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# Journal of Chemical Ecology

## SEX PHEROMONE OF THE ALFALFA PLANT BUG, ADELPHOCORIS LINEOLATUS: PHEROMONE COMPOSITION AND ANTAGONISTIC EFFECT OF 1-HEXANOL (HEMIPTERA: MIRIDAE)

--Manuscript Draft--

Manuscript Number:	JOCE-D-20-00288R	
Full Title:	SEX PHEROMONE OF THE ALFALFA PLANT BUG, ADELPHOCORIS LINEOLATUS: PHEROMONE COMPOSITION AND ANTAGONISTIC EFFECT OF 1-HEXANOL (HEMIPTERA: MIRIDAE)	
Article Type:	Original Research	
Keywords:	- Adelphocoris lineolatus; sex pheromone; field attraction; electroantennography; Miridae	
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Funding Information:	Nemzeti Kutatási Fejlesztési és Innovációs Hivatal (FK134744) Biotechnology and Biological Sciences Research Council's Industrial Strategy Challenge Fund (BBS/OS/CP/000001)	Not applicable Not applicable
Abstract:	Abstract – The sex pheromone composition of alfalfa plant bugs, Adelphocoris lineolatus (Goeze, 1778), from Central Europe was investigated to test the hypothesis that insect species across a wide geographical area can vary in their pheromone composition. Potential interactions between the pheromone and a known attractant, (E)-cinnamaldehyde, were also assessed. Coupled gas chromatography-electroantennography (GC-EAG) using male A. lineolatus antennae and volatile extracts collected from females, which had been shown to attract males in earlier field experiments, revealed the presence of three physiologically active compounds, which were identified by coupled GC-mass spectrometry (GC-MS) and GC peak enhancement as hexyl butyrate, (E)-2-hexenyl butyrate and (E)-4-oxo-2-hexenal. A	

	<p>ternary blend of these compounds in 5.4:9.0:1.0 ratio attracted male <i>A. lineolatus</i> in field trials in Hungary. Omission of either (E)-2-hexenyl-butyrate or (E)-4-oxo-2-hexenal from the ternary blend or substitution of (E)-4-oxo-2-hexenal by (E)-2-hexenal resulted in loss of activity. These results indicate that the studied Central European population is similar in its pheromone composition to that of a previously reported East Asian population. Interestingly, another EAG-active compound, 1-hexanol, was also present in extracts of females. When tested in combination with the ternary pheromone blend, 1-hexanol significantly reduced male catches. This antagonism produced a dose-response effect with relatively small doses showing remarkable influence, which suggests that 1-hexanol may act as a sex pheromone antagonist for <i>A. lineolatus</i>. Furthermore, when field tested in combination with the sex pheromone, (E)-cinnamaldehyde did not increase male catches significantly; however, the combination attracted both males and females. Prospects for practical applications are discussed.</p>
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Responses to comments on manuscript:

JOCE-D-20-00288, "STUDIES ON THE SEX PHEROMONE OF THE ALFALFA PLANT BUG, ADELPHOCORIS LINEOLATUS: ANTAGONISTIC EFFECT OF 1-HEXANOL(HEMIPTERA: MIRIDAE)"

Dear Editor,

We are grateful for your and the reviewers' comments and suggestions. We have revised the manuscript based on the recommendations: we have changed the title to better describe the study, supplemented the Introduction in order to provide a more comprehensive rationale for the study, added a table on experimental design to provide a more concise Material and Methods section, and made corrections to the Results and the Discussion. Along with those we have checked the manuscript again to better match format requirements and made grammatical corrections. Based on the suggestions, we have added a photo of the dispensers applied in the study as Electronic Supplementary Material. We believe the changes resulted in an overall more focused manuscript, we hope the revised version shall prove satisfactory.

With kindest regards

Sandor Koczor

**Editorial comment:**

Both reviews are favorable, indicating some revision before publication. I concur with reviewer #2 that the manuscript is a little unfocused. It could do with a tighter ecological rationale for the work. For instance, in the Introduction, there is no rationale for testing cinnamaldehyde. Why test this compound in particular? What is its ecological function and how would responses to this compound expect to interact with responses to the sex pheromone response? Try to set up some hypotheses that are tested by the experiments.

RESPONSE: Thank you for your comment. Based on your and the reviewer's recommendations we have supplemented the Introduction in order to provide a more comprehensive rationale for the study. With the improvement of Introduction to avoid unnecessary duplication the respective parts were omitted or changed in Discussion.

**Comments of Reviewer #1:** "STUDIES ON THE SEX PHEROMONE OF THE ALFALFA PLANT BUG, ADELPHOCORIS LINEOLATUS: ANTAGONISTIC EFFECT OF 1-HEXANOL (HEMIPTERA: MIRIDAE)" by Koczor et al. is a well-done and by and large well-written research manuscript. To me the most interesting aspect of the study, aside from verifying the pheromone of the species in Central Europe is essentially the same as that for the species in Asia, is the discovery the 1-hexanol is a apparently a naturally produced antagonist. The discussion is easily understood, and the recognition that the oxo-hexenal is an

unstable and irritating compound is of practical importance for which 1-hexanol may provide a practical alternative to control of this pest mirid. An edited Word document is attached for various grammatical suggestions are offered.

RESPONSE: We are grateful for the reviewer's comments and for the suggestions marked in the text, for these please the responses below.

Responses to specific comments of Reviewer #1:

Thank you for your grammatical suggestions marked in the text, we have corrected the manuscript accordingly.

Page. 18. Lines 7-9: Do you think that 1-hexanol is part of the natural communication system?

RESPONSE: Thank you for the question. As we have emphasized in the manuscript the ecological role of 1-hexanol for *A. lineolatus* is uncertain, however, our studies indicate that the effect of 1-hexanol is due to a biological, behavioral response, thus, we believe it is part of the natural communication system. Further research may clarify the role of this compound.

**Comments of Reviewer #2:** This is an interesting and generally well-written paper on the sex pheromone of an important crop pest. In the attached word file are track changes edits and comments, most of which deal with minor grammar/editing issues. My major suggestion (also mentioned in the last comment in the discussion) is to rewrite portions of the intro, M and M, and results to clarify the rationale of the study and clearly state specific objectives. Then in the M and M and results follow through with the relevant progression of experiments and the findings. As written, the paper seemed a little unfocused. I hope these comments are helpful.

RESPONSE: We are grateful for the reviewer's comments and suggestions, we have corrected the manuscript accordingly. We have supplemented Introduction in order to provide a more clearly defined rationale for the study. We have added a table on experimental setup thereby providing a more concise Material and Methods section. With the changes in Introduction to avoid unnecessary duplication the respective parts were omitted or changed in Discussion. We believe the changes resulted in an overall more focused manuscript.

Responses to specific comments of Reviewer #2:

Thank you for your grammatical suggestions marked in the text, we have corrected the manuscript accordingly, for other specific comments please find the responses below.

Page 1. Lines 1-3: Consider changing the title to something more descriptive of the study.

RESPONSE: Thank you for your comment, we have changed the title to: 'Sex pheromone of the alfalfa plant bug, *Adelphocoris lineolatus*: pheromone composition and antagonistic effect of 1-hexanol (Hemiptera: Miridae)'

Page 1. Lines 1-3: Number pages consecutively throughout the paper, instead of starting over on each page.

RESPONSE: We have changed line numbering to continuous.

Page 6, Line 3: On line 3 above it's not clear what the two methods were. Please rewrite for clarity.

RESPONSE: Thank you for your comment, we have rephrased the respective sentence for more clarity: 'For preparation of headspace collections two methods were used.'

Page 6, Line 10: Please include light and RH

RESPONSE: We have added the requested information: '...for 24 h at 14:10 light:dark period, 20°C and ca. 50% relative humidity...'

Page 9 Line 2: Please give manufacturer information for the transparent PVC foil.

RESPONSE: We have added the requested information.

Page 9, Line 17: I saw the callout for experiment 1, but not for experiment 2. Did I miss it?

RESPONSE: Thank you for your comment, we have rephrased the respective sentence: 'Binary combinations in Experiment 2 were prepared with the same load of the respective compounds.'

Page 9, Line 19: Adding a figure with of photo of this would be great.

RESPONSE: We have added a photo of the dispensers applied in the study as Electronic Supplementary Material

Page 10, Line 16: Please clearly define what you mean by block, i.e., is it a certain portion of a field?

RESPONSE: Thank you for your comment, we have clarified this point: 'The experiments were performed in randomized complete block design, that is, one replicate of each treatment was incorporated into a block, so that individual treatments...'

Page 11, Line 8: Please tabulate the treatments in these experiments. They will fit into one table that consolidates them and eliminates a lot of tedious text in the body of the ms.

RESPONSE: Based on your suggestions, we have added a table containing treatments of the respective experiments, and omitted their description from the text.

Page 19, Lines 9-11: This topic should be brought up in the introduction. Doing so would more clearly frame the rationale of your study and give your experiments more direction. As written, your study, while interesting, seems to be a collection of slightly disconnected experiments. As a reader I didn't always know where the ship was sailing. Please consider restructuring the introduction, as well as portions of the M and M, and results to make the paper more cohesive.

RESPONSE: Thank you for your comments. Based on your suggestions, we have supplemented the Introduction and provided a more concise Material and Methods section by presenting treatments of experiments in a table, furthermore, we supplemented Results with information on female catches. In accordance with changes in the Introduction, to avoid unnecessary duplication the Discussion was also modified, we believe the changes resulted in an overall more focused manuscript.

Page 24, Line 8: Please describe all the components of the box plots in the legend for each figure.

RESPONSE: We have added the requested information to the respective figure legends: 'box plot diagram indicating median, minimum, maximum, the 1st and 3rd quartiles of catches of the respective treatments'

SEX PHEROMONE OF THE ALFALFA PLANT BUG, *ADELPHOCORIS LINEOLATUS*:  
PHEROMONE COMPOSITION AND ANTAGONISTIC EFFECT OF 1-HEXANOL  
(HEMIPTERA: MIRIDAE)

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ANDRÉ SARRIA<sup>2, 3</sup>, JOHN A. PICKETT<sup>2, 4</sup>, MICHAEL A. BIRKETT<sup>2</sup>, ÉVA BÁLINTNÉ  
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**Abstract** – The sex pheromone composition of alfalfa plant bugs, *Adelphocoris lineolatus* (Goeze, 1778), from Central Europe was investigated to test the hypothesis that insect species across a wide geographical area can vary in their pheromone composition. Potential interactions between the pheromone and a known attractant, (*E*)-cinnamaldehyde, were also assessed. Coupled gas chromatography-electroantennography (GC-EAG) using male *A. lineolatus* antennae and volatile extracts collected from females, which had been shown to attract males in earlier field experiments, revealed the presence of three physiologically active compounds, which were identified by coupled GC-mass spectrometry (GC-MS) and GC peak enhancement as hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal. A ternary blend of these compounds in 5.4:9.0:1.0 ratio attracted male *A. lineolatus* in field trials in Hungary. Omission of either (*E*)-2-hexenyl-butyrate or (*E*)-4-oxo-2-hexenal from the ternary blend or substitution of (*E*)-4-oxo-2-hexenal by (*E*)-2-hexenal resulted in loss of activity. These results indicate that the studied Central European population is similar in its pheromone composition to that of a previously reported East Asian population. Interestingly, another EAG-active compound, 1-hexanol, was also present in extracts of females. When tested in combination with the ternary pheromone blend, 1-hexanol significantly reduced male catches. This antagonism produced a dose-response effect with relatively small doses showing remarkable influence, which suggests that 1-hexanol may act as a sex pheromone antagonist for *A. lineolatus*. Furthermore, when field tested in combination with the sex pheromone, (*E*)-cinnamaldehyde did not increase male catches significantly; however, the combination attracted both males and females. Prospects for practical applications are discussed.

**Key Words** – *Adelphocoris lineolatus*, sex pheromone, field attraction, electroantennography, Miridae.

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**Declarations:**

**Funding.** The current research was partially financed by the National Research Development and Innovation Office (NKFIH, grant FK134744). The work at Rothamsted Research formed part of the Smart Crop Protection (SCP) strategic programme (BBS/OS/CP/000001) funded through Biotechnology and Biological Sciences Research Council's Industrial Strategy Challenge Fund.

**Conflicts of interest/Competing interests.** Not applicable.

**Ethics approval.** Not applicable.

**Consent to participate.** Not applicable.

**Consent for publication.** Not applicable.

**Availability of data and material.** Not applicable.

**Code availability.** Not applicable.

**Author contributions.** S. Koczor, J.A. Pickett, M.A. Birkett, M. Tóth and J. Vuts conceived and designed the experiments. É. Bálintné Csonka, A. Sarria and J. Vuts performed volatile collections, GC-EAG was done by S. Koczor. Chemical analysis of the samples and identification of compounds was done by J. Vuts, J.C. Caulfield, M.A. Birkett and A. Sarria. D.M. Withall performed the synthesis of pheromone compounds. S. Koczor conducted the field experiments, determined the collected material and analysed data. S. Koczor wrote a first draft of the manuscript. All authors read, contributed to and approved the final manuscript.

## INTRODUCTION

Plant bugs (Heteroptera: Miridae) represent the most species-rich family of true bugs. Several species are pests, and some have an extremely wide spectrum of potential hosts (e.g. Holopainen and Varis 1991). Due to new pest control technologies and recent changes in regulation, there is a marked and continuous decrease in the use of broad-spectrum insecticides in agriculture. As a consequence, pests considered previously to be of minor importance become more damaging, as observed for genetically engineered lepidopteran-resistant crops, such as Bt-cotton (Lu et al. 2010). Furthermore, this effect may reach beyond the crop initially affected. Lu et al. (2010) found that broad-spectrum insecticide sprayings may result in 'sink' populations of a particular pest, but without such treatments, they can reach high abundance and create 'source' populations, which results in higher levels of damage in other crops as well. *Adelphocoris* species are among those pests that have gained increasing economic importance with the decrease in broad-spectrum insecticide use (Lu et al. 2008).

The alfalfa plant bug, *Adelphocoris lineolatus* Goeze (1778), occurs widely in the Palearctic, where it is a major pest of alfalfa (*Medicago sativa* L., Fabaceae) (Benedek et al. 1970); however, several other potential hosts have also been reported (Golledge 1944; Peterson et al. 1992). Currently, the most serious economic impact of *Adelphocoris* spp., including *A. lineolatus*, is associated with the damage caused to Bt-cotton in China (Wu et al. 2002; Lu et al. 2008).

Partially due to their increased economic importance, several reports on the chemical ecology of *Adelphocoris* species have been published recently, including pheromone identification of major pests of Bt-cotton in China, such as *A. fasciaticollis* Reuter, 1903 (Zhang et al. 2015b), *A. suturalis* (Jakovlev, 1882) (Zhang et al. 2016) and *A. lineolatus* (Zhang et al. 2015a), with

the aim to develop species-specific detection and monitoring traps to aid their pest management. Among these species, *A. lineolatus* has the widest distribution in the Palearctic, and it has also been introduced to the Nearctic. Zhang et al. (2015a) identified hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal as components of the female sex pheromone of an east Asian population. Nevertheless, as has been found in other pest insects with wide distributions, pheromone composition can vary throughout the distribution range. A remarkable example for that is *Agrotis segetum* (Denis & Schiffermüller, 1775), where sex pheromone composition of populations in different geographic regions consisted of different combinations of the components (Tóth et al. 1992).

(*E*)-4-Oxo-2-hexenal, a common component of plant bug pheromones, is highly sensitive to environmental conditions, including heat, light and oxidation. Thus, in previous studies on the chemical ecology of the Miridae, special caution was taken during application, for instance the compound was applied in separate bait dispensers and replaced on a daily basis to maintain its activity (Byers et al. 2013). Yasuda and Higuchi (2012) reported that the level of (*E*)-4-oxo-2-hexenal decreased quickly in dispensers, and they found that an increased dose of the compound attracted more males of *Stenotus rubrovittatus* (Matsumura, 1913), another pestiferous plant bug species. Thus, in our study, we tested the compound in increased dosage. We also tested (*E*)-2-hexenal, a much more stable compound, for potential analogous activity.

Plant volatiles are known to affect sex pheromone production and activity in several insect species (Landolt & Phillips 1997). For example, in a closely related plant bug, *Lygus rugulipennis* (Poppius, 1911), host plant odors evoked increased sex pheromone production in females (Frati et al. 2009). Based on this, we aimed to assess potential interactions between the sex pheromone and (*E*)-cinnamaldehyde, a floral volatile, which attracts *A. lineolatus* (Koczor et al. 2012).

Thus, the aims of this paper were 1) to determine the pheromone composition of a central European population of *A. lineolatus*, representing a different geographic region, 2) to test increased dosage of (*E*)-4-oxo-2-hexenal, 3) to test (*E*)-2-hexenal as a more stable, potential analogue for (*E*)-4-oxo-2-hexenal, and 4) to assess potential interactions between the sex pheromone and (*E*)-cinnamaldehyde.

## METHODS AND MATERIALS

*Insects for Experiments.* Virgin *A. lineolatus* males and females were reared in the laboratory at 18:6 light:dark period, 26°C and ca. 40% relative humidity. Nymphs were collected by sweep-netting at alfalfa fields in Halásztelek, Pusztazámor and Tököl (Hungary), and taken to the laboratory where they were reared on green bean pods in 12.5 × 17.5 cm glass jars covered with fine mesh. Freshly molted adults were removed from the rearing containers, identified to species, sexed and kept separately to ensure they were virgin. Adult bugs were kept in the same conditions as nymphs.

*Volatile Collection from Live Females.* As field cage experiments with live bugs indicated the presence of a female-produced sex pheromone, headspace collections were performed with single *A. lineolatus* females on green bean pods, and with green bean pods alone as control, for 1 day (20-24 h) or 3 days (71-72 h), as the daily rhythm of pheromone emission was unknown in this species. For preparation of headspace collections, two methods were used. The bugs and green bean pods were placed into 200 mL glass containers of a closed-loop stripping apparatus (CLSA, Boland et al. 1984), equipped with a DC12/16NK vacuum pump (Erich Fürgut GmbH, Tannheim, Germany) with an airflow of ca. 5.0 L/min, and a collection filter containing 5 mg activated charcoal (Brechtbühler AG, Schlieren, Switzerland). Trapped

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142 volatiles were eluted from the charcoal filter with 25 µL dichloromethane (Merck KGaA,  
143 Darmstadt, Germany).

144 To determine pheromone emission patterns, dynamic headspace collection (air entrainment)  
145 (Birkett 2010) was also done with single *A. lineolatus* females on green bean pods for 24 h at  
146 14:10 light:dark period, 20°C and ca. 50% relative humidity. The material to be sampled was  
147 placed in a 380 mL glass jar, and activated charcoal-filtered (Capillary-Grade Hydrocarbon  
148 Trap with 1/8 in. compression fittings; Thames Restek Ltd., High Wycombe, UK) air was  
149 supplied by a pump system (Pye volatile collection kits, Kings Walden, UK) through the inlet  
150 port at a rate of 600 mL/min. Air subsequently passed over the material in the jar and  
151 headspace volatiles were adsorbed on Porapak Q filters (0.05 g, 50/80 mesh; Supelco) that  
152 were fitted on the outlet port, through which air was drawn at a rate of 500 mL/min. All  
153 connections in the air entrainment setup were made using PTFE tubing. Prior to entrainment,  
154 Porapak Q filters were washed with diethyl ether and conditioned by heating to 132°C in an  
155 activated charcoal-filtered nitrogen stream for 2 h. Entrained volatiles were eluted with 750  
156 µL redistilled diethyl ether and stored in 1.1 mL glass microvials at –20°C until analysis.  
157 Glass jars were washed with detergent (Teepol), acetone and distilled water, and baked  
158 overnight at 140°C. The sampling was replicated four times.

159  
160 *Coupled Gas Chromatography-Electroantennography (GC-EAG)*. Female air entrainment  
161 extracts were tested for electroantennographic activity on antennae from males by coupled  
162 GC-EAG using an Agilent 6890N gas chromatograph equipped with a DB-WAX column with  
163 polyethylene glycol phase (30 m × 0.32 mm i.d.). Helium was used as carrier gas, injection  
164 was performed in splitless mode. Temperature program started at 60°C and increased to  
165 220°C by 10°C/min. The effluent was split between the GC-FID and a heated transfer line to  
166 the EAG apparatus. For each test, 1 µL aliquots of the air entrainment extracts and 10 ng

tetradecyl acetate as internal standard in 1  $\mu$ L dichloromethane solution were co-injected. For EAG, the male antenna was freshly cut at the base from a live bug, and the tip of the last segment was cut off to ensure better connection. The antenna was mounted between two glass capillaries containing Ringer solution. One of the electrodes was grounded, while the other was connected to a high-impedance DC amplifier (IDAC-232, Ockenfels Syntech GmbH, Kirchzarten, Germany). A compound was defined as EAG-active if it evoked an antennal response, distinguishable from background noise, in at least three coupled runs.

*Identification of EAG-Active Compounds.* For the identification of electrophysiologically active compounds in air entrainment samples, a Hewlett-Packard 5890 series II GC fitted with a capillary DB-WAX GC column (30 m  $\times$  0.32 mm i.d., 0.5  $\mu$ m film thickness; J&W Scientific, Folsom, CA) and equipped with a cool on-column injector was directly coupled to a mass spectrometer (Hewlett-Packard 5972 mass-selective detector). Ionisation was by electron impact at 70 eV, 220°C. The oven temperature was maintained at 40°C for 1 min and then programmed at 5°C/min to 250°C (hold time 17.2 min). The carrier gas was helium. Tentative identification by GC-MS was confirmed by comparing retention indices of peaks with those of synthetic standards and by peak enhancement on GC by coinjection with authentic compounds (Pickett 1990), using an Agilent 7890A GC equipped with a cool on-column injector, FID and a 30 m  $\times$  0.32 mm i.d., 0.52  $\mu$ m film thickness DB-WAX column. The oven temperature was maintained at 30°C for 0.5 min and then programmed at 5°C/min to 150°C for 0.1 min, then 10°C/min to 230°C for 25 min. The carrier gas was hydrogen. Quantification of compounds was achieved using the multiple-point external standard method, whereby concentration ranges of synthetic standards of the pheromone compounds provided calibration curves.

*Chemicals.* Hexyl butyrate, (*E*)-2-hexenyl butyrate, (*E*)-cinnamaldehyde and 1-hexanol ( $\geq 96\%$  purity as per the manufacturer) were obtained from Sigma-Aldrich Kft (Budapest, Hungary). (*E*)-4-Oxo-2-hexenal was synthesized as follows: To a solution of 2-ethylfuran (10.00 g, 104.03 mmol) in a mixture of THF (100 mL), acetone (80 mL) and water (40 mL), cooled to  $-15\text{ }^{\circ}\text{C}$  under nitrogen, was added *N*-bromosuccinimide (27.78 g, 156.04 mmol), followed by pyridine (16.8 mL, 208.06 mmol). The reaction mixture was stirred for 30 mins before being warmed to  $0^{\circ}\text{C}$  for a further 3 hours. The reaction mixture was poured into 0.5M HCl and extracted with EtOAc. The combined organics were washed with water, dried ( $\text{MgSO}_4$ ) and concentrated under vacuum. The crude product was purified on silica gel (20% EtOAc in pet ether) to give (*E*)-4-oxo-2-hexenal (4.42 g, 37% yield) as an orange oil.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 500 MHz): 9.79 (d, 1H,  $J = 7.2\text{ Hz}$ ), 6.90 (d, 1H,  $J = 16.2\text{ Hz}$ ), 6.80 (dd, 1H,  $J = 16.2$  and  $7.2\text{ Hz}$ ), 2.75 (qu, 2H,  $J = 7.3\text{ Hz}$ ), 1.18 (t, 3H,  $J = 7.2\text{ Hz}$ ).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 500 MHz): 200.38, 193.46, 144.78, 137.30, 34.54 & 7.55. Due to its inherent instability, the compound was stored as a 1:1 solution in dichloromethane at  $-80^{\circ}\text{C}$  until required for use.

## Field tests

*Field Experiment with Live Virgin A. lineolatus.* Experiment 1. This test was performed at Pusztazámor, Hungary, at the edge of an alfalfa field from July 15 to August 8, 2013. Four different treatments were applied: three virgin females on a green bean pod, three virgin males on a green bean pod, a green bean pod without insects and an empty control. Traps consisted of a plastic roof ( $27 \times 24\text{ cm}$ ) with its upper side covered with aluminum foil (I.S.X.-TRADE Kft., Budapest, Hungary) to prevent insolation. To the roof, a transparent sticky PVC sheet ( $23 \times 36\text{ cm}$ ) was attached with pegs, its sticky side facing inwards. The bugs and pods were placed in  $9.5 \times 4\text{ cm}$  cylindrical containers made of transparent PVC foil, and closed at both ends with fine mesh. The containers were fixed to the bottom side of the roof. At each



inspection, bean pods and bugs were replaced with fresh ones. One replicate of each treatment was incorporated into a block, within which individual treatments were 5-8 m apart in a randomized arrangement. The distance between blocks was 10-15 m. The experiment was run with 4 blocks. Traps were inspected twice weekly and insects caught in the sticky insert were removed and taken to the laboratory for identification.

*Field Experiments with Synthetic Compounds.* Ternary pheromone baits were prepared as follows: hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal were formulated into 0.7 mL polyethylene vials with lid (No. 730, Kartell Co., Italy) in 5.4:9.0:1.0 ratio, respectively. Total load of baits was kept at 50 mg. Binary combinations in Experiment 2 were prepared with the same load of the respective compounds. For Experiment 3, 0.1, 1 or 10 mg of 1-hexanol was added to the ternary pheromone blend. The lids of the vials were closed and the dispensers were attached to 8×1 cm plastic handles for easy handling when assembling the traps. The dispensers were kept in the shade under the roof of traps and equipped with a loosely applied aluminum foil to provide protection from light, since (*E*)-4-oxo-2-hexenal is known to be light-sensitive (Fountain et al. 2014). For Experiment 4, 10 mg of 1-hexanol was added to the ternary pheromone blend as described above and separate polyethylene vial baits were also prepared with 10 mg load of 1-hexanol. These were also closed but no shading was added to these baits.

(*E*)-Cinnamaldehyde, a known attractant for *A. lineolatus* (Koczor et al. 2012), was also tested as positive control. Baits were prepared as follows: 100 mg (*E*)-cinnamaldehyde was loaded onto a 1 cm piece of dental roll (Celluron<sup>®</sup>, Paul Hartmann AG, Heidenheim, Germany), which was put into a polyethylene bag (ca 1.0×1.5 cm) made of 0.02 mm linear polyethylene foil (FS471-072, Phoenixplast BT, Pécs, Hungary). The dispensers were heat-sealed and attached to 8×1 cm plastic handles for easy handling when assembling the traps. In the field,

polyethylene vial dispensers were replaced at 4-5 week intervals and polyethylene bag dispensers were replaced at 3-4 week intervals, as previous experience showed that they do not lose their attractiveness during this period (Koczor et al. 2012, 2015). For storage, all dispensers used in the experiments were wrapped singly in pieces of aluminum foil and stored at -18°C until used. For field testing of synthetic compounds, CSALOMON® VARL+ funnel traps were used (produced by the Plant Protection Institute, CAR, Budapest, Hungary), which proved to be suitable for catching plant bugs (Koczor et al. 2012). A small piece (1×1 cm) of household anti-moth strip (Chemotox®, Sara Lee; Temana Intl. Ltd, Slough, UK; active ingredient 15% dichlorvos) was placed in the containers to kill captured insects. The experiments were performed in randomized complete block design, that is, one replicate of each treatment was incorporated into a block, so that individual treatments were 5-8 m apart in a randomized arrangement. Distance between blocks was 10-15 meters. To avoid positional effects, trap positions were changed regularly on a fortnightly basis. As a rule, traps were inspected weekly, and catches were brought to the laboratory, where collected individuals were sexed and determined to species.

#### Details of individual experiments:

Experiment 2: The aim of this experiment was to test ternary and binary combinations of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal (Table 1). Traps were set up at the edge of an alfalfa field in the vicinity of Cegléd (Hungary). The experiment was run from 12 July to 24 September, 2018, with 4 blocks.

Experiment 3: The aim of this experiment was to test addition of 1-hexanol to the ternary pheromone blend, containing hexyl butyrate + (*E*)-2-hexenyl butyrate + (*E*)-4-oxo-2-hexenal. 1-Hexanol was loaded in 0.1, 1, or 10 mg dose in the same bait dispensers (Table 1). Traps

were set up at the edge of an alfalfa field in the vicinity of Cegléd (Hungary). The experiment was run from 12 July to 24 September, 2018, with 4 blocks.

Experiment 4: The aim of this experiment was to test addition of 1-hexanol to the pheromone blend in the same or in separate dispensers to assess if the inhibition by 1-hexanol of *A. lineolatus* catches was a result of its chemical interactions with pheromone constituents (Table 1). Traps were set up at the edge of an alfalfa field in Érd-Elvira major (Hungary). The experiment was run from 15 July to 19 September, 2019, with 5 blocks.

Experiment 5: The aim of this experiment was to test the effect on *A. lineolatus* catches of the addition of (*E*)-cinnamaldehyde to the ternary pheromone blend (Table 1). (*E*)-Cinnamaldehyde was added in a separate dispenser. Traps were set up at the edge of an alfalfa field in the vicinity of Cegléd (Hungary). The experiment was run from 12 July to 24 September, 2018, with 4 blocks.

Experiment 6: The aim of this experiment was to test increased doses of (*E*)-4-oxo-2-hexenal in the pheromone blend based on findings of Yasuda and Higuchi (2012), and to test if (*E*)-4-oxo-2-hexenal can be substituted with (*E*)-2-hexenal in the pheromone blend (Table 1). Traps were set up at the edge of an alfalfa field in Érd-Elvira major (Hungary). The experiment was run from 15 July to 19 September, 2019, with 5 blocks.

*Statistics.* Trap catch data were tested for normality by Shapiro-Wilk test. Since experimental data were not normally distributed, nonparametric tests were used. Inspections with low catches, accounting for less than 5% of total catches of the respective experiment, were excluded from the analysis. Catch data were analyzed by Kruskal-Wallis test, and differences

between treatments were evaluated by pairwise Wilcoxon test with Benjamini-Hochberg correction. Statistical procedures were conducted using the software R (R Core Team 2016).

## RESULTS

*Field Experiment with Live Virgin A. lineolatus.* In Experiment 1, significantly more *A. lineolatus* males were found in traps baited with live virgin females than in any other treatments, and catches in other treatments did not differ from those in unbaited traps (Fig 1). No significant difference was found among treatments for female catches (total female catch: 11, Kruskal-Wallis chi-squared=4.369, P=0.224, data not shown).

*Analyses of Female Extracts and Identification of EAG-Active Constituents.* The compounds in an *A. lineolatus* extract of a female that consistently elicited male antennal responses in GC-EAG were identified by GC-MS and GC peak enhancement by co-injecting with authentic standards as hexyl butyrate [Kováts index (KI) on a polar DB-WAX column = 1420], (*E*)-2-hexenyl butyrate (KI=1478) and (*E*)-4-oxo-2-hexenal (KI=1592) (Fig. 2). Beside these compounds, a further compound elicited stable EAG responses from antennae of males, which was identified as 1-hexanol (KI=1360) (Fig. 2). Based on air entrainment samples, the average emission of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal was found to be  $0.27 \pm 0.09$ ,  $0.45 \pm 0.44$  and  $0.05 \pm 0.02$   $\mu\text{g/h/female}$ , respectively.

*Field Experiments with Identified Compounds.* In Experiment 2, the ternary blend attracted more *A. lineolatus* males than did unbaited traps (Fig. 3). Catches with the binary combination of (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal were numerically lower, but did not differ significantly from those by the ternary combination. Only very few females were caught,

treatments not differing significantly from each other (total female catch: 15, Kruskal-Wallis chi-squared=4.077, P=0.396, data not shown).

In Experiment 3, addition of 1-hexanol to the ternary pheromone blend decreased male catches significantly at 1 and 10 mg doses (Table 2). For females, no difference was found among treatments.

In Experiment 4, addition of 1-hexanol to the ternary pheromone blend reduced the catches of *A. lineolatus* males considerably both if it was loaded into the same or into separate dispensers. Only the treatments containing the ternary pheromone blend alone attracted more *A. lineolatus* males than did unbaited traps (Fig 4). For females, treatments did not differ significantly (total female catches: 6, Kruskal-Wallis chi-squared=2.106, P=0.551, data not shown).

In Experiment 5, treatments containing the ternary pheromone blend attracted more *A. lineolatus* males than unbaited traps (Fig 5). Addition of (*E*)-cinnamaldehyde to the ternary pheromone blend resulted in a non-significant increase in male catches, compared to the ternary pheromone blend. For females, treatments containing (*E*)-cinnamaldehyde attracted more individuals than unbaited traps, irrespective of the presence or absence of pheromone baits.

In Experiment 6, treatments containing the ternary blend attracted more *A. lineolatus* males than did unbaited traps, and baits with the increased dosage of (*E*)-4-oxo-2-hexenal did not attract more *A. lineolatus* males than those with the dosage of the compound in the original ternary blend (Fig. 6). Catches of traps baited with the substituted blend containing (*E*)-2-hexenal did not catch more males than did unbaited traps (Fig. 6). For females, no difference was found among treatments (total female catch: 5, Kruskal-Wallis chi-squared=2.038, P=0.564, data not shown).

## DISCUSSION

Our results on central European populations of *A. lineolatus* confirm the identity of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal as female-produced pheromone components of *A. lineolatus*, as reported previously from east Asian populations of the species (Zhang et al. 2015a). The relative importance of the compounds identified was also similar in the present study, as binary blends from which either (*E*)-2-hexenyl butyrate or (*E*)-4-oxo-2-hexenal was missing did not show activity, whereas binary combination of these compounds and the ternary blend showed similar attractiveness. Thus, it appears that populations from central Europe and east Asia are similar in respect of their pheromone communication.

The above three compounds are known as sex pheromone components of several other plant bug species, for instance *L. rugulipennis* (Innocenzi et al. 2005) and *L. pratensis* (Linnaeus, 1758) (Fountain et al. 2014), which may occur in the same habitats as *A. lineolatus*. Fountain et al. (2014) reported lesser importance of ratios for closely related *Lygus*, *Lygocoris* and *Liocoris* species, the sex pheromones of which also consist of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal. Thus, it is probable that other means of sexual communication may also be of importance for mate recognition in *A. lineolatus*, as it was found in *Lygocoris pabulinus* (Linnaeus, 1761) (Drijfhout and Groot 2001) and *L. rugulipennis* (Koczor and Cokl 2014).

Based on the findings of Yasuda and Higuchi (2012) on *S. rubrovittatus*, we tested increased dosage of (*E*)-4-oxo-2-hexenal in the pheromone blend; however, the blend with the increased dosage did not attract more *A. lineolatus* males than the original blend.

Since a compound may have multiple functions and (*E*)-4-oxo-2-hexenal is known to be an irritating compound with potential importance in defence (Moreira and Millar 2005), we tested blends where (*E*)-4-oxo-2-hexenal was substituted with (*E*)-2-hexenal, a much more

stable compound, for potential analogous effects. Nevertheless, we found that the substituted blend did not show activity.

1-Hexanol was also found in air entrainment samples of female *A. lineolatus* and elicited conclusive EAG responses from the antennae of males in this study. Surprisingly, when the compound was tested in combination with the ternary pheromone blend, it significantly decreased the number of males caught. Subsequent field experiments showed that this was the result of a biological response and was not due to a chemical reaction between 1-hexanol and the sex pheromone components, as the effect was found both if the compound was loaded in the same or in separate dispensers as the ternary pheromone blend. Conceivably, the compounds could be reacting in the air as well, even if they originate from separate lures, however likely with considerably lesser probability. Nevertheless, in our study, no difference was found between treatments in which 1-hexanol was added in the same or in separate dispensers, as catches of both were similar to those of unbaited traps.

In their laboratory study on host plant volatiles, Sun et al. (2013) found that more *A. lineolatus* adults chose solvent control over 1-hexanol in Y-tube olfactometer tests, indicating a repellent-like effect. Our study confirmed this finding for *A. lineolatus* males in field experiments, where 1-hexanol showed a remarkably strong antagonistic effect against the sex pheromone.

The ecological role of 1-hexanol for *A. lineolatus* is uncertain. Host plant volatiles are known to affect sex pheromone production and activity in insects (Landolt and Phillips 1997); for instance in *L. rugulipennis*, a closely related plant bug species, Frati et al. (2009) found that host plant odors evoked increased sex pheromone production in females. Thus, it is possible that a compound indicating unfavorable conditions of the host may negatively affect attraction of males to the sex pheromone. As another potential hypothesis, this strong antagonistic effect of 1-hexanol on male *A. lineolatus* response to the sex pheromone could originate in the

evolutionary past of speciation in the taxon. For instance, if an ancestor of *A. lineolatus* was  
 using 1-hexanol as a simple pheromone, or a pheromone component, the compound might  
 have become antagonistic during speciation as a representative of the earlier species.  
 Interestingly, 1-hexanol was found in gland extracts of a closely related eastern Asian species,  
*A. suturalis* (Zhang et al. 2014). Nevertheless, since air entrainment extracts in this study were  
 prepared from live bugs kept on green bean pods, the compound may also be connected to  
 other activities, e.g. feeding.  
 Several previous reports demonstrate the synergistic effect of plant volatiles on insect  
 attraction to sex pheromones (Landolt and Phillips 1997). This, however, was not the case for  
*A. lineolatus* males, which were not attracted to the combination of the ternary pheromone  
 blend and a previously published floral attractant, (*E*)-cinnamaldehyde, stronger than to the  
 pheromone alone. On the other hand, the presence of the sex pheromone blend did not affect  
 attraction of females to (*E*)-cinnamaldehyde, which may open up opportunities for the  
 monitoring of both sexes of this pest using a combination of sex pheromone and (*E*)-  
 cinnamaldehyde baits.  
 Insect pheromones have special importance from a practical point of view and may be applied  
 for monitoring or direct control, for instance by mating disruption (Witzgall et al. 2010).  
 However, whereas monitoring of plant bugs may be an important tool for agricultural  
 practice, mating disruption for control could have very high costs, as suggested by Yasuda  
 and Higuchi (2012). An important factor in that may be the high instability of (*E*)-4-oxo-2-  
 hexenal affecting storage and bait longevity. A further problem can be the irritative nature of  
 this compound, which highlights health and safety issues to be considered. Substitution of the  
 compound with a more stable alternative may potentially be a solution; however, as our study  
 has showed, more detailed work is needed to screen for feasible substitutes. 1-Hexanol as a  
 simple, stable and inexpensive compound may be suitable for practical use as a sex



pheromone antagonist, for example in mating disruption. Experiments are underway to assess its potential in agricultural practice.

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## REFERENCES

- Benedek P, Erdélyi Cs, Jászai VE (1970) Seasonal activity of Heteropterous species injurious to lucerne and its relations to the integrated pest control of lucerne grown for seed. *Acta Phytopathol Hun* 5:81-93
- Birkett MA (2010) The chemistry of plant signalling. In: BALUŠKA F AND NINKOVIC V (eds): *Plant communication from an ecological perspective*. Springer, Berlin-Heidelberg, pp 21-42
- Boland W, Ney P, Jaenicke L, Gassmann G (1984) A “closed-loop-stripping” technique as a versatile tool for metabolic studies of volatiles. In: SCHREIER P (ed): *Analysis of volatiles*. Walter de Gruyter, Berlin, pp 371-380
- Byers JA, Fefer D, Levi-Zada A (2013) Sex pheromone component ratios and mating isolation among three *Lygus* plant bug species of North America. *Naturwissenschaften* 100:1115-1123

- 444
- 1
- 2 445 Drijfhout FP, Groot AT (2001) Close-range attraction in *Lygocoris pabulinus* (L.). J Chem
- 3
- 4 446 Ecol 27:1133-1149
- 5
- 6
- 7 447
- 8
- 9 448 Fountain M, Jåstad G, Hall D, Douglas P, Farman D, Cross J (2014) Further studies on sex
- 10
- 11 449 pheromones of female *Lygus* and related bugs: development of effective lures and
- 12
- 13 450 investigation of species-specificity. J Chem Ecol 40:71-83
- 14
- 15
- 16 451
- 17
- 18 452 Frati F, Chamberlain K, Birkett M, Dufour S, Mayon P, Woodcock C, Wadhams L, Pickett J,
- 19
- 20 453 Salerno G, Conti E, Bin F (2009) *Vicia faba*-*Lygus rugulipennis* interactions: induced plant
- 21
- 22 454 volatiles and sex pheromone enhancement. J Chem Ecol 35:201-208
- 23
- 24
- 25 455
- 26
- 27 456 Golledge CJ 1944 The food plants of *Adelphocoris lineolatus*, Goeze. J Kansas Entomol Soc
- 28
- 29 457 17:80
- 30
- 31
- 32 458
- 33
- 34 459 Holopainen JK, Varis AL (1991) Host plants of the European tarnished plant bug *Lygus*
- 35
- 36 460 *rugulipennis* Poppius (Het., Miridae). J Appl Ent 111:484-498
- 37
- 38
- 39 461
- 40
- 41 462 Innocenzi PJ, Hall D, Cross JV, Hesketh H (2005) Attraction of male European tarnished
- 42
- 43 463 plant bug, *Lygus rugulipennis* to components of the female sex pheromone in the field. J
- 44
- 45 464 Chem Ecol 31:1401-1413
- 46
- 47
- 48 465
- 49
- 50 466 Koczor S, Cokl A (2014) Percussion signals of *Lygus rugulipennis* Poppius (Heteroptera:
- 51
- 52 467 Miridae). Cent Eur J Biol 9:543-549
- 53
- 54
- 55 468
- 56
- 57
- 58
- 59
- 60
- 61
- 62
- 63
- 64
- 65

- Koczor S, Vuts J, Tóth M (2012) Attraction of *Lygus rugulipennis* and *Adelphocoris lineolatus* to synthetic floral odour compounds in field experiments in Hungary. J Pest Sci 85:239-245
- Koczor S, Szentkirályi F, Pickett JA, Birkett MA, Tóth M (2015) Aphid sex pheromone compounds interfere with attraction of common green lacewings to floral bait. J Chem Ecol 41:550-556
- Landolt PJ, Phillips TW (1997): Host plant influences on sex pheromone behavior of phytophagous insects. Annu Rev Entomol 42:371-391
- Lu YH, Qiu F, Feng HQ, Li HB, Yang ZC, Wyckhuys KAG, Wu KM (2008) Species composition and seasonal abundance of pestiferous plant bugs (Hemiptera: Miridae) on Bt cotton in China. Crop Prot 27:465-472
- Lu Y, Wu K, Jiang Y, Xia B, Li P, Feng H, Wyckhuys KAG, Guo Y (2010) Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. Science 328:1151-1154
- Moreira JA, Millar JG (2005) Short and simple syntheses of 4-oxo-(E)-2-hexenal and homologs: Pheromone components and defensive compounds of Hemiptera. J Chem Ecol 31:965-968
- Peterson SS, Wedberg JL, Hogg DB (1992) Plant bug (Hemiptera: Miridae) damage to birdsfoot trefoil seed production. J Econ Ent 85:250-255

- Pickett JA (1990) GC-MS in insect pheromone identification: three extreme case histories, in *Chromatography and Isolation of Insect Hormones and Pheromones*, ed. by McCaffery AR and Wilson ID. Plenum Press, New York/London, pp 299–309
- R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Sun L, Gu S-H, Xiao H-J, Zhou J-J, Guo Y-Y, Liu Z-W, Zhang Y-J (2013) The preferential binding of a sensory organ specific odorant binding protein of the alfalfa plant bug *Adelphocoris lineolatus* AlinOBP10 to biologically active host plant volatiles. *J Chem Ecol* 39:1221-1231
- Tóth M, Löfstedt C, Blair BW, Cabello T, Farag AI, Hansson BS, Kovalev BG, Maini S, Nesterov EA, Pajor I, Sazonov AP, Shamshev IV, Subchev M, Szőcs G (1992) Attraction of male turnip moths *Agrotis segetum* (Lepidoptera: Noctuidae) to sex pheromone components and their mixtures at 11 sites in Europe, Asia, and Africa. *J Chem Ecol* 18:1337-1347
- Witzgall P, Kirsch P, Cork A (2010) Sex pheromones and their impact on pest management. *J Chem Ecol* 36:80-100
- Wu K, Li W, Feng H, Guo Y (2002) Seasonal abundance of mirids, *Lygus lucorum* and *Adelphocoris* spp. (Hemiptera: Miridae) on Bt cotton in northern China. *Crop Prot* 21:997-1002

Yasuda T, Higuchi H (2012) Sex pheromones of *Stenotus rubrovittatus* and *Trigonotylus*  
*caelestialium*, two mirid bugs causing pecky rice, and their application to insect monitoring in  
 Japan. Psyche Article ID 435640

Zhang Z, Luo J, Wang Y, Chen L, Chen L, Lei C (2014) Morphology and chemical analysis  
 of the metathoracic scent glands system in *Adelphocoris suturalis* (Hemiptera: Miridae). J  
 Insect Sci 14:293

Zhang T, Mei X-D, Li Y-F, Zhang K, Wu K-M, Ning J (2015a) Sex pheromone of the alfalfa  
 plant bug, *Adelphocoris lineolatus*. Entomol Exp Appl 156:263-270

Zhang T, Mei X, Zhang L, Wu K, Ning J (2015b) Identification of female sex pheromone of a  
 plant bug, *Adelphocoris fasciaticollis* Reuter (Hemiptera: Miridae). J Appl Ent 139:87-93

Zhang Z, Zhang T, Zhang A, Luo J, Chen L, Wang M, Ning J, Lei C (2016) Identification and  
 field verification of sex pheromone from the mirid bug, *Adelphocoris suturalis*.  
 Chemoecology 26:25-31

Figure legends

Fig. 1 Catches of *Adelphocoris lineolatus* males in traps baited either with live virgin *A. lineolatus* males on green bean pods, live virgin *A. lineolatus* females on green bean pods, green bean pods alone and in unbaited traps. Treatments marked with the same letter are not significantly different (Kruskal-Wallis test, pairwise comparison by Wilcoxon test with Benjamini-Hochberg correction at  $p=0.05$ )  $\Sigma$ = total number of *A. lineolatus* males caught in the experiment (box plot diagram indicating median, minimum, maximum, the 1st and 3rd quartiles of catches of the respective treatments)

Fig. 2 Coupled GC-EAG analysis of a female *Adelphocoris lineolatus* headspace extract on a male antenna, with bioactive peaks labelled. The extract used for GC-EAG was prepared by the CLSA method and shows a ratio of pheromone constituents different from that in air entrainment samples, which were used for quantitative analysis

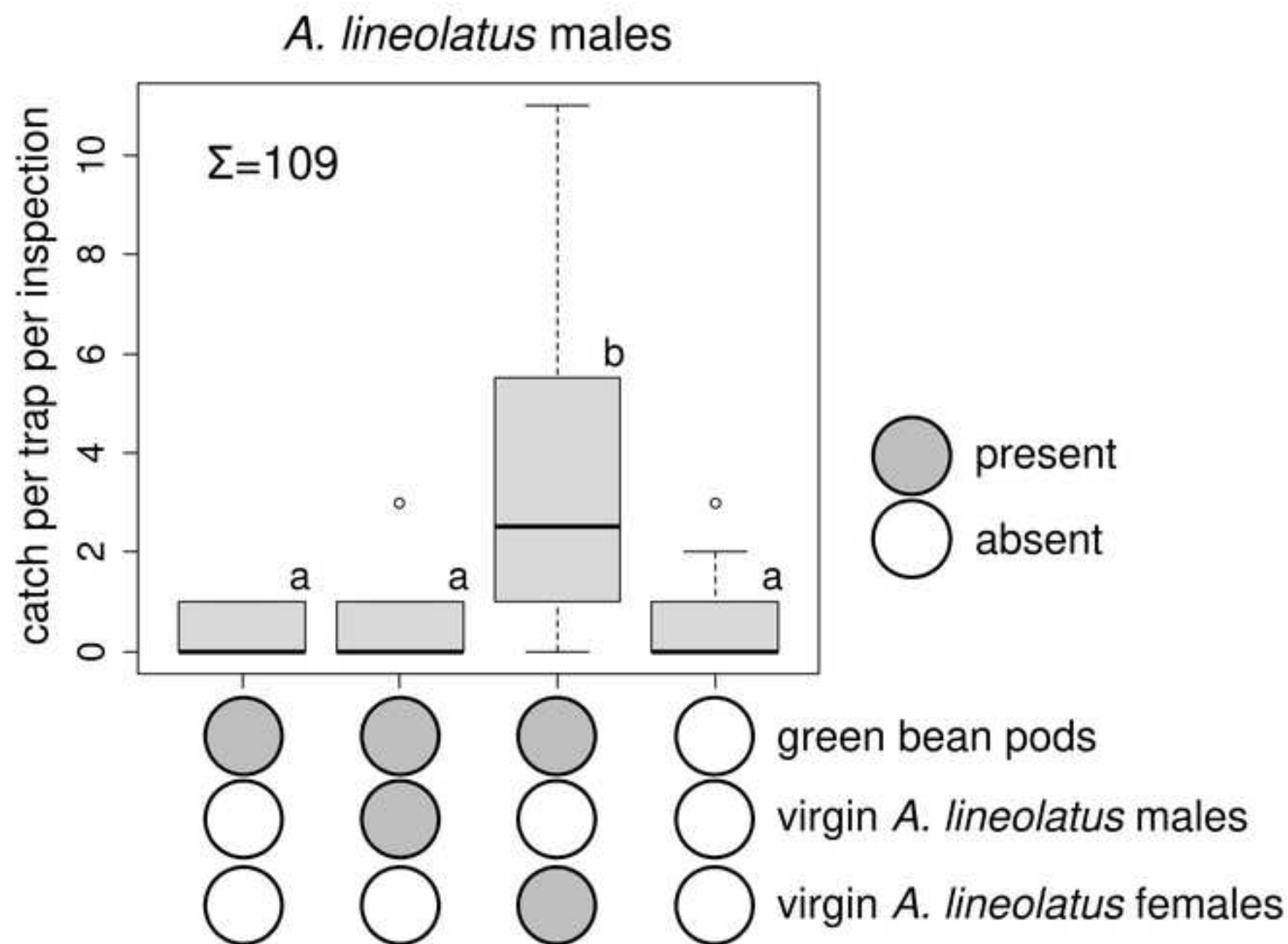
Fig. 3 Catches of *Adelphocoris lineolatus* males in traps baited with ternary and binary combinations of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal and in unbaited traps. Treatments marked with the same letter are not significantly different (Kruskal-Wallis test, pairwise comparison by Wilcoxon test with Benjamini-Hochberg correction at  $p=0.05$ )  $\Sigma$ = total number of *A. lineolatus* males caught in the experiment (box plot diagram indicating median, minimum, maximum, the 1st and 3rd quartiles of catches of the respective treatments)

Fig. 4 Catches of *Adelphocoris lineolatus* males in traps baited with ternary pheromone blend alone, with addition of 1-hexanol in the same dispenser, with addition of 1-hexanol in

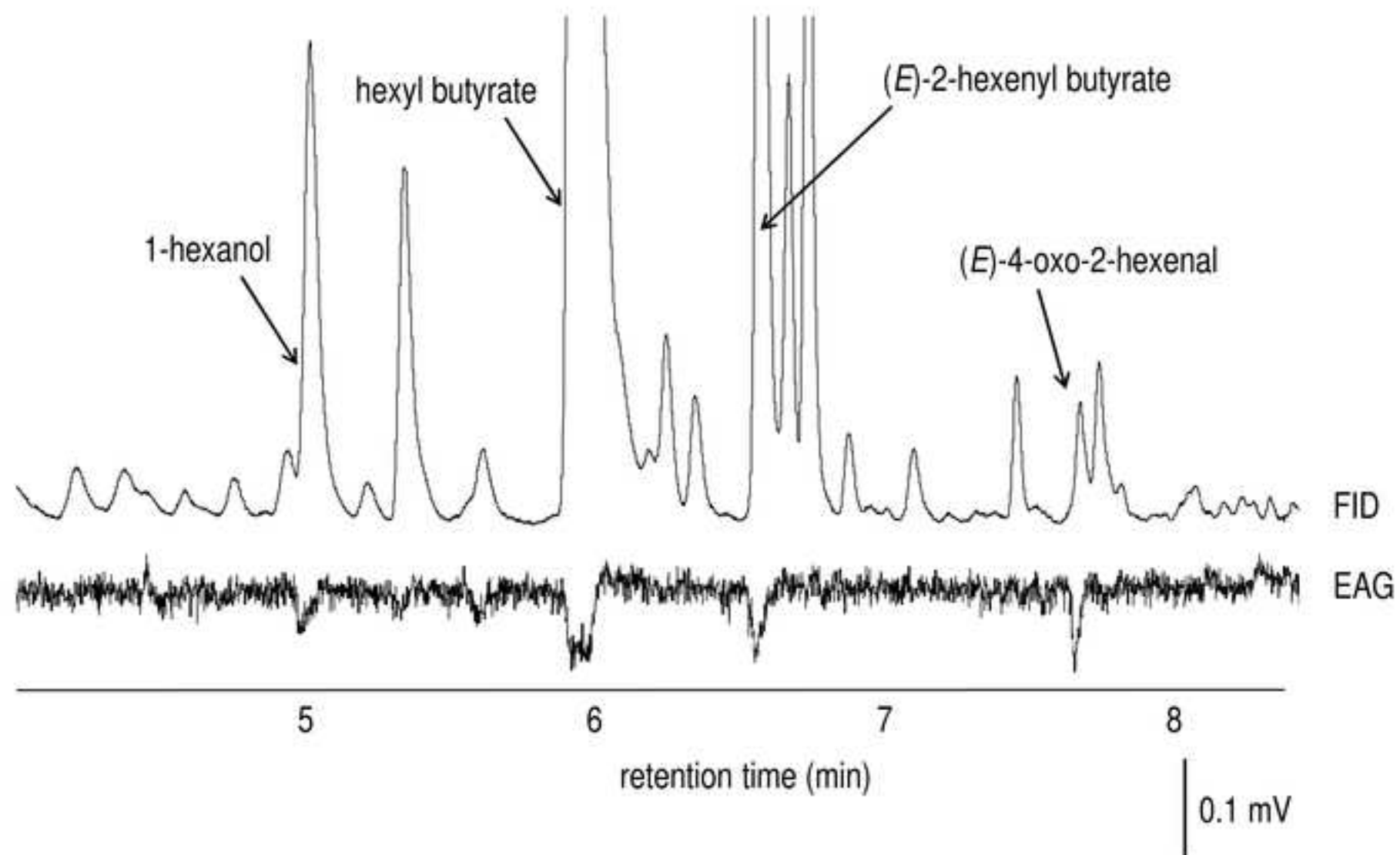
separate dispensers and in unbaited traps. Treatments marked with the same letter are not significantly different (Kruskal-Wallis test, pairwise comparison by Wilcoxon test with Benjamini-Hochberg correction at  $p=0.05$ )  $\Sigma$ = total number of *A. lineolatus* males caught in the experiment (box plot diagram indicating median, minimum, maximum, the 1st and 3rd quartiles of catches of the respective treatments)

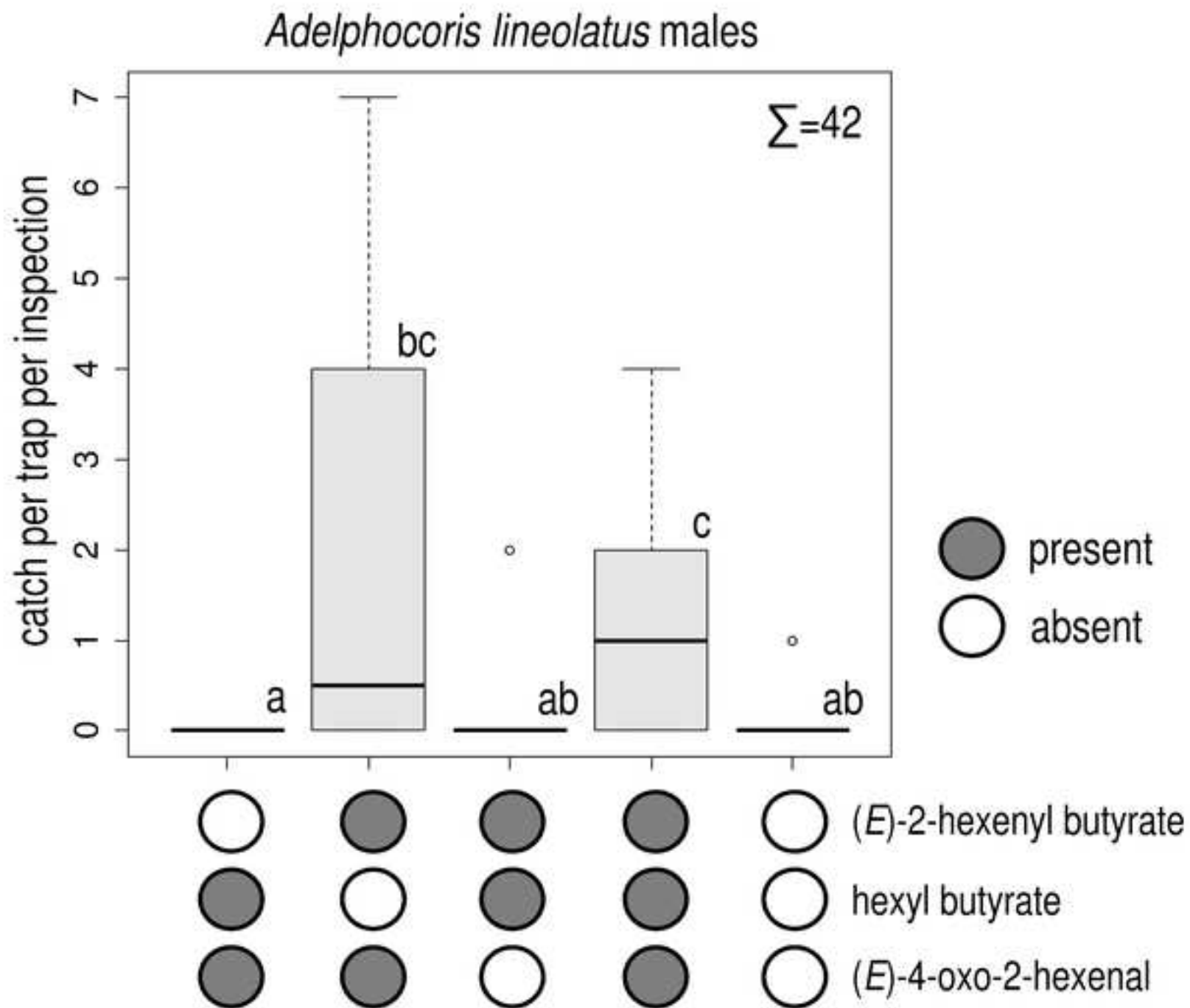
Fig. 5 Catches of *Adelphocoris lineolatus* males and females in traps baited with ternary pheromone blend, (*E*)-cinnamaldehyde, their combinations and in unbaited traps. Treatments marked with the same letter are not significantly different (Kruskal-Wallis test, pairwise comparison by Wilcoxon test with Benjamini-Hochberg correction at  $p=0.05$ )  $\Sigma$ = total number of *A. lineolatus* males/females caught in the experiment (box plot diagram indicating median, minimum, maximum, the 1st and 3rd quartiles of catches of the respective treatments)

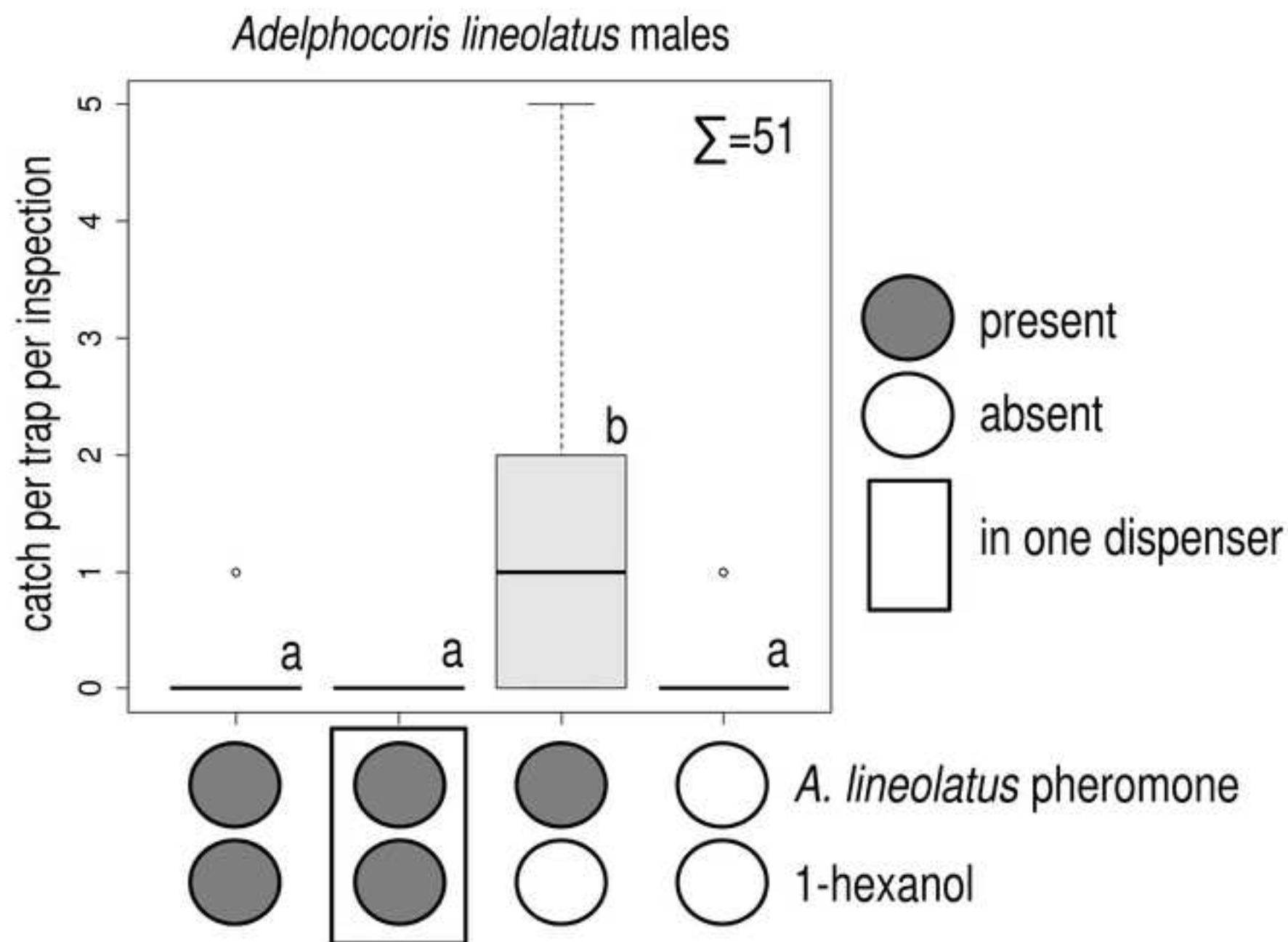
Fig. 6 Catches of *Adelphocoris lineolatus* males in traps baited with the ternary pheromone blend with original dose of (*E*)-4-oxo-2-hexenal, with 5-fold increased dose of (*E*)-4-oxo-2-hexenal, with substituted blend ((*E*)-4-oxo-2-hexenal substituted with (*E*)-2-hexenal) and in unbaited traps. Treatments marked with the same letter are not significantly different (Kruskal-Wallis test, pairwise comparison by Wilcoxon test with Benjamini-Hochberg correction at  $p=0.05$ )  $\Sigma$ = total number of *A. lineolatus* males caught in the experiment (box plot diagram indicating median, minimum, maximum, the 1st and 3rd quartiles of catches of the respective treatments)

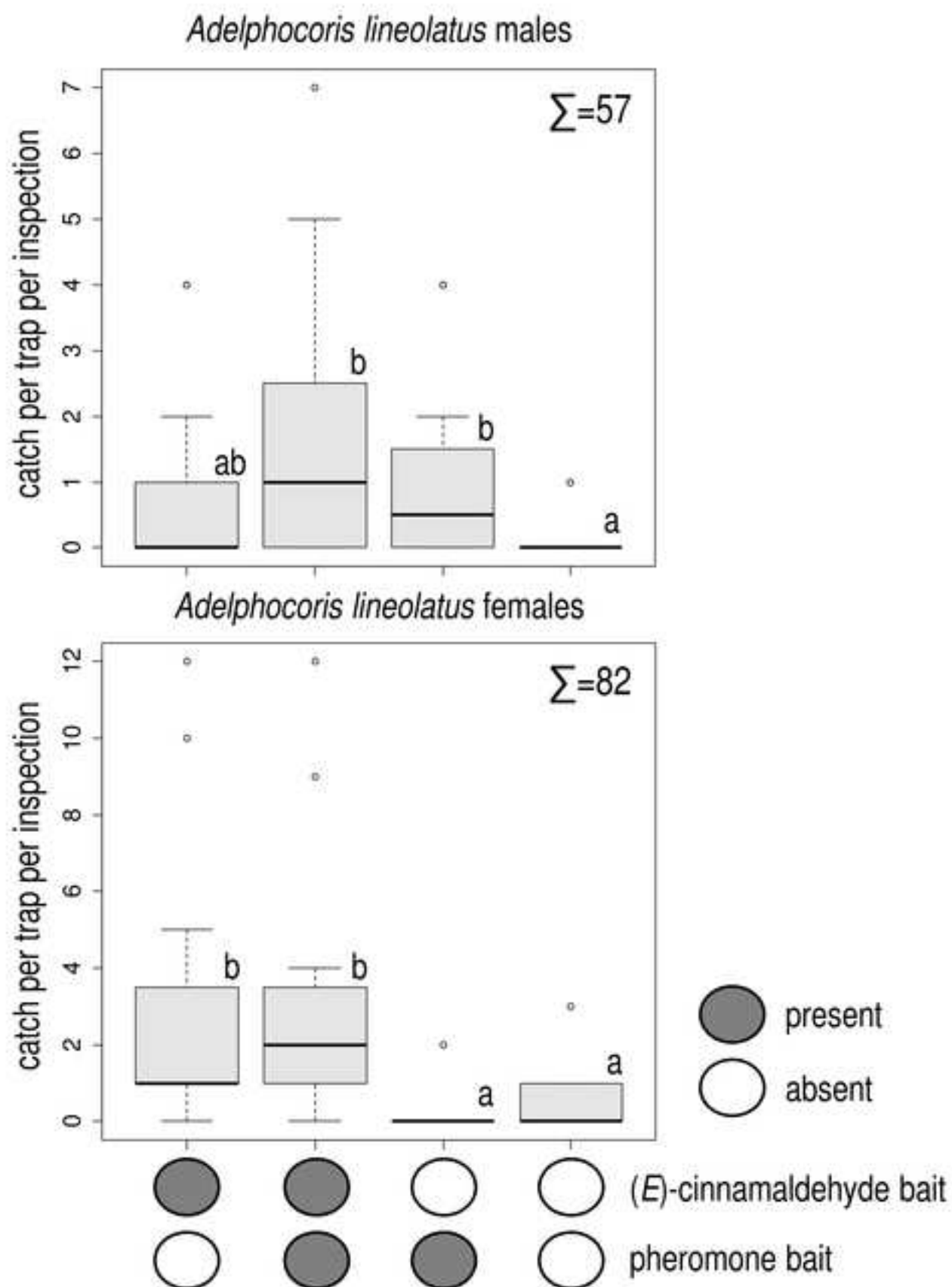












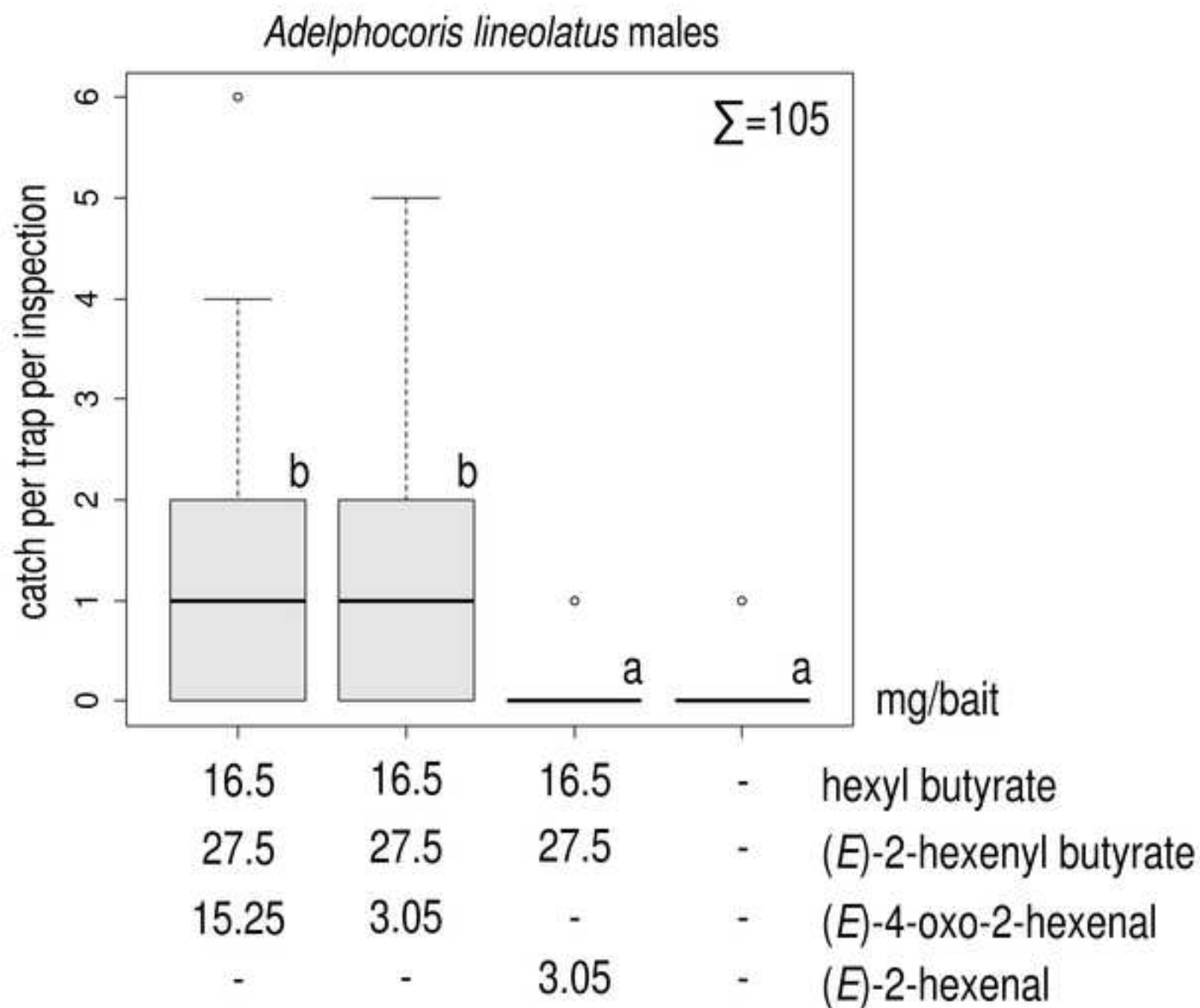


Table 1. Treatments of Experiments 2-6. '+' marks indicate the presence of a treatment in a respective experiment

treatment/bait composition*	Experiment 2	Experiment 3	Experiment 4	Experiment 5	Experiment 6
HB + E4O2H	+	–	–	–	–
E2HB + E4O2H	+	–	–	–	–
HB + E2HB	+	–	–	–	–
HB + E2HB + E4O2H	+	+	+	+	+
HB + E2HB + E4O2H + 0.1 mg 1-hexanol	–	+	–	–	–
HB + E2HB + E4O2H + 1 mg 1-hexanol	–	+	–	–	–
HB + E2HB + E4O2H + 10 mg 1-hexanol	–	+	+	–	–
HB + E2HB + E4O2H and 10 mg 1-hexanol baits	–	–	+	–	–
( <i>E</i> )-cinnamaldehyde	–	–	–	+	–
HB + E2HB + E4O2H and ( <i>E</i> )-cinnamaldehyde baits	–	–	–	+	–
HB + E2HB + 5× increased dose of E4O2H	–	–	–	–	+
HB + E2HB + ( <i>E</i> )-2-hexenal	–	–	–	–	+
no bait	+	–	+	+	+

\* Abbreviations: HB: hexyl butyrate, E2HB: (E)-2-hexenyl butyrate, E4O2H: (E)-4-oxo-2-hexenal

Table 2. Catches of *Adelphocoris lineolatus* males and females in traps baited with the ternary pheromone blend and different doses of 1-hexanol (total catch: 45 *A. lineolatus*)

		catch per trap per inspection $\pm$ SE*	
		<i>Adelphocoris lineolatus</i>	
pheromone blend	dose of 1-hexanol	males	females
present	–	1.25 $\pm$ 0.43 b	0.62 $\pm$ 0.32 a
present	0.1 mg	0.75 $\pm$ 0.37 b	0 $\pm$ 0 a
present	1 mg	0 $\pm$ 0 a	0 $\pm$ 0 a
present	10 mg	0 $\pm$ 0 a	0 $\pm$ 0 a

\* Treatments marked with the same letter are not significantly different (Kruskal-Wallis test, pairwise Wilcoxon test with Benjamini-Hochberg correction at P=0.05)



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**Supplementary Material**

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