Project title:	**Identification of the sex pheromone of *Nesidiocoris tenuis,* a damaging pest of commercial tomato**
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Project leader:	Michelle Fountain, NIAB EMR
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Key staff:	**NIAB EMR** Michelle Fountain, Celine Silva, James Woodward **NRI** David Hall, Dan Bray, Steven Harte, Dudley Farman **Thanet Earth** Robert James
Location of project:	NIAB EMR Natural Resources Institute, University of Greenwich
Industry Representative:	Robert James, Thanet Earth
Date project commenced:	1 November 2019

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The results and conclusions in this report are based on an investigation conducted over a 4-four-month 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.

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.

Michelle Fountain

Deputy Head of the Pest & Pathogen Ecology Department

NIAB EMR

Signature .....MT Fountain ..... Date 24 Feb 2020

[Name]

[Position]

[Organisation]

Signature ..... Date .....

Report authorised by:

[Name]

[Position]

[Organisation]

Signature ..... Date .....

[Name]

[Position]

[Organisation]

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GROWER SUMMARY

Headline

Two components of the female sex pheromone of the mirid pest of tomatoes, *Nesidiocoris tenuis,* have been identified and traps baited with the synthetic pheromone have been shown to be highly attractive to males in commercial glasshouses.

Background

Nesidiocoris tenuis (Reuter) (Heteroptera: Miridae) is a tropical mirid bug. Both adults and nymphs are predators and can make a useful contribution to integrated pest management in tomato crops, but, in the absence of insect or mite prey, the bug will turn its attention to the tomato plants and can cause serious economic damage. In southern European countries it is used as a biocontrol agent in protected crops, including tomatoes, e.g. Nesidiocoris-System from Biobest and Nesibug from Koppert. It is not approved for use in the UK, but the closely related *Macrolophus pygmaeus* (Rambur) (formerly *M. caliginosus* Wagner) is widely used. Although *N. tenuis* predates important insect pests, especially whitefly and larvae of *Tuta absoluta*, it also causes damage by feeding on tomato plants (Sanchez 2008), particularly when prey populations decline. Feeding causes necrotic rings on stems and leaf petioles, flower abortion and punctures on fruits (Calvo et al. 2009). *Nesidiocoris tenuis* causes economic losses due to reductions in yield through feeding on trusses (Arnó et al 2009), as well as increased costs for additional crop protection products, labour to spot and manage the pests and cleaning out glasshouses between cropping. *Nesidiocoris tenuis* has already become established in glasshouses growing all-year-round tomato crops in some northern European countries (Jacobson, 2019) and is threatening the 180 ha of tomatoes grown in the UK. Control requires application of pesticides incompatible with IPM programmes, leading to resurgence of whitefly populations and associated viruses, and disruption of other biocontrol systems such as use of predatory mites against spider mites.

This project aimed to identify, synthesise and field test the sex pheromone of *N. tenuis.* This will pave the way for development of pheromone traps for better monitoring of pest numbers and more efficient use of control agents. The pheromone could potentially also be used for control of *N. tenuis* by mass trapping or mating disruption and hence reduce or avoid the use of conventional pesticides against this pest on protected tomato crops in the UK.

Summary

In this project, *Nesidiocoris tenuis* was reared through several generations in the laboratory to provide mated and unmated adults for further work. Volatiles were collected from groups

and individuals of mated and unmated females and males separately. Whole-body extracts were also made. In analyses of volatiles from females by gas chromatography (GC) coupled to electroantennographic (EAG) recording from the antennae of male *N. tenuis*, two components were detected that elicited consistent EAG responses. These were identified as 1-octanol and octyl hexanoate following analyses by GC coupled to mass spectrometry (MS), and the synthesised compounds had identical GC retention times and mass spectra to the natural compounds. These compounds were thus candidate components of the female sex pheromone. An analogue of one of the pheromone components was synthesised and this elicited an EAG response from the antennae of male *N. tenuis.* This compound is a potential pheromone inhibitor or even repellent that may have applications in control of this pest.

Polyethylene vials were shown to be suitable controlled release dispensers for the pheromone components. The release rate could be modified by dilution in sunflower oil and release continued for over two months under laboratory conditions. Several different laboratory bioassays were trialled, but none showed conclusive attraction of *N. tenuis* males to natural or synthetic sources of pheromone. However, in two experiments carried out in commercial glasshouses, traps baited with a blend of the synthetic pheromone components caught large numbers of male *N. tenuis*. Traps baited with the blend caught significantly more males than traps baited with one component, although the latter caught significantly more than unbaited traps, which caught very few bugs. Reducing the release rate of the blend ten-fold reduced catches, but not significantly so. There was some indication that traps at plant height caught more than traps below plant height or significantly above. The trap catches were thought to provide a good indication of population levels of *N. tenuis* and were greatly reduced following an application of insecticide.

Although good progress has been made in this short project, further research is required to optimise the blend of pheromone components, the release rate of dispensers, trap design and trap positioning, and then to correlate catches in pheromone traps with population levels and to develop thresholds for intervention. Use of the pheromone components and the potential inhibitor/repellent in control of *N. tenuis* should also be investigated.

Financial Benefits

If *Nesidiocoris tenuis* became established in UK glasshouses, then additional costs to avoid crop losses estimated by Rob James (Thanet Earth) could be for additional crop protection products (£30k/ha), labour to spot and manage the pests (£10k/ha) and cleaning out glasshouses between cropping (£14k/ha), totalling just over £9.7M per crop cycle for the 180 ha of tomatoes grown in the UK. Moreover, control in other European countries has required

application of pesticides incompatible with IPM programmes or organic programmes, leading to resurgence of whitefly populations and associated viruses, and disruption of other biocontrol systems such as use of predatory mites against spider mites (Jacobson, 2019).

Pheromone traps should provide a means of detecting infestations of *N. tenuis* more accurately and cost-effectively in order to minimise unnecessary applications of conventional pesticides, thereby reducing costs and disruption of IPM programmes. The pheromone could also be used for control of the pest by mass trapping or mating disruption, which are compatible with IPM programmes. A candidate inhibitor or repellent was also identified during this project and this may also have application in control of this pest.

Action Points

- Two components of the female sex pheromone of *Nesidiocoris tenuis* have been identified, and traps baited with a blend of the two components have been shown to catch large numbers of *N. tenuis* adult males in commercial greenhouses. Traps and lures are now available for further evaluation by growers and researchers.

SCIENCE SECTION

Introduction

Nesidiocoris tenuis (Reuter) (Heteroptera: Miridae) is an omnivorous mirid bug, used as a biological control agent in southern European countries, for vegetable crops, and is found predominantly in the Mediterranean region (Biondi et al., 2016). It is not approved for use in the UK, but the closely related *Macrolophus pygmaeus* (Rambur) (formerly *M. caliginosus* Wagner) is widely used. *N. tenuis* is deployed to predate on a range of economically important crop pests such as whiteflies (family Aleyrodidae), aphids (superfamily Aphidoidea), thrips (order Thysanoptera) and moths (order Lepidoptera) (Kim et al., 2016).

However, the use of *N. tenuis* as a biological control agent is controversial. Although this bug prefers prey, it can start feeding on the crop when prey is absent (Sanchez 2008; Nakaishi et al. 2011; Pérez-Hedo and Urbaneja, 2016). It does this by inserting its stylet into the phloem to derive nutrients, which can lead to brown necrotic rings around stems and petioles followed by the drying of flower stalks and flower abortion (Raman and Sanjayan, 1948; Calvo et al. 2009; Arnó et al 2009, Castañé et al., 2011). In some northern European countries, *N. tenuis* has become invasive in glasshouses (AHDB, 2019). Although not native to the UK, *N. tenuis* is threatening the 180 ha of tomatoes grown in the UK, at an estimated cost to the industry of over £9.7M p a. including economic losses, costs for additional crop protection products, labour to spot and manage the pests and cleaning out glasshouses between cropping (pers. Comm. Robert James).

In circumstances where *N. tenuis* needs controlling, traditional control options might not be suitable. Although the bug cannot complete development to adulthood if feeding exclusively on tomato plants, *Solanum lycopersicum* (Urbaneja et al., 2005), growers may resort to the use of chemical plant protection products (PPP's) incompatible with IPM programmes to prevent further crop damage. This can lead to a resurgence of whitefly populations and associated viruses, and disruption of other biocontrol systems such as use of predatory mites against spider mites. Moreover, a number of PPP's have been shown to have lethal and sublethal effects on pollinators (Feltham et al. 2014; Cloyd, 2012), and it is recommended that their use is avoided unless necessary (PAN Europe, 2013).

Semiochemicals offer an IPM-compatible control option for *N. tenuis*. *Nesidocoris tenuis* belongs to the Bryocorinae sub-family of mirids, and female-produced sex pheromones have been identified in several species of this sub-family by NRI. These include the cocoa mirids *Distantiella theobroma* Dist. and *Sahlbergella singularis* Haglund (Mahob et al. 2011; Sarfo 2013; Sarfo et al. 2018a, 2018b; Mahot et al. 2020), and the aphidophagous mirid,

Macrolophus caliginosus Wagner (Gemeno et al. 2006; see also Castañé et al. 2007; Gemeno et al. 2007, 2012, 2015). The components of these pheromones are derivatives of the hexyl ester of 3-hydroxybutyric acid, and rather different from those identified in mirid species from other subfamilies. For cocoa mirids, the synthetic pheromone has been used for monitoring (Mahob et al. 2011; Sarfo 2013; Sarfo et al. 2018a; Mahot et al. 2020) and mass trapping (Sarfo et al. 2018b).

The aim of this project was to identify the female-produced sex pheromone of *N. tenuis* and demonstrate attraction of male *N. tenuis* to the synthesised sex pheromone in commercial glasshouses. This will pave the way for development of pheromone traps for better monitoring of pest numbers and more efficient use of control agents. The pheromone could also be used for control of *N. tenuis* by mass trapping or mating disruption and hence reduce or avoid the use of conventional pesticides against this pest on protected tomato crops in the UK.

Materials and Methods

WP1. Rearing of *Nesidiocoris tenuis* and collection of pheromone (NIAB EMR)

Preparing Host Plants

Young tobacco (*Nicotiana* spp.) and aubergine (*Solanum melongena*) plants were grown and provided by colleagues at NRI. Tomato (*Solanum lycopersicum*) and cucumber (*Cucumis sativus*) were grown at NIAB EMR. Plants were sown separately in 11 x 11 x 11 cm pots containing standard compost and kept in a glasshouse at 26±3 °C and 16:8 h (light:dark) photoperiod. Dwarf organic beans, used in bioassays and to rear virgin adult cultures, were obtained from Sainsbury's.

Culturing Nesidiocoris tenuis

Nesidiocoris tenuis adults were purchased from Bioline AgroSciences Ltd (Nesiline bottle - 500 individuals) and a culture established in the quarantine facility at NIAB EMR. Insects were reared in BugDorms (50x50x50 cm) (MegaView Science, Taichung, Taiwan) on tobacco, aubergine, cucumber or tomato plants supplemented with sterile *Ephestia kehniella* eggs (Nutrimac, Biobest) applied to the plant every Monday and Friday, ~1 g of pollen per month and 5 ml of sugar solution (5% dextrose) per week. Cultures were maintained at 20 to 26°C and 55 to 65% relative humidity 16:8 h (light:dark) photoperiod. BugDorms floors were lined with a layer of damp tissue to maintain humidity.

Rearing Virgin Nesidocoris tenuis

Third to fifth instar nymphs were collected from BugDorm cultures using a handheld vacuum and placed individually into separate ventilated Perspex rearing boxes (8 x 6 x 14 cm). Nymphs were reared on sterile *E. kehniella* eggs (~0.5 g), a dwarf organic bean and 2.5 ml of sugar solution (5% dextrose) under the same conditions as BugDorm cultures. Virgin adults were kept in the rearing boxes until use in bioassays. Live adults were sexed by chilling at 4°C for 1 minute and then identifying the obvious ovipositor on female bugs. Killed adults (sticky traps – see later) were sexed by dissecting open the posterior abdomen and identifying the paramere in the males and ovipositor in the females (Kim et al. 2016).

Volatile collection

Volatiles were collected in the quarantine facility at NIAB EMR. Virgin males and females were placed in separate entrainment chambers (12 cm x 4 cm) containing a dwarf organic bean for sustenance (Fig. 1). Chambers containing dwarf organic beans without adults were used as controls. The airflow pump supplying the entrainment apparatus was set to 200 ml/min and air was filtered through an activated charcoal column at the entry point. Insects were left to settle in the chambers for at least 30 minutes prior to volatile collection. A Pasteur pipette (4 mm i.d.) containing Porapak Q (50-80 mesh; 200 mg; Supelco, Gillingham, Dorset, UK) held between silanised glass wool plugs was placed at the downwind end of each chamber to collect volatiles. The Porapak was purified by Soxhlet extraction with chloroform and washing with dichloromethane before use. Volatiles were collected for 24 or 48 h, after which the Porapak filters were wrapped in aluminium foil and stored in a fridge before transfer to NRI.

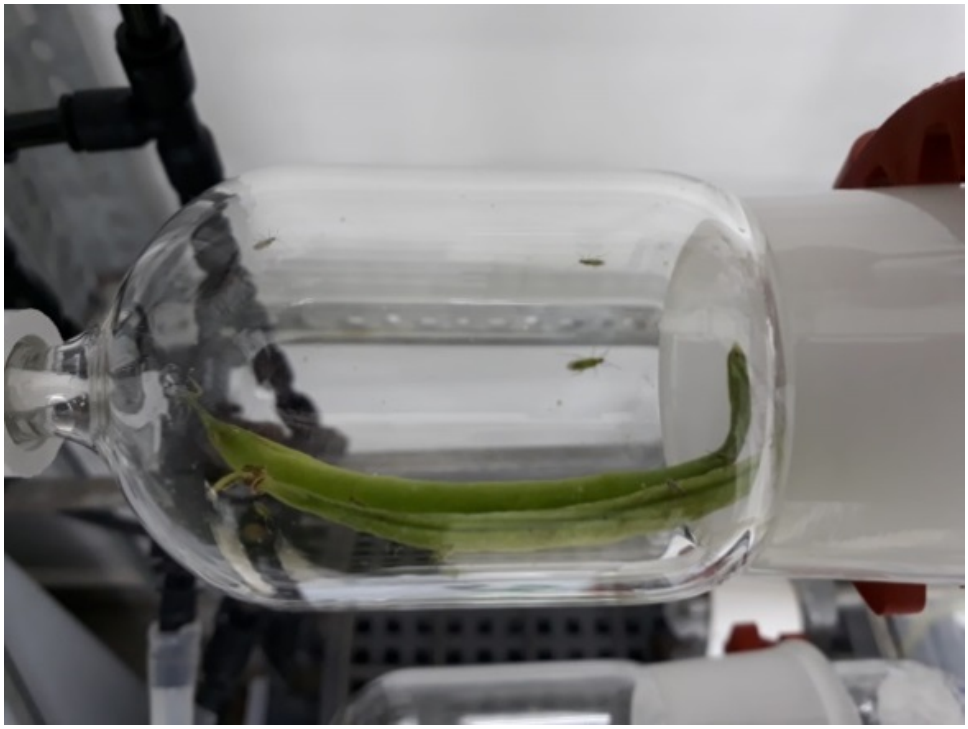

Fig. 1. Entrainment chamber containing adult *Nesidiocoris tenuis* (males or females) and organic dwarf beans.

Volatiles were first collected (entrainment) from groups of 20 adult females, 20 adult males, and a control with no *N. tenuis*. These entrainments were done once with mated adults and twice with virgin adults. Subsequently, volatile samples were also collected from isolated, individual, virgin adults. In the latter, 6 chambers were used; 3 with 1 virgin female and organic bean, 2 with 1 virgin male and organic bean and 1 control with organic bean only. This was repeated three times.

Whole-body Extraction

Whole-body extracts were made at NRI by immersing individual *N. tenuis* adults in diethyl ether (0.5 ml; SLR grade containing BHT antioxidant) for 15 min, then removing the ether, drying with a few grains of anhydrous magnesium sulphate and storing at 4°C until analysis. Diethyl ether was used because potential compounds of interest such as (*E*)-4-oxo-2-hexenal are poorly soluble in hexane and are relatively stable in diethyl ether containing BHT (Fountain et al., 2014).

WP2. Pheromone identification and synthesis (NRI)

Analysis of Pheromone Collections

Collections of volatiles were eluted from the Porapak with dichloromethane (1 ml; Pesticide Residue Grade). Extracts were concentrated to approx. 100 µl and stored at 4°C before analysis. Samples were analysed quantitatively by GC-FID using external standards to avoid contaminating them.

Whole body extracts were analysed quantitatively by GC-FID after addition of an internal standard of decyl acetate (5 µg).

Analysis by Gas Chromatography Coupled to Electroantennographic Recording (GC-EAG)

GC-EAG Analyses were carried out on a HP6890 GC (Agilent Technologies) fitted with flame ionization detector (FID) and fused silica capillary columns (30 m x 0.32 mm i.d. x 0.25 µm film thickness) coated with DBWax and DB5 (Supelco). Injections onto the DBWax column were in splitless mode (220 °C), carrier gas was helium (2.4 ml/min) and the oven temperature was programmed from 50 °C for 2 minutes and then at 20 °C/min. to 250 °C for 3 min. The effluents of the two columns were combined with a glass push-fit Y-tube connector (Agilent Technologies) connected to a second Y-tube connector with deactivated fused silica tubing (10 cm x 0.32 mm i.d.). One arm of this connector was connected with fused silica tubing (50 cm x 0.32 mm i.d.) to the FID (250 ˚C) and the other to an equal length of deactivated silica tubing passing through a heated transfer line (250°C; Syntech, Hilversum, The Netherlands, now Kirchzarten, Germany) into a glass tube (4 mm i.d.) through which air passed (500 ml/min) over the EAG preparation. Both the FID and EAG signals were collected and analysed with EZChrom software (Elite v3.0; Agilent Technologies).

For EAG recordings, adult *N. tenuis* were anesthetised using carbon dioxide, and the head and one antenna removed under a dissecting microscope with a razor blade. A borosilicate glass capillary electrode (ID 0.86mm, Warner instruments), pulled to a fine tip and filled with Beadle-Ephrussi Ringer containing 1% polyvinylpyrrolidine as electrolyte, was inserted into the back of the head. The electrode and head were then mounted onto a silver wire held within an electrode holder connected to the earth probe of a portable EAG amplifier (INR-2, Syntech, formerly Hilversum, The Netherlands, now Kirchzarten, Germany). A similar electrode mounted onto the x10 recording preamplifier was then brought into contact with the distal tip of the antenna (Fig. 2). Straightening the antenna between the electrodes reduced background noise from the preparation.

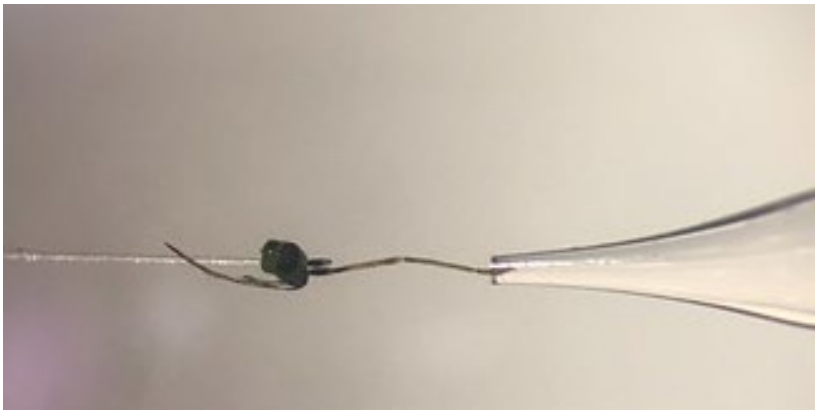

Fig. 2. GC-EAG preparation of male *Nesidiocoris tenuis*

Analysis by Gas Chromatography Coupled to Mass Spectrometric Recording (GC-MS)

GC-MS Analyses were carried out on a CP3500 GC coupled to a CP2200 Saturn Ion Trap Detector (Varian, now Agilent Technologies, Cheadle, UK). The GC was fitted with fused silica capillary columns (30 mm x 0.25 mm i.d. x 0.25 µm film thickness) coated with DBWax (Supelco) and VF5 (Varian) with a switching device to select the column used. Carrier gas was helium (1 ml/min) and manual injections (1 µl) were made in splitless mode (220 °C), with oven temperature programmed from 40°C for 2 min then at 10°C/min to 240°C. Compounds were identified according to their mass spectrum, retention index relative to retention times of *n*-alkanes, and co-chromatography with authentic compounds.

Analysis by Gas Chromatography with Flame Ionisation Detection (GC-FID)

GC-FID Analyses were carried out on HP6850 instruments (Agilent) fitted with fused silica capillary columns (30 m x 0.32 mm i.d. x 0.25 µm film thickness) coated with polar DBWax (Supelco) and non-polar HP5 (Agilent) and FID detectors (250°C). Carrier gas was helium (2.4 ml/min), injection was splitless (220°C) and the oven temperature was programmed from 50°C for 2 min then at 10°C/min to 250°C. Data were collected and analysed with EZChrom software (Elite v3.0; Agilent).

Chemicals

Unless otherwise stated, chemicals were purchased from SigmaAldrich (Gillingham, Dorset, UK) and were at least 99% pure.

Octyl hexanoate was synthesised by reaction of 1-octanol with hexanoic acid in dichloromethane in the presence of N,N'-dicyclohexylcarbodiimide and a catalytic amount of 4-N,N-dimethylaminopyridine (Neises and Steglich 1978). The product was purified by flash chromatography on silica gel eluted with 2% diethyl ether in petroleum spirit (bp 40-60°C) followed by Kugelrohr distillation (100°C/0.06 mm Hg) in 90% yield.

Hexyl octanoate was prepared similarly by reaction of 1-hexanol and octanoic acid in 93% yield. Both compounds were characterised by their mass spectra and ^{1}H and ^{13}C NMR spectra.

Controlled Release Dispensers

Two types of controlled-release dispenser for the pheromone components were investigated (Fig. 3). The first were opaque, polypropylene pipette tips (1 ml; Fisher Scientific) with a 0.2 mm aperture, sealed with a Teflon-lined crimp seal (11 mm; Chromacol, Welwyn Garden City, UK) as developed for the pheromone of *Lygus* bugs by Fountain et al. (2014). The second were sealed low-density polyethylene vials (22 mm x 8 mm x 1.5 mm thick; Just Plastics, London, UK).

A mixture of equal weights of 1-octanol and octyl hexanoate was formulated as the neat material (25 µl) or a 10% solution in sunflower oil (100 µl) and applied to cellulose acetate cigarette filters (14 mm x 6 mm; Swan) in the dispensers.

Fig. 3. Controlled release pheromone dispensers: polyethylene vials (left) and pipette tips (right)

Two vials for each type of dispenser and loading were maintained in a windtunnel (27°C and 8 km/h windspeed) and release rates were measured by periodic trapping of volatiles on Porapak followed by quantitative GC-FID analysis using decyl acetate (5µg) as internal standard, as described for collection of pheromone from insects.

WP3. Bioassay of pheromone (NIAB EMR/NRI/Thanet Earth)

Attraction of Male Nesidiocoris tenuis *to Virgin Females with Airflow in Laboratory Wind Tunnel*

A large wind tunnel (60 cm x 60 cm x 180 cm) was used with two tea infusers in neighbouring corners of the upwind end (Fig. 4). One infuser contained virgin females and a section of dwarf organic bean, the other a section of dwarf organic bean only (control). The floor of the tunnel was covered with tissue kept moist throughout the experiment using a water spray bottle. White sticky traps (40 cm x 20 cm) were placed below or behind the tea infusers. A sugar feeder (5% dextrose) was placed at the male release point for food, and a temperature data logger was secured to a clamp stand. The wind tunnel was subjected to the same L:D 16:8 h photoperiod as the cultures. Males were released at the downwind end of the wind tunnel (opposite end to tea infusers). The number of males on each sticky trap was recorded at each assessment time.

Variations of this experiment were tested to determine the ideal conditions to assess the responses of males to female pheromone. Details of each variation are described in Table 1. Experiment 3 was repeated by switching the infuser containing females from one neighbouring corner to the other to account for any factors that may attract insects in the tunnel (e.g. lighting). In this variation we replaced the sugar feeder with an organic bean and used smaller white sticky traps (18 cm x 18) cm perforated at the infuser level to allow airflow.

Table 1. Details of variations of the wind tunnel experiments to test male attraction of male *Nesidiocoris tenuis* to virgin females with airflow conditions. All experiments were performed in a wind tunnel with variable airflow and white sticky traps.

Expt	*N*	Trap position	No. Insects		Wind speed (m/s)	Assessments (h after release)
			Females	Males		
1 (Fig. 4a)	1	Beneath infusers	1 virgin	20 mated	~0.3	0, 6, 24, 30, 48, 54
2	1	Beneath infusers	4 virgin	30 mated	~0.18	0, 6, 24, 30
3 (Fig. 4b)	2	Vertically behind infusers	4 virgin	30 mated	~0.22	0, 6, 24, 30, 48

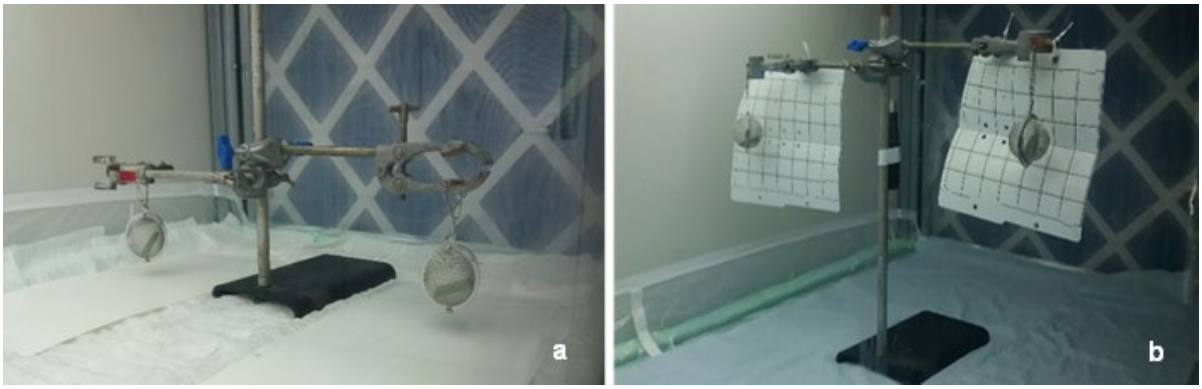

Fig. 4. Set up of wind tunnel experiments: (a) variation 1 - white sticky traps beneath tea infusers (upwind); (b) variation 3 - sticky traps behind tea infusers (upwind)

Attraction of Male Nesidiocoris tenuis *to Females in Still-air Conditions in Laboratory*

Experiment 4

Using a large BugDorm (50 cm x 50 cm x 100 cm), two tea infusers each containing 4 mated females and a section of dwarf bean were placed on top of a white sticky trap (18 cm x 18 cm) on one end of the BugDorm floor. At the other end, two infusers each containing a section of dwarf bean only, were placed on top of another white sticky trap (Fig. 5). Damp tissue was placed under the sticky traps to maintain moisture. Two dwarf organic beans were placed in between the sticky traps as a food source. The BugDorm was kept in the culture room under a photoperiod of L:D 16:8 h, then 20 mated males were released in the centre. The number of males on each sticky trap was recorded at 0, 18, 24, 42, and 48 h after release.

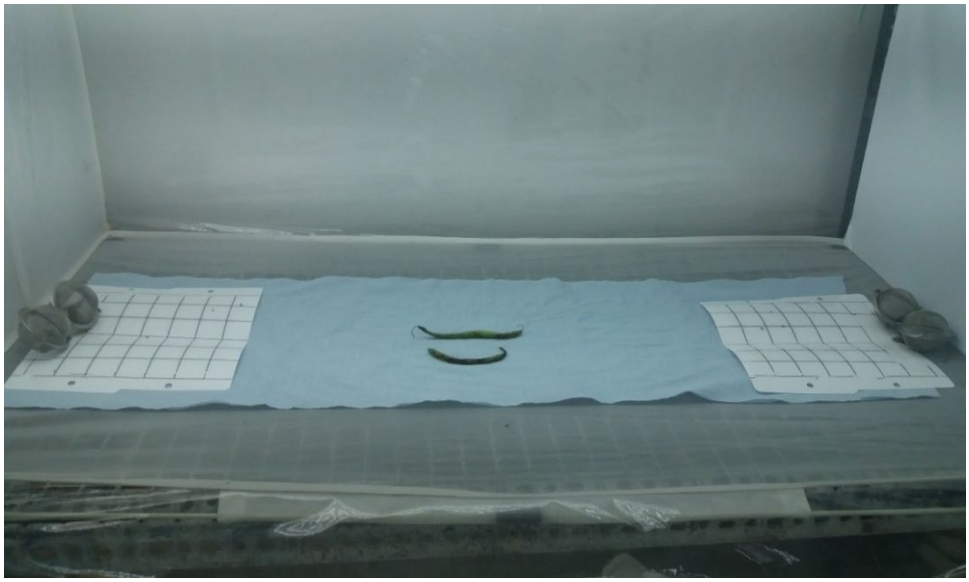

Fig. 5. Larger BugDorm setup testing attraction of *Nesidiocoris tenuis* males to mated females in two tea infusers at one end, compared to a control consisting of two tea infusers without females at the other end of the cage. Males were released in the centre of the cage where two dwarf organic beans are positioned

Experiment 5

A no choice setup was tested with smaller BugDorms (32.5 cm x 32.5 cm x 32.5 cm). Three replicate treatment BugDorms were set up, each with a tea infuser containing 3 virgin females and section of dwarf organic bean, hanging in front of a blue sticky trap (25 cm x 10 cm; Russell IPM) (Fig. 6). Three replicate control cages were also set up, each with a tea infuser containing a section of dwarf organic bean only hanging in front of a blue sticky trap. BugDorms were kept moist using dampened tissue. Eight *N. tenuis* mated males were released into each BugDorm. The number of males on each sticky trap was recorded every 30 min for 6 h.

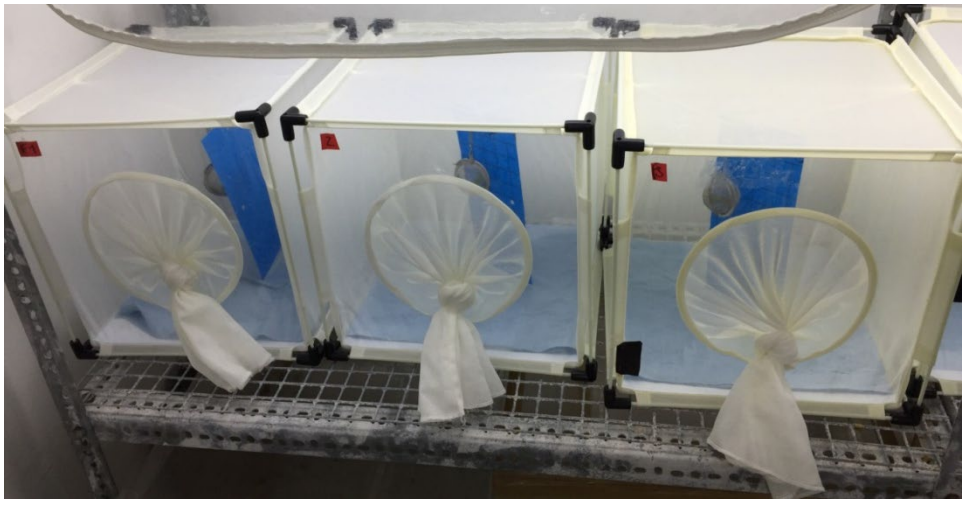

Fig. 6. Small BugDorm setup for the no choice experiment testing attraction of *Nesidiocoris tenuis* mated males to virgin females. Blue sticky traps were hung behind tea infusers to catch males. Eight mated males were released into each BugDorm

Experiment 6

In a large wind tunnel with no airflow, yellow sticky traps (25 cm x 10 cm) were hung behind tea infusers. An infuser containing 5 virgin females and section of dwarf organic bean was placed at one end of the tunnel and another infuser with only a section of dwarf organic bean at the opposite end (Fig. 7).

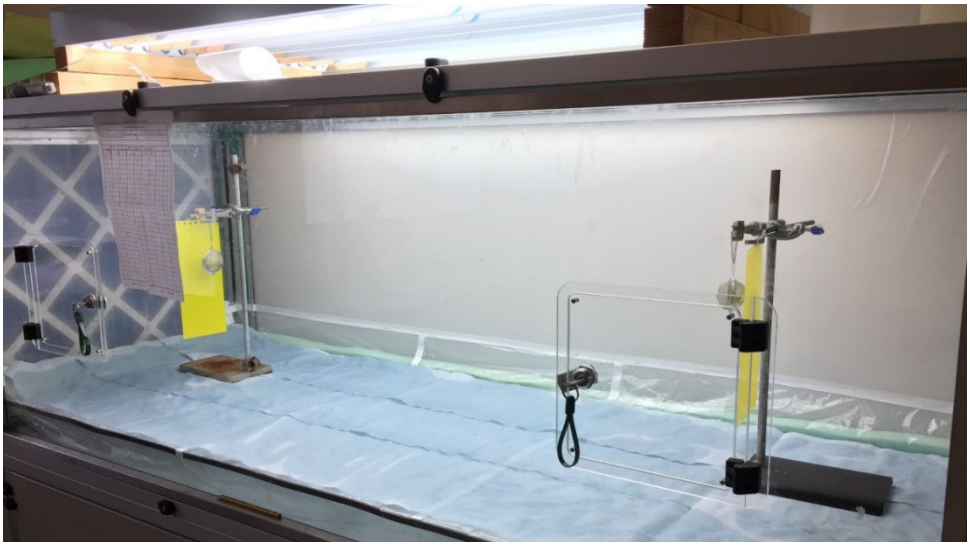

Fig. 7. Larger wind tunnel setup testing attraction of mated *Nesidiocoris tenuis* males to virgin females under still air conditions. At one end, a tea infuser containing five virgin females with dwarf organic bean, at the other end a control consisting of tea infuser containing dwarf organic bean only. Yellow sticky traps were hung behind each tea infuser to catch males. Thirty mated males were released in the wind tunnel centre

Thirty mated males were released in between the sticky traps. The floor of the tunnel was covered with tissue and kept moist throughout the experiment with a water spray bottle. No food was provided to the males as the experiment only lasted for 6 h. The number of males on each sticky trap was recorded every 30 minutes for a period of 6 h.

Experiment 7

The same setup as in experiment 6 was used in a smaller wind tunnel (31 cm x 31 cm x 90 cm) (Fig. 8). The only variation being the release of 20 virgin males instead of 30 mated males.

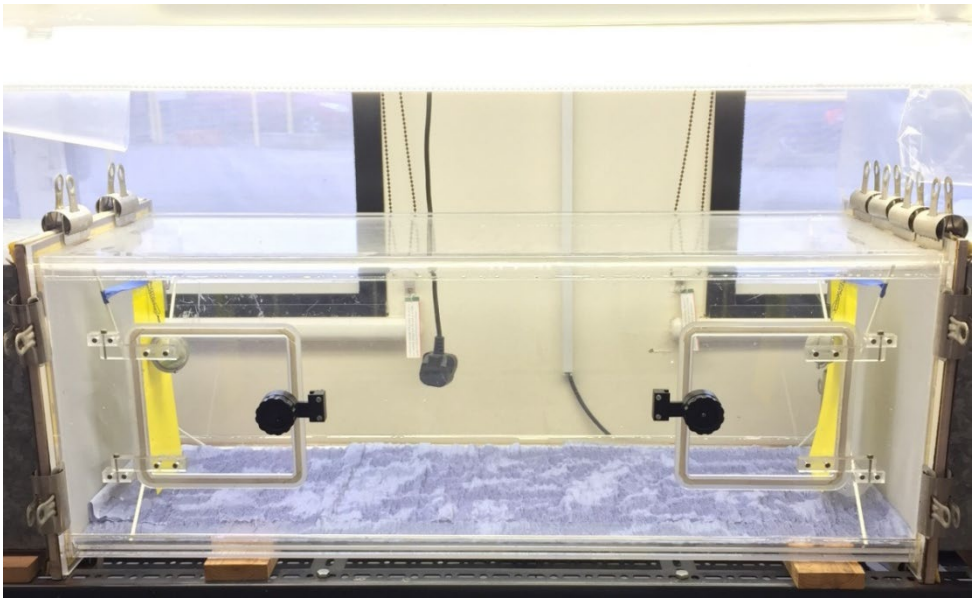

Fig. 8. Smaller wind tunnel setup testing attraction of virgin *Nesidiocoris tenuis* males to virgin females under still air conditions. At one end a tea infuser containing 5 virgin females with dwarf organic bean, at the other end a control consisting of tea infuser containing dwarf organic bean only. Yellow sticky traps were hung behind each tea infuser to catch males. Twenty virgin males were released in the wind tunnel centre

Laboratory Bioassay of Synthetic Sex Pheromone

BugDorms (50 cm x 50 cm x 100 cm) were used with a blue sticky trap (25 cm x 10 cm) fastened to an inside wall (Fig. 9). In one BugDorm the sticky trap was baited with a polyethylene vial containing the two-component blend of 1-octanol and octyl hexanoate (25 µl) stuck to the centre. In the other, a blank vial was stuck to the trap. Eight *N. tenuis* mated males were released into each BugDorm. The floor was covered with damp tissue and a plastic sheet was placed on top of the BugDorm to maintain humidity, temperature was 22 ±1°C, relative humidity 75 ±5% and photoperiod of L:D 16:8 h. Subsequently the number of males on each sticky trap was recorded every hour after release for 5 h. This experiment was repeated five times.

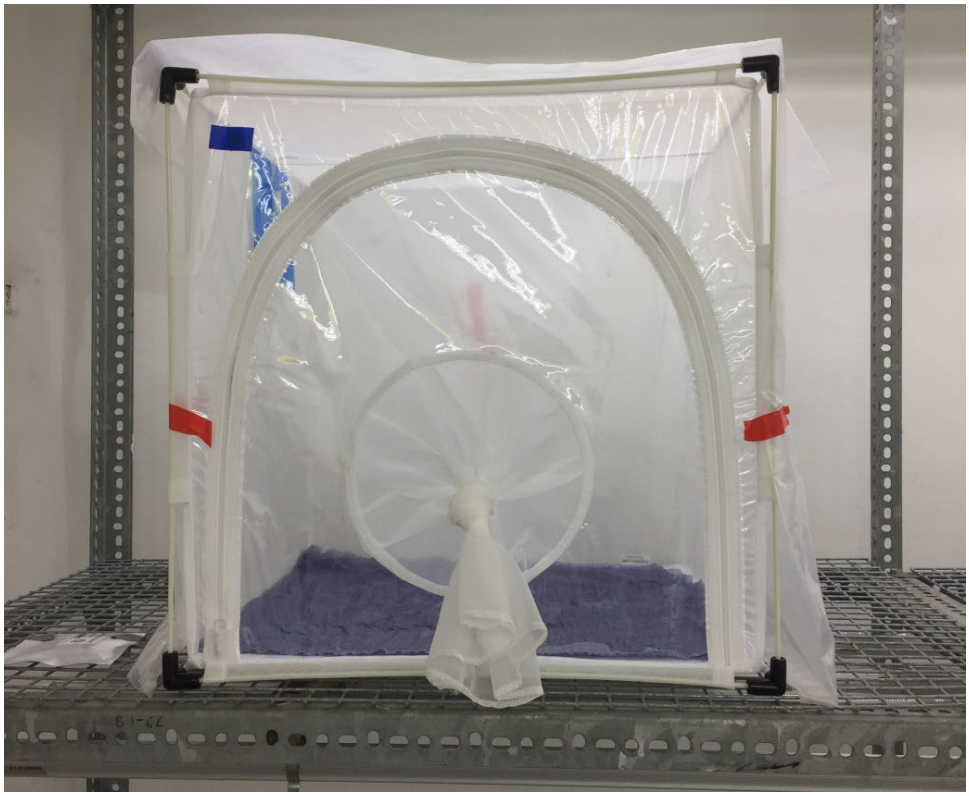

Fig. 9. Bioassay of synthetic sex pheromone, consisting of BugDorm containing a baited or unbaited blue sticky trap (Russell IPM) fastened to inside wall to catch males. Eight *N. tenuis* mated males were released into each BugDorm

Field Trials in Commercial Glasshouses

Two field trapping experiments were carried out on tomato in commercial glasshouses in Kent. Varieties included Piccolo, Summer Sun and Sun Stream. Traps were yellow “Drystick” (dry glue) sticky traps (25 cm x 10 cm; Koppert) with the lure attached to the centre by a twist-tie (Fig. 10).

In the first trial (12 December 2019 – 09 January 2020) there were 10 replicates of three treatments in polyethylene vials: (A) a 1:1 blend of neat 1-octanol and octyl hexanoate (25 µl); (B) neat octyl hexanoate only (12.5 µl); (C) blank control consisting of an empty vial with cigarette filter. In eight blocks traps were positioned at a height of 6 m, above the crop canopy, and in the other 2 blocks traps were positioned at 1 m, within the crop canopy. Traps were spaced >10 m apart to minimize interference between treatments. Initially white sticky traps were used with standard polybutene glue, but laboratory and field studies indicated the bugs could escape from this, and these traps were replaced with the yellow dry glue traps on 19 December 2019. The glasshouse was treated with insecticide on 4 January 2020. Trap catches were recorded weekly on 27 December 2019, 3 January and 9 January 2020.

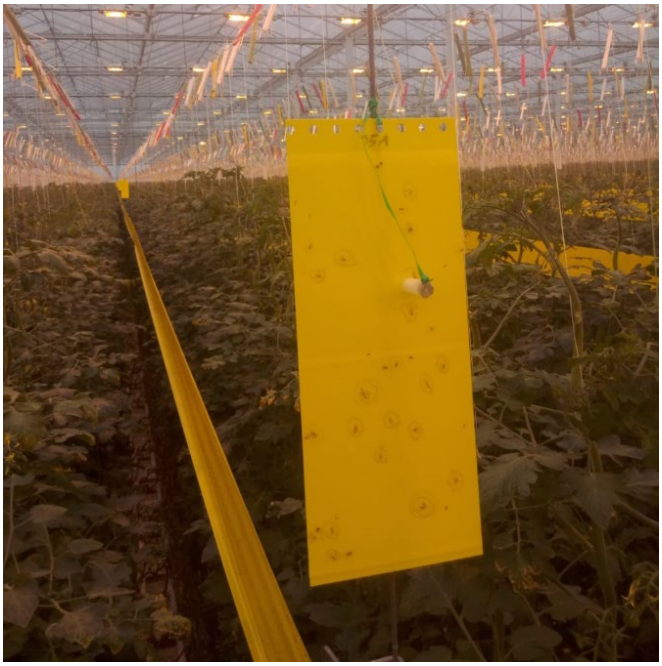

Fig. 10. Yellow “Drystick” trap positioned above the crop canopy with the lure secured to the centre by twist tie

In the second experiment (10 January – 30 January 2020), the three treatments were (A) and (C) as above, and treatment (D) containing a 1:1 blend of 1-octanol and octyl hexanoate as a 10% solution in sunflower oil, giving an approximately 10-fold reduction in release rate. There were 15 replicates with 8 blocks positioned immediately above the crop canopy, 4 blocks 1 m above the crop canopy and 4 blocks just above plant base. Catches were recorded on 18, 25 and 30 January 2020.

The same sticky trap was used throughout each experiment. *N. tenuis* captured were counted and sexed on the trap at Thanet Earth and circled with a marker pen to differentiate them for new catches the following week. Sticky traps were collected from the glasshouse at the end of experiment and sex was confirmed under a microscope (Leica M165C) at NIAB EMR according to Kim et al. (2016).

For each experiment, mean catches in each replicate over the whole experimental period were transformed to log(x+1) to normalise the variance and then subjected to analysis of variance. Where the *F* value indicated significant differences, differences between means were tested for significance by the Least Significant Difference (LSD) test at $P < 0.05$.

Results

WP1. Rearing of *Nesidiocoris tenuis* and collection of pheromone (NIAB EMR)

Adults of *Nesidiocoris tenuis* were purchased and successfully reared through several generations at NIAB EMR. Virgin female and male adults were obtained by isolating individual third-fifth instar nymphs and rearing these through to adults.

A total of 31 collections of volatiles was made from *N. tenuis* adults, including collections from groups (5-20) and individuals of mated and unmated males and females. In addition, 7 blank collections from the food source (aubergine leaf or green bean) with no insects were also made.

Whole-body extracts were made in diethyl ether of individual virgin females (2), virgin males (2), mated females (9) and mated males (8).

WP2. Pheromone identification and synthesis (NRI)

Pheromone Identification

Initial GC-EAG analyses of volatile collections from mated female *N. tenuis* showed two peaks (A and B, Fig.11A) eliciting consistent EAG responses from antennae of mated male *N. tenuis*. The same result was obtained in analyses of volatiles from a single unmated female with antennae of unmated males (Fig. 11B).

In analyses of these collections by GC-MS, the two EAG-active components (A) and (B) were present in volatiles from both mated and unmated female *N. tenuis* and in volatiles from both mated and unmated males, but not detectable in volatiles from aubergine leaves or green beans used as food source during the volatile collections (Fig. 12). The mass spectra indicated these two peaks corresponded to 1-octanol (I) and octyl hexanoate (II) respectively (structures in Fig. 13), and the corresponding synthetic compounds had identical mass spectra and retention times on both polar and non-polar GC columns (Fig. 14 and Table 2).

Detailed examination of the GC-MS traces failed to detect the pheromone components identified in closely related species: hexyl 3-hydroxybutyrate and hexyl 3-[(*E*)-2-butenoyl]-butyrate from cocoa mirids, or hexyl 3-acetoxybutanoate, the proposed pheromone of *Macrolophus caliginosus* (Table 2).

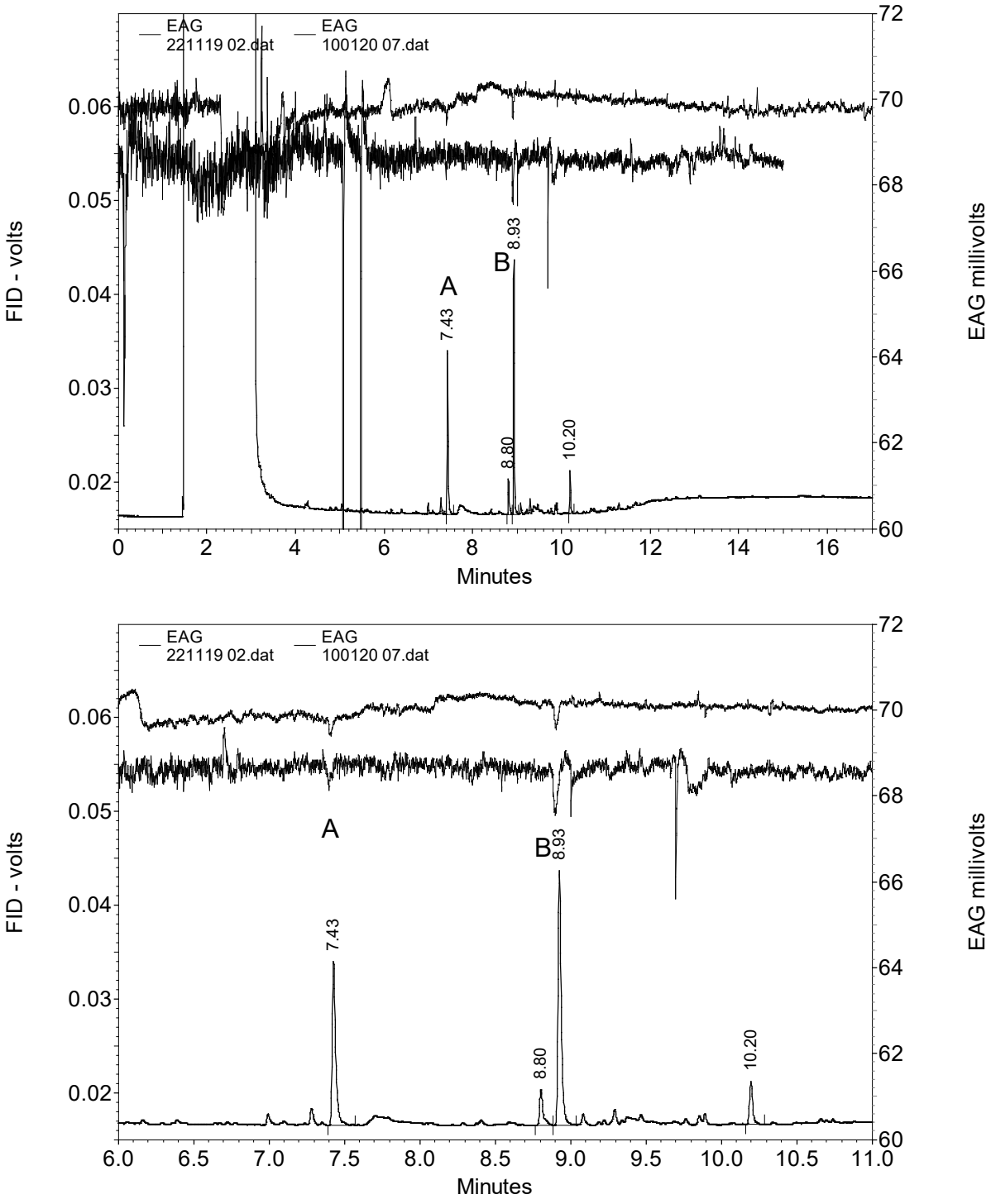

Fig. 11. GC-EAG Analyses of volatile collection from virgin female *Nesidiocoris tenuis* with male antennal EAG preparation on polar GC column showing EAG responses to components (A) at 7.43 min and (B) at 8.93 min; lower trace is expansion of upper; in each, upper EAG trace is responses to volatiles from mated females, lower is responses to volatiles from unmated females (other compounds 7.00 min 2-ethylhexanol; 7.28 min benzaldehyde; 8.80 min methyl salicylate; 9.30 and 10.20 min Porapak impurities)

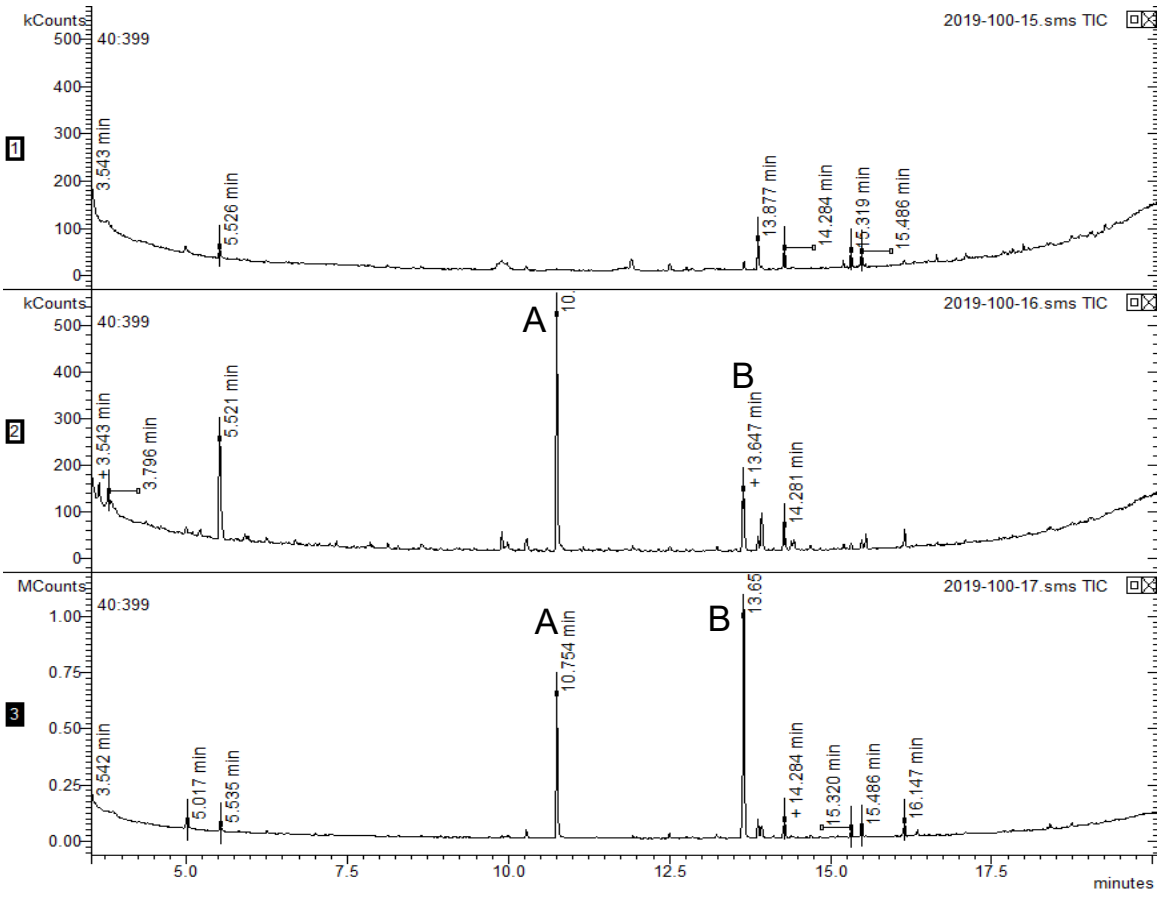

Fig. 12. GC-MS analyses on polar GC column of volatile collections from green bean (upper), single virgin male *Nesidiocoris tenuis* (middle), and single virgin female *N. tenuis*, showing peaks A and B corresponding to EAG responses

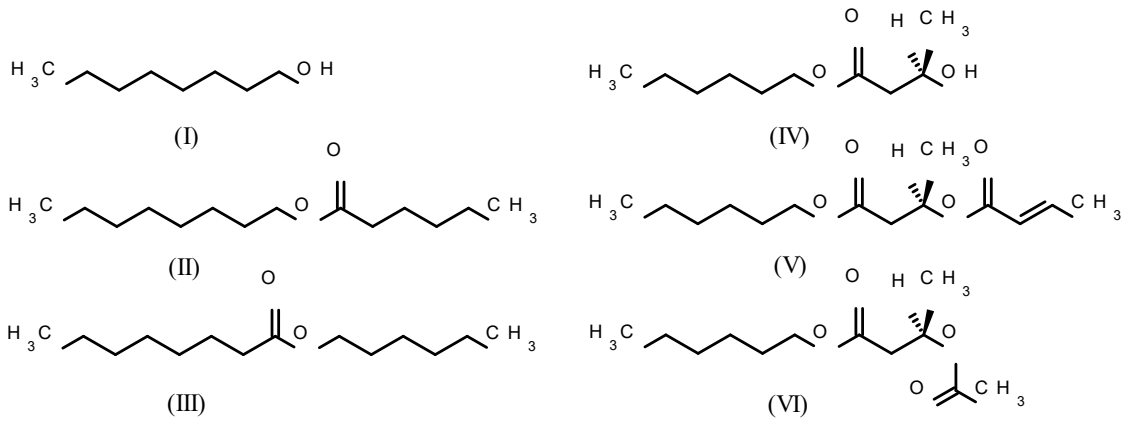

Fig. 13. Structures of pheromone components and analogues: (I) 1-octanol (component A); (II) octyl hexanoate (component B); (III) hexyl octanoate; (IV) hexyl 3-hydroxybutyrate; (V) hexyl 3-acetoxybutanoate; (VI) hexyl 3-[(*E*)-2-butenoyl]-butyrate)

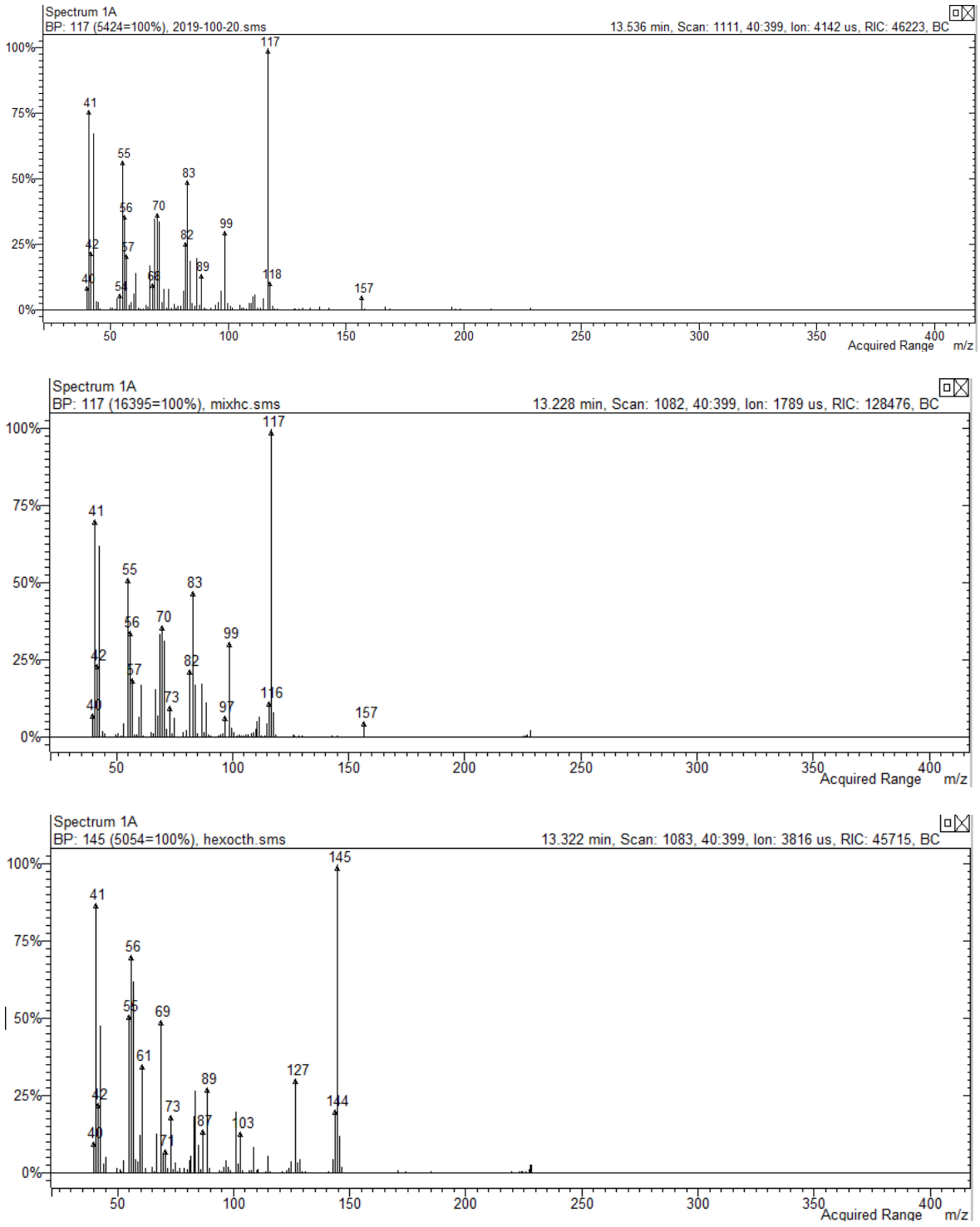

Fig. 14. Mass spectra of pheromone component (B) (upper) octyl hexanoate (II) (middle) and hexyl octanoate (III) (lower)

Table 2. GC Retention Indices (RI) of compounds in this study (relative to the retention times of *n*-alkanes)

	Retention Index				
	Polar			Non-polar	
Compound	GCEAG	FID	GCMS	FID	GCMS
EAG-active (A)	1557	1556	1566	1071	1074
EAG Active (B)	1815	1813	1820	1584	1584
1-octanol (I)	1557	1556	1566	1071	1074
octyl hexanoate (II)	1815	1813	1820	1584	1584
hexyl octanoate (III)	1815	1813	1820	1584	1584
hexyl 3-hydroxybutyrate (IV);	1912	1907	1921	1333	1338
hexyl 3-acetoxybutanoate (V)	1942	1941	19.57	1493	1487
hexyl 3-[(*E*)-2-butenoyl]-butyrate (VI)	2232	2225	2242	1713	1709

In GC-EAG analyses, synthetic 1-octanol (I) and octyl hexanoate (II) elicited EAG responses from antennae from both male and female *N. tenuis*. No EAG response was observed to the cocoa mirid pheromone components, hexyl 3-hydroxybutyrate (IV) and hexyl 3-[(*E*)-2-butenoyl]-butyrate) (VI), but a small response was sometimes observed to the *Macrolophus* pheromone component, hexyl 3-acetoxybutanoate (V) (Fig. 15).

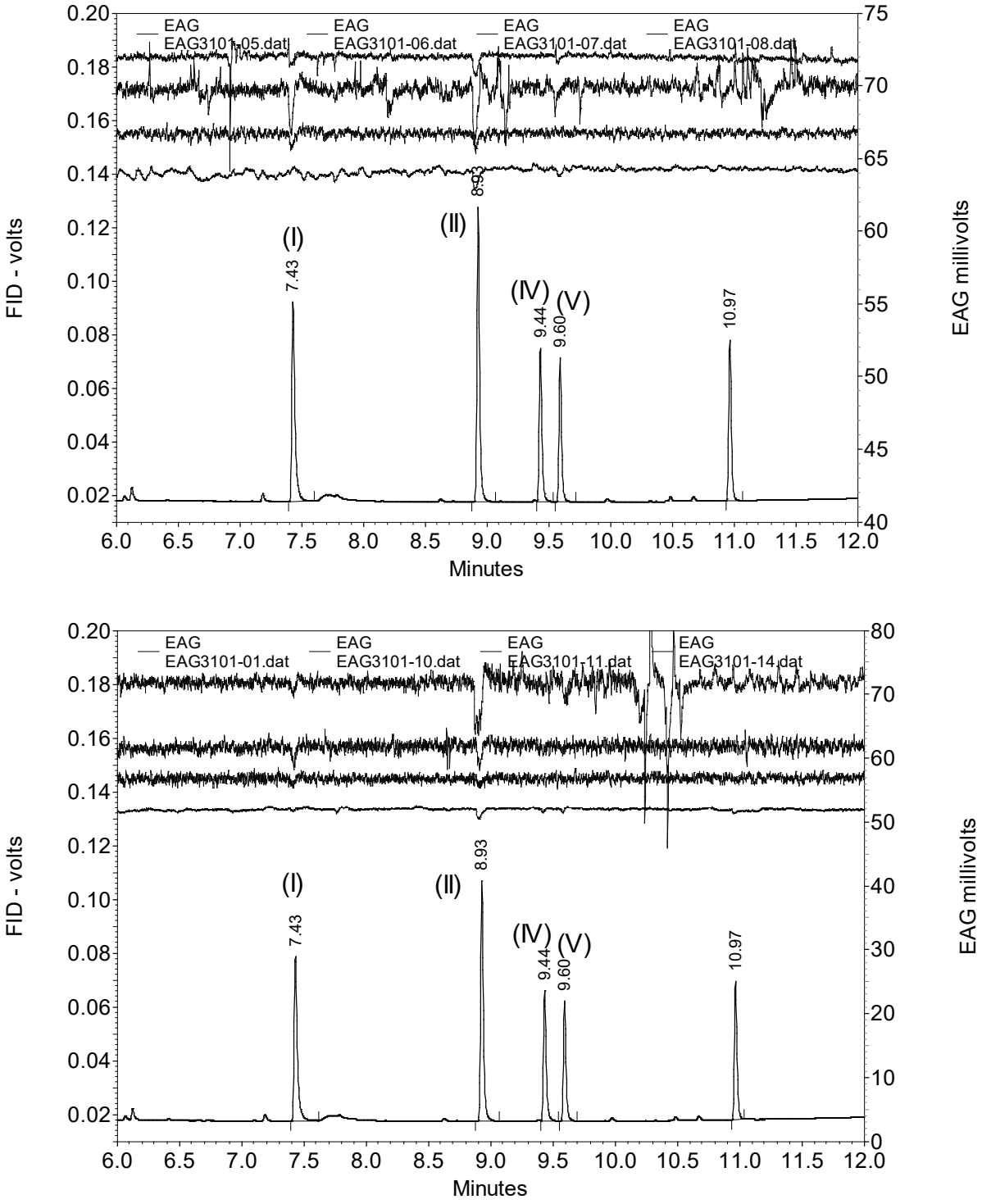

Fig. 15. GC-EAG Analyses of synthetic compounds (20 ng injected) with male (upper) or female (lower) *Nesidiocoris tenuis* antennal EAG preparation on polar GC column showing EAG responses to 1-octanol (I, component A) at 7.43 min and octyl hexanoate (II, compound B) at 8.93 min and also possibly at to hexyl 3-acetoxybutanoate (IV) at 9.60 min but not to hexyl 3-hydroxybutyrate (IV) at 9.44 min; or hexyl 3-[(*E*)-2-butenoyl]-butyrate) (VI) at 10.97 min

The analogue of octyl hexanoate, hexyl octanoate had identical retention times to octyl hexanoate on both polar and non-polar GC columns (Table 2), but clearly different mass spectrum (Fig. 14). The mass spectrum of octyl hexanoate shows the anticipated diagnostic base peak at *m/z* 117, corresponding to protonated hexanoic acid ($C_5H_{11}COOH_2^+$), while that of hexyl octanoate shows a similarly diagnostic base peak at *m/z* 145 corresponding to protonate octanoic acid ($C_7H_{15}COOH_2^+$).

In GC-EAG analyses, the analogue, hexyl octanoate, elicited a consistent EAG response from the antennae of male *N. tenuis* (Fig. 16)

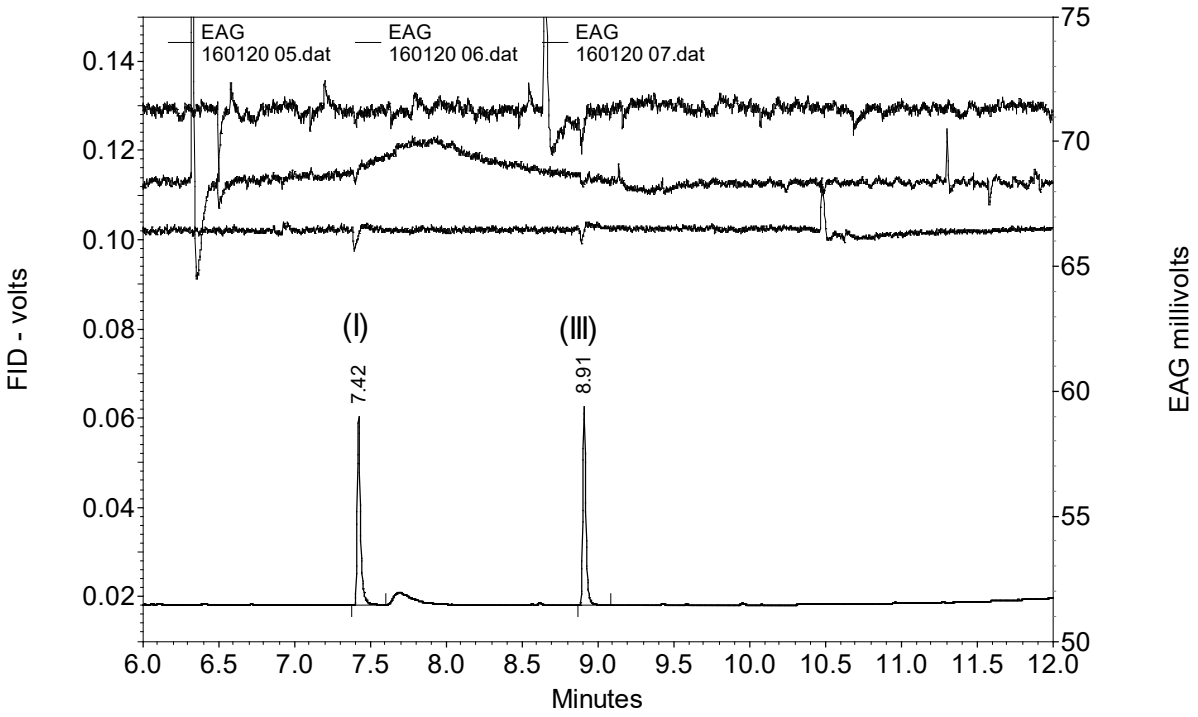

Fig. 16. GC-EAG Analyses of synthetic compounds (20 ng injected) with male *Nesidiocoris tenuis* antennal EAG preparation on polar GC column showing EAG responses to 1-octanol (I) at 7.42 min and the pheromone analogue hexyl octanoate (III) at 8.91 min

Quantification of Pheromone Components

Of the 31 collections of volatiles made from *N. tenuis* adults, only two collections – one virgin female and one virgin male – contained no detectable pheromone components. The rest contained detectable amounts of both pheromone component (A), 1-octanol (I), and pheromone component (B), octyl hexanoate (II), as shown in Table 3.

In collections from groups of 5-20 insects, the ratio of octyl hexanoate/octanol was higher in those from females than in males for both mated (0.6 ± 0.2 SE and 0.1 ± 0.01 respectively) and unmated (0.9 and 0.1 respectively) insects. For single unmated insects, the ratios were the same (1.1) although more of both components was produced by females than males (Table 3).

Unexpectedly, whole body extracts of mated and unmated males and females contained only octyl hexanoate and 1-octanol could not be detected (<<0.01%). For both females and males, amounts in unmated insects seemed to be higher than in mated, although only two unmated insects were extracted.

Table 3. Amounts and relative ratio of pheromone components in collections of volatiles and whole-body extracts from *Nesidiocoris tenuis* (analysis GC-FID on non-polar GC column against external standard for volatiles, internal standard 5 µg decyl acetate for extracts)

	Mean (SE or range)		
Source	Component A Octanol (I)	Component B Octyl hexanoate (II)	Ratio octyl hexanoate/octanol
Volatile Collections	(ng/h/insect)		
5-20 mated female (N=5)	1.3 (0.2)	0.8 (0.4)	0.6 (0.2)
5-20 mated male (N=5)	3.7 (0.5)	0.3 (0.04)	0.1 (0.01)
20 virgin female (N=2)	4.6 (2.3-6.9)	3.3 (2.7-3.9)	0.9 (0.6-1.2)
20 virgin male (N=2)	3.7 (3.0-4.4)	0.4 (0.4-0.4)	0.1 (0.1-0.1)
single virgin female (N=8)	2.9 (1.1)	2.5 (0.8)	1.1 (0.3)
single virgin male (N=5)	0.7 (0.1)	0.6 (0.2)	1.1 (0.6)
Extracts	(ng/insect)		
virgin female (N=2)		1,826 (1,292-2,360)	
virgin male (N=2)		1,712 (993-2,432)	
mated female (N=6)		750 (197)	
mated male (N=6)		821 (215)	

Controlled Release Dispensers

Pipette tips and sealed polyethylene vials were investigated as controlled release dispensers for the synthetic pheromone components, using a 1:1 blend of 1-octanol and octyl hexanoate either neat or as a 10% solution in sunflower oil.

Release of the octyl hexanoate from the pipette tips was negligible (Fig. 17) and these were considered unsuitable.

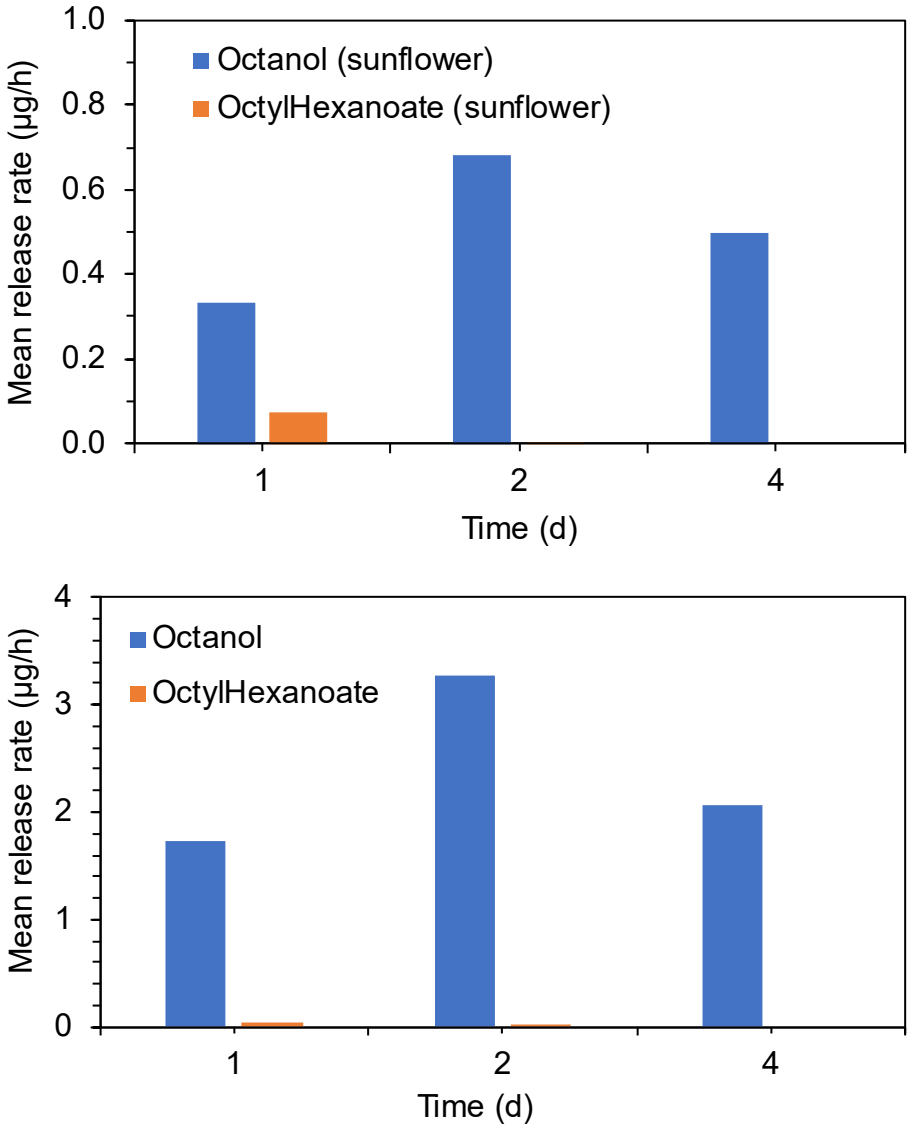

Fig. 17. Mean release rates (*N* = 2) of 1-octanol and octyl hexanoate from pipette tip dispensers containing a 1:1 blend of the components as a 10% solution in sunflower oil (100 µl; upper) or neat (25 µl; lower); dispensers maintained in wind tunnel at 27°C and 8 km/h windspeed

Polyethylene vials gave much better release rates of both components (Fig. 18) for over six weeks at 27°C and 8 km/h windspeed. The release rates for the neat material were approximately 10x those for the solution in sunflower oil. The ratio of octyl hexanoate/ octanol during the first month of exposure was approximately 0.8 for the neat material and 0.5 for the sunflower solution (Fig. 19).

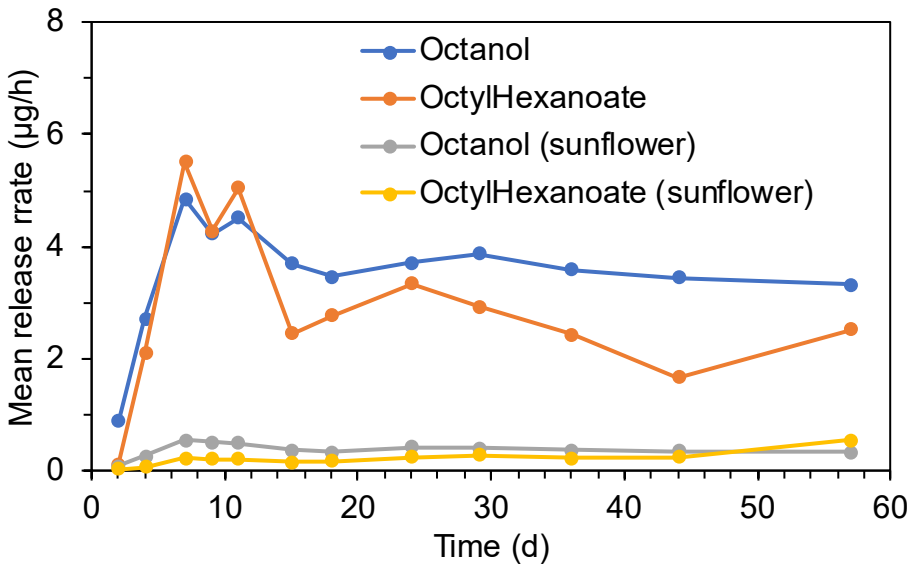

Fig. 18. Mean release rates (*N* = 2) of 1-octanol and octyl hexanoate from polyethylene vial dispensers containing a 1:1 blend of the components as a 10% solution in sunflower oil (100 µl) or neat (25 µl); dispensers maintained in windtunnel at 27°C and 8 km/h windspeed although temperature dropped to 18-20°C during days 10-20

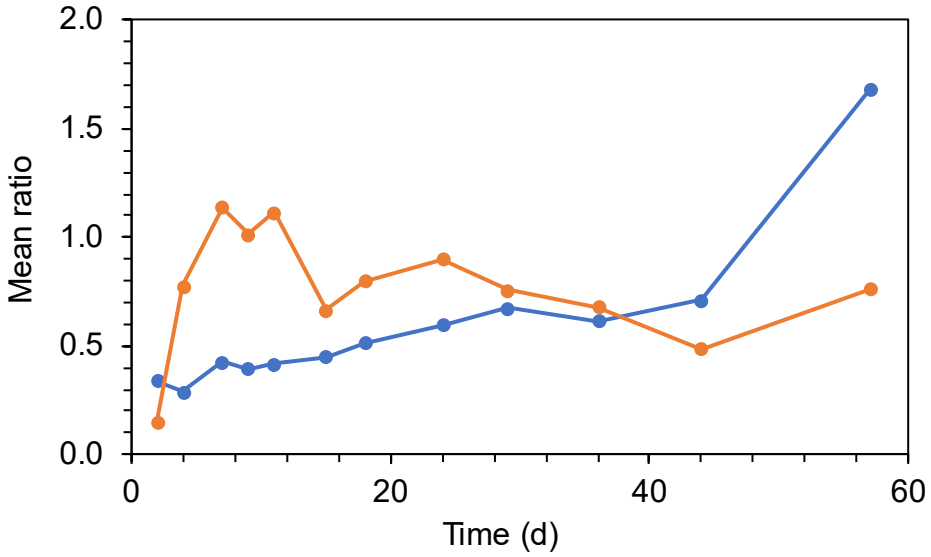

Fig. 19. Mean ratio of octyl hexanoate/octanol (*N* = 2) emitted from polyethylene vial dispensers containing a 1:1 blend of the components as a 10% solution in sunflower oil (100 µl) or neat (25 µl); dispensers maintained in windtunnel at 27°C and 8 km/h windspeed although temperature dropped to 18-20°C during days 10-20

WP3. Bioassay of pheromone (NIAB EMR/NRI/Thanet Earth)

Attraction of Male Nesidiocoris tenuis *to Virgin Females with Airflow in Wind Tunnel*

In Experiment 1 and 2 of the wind tunnel bioassay only 1 of 20 and 30 males, respectively, released in the wind tunnel became stuck to the white sticky trap beneath the tea strainer containing the virgin female, compared to no catches on the control. In these bioassays sticky traps were position horizontally beneath the tea infuser. It was observed that *N. tenuis* individuals were able to slowly walk on the trap and be able to free themselves.

In Experiment 3, sticky traps were hung behind the tea infuser. Small holes were made on the trap, at the infuser height, to allow airflow through the trap and tea infuser. No effect was recorded between the sticky trap with virgin females and the control trap after 24 h of exposure.

Attraction of Male Nesidiocoris tenuis *to Virgin Females in Still-Air Conditions*

In Experiment 4 of the still-air bioassay sticky traps were placed horizontally beneath the tea infusers. After 24 h we observed that the sticky trap with virgin females had 2 captured males and the control trap 3 males. We also observed individuals walking across the sticky traps and freeing themselves. No significant differences between males caught on the virgin female sticky trap and the control trap were recorded in Experiment 5 bioassay.

No response from the males was observed in Experiment 6 and 7 wind tunnel bioassays.

In all bioassays there was no difference in numbers of males attracted to sticky traps accompanying virgin females and control (without virgin females). Males showed no preference in airflow (wind tunnel) or still air conditions. Under airflow, males remained on the downwind mesh of the wind tunnel with very little activity even at low air speeds. In experiments where a sugar solution or dwarf organic bean food source was provided, males remained near to the food source.

Field Trapping Tests

Two trapping tests were carried out in commercial greenhouses at Thanet Earth. In the first, traps baited with the two-component blend of 1-octanol and octyl hexanoate formulated as the neat material in polyethylene vials caught significantly more male *N. tenuis* than traps baited with octyl hexanoate alone which caught more than unbaited traps ($N = 10$; $F = 17.73$; df = 2,18; $P < 0.001$) (Fig. 20). Unbaited traps caught very few males and catches of female *N. tenuis* were very low and similar in all traps.

Results shown are for counts of the first two weeks using yellow traps (19 December 2019 – 3 January 2020) as the glass house was treated with insecticide after that and numbers

caught were much lower in the third and last count on 9 January 2020: total numbers of male *N. tenuis* caught in 10 traps baited with lure A were 38, 31 and 6 on the three counts.

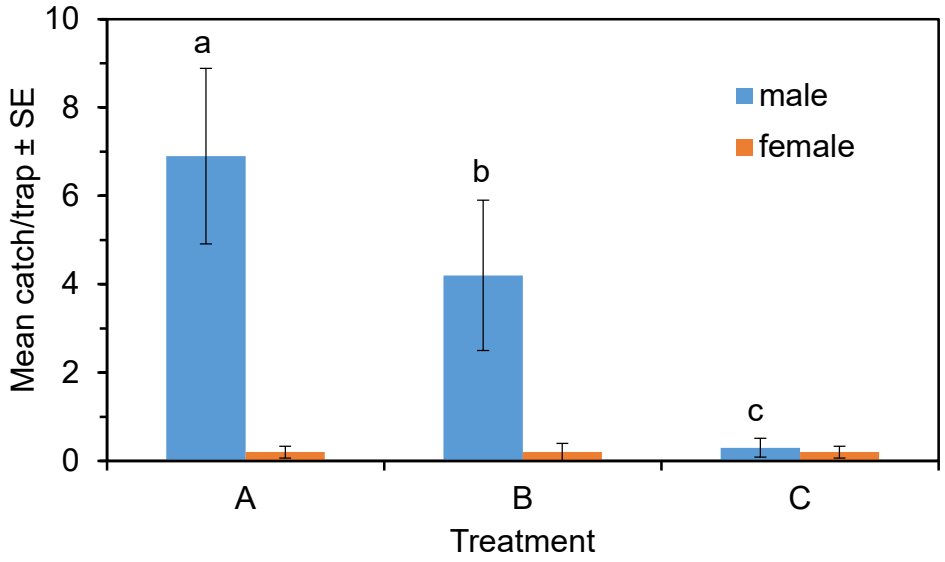

Fig. 20. Mean catches of *Nesidiocoris tenuis* adults in traps during first two weeks of Experiment 1 (19/12/2019 3/1/2020; (A) two-component high release lure; (B) single-component high release lure; (C) unbaited control; *N* = 10; means with different letters are significantly different *P* < 0.05)

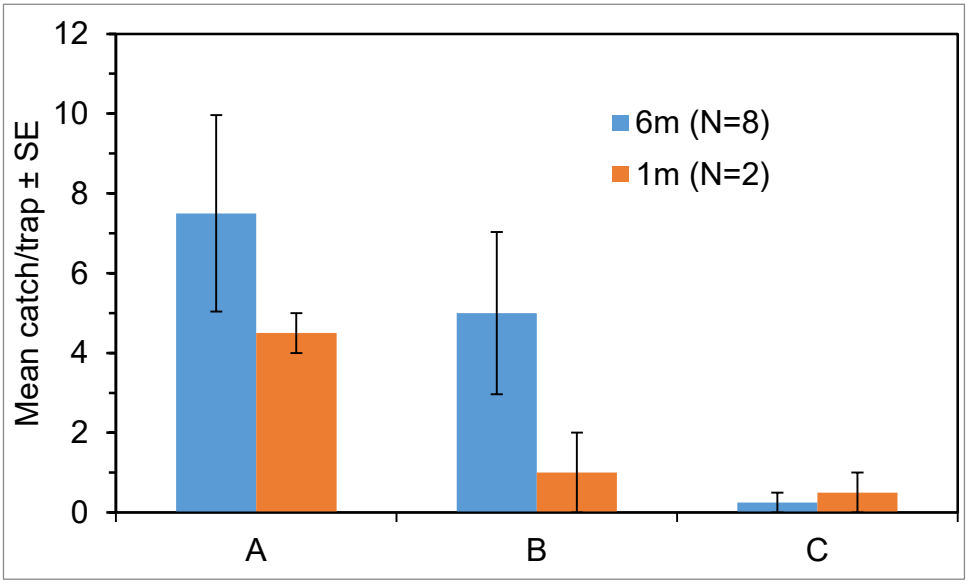

Fig. 21. Mean catches of *Nesidiocoris tenuis* males in traps at different heights during first two weeks of Experiment 1 (19/12/2019 - 3/1/2020; (A) two-component high release lure; (B) single-component high release lure; (C) unbaited control)

In this experiment, eight of the sets of traps were positioned at 6 m height, above the crop canopy, and two at 1 m, within the canopy and data were combined for analyses. Results indicated that catches were higher in traps positioned above the crop, but it was not possible to do a useful statistical analysis with the low number of replicates of traps within the crop (Fig. 21).

In the second experiment, catches of *N. tenuis* males in traps baited with the two-component blend of 1-octanol and octyl hexanoate formulated neat in polyethylene vials were higher, but not significantly so ($P > 0.05$) than in traps baited with the blend formulated as a 10% solution in sunflower oil in polyethylene vials, i.e. with release rate approximately one tenth of that from the neat material ($N = 15$; $F = 27.80$; df = 2,28; $P < 0.001$) (Fig. 22). For both treatments, significantly more male *N. tenuis* were caught than in unbaited traps, although the mean catch in the latter was quite high due to a few occasions when the unbaited traps caught similar numbers to those in the baited traps.

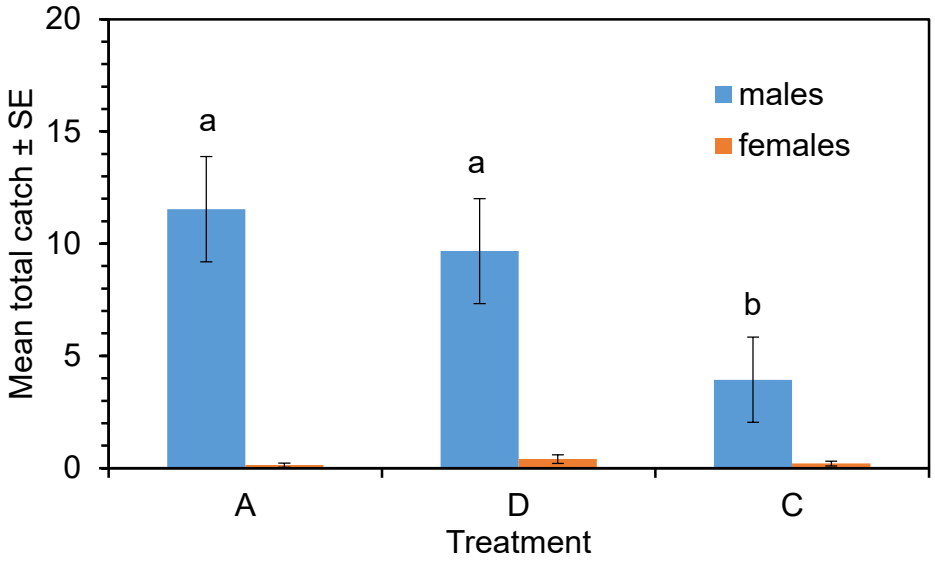

Fig. 22. Mean catches of *Nesidiocoris tenuis* adults in traps during Experiment 2 (10-30/1/2020; (A) two-component high release lure; (D) two-component low release lure; (C) unbaited control; $N = 15$; means with different letters are significantly different $P < 0.05$)

In this experiment, eight of the replicates were positioned just above the tops of the plants, four at 1 m above the plants and three above the gutter at the base of the plants. Highest catches were observed in the traps at the tops of the plants (Fig. 23).

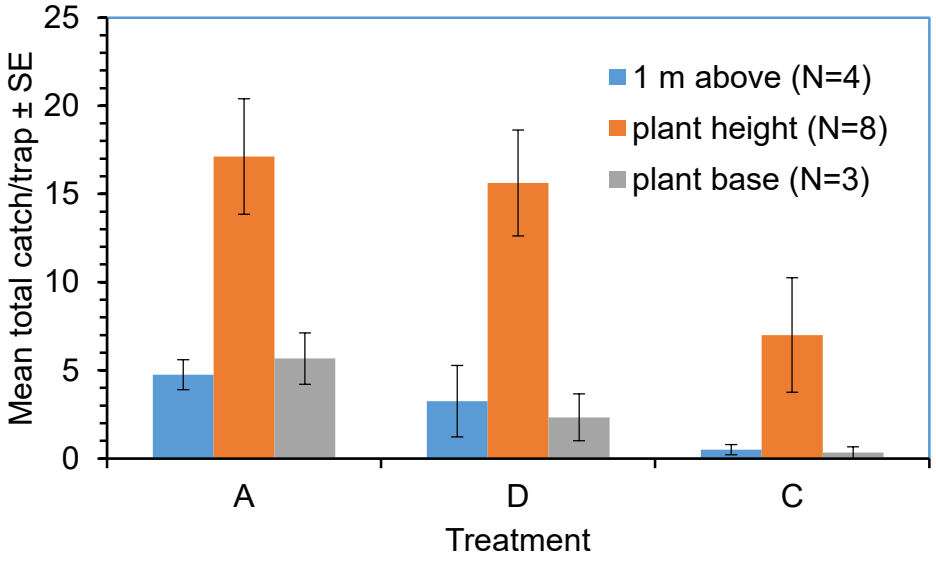

Fig. 23. Mean catches of *Nesidiocoris tenuis* males in traps at different heights during Experiment 2 (10-30/1/2020; (A) two-component high release lure; (D) two-component low release lure; (C) unbaited control)

In both experiments, Individual trap catches were quite variable with respect to position and time (Fig 24). Some traps generally caught more than others, but this was not consistent across time and may reflect local variation in numbers of bugs and the location and timing of "hot-spots".

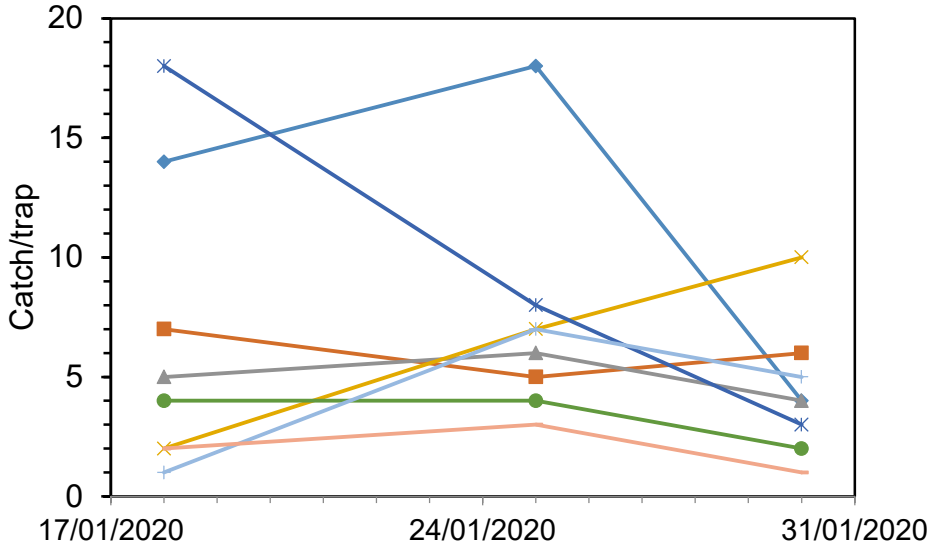

Fig. 24. Catches of individual traps at plant height (*N* = 8) baited with the most attractive lure (A) during the second trapping experiment 10-30/1/2020).

Discussion

Pheromone Identification

Two components of the sex pheromone produced by adult female *Nesidiocoris tenuis* have been identified as 1-octanol and octyl hexanoate. In trapping tests carried out in commercial glasshouses, traps baited with octyl hexanoate alone caught significantly more male *N. tenuis* than unbaited traps, but significantly less than traps baited with a 1:1 blend of the two components.

Octyl hexanoate was reported as a minor component (5-7% of major component, hexyl hexanoate) in volatiles produced by females of another mirid bug, *Trigonotylus caelestialium* Kirkaldy, but was not important for attraction of males (Kakizaki and Sugie, 2001). It is also reported to be involved in the chemical ecology of various stingless bees, e.g. of the genus *Trigona* (Hymenoptera; Apidae). For example, it is a component of a trail pheromone and labial gland secretion (Jarau et al., 2010), of a recruitment pheromone (Lichtenberg et al., 2011), and of the cephalic secretion (Francke et al., 2000). The isomer, hexyl octanoate has also been reported to be present in these stingless bee secretions, but 1-octanol has not previously been reported as an insect semiochemical. This compound was shown to be produced by female *Macrolophus caliginosus,* but its role was not established (Gemeno et al. 2006).

Nesidocoris tenuis belongs to the Bryocorinae sub-family of mirids, and female-produced sex pheromones have been identified in several species of this sub-family by NRI. These include the cocoa mirids *Distantiella theobroma* and *Sahlbergella singularis* Haglund (Mahob et al. 2011; Sarfo 2013; Sarfo et al. 2018a, 2018b; Mahot et al. 2020), and the aphidophagous mirid, *Macrolophus caliginosus* (Gemeno et al. 2006; see also Castañé et al. 2007; Gemeno et al. 2007, 2012, 2015). The components of these pheromones are derivatives of the hexyl ester of 3-hydroxybutyric acid, and rather different from those identified in mirid species from other subfamilies. The pheromone components identified in *N. tenuis*, 1-octanol and octyl hexanoate, do not follow this pattern, and the cocoa mirid and *Macrolophus* pheromone components could not be detected in volatiles or extracts from female or male *N. tenuis*. Furthermore, the synthetic compounds did not elicit a consistent EAG response from antennae of male *N. tenuis*.

It was also unexpected that both pheromone components are released by both mated and unmated male and female *N. tenuis* when in groups or as isolated individuals. For many species of mirid, the same compounds typically serve as pheromone components and also as defensive compounds (Staddon 1986; Aldrich 1988; Fountain et al. 2014). Thus in four *Lygus* species, Fountain et al. (2014) showed that three compounds could be extracted from

both females and males, and when volatiles were collected from groups of insects, all three compounds were produced in a similar ratio from females and males. However, when volatiles were collected from individual, undisturbed virgin insects, the three compounds were only produced by females and in a species-specific ratio that constituted the sex pheromone and was different from that produced by groups of insects.

Results with *N. tenuis* were rather different in that collections from insects in groups, the ratio of octyl hexanoate/1-octanol was higher from females than males whether mated (0.6 and 0.1 respectively) or unmated (0.9 and 0.1 respectively). In collections from individual insects the ratios from females and males were essentially the same (1.1), although results from individuals were very variable at 0.4 – 3.0 in females and 0.2 – 3.4 in males.

Even more surprising was the observation that, although the two compounds were found in volatiles from mated and unmated, groups and individuals of male and female *N. tenuis*, whole body extracts in diethyl ether of mated and unmated males and females contained only octyl hexanoate with no 1-octanol detectable. This result is difficult to explain unless the 1-octanol is contained in some gland not extracted by the solvent. Significant crushing of the bodies was avoided in order not to extract extraneous body materials that could damage the GC column used for analysis, but light crushing of the bodies failed to change the result. Furthermore, the same technique has been used on several other mirid species and given extraction of all the pheromone components.

Trapping Studies

In the initial studies of volatiles released by mated insects, the amount of 1-octanol was relatively higher than that of octyl hexanoate. Nevertheless, it was thought that 1-octanol was possibly a biosynthetic precursor and that octyl hexanoate was the “major” pheromone component. Thus, in the first trapping experiment, because of the limited resources available, only lures containing the two-component blend or octyl hexanoate alone were tested. Traps baited with the latter did catch more male *N. tenuis* than unbaited traps, but those baited with the two-component blend caught significantly more. It was not possible to test the 1-octanol alone during the short timeframe of this project, but obviously, this should be done in future work.

It is anticipated that 1-octanol will only be weakly attractive or unattractive, in which case it would provide an interesting example of the ambiguity of referring to “major” and “minor” pheromone components. In most other cases, the ”major” component of an insect pheromone is that which is both present in highest amount in the blend and also shows attractiveness alone. The “minor” components are present in smaller amounts and may synergise the attractiveness of the “major” component but are not attractive alone. In fact, on

average, single virgin female *N. tenuis* released marginally more octyl hexanoate than 1-octanol, and solvent extracts of both males and females contained only octyl hexanoate.

Polyethylene vials provided convenient dispensing systems for the two pheromone components. The release rate could be adjusted by diluting the components in an involatile diluent such as sunflower oil, and the ratio in the blend released could be modified by changing the ratio in the blend loaded. The blend released with the 1:1 blend loading used in the trapping tests approximated to the mean ratio produced by virgin females, but the latter was very variable and there is scope to optimise the blend and release rate from the vials.

In the first trapping experiment, lures were used with release rates of approximately 4 µg/h. This is three orders of magnitude greater than the amounts recorded from individual *N. tenuis* females. Heteropteran bugs often use similar compounds as pheromones and as defence compounds with the latter produced at much higher release rates than the former (Staddon 1986; Aldrich 1988; Fountain et al. 2014). Thus, in the second trapping experiment lures with a 10-fold lower release rate were tested. Catches of *N. tenuis* males with the lower release rate were lower than those with the higher rate, although not significantly so because of the high variability in catches in this experiment. However, it would seem that use of very low release rates is not necessary with *N. tenuis*.

Catches in individual traps were very variable. For instance, in a period of overall high catches in the second trapping experiment, catches with the most attractive lure varied from 3 to 33. There was some suggestion that certain traps caught higher numbers than others, but this was not always consistent from week to week. This may reflect fluctuations in local populations, and the effect of spraying insecticide during the first trapping experiment was accurately reflected in a marked drop in trap catches. However, catches in pheromone traps are influenced by a number of factors and further research is required to understand the reasons for these fluctuations. For example, at high populations trap catches may be reduced due to female insects competing with the traps as has been found in the mirid bug *Lygus hesperus* (Hall, Millar and Daane, unpublished).

Furthermore, in the second trapping experiment, on two occasions the unbaited traps caught as many male *N. tenuis* as the baited traps. Occasional high catches have been observed in routine use of unbaited monitoring traps (Janos Domsodi, pers comm), and this has been attributed to capture of a virgin female bug on the trap which emitted pheromone and attracted males. In the present trial, the lures were checked at the end of the experiment and shown to be both correctly labelled and free of contamination. A female was found on the trap in one case, but not in the other, although the bugs are known to be capable of escaping from the glue on occasions.

Conclusions

- Two components of the female sex pheromone of *Nesidiocoris tenuis* have been identified as 1-octanol and octyl hexanoate, and traps baited with a blend of the two components have been shown to catch large numbers of *N. tenuis* adult males in commercial glasshouses. Traps and lures are now available for further evaluation by growers and researchers.
- Polyethylene vials were shown to be effective, long-lived dispensers for the pheromone components, but further research is required to optimise the blend of pheromone components, the release rate of dispensers, trap design and trap positioning.
- Trap catches in a commercial glasshouse were variable over time and space. Significant interventions such as an insecticide spray were reflected in lower catches, and it is anticipated that trap catches will give some indication of fluctuations in local populations of the bug. However, further research is required to correlate catches in pheromone traps with population levels and to develop thresholds for intervention.
- One of the two pheromone components is commercially available and the other is relatively straightforward to produce, so their use in controlling *N. tenuis* by mass trapping, lure-and-kill or mating disruption could be considered in further research. A potential pheromone inhibitor/repellent was also identified during this project and that could also be investigated for control of the pest.

Knowledge and Technology Transfer

Fountain MT, Silva C, Woodward J, Hall D, Bray D, Farman D. Identification of the sex pheromone of *Nesidiocorins tenuis*, a damaging pest of commercial tomato. HAPI Meeting 10 Dec 2019.

Fountain MT, Silva C, Woodward J, Hall D, Bray D, Farman D. Identification of the sex pheromone of Nesidiocoris – a damaging pest of tomato. AHDB Protected Edibles Day 2020. Warwickshire, 11 Mar 2020 – **CANCELLED** to be replaced by…

Fountain MT, Silva C, Woodward J, Hall D, Bray D, Farman D. Identification of the sex pheromone of Nesidiocoris – a damaging pest of tomato. Webinar: Tackling key pests in horticulture. 07 May 2020. https://ahdb.org.uk/events/webinar-tackling-key-pests-in-horticulture

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