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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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	Biotechnology and Biological Sciences Research Council's Industrial Strategy Challenge Fund (BBS/OS/CP/000001)	Not applicable
Abstract:	Abstract – The sex pheromone composition of alfalfa plant bugs, <i>Adelphocoris lineolatus</i> (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 <i>A. lineolatus</i> 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-butylate 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.

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'



[Click here to view linked References](#)

1 SEX PHEROMONE OF THE ALFALFA PLANT BUG, *ADELPHOCORIS LINEOLATUS*:
2 PHEROMONE COMPOSITION AND ANTAGONISTIC EFFECT OF 1-HEXANOL
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5 3 (HEMIPTERA: MIRIDAE)
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18 **Abstract** – The sex pheromone composition of alfalfa plant bugs, *Adelphocoris lineolatus*
19 (Goeze, 1778), from Central Europe was investigated to test the hypothesis that insect species
20 across a wide geographical area can vary in their pheromone composition. Potential
21 interactions between the pheromone and a known attractant, (*E*)-cinnamaldehyde, were also
22 assessed. Coupled gas chromatography-electroantennography (GC-EAG) using male *A.*
23 *lineolatus* antennae and volatile extracts collected from females, which had been shown to
24 attract males in earlier field experiments, revealed the presence of three physiologically active
25 compounds, which were identified by coupled GC-mass spectrometry (GC-MS) and GC peak
26 enhancement as hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal. A ternary
27 blend of these compounds in 5.4:9.0:1.0 ratio attracted male *A. lineolatus* in field trials in
28 Hungary. Omission of either (*E*)-2-hexenyl-butyrate or (*E*)-4-oxo-2-hexenal from the ternary
29 blend or substitution of (*E*)-4-oxo-2-hexenal by (*E*)-2-hexenal resulted in loss of activity.
30 These results indicate that the studied Central European population is similar in its pheromone
31 composition to that of a previously reported East Asian population. Interestingly, another
32 EAG-active compound, 1-hexanol, was also present in extracts of females. When tested in
33 combination with the ternary pheromone blend, 1-hexanol significantly reduced male catches.
34 This antagonism produced a dose-response effect with relatively small doses showing
35 remarkable influence, which suggests that 1-hexanol may act as a sex pheromone antagonist
36 for *A. lineolatus*. Furthermore, when field tested in combination with the sex pheromone, (*E*)-
37 cinnamaldehyde did not increase male catches significantly; however, the combination
38 attracted both males and females. Prospects for practical applications are discussed.

39
40 **Key Words** – *Adelphocoris lineolatus*, sex pheromone, field attraction, electroantennography,
41 Miridae.

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1
2 44 **Declarations:**
3

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5
6 and Innovation Office (NKFIH, grant FK134744). The work at Rothamsted Research formed
7 46
8 part of the Smart Crop Protection (SCP) strategic programme (BBS/OS/CP/000001) funded
9 47
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11 48
12 Challenge Fund.
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14 49
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16 50
17
18 51 **Conflicts of interest/Competing interests.** Not applicable.
19
20

21 52 **Ethics approval.** Not applicable.
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23

24 53 **Consent to participate.** Not applicable.
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27 54 **Consent for publication.** Not applicable.
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29

30 55 **Availability of data and material.** Not applicable.
31
32

33 56 **Code availability.** Not applicable.
34
35

36 57 **Author contributions.** S. Koczor, J.A. Pickett, M.A. Birkett, M. Tóth and J. Vuts conceived
37 58 and designed the experiments. É. Bálintné Csonka, A. Sarria and J. Vuts performed volatile
38 59 collections, GC-EAG was done by S. Koczor. Chemical analysis of the samples and
40 identification of compounds was done by J. Vuts, J.C. Caulfield, M.A. Birkett and A. Sarria.
41 60
42 D.M. Withall performed the synthesis of pheromone compounds. S. Koczor conducted the
43 61
44 field experiments, determined the collected material and analysed data. S. Koczor wrote a first
45 62
46 draft of the manuscript. All authors read, contributed to and approved the final manuscript.
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INTRODUCTION

70 Plant bugs (Heteroptera: Miridae) represent the most species-rich family of true bugs. Several
71 species are pests, and some have an extremely wide spectrum of potential hosts (e.g.
72 Holopainen and Varis 1991). Due to new pest control technologies and recent changes in
73 regulation, there is a marked and continuous decrease in the use of broad-spectrum
74 insecticides in agriculture. As a consequence, pests considered previously to be of minor
75 importance become more damaging, as observed for genetically engineered lepidopteran-
76 resistant crops, such as Bt-cotton (Lu et al. 2010). Furthermore, this effect may reach beyond
77 the crop initially affected. Lu et al. (2010) found that broad-spectrum insecticide sprayings
78 may result in 'sink' populations of a particular pest, but without such treatments, they can
79 reach high abundance and create 'source' populations, which results in higher levels of
80 damage in other crops as well. *Adelphocoris* species are among those pests that have gained
81 increasing economic importance with the decrease in broad-spectrum insecticide use (Lu et al.
82 2008).

83 The alfalfa plant bug, *Adelphocoris lineolatus* Goeze (1778), occurs widely in the Palearctic,
84 where it is a major pest of alfalfa (*Medicago sativa* L., Fabaceae) (Benedek et al. 1970);
85 however, several other potential hosts have also been reported (Golledge 1944; Peterson et al.
86 1992). Currently, the most serious economic impact of *Adelphocoris* spp., including *A.*
87 *lineolatus*, is associated with the damage caused to Bt-cotton in China (Wu et al. 2002; Lu et
88 al. 2008).

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

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

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

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

118 Thus, the aims of this paper were 1) to determine the pheromone composition of a central
119 European population of *A. lineolatus*, representing a different geographic region, 2) to test
120 increased dosage of (*E*)-4-oxo-2-hexenal, 3) to test (*E*)-2-hexenal as a more stable, potential
121 analogue for (*E*)-4-oxo-2-hexenal, and 4) to assess potential interactions between the sex
122 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

142 volatiles were eluted from the charcoal filter with 25 μ L dichloromethane (Merck KGaA,
1
2 143 Darmstadt, Germany).

3
4 144 To determine pheromone emission patterns, dynamic headspace collection (air entrainment)
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7 145 (Birkett 2010) was also done with single *A. lineolatus* females on green bean pods for 24 h at
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9 146 14:10 light:dark period, 20°C and ca. 50% relative humidity. The material to be sampled was
10
11 147 placed in a 380 mL glass jar, and activated charcoal-filtered (Capillary-Grade Hydrocarbon
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13 148 Trap with 1/8 in. compression fittings; Thames Restek Ltd., High Wycombe, UK) air was
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15 149 supplied by a pump system (Pye volatile collection kits, Kings Walden, UK) through the inlet
16
17 150 port at a rate of 600 mL/min. Air subsequently passed over the material in the jar and
18
19 151 headspace volatiles were adsorbed on Porapak Q filters (0.05 g, 50/80 mesh; Supelco) that
20
21 152 were fitted on the outlet port, through which air was drawn at a rate of 500 mL/min. All
22
23 153 connections in the air entrainment setup were made using PTFE tubing. Prior to entrainment,
24
25 154 Porapak Q filters were washed with diethyl ether and conditioned by heating to 132°C in an
26
27 155 activated charcoal-filtered nitrogen stream for 2 h. Entrained volatiles were eluted with 750
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29 156 μ L redistilled diethyl ether and stored in 1.1 mL glass microvials at -20°C until analysis.
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31 157 Glass jars were washed with detergent (Teepol), acetone and distilled water, and baked
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33 158 overnight at 140°C. The sampling was replicated four times.
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43 160 *Coupled Gas Chromatography-Electroantennography (GC-EAG)*. Female air entrainment
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45 161 extracts were tested for electroantennographic activity on antennae from males by coupled
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47 162 GC-EAG using an Agilent 6890N gas chromatograph equipped with a DB-WAX column with
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49 163 polyethylene glycol phase (30 m \times 0.32 mm i.d.). Helium was used as carrier gas, injection
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51 164 was performed in splitless mode. Temperature program started at 60°C and increased to
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53 165 220°C by 10°C/min. The effluent was split between the GC-FID and a heated transfer line to
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55 166 the EAG apparatus. For each test, 1 μ L aliquots of the air entrainment extracts and 10 ng
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167 tetradecyl acetate as internal standard in 1 μ L dichloromethane solution were co-injected. For
168 EAG, the male antenna was freshly cut at the base from a live bug, and the tip of the last
169 segment was cut off to ensure better connection. The antenna was mounted between two glass
170 capillaries containing Ringer solution. One of the electrodes was grounded, while the other
171 was connected to a high-impedance DC amplifier (IDAC-232, Ockenfels Syntech GmbH,
172 Kirchzarten, Germany). A compound was defined as EAG-active if it evoked an antennal
173 response, distinguishable from background noise, in at least three coupled runs.

174
175 *Identification of EAG-Active Compounds.* For the identification of electrophysiologically
176 active compounds in air entrainment samples, a Hewlett-Packard 5890 series II GC fitted with
177 a capillary DB-WAX GC column (30 m \times 0.32 mm i.d., 0.5 μ m film thickness; J&W
178 Scientific, Folsom, CA) and equipped with a cool on-column injector was directly coupled to
179 a mass spectrometer (Hewlett-Packard 5972 mass-selective detector). Ionisation was by
180 electron impact at 70 eV, 220°C. The oven temperature was maintained at 40°C for 1 min and
181 then programmed at 5°C/min to 250°C (hold time 17.2 min). The carrier gas was helium.
182 Tentative identification by GC-MS was confirmed by comparing retention indices of peaks
183 with those of synthetic standards and by peak enhancement on GC by coinjection with
184 authentic compounds (Pickett 1990), using an Agilent 7890A GC equipped with a cool on-
185 column injector, FID and a 30 m \times 0.32 mm i.d., 0.52 μ m film thickness DB-WAX column.
186 The oven temperature was maintained at 30°C for 0.5 min and then programmed at 5°C/min
187 to 150°C for 0.1 min, then 10°C/min to 230°C for 25 min. The carrier gas was hydrogen.
188 Quantification of compounds was achieved using the multiple-point external standard method,
189 whereby concentration ranges of synthetic standards of the pheromone compounds provided
190 calibration curves.

192 *Chemicals*. Hexyl butyrate, (*E*)-2-hexenyl butyrate, (*E*)-cinnamaldehyde and 1-hexanol
193 ($\geq 96\%$ purity as per the manufacturer) were obtained from Sigma-Aldrich Kft (Budapest,
194 Hungary). (*E*)-4-Oxo-2-hexenal was synthesized as follows: To a solution of 2-ethylfuran
195 (10.00 g, 104.03 mmol) in a mixture of THF (100 mL), acetone (80 mL) and water (40 mL),
196 cooled to $-15\text{ }^{\circ}\text{C}$ under nitrogen, was added *N*-bromosuccinimide (27.78 g, 156.04 mmol),
197 followed by pyridine (16.8 mL, 208.06 mmol). The reaction mixture was stirred for 30 mins
198 before being warmed to $0\text{ }^{\circ}\text{C}$ for a further 3 hours. The reaction mixture was poured into 0.5M
199 HCl and extracted with EtOAc. The combined organics were washed with water, dried
200 (MgSO_4) and concentrated under vacuum. The crude product was purified on silica gel (20%
201 EtOAc in pet ether) to give (*E*)-4-oxo-2-hexenal (4.42 g, 37% yield) as an orange oil. $^1\text{H-NMR}$
202 (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, $J = 16.2$
203 and 7.2 Hz), 2.75 (qu, 2H, $J = 7.3\text{ Hz}$), 1.18 (t, 3H, $J = 7.2\text{ Hz}$). $^{13}\text{C-NMR}$ (CDCl_3 , 500 MHz):
204 200.38, 193.46, 144.78, 137.30, 34.54 & 7.55. Due to its inherent instability, the compound
205 was stored as a 1:1 solution in dichloromethane at $-80\text{ }^{\circ}\text{C}$ until required for use.

207 Field tests

208 *Field Experiment with Live Virgin A. lineolatus*. Experiment 1. This test was performed at
209 Pusztazámor, Hungary, at the edge of an alfalfa field from July 15 to August 8, 2013. Four
210 different treatments were applied: three virgin females on a green bean pod, three virgin males
211 on a green bean pod, a green bean pod without insects and an empty control. Traps consisted
212 of a plastic roof ($27\times 24\text{ cm}$) with its upper side covered with aluminum foil (I.S.X.-TRADE
213 Kft., Budapest, Hungary) to prevent insolation. To the roof, a transparent sticky PVC sheet
214 ($23\times 36\text{ cm}$) was attached with pegs, its sticky side facing inwards. The bugs and pods were
215 placed in $9.5\times 4\text{ cm}$ cylindrical containers made of transparent PVC foil, and closed at both
216 ends with fine mesh. The containers were fixed to the bottom side of the roof. At each

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

222
223 *Field Experiments with Synthetic Compounds.* Ternary pheromone baits were prepared as
224 follows: hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal were formulated into
225 0.7 mL polyethylene vials with lid (No. 730, Kartell Co., Italy) in 5.4:9.0:1.0 ratio,
226 respectively. Total load of baits was kept at 50 mg. Binary combinations in Experiment 2
227 were prepared with the same load of the respective compounds. For Experiment 3, 0.1, 1 or
228 10 mg of 1-hexanol was added to the ternary pheromone blend. The lids of the vials were
229 closed and the dispensers were attached to 8×1 cm plastic handles for easy handling when
230 assembling the traps. The dispensers were kept in the shade under the roof of traps and
231 equipped with a loosely applied aluminum foil to provide protection from light, since (*E*)-4-
232 oxo-2-hexenal is known to be light-sensitive (Fountain et al. 2014). For Experiment 4, 10 mg
233 of 1-hexanol was added to the ternary pheromone blend as described above and separate
234 polyethylene vial baits were also prepared with 10 mg load of 1-hexanol. These were also
235 closed but no shading was added to these baits.

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

242 polyethylene vial dispensers were replaced at 4-5 week intervals and polyethylene bag
243 dispensers were replaced at 3-4 week intervals, as previous experience showed that they do
244 not lose their attractiveness during this period (Koczor et al. 2012, 2015).
245 For storage, all dispensers used in the experiments were wrapped singly in pieces of
246 aluminum foil and stored at -18°C until used. For field testing of synthetic compounds,
247 CSALOMON® VARL+ funnel traps were used (produced by the Plant Protection Institute,
248 CAR, Budapest, Hungary), which proved to be suitable for catching plant bugs (Koczor et al.
249 2012). A small piece (1×1 cm) of household anti-moth strip (Chemotox®, Sara Lee; Temana
250 Intl. Ltd, Slough, UK; active ingredient 15% dichlorvos) was placed in the containers to kill
251 captured insects. The experiments were performed in randomized complete block design, that
252 is, one replicate of each treatment was incorporated into a block, so that individual treatments
253 were 5-8 m apart in a randomized arrangement. Distance between blocks was 10-15 meters.
254 To avoid positional effects, trap positions were changed regularly on a fortnightly basis. As a
255 rule, traps were inspected weekly, and catches were brought to the laboratory, where collected
256 individuals were sexed and determined to species.

257

258 Details of individual experiments:

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

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264 Experiment 3: The aim of this experiment was to test addition of 1-hexanol to the ternary
265 pheromone blend, containing hexyl butyrate + (*E*)-2-hexenyl butyrate + (*E*)-4-oxo-2-hexenal.
266 1-Hexanol was loaded in 0.1, 1, or 10 mg dose in the same bait dispensers (Table 1). Traps

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

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

275
276 Experiment 5: The aim of this experiment was to test the effect on *A. lineolatus* catches of the
277 addition of (*E*)-cinnamaldehyde to the ternary pheromone blend (Table 1). (*E*)-
278 Cinnamaldehyde was added in a separate dispenser. Traps were set up at the edge of an alfalfa
279 field in the vicinity of Cegléd (Hungary). The experiment was run from 12 July to 24
280 September, 2018, with 4 blocks.

281
282 Experiment 6: The aim of this experiment was to test increased doses of (*E*)-4-oxo-2-hexenal
283 in the pheromone blend based on findings of Yasuda and Higuchi (2012), and to test if (*E*)-4-
284 oxo-2-hexenal can be substituted with (*E*)-2-hexenal in the pheromone blend (Table 1). Traps
285 were set up at the edge of an alfalfa field in Érd-Elvira major (Hungary). The experiment was
286 run from 15 July to 19 September, 2019, with 5 blocks.

287
288 *Statistics.* Trap catch data were tested for normality by Shapiro-Wilk test. Since experimental
289 data were not normally distributed, nonparametric tests were used. Inspections with low
290 catches, accounting for less than 5% of total catches of the respective experiment, were
291 excluded from the analysis. Catch data were analyzed by Kruskal-Wallis test, and differences

292 between treatments were evaluated by pairwise Wilcoxon test with Benjamini-Hochberg
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2 293 correction. Statistical procedures were conducted using the software R (R Core Team 2016).

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RESULTS

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12 297 *Field Experiment with Live Virgin A. lineolatus*. In Experiment 1, significantly more *A.*
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14 298 *lineolatus* males were found in traps baited with live virgin females than in any other
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16 299 treatments, and catches in other treatments did not differ from those in unbaited traps (Fig 1).
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19 300 No significant difference was found among treatments for female catches (total female catch:
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21 301 11, Kruskal-Wallis chi-squared=4.369, P=0.224, data not shown).

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26 303 *Analyses of Female Extracts and Identification of EAG-Active Constituents*. The compounds
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28 304 in an *A. lineolatus* extract of a female that consistently elicited male antennal responses in
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30 305 GC-EAG were identified by GC-MS and GC peak enhancement by co-injecting with
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34 306 authentic standards as hexyl butyrate [Kováts index (KI) on a polar DB-WAX column =
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36 307 1420], (*E*)-2-hexenyl butyrate (KI=1478) and (*E*)-4-oxo-2-hexenal (KI=1592) (Fig. 2). Beside
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38 308 these compounds, a further compound elicited stable EAG responses from antennae of males,
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41 309 which was identified as 1-hexanol (KI=1360) (Fig. 2). Based on air entrainment samples, the
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44 310 average emission of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal was
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46 311 found to be 0.27 ± 0.09 , 0.45 ± 0.44 and 0.05 ± 0.02 $\mu\text{g/h/female}$, respectively.

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51 313 *Field Experiments with Identified Compounds*. In Experiment 2, the ternary blend attracted
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53 314 more *A. lineolatus* males than did unbaited traps (Fig. 3). Catches with the binary combination
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56 315 of (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal were numerically lower, but did not differ
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58 316 significantly from those by the ternary combination. Only very few females were caught,

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2 317 treatments not differing significantly from each other (total female catch: 15, Kruskal-Wallis
3 318 chi-squared=4.077, P=0.396, data not shown).

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5 319 In Experiment 3, addition of 1-hexanol to the ternary pheromone blend decreased male
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7 320 catches significantly at 1 and 10 mg doses (Table 2). For females, no difference was found
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9 321 among treatments.

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11 322 In Experiment 4, addition of 1-hexanol to the ternary pheromone blend reduced the catches of
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13 323 *A. lineolatus* males considerably both if it was loaded into the same or into separate
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15 324 dispensers. Only the treatments containing the ternary pheromone blend alone attracted more
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17 325 *A. lineolatus* males than did unbaited traps (Fig 4). For females, treatments did not differ
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19 326 significantly (total female catches: 6, Kruskal-Wallis chi-squared=2.106, P=0.551, data not
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21 327 shown).

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23 328 In Experiment 5, treatments containing the ternary pheromone blend attracted more *A.*
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25 329 *lineolatus* males than unbaited traps (Fig 5). Addition of (*E*)-cinnamaldehyde to the ternary
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27 330 pheromone blend resulted in a non-significant increase in male catches, compared to the
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29 331 ternary pheromone blend. For females, treatments containing (*E*)-cinnamaldehyde attracted
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31 332 more individuals than unbaited traps, irrespective of the presence or absence of pheromone
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33 333 baits.

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35 334 In Experiment 6, treatments containing the ternary blend attracted more *A. lineolatus* males
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37 335 than did unbaited traps, and baits with the increased dosage of (*E*)-4-oxo-2-hexenal did not
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39 336 attract more *A. lineolatus* males than those with the dosage of the compound in the original
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41 337 ternary blend (Fig. 6). Catches of traps baited with the substituted blend containing (*E*)-2-
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43 338 hexenal did not catch more males than did unbaited traps (Fig. 6). For females, no difference
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45 339 was found among treatments (total female catch: 5, Kruskal-Wallis chi-squared=2.038,
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47 340 P=0.564, data not shown).

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DISCUSSION

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

352 The above three compounds are known as sex pheromone components of several other plant
353 bug species, for instance *L. rugulipennis* (Innocenzi et al. 2005) and *L. pratensis* (Linnaeus,
354 1758) (Fountain et al. 2014), which may occur in the same habitats as *A. lineolatus*. Fountain
355 et al. (2014) reported lesser importance of ratios for closely related *Lygus*, *Lygocoris* and
356 *Liocoris* species, the sex pheromones of which also consist of hexyl butyrate, (*E*)-2-hexenyl
357 butyrate and (*E*)-4-oxo-2-hexenal. Thus, it is probable that other means of sexual
358 communication may also be of importance for mate recognition in *A. lineolatus*, as it was
359 found in *Lygocoris pabulinus* (Linnaeus, 1761) (Drijfhout and Groot 2001) and *L.*
360 *rugulipennis* (Koczor and Cokl 2014).

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

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

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2 367 stable compound, for potential analogous effects. Nevertheless, we found that the substituted
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4 368 blend did not show activity.
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6 369 1-Hexanol was also found in air entrainment samples of female *A. lineolatus* and elicited
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8 370 conclusive EAG responses from the antennae of males in this study. Surprisingly, when the
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10 371 compound was tested in combination with the ternary pheromone blend, it significantly
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12 372 decreased the number of males caught. Subsequent field experiments showed that this was the
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14 373 result of a biological response and was not due to a chemical reaction between 1-hexanol and
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16 374 the sex pheromone components, as the effect was found both if the compound was loaded in
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18 375 the same or in separate dispensers as the ternary pheromone blend. Conceivably, the
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20 376 compounds could be reacting in the air as well, even if they originate from separate lures,
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22 377 however likely with considerably lesser probability. Nevertheless, in our study, no difference
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24 378 was found between treatments in which 1-hexanol was added in the same or in separate
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26 379 dispensers, as catches of both were similar to those of unbaited traps.
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29 380 In their laboratory study on host plant volatiles, Sun et al. (2013) found that more *A.*
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31 381 *lineolatus* adults chose solvent control over 1-hexanol in Y-tube olfactometer tests, indicating
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33 382 a repellent-like effect. Our study confirmed this finding for *A. lineolatus* males in field
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35 383 experiments, where 1-hexanol showed a remarkably strong antagonistic effect against the sex
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37 384 pheromone.
38
39 385 The ecological role of 1-hexanol for *A. lineolatus* is uncertain. Host plant volatiles are known
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41 386 to affect sex pheromone production and activity in insects (Landolt and Phillips 1997); for
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43 387 instance in *L. rugulipennis*, a closely related plant bug species, Frati et al. (2009) found that
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45 388 host plant odors evoked increased sex pheromone production in females. Thus, it is possible
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47 389 that a compound indicating unfavorable conditions of the host may negatively affect attraction
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49 390 of males to the sex pheromone. As another potential hypothesis, this strong antagonistic effect
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51 391 of 1-hexanol on male *A. lineolatus* response to the sex pheromone could originate in the
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2 392 evolutionary past of speciation in the taxon. For instance, if an ancestor of *A. lineolatus* was
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4 393 using 1-hexanol as a simple pheromone, or a pheromone component, the compound might
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6 394 have become antagonistic during speciation as a representative of the earlier species.
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8 395 Interestingly, 1-hexanol was found in gland extracts of a closely related eastern Asian species,
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10 396 *A. suturalis* (Zhang et al. 2014). Nevertheless, since air entrainment extracts in this study were
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12 397 prepared from live bugs kept on green bean pods, the compound may also be connected to
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14 398 other activities, e.g. feeding.
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16 399 Several previous reports demonstrate the synergistic effect of plant volatiles on insect
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18 400 attraction to sex pheromones (Landolt and Phillips 1997). This, however, was not the case for
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20 401 *A. lineolatus* males, which were not attracted to the combination of the ternary pheromone
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22 402 blend and a previously published floral attractant, (*E*)-cinnamaldehyde, stronger than to the
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24 403 pheromone alone. On the other hand, the presence of the sex pheromone blend did not affect
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26 404 attraction of females to (*E*)-cinnamaldehyde, which may open up opportunities for the
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28 405 monitoring of both sexes of this pest using a combination of sex pheromone and (*E*-
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30 406 cinnamaldehyde baits.
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36 407 Insect pheromones have special importance from a practical point of view and may be applied
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38 408 for monitoring or direct control, for instance by mating disruption (Witzgall et al. 2010).
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41 409 However, whereas monitoring of plant bugs may be an important tool for agricultural
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43 410 practice, mating disruption for control could have very high costs, as suggested by Yasuda
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45 411 and Higuchi (2012). An important factor in that may be the high instability of (*E*)-4-oxo-2-
46
47 412 hexenal affecting storage and bait longevity. A further problem can be the irritative nature of
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49 413 this compound, which highlights health and safety issues to be considered. Substitution of the
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51 414 compound with a more stable alternative may potentially be a solution; however, as our study
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53 415 has showed, more detailed work is needed to screen for feasible substitutes. 1-Hexanol as a
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55 416 simple, stable and inexpensive compound may be suitable for practical use as a sex
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2 417 pheromone antagonist, for example in mating disruption. Experiments are underway to assess
3 418 its potential in agricultural practice.

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20
21 428 REFERENCES

22
23 429 Benedek P, Erdélyi Cs, Jászai VE (1970) Seasonal activity of Heteropterous species injurious
24
25 430 to lucerne and its relations to the integrated pest control of lucerne grown for seed. Acta
26
27 431 Phytopathol Hun 5:81-93

28
29
30 432

31
32 433 Birkett MA (2010) The chemistry of plant signalling. In: BALUŠKA F AND NINKOVIC V
33
34
35 434 (eds): Plant communication from an ecological perspective. Springer, Berlin-Heidelberg, pp
36
37 435 21-42

38
39
40 436

41
42 437 Boland W, Ney P, Jaenickea L, Gassmann G (1984) A “closed-loop-stripping” technique as a
43
44
45 438 versatile tool for metabolic studies of volatiles. In: SCHREIER P (ed): *Analysis of volatiles*.
46
47 439 Walter de Gruyter, Berlin, pp 371-380

48
49
50 440

51
52 441 Byers JA, Fefer D, Levi-Zada A (2013) Sex pheromone component ratios and mating
53
54
55 442 isolation among three *Lygus* plant bug species of North America. *Naturwissenschaften*
56
57 443 100:1115-1123

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1
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65

445 Drijfhout FP, Groot AT (2001) Close-range attraction in *Lygocoris pabulinus* (L.). J Chem
446 Ecol 27:1133-1149

447
448 Fountain M, Jåstad G, Hall D, Douglas P, Farman D, Cross J (2014) Further studies on sex
449 pheromones of female *Lygus* and related bugs: development of effective lures and
450 investigation of species-specificity. J Chem Ecol 40:71-83

451
452 Frati F, Chamberlain K, Birkett M, Dufour S, Mayon P, Woodcock C, Wadhams L, Pickett J,
453 Salerno G, Conti E, Bin F (2009) *Vicia faba*-*Lygus rugulipennis* interactions: induced plant
454 volatiles and sex pheromone enhancement. J Chem Ecol 35:201-208

455
456 Golledge CJ 1944 The food plants of *Adelphocoris lineolatus*, Goeze. J Kansas Entomol Soc
457 17:80

458
459 Holopainen JK, Varis AL (1991) Host plants of the European tarnished plant bug *Lygus*
460 *rugulipennis* Poppius (Het., Miridae). J Appl Ent 111:484-498

461
462 Innocenzi PJ, Hall D, Cross JV, Hesketh H (2005) Attraction of male European tarnished
463 plant bug, *Lygus rugulipennis* to components of the female sex pheromone in the field. J
464 Chem Ecol 31:1401-1413

465
466 Koczor S, Cokl A (2014) Percussion signals of *Lygus rugulipennis* Poppius (Heteroptera:
467 Miridae). Cent Eur J Biol 9:543-549

- 1
2
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47
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49
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51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
- 469 Koczor S, Vuts J, Tóth M (2012) Attraction of *Lygus rugulipennis* and *Adelphocoris*
470 *lineolatus* to synthetic floral odour compounds in field experiments in Hungary. J Pest Sci
471 85:239-245
472
473 Koczor S, Szentkirályi F, Pickett JA, Birkett MA, Tóth M (2015) Aphid sex pheromone
474 compounds interfere with attraction of common green lacewings to floral bait. J Chem Ecol
475 41:550-556
476
477 Landolt PJ, Phillips TW (1997): Host plant influences on sex pheromone behavior of
478 phytophagous insects. Annu Rev Entomol 42:371-391
479
480 Lu YH, Qiu F, Feng HQ, Li HB, Yang ZC, Wyckhuys KAG, Wu KM (2008) Species
481 composition and seasonal abundance of pestiferous plant bugs (Hemiptera: Miridae) on Bt
482 cotton in China. Crop Prot 27:465-472
483
484 Lu Y, Wu K, Jiang Y, Xia B, Li P, Feng H, Wyckhuys KAG, Guo Y (2010) Mirid bug
485 outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. Science
486 328:1151-1154
487
488 Moreira JA, Millar JG (2005) Short and simple syntheses of 4-oxo-(E)-2-hexenal and
489 homologs: Pheromone components and defensive compounds of Hemiptera. J Chem Ecol
490 31:965-968
491
492 Peterson SS, Wedberg JL, Hogg DB (1992) Plant bug (Hemiptera: Miridae) damage to
493 birdsfoot trefoil seed production. J Econ Ent 85:250-255

494

1
2
3
4
5
6
7
8
9
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11
12
13
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15
16
17
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47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

495 Pickett JA (1990) GC-MS in insect pheromone identification: three extreme case histories, in
496 *Chromatography and Isolation of Insect Hormones and Pheromones*, ed. by McCaffery AR
497 and Wilson ID. Plenum Press, New York/London, pp 299–309

498
499 R Core Team (2016) R: A language and environment for statistical computing. R Foundation
500 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

501
502 Sun L, Gu S-H, Xiao H-J, Zhou J-J, Guo Y-Y, Liu Z-W, Zhang Y-J (2013) The preferential
503 binding of a sensory organ specific odorant binding protein of the alfalfa plant bug
504 *Adelphocoris lineolatus* AlinOBP10 to biologically active host plant volatiles. *J Chem Ecol*
505 39:1221-1231

506
507 Tóth M, Löfstedt C, Blair BW, Cabello T, Farag AI, Hansson BS, Kovalev BG, Maini S,
508 Nesterov EA, Pajor I, Sazonov AP, Shamshev IV, Subchev M, Szócs G (1992) Attraction of
509 male turnip moths *Agrotis segetum* (Lepidoptera: Noctuidae) to sex pheromone components
510 and their mixtures at 11 sites in Europe, Asia, and Africa. *J Chem Ecol* 18:1337-1347

511
512 Witzgall P, Kirsch P, Cork A (2010) Sex pheromones and their impact on pest management. *J*
513 *Chem Ecol* 36:80-100

514
515 Wu K, Li W, Feng H, Guo Y (2002) Seasonal abundance of mirids, *Lygus lucorum* and
516 *Adelphocoris* spp. (Hemiptera: Miridae) on Bt cotton in northern China. *Crop Prot* 21:997-
517 1002

519 Yasuda T, Higuchi H (2012) Sex pheromones of *Stenotus rubrovittatus* and *Trigonotylus*
1
2 520 *caelestialium*, two mirid bugs causing pecky rice, and their application to insect monitoring in
3
4
5 521 Japan. Psyche Article ID 435640
6
7 522
8
9 523 Zhang Z, Luo J, Wang Y, Chen L, Chen L, Lei C (2014) Morphology and chemical analysis
10
11 524 of the metathoracic scent glands system in *Adelphocoris suturalis* (Hemiptera: Miridae). J
12
13 525 Insect Sci 14:293
14
15
16
17 526
18
19 527 Zhang T, Mei X-D, Li Y-F, Zhang K, Wu K-M, Ning J (2015a) Sex pheromone of the alfalfa
20
21 528 plant bug, *Adelphocoris lineolatus*. Entomol Exp Appl 156:263-270
22
23
24 529
25
26 530 Zhang T, Mei X, Zhang L, Wu K, Ning J (2015b) Identification of female sex pheromone of a
27
28 531 plant bug, *Adelphocoris fasciaticollis* Reuter (Hemiptera: Miridae). J Appl Ent 139:87-93
29
30
31 532
32
33
34 533 Zhang Z, Zhang T, Zhang A, Luo J, Chen L, Wang M, Ning J, Lei C (2016) Identification and
35
36 534 field verification of sex pheromone from the mirid bug, *Adelphocoris suturalis*.
37
38 535 Chemoecology 26:25-31
39
40
41 536
42
43
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538 Figure legends

539

540 Fig. 1 Catches of *Adelphocoris lineolatus* males in traps baited either with live virgin *A.*

541 *lineolatus* males on green bean pods, live virgin *A. lineolatus* females on green bean pods,

542 green bean pods alone and in unbaited traps. Treatments marked with the same letter are not

543 significantly different (Kruskal-Wallis test, pairwise comparison by Wilcoxon test with

544 Benjamini-Hochberg correction at $p=0.05$) Σ = total number of *A. lineolatus* males caught in

545 the experiment (box plot diagram indicating median, minimum, maximum, the 1st and 3rd

546 quartiles of catches of the respective treatments)

547

548 Fig. 2 Coupled GC-EAG analysis of a female *Adelphocoris lineolatus* headspace extract on a

549 male antenna, with bioactive peaks labelled. The extract used for GC-EAG was prepared by

550 the CLSA method and shows a ratio of pheromone constituents different from that in air

551 entrainment samples, which were used for quantitative analysis

552

553 Fig. 3 Catches of *Adelphocoris lineolatus* males in traps baited with ternary and binary

554 combinations of hexyl butyrate, (*E*)-2-hexenyl butyrate and (*E*)-4-oxo-2-hexenal and in

555 unbaited traps. Treatments marked with the same letter are not significantly different

556 (Kruskal-Wallis test, pairwise comparison by Wilcoxon test with Benjamini-Hochberg

557 correction at $p=0.05$) Σ = total number of *A. lineolatus* males caught in the experiment (box

558 plot diagram indicating median, minimum, maximum, the 1st and 3rd quartiles of catches of

559 the respective treatments)

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561 Fig. 4 Catches of *Adelphocoris lineolatus* males in traps baited with ternary pheromone blend

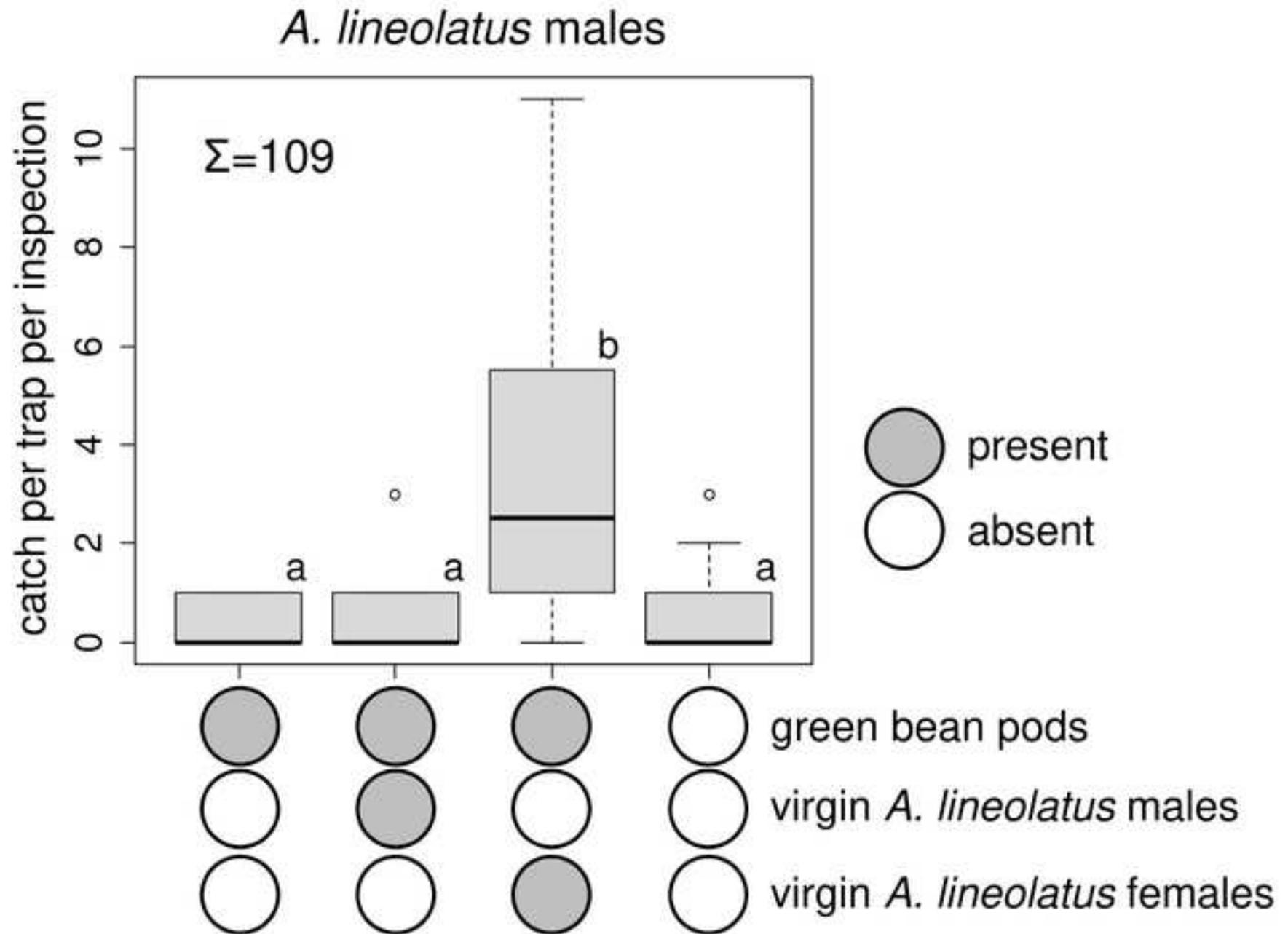
562 alone, with addition of 1-hexanol in the same dispenser, with addition of 1-hexanol in

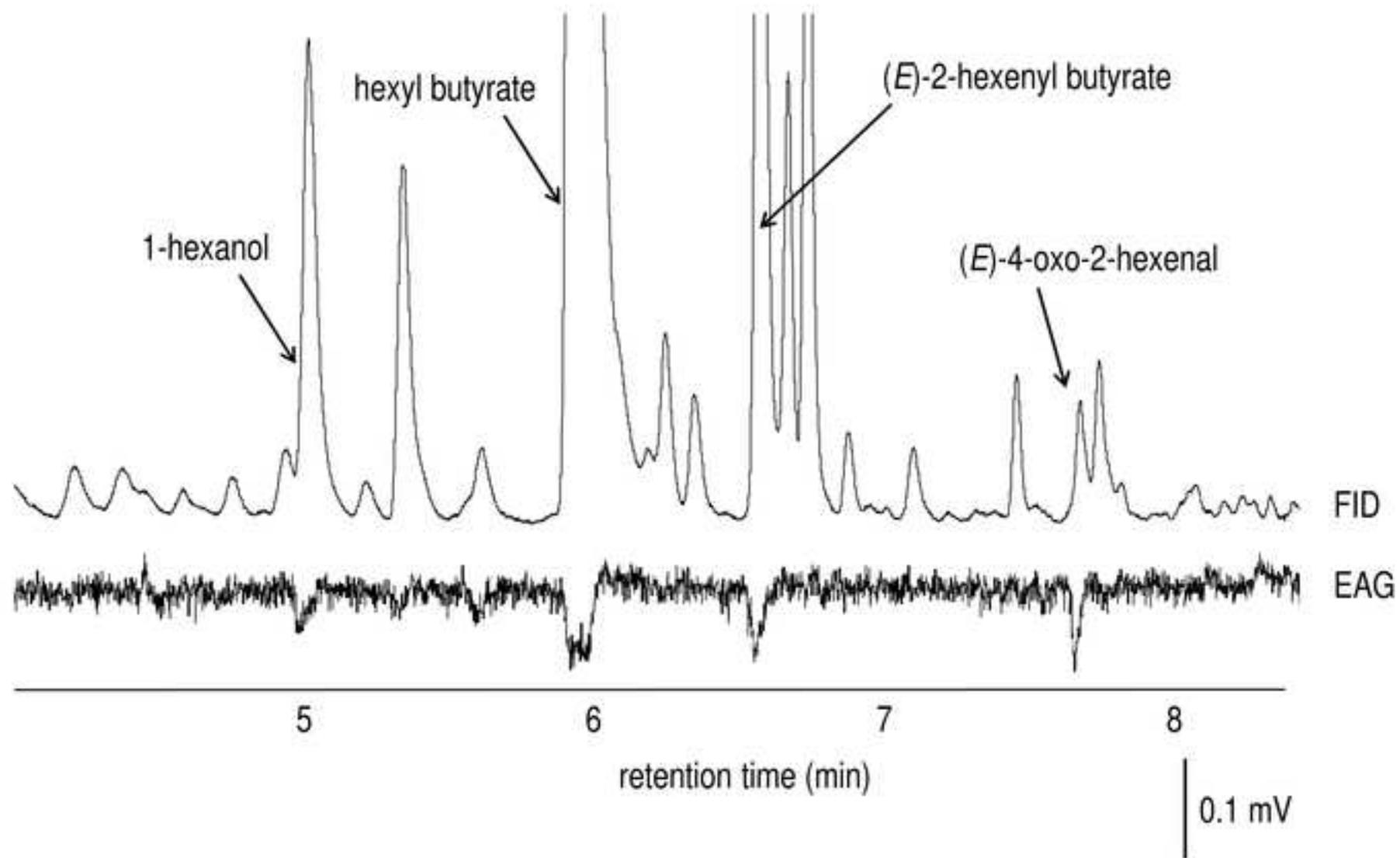
563 separate dispensers and in unbaited traps. Treatments marked with the same letter are not
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2 564 significantly different (Kruskal-Wallis test, pairwise comparison by Wilcoxon test with
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4 565 Benjamini-Hochberg correction at $p=0.05$) Σ = total number of *A. lineolatus* males caught in
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7 566 the experiment (box plot diagram indicating median, minimum, maximum, the 1st and 3rd
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9 567 quartiles of catches of the respective treatments)
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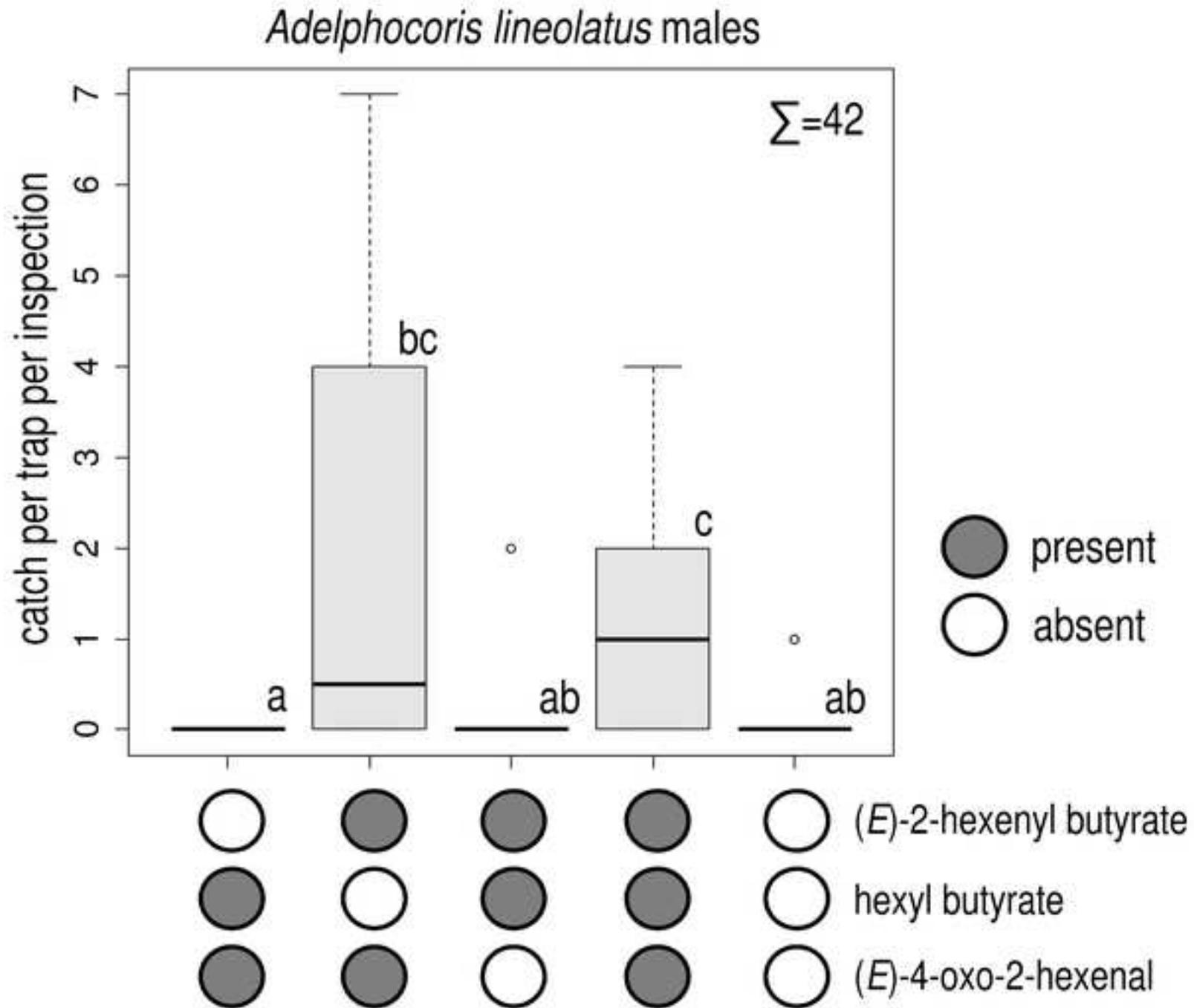
11 568
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14 569 Fig. 5 Catches of *Adelphocoris lineolatus* males and females in traps baited with ternary
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16 570 pheromone blend, (*E*)-cinnamaldehyde, their combinations and in unbaited traps. Treatments
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19 571 marked with the same letter are not significantly different (Kruskal-Wallis test, pairwise
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21 572 comparison by Wilcoxon test with Benjamini-Hochberg correction at $p=0.05$) Σ = total
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23 573 number of *A. lineolatus* males/females caught in the experiment (box plot diagram indicating
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25 574 median, minimum, maximum, the 1st and 3rd quartiles of catches of the respective
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27 575 treatments)
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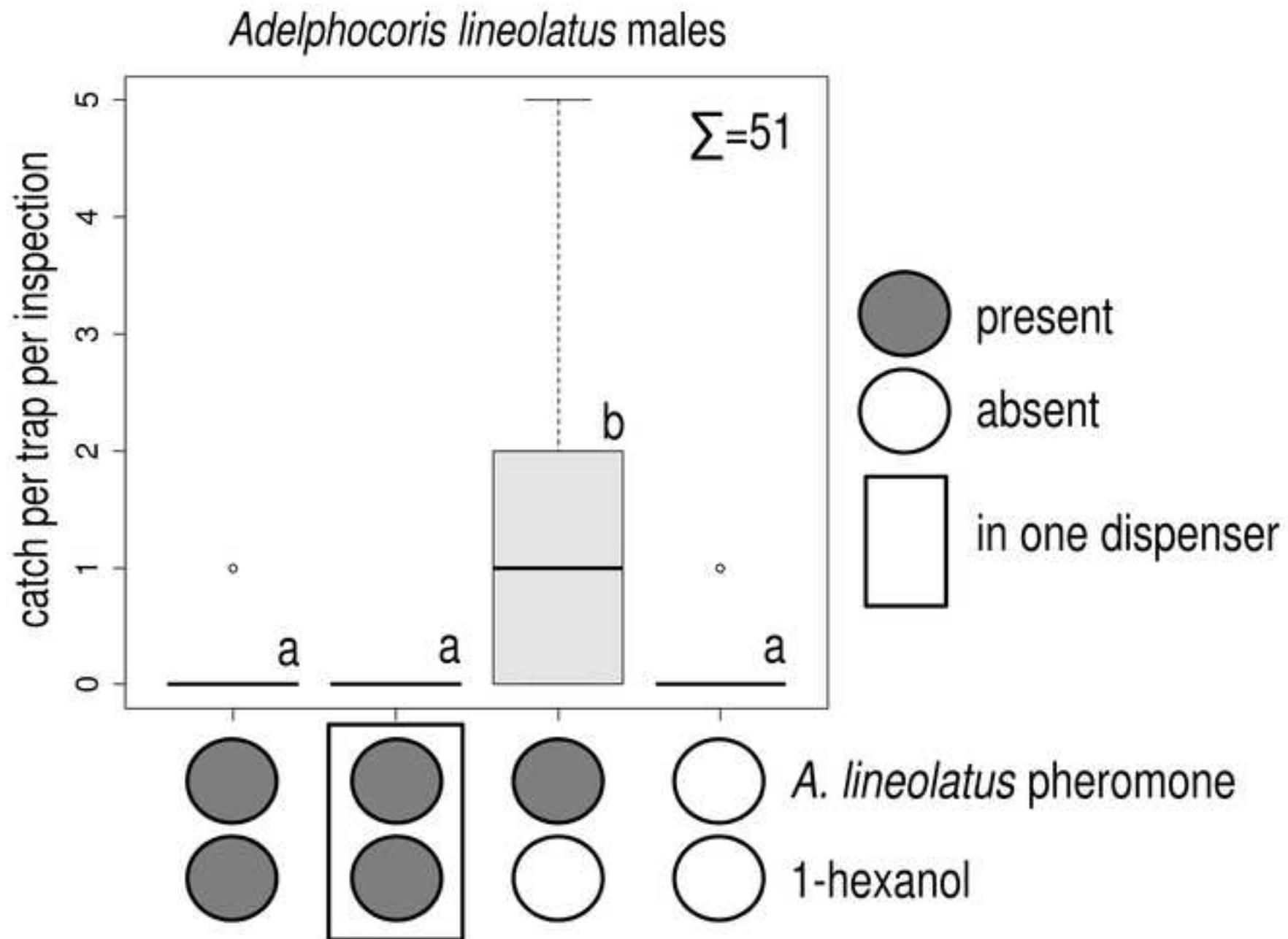
31 576
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34 577 Fig. 6 Catches of *Adelphocoris lineolatus* males in traps baited with the ternary pheromone
35
36 578 blend with original dose of (*E*)-4-oxo-2-hexenal, with 5-fold increased dose of (*E*)-4-oxo-2-
37
38 579 hexenal, with substituted blend ((*E*)-4-oxo-2-hexenal substituted with (*E*)-2-hexenal) and in
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41 580 unbaited traps. Treatments marked with the same letter are not significantly different
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43 581 (Kruskal-Wallis test, pairwise comparison by Wilcoxon test with Benjamini-Hochberg
44
45 582 correction at $p=0.05$) Σ = total number of *A. lineolatus* males caught in the experiment (box
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47 583 plot diagram indicating median, minimum, maximum, the 1st and 3rd quartiles of catches of
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50 584 the respective treatments)
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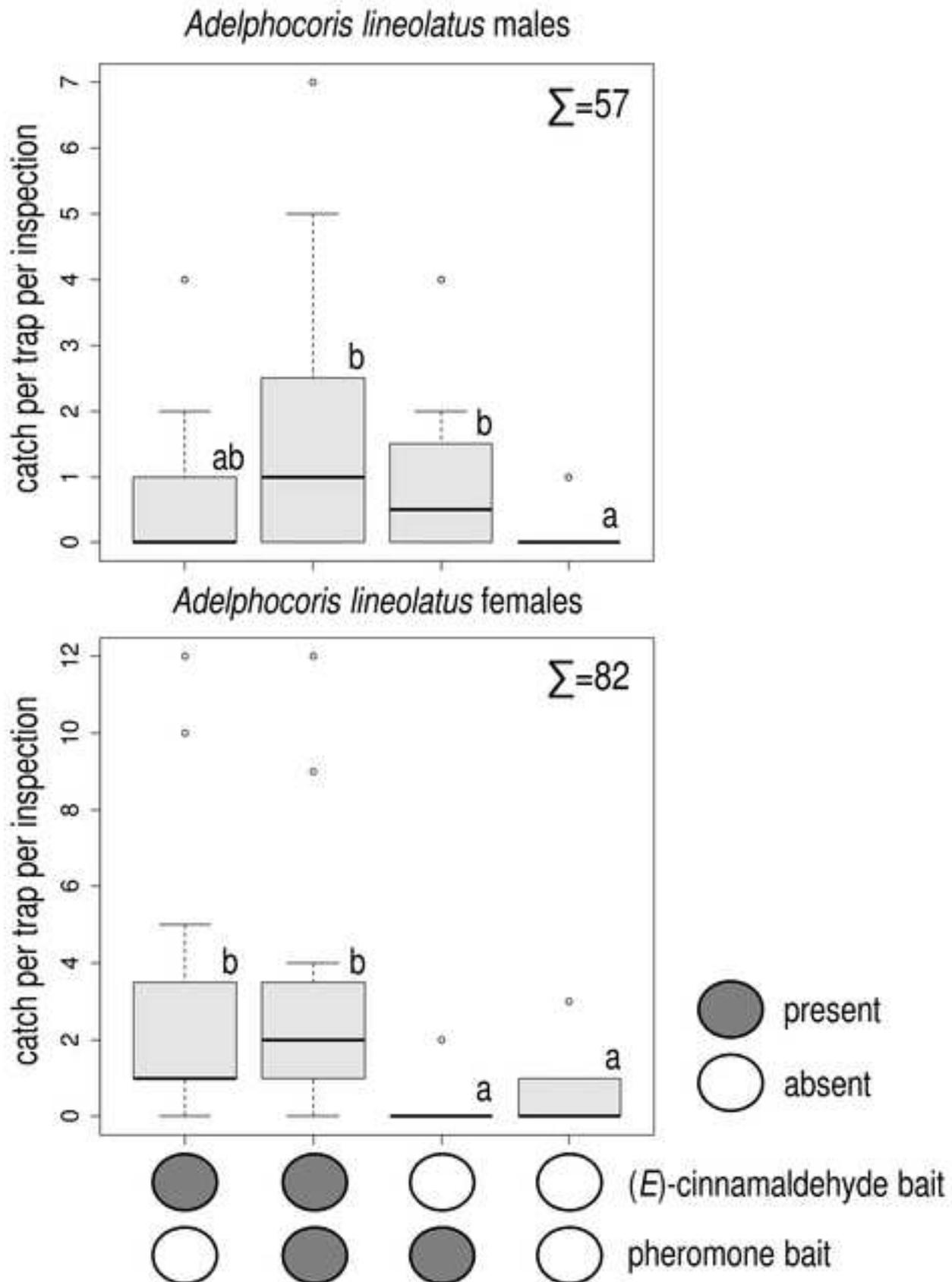
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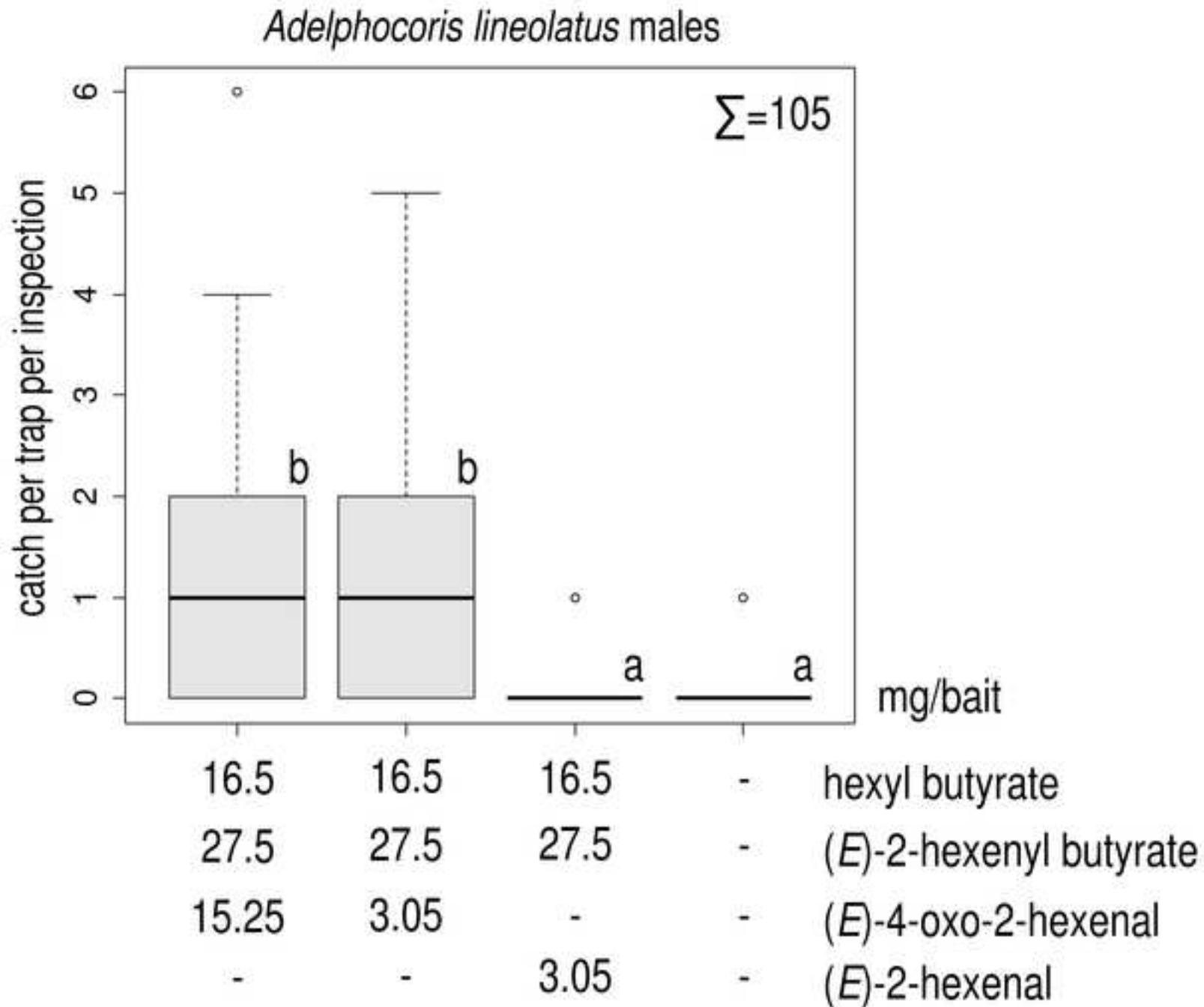


Table 1. Treatments of Experiments 2-6. '+' marks indicate the presence of a treatment in a respective experiment

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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