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Project title:	Pheromone technology for management of capsid pests to reduce pesticide use in horticultural crops – 2 year extension
Project number:	HL0184 (PC/SF 276)
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

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The following are members of the Consortium for LINK project HL 0184:

East Malling Research

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Natural Resources Institute

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Agirsense BCS Ltd

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Cucumber Growers Association

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AUTHENTICATION

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

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GROWER SUMMARY

Headlines

- A European tarnished plant bug trap has been successful at predicting the invasion of the pest into strawberry and cucumber crops.

- For the first time, the common green capsid has been attracted to a synthetic sex pheromone lure.

Background and expected deliverables

Capsid bugs are important pests of several high-value horticultural crops in the UK and many more worldwide. In the UK, the common green capsid, *Lygocoris pabulinus,* and the European tarnished plant bug, *Lygus rugulipennis* are the most important species. *L. pabulinus* is a sporadic but very damaging pest of apples, pears, blackcurrants, strawberries, blackberries and raspberries. *L. rugulipennis* is an important pest of late season strawberries and of various glasshouse salad crops, notably cucumber.

Crop invasion by capsids is sporadic and unpredictable, and, in the absence of effective control measures, capsid bugs cause severe economic losses. They cause damage at low population densities and are difficult to detect at such levels in normal crop inspections.

In conventional crops, capsids are controlled by sprays of broad-spectrum insecticides, organophosphorus insecticides being the most effective and frequently used. Neonicotinoids and other modern insecticide groups are only partially effective against capsids whilst insect growth regulators are totally ineffective. In the future, chlorpyrifos and thiacloprid, the main control methods for capsids, are likely to be withdrawn from use in many edible crops. In organic crops the pests cause high levels of damage because the insecticides available are inadequate and of short persistence. Capsids have few natural enemies and effective biocontrol methods have not yet been developed.

Without accurate monitoring information, growers are forced to use remedial applications of broad spectrum insecticides. Although these treatments can be effective against capsids, they disrupt the biological control of other pests and can lead to the application of further sprays. The recent outbreaks of pesticide-resistant western flower thrips on strawberry are probably due, at least in part, to routine spraying against capsids.

The need to use broad spectrum insecticides for control of capsid bugs is a major obstruction to the implementation of IPM and the quest towards pesticide-free foods.

Effective monitoring systems for capsid pests would help to ensure that pesticides are only used when necessary thereby reducing routine applications of broad-spectrum pesticides that disrupt the IPM of these and other pests. They would also enable the use of more selective insecticides and biological approaches for which timing of sprays is critical.

Summary of project and main conclusions

Progress on each objective of the project is summarised below;

1. Improve and test the lure for *L. rugulipennis* so that it is long lasting and practical for use by growers (Yr 1)

The life and release rate of pheromone components from the pipette tip lure have been enhanced. Shielding the pipette tips from sunlight by wrapping them in duct tape extended the life of the lure to over 4 weeks in the field.. The use of larger pipette tips also gave a more consistent release rate.

In the laboratory windtunnel the 1 ml pipette tips proved much more reliable than the 0.2 ml tips, releasing a blend very similar to that loaded into the dispenser for up to 2 months at 27°C and 8 km/h windspeed. They also released at a higher rate than the 0.2 ml pipette tips. Furthermore, the 1 ml pipettes were easier to load with the pheromone blend and to seal with the crimp cap. The results have confirmed that disposable pipette tips are suitable dispensers for the three candidate components of the *Lygus* bug pheromone trap.

The Agrisense sachets proved unsatisfactory for dispensing the pheromones (*E*)-4-oxo-2-hexenal (KA), hexyl butyrate (HB), and (*E*)-2-hexenyl butyrate (E2HB).. The components diffuse through a polyethylene disc such that release of KA is proportionately faster than that of HB and E2HB. This results in a very high relative amount of KA initially which drops to a very low level within 10 days under windtunnel conditions. Thus, in the field the sachet performed well in comparison with the pipette tip during the first 5 days but much less well subsequently.
The pipette tip lure was also shown to be as attractive as live female *L. rugulipennis*.
Improvements have been confirmed using field trapping tests.

The trap was further tested by adding Fluon to the cross vanes. This increased the catch by more than a third in week one, but catches of males decreased subsequently – probably because of contamination by debris on the cross vanes over time (enables the insects to grip the surface more easily). Products such as Teflon should be considered as an alternative

coating for the cross vanes.

Traps that combined the lures of *L. rugulipennis* and/or *Anthonomus rubi* with either white or green cross vanes showed that white cross vanes cannot be used as they reduce the catch of *L. rugulipennis* in the traps. In addition, the grid designed for preventing capture of bees attracted to white cross vanes prevents the *Lygus* bugs falling into the bucket of the trap.

2. Calibrate the trap for *L. rugulipennis* for use in pest monitoring to establish a treatment threshold for its use in late season strawberry and/or cucumber (Yrs 1 and 2)

Extensive trapping in both cucumber and strawberry crops has proven the monitoring trap to be an excellent early warning system of invasion into the crops. The pest is detected in high numbers in the trap at least 2 weeks before detection in cucumber and up to 2 months in strawberry compared to using traditional monitoring methods. More than 3 sites of each crop were monitored. Pheromone baited traps positioned outside cucumber greenhouses appeared to provide useful prior warning of crop invasion and plant damage by *L. rugulipennis*. Those positioned within the crops were of little value.

3. Develop an effective lure and trap for *L. pabulinus* with associated data for pest monitoring (Yrs 1 and 2)

For the first time, significant numbers of male *L. pabulinus* have been trapped using synthetic sex pheromone lures. Trap design is of major importance and the green cross vane and delta traps were found to be ineffective at catching males. The lure was more attractive than caged virgin females at attracting males to sticky stake traps. These traps are not practical for use by growers. Sticky platform and water traps were also tested, but were not found to be more effective than sticky stake traps. There should be more focus on trap design in year 2 of the extension of the project.

Camera recordings have been made using pheromone lures and virgin females as bait. However, no direct observations were made of the interactions. This is believed to be because recordings were made between generations, so another attempt will be made next year with improved equipment.

4. **Encourage commercial production of traps and lures and produce grower information sheets on the use of the traps for monitoring capsids**

It is the intension of the consortium to make available the *L. rugulipennis* trap for testing by strawberry and cucumber growers in 2011 in order to establish an action threshold.

Future research

In the final year of the project, research will focus on;

- Determining the best height for the traps in strawberry and cucumber crops
- Determining a trap threshold for *L. rugulipennis*
- Filming the behaviour of *L. pabulinus* around the synthetic lure
- Investigating grower convenient trap designs for monitoring *L. pabulinus*

Financial benefits

The financial benefits for growers will be realised in more accurate predictions of a capsid attack and more focused, not prophylactic, control measures.

Action points for growers

Growers interested in trialling the European tarnished plant bug trap in 2011 for monitoring the pest in strawberry or cucumber crops should contact Michelle Fountain (michelle.fountain@emr.ac.uk; 01732 523 749).

SCIENCE SECTION

Background

The 3 year HortLINK project HL0184 (HDC project PC/SF 276) was very ambitious with several very challenging objectives. However, outstanding progress was made in developing a highly attractive sex pheromone trap for the European tarnished plant bug, *Lygus rugulipennis*. This is the first time an attractive lure and effective trap have been developed for any *Lygus* species in the world, despite long term intensive efforts by several internationally renowned research institutes over many years at substantial cost. The project was due to end on 31 March 2010 but has been extended for a further two years.

Overall Aim

The overall aim of this project is to reduce use of broad-spectrum insecticides against capsid pests on a range of horticultural crops and to maintain or improve the level of control in both conventional and organic crops. This will permit greater use of IPM approaches against these and other pests ad contribute towards production of pesticide-free fresh produce demanded by retailers and consumers.

This will be done by developing pheromone traps for monitoring the two main species, *L. rugulipennis* and *L. pabulinus,* to assess the need and to time application of interventions against the pests.

Specific Objectives of the extension

1. Improve and test the lure for *L. rugulipennis* so that is long lasting and practical for use by growers
2. Calibrate the trap for *L. rugulipennis* for use in pest monitoring to establish a treatment threshold for its use in late season strawberry and/or cucumber crops
3. Develop an effective lure and trap for *L. pabulinus* with associated data for pest monitoring.
4. Arrange commercial production of traps and lures and produce information sheets on use of the traps for monitoring capsids for use by growers.

Methods and results

Objective 1. Improve and test the lure for *L. rugulipennis* so that it is long lasting and practical for use by growers.

- Test the effect of Fluon for increasing trap catch
- Compare the catch of the pipette lure compared to virgin females and sachets
- Determine whether or not pheromone traps for the European tarnished plant bug and the strawberry blossom weevil can be effectively combined into one
    - determine whether the lures for the two species interact
    - determine which of the trap designs used for the two pests is the best

Small scale randomised block field experiments compared trap designs and lure types. The test site was an organic apple orchard at East Malling Research (WM 144.) that had *Chenopodium album* (fat-hen)_and *Tripleurospermum inodorum* (scentless mayweed) growing in the alleyways and between the trees. Traps were baited with *L. rugulipennis* sex pheromone lures (pipettes or sachets) or with females collected from the field and isolated or virgin females, and 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 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 through the hole in the top of the trap. Green cross vane bucket traps (Agralan) were used as these had been shown to be the most effective at trapping this species in previous trials. Water and a drop of detergent were added to the bucket as a trapping agent. Traps were hung from the wire system 50 cm above ground 20 m apart. Experiments were set up as randomised block designs. Plots were individual traps. There were 10 replicates of each treatment.

<u>Fluon test</u>

The Fluon test ran between 23 Jul and 12 Aug. Cross vanes of the green bucket traps were coated with a thin layer of Fluon and compared to non-coated cross vane traps. One ml pipette tip lures were used at bait (labelled Batch 2010.055 (July 2010)).

<u>Sachet vs Pipette tip vs Female</u>

The test was run between 12 Aug and 2 Sep. Sachets (Sachet Batch 2010.063 August 2010) were compared to pipette tips (Pipette tip 1 ml Batch 2010.055 July 2010) and laboratory reared unmated females.

Assessments were weekly counts of male *L. rugulipennis* in the traps. The female baits were

changed weekly and found to survive in the cages for that period of time. The tests continued until there were enough data to show a significant difference between the treatments. Data was transformation as necessary and analysed with ANOVA.

Combined trap

The trial was at Haygrove Ltd, Redbank Farm, Little Marcle Rd, Ledbury, Hereford HR8 2JL by kind agreement of Graham Moor in 'Southfield' organic plantation. This plantation had moderate levels of blossom weevil, and was replanted with potted Evie 2 (everbearer) in March 2010. The experimental plot consisted of 12 tunnels (9-20 from the west; tunnels 1-4 are planted with raspberries). The tunnels were 7.4 m width. In each tunnel there were 4 beds (each containing 3 rows of strawberries).

Traps were deployed on 13 July. The treatments were a factorial comparison of trap design (2 levels), and lure composition (3 levels) (Table 1.1). A Latin square design comprising 6 replicates of the 6 treatments was used.

Table 1.1. Combined strawberry blossom weevil and European tarnished plant bug trap treatments

Treatment no.	**Factor 1: Trap design**	**Factor 2 Lure(s)**
1. GA	Green cross vane no grid	*A. rubi*
2. GL	Green cross vane no grid	*L. rugulipennis*
3. GLA	Green cross vane no grid	*A. rubi* + *L. rugulipennis*
4. WA	White cross vane with grid	*A. rubi*
5. WL	White cross vane with grid	*L. rugulipennis*
6. WLA	White cross vane with grid	*A. rubi* + *L. rugulipennis*

Traps were Agrisense funnel traps with either white or green cross vanes. The white cross vane traps were deployed with a bee excluder grid over the funnel. This is because the white cross vane traps attract non-target insects, such as honeybees and bumblebees. This was not necessary with the green cross vane traps because they do not attract bees. Lures were either the standard *Anthonomus rubi* sachet containing 100 µl of the normal 1:4:1 blend of Grandlure I: Grandlure 2: lavandulol plus 1 g of the strawberry flower volatile 2, 4-dimethoxybenzene, provided by International Pheromone Systems Ltd or *Lygus rugulipennis* pipette tips containing 100 µl of the standard blend of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal (10% in sunflower oil). Plots were single traps deployed in a square grid, spaced 2 tunnels (= 14.8 m) apart in the leg rows of the Spanish tunnel protected strawberry field.

The traps were stood on the ground and held in place with a wire hoop, and contained water plus a few drops of detergent to break the surface tension. *L. rugulipennis* lures were renewed on each visit.

The grower was requested to avoid spraying the field for the two target pests for as long as possible. A temperature/humidity data logger was deployed in a Stevenson's screen in the field to take half hourly records.

Counts of the number of male *L. rugulipennis* and *Anthonomus rubi* in each trap were made.

Laboratory studies

In previous work, virgin females of four species of *Lygus* bugs were shown to produce hexyl butyrate (HB), (*E*)-2-hexenyl butyrate (E2HB) and the ketoaldehyde (*E*)-4-oxo-2-hexenal (KA) which were proposed to be components of the female sex pheromones. Each species was shown to produce a different blend of these compounds and the compositions of these were determined for each species (Table 1.2).

Table 1.2. Relative amounts of pheromone components produced by four species of *Lygus* bug

	Ratio HB=100 (SE)	
Species	**E2HB/HB**	**KA/HB**
Lygus rugulipennis	3.1 (0.8)	15.8 (6.1)
Lygocoris pabulinus	3.8 (0.2)	8.2 (0.3)
Liocoris tripustulatus	7.2 (0.5)	13.9 (1.2)
Lygus pratensis	25.7 (1.4)	23.9 (2.9)

Standard rubber septa and polyethylene vial dispensers are not suitable for the three candidate pheromone components due to the difference in volatility and polarity of the KA compared with HB and E2HB. Disposable pipette tips were found to be suitable dispensers for these compounds, maintaining a reasonably steady release rate and ratio of the components for up to three months in a laboratory windtunnel. In the field they had a much shorter lifetime and this was suspected to be due to the degradation of the unstable KA in sunlight.

The objectives of the work in 2010 were to produce a lure lasting at least one month in the

field, to investigate other more convenient, commercially-available systems and to investigate further lures for *L. pabulinus*.

Chemicals

HB and E2HB were commercially available (Aldrich). KA was prepared from 2-ethylfuran at NRI as described in previous reports. Blends were made up, assayed by GC and then diluted in sunflower oil so that the HB was 10% of the mixture.

Dispensers

Pipette tip dispensers were clear plastic 0.2 ml or 1 ml disposable pipette tips (Fisher). The pheromone mixture (100 µl) was applied to a cellulose acetate cigarette filter (15 mm x 6 mm) and the pipette tip was sealed with a crimp seal. Sachet dispensers were impermeable sachets (8 cm x 5 cm) containing a glass fibre pad on which the pheromone blend was deposited (Agrisense). The standard commercially-available lure had a circular, black polyethylene-covered opening (7.5 mm diameter) through which the pheromone was released. Sachets with a hole (3 mm) punched in the wall were also tested.

Measurement of release rate

Dispensers were maintained in a windtunnel at 27°C with wind speed 8 km/h. At intervals release rates were measured trapping volatiles on Porapak. Individual dispensers were placed in a glass vessel (6 cm x 4 cm diameter) and air drawn (2 l/min) in through an activated charcoal filter and out through a filter consisting of a Pasteur pipette (4 mm diameter) packed with purified Porapak Q (200 mg) held between glass wool plugs. Collections were carried out for approximately 2 h and two replicates were run simultaneously.

Volatiles trapped on the Porapak filters were eluted with dichloromethane (Pesticide Residue Grade; 3 x 0.5 ml) and decyl acetate (2 µg) added as internal standard. The resulting solutions were analysed by gas chromatography (GC) using a fused silica capillary column (30 m x 0.32 mm i.d. x 0.25 µm film thickness) coated with polar DBWax (Supelco) and flame ionisation detection. Injection was splitless (220°C), carrier gas helium (2.4 ml/min) and the oven temperature was programmed from 50°C for 2 min then at 10°C/min to 250°C. Quantification was by comparison of peak area with that of the internal standard and results are the means of two replicates.

Results

Fluon test

The numbers of male *L. rugulipennis* trapped in the Fluon coated cross vane traps was

significantly higher than traps not coated with Fluon in the first week of the test (ANOVA, Log_{10} transformed data P=0.027, Fig. 1.1). However, by week 2 the effect had diminished. This is believed to be due to the Fluon washing off with heavy rainfall and the coating of the cross vanes in soil from rain splash.

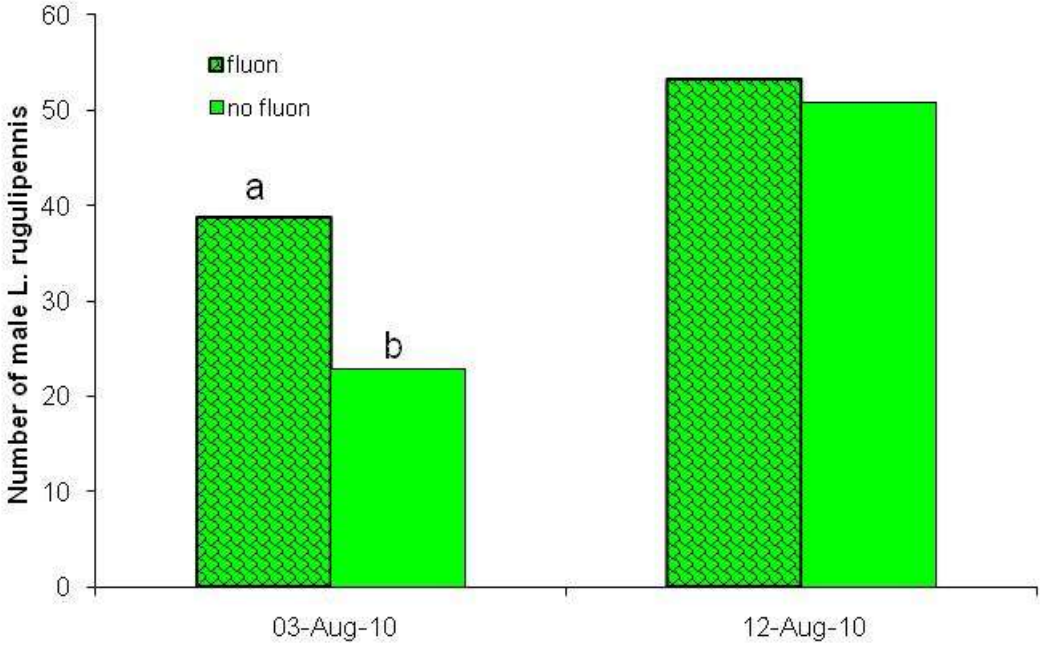


Figure 1.1. Mean number of male *L. rugulipennis* trapped in fluon coated and non-coated green cross vane traps (n=10). Pipette lures used as bait.

Sachet vs Pipette tip vs Female

In the first week of trapping, almost double the numbers of males were caught in the sachet baited traps compared to the pipette and unmated female baited traps (ANOVA, untransformed data P<0.001, Fig. 1.2). In the second week, the 1 ml pipette tips were more effective at catching males than unmated females or sachets. Sachets were known to be releasing pheromone 5 times faster than the pipettes, so were probably running out of synthetic pheromone by week 2. Pipette tips performed as well or better than the unmated females throughout the whole experiment (periods of inclement weather may affect the behaviour of females releasing pheromone and, hence, trap catches). By the last week of the experiment populations of *L. rugulipennis* had dropped in the field and overwintering adults were becoming more common.

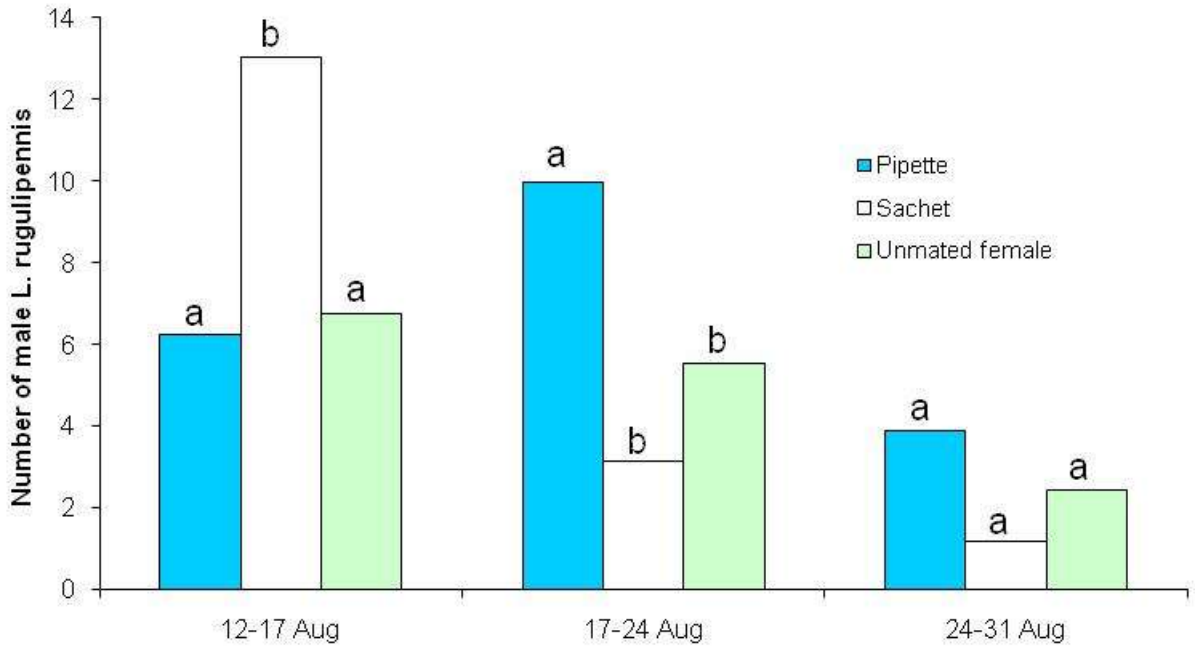


Figure 1.2. Mean numbers of male *L. rugulipennis* trapped in green cross vane traps with different baits (n=10). Letters show significant differences within a week.

Combined trap

Significantly more *L. rugulipennis* males were captured in green cross vane traps than white cross vane traps (ANOVA P<.001) and more were caught in traps baited with *L. rugulipennis* pheromone than *A. rubi* pheromone baited traps (ANOVA, P=0.009). The *A. rubi* lures did not interfere with catches of *L. rugulipennis*. In previous experiments *L. rugulipennis* was less attracted to white cross vane traps and was also impeded by the grids used as bee excluders. *A. rubi* was observed in all traps regardless of whether there were *Anthonomus* baits or not. However, the numbers were very low and probably not high enough for differences to be seen. Any future combined monitoring trap should not have white cross vanes or a grid. The ideal trap would be a green cross vane that attracts *L. rugulipennis* and *A. rubi*.

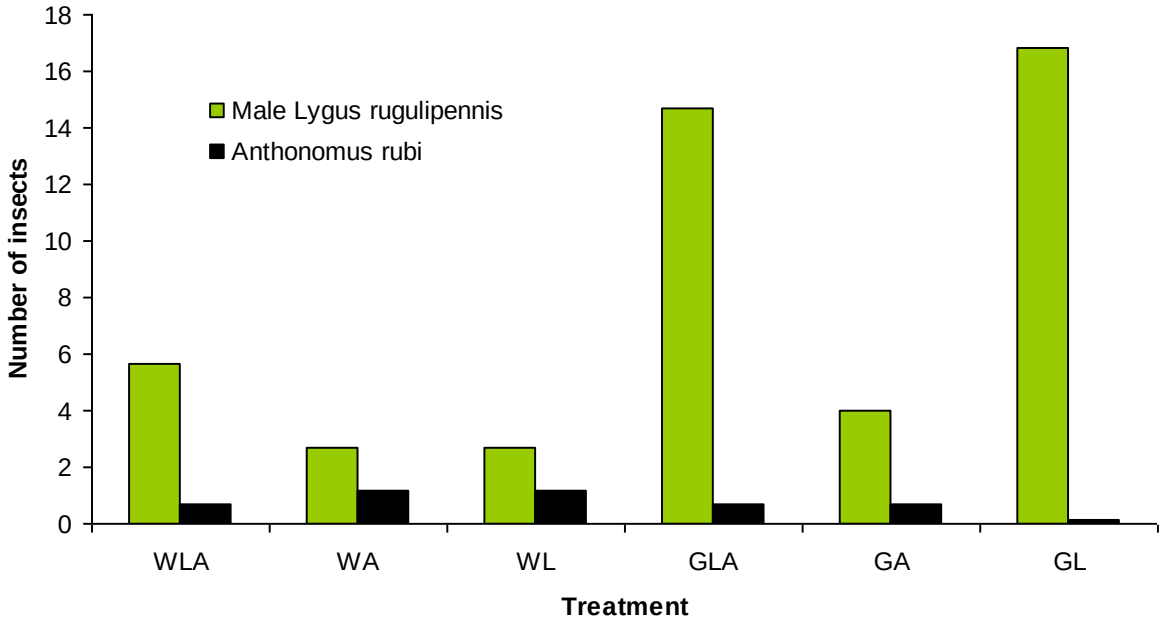


Figure 1.3. Mean number of male *L. rugulipennis* and *Anthonomus rubi* trapped in green (G) or white (W) cross vane traps with *Lygus* (L) and/or *Anthonomus* (A) lures (n=6).

Pipette tip dispensers

An initial study of release of the *L. rugulipennis* blend in the windtunnel showed that release from both 1 ml (Fig. 1.4) and 0.2 ml (Fig. 1.5) disposable pipette tips was reasonably uniform for at least 40 days, and the blend released was similar to that put in the dispenser. In particular, the relative amount of KA/HB was maintained at 15-20%, as required (Table 1.2). The release rate of HB was approximately 1.2 µg/h from the 1 ml pipettes and 0.7 µg/h from the 0.2 ml pipettes. These rates are similar to the approximately 0.5 µg/h produced by a female insect.

However, in subsequent tests, the 0.2 ml disposable pipettes gave much more variable release rates, e.g. Fig. 1.6 for *L. rugulipennis*, showing a much higher proportion of the KA than expected. Similarly for the *L. pabulinus* blend, the results in Fig. 1.8 with the 1 ml pipette show that the relative amount of KA/HB was maintained at 8-12% for at least 60 days as required (Table 1.2). With the 0.2 ml pipette (Fig. 1.7) the relative amount of KA/HB was too high at 15-20% and this dropped rapidly after 40 days as the relative amount of KA remaining was reduced.

The satisfactory performance of the 1 ml pipettes with the blends for both species was confirmed in a further set of tests for *L. rugulipennis* (Fig. 1.9) and *L. pabulinus* (Fig. 1.10).

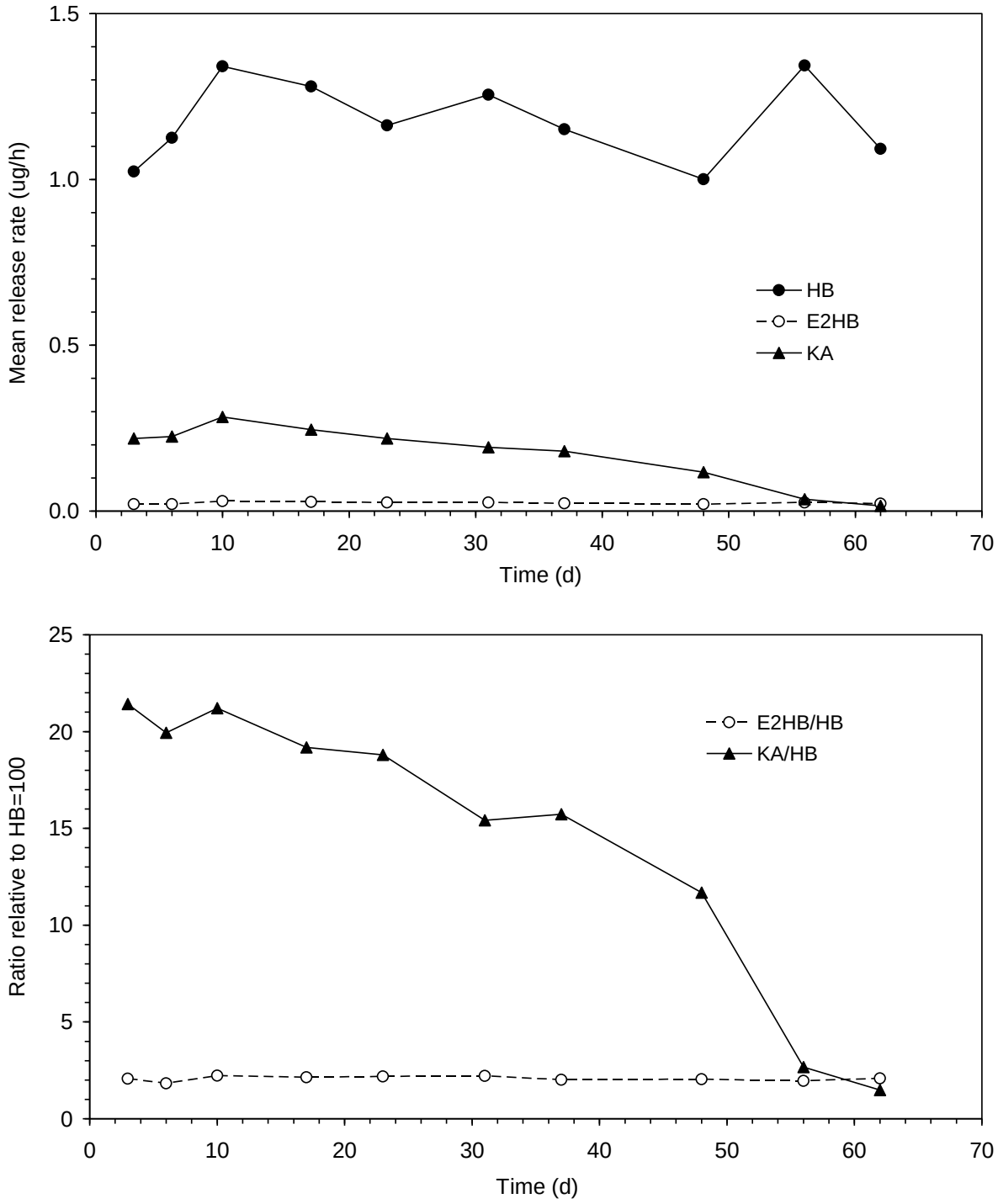


Figure 1.4. Release of *L. rugulipennis* blend from 1 ml pipette in wind tunnel showing actual release rates (upper) and relative amounts of E2HB and KA (lower) (27°C, 8 km/h windspeed; starting blend 100:2.7:19.9 HB:E2HB:KA)

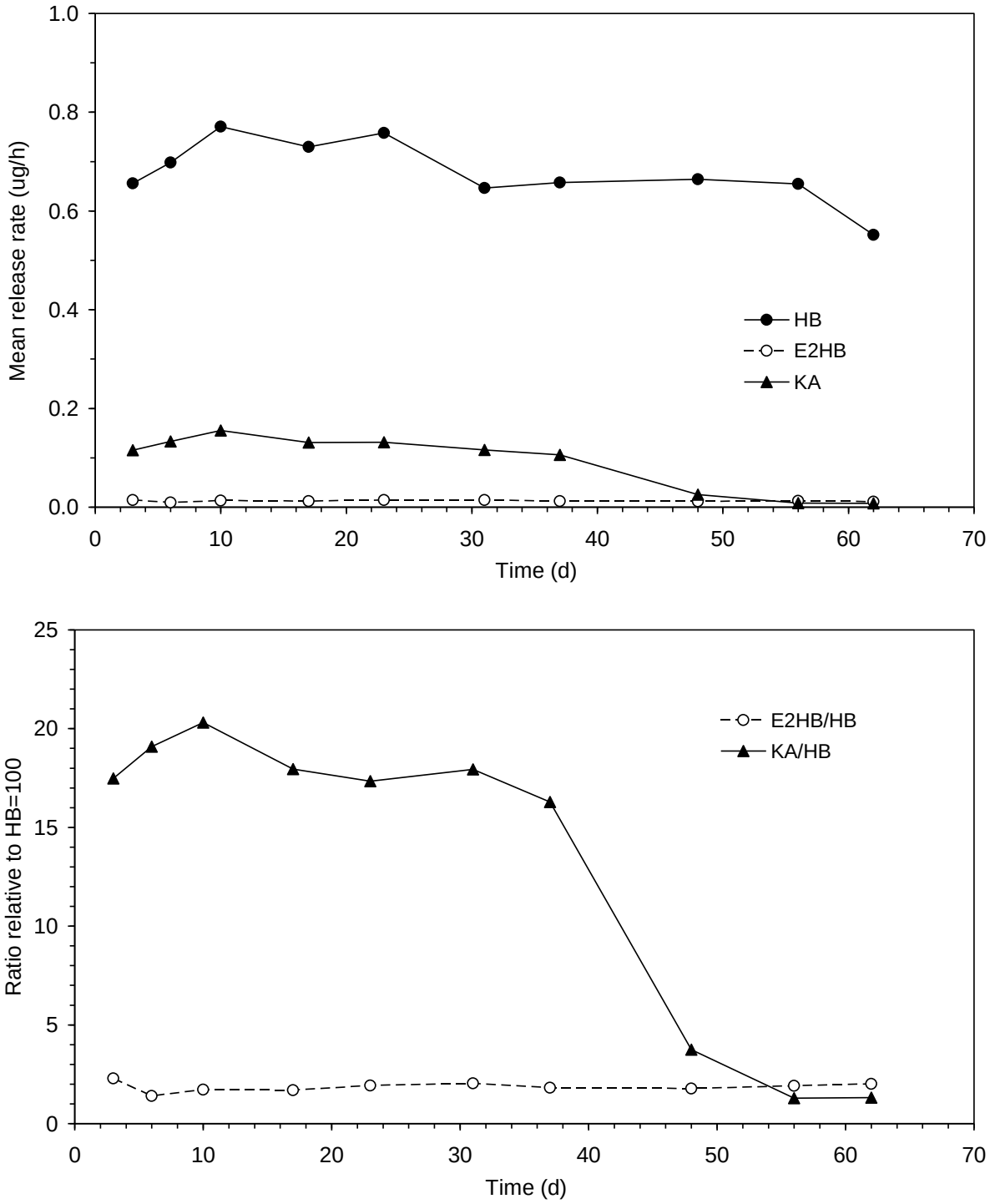


Figure 1.5. Release of *L. rugulipennis* blend from 0.2 ml pipette in wind tunnel showing actual release rates (upper) and relative amounts of E2HB and KA (lower) (27°C, 8 km/h windspeed; starting blend 100:2.7:19.9 HB:E2HB:KA)

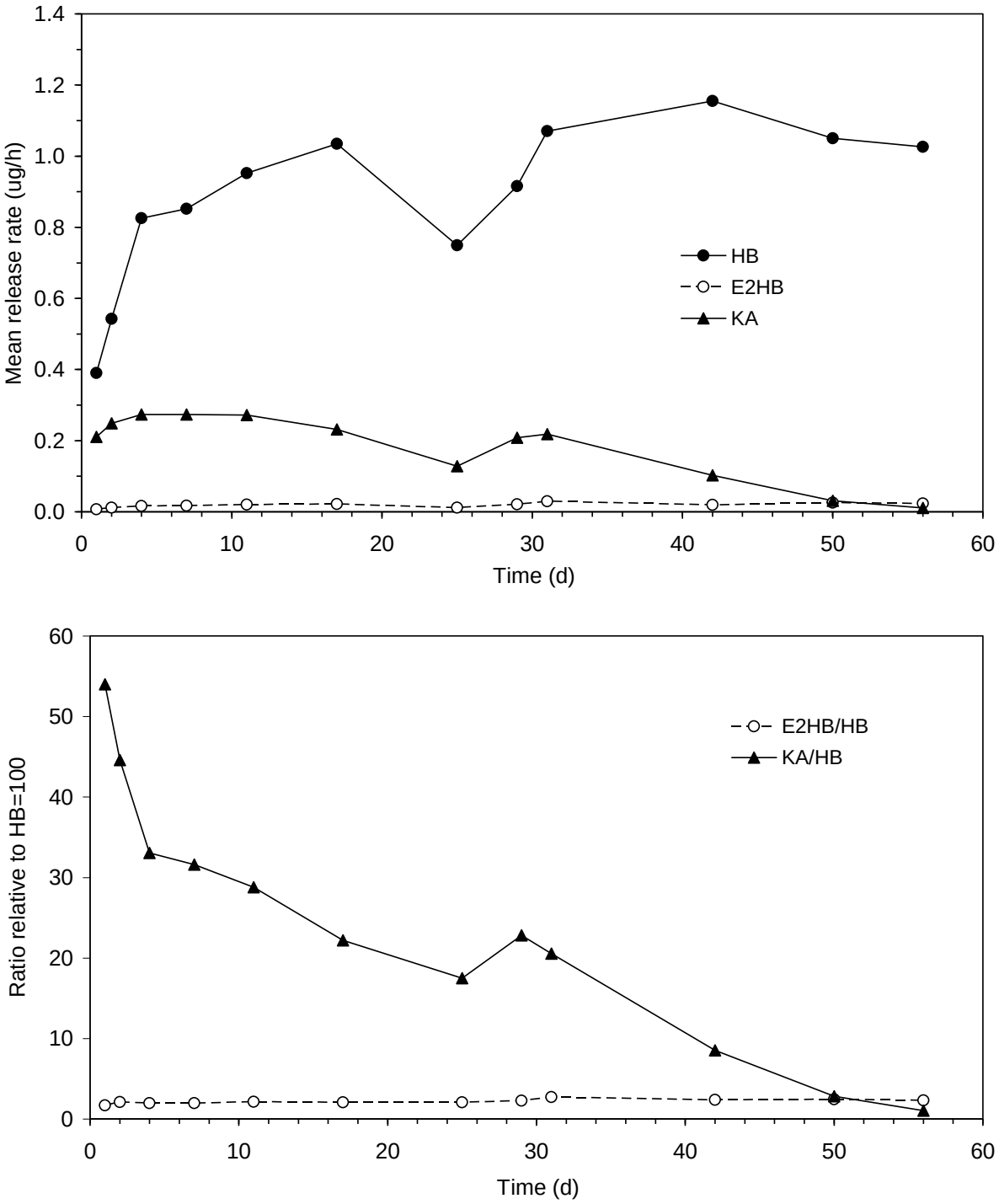


Figure 1.6. Release of *L. rugulipennis* blend from 0.2 ml pipette in wind tunnel showing actual release rates (upper) and relative amounts of E2HB and KA (lower) (27°C, 8 km/h windspeed; starting blend 100:2.7:19.9 HB:E2HB:KA)

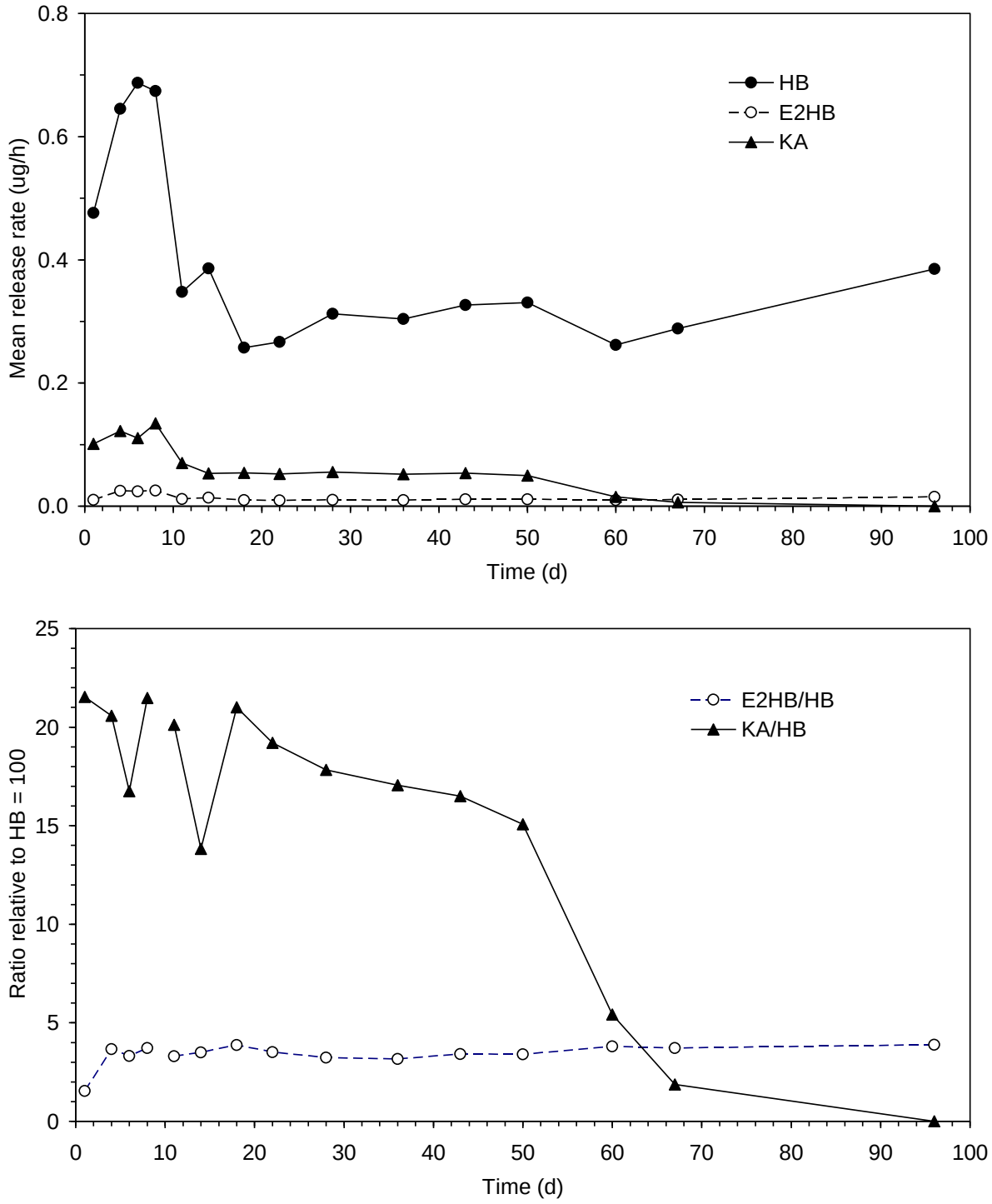


Figure 1.7. Release of *L. pabulinus* blend from 0.2 ml pipette in wind tunnel showing actual release rates (upper) and relative amounts of E2HB and KA (lower) (27°C, 8 km/h windspeed; starting blend 100:4.4:11.1 HB:E2HB:KA)

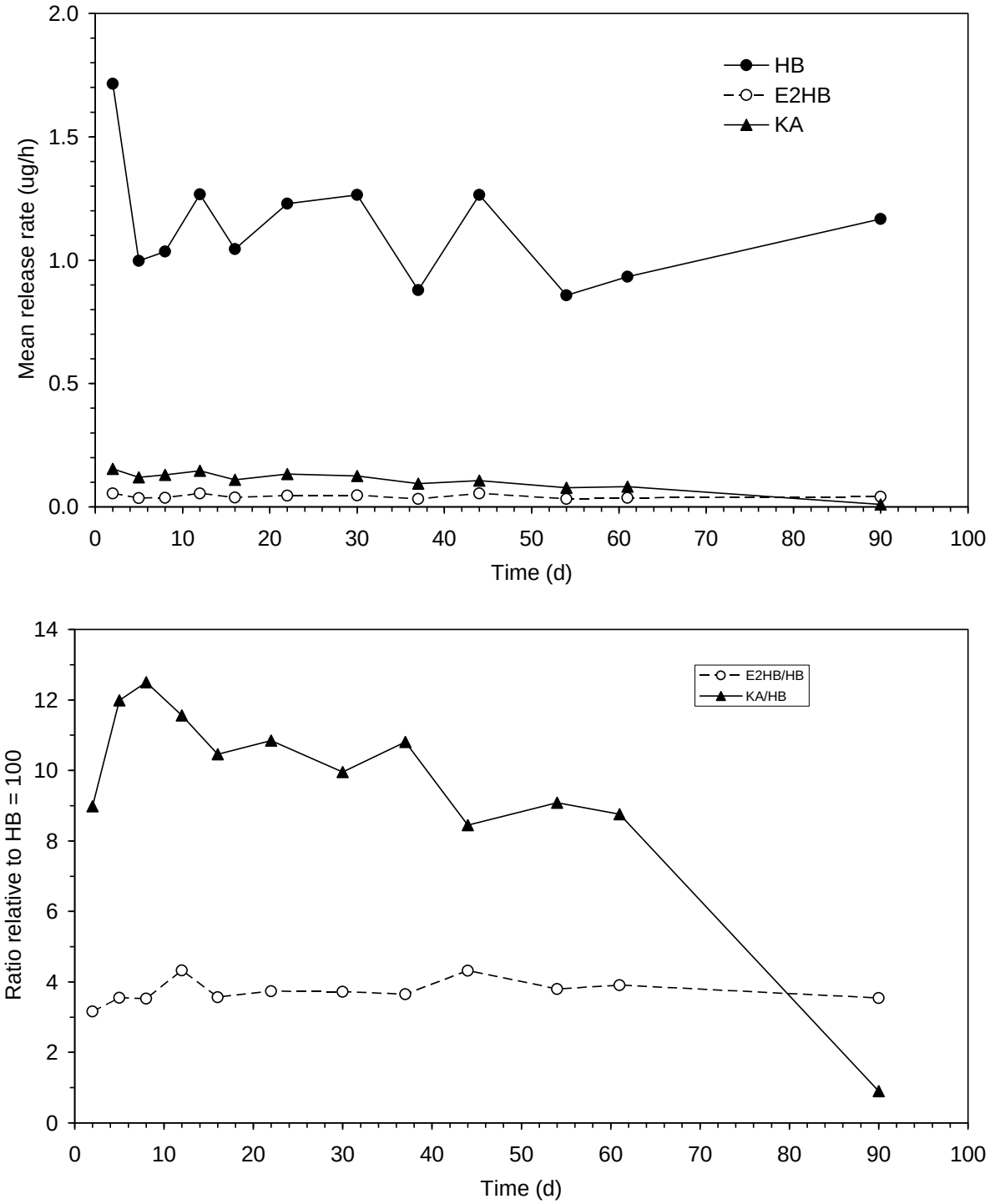


Figure 1.8. Release of *L. pabulinus* blend from 1 ml pipette in wind tunnel showing actual release rates (upper) and relative amounts of E2HB and KA (lower) (27°C, 8 km/h windspeed; starting blend 100:4.4:11.1 HB:E2HB:KA)

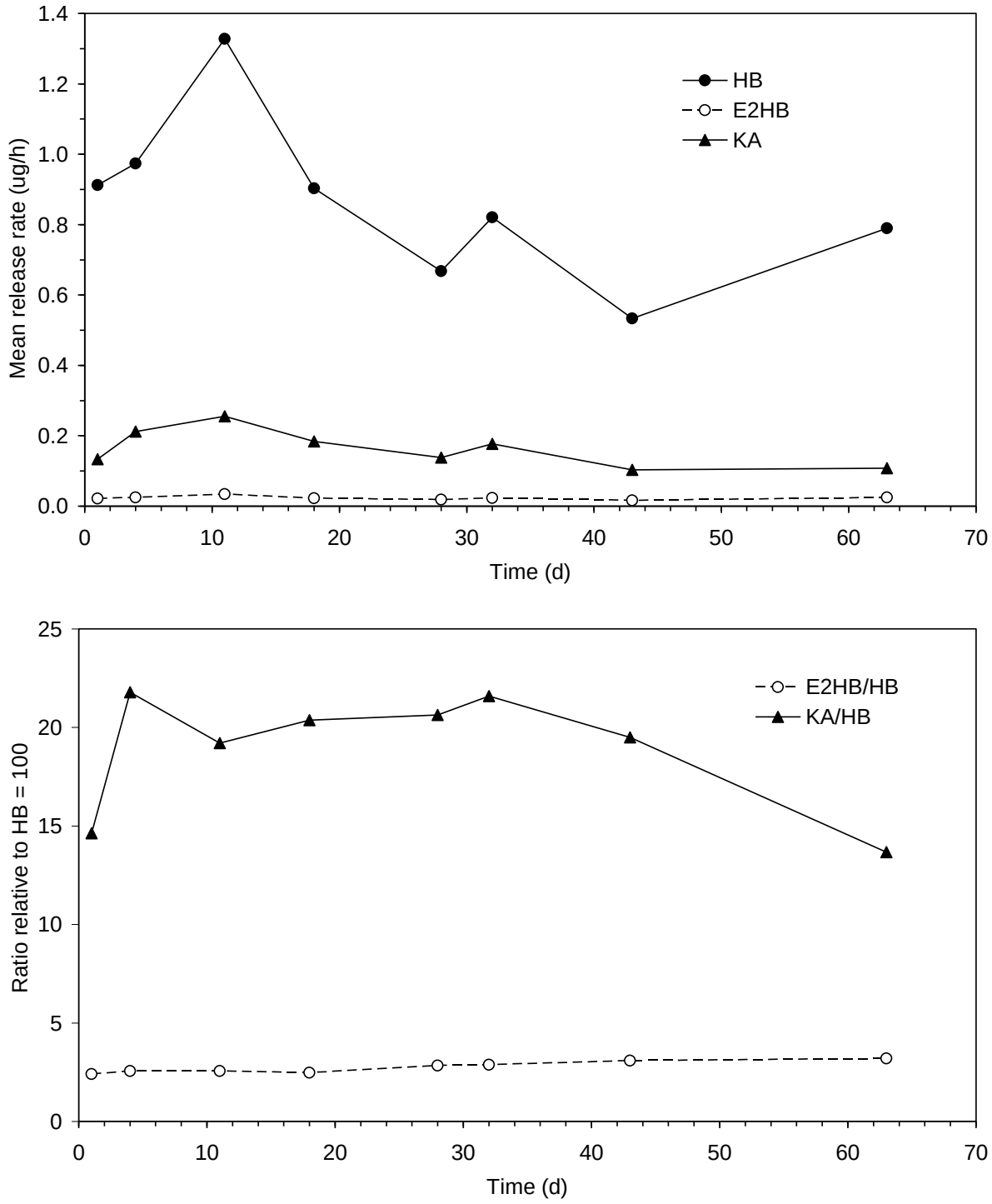


Figure 1.9. Release of *L. rugulipennis* blend from 1 ml pipette in wind tunnel showing actual release rates (upper) and relative amounts of E2HB and KA (lower) (27°C, 8 km/h windspeed; starting blend 100:3.4:19.9 HB:E2HB:KA)

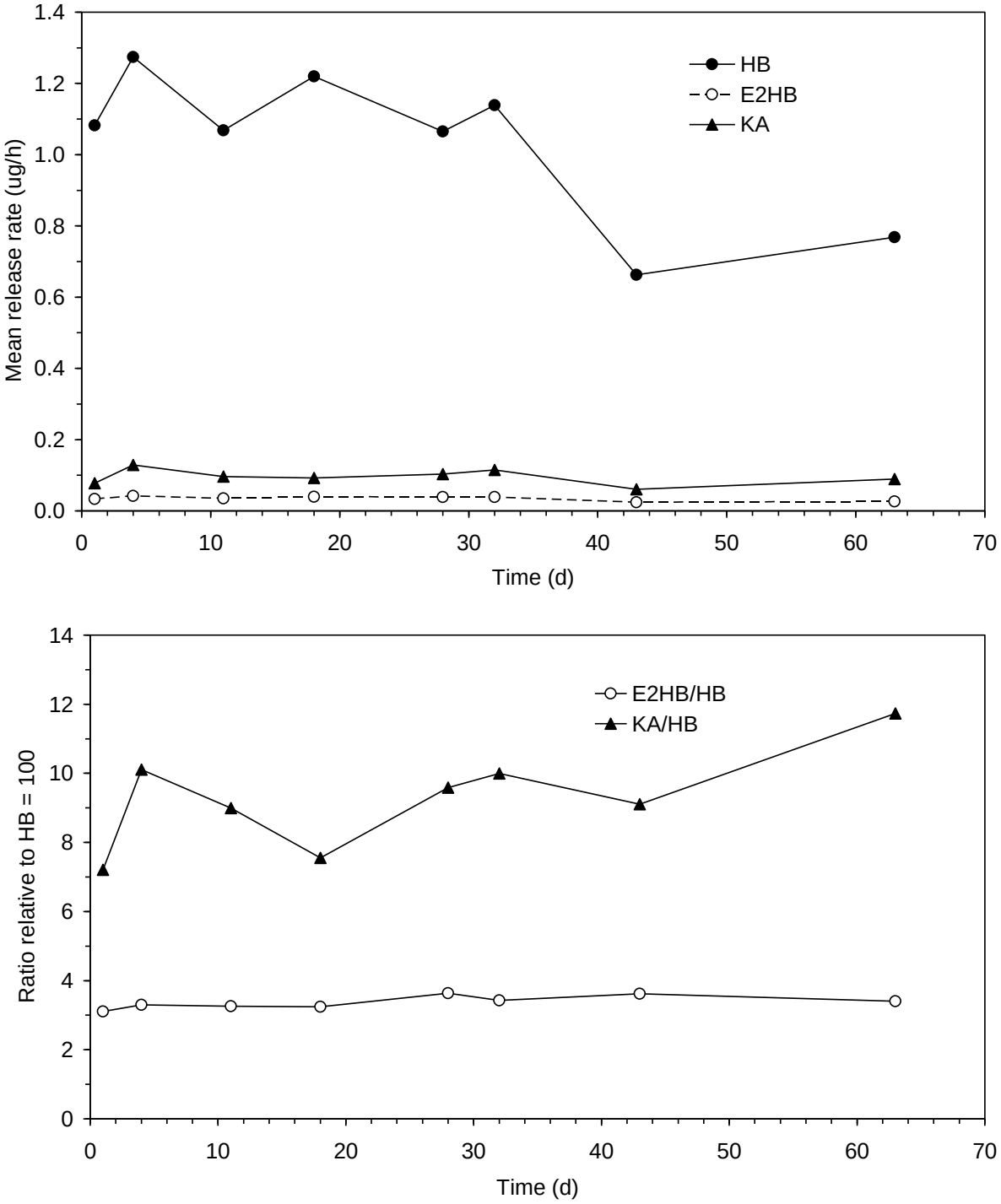


Figure 1.10. Release of *L. pabulinus* blend from 1 ml pipette in wind tunnel showing actual release rates (upper) and relative amounts of E2HB and KA (lower) (27°C, 8 km/h windspeed; starting blend 100:4.3:9.4 HB:E2HB:KA)

Persistence under field conditions

Previous results showed that the pipette tip lures remained attractive for a much shorter time under field conditions than was anticipated from their performance in the laboratory

windtunnel. This was thought to be due to the degradation of the unstable KA in sunlight and the lures used during 2010 were shielded by wrapping in duct tape.

Release rates were measured from 0.2 ml pipette tip dispensers used in field tests with funnel traps for attraction of *L. rugulipennis* during 15 April – 15 May 2010. Results (Fig. 1.11) showed that the blend released was similar to that found in the windtunnel (Fig. 1.12) and suitable for attraction of *L. rugulipennis* males. Relative amounts of HB:E2HB:KA in Fig. 1.11 were 100:2.3:16.9 and 100:2.3:19.1 on the two measurements.

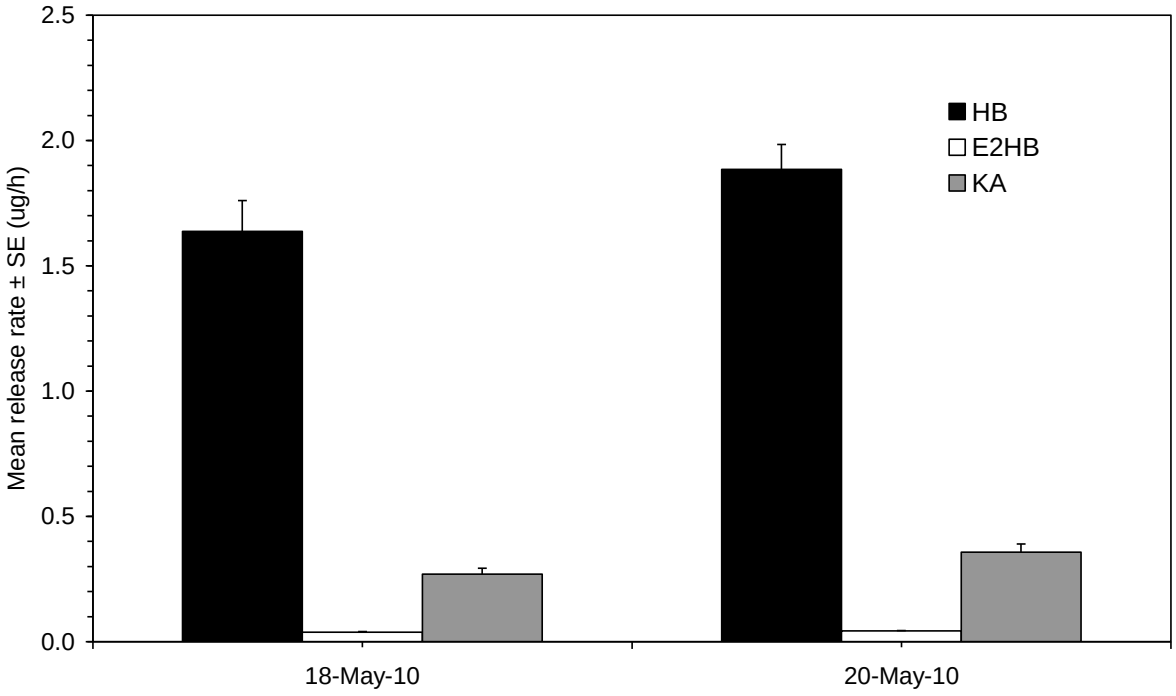


Figure 1.11. Release rates from 0.2 ml pipette dispensers for *L. rugulipennis* returned from exposure in field tests with funnel traps (15 April – 15 May 2010) in the laboratory windtunnel and measured on 18 May and 20 May 2010 (27°C, 8 km/h windspeed; n=5)

Release rates were also checked for 1 ml pipette tip dispensers used in the trap design experiment with *L. pabulinus* (7-30 September 2010). In this experiment there was particular concern over the platform and water traps where the dispenser was positioned in full sunlight vertically with the open tip upwards. Results (Fig. 1.12) showed that the proportion of KA in the blend was satisfactory for the sticky stake and water pan traps (5.0% and 7.1%, respectively) although low for the platform trap (2.5%).

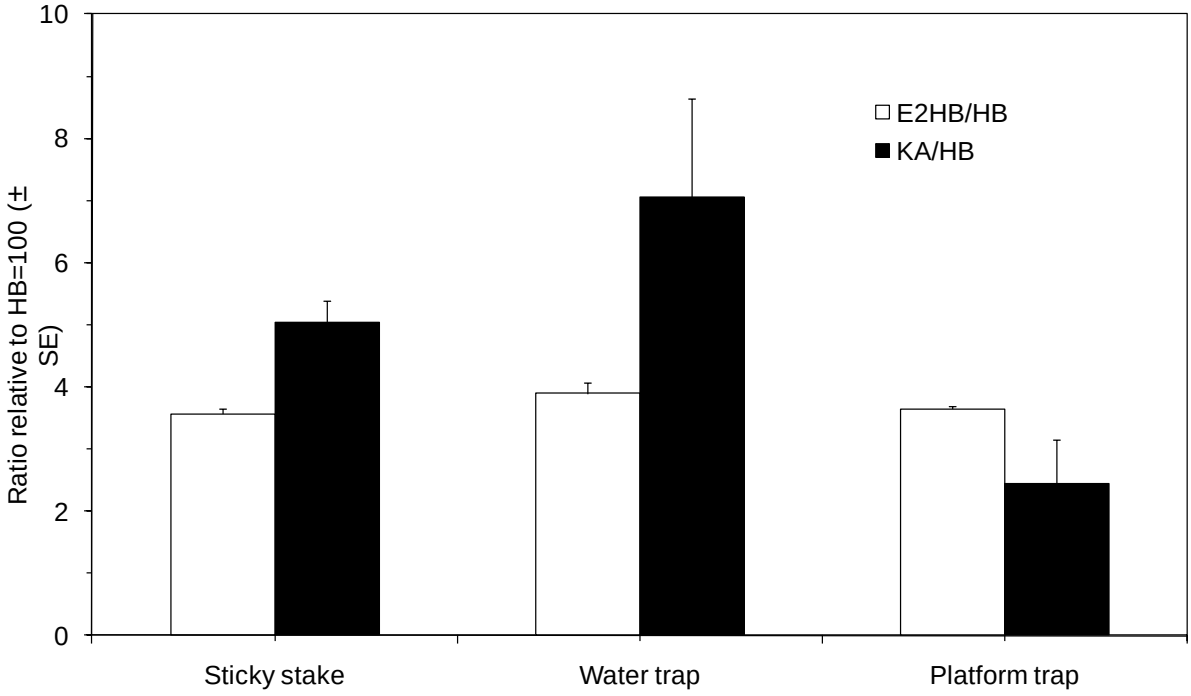


Figure 1.12. Release rates from 1 ml pipette dispensers for *L. pabulinus* returned from exposure in field tests with different trap designs (7-30 September 2010) in the laboratory windtunnel (27°C, 8 km/h windspeed; n=4)

Sachet dispensers

Sachets supplied by Agrisense were loaded with a standard *L. rugulipennis* blend, 10% in sunflower oil. The release rate of HB was higher than from the pipette tips (approx 5 µg/h from the sachets and 1 µg/h from the 1 ml pipette tips (Fig. 1.13)). More importantly the initial release rate of the KA was much higher (49% relative to HB from sachet, 20% in blend loaded) such that within 10 days the relative amount released had dropped to 1% relative to the HB, the required rate being 15% (Table 1.2).

Further experiments were carried out using the cigarette filter as in the pipette tips rather than the fibre discs supplied and also using impermeable sachets with an open hole. Results for a sachet with 3 mm diameter hole are shown in Fig. 1.14. The release rate of HB dropped from 4.5 to 1.3 µg/h over 7 days and the relative amount of KA/HB dropped from 18.5% to 1.5% over the same period.

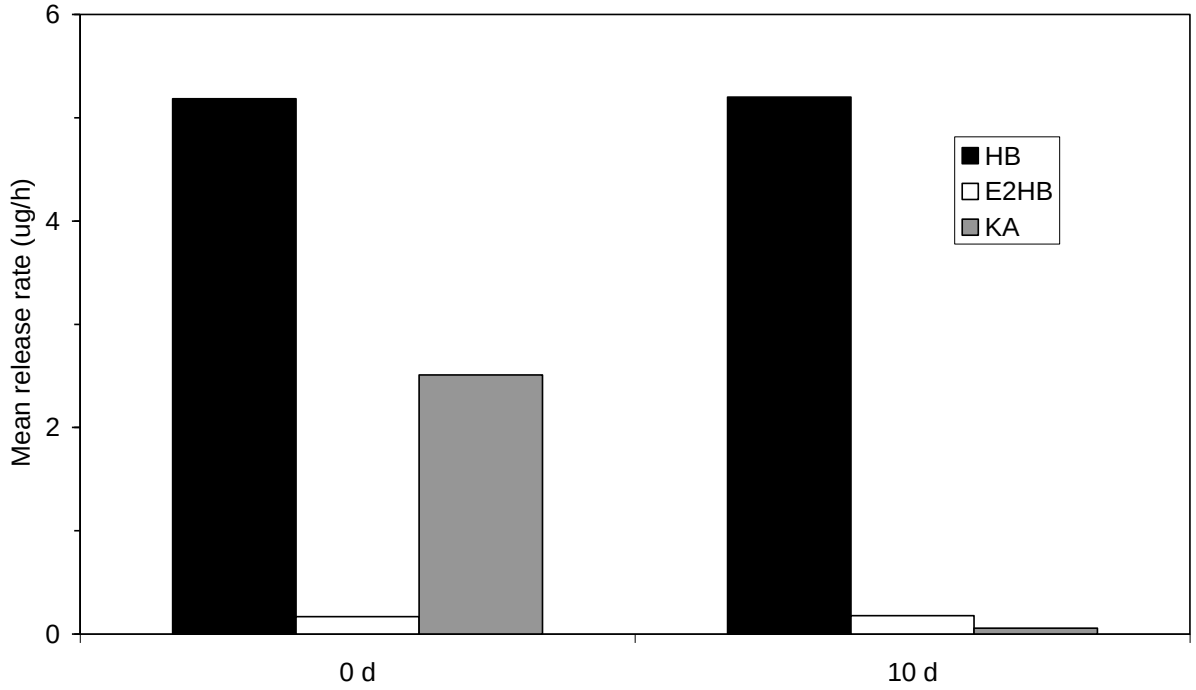


Figure 1.13. Release rates for *L. rugulipennis* blend in Agrisense sachets maintained in laboratory windtunnel (27°C, 8 km/h windspeed; starting blend 100:3.4:19.9 HB:E2HB:KA)

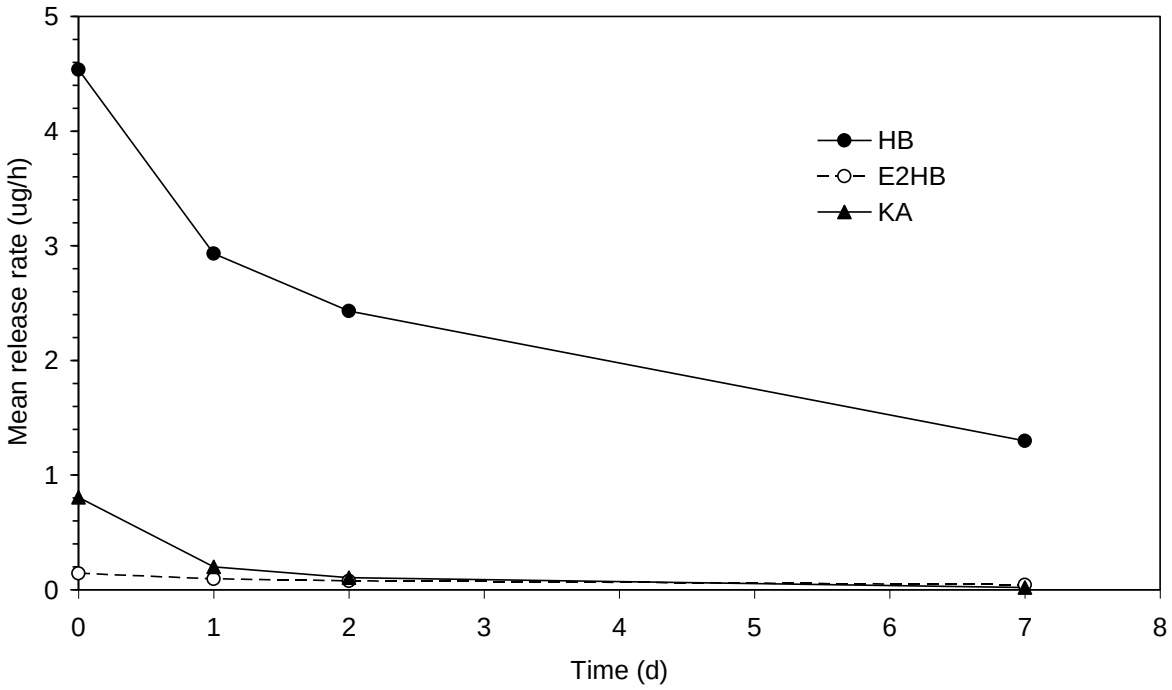


Figure 1.14. Release rates for *L. rugulipennis* blend in Agrisense sachets with 3 mm hole maintained in laboratory windtunnel (27°C, 8 km/h windspeed; starting blend 100:3.4:19.9 HB:E2HB:KA)

Objective 2. Calibrate the trap for *L. rugulipennis* for use in pest monitoring to establish a treatment threshold for its use in late season strawberry and cucumber.

- investigate correlations between catches of male capsids in pheromone traps, and capsid populations in strawberry and cucumber crops

Trap catches with lures containing synthetic pheromone compounds were compared to traditional crop sampling techniques for capsids.

Site 1: Strawberry: 'Owens 3' everbearer strawberry plantation (cv. Elsinore) at Langdon Manor Farm, Goodnestone, Faversham, Kent ME13 9DA by kind agreement of Alastair Brooks and Andrew Reeve. The plantation was located at NGR TQ 024 593. It was planted in April 2009 and cropped as an everbearer (cv. Elsinor) in 2009 and 2010. The plantation was 88 x 90 m and consisted of 11 tunnels with 5 row beds. The traps were located in the 4th and 8th tunnels (Figs. 2.1, 2.2).

Figure 2.1. Site 1: Plot and tunnel location of the two blocks of 11 Elsinor tunnels (marked in green with red surround).

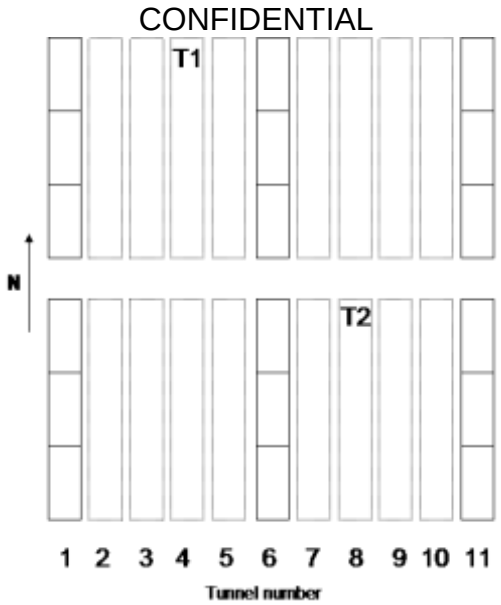


Figure 2.2. Site 1: Location of traps in tunnels at Langdon Manor Farm, Goodnestone

Site 2: Strawberry: Park West strawberry plantation (Cv Albion) at Robert Boucher and Son, Newlands Farm, Teynham, Sittingbourne, Kent ME9 9JQ by kind agreement of Hugh Boucher. The plantation was located at NGR TQ 956 622. The rows were 69 m long (Figs. 2.3, 2.4) and surrounded by Elsanta and Flamenco strawberries. The row spacing was 1.9 m. The plot contained cv. Elsanta.

Figure 2.3. Site 2: Newlands Farm, map of the experimental plot location and tunnel (red bar)

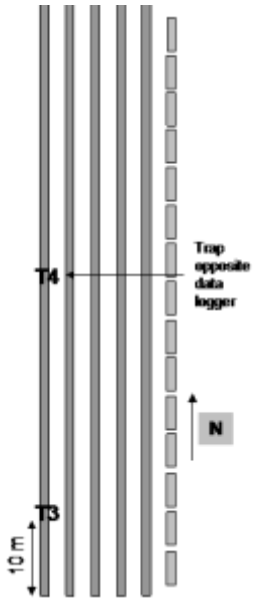


Figure 2.4. Site 2: Location of traps in tunnels at Newlands Farm

Site 3: Strawberry: Peaches Valley strawberry field at Mansfield's, Middle Pett Farm, Bridge, Canterbury, Kent by kind agreement of David Stockbury. The plantation was located at NGR TR 165542. Variety Albion (everbearer), planted September 2008, 8.4 ha, 42500 plants/ha, bed spacing = 1.5 m, plant spacing in bed 30 cm in zig-zag row, 4 rows/tunnel (Fig. 2.5).

Figure 2.5. Site 3: Location of plantation at Middle Pett Farm. Traps were located at either end of the plot.

Site 4: Strawberry: Field 2B. cv Florence (June bearer) in second year. At Hall-Hunter Farms, organic site at Tuesley Farm, Milford, near Godalming by kind agreement of Harry Hall. The plantation consisted of 115 beds (5 bed tunnels). The traps were in rows 2 & 20 (Fig. 2.6).

Figure 2.6. Site 4: Location of traps in tunnels at Tuesley Farm. T7 and T8 are *L. rugulipennis* monitoring traps

For the cucumber crops the traps were evaluated at three sites which had suffered serious damage by capsid bugs in previous years. At each site, two traps were placed above the plants within a production glasshouse and one was placed outside (e.g. Fig 2.7). Those within glasshouses were moved between adjacent crops as they were replanted so that they were always monitoring a high risk situation. The sites were:

Site 4: Cucumber: Hedon Salads, Burstwick (east of Hull) by kind agreement of Mr Phil Clarkson. The traps were originally placed at the end of April and the lures were changed in weeks 24, 30 and 35 2010.

Site 5: Cucumber: Halsham Farms, Cottingham (west of Hull) by kind agreement of Mr Les Deeley. The traps were originally placed at the end of April and the lures were changed in weeks 24, 27, 30 and 35 2010.

Site 6: Cucumber: Stubbins Marketing, Fen Drayton (Cambridgeshire) by kind agreement of Mr Steve Clarkson. The traps were originally placed at the end of April and the lures were changed in weeks 23, 30 and 35 2010.

Figure 2.7. Typical trap positions at the cucumber sites; left – inside the glasshouses, right – outside the glasshouse

One ml pipette tips loaded with the 3-component mix of hexyl butyrate ('HB') : (*E*)-2-hexenyl butyrate ('E2HB') : (*E*)-4-oxo-2-hexenal ('KA') (10% in sunflower oil; 100 µl) which is attractive to *L. rugulipennis* males and with a release rate of approximately 1 µg/h was used in combination with funnel bucket traps with pre-moulded green cross vanes.

Strawberry

The contents of the pheromone traps were sieved and numbers of male *L. rugulipennis* counted. In addition, 40 strawberry plants from each plot were tap sampled over a white tray. Capsids landing on the tray were identification in the laboratory to instar (N1-N5) and adults were sexed. It is known that 1 capsid in 40 strawberry plants is enough to cause economic damage so this could be correlated with pheromone trap catches. Traps were in place on 15 April 2010.

Cucumber

The traps were examined at approximate weekly intervals between weeks 20 and 38 2010. On each occasion, the contents were sieved and the numbers of adult male and female *L. rugulipennis* were recorded separately per trap.

There is no established method of monitoring capsid numbers in cucumber crops that will provide a reliable indication of the size of the population or the risk of plant damage. It is usual for crop workers to look for the insects and / or damage symptoms while doing their routine crop work and immediately report any sightings to the nursery manager. The grower will then decide on the need for treatment depending on the growth stage and vulnerability of the crop. In addition, Mr Derek Hargreaves, who is the Cucumber Growers' Association (CGA) Technical Officer and the principal cucumber crop consultant in the UK, will alert CGA members when the pest becomes active in his clients' crops. This is likely to instigate more widespread precautionary treatments.

Results

Strawberry

The populations at Tuesley Farm were relatively low and by the end of July the strawberry crop had been grubbed (Fig. 2.8). Adult populations peaked at the end of August and nymphs in early to mid September. The pheromone traps were catching adult male *L. rugulipennis* in the strawberry crops weeks before adults could be detected in the field. For example, one 1st instar nymph was sampled on 22 Jul at Langdon Manor Farm and by

the next sampling date (5 Aug) 6 nymphs were trapped (Fig. 2.8). The pheromone traps generally gave a 4 week warning of second generation nymph emergence in strawberry. At Newlands Farm, there was a peak of males in the pheromone traps, but then the polythene was removed and nymph numbers never increased (Fig. 2.8).

Data was plotted with temperature and humidity recordings (Fig. 2.9). At Langdon Manor Farm the numbers of trapped males in the pheromone traps closely followed temperature increases in the two adult generations.

Cucumber

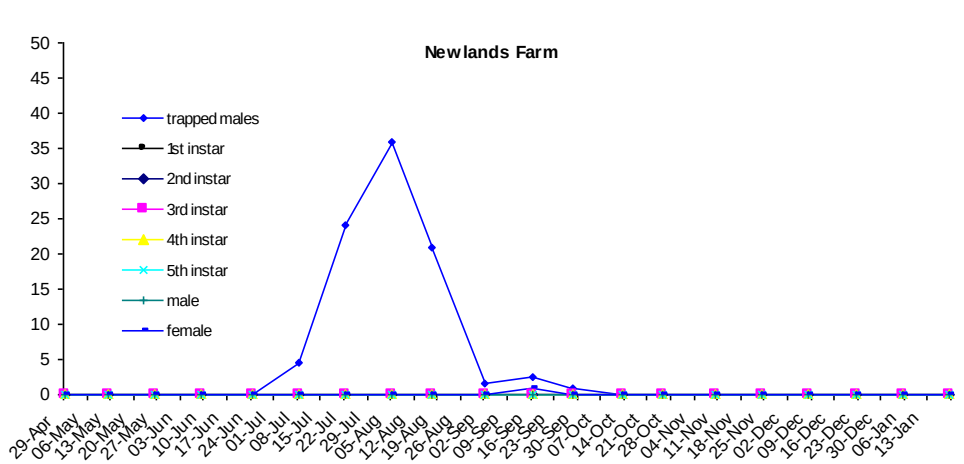
Only 1% of the *L. rugulipennis* caught in traps in and around cucumber crops were female and so the present results focus solely on males. The numbers of males caught, per trap per week, inside and outside glasshouses at each of the three sites are shown in Fig. 2.10.

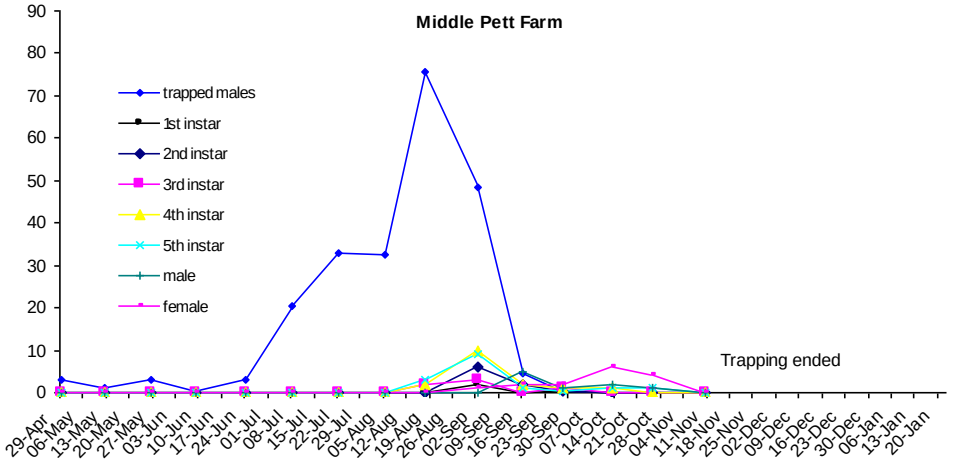
There was a clear peak in the numbers of *L. rugulipennis* caught outside the glasshouses at two sites between late July and late August, which coincided with the anticipated summer generation. Crop workers first reported capsid activity in crops at sites 4 and 6 during weeks 31 and 30 respectively. Plant damage was recorded at both of these sites during the subsequent 4-5 week period. Mr Hargreaves issued the general 'capsid alert' to CGA members in week 31.

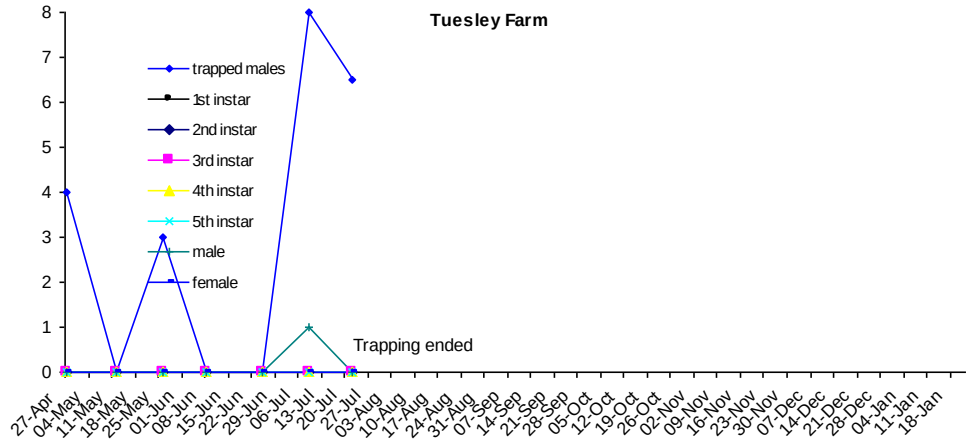
The increase in trap catches outside the glasshouses at sites 4 and 6 pre-empted the reports of capsid activity within those crops and the general capsid alert by 7-14 days.

Very few *L. rugulipennis* were caught in traps at site 5 and only slight crop damage was reported by the nursery manager. However, the most vulnerable crops were treated with pymetrozine (an anti-feedant) as a precautionary measure in response to the general capsid alert in week 31. Site 5 was surrounded by oil seed rape crops, whereas sites 4 and 6 had mixed crops adjacent to them.

Pheromone baited traps positioned outside cucumber greenhouses appear to provide useful prior warning of crop invasion and plant damage by *L. rugulipennis*. Those positioned within the crops were of little value.







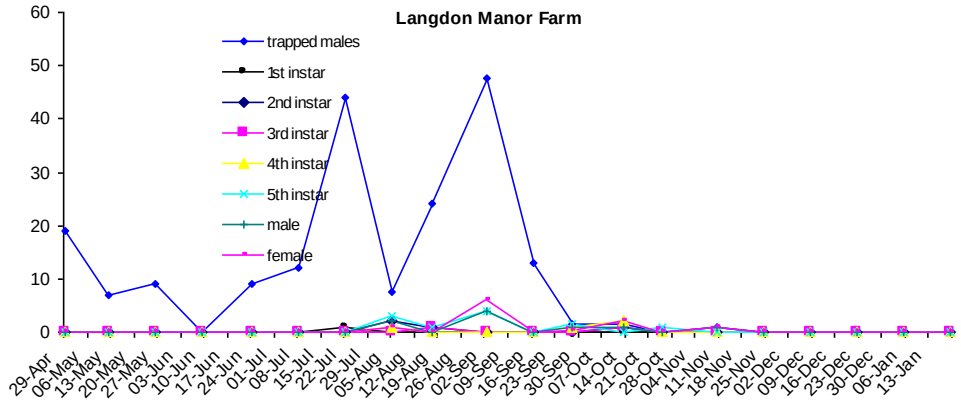
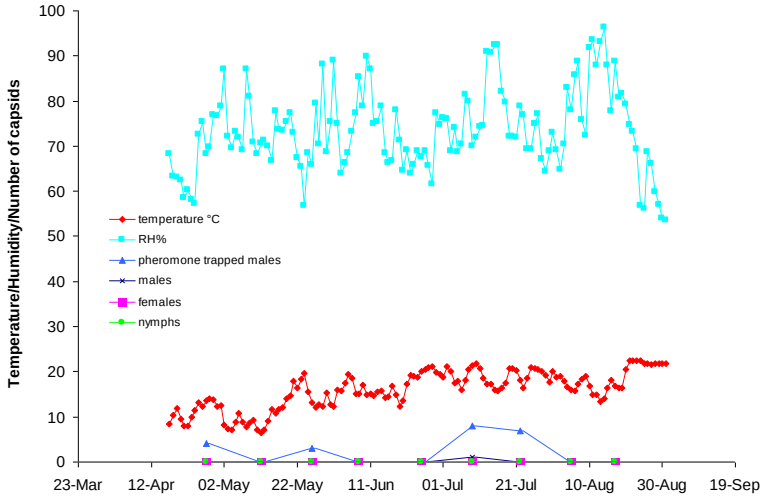
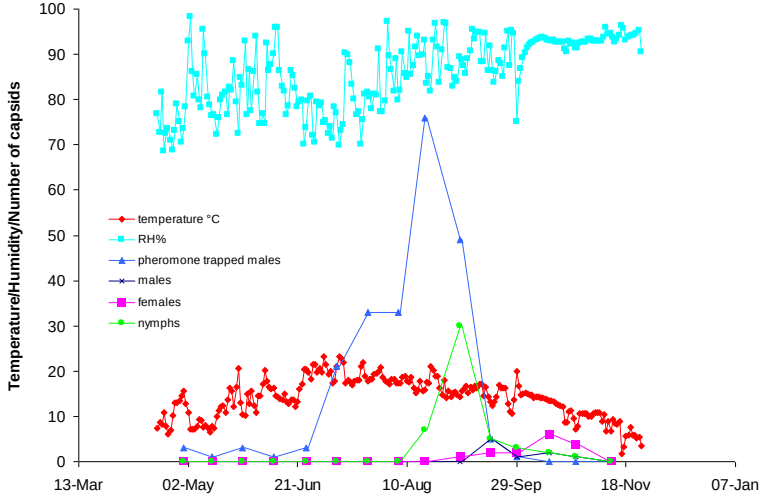


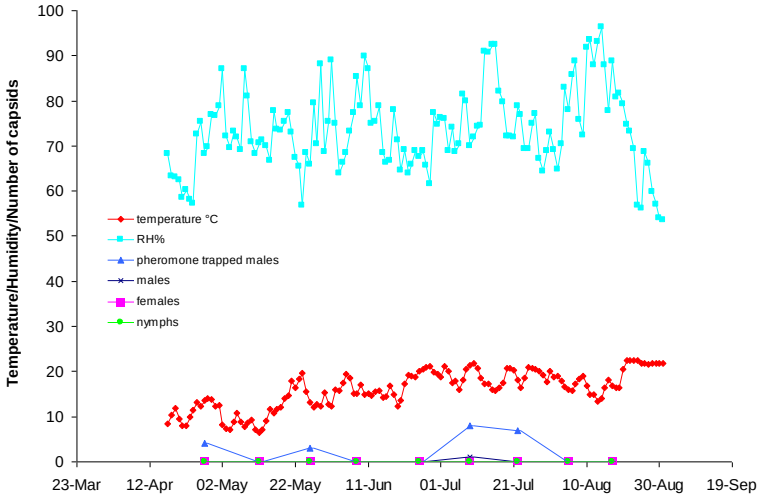
Figure 2.8. Numbers of pheromone trapped male *L. rugulipennis*, and tap sampled males, females and all 5 stages of nymphs in the 4 strawberry crops



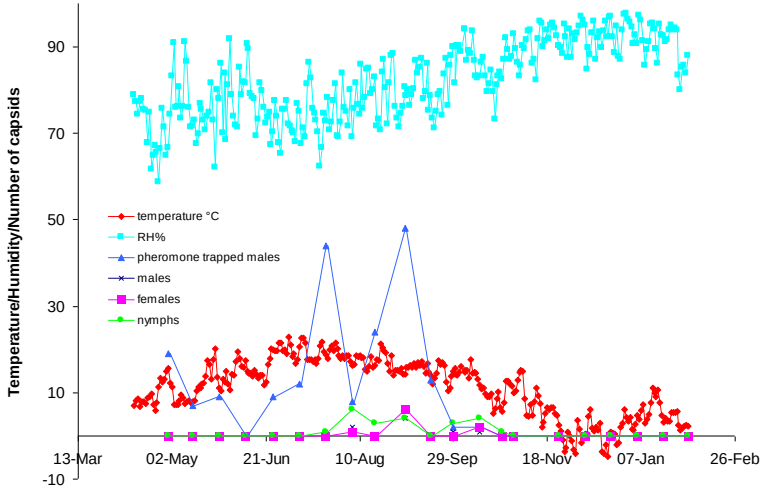
Newlands Farm



Middle Pett Farm



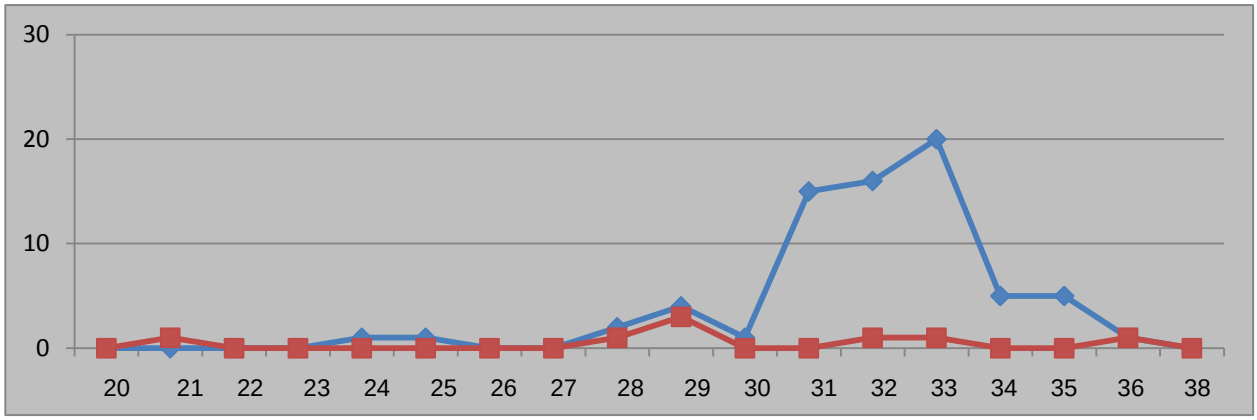
Tuesley Farm



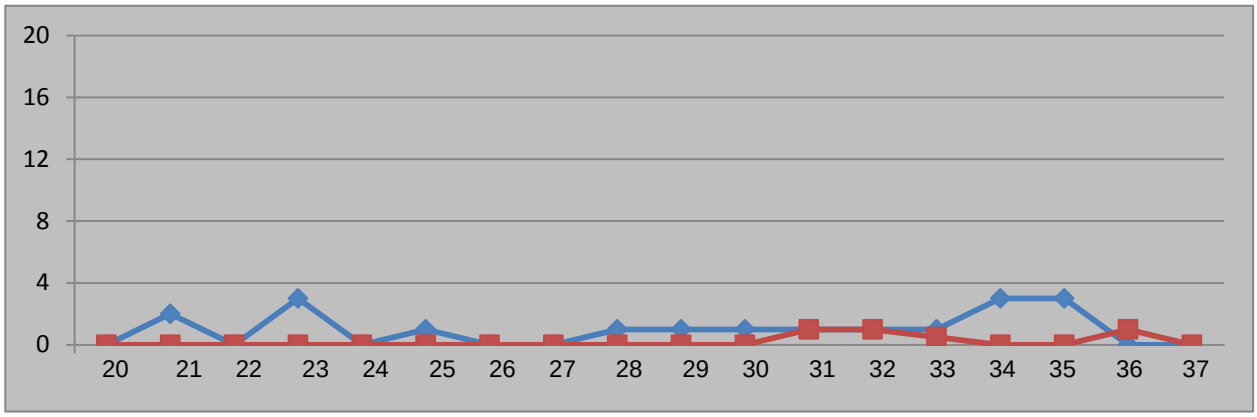
Langdon Manor Farm

Figure 2.9. Numbers of pheromone trapped male *L. rugulipennis*, and tap sampled males, females and total nymphs in the 4 strawberry crops related to temperature and humidity.

Site 4. Hedon Farms



Site 5. Halsham Farms



Site 6. Stubbins Marketing

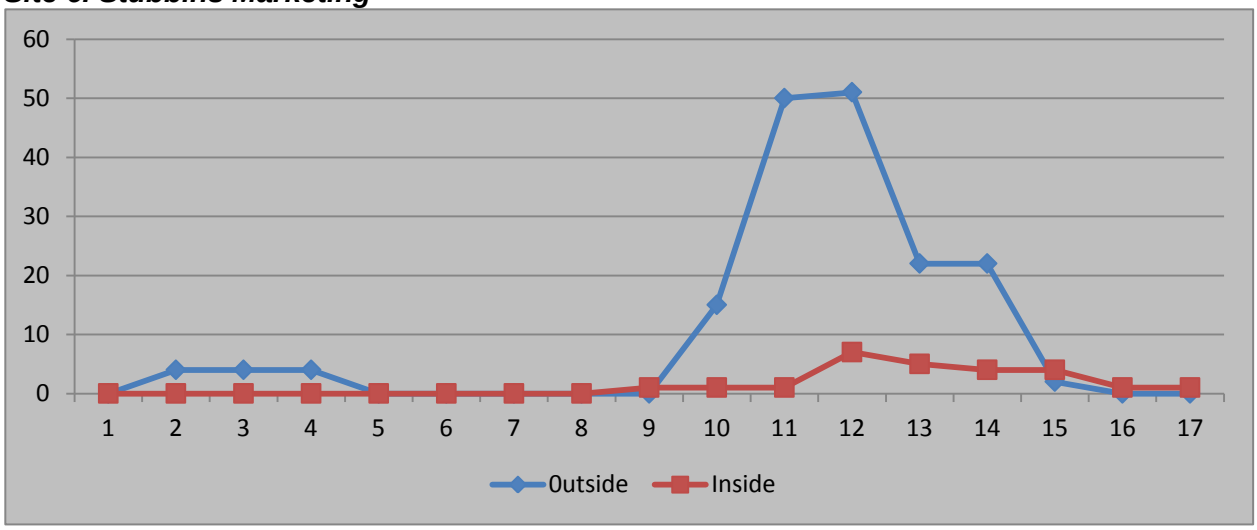


Figure 2.10. Number of male *L. rugulipennis* per trap per week inside and outside the cucumber glasshouses at three sites between week 20 (mid-May) and week 38 (late-September)

Objective 3. Develop an effective lure and trap for *L. pabulinus* with associated data for pest monitoring.

In 2008, from 9-30 June, three males were captured in three female baited traps and 1 male was captured in 1 male baited trap. Three of the four males were captured in clear plastic delta traps. The attraction of males to females was not very successful, was probably due to trap design.

- Confirm females attracting males
- Identify effective trap design

Most of the experiments, except the final trap design test, were done in a Ben Alder blackcurrant plantation at Stonebridge, Horsmonden, Location NGR TQ 719 399 by kind permission of Tom Maynard. All were small scale field trials using either caged female *L. pabulinus* in various trap designs or 1 ml pipette tips with the synthetic pheromone. The trials were either fully randomised or set out in sequential order. The first *L. pabulinus* nymph was found on willow (*Salix*) on 6 May, but nymphs were not found in blackcurrant until 28 May. Nymphs were collected and cultured at 20^{o}C for use in field tests.

Experiment 1

This test compared trap design using laboratory reared unmated females as bait. Insects were caged as previously described for *L. rugulipennis*. The traps were either clear 20 x 20 cm delta traps with a clear sticky insert; green cross vane funnelled bucket traps (Agralan); or sticky stake traps (Fig. 3.1). The traps were suspended (funnel and delta trap) or hammered into the soil beneath the crop (stake trap), at crop height. Traps were set up (21 June – 8 July), checked every 7 days and capsids replaced with new virgin individuals. There were 5 replicates and traps were 10 m apart.

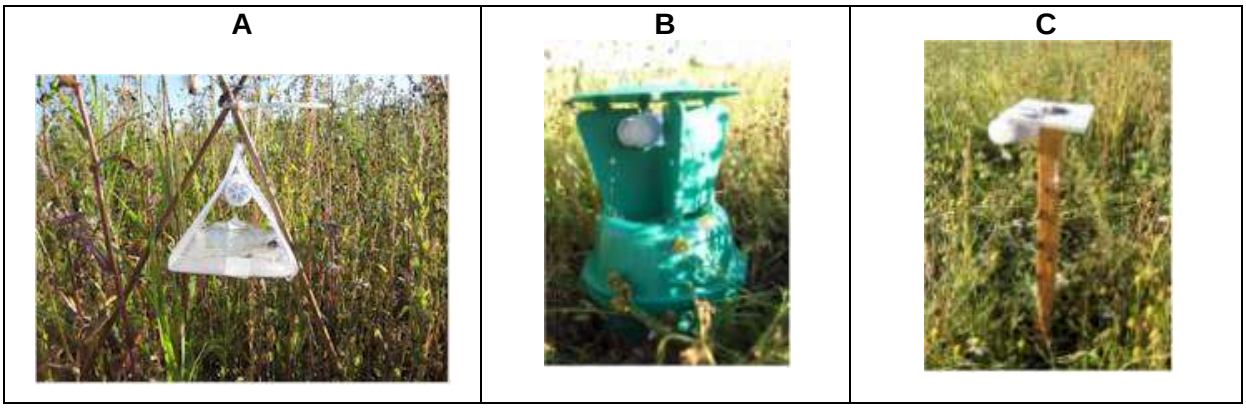


Figure 3.1. Trap designs used in field test. A – Clear delta, B – Green funnelled cross vane trap, C – Sticky stake trap (n=5)

Experiment 2

From 20 July – 1 September a field test was set up using 1 ml pipette tips as bait on sticky stake traps (n=7). The lures were changed on 12 August (Batch 2010.056, July 2010).

Experiment 3

From 12 August – 1 September pipette tip lures were compared to sachets previously described for *L. rugulipennis* containing the ratio pheromone blend for *L. pabulinus*. Lures were attached to sticky stake traps (n=7).

Experiment 4

Between 14 - 30 June a test comparing clear delta traps, green cross vane funnelled bucket traps and sticky stake traps, baited with unmated female *L. pabulinus* was compared to traps with no bait (n=5).

Experiment 5

Different trap designs were compared to the sticky stake trap (September 2010). Green sticky horizontal platform traps (20 x 20 cm) were compared with water traps (Fig. 3.2) (n=10). A drop of detergent was added to the water in the water traps. The experiment was set up between 7 - 30 September in the blackcurrant plantation and a blackberry plantation (Belks No. 5) where capsid damage and nymphs had been seen. The site was a protected crop of blackberry at A. Belks Farm, Otham, Kent ME15 8RL by kind permission of Tim Chambers. Traps were baited with 1 ml pipette tip lures (Batch No 2010.071).

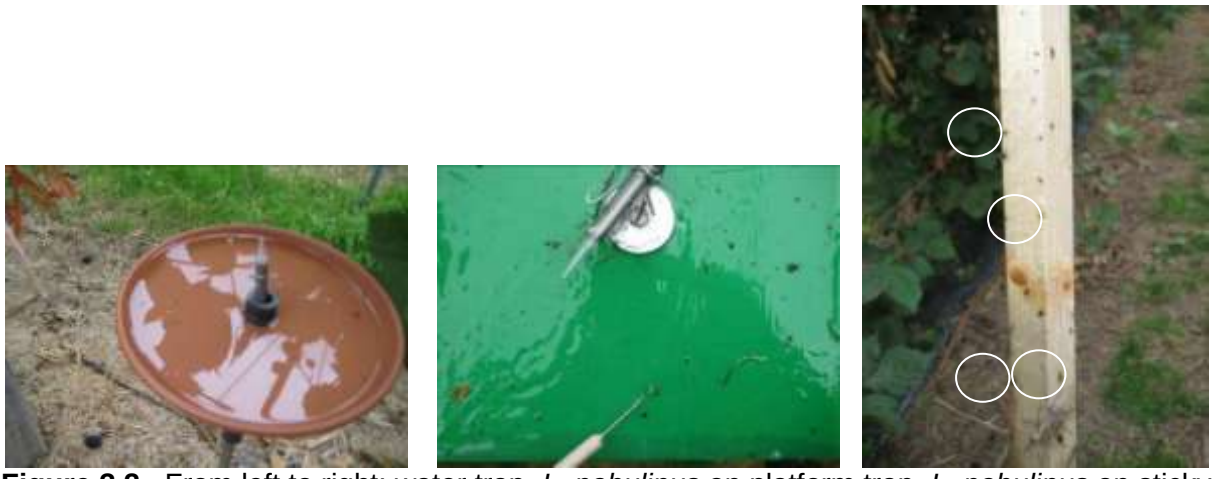


Figure 3.2. From left to right; water trap, *L. pabulinus* on platform trap, *L. pabulinus* on sticky stake traps.

Counts of the number and sex of *L. pabulinus* were made weekly for all experiments.

Video recording of behaviour

Using a remote security camera and hard drive recorder a series of experiments was set up to observe the behaviour of males *L. pabulinus* approaching females or pheromone lures (Fig. 3.3, Table 3.1).

Table 3.1. Video recording experiments for observing male capsid behaviour.

Trap	Set up		Bait	No. males released into tunnel	Test ended	
White correx platform	14-Jun	15:15	Female (11-Jun)	10 (11-14 Jun)	21-Jun	
White correx platform	21-Jun		Female (14-Jun)	17 (11-14 Jun)	25-Jun	13:00
Wooden stake	1-Jul	15:35	pipette tip lure	Open field Horsmonden	1-Jul	16:19
Wooden stake	5-Jul	9:45	pipette tip lure	Open field Horsmonden	5-Jul	20:33
Wooden stake	21-Jul	12:24	pipette tip lure	Open field EMR blackcurrant	2-Aug	14:40

Figure 3.3. Photograph of camera and lure set up in field to record male *L. pabulinus* behaviour

Results

Experiment 1

Significantly more male *L. pabulinus* were trapped on the sticky stake traps compared to clear delta traps or green cross vane traps between 21 June – 8 July (ANOVA, square root transformed data, P<.001, Fig. 3.4).

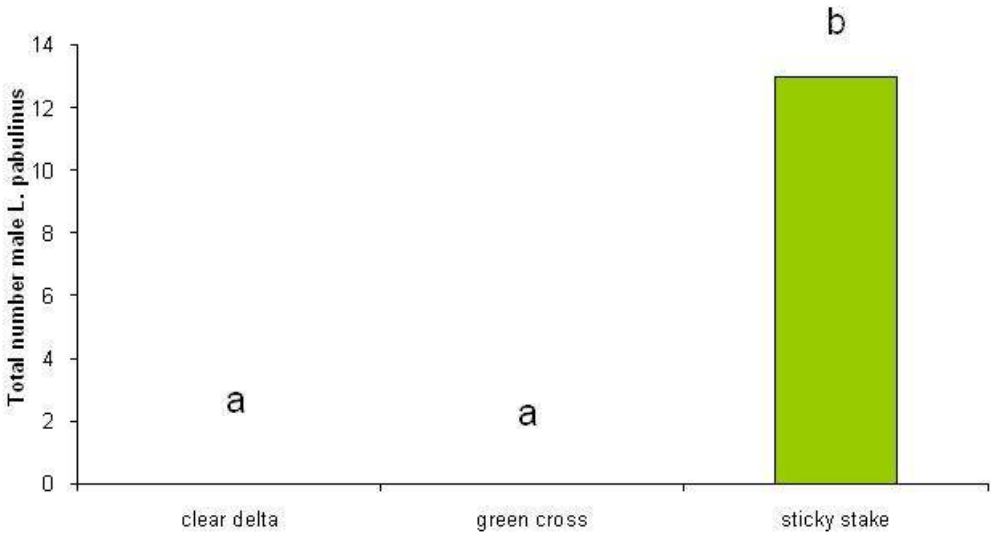


Figure 3.4. Number of male *L. pabulinus* trapped in different trap designs

Experiment 2

Nine male *L. pabulinus* were captured on sticky stake traps using pipette tips as lures (20 July – 1 September) compared to only 2 on the control traps (no lure).

Experiment 3

Only one male *L. pabulinus* was trapped on a pipette baited sticky stake trap (12 August – 1 September). None were trapped on the sachet baited sticky stakes.

Experiment 4

The results of the trap design test (14 - 30 June) indicated that sticky stake traps were more effective at trapping male *L. pabulinus,* but numbers captured during this time were very low (Fig. 3.5).

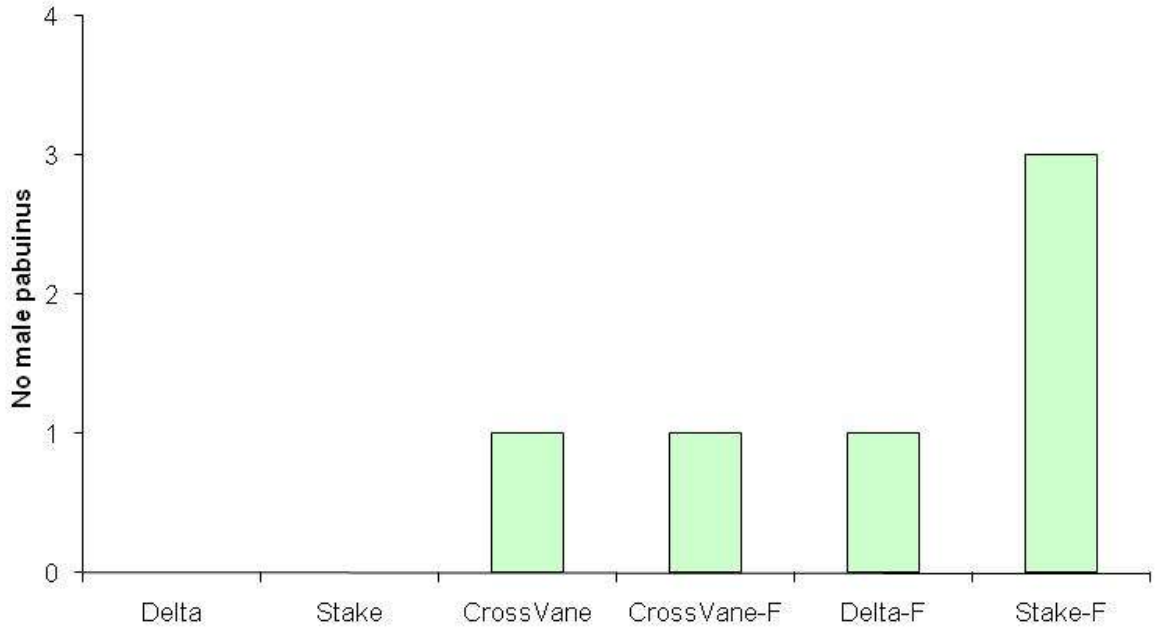


Figure 3.5. Numbers of male *L. pabulinus* captured in different trap designs with or without unmated females as bait (14 - 30 June). F = female as bait

Experiment 5

Significantly more males were captured in sticky stake traps compared to platform or water traps in both the blackcurrant (open) and blackberry (protected) crops between 7 - 30 September (ANOVA, Log_{10} transformed data P <.001, Fig. 3.6).

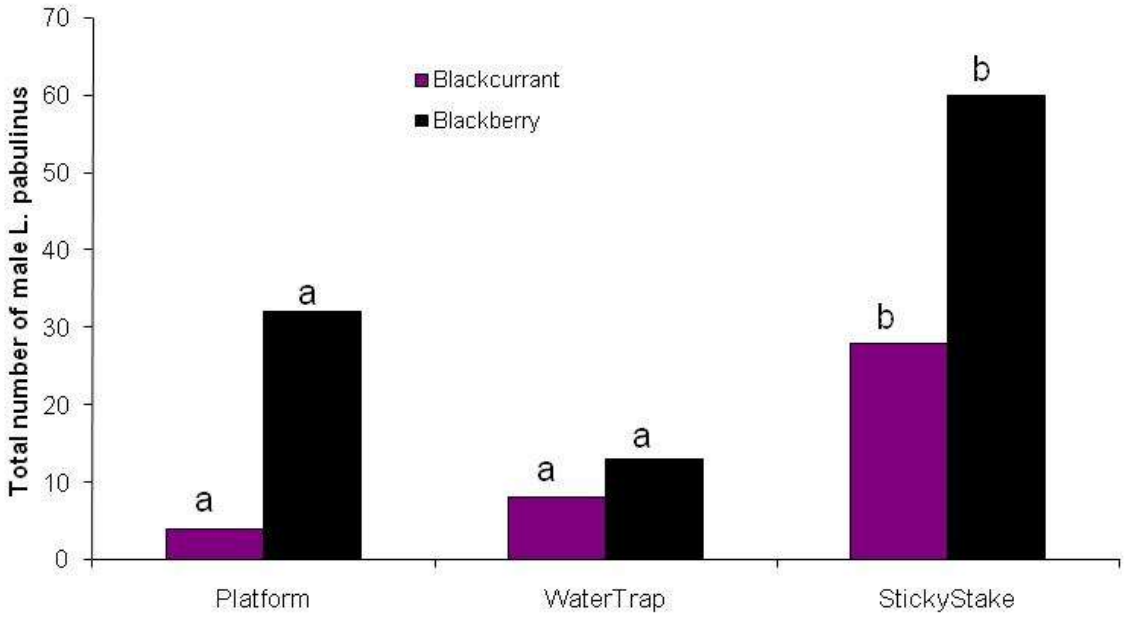


Figure 3.6. Numbers of male *L. pabulinus* trapped in 3 different trap designs in blackberry and blackcurrant plantations (n=5 /site)

Video recording of behaviour

No definite recordings of the males being attracted to the females were observed. Firstly, the resolution of the camera was not high enough to distinguish species of capsid. Secondly, most of the video recording was done in-between the two generation of adults, so it is likely that this is the main reason for not observing significant attraction to the baits (female or synthetic lures). Very few males were captured in July and August in any of the experiments indicating that mid July was the main period of eggs and nymphs.

Objective 4. Encourage commercial production of traps and lures and produce grower information sheets on the use of the traps for monitoring capsids

This will be done in year 2 of the extension.

Appendix 1

SIX MONTHLY REPORT TO HORTICULTURE LINK PROGRAMME MANAGEMENT COMMITTEE

Project Number:	HL0184 (PC/SF 276)
Project Title:	Pheromone technology for management of capsid pests to reduce pesticide use in horticultural crops – 2 year extension
Project Partners:	*SCIENCE BASED PARTNERS* East Malling Research Natural Resources Institute East Malling Research Associate (Mr R Jacobson) *INDUSTRY PARTNERS* Horticultural Development Company (GlaxoSmithKline Blackcurrant growers research fund) GlaxoSmithKline East Malling Trust East Malling Ltd Agrisense Cucumber Growers Association K G Growers Ltd Donald J Moor, Nichol Farm, Teynham
Report Written by:	Michelle Fountain and Jerry Cross
Project Start/Completion Dates:	Project extension: (1 April 2010 - 31 March 2012)
Reporting Period:	1 April 2010 – 31 March 2011
Number of Months Since Commencement:	12
Date of Last Management Meeting:	13 December 2010, 3 March 2011

1. Project objectives: (from project proposal, or other more recently approved planning document)

Objectives Proposed in the Extension

1. Improve and test the lure for *L. rugulipennis* so that it is long lasting and practical for use by growers.
2. Calibrate the trap for *L. rugulipennis* for use in pest monitoring to establish a treatment threshold for its use in late season strawberry and cucumber.
3. Develop an effective lure and trap for *L. pabulinus* with associated data for pest monitoring.
4. Encourage commercial production of traps and lures and produce grower information sheets on the use of the traps for monitoring capsids.

2. Table showing overview of progress against milestones for project as a whole (from project proposal, or other more recently approved planning document)

Milestone	Target year	Title
P1	1	Lure for *L. rugulipennis* developed which lasts for at least one month under field conditions Y
P2	2	Action thresholds developed and validated for monitoring *L. rugulipennis*
P3	2	Trap and lure for *L. pabulinus* developed and validated
P4	2	Information on use of traps for monitoring capsids available to growers

3. Milestones for the six month period: (from project proposal, or other more recently approved planning document)

NA

4. Research report: (concise account including comments on whether targets are being met)

1. Improve and test the lure for *L. rugulipennis* so that it is long lasting and practical for use by growers (Yr 1)

The life and release rate of pheromone components from the pipette tip lure have been enhanced. The lure now lasts over 4 weeks in the field having been shielded from sunlight and the use of larger pipette tips is giving a more consistent release rate. Wrapping the pipette tips in duct tape provided effective screening from sunlight in the field. In the laboratory windtunnel the 1 ml pipette tips proved much more reliable than the 0.2 ml tips, releasing a blend very similar to that loaded into the dispenser for up to 2 months at 27°C and 8 km/h windspeed. They also released at a higher rate than the 0.2 ml pipette tips. Furthermore, the 1 ml pipettes were easier to load with the pheromone blend and to seal with the crimp cap. The results have confirmed that disposable pipette tips are suitable dispensers for the three candidate pheromone components of *Lygus* bugs. The Agrisense sachets proved unsatisfactory for dispensing the pheromone components. The components diffuse through a polyethylene disc such that release of the KA is proportionately faster than the HB and E2HB. This results in a very high relative amount of KA initially which dropped to a very low level within 10 days under windtunnel conditions. Thus, in the field the sachet performed well in comparison with the pipette tip during the first 5 days but much less well subsequently. The pipette tip lure was also shown to be as attractive as live female *L. rugulipennis*. Improvements have been confirmed using field trapping tests. The trap was further tested by adding Fluon to the cross vanes. This increased the catch by more than a third in week one, but catches of males decreased subsequently – probably because of contamination by debris on the cross vanes over time (enables the insects to grip the surface more easily). Products such as Teflon should be considered as an alternative coating for the cross vanes. Traps that combined the lures of *L. rugulipennis* and/or *Anthonomus rubi* with either white or green cross vanes showed that white cross vanes cannot be used as they reduce the catch of *L. rugulipennis* in the traps. In addition, the grid designed for preventing capture of bees attracted to white cross vanes prevents the *Lygus* bugs falling into the bucket of the trap.

2. Calibrate the trap for *L. rugulipennis* for use in pest monitoring to establish a treatment threshold for its use in late season strawberry and/or cucumber (Yrs 1 and 2)

Extensive trapping in both cucumber and strawberry crops has proven the monitoring trap to be an excellent early warning system of invasion into the crops. The pest is detected in high numbers in the trap at least 2 weeks before detection in cucumber and up to 2 months in strawberry compared to using traditional monitoring methods. More than 3 sites of each crop were monitored. Pheromone baited traps positioned outside cucumber greenhouses appeared to provide useful prior warning of crop invasion and plant damage by *L. rugulipennis*. Those positioned within the crops were of little value.

3. Develop an effective lure and trap for *L. pabulinus* with associated data for pest monitoring (Yrs 1 and 2)

For the first time, significant numbers of male *L. pabulinus* have been trapped using synthetic sex pheromone lures. Trap design is of major importance and the green cross vane and delta traps were found to be ineffective at catching males. The lure was more attractive than caged virgin females at attracting males to sticky stake traps. These traps are not practical for use by growers. Sticky platform and water traps were also tested, but were not found to be more effective than sticky stake traps. There should be more focus on trap design in year 2 of the extension of the project. Camera recordings have been made using pheromone lures and virgin females as bait. However, no direct observations were made of the interactions. This is believed to be because recordings were made between generations and another attempt will be made next year with improved equipment.

4. Encourage commercial production of traps and lures and produce grower information sheets on the use of the traps for monitoring capsids

It is the intension of the consortium to make available the *L. rugulipennis* trap for testing by strawberry and cucumber growers in 2011 in order to establish an action threshold.

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

No changes have been made to the proposed extension milestone and objectives.

6. Publications and technology transfer outputs: (including public presentations/talks given. Indicate additions since last report by use of bold type)

Technology transfer activities

10 November 2009. J Cross gave a 40 minute invited plenary lecture at the Nordo Baltic Soft Fruit conference entitled 'UK research into monitoring and control of European Tarnished Plant Bug, *Lygus rugulipennis'.*

Rob Jacobson (6 October 2010) Invited presentation to the Cucumber Growers' Association Annual Conference, Peterborough.

Michelle Fountain 29 June 2010. Talk to the Strawberry Growers Club at East Malling Research on the use of the trap for monitoring capsids in strawberry. HDC/EMRA meeting.

Michelle Fountain 22 October 2010. Talk to the Food and Plant Research, Lincoln, New Zealand entomology group Pheromones and management of capsid bugs in the UK.

Michelle Fountain 24 November 'Novel technology for controlling capsids in soft fruit', EMRA/HDC Soft Fruit Day at East Malling Research.

Publications

Michelle Fountain, Jerry Cross, Dudley Farman, David Hall (2009) Developing an effective trap and lure to monitor capsids in UK horticultural crops. EMRA/HDC bulletin.

Cross J V. (2010) To spray, or not to spray: That is the question. Horticultural Entomology in the 21st century. Inaugural professorial lecture 11 February 2010, P 42-43 and p66-67.

Michelle Fountain, Jerry Cross, David Hall (2009) Using pheromones to monitor *Lygus* populations in fruit crops in the UK IOBC Bulletin, IOBC semiochemical conference proceedings, Budapest 2009.

Cross, JV, Fountain, M.T., Hall, D.R. (2010) Management of European tarnished plant bug in late season strawberries. "Integrated Plant Protection in Fruit Crops" Subgroup "Soft Fruits". "Workshop on Integrated Soft Fruit Production" 7th Meeting in Budapest, Hungary, Monday 20th – Thursday 23rd September 2010.

Hall. DR, Article in Kent Profile column (2009) Sex appeal solves fruity problem.

Article in HDC Annual Soft Fruit publication (2009) Enhancing pheromone technology for managing capsids in horticultural crops.

Article in HDC Soft Fruit Review Magazine (2010/11) Modern technology for improved capsid management.

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

Agrisense, and/or other UK suppliers (e.g., Russell, International Pheromone Systems LTD.) will be encouraged to take up commercial production of traps and lures. An information sheet for growers on the use of the traps for pest monitoring will be developed.

