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Corresponding Author	Family Name	**Koczor**
	Particle	
	Given Name	**Sándor**
	Suffix	
	Division	
	Organization	Plant Protection Institute, HAS
	Address	Herman O. u. 15, Budapest, 1022, Hungary
	Email	koczor@julia-nki.hu
Author	Family Name	**Vuts**
	Particle	
	Given Name	**József**
	Suffix	
	Division	
	Organization	Rothamsted Research
	Address	Harpenden, Hertfordshire, AL52JQ, UK
	Email	
Author	Family Name	**Tóth**
	Particle	
	Given Name	**Miklós**
	Suffix	
	Division	
	Organization	Plant Protection Institute, HAS
	Address	Herman O. u. 15, Budapest, 1022, Hungary
	Email	

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Abstract

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Keywords (separated by '-')	Heteroptera - Miridae - Phenylacetaldehyde - (*E*)-cinnamaldehyde - Synthetic floral odour compounds - Field trapping
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ORIGINAL PAPER

Attraction of *Lygus rugulipennis* and *Adelphocoris lineolatus* to synthetic floral odour compounds in field experiments in Hungary

Sándor Koczor · József Vuts · Miklós Tóth

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Abstract Field experiments were carried out to ascertain whether synthetic floral odour compounds were attractive for two pest bug species. The European tarnished plant bug (*Lygus rugulipennis* Poppius) has been reported to damage various crops (e.g. strawberry, sugarbeet, alfalfa, cucumber), and the alfalfa plant bug (*Adelphocoris lineolatus* (Goeze)) is considered as a pest of alfalfa and Bt-cotton. In our field tests, traps baited with phenylacetaldehyde caught significantly more *L. rugulipennis* than unbaited traps. In addition, *A. lineolatus* was also attracted to phenylacetaldehyde-baited traps. When testing other, EAG active compounds, (*E*)-cinnamaldehyde attracted *A. lineolatus* as well. This compound was also attractive for *L. rugulipennis*, however, to a lesser extent than phenylacetaldehyde. When the two compounds were presented in combination, no synergistic or inhibitory effect was detected in either species. By attracting both sexes of both species, these new attractants may prove to be useful and provide the basis for further development of new lures for agricultural use.

Keywords Heteroptera · Miridae · Phenylacetaldehyde · (*E*)-cinnamaldehyde · Synthetic floral odour compounds · Field trapping

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S. Koczor (✉) · M. Tóth
Plant Protection Institute, HAS, Herman O. u. 15, Budapest 1022, Hungary
e-mail: koczor@julia-nki.hu

Present Address:
J. Vuts
Rothamsted Research, Harpenden, Hertfordshire AL52JQ, UK

Introduction

In the Palaearctic, several species of *Lygus* are present. Among these, the European tarnished plant bug (*Lygus rugulipennis* Poppius) is the most common species. This species is highly polyphagous (Holopainen and Varis 1991), and was reported to damage several crops, e.g. strawberry (Jay et al. 2004; Labanowska 2007), alfalfa (Benedek et al. 1970; Cs et al. 1994), sugarbeet (Varis 1972), wheat (Varis 1991) and glasshouse cucumber (Jacobson 2002).

Plant volatiles have been reported to influence behaviour of insects either by affecting sex pheromone production, release, or by increasing attraction (Landolt and Phillips 1997). Also, in case of different insect species which use plant volatile cues to locate hosts, reports of effective synthetic baits are available (e.g. Tóth et al. 2009; Vuts et al. 2010). Behavioural response to plant volatiles have also been reported in mirid species (e.g. Fujii et al. 2010), including the North American *Lygus* species as well (Blackmer et al. 2004; Whitbey 1999). Also, for the European tarnished plant bug, it was shown in olfactometer and wind tunnel experiments, that host plant volatiles provided an important stimulus for the species (Frati et al. 2008). Some of our previous findings indicated that phenylacetaldehyde, a general floral odour compound may attract the European tarnished plant bug (unpublished data).

The alfalfa plant bug (*Adelphocoris lineolatus* (Goeze)) is another pest species in the family Miridae. This species has been reported to damage alfalfa (Benedek et al. 1970; Cs et al. 1994), birdsfoot trefoil (Peterson et al. 1992) and also Bt-cotton (Wu et al. 2002).

In this study, general floral compounds (including phenylacetaldehyde) were tested in field experiments. The aim of this study was to confirm attractive activity of phenylacetaldehyde to *L. rugulipennis* and to test whether other

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synthetic floral odour compounds are attractive in the field to *L. rugulipennis* and *A. lineolatus*.

Materials and methods

Baits

All synthetic compounds (>95% chemical purity as per the manufacturer) were obtained from Sigma-Aldrich Kft (Budapest, Hungary). For preparing baits, compounds were loaded onto a 1 cm piece of dental roll, prepared of pure cotton (Celluron®, 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. Dispensers were wrapped singly in pieces of aluminium foil and stored at −18°C until used. In the field, baits were changed at 2- to 3-week intervals, as previous experience showed that they do not lose their attractiveness during this period (unpublished data). The load of baits were the following for the different experiments:

Experiment 1, the load of phenylacetaldehyde was 100 mg, dissolved in the same amount of dichloromethane.

Experiment 2, the load of different compounds was 100 mg each, dissolved in the same amount of dichloromethane. When using multiple compound baits, test chemicals were loaded in the same dispenser.

Experiment 3, the load of phenylacetaldehyde and (*E*)-cinnamaldehyde was 0, 10 or 100 mg depending on treatment, dissolved in 200 mg dichloromethane. In the case of binary lures, the test chemicals were loaded in the same dispenser.

Experiment 4, the load of phenylacetaldehyde and (E)-cinnamaldehyde was 20, 60, 200, 600 mg and no solvent was added.

Trap types tested

Three different trap designs were tested, all belonging to the CSALOMON® trap family (produced by the Plant Protection Institute, HAS, Budapest, Hungary): a sticky delta trap design, a funnel trap design and a "hat" trap design. The sticky delta trap design (code named RAG) is generally used for the capture of many moth species (Szőcs 1993; Tóth and Szőcs 1993). The funnel trap design (code named VARL+) was originally developed for catching larger moths (i.e. noctuids, geometrids, etc.) (Tóth et al. 2000; Subchev et al. 2004). The special "hat" trap design (code named KLP+) with a combination of vertical landing panel and an upper funnel container, was originally developed to catch selected beetles (Tóth et al. 2006b). Photographs of all traps can be viewed at http://www.julia-nki.hu/traps/index.html.

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 container of KLP+ and VARL+ traps to kill the captured insects. Sticky inserts of RAG traps were replaced when *Lygus* or *Adelphocoris* bugs were caught or when it became necessary to prevent the surface from becoming completely covered with dead insects.

Electrophysiological studies

Alfalfa plant bug adults for electroantennographic (EAG) analyses were collected by sweep netting from alfalfa fields at Pusztazámor (Fejér county, Hungary) and Julianna major (Budapest, Hungary). Altogether 16 individuals were used for EAG screenings. For presenting the stimuli to the antenna, a stainless steel tube (Teflon coated inside) with a constant humidified airflow of ca. 0.7 l/min was set up. An antenna was freshly amputated at the base from a live bug and mounted between two glass capillaries containing 0.1 N KCl solution. The mounted antenna was placed at ca. 3 mm distance from the outcoming airflow. One of the electrodes was grounded while the other was connected to a high impedance DC amplifier (IDAC-232, Syntech, Hilversum, The Netherlands). All synthetic compounds (>95% chemical purity as per the manufacturer) were obtained from Sigma-Aldrich Kft (Budapest, Hungary). Test compounds (10 µg each) were administered in hexane solution to a 10 × 10 mm piece of filter paper inside a Pasteur pipette. Tested compounds included synthetic plant odour compounds, 1-phenylethanol as a common standard, solvent (hexane) and air (tested compounds are listed in Fig. 1). Stimuli consisted of pushing 1 ml of air through the Pasteur pipette into the airstream flowing towards the antenna. Response amplitudes were normalized against the mean of responses to the standard (1-phenylethanol), which was tested before and after other test compounds. Stimuli were administered at ca. 20–30 s intervals.

Field trapping experiments

All experiments were conducted at Pusztazámor, Fejér county, Hungary. Traps were placed on the edge of an alfalfa field at ground level. One trap of each treatment was incorporated into a block, so that individual treatments were 5–8 m apart. Within each block, the arrangement of treatments was randomized. As a rule, traps were checked weekly twice. Insect material caught was determined according to the work of Wagner (1952) and following the suggestions of Dr. Dávid Rédei (Hemiptera Collection,

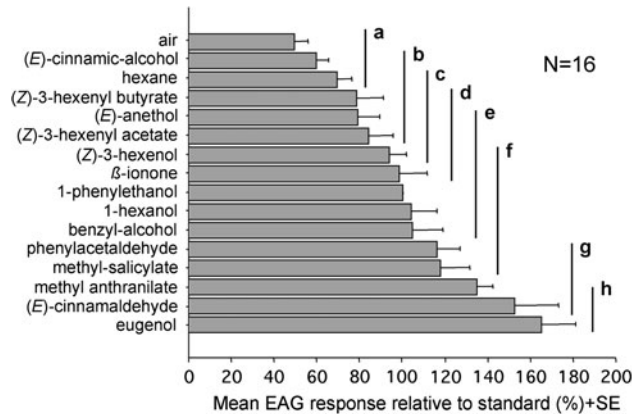

Fig. 1 EAG responses of *A. lineolatus* antennae relative to the common standard 1-phenylethanol. Columns with *same letter* are not statistically different at $p = 5\%$ by ANOVA, Fisher's Protected LSD

Hungarian Natural History Museum, Budapest). Individuals caught were sexed in all experiments, except for experiment 1. Some individuals were damaged and could not be sexed, these were taken in consideration in calculation of catches of males and females together of the respective species.

Description of single experiments

Experiment 1

The objective of this test was to confirm the attractive activity of phenylacetaldehyde for *L. rugulipennis* in different, commercially available trap designs. The test period was May 25–August 27, 2007 and three replicates of each treatment were used. Treatments included the KLP+, RAG and VARL+ trap designs with or without phenylacetaldehyde as a bait.

Experiment 2

The objective of this test was to determine the field activity of compounds found active in preliminary EAG screening of synthetic floral odour compounds on *A. lineolatus* antennae. The test period was June 10–July 8, 2008. VARL+ traps were used for all treatments and the test was conducted with 5 blocks of traps. The treatments were

- phenylacetaldehyde alone
- phenylacetaldehyde + eugenol
- eugenol alone
- phenylacetaldehyde + (*E*)-cinnamaldehyde
- (*E*)-cinnamaldehyde alone
- phenylacetaldehyde + methyl anthranilate
- methyl anthranilate alone
- unbaited traps

Experiment 3

The objective was to ascertain whether there was an interaction between phenylacetaldehyde and (*E*)-cinnamaldehyde when presented together in the same trap. The test period was July 8–September 17, 2008. VARL+ traps were used and the test was conducted with 5 blocks of traps. The treatments included

- 100 mg phenylacetaldehyde
- 100 mg phenylacetaldehyde + 10 mg (*E*)-cinnamaldehyde
- 100 mg phenylacetaldehyde + 100 mg (*E*)-cinnamaldehyde
- 10 mg phenylacetaldehyde + 100 mg (*E*)-cinnamaldehyde
- 100 mg (*E*)-cinnamaldehyde
- unbaited traps

Experiment 4

The objective was to test responses of bugs to increasing doses of phenylacetaldehyde and (*E*)-cinnamaldehyde. The test period was August 7–September 17, 2008. VARL+ traps were used and the test was conducted with 4 blocks of traps. Treatments included 20, 60, 200 or 600 mg of either single compound and unbaited traps.

Statistics

Catch and EAG response data were transformed using $(x + 0.5)^{1/2}$ as suggested by Roelofs and Cardé (1977) and analysed by one-way ANOVA. Treatment means were separated by Games–Howell test (Games and Howell 1976; Jaccard et al. 1984) and means of EAG responses relative to standard were separated by Fisher's Protected LSD. The level of significance was $p = 0.05$. If one of the treatments caught no insects, the Bonferroni–Dunn test (Dunn 1961) was used to check whether mean catches in other treatments were significantly different from zero. All statistical procedures were conducted using the software packages StatView® v4.01 and SuperANOVA® v1.11 (Abacus Concepts, Inc., Berkeley, USA, 1991-93).

Results

Electroantennography

In the preliminary EAG screenings, antennae of both sexes of *A. lineolatus* gave high responses to (*E*)-cinnamaldehyde, eugenol and methyl anthranilate (Fig. 1). These compounds were also tested in field experiments as single

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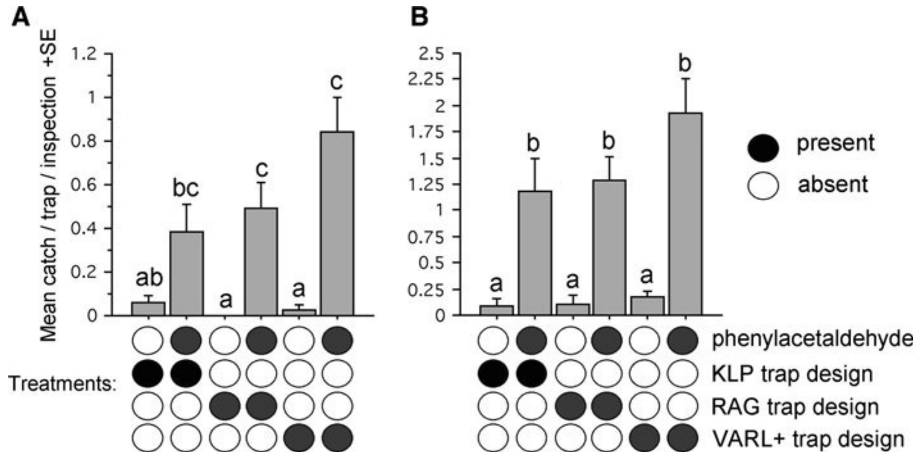

Fig. 2 Captures of bugs (both sexes together) in different trap designs baited with phenylacetaldehyde and in unbaited traps. **a** *L. rugulipennis*, total caught 77 bugs; **b** *A. lineolatus*, total caught 254 bugs. Columns with same letter within one diagram are not statistically different at $p = 5\%$ by ANOVA, Games–Howell, Bonferroni–Dunn

compounds or in combination with phenylacetaldehyde (Exp. 2).

Field trappings

In Exp. 1, phenylacetaldehyde-baited traps caught significantly more *L. rugulipennis* than unbaited traps in RAG and VARL+ trap designs, however, difference between baited and unbaited KLP+ traps was not statistically significant. (Fig. 2a). In the same experiment, all phenylacetaldehyde-baited traps caught significantly more individuals of *A. lineolatus* than unbaited traps (Fig. 2b). No significant difference was observed among catches of baited traps for either species.

In Exp. 2, very few individuals of *L. rugulipennis* were caught. However, traps baited with phenylacetaldehyde alone or with phenylacetaldehyde plus (*E*)-cinnamaldehyde caught significantly more females and more of both sexes in total than unbaited traps or other treatments (Table 1). Phenylacetaldehyde alone attracted more individuals than combinations of phenylacetaldehyde and either eugenol or methyl anthranilate in case of females and total catches.

For females and total catches, including both sexes of *A. lineolatus*, all treatments except for eugenol alone caught significantly more individuals than unbaited traps (Table 1). For males, all treatments except for eugenol alone and methyl anthranilate alone caught more than unbaited. Traps baited with phenylacetaldehyde plus (*E*)-cinnamaldehyde caught the highest number of individuals of both sexes, although the mean catch did not differ significantly from the treatment with phenylacetaldehyde alone (Table 1).

In Exp. 3, all treatments caught more *L. rugulipennis* than unbaited traps (Table 2). Traps baited with phenylacetaldehyde alone caught more bugs than traps baited with (*E*)-cinnamaldehyde alone in case of males and in total catches including both sexes. Blends, generally, did not differ from catches with single compounds. All baited traps caught more *A. lineolatus* than unbaited traps (Table 2). There was no significant difference between treatments with different bait compositions.

In Exp. 4, traps baited with 200 or 600 mg of (*E*)-cinnamaldehyde caught more *L. rugulipennis*, than unbaited traps (Fig. 3a). At the same time, phenylacetaldehyde-baited traps caught more than unbaited traps in all doses. Traps baited with the 200 mg dose of phenylacetaldehyde caught more than those baited with either 20 or 60 mg of (*E*)-cinnamaldehyde, however, this was not the case for 600 mg of phenylacetaldehyde (Fig. 3a). For *A. lineolatus*, both compounds in all doses caught more bugs than unbaited traps. Catches showed an increasing tendency with dose, up to 200 mg, however, the difference between mean catches was not significant (Fig. 3b).

Discussion

In our studies, phenylacetaldehyde and (*E*)-cinnamaldehyde were found attractive to *L. rugulipennis* and *A. lineolatus*. To our best knowledge, these compounds have not been reported as attractants of these species before.

The occurrence of both phenylacetaldehyde and (*E*)-cinnamaldehyde has been reported from various plant families, including Apiaceae, Fabaceae and Rosaceae (Knudsen et al. 2006). (*E*)-cinnamaldehyde has been reported as an attractant for *Diabrotica* beetles (Lance and Sutter 1991; Herbert et al. 1996), and attractancy of phenylacetaldehyde has been reported for several taxa including moths (Cantelo and Jacobson 1979; Creighton et al. 1973), the common green lacewings (Tóth et al. 2006a) and also for the nearctic *Lygus lineolaris* (Palisot de Beauvois) (Cantelo and Jacobson 1979), however, this was

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Table 1 Captures of *L. rugulipennis* and *A. lineolatus* in VARL+ traps baited with synthetic floral odour compounds and in unbaited traps in Exp. 2

Treatment				Mean catch/trap/inspection ± SE					
				L. rugulipennis			*A. lineolatus*		
Phenylacetaldehyde	Eugenol	(*E*)-cinnamaldehyde	Methyl anthranilate	Males	Females	Total	Males	Females	Total
+	–	–	–	0.13 ± 0.06a	0.23 ± 0.08b	0.28 ± 0.08b	1.40 ± 0.32bc	2.30 ± 0.49bc	3.70 ± 0.74bc
+	+	–	–	0.03 ± 0.03a	0.17 ± 0.07a	0.15 ± 0.07a	1.55 ± 0.39bc	1.90 ± 0.35bc	3.45 ± 0.71bc
–	+	–	–	0.00 ± 0.00a	0.03 ± 0.03a	0.03 ± 0.03a	0.73 ± 0.25abc	0.90 ± 0.30ab	1.62 ± 0.54ab
+	–	+	–	0.07 ± 0.05a	0.23 ± 0.08b	0.22 ± 0.08b	2.42 ± 0.58c	3.35 ± 0.74c	5.78 ± 1.27c
–	–	+	–	0.00 ± 0.00a	0.10 ± 0.06a	0.08 ± 0.04a	1.58 ± 0.39bc	2.00 ± 0.53bc	3.58 ± 0.90bc
+	–	–	+	0.00 ± 0.00a	0.03 ± 0.03a	0.03 ± 0.03a	1.33 ± 0.40bc	2.25 ± 0.54bc	3.58 ± 0.88bc
–	–	–	+	0.07 ± 0.05a	0.03 ± 0.03a	0.08 ± 0.04a	0.62 ± 0.24ab	1.23 ± 0.31bc	1.85 ± 0.51bc
–	–	–	–	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.08 ± 0.04a	0.28 ± 0.08a	0.35 ± 0.10a
Total caught				9	25	34	388	568	956

Means with same letter within one column are not statistically different at $p = 0.05$ by ANOVA, Games–Howell

+ present, – absent

not confirmed in recent field experiments on North American *Lygus* species (Blackmer and Byers 2009). Our findings together with the indication of Cantelo and Jacobson (1979) suggest that phenylacetaldehyde may be an important chemical stimulus in the *Lygus* genus.

It is usually hypothesized that these floral compounds act as host localizing stimuli for these taxa, and in case of the two mirids in this study this can also be suggested, since both of our bugs feed on generative parts of many plant species (Benedek et al. 1970; Cs et al. 1994; Jacobson 2002; Jay et al. 2004; Labanowska 2007; Peterson et al. 1992; Wu et al. 2002).

For *L. rugulipennis*, some compounds were identified as components of the sex pheromone (Innocenzi et al. 2004), and attraction of males to these compounds has been reported (Innocenzi et al. 2005; Fountain et al. 2010). However, in traps baited with the two floral attractants discovered in our present study, both sexes of *L. rugulipennis* were caught, thus these compounds may show practical advantages over the use of sex pheromones.

Naturally, it could also be rewarding to test synthetic sex pheromone and floral compounds in combination to study possible interactions and to see whether they provide a more attractive stimulus when presented together, as amply documented in case of other taxa (Landolt and Phillips 1997). Preliminary studies in this direction are underway (personal communication Michelle Fountain, EMR, UK).

As for *A. lineolatus*, there was only very limited knowledge on its chemical ecology. Although a recent study reported of the high binding specificity of an odorant binding protein to a plant volatile compound (α-phellandrene) and a sex pheromone compound of related species (hexyl-butyrate) (Gu et al. 2010), to date there were no reports available on the behavioural responses of the species to chemical stimuli neither in lab experiments nor in the field. Thus, to our best knowledge, this is the first report on any synthetic attractant for *A. lineolatus*.

Both *L. rugulipennis* and *A. lineolatus* have been reported to damage various crops (e.g. Benedek et al. 1970; Jacobson 2002; Jay et al. 2004; Labanowska 2007; Varis 1972; Varis 1991; Wu et al. 2002), therefore monitoring of these bugs could yield benefits for agriculture. Although there were attempts to provide effective, practicable means for monitoring *L. rugulipennis* (e.g. Fountain et al. 2010), to our knowledge to date no such method is available for public use. Methods currently available for monitoring these pests include light trapping (Benedek et al. 1970), coloured sticky plates (Holopainen et al. 2001), beating tray (Jay et al. 2004) and probably the most commonly used sweep netting (Varis 1995). These methods even if effective (e.g. sweep netting) may be rather labour-intensive or impractical for everyday agricultural use.

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Table 2 Captures of *L. rugulipennis* and *A. lineolatus* in VARL + traps baited with phenylacetaldehyde and (*E*)-cinnamaldehyde at different ratios and in unbaited traps in Exp. 3

Bait composition (mg)		Mean catch/trap/inspection ± SE					
		L. rugulipennis			*A. lineolatus*		
Phenylacetaldehyde	(*E*)-cinnamaldehyde	Males	Females	Total	Males	Females	Total
100	0	0.57 ± 0.09c	0.41 ± 0.06b	0.98 ± 0.11c	0.84 ± 0.20b	0.63 ± 0.12b	1.44 ± 0.28b
100	10	0.42 ± 0.08bc	0.22 ± 0.06b	0.64 ± 0.11bc	0.97 ± 0.18b	0.52 ± 0.13b	1.46 ± 0.27b
100	100	0.36 ± 0.09bc	0.26 ± 0.07b	0.62 ± 0.12bc	1.20 ± 0.21b	0.74 ± 0.15b	1.91 ± 0.33b
10	100	0.35 ± 0.08bc	0.24 ± 0.05b	0.59 ± 0.09bc	0.79 ± 0.13b	0.66 ± 0.11b	1.41 ± 0.21b
0	100	0.18 ± 0.04b	0.19 ± 0.05b	0.37 ± 0.07b	0.93 ± 0.15b	0.54 ± 0.13b	1.44 ± 0.24b
0	0	0.03 ± 0.02a	0.01 ± 0.01a	0.04 ± 0.03a	0.15 ± 0.05a	0.13 ± 0.04a	0.27 ± 0.07a
Total caught		181	127	308	463	291	754

Means with same letter within one column are not statistically different at $p = 0.05$ by ANOVA, Games–Howell

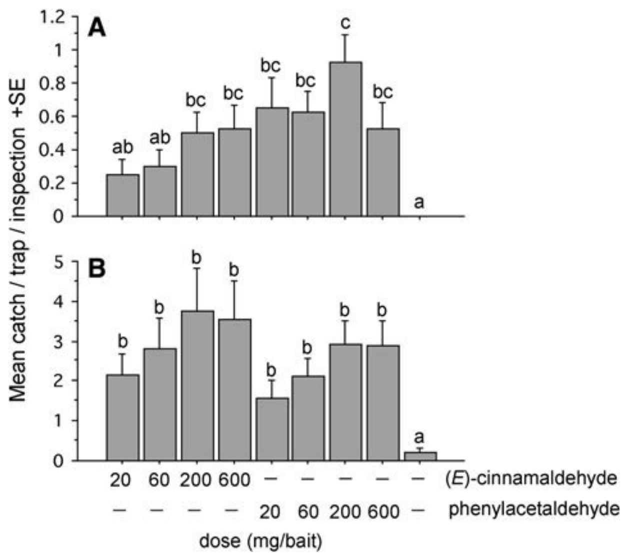

Fig. 3 Captures of bugs (both sexes together) in VARL + traps baited with phenylacetaldehyde or (*E*)-cinnamaldehyde in different doses. **a** *L. rugulipennis*, total caught 172 bugs; **b** *A. lineolatus*, total caught 878 bugs. Columns with *same letter* within one diagram are not statistically different at $p = 5\%$ by ANOVA, Games–Howell, Bonferroni–Dunn

Semiochemical-baited traps could serve as a practicable method for monitoring these species. Especially baits attractive for both sexes of the pest could yield high benefits by providing information on the abundance of females. We believe that our findings may contribute to achievement of this goal.

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