (b) 3 October and (c) 31 October-2 November. Traps were set up the previous day on each of

the three occasions with 5 males added on the first, 4 on the second and 2 on the third date.

L. tripustulatus

The number of females caught on the sticky traps containing males was higher than on the control traps on both dates (Fig. 3.1.8).



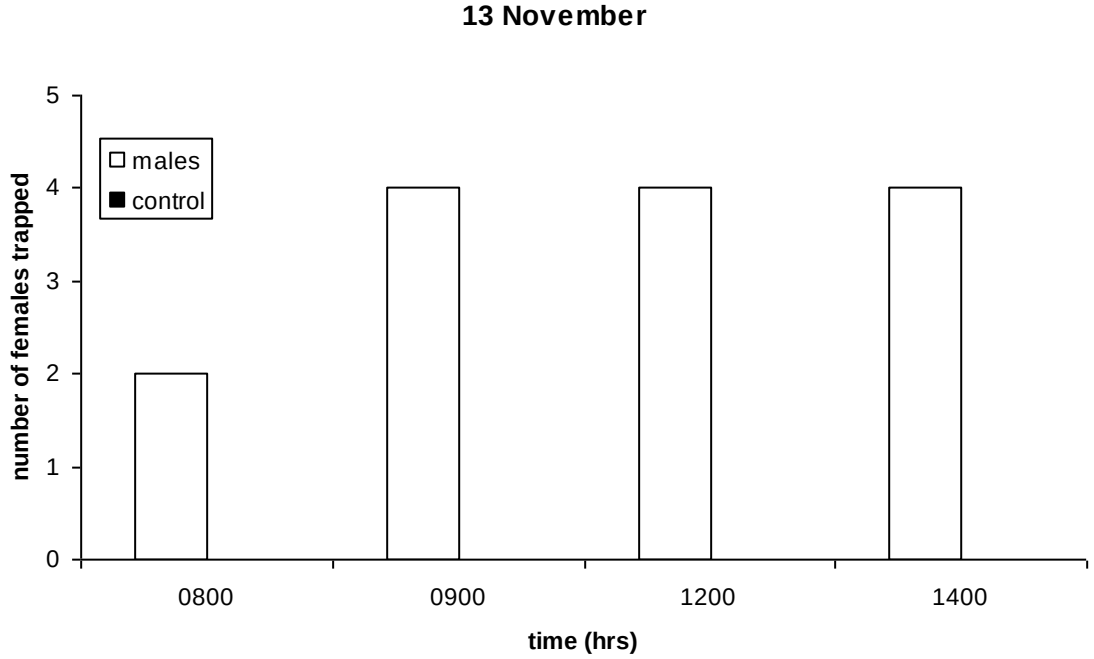


Figure 3.1.8. The cumulative number of females observed on the male and control non-sticky traps (20 replicates) on 2 dates. Traps were set up the previous day and 3 females were added to each replicate.

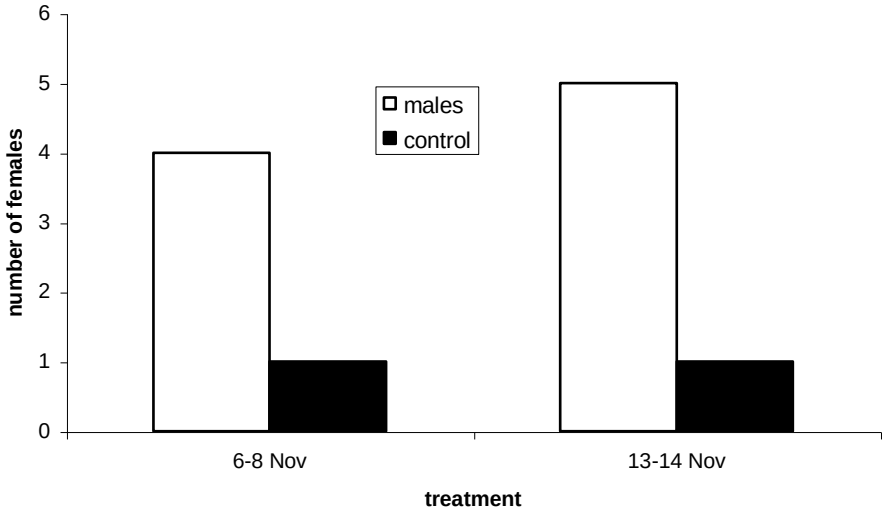


Figure 3.1.9. The cumulative number of females observed on the male and control sticky traps (20 replicates) on two test dates. Traps were set up the previous day and 2-3 females were added to each replicate.

Conclusions

- Windtunnel experiments were unsuccessful at demonstrating male attraction of *L. rugulipennis* to lures or unmated females.
- The first still air bioassay with *L. rugulipennis* was promising, with more males captured in female traps compared to control traps. The failure of the next two bioassays was believed to be due to the physiological condition of the mirids (entering diapause).
- Some limited evidence was obtained that *L. tripustulatus* females are attracted to males. This suggests that if sex pheromone attraction is involved, males may be releasing pheromones. However, more studies are needed to confirm this.

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.

This work is ongoing (see Obj. 3); however, more studies are needed to identify the ratio of the 3 pheromone components released from *L. rugulipennis*, *L. pabulinus* and *L. tripustulatus*. Analysing collected samples with GC-EAD will make it possible to confirm both the attractive components and the ratio of these components for all three species.

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 2.1) and GC analysis was performed at NRI. The entrainment apparatus was set up on 11 September. Single unmated females and males (> 6 d old) were used. Capsids were provided with bean during entrainment.

Each 24 hour period of pheromone collection was divided into three periods, based on data gathered from the field experiments (see Obj 5);

- night (dark for 8 h) (Period I) (code: black)
- first part of day (8 h light, morning) (Period II) (code: white)
- second part of day (light for 8 h, afternoon) (Period III) (code: blue)

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 3.2.1). 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. 3.2.1). 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. Pumps were sampled from: 0130–0800 (Period I), 0800-1600 (Period II) and 1600-0130 (Period III). All filters were changed at the same time. Collections were made for different lengths of time (one or several days).

In the second round of sampling the times were changed slightly to include 1 h of dark in period II. The pumps were timed to come on between 2000–0400 (Period I, dark), 0400-1200 (Period II, 1 h dark, 7 h light) and 1200-2000 (Period III, light). For both tests, capsids were placed into the chambers approximately 2 h before the filters were in place to give them time to settle, in order to avoid a build up of any defensive volatiles. At the end of the tests, filters were wrapped in

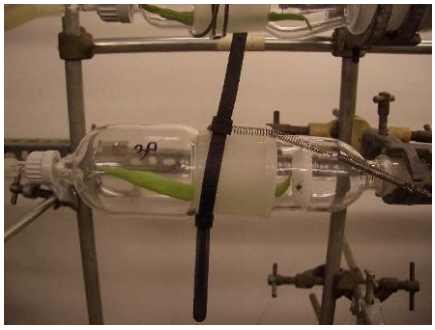
aluminum foil and transported to NRI for analysis. Capsids were checked at the end of the entrainment to ensure they had survived. Each sample was labeled with species (L.r.), female number (1-), time interval (I, II, III), charcoal filter number (1-6), and date sampling began (12/09/07-), for example: Lr-1-I-1-11/09/07 or Lr-15-I-3-27/09/07. Entrainment dates were 12-17, 18-19, 19-20, 20-21 September, 2-3, 3-4 and 4-8 October. The details of the capsids used in the entrainment are given in Table 3.2.1.

The collection filters were extracted at NRI and analysed by GC-MS as described in the pheromone analyses by GC in Task 2.1.

a

b

c



d

Figure 3.2.1. Entrainment apparatus used for sampling pheromones released by *L. rugulipennis* at three time periods in the day. (a) pump and piping from glass chambers (b) entrainment apparatus (c) glass chamber containing capsid and bean (d) capsid feeding on bean in glass chamber.

Table 3.2.1. Details of the *L. rugulipennis* capsids used in the entrainment. * date not known.

Capsid i.d.	Sex	Filter	Emerged as adult	date in	time in	date finished	time finished	dead/alive at end
20	f	1	16-28/08/07	12/09/2007	2000	17/09/2007	0730	a
29	f	2	28/08 - 3/09/07	12/09/2007	2000	17/09/2007	0730	d
30	f	3	28/08 - 3/09/07	12/09/2007	2000	17/09/2007	0730	a
31	f	4	28/08 - 3/09/07	12/09/2007	2000	17/09/2007	0730	a
32	m	5	16-28/08/07	12/09/2007	2000	17/09/2007	0730	a
33	m	6	16-28/08/07	12/09/2007	2000	17/09/2007	0730	a
34	f	1	07/09/2007	18/09/2007	2000	19/09/2007	0730	a
35	f	2	14/09/2007	18/09/2007	2000	19/09/2007	0730	a
36	f	3	14/09/2007	18/09/2007	2000	19/09/2007	0730	a
37	f	4	14/09/2007	18/09/2007	2000	19/09/2007	0730	a
38	f	5	07/09/2007	18/09/2007	2000	19/09/2007	0730	a
39	f	6	07/09/2007	18/09/2007	2000	19/09/2007	0730	a
34	f	1	07/09/2007	19/09/2007	2000	20/09/2007	0730	a
35	f	2	14/09/2007	19/09/2007	2000	20/09/2007	0730	a
36	f	3	14/09/2007	19/09/2007	2000	20/09/2007	0730	a
37	f	4	14/09/2007	19/09/2007	2000	20/09/2007	0730	a
38	f	5	07/09/2007	19/09/2007	2000	20/09/2007	0730	a
39	f	6	07/09/2007	19/09/2007	2000	20/09/2007	0730	a
34	f	1	07/09/2007	20/09/2007	2000	21/09/2007	0730	a
35	f	2	14/09/2007	20/09/2007	2000	21/09/2007	0730	a
36	f	3	14/09/2007	20/09/2007	2000	21/09/2007	0730	a
37	f	4	14/09/2007	20/09/2007	2000	21/09/2007	0730	a
38	f	5	07/09/2007	20/09/2007	2000	21/09/2007	0730	a
39	f	6	07/09/2007	20/09/2007	2000	21/09/2007	0730	a
20	f	1	16-28/08/07	02/10/2007	1800	03/10/2007	1600	a
46	f	2	21/09/2007	02/10/2007	1800	03/10/2007	1600	a
47	f	3	17/09/2007	02/10/2007	1800	03/10/2007	1600	a
2	f	4	16-28/08/07	02/10/2007	1800	03/10/2007	1600	a
48	f	5	17/09/2007	02/10/2007	1800	03/10/2007	1600	a
18	f	6	*	02/10/2007	1800	03/10/2007	1600	a
20	f	1	16-28/08/07	03/10/2007	1800	04/10/2007	1600	a
46	f	2	21/09/2007	03/10/2007	1800	04/10/2007	1600	a

47	f	3	17/09/2007	03/10/2007	1800	04/10/2007	1600	a
2	f	4	16-28/08/07	03/10/2007	1800	04/10/2007	1600	a
48	f	5	17/09/2007	03/10/2007	1800	04/10/2007	1600	a
18	f	6	*	03/10/2007	1800	04/10/2007	1600	a
20	f	1	16-28/08/07	04/10/2007	1800	08/10/2007	1200	a
46	f	2	21/09/2007	04/10/2007	1800	08/10/2007	1200	a
47	f	3	17/09/2007	04/10/2007	1800	08/10/2007	1200	d
2	f	4	16-28/08/07	04/10/2007	1800	08/10/2007	1200	d
48	f	5	17/09/2007	04/10/2007	1800	08/10/2007	1200	d
18	f	6	*	04/10/2007	1800	08/10/2007	1200	d

Results

The time of day that females released pheromone compounds was mainly between 2000-1200, i.e. night and morning (5 day collection, 12-17 September) (Fig. 3.2.2). The quantity of each pheromone compound released varied from female to female. When females were sampled for one day the variation between females became more obvious. Some females released the compounds throughout a 24 hour period and other females did not release any compound for the 3 days (female No 48). In addition, not all females were releasing the 3 compounds every day (Fig. 3.2.3). The ratio of the 3 compounds and time of day released also differed depending on the individual.

Conclusions

- The amount, ratio, time of day and day a female released pheromone depended on the individual female.

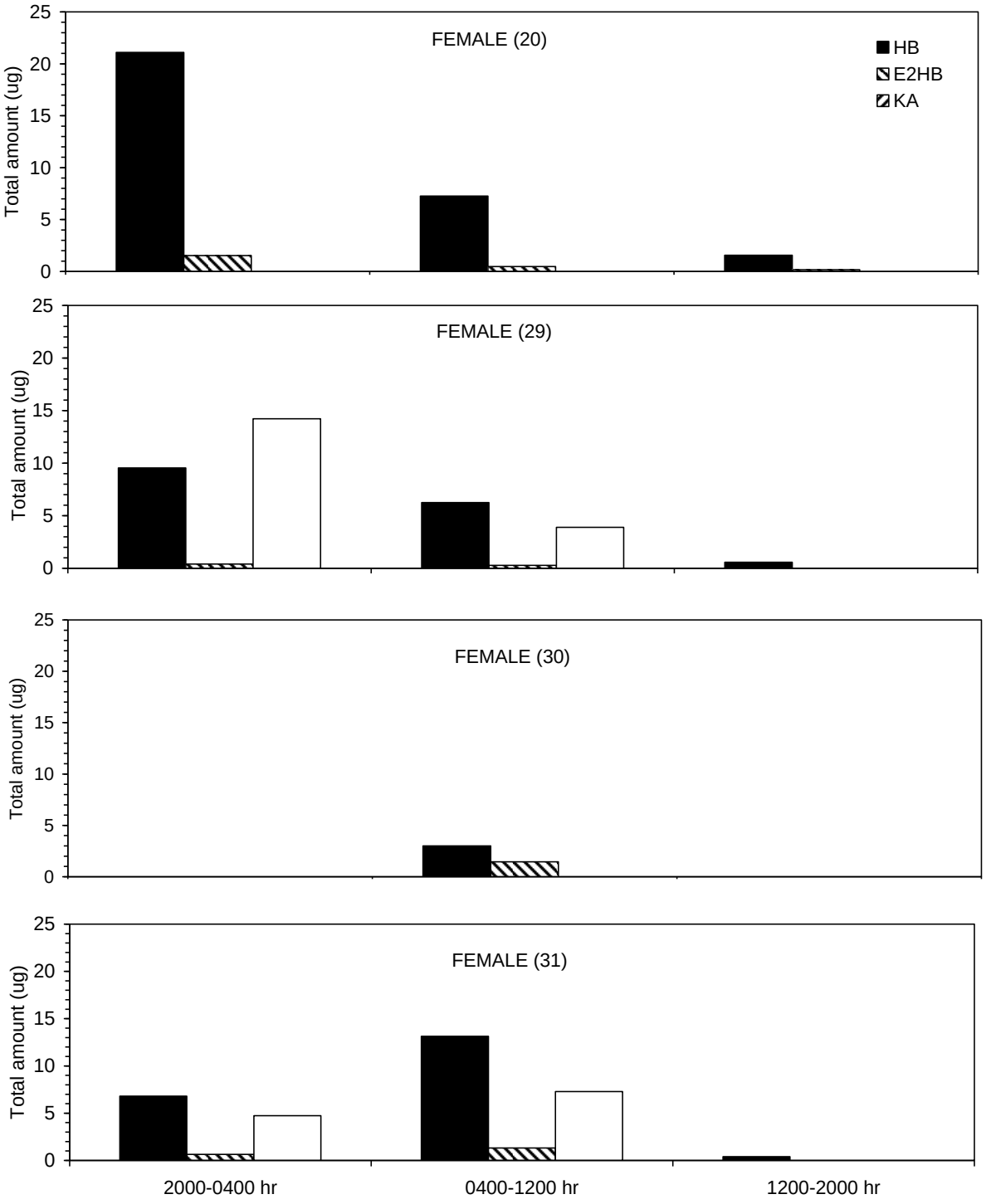


Figure 3.2.2. Quantity of pheromone compounds released by single female *L. rugulipennis* over 5 d separated into the 3 times of the day on females from 12–17 September. Hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA). 2 males were also entrained, but none of these compounds was produced.

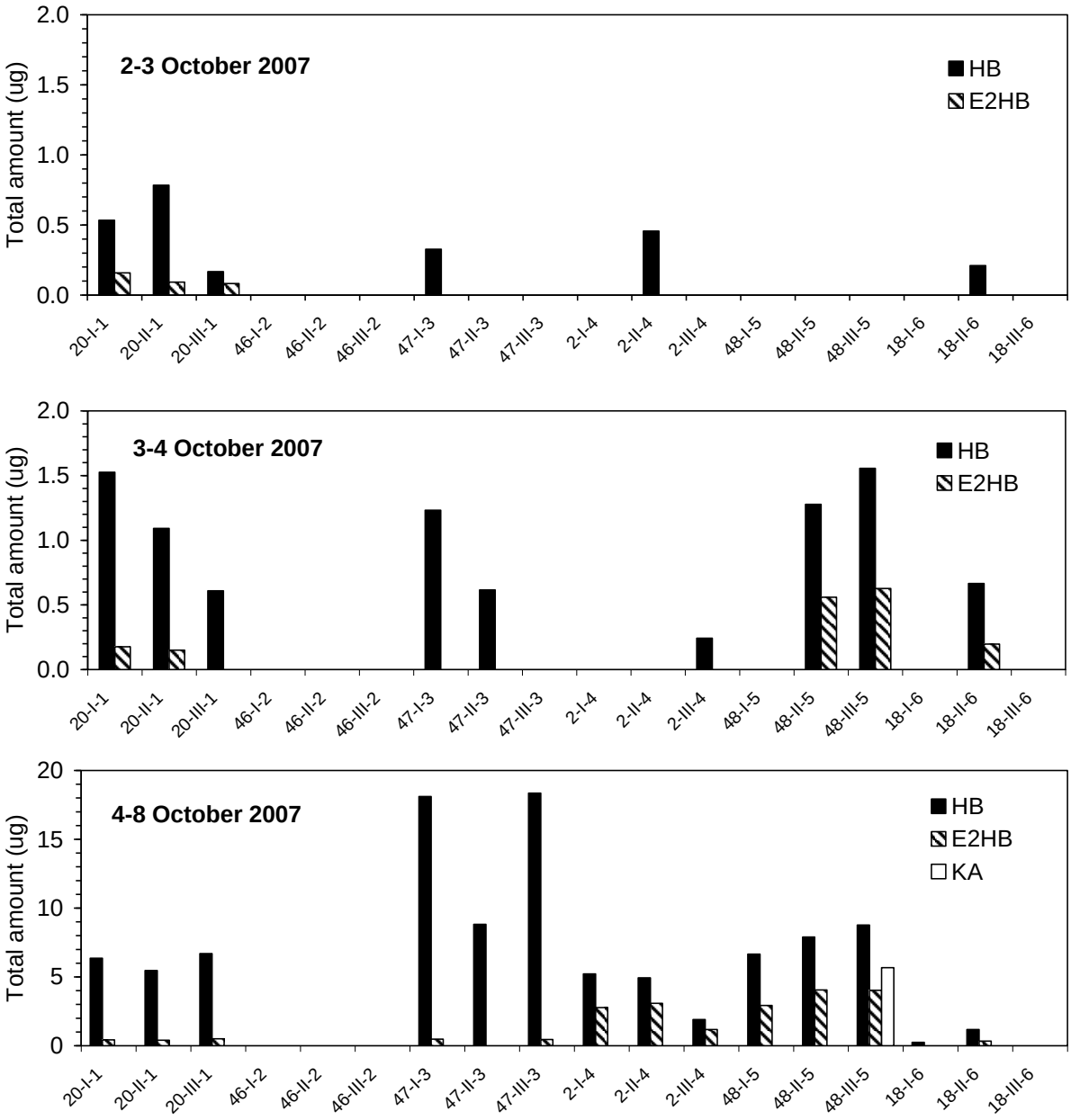


Figure 3.2.3. Quantity of pheromone compounds released by single female *L. rugulipennis* over a 1 and 4 d period separated into the 3 times of the day (codes are female i.d., time of day (I = dark, II = morning, III = afternoon), entrainment line No). Hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA).

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 laboratory windtunnel 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, in comparison with virgin females where possible.

Materials and methods

1. Micro-capillary reservoir lure

Large scale randomised block experiments comparing catches of capsids in green delta traps bated with different lure blends/types were done in plots of apple, blackcurrant, cucumber, strawberry and weeds (Table 3.3.1).

At sites 1-4, four different treatments (A-D) comprising micro-capillary lures containing different blends of hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA) and an untreated (treatment E) were included (Table 3.3.2). At site 5, an additional treatment comprising a caged unmated *L. rugulipennis* female was included. In a previous study, the ratio of release of the components from a single *L. rugulipennis* female was 1.5:1.0:0.08, HB:E2HB:KA. Two way and 3 way blends were made by combining components in the same ratio. 10% BHT was included as an antioxidant.

Dispensers were sample vials fitted with a 5µl glass micro-capillary. A hole was made in the septum with a GC ferrule reamer and then the capillary was inserted carefully from the Teflon side in order to ensure a good seal of the septum round the capillary (Fig. 3.3.1). The total release was approximately 1µg/hr (Fig. 3.3.2). 10 mg of the blend was dispensed into each lure, which should have lasted approximately 400 days. The lures were made on 17 July.

A 5 x 5 Latin Square design was used for sites 1-4 and a 6 x 6 Latin square was used at site 5 where the unmated female treatment was included. Plots were individual delta traps each containing a single lure (no lure in untreated) (Fig. 3.3.1). The lures (or unmated female in cage) were suspended vertically inside a green 20 x 20 cm delta trap from a wire twist tie. Sticky bases had additional Ecotac applied. The traps were deployed at sites 2, 3 and 5 on 23 July and at site 1 on 31 July. At sites 2, 3 and 5 the traps were deployed in a grid with each treatment on each row. At the apple orchard (site 1) traps were deployed in a row along the edge of the orchard. They were suspended at mid crop height (Fig. 3.3.1). At the strawberry site (site 3) the traps were between the strawberry plants, fixed on top of the grow bags (Fig. 3.3.1). At site 4 (cucumber) each

trap was placed in a separate protected crop and the grower checked the traps on 8 and 24 August, and 12 September. The traps at this site were placed above the crop. Traps were spaced at least >10 m apart on all sites and the position was allocated at random.

The 5µl micro-capillaries at the blackcurrant, strawberry and vine sites were replaced with 10µl micro-capillaries on 3 August to increase the release rate. In addition, 4 of the lures on row 5 at the blackcurrant site were replaced with new lures so an analysis could be done to test the longevity of the compounds in the field. On this date, 5 field collected (isolated for 2 weeks) females were introduced into cages at the vine site in delta traps (treatment F). Later 5 laboratory reared unmated females were placed in the traps. The females were held in small gauze cages (made from hair rollers with caps at each end holding the gauze in place). Damp cotton wool and a piece of bean were also included in the cage for humidity and food, respectively. The trials ended on 10 August.

Weekly counts of the numbers of males and females of each species of capsid caught in each trap were made. Sticky bases were stored in pizza boxes and capsid identification confirmed using a microscope in the laboratory.

Table 3.3.1. Sites used for testing the efficacy of the micro-capillary 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 20 m apart.	*L. pabulinus*	Nichol Farm, Deerton Street, Teynham ME9 9LJ	John Moor
2	Blackcurrant	23 rows of blackcurrant. Used 2nd row from edge then every 4th row 12 m apart in a grid. 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 B Calcutt by T Maynard at NGR TQ 718 398	Rob Saunders & Bill Calcutt
3	Strawberry	Protected table top strawberries. Used 3rd row in the 1st and then every 3rd tunnel. Traps spaced 20 m apart.	*L. rugulipennis*	Robert Pascal, Clockhouse Farm, Linton	Robert Pascal
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	Weedy vineyard (organic)	Traps spaced 10 m between rows and 10.8 m within a row between vines. Weeds: fat-hen, scentless mayweed, Borage, Chickweed, Scarlet pimpernel,	*L. rugulipennis*	Poultry Farm, Marden NGR TQ 742 434	Nick Hall & Peter Hall

Germander speedwell and thistle.

Table 3.3.2. Treatments, hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA).

	Ratios of components in blends			
Treatment	HB (mg)	E2HB (mg)	KA (mg)	Virgin female *L. rugulipennis*
A	300	200	0	0
B	470	0	30	0
C	0	450	50	0
D	290	190	20	0
E	0	0	0	0
F (site 5 only)	0	0	0	1

Notes:

All except E and F had 10% BHT as antioxidant.

Ratio from single *L. rugulipennis* female 1.5 : 1 : 0.08 HB : E2HB : KA

a

b

c

d

Figure 3.3.1. (a) micro-capillary reservoir lure, (b) lure suspended in delta trap (c) delta trap at vine site (d) delta trap deployed in strawberry crop.

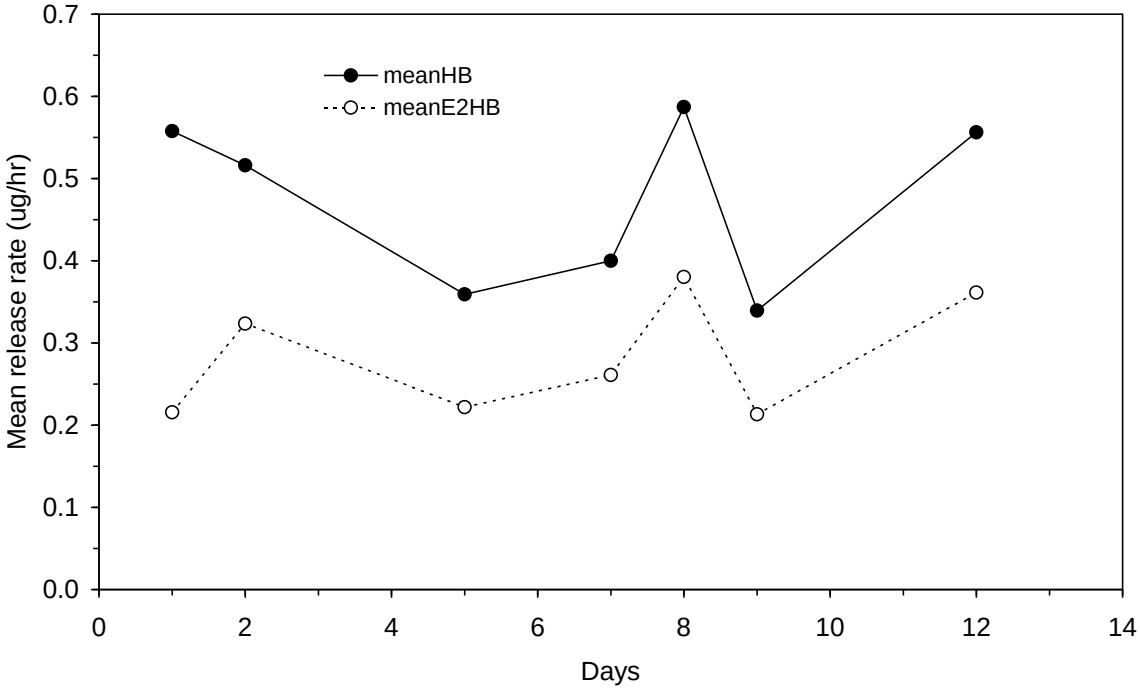


Figure 3.3.2. Release rates of a 3:2 HB/E2HB mixture (10 mg total) from reservoir microcapillary in a windtunnel at 27°C and 8 km/h windspeed (volatiles trapped on Porapak Q as for live insects and assayed by GC on SupelcoWax column with decyl acetate as internal standard).

2. Micro-capillary lure

This test was set up to test the attractiveness of blends of hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA) dispensed separately and mixed in glass micro-capillaries to *L. rugulipennis* in the field, in comparison with unmated females and a reservoir of all three components (Table 3.3.3). A small scale randomised block experiment was set up at EMR. The site was a triangular shaped weedy area (170x150x75m), 'Ditton Field', with herbs including fat-hen, sow-thistle and scentless mayweed. There was an Italian poplar windbreak to the west, two rows of pears to the south and raspberries to the east.

The treatments were green 20x20 cm delta traps with white sticky bases (additional Ecotac added). The traps were suspended at weed height on two canes taped together (Fig. 3.3.3). All traps, with the exception of the controls contained one of the following treatments: a lab reared unmated female *L. rugulipennis*, micro-capillary lures with components in combination or separate, or a high ratio component reservoir lure (Table 3.3.3).

The trial was set up on 10 September. Females were contained in a cage consisting 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 cotton wool at the bottom to maintain the humidity and a section of bean as food (food alone was not found to attract males in a previous study, see Obj. 5). The cage was placed vertically through a hole made in the top of the delta trap, so that the cage was not touching the sticky base (Fig. 3.3.3). Females known to be calling from the last experiment were used initially (numbers 1, 4, 8 and 17). The females were renewed on 14 September (numbers 2, 6, 11 and 23).

The 5 µl glass micro-capillary tubes were suspended on a piece of electrical tape (5mm diameter, 70mm long, 4mm apart) with a paperclip from the top of the delta trap (Fig. 3.3.3) and renewed every two hours during the observation period. The micro-capillary tubes are expected to last for only approximately 2 hours, so, the tubes were re-filled at 7am and 9am each morning. To fill the micro-capillaries, the tubes were held at a 90 degree angle in the liquid until the fluid reached the top by capillary action. The reservoir dispensers (sample vials fitted with a 5µl glass micro-capillary) were suspended from a wire twist tie (see previous micro-capillary reservoir lure trial). The total release approx was expected to be 1µg/hr, hence, 5 mg of the blend should last approximately 200 days.

A randomised block design with 4 replicates of each treatment was used, with each treatment in each of 4 rows. Plots were individual delta traps each containing a single lure (no lure in untreated/control). Traps were spaced 10 m apart in a grid system. Counts of mirids captured in the traps were made 2 hours after the micro-capillary tubes had been filled, 9am and 11am each day. The sticky bases were examined under a microscope after the trial to confirm the sex and species of mirid.

Table 3.3.3. Treatments, hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA)

Treatment	Components tested	Concentration of components (mg)	Vial label
1	(HB) + (KA)	(10)+(10)	E and F
2	(HB) + (E2HB + KA)	(10)+(450+50)	E and C
3	(HB + KA)	(300+200)	A
4	(HB + E2HB + KA)	(290+190+20)	D
5	Reservoir with high ratio of all 3 components	(10+10+10)	H

	(1:1:1)		
6	Virgin females	-	-
7	Untreated control	-	-
Notes: All except treatments 6 and 7 had 10% BHT as antioxidant. Ratio from single *L. rugulipennis* female 1.5 : 1 : 0.08 HB : E2HB : KA			

a

b

c

d

Figure 3.3.3. Photographs of the micro-capillary lure test (a) micro-capillary lure, (b) lure inside delta trap, (c) live capsid cage in delta trap, (d) 'Ditton Field' where the test was set up.

Results

1. Micro-capillary reservoir lure

At site 1 (apple) only three female *L. rugulipennis* were found in two delta traps. No capsids were captured at site 3 (strawberry) for the duration of the test. The strawberry site had very few insects of any species on the sticky bases. Equally, no mirids were captured at site 4 (cucumber).

At site 2 (blackcurrant) only one male *L. pabulinus* was found in one replicate of treatment D. Although *L. rugulipennis* was abundant at site 5 (demonstrated by sweep netting) only one male *L. pratensis* was trapped in treatment B and D, one male *L. rugulipennis* in treatment E (control), two male *L. rugulipennis* in treatment D and one *L. rugulipennis* female in treatment A. The female cages trapped 4 male *L. rugulipennis* and one male *L. pratensis*. Changing the microcapillaries at three of the sites to 10µl did not result in any males being captured.

2. Micro-capillary lure

Between 11-18 September very few mirids were captured (Table 3.3.4). However, the majority of the male *L. rugulipennis* were found in the unmated female traps. No males were found in the micro-capillary tube traps.

Table 3.3.4. Number of adult *L. rugulipennis* trapped in green delta traps with white sticky bases with different treatments. Hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and (*E*)-4-oxo-2-hexenal (KA).

Treatment	males	females
(HB) + (KA)	0	0
(HB) + (E2HB + KA)	0	0
(HB + KA)	0	0
(HB + E2HB + KA)	0	0
Reservoir	2	1
Virgin females	5	1
Untreated	0	3

Conclusions

- The ratios and blends in the micro-capillary reservoir and micro-capillary lures did not attract sufficient numbers of male capsids compared to unmated females lures.

It is possible that the point source, plume, concentration, blend or ratio were not correct.

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

The release rates of the dispensers collected in from field are to be analysed.

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)

This work will begin in year 2.

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

1. Experiment one

A small scale field trial using caged females in delta traps with sticky bases was done to determine the efficacy of delta traps for trapping *L rugulipennis* males and establish what time of day females were 'calling' (releasing pheromone). The site was an organic apple orchard GE186 (1.39 ha) at EMR. The plot was a mixture of young Rajka, Rubinola and Ceeval trees on M9 rootstocks. The trees were 1.83 m apart and the rows were 3.05 m apart and interspersed with mayweed. The traps were green 20x20 cm green delta traps with a white sticky base. The traps were suspended at weed height on two canes tied together with tape. The traps were arranged in 3 rows of 8 (plot row numbers 15, 27 and 35), 7 trees apart and numbered 1-24. Traps 3, 7, 12, 16, 17 and 21 were control traps with no female inside (2 on each row).

All traps, with the exception of the controls, contained a field collected female *L. rugulipennis* that had been isolated from males for over one week. Females 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 cotton wool on the bottom to maintain the humidity and a mayweed flower as food (*Matricaria*). The cage was placed vertically on the sticky base (Fig. 5.1.1).

a

b

Figure 5.1.1. (a) Female cage on sticky base inside delta trap and (b) traps set up in organic apple orchard at EMR.

Counts of the number, sex and species of mirids caught in the traps were made between 1-3 h intervals between 7-17 August. On 8 August, any females not attracting males were replaced with new females. On 10 August sticky bases with extra glue (Ecotac) were made and old sticky bases replaced. Records of wind speed, light (fraction of hour which was sunlight), temperature and humidity were obtained from the Water Centre records, courtesy of Olga Grant, EMR.

2. Experiment two

A small scale trial using caged females in delta traps with sticky bases was used to determine the attractiveness of female mirids to males and identify the time that females are calling. This was to enable future testing to be done at the appropriate time of day. The site was a triangular shaped area (170x150x75m) of abandoned weeds ('Ditton Field' at EMR) with herbs including fat-hen, scentless mayweed and sow-thistle. There was an Italian poplar windbreak to the west, two rows of pears to the south and raspberries to the east.

The traps were green 20x20 cm delta traps with a brown sticky base and were suspended at weed height on two canes taped together at the top. Extra Ecotac was added to the sticky bases to ensure trapping of attracted males. All traps, with the exception of the control traps, contained either a laboratory reared unmated female (matured to adult 16/08/07-28/08/07) or a newly captured field collected female *L. rugulipennis*. Females 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 cotton wool on the bottom to maintain the humidity and a section of bean as food. 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 (Fig. 5.1.2). The traps were arranged in 4 rows of 7, 10 m apart in a grid (numbered 1-28) and 10 m in from the edge of the plot. Traps 5, 12, 16 and 24 were control traps with no female inside (only cotton wool and a section of bean). Counts of the number, sex and species of mirid caught in the traps was made at approximately 0500, 0800, noon, 1800 and 2100 h each day between 30 August – 7 September. Records of wind speed, light (fraction of hour which was sunlight), temperature and humidity obtained from the Water Centre records, courtesy of Olga Grant, EMR.

a	b

Figure 5.1.2. (a) Female cage inserted into top of delta trap and (b) top view of delta trap with female trap lid visible.

Results

1. Experiment one

On the first night of records it was noted that the capsids were able to walk across the glue on new sticky bases, so extra Ecotac glue was added. A thunder storm and cold cloudy days were the likely cause of a low number of capsids captured in the first few days (Fig. 5.1.3a&b). Most of the insects were trapped between the 10 – 14 August when the hourly observations had stopped and the weather had improved (Fig. 5.1.4). It was decided to repeat the test under warmer weather conditions.

2. Experiment two

Unmated females attracted more males than newly captured field females. This may have been because newly caught field females had been recently mated. In *experiment one* field caught females that attracted males had been isolated for over a week prior to the experiment. Because of warmer weather conditions and less cloud cover (Fig. 5.1.5a&b) this experiment yielded more captured male *L. rugulipennis* than the previous test. More males were captured in the delta traps in the morning compared to the nighttime and afternoon. This could be further extrapolated to more males captured between 0500-0900/1030 compared to 0830-1200/1300.

Conclusions

- Male *L. rugulipennis* are attracted to females in the early hours of the morning – after dawn.
- The delta traps were effective at capturing capsids and growers are used to using these traps with other pest insects. However, the capsids were able to walk off the sticky bases (designed for capturing moths), and so more glue or a different formulation needs to be used.

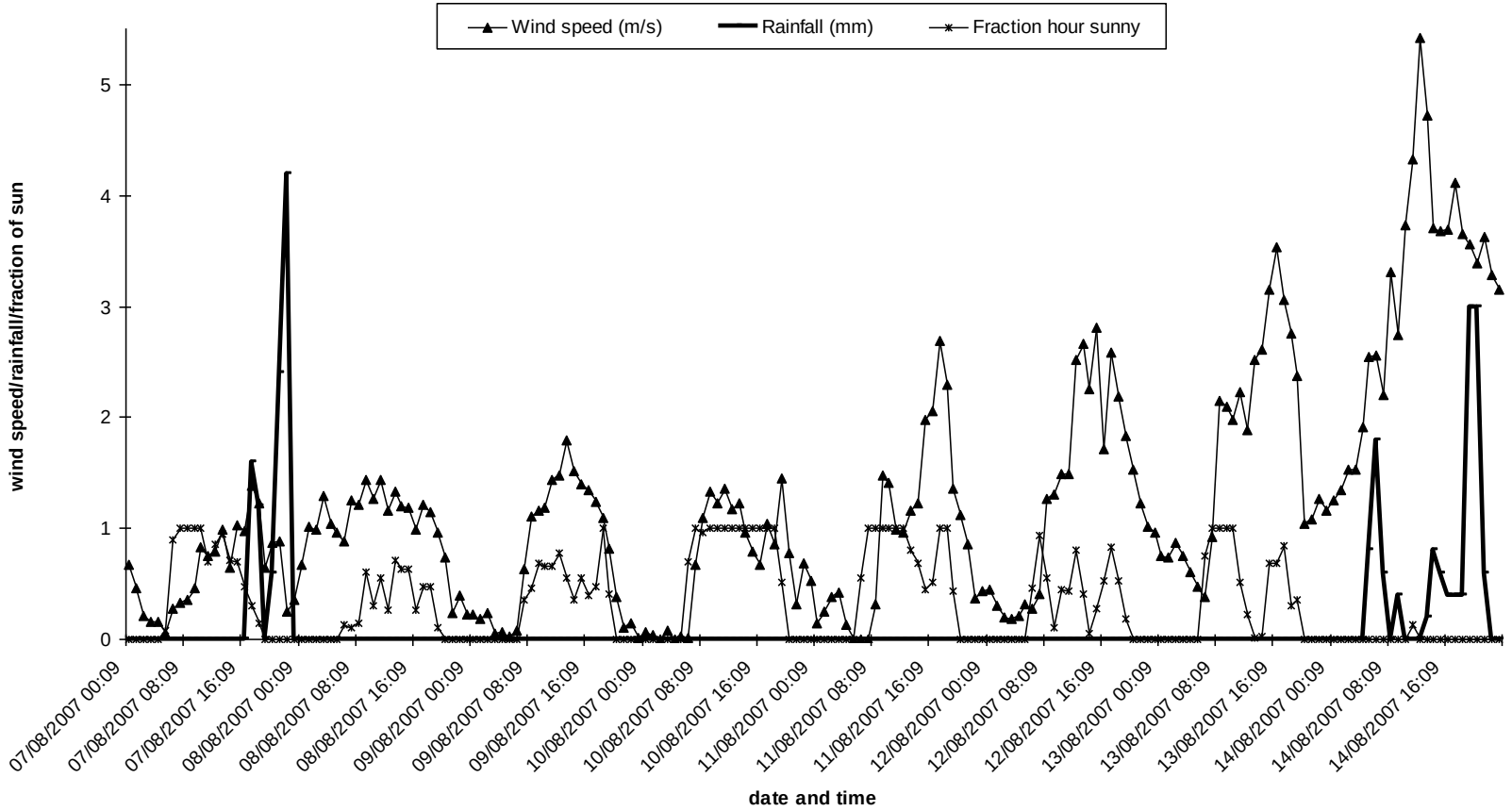


Figure 5.1.3a. Wind speed, rainfall and fraction of hour that was sunny for the period of the first female calling test on 10 – 14 August.

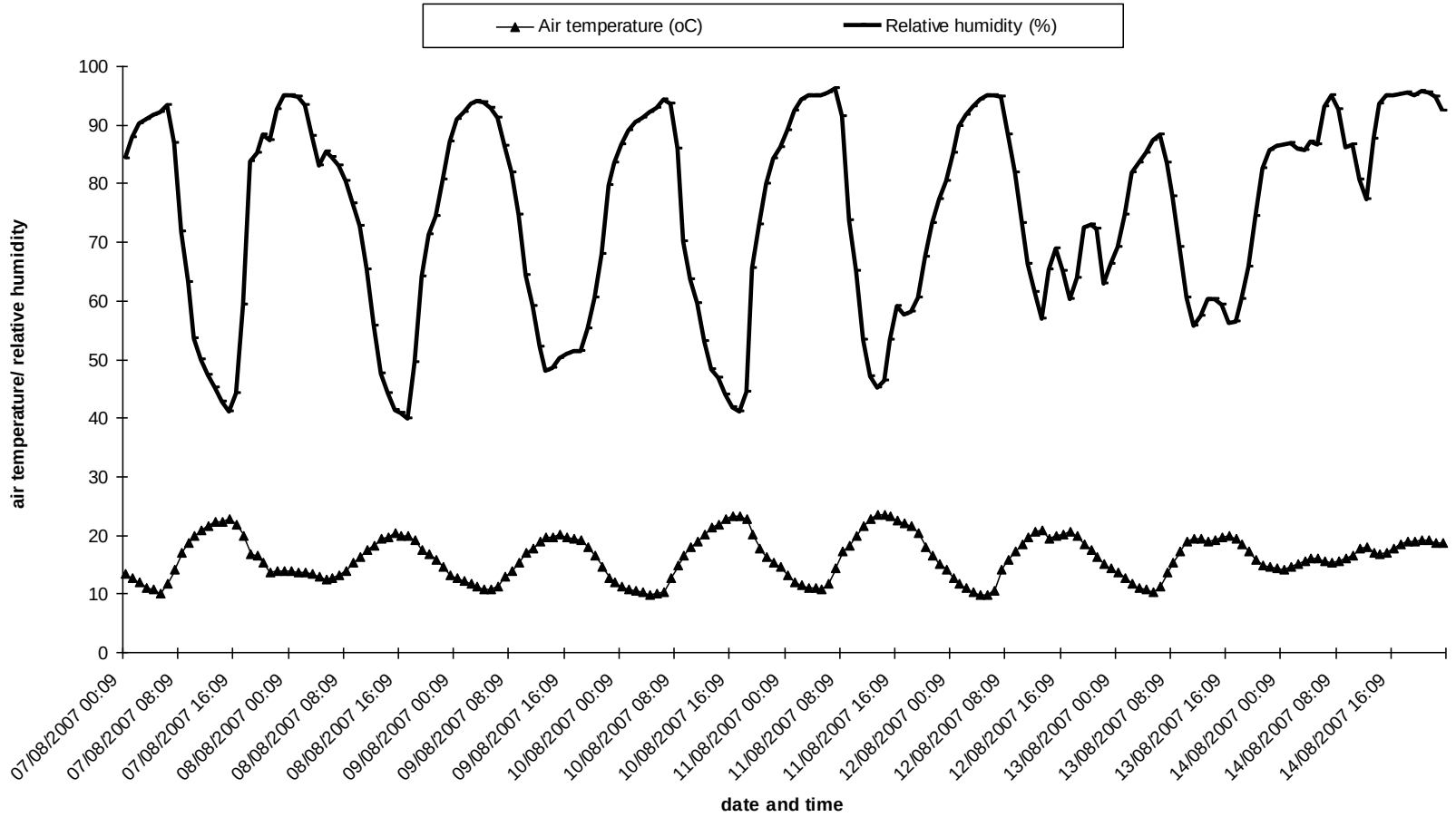


Figure 5.1.3b. Air temperature and relative humidity data for the period of the first female calling test on 10 – 14 August.

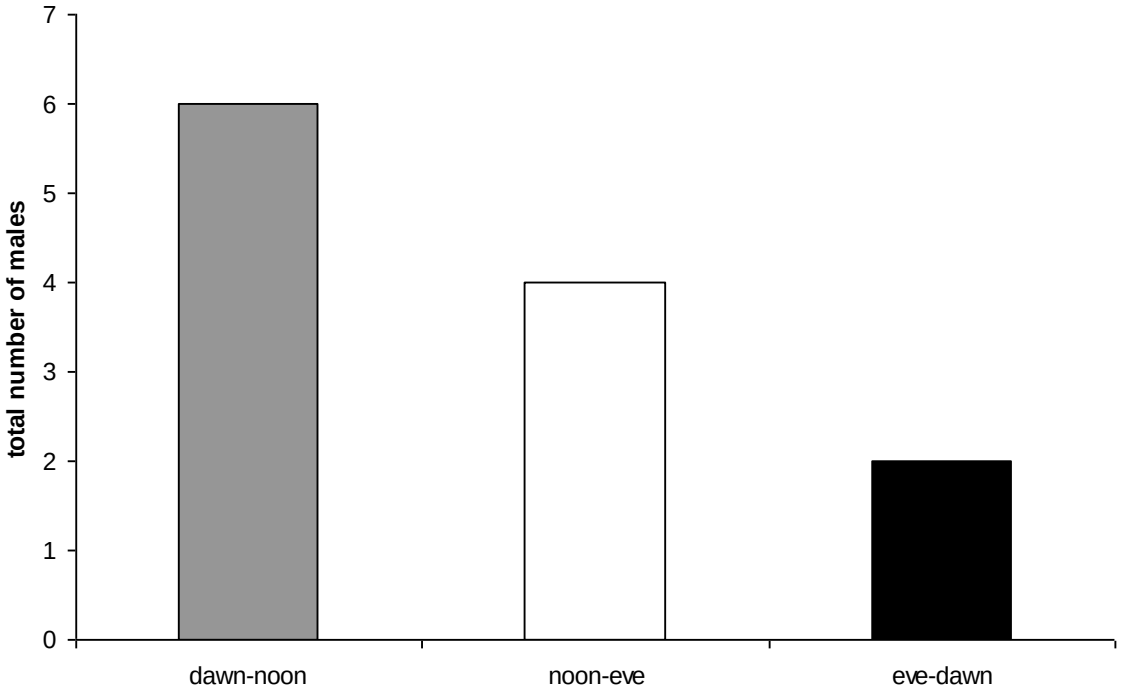


Figure 5.1.4. Number of male *L. rugulipennis* captured in sticky base delta traps between 10 - 14 August.

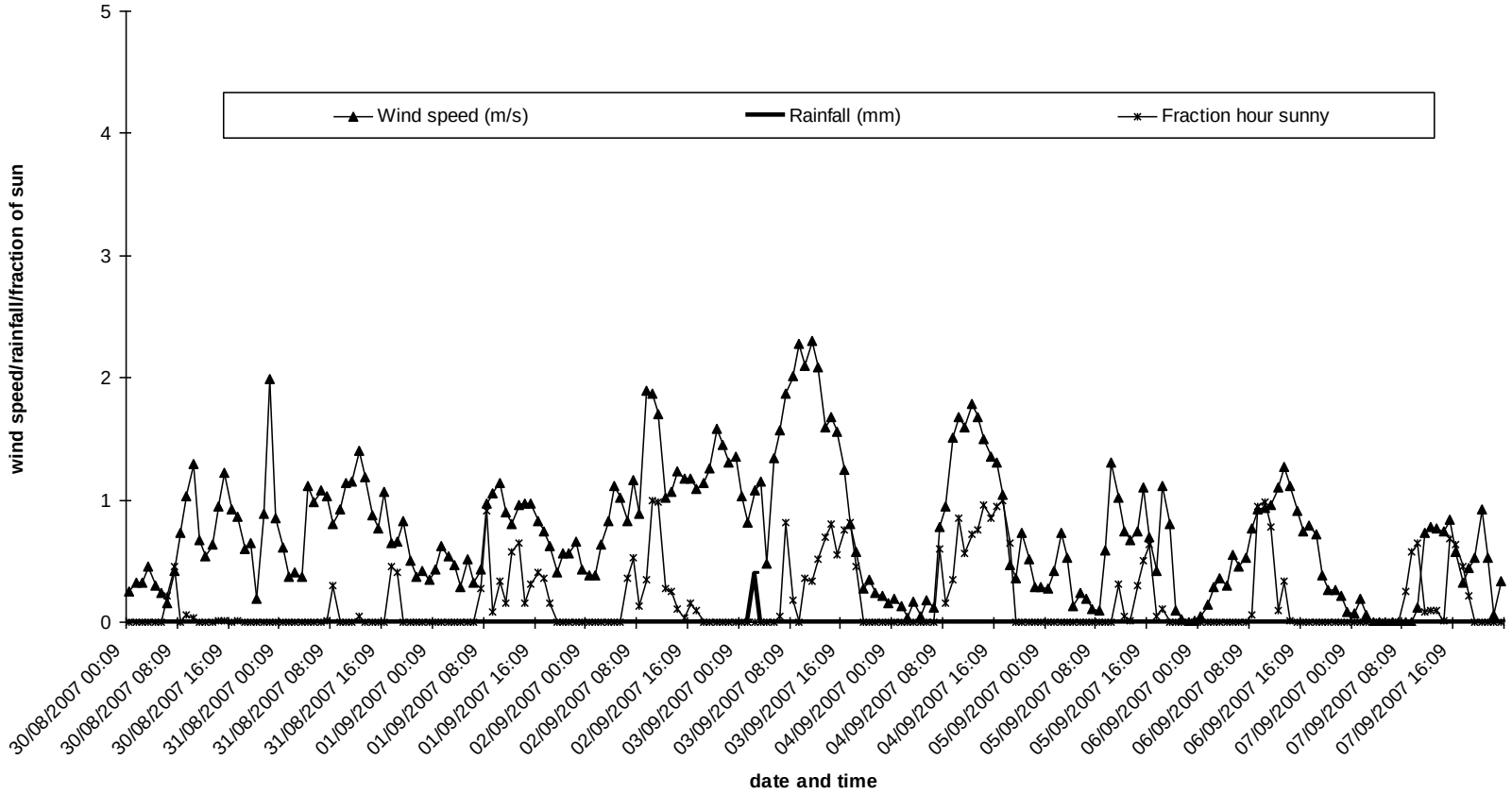


Figure 5.1.5a. Wind speed, rainfall and fraction of hour sunny for the period of the second female calling test 8 August – 9 September.

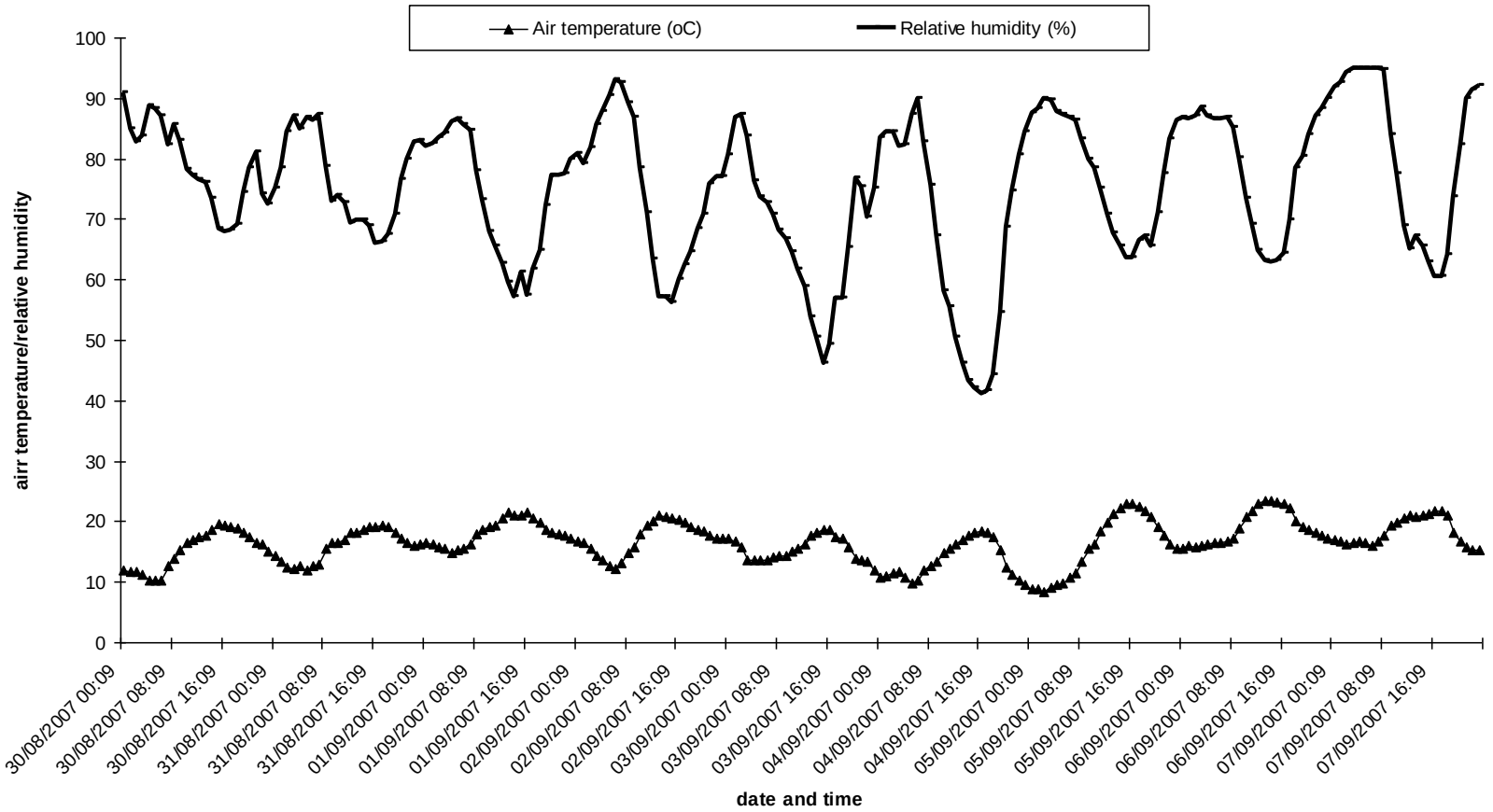


Figure 5.1.5b. Air temperature and relative humidity data for the period of the second female calling test 8 August – 9 September.

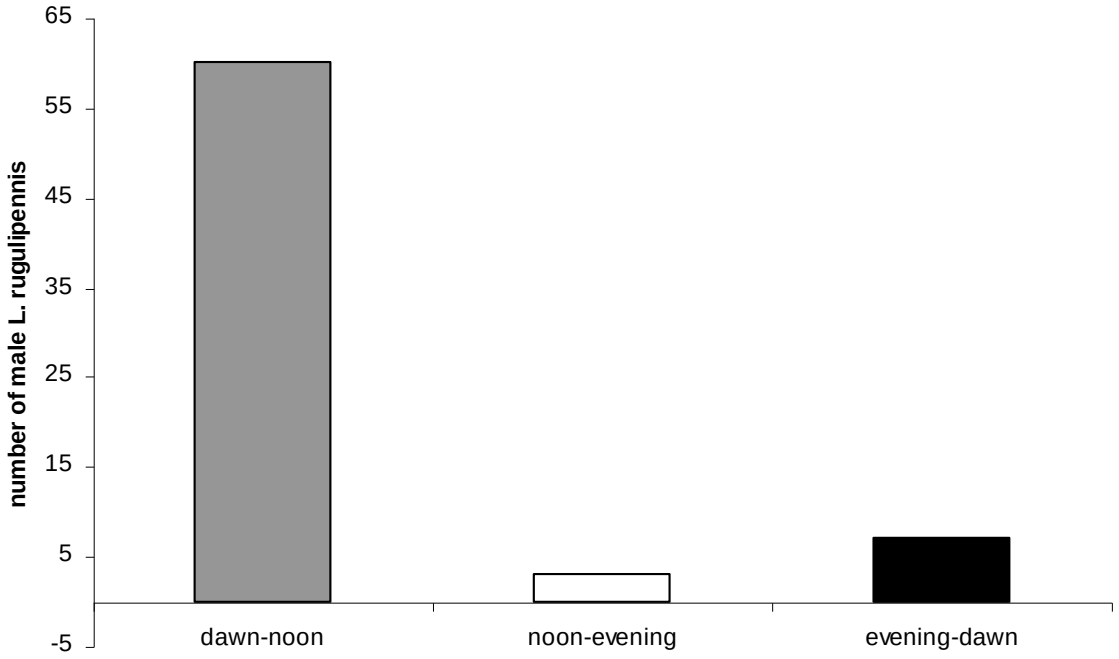


Figure 5.1.6. Number of male *L. rugulipennis* captured in sticky base delta traps between 8 August – 9 September at different times of the day.

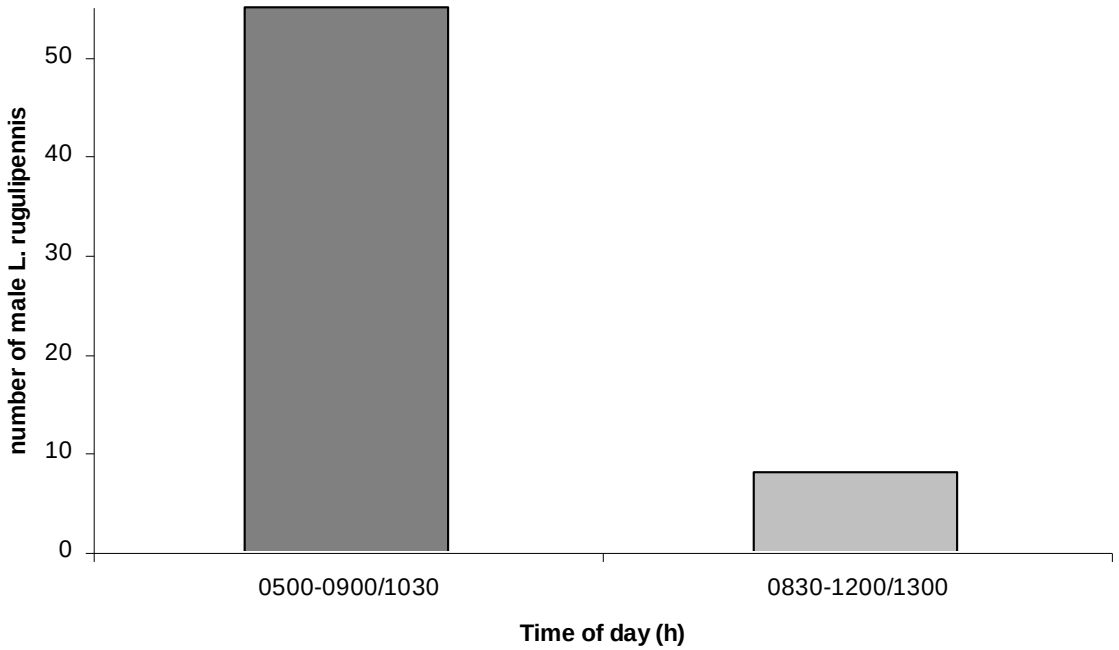


Figure 5.1.7. Number of male *L. rugulipennis* captured in sticky base delta traps between 8 August – 9 September at two time periods during the morning.

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)

The green delta trap appears to be effective at trapping *L. rugulipennis* and delta traps are used routinely by growers to monitor other pests. However, some modification is needed to maximize the effectiveness of the glue at trapping capsids.

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)

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 2008

- Sourcing *L. pabulinus* from the field, establishing cultures and initiating work on pheromone identification and laboratory bioassays. Establish time-of-day of attraction for more accurate entrainment.
- Test field attraction of males and females of *L. tripustulatus* to opposite sex. Time-of-day of attraction should also be established for more accurate entrainment.
- Further develop the still-air large cage bioassay for *L. rugulipennis* and *L. tripustulatus* using insects that are highly reproductively active (May for *tripustulatus* and July for *rugulipennis*).
- Following the success of 2008 milestone 2, test synthetic lures using the still air bioassay.
- Field test blends and release rates of 3 known compounds using modified micro-capillary lures and a new piezoelectric sprayer in prepared capsid abundant weed fields (Palmer's Rough at EMR). The piezoelectric sprayer will allow us to test numerous combinations and release rates of the 3 main pheromone components to see which is most attractive in the field.
- Collect and analyze pheromones from *L. rugulipennis* and *L. tripustulatus* when insects are highly reproductively active in the main season, including entrainment at different times of day.
- Improve and further test GC-EAG on all three species of capsid.
- Improve sticky bases for delta traps to minimize escape and consider mass production with Agrisense.

Milestone status at end of Year 1 (2007)

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	No	No	*L. rugulipennis* and *L. tripustulatus* cultures established as
1.2	Identification of components of sex pheromone of *L. pabulinus* completed	31 Mar 08	No	No	Populations of *L. pabulinus* were very low in 2007. This milestone will be one of the main goals for 2008
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	In progress. A still air bioassay looks promising and will be further developed in 2008 using reproductively active individuals. The windtunnel approach will be abandoned for the foreseeable future.
Year 2					
2.1	Identification of components of pheromone of *L. tripustulatus* completed	31 Mar 09	-	-	Work has begun and will be optimized in 2008
3.2	Pheromone lures attractive to *L. rugulipennis* and *L. pabulinus* in laboratory and field developed	31 Mar 09	-	-	In progress. We aim to test a modified capillary lure alongside the piezoelectric sprayer.
4.1	Effect of adding selected host plant volatiles to pheromone lures for *L. rugulipennis* and *L. pabulinus*	31 Mar 09	-	-	Collaboration with The Pheromone Group in Budapest, Hungary is expected to result in advances with this milestone in 2008.

	determined in laboratory and field				
~~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 developed	31 Mar 10	-	-	-
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	31 Mar 10 (new	-	-	The delta trap is considered effective and practical for growers (males captured in virgin female baited traps). Lures are still to

	adequate for evaluation as monitoring tools for capsid populations in the field	target)			be developed.
Secondary milestones					
Year 1					
1.1	Methods for collecting pheromone from capsids optimised	31 Mar 08	No	No	In progress
1.2	Methods for carrying out GC-EAG analyses on capsids further improved	31 Mar 08	No	No	Ongoing
3.1	Fields of weeds prepared at EMR for field trapping trials	31 Mar 08	Yes	Yes	'Ditton Field', EMR
3.2	Release rates of pheromone components from dispensers used in bioassays determined	31 Mar 08	No	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	Ongoing. Also collaboration with Hungarian scientists.
5.1	Trials initiated in growers' fields	31 Mar 08	Yes	Yes	An attempt was made in 2007. In 2008 we will concentrate on achieving an attractive combination of pheromone compounds.
Year 2					
2.1	Volatiles collected from *L. tripustulatus* males and females	31 Mar 09	Yes	Yes	A start was made in 2007. This will be further tested in 2008.
2.2	EAG recordings made from *L. tripustulatus*	31 Mar	Yes	Yes	A start was made in 2007. This will be further tested in

	males and females	09			2008.
3.1	Fields of weeds prepared at EMR for field trapping trials	31 Mar 09	Yes	Yes	'Palmer's Rough' at EMR
3.2	Release rates of pheromone components from dispensers used in bioassays determined	31 Mar 09	-	-	Ongoing
5.1	Feedback from growers on use of lures and traps gathered and evaluated	31 Mar 09	-	-	-
Year 3					
5.1	Feedback from growers on use of lures and traps gathered and evaluated	31 Mar 10	-	-	-