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Responses of *Pseudovadonia livida* adults to olfactory and visual cues

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Abstract

Pseudovadonia livida (F.) (Coleoptera Cerambycidae Lepturinae) is a widely distributed and common species across Europe. This study was undertaken to investigate some aspects of the sensory and behavioural ecology of *P. livida* adults in relation to flowering plants they visit. First, their electroantennogram (EAG) responses to 42 synthetic plant volatile compounds were recorded. The antennae gave the strongest EAG responses to methyl anthranilate, methyl salicylate and 2-phenylethyl alcohol. In a field trapping experiment, *P. livida* beetles preferred fluorescent yellow and yellow traps over white, blue and transparent traps. When we compared different chemical lures, loaded with EAG-active compounds and their blends, in fluorescent yellow traps, we found that the beetles responded stronger to the two-component blend of methyl anthranilate and 2-phenylethyl alcohol than to other lures tested. In a subsequent experiment testing different ratios of these two compounds, the highest number of *P. livida* adults was recorded in traps baited with a ratio of 1:1 (100 mg of each compound) of methyl anthranilate and 2-phenylethyl alcohol, followed by traps with the 10:1 ratio. Thus, 1:1 and 10:1 blends of methyl anthranilate and 2-phenylethyl alcohol in fluorescent yellow traps are suitable for detection and monitoring of *P. livida*.

Key words: *Pseudovadonia livida*, Cerambycidae, Lepturinae, floral compounds, electrophysiology, field responses, visual cues, 2-phenylethyl alcohol, methyl anthranilate, attractant trap.

Introduction

Chemical communication of Cerambycidae has been a subject of numerous studies and resulted in the discovery of chemical signals for several hundred species, including attractants (floral, trunk, leaf and smoke volatiles, bark beetle pheromones), repellents and deterrents, sex pheromones (short-range or contact pheromones and long-range sex pheromones), aggregation pheromones, trail pheromones, oviposition stimulants, marking pheromones and defensive compounds (Allison *et al*., 2004; Lacey *et al*., 2008; Francke and Dettner, 2005; Pajares *et al*., 2010; Teale *et al*., 2011; Hoover *et al*., 2014). Most studies on the chemical ecology of Cerambycidae relate to species of economic importance, mainly those regarded as pests in the subfamilies Cerambycinae and Lamiinae, which attack living or recently killed trees. Such efforts aim to develop semiochemical-based tools for detection (including detection of invasive species), monitoring and control purposes (Allison *et al*., 2004; 2014).

Presently, little is known about the chemical signals involved in inter- and intraspecific communication of Lepturinae species. Long-range female-produced sex pheromones have been identified for *Ortholeptura valida* (LeConte) (Ray *et al*., 2011) [(*Z*)-11-octadecen-1-yl acetate], *Desmocerus californicus californicus* Horn (Ray *et al.*, 2012) and *Desmocerus aureipennis aureipennis* Chevrolat (Ray *et al*., 2014) [(*R*)-desmolactone]. Ray *et al*. (2014) demonstrated that several *Desmocerus* species and subspecies have been attracted to the same sex attractant in the field, (*R*)-desmolactone. According to the literature, some Lepturinae species are attracted to monoterpenes (α-pinene, β-pinene, 3-carene, camphene, limonene and myrcene) and other host plant volatiles (ethanol, benzyl acetate, 2-methyl-3-buten-2-ol), as well as turpentine and bark beetle pheromones. Also, there are species which use host volatiles in combination with bark beetle pheromones (Sweeney *et al*., 2014; Handley *et al*., 2015; El-Sayed, 2016). Attraction to floral scent compounds (benzyl acetate, methyl benzoate, methyl phenylacetate and linalool) have been reported for several species in the *Pidonia* genus in Japan (Sakakibara *et al*., 1998).

Pseudovadonia livida (F) (Lepturinae Lepturini) occurs in the Palaearctic region and its distribution covers almost the whole of Europe (except Finland, Norway, Sweden), Siberia, North-western China, Caucasus, Transcaucasia and the Middle East (Syria, Lebanon, Israel, Iran) (Özdikmen and Turgut, 2009; Shapovalov, 2012). The soil-dwelling larvae feed externally between dead or dying roots and root stalks of grasses, and on hyphae of underground parts of the saprophytic fungus *Marasmius oreades* (Bolton) Fries (Agaricales Marasmiaceae) (Burakowski, 1979). *P. livida* adults visit flowers of members in the Adoxaceae (*Sambucus*), Apiaceae (*Aegopodium*, *Chaerophyllum*, *Daucus*, *Heracleum*), Asteraceae (*Achillea*, *Anthemis*, *Matricaria*, *Leucanthemum*, *Tanacetum*), Cistaceae (*Cistus*), Dipsacaceae (*Scabiosa*), Euphorbiaceae (*Euphorbia*), Hydrophyllaceae (*Phacelia*), Rosaceae (*Aruncus*, *Filipendula*, *Rosa, Rubus*) and Rubiaceae (*Gallium*) families, where

they mate and feed (Nikolova, 1968; Feldmann, 2001; Filimonov and Udanov, 2002; Petanidou, 2003; Martinov and Pisarenko, 2004; Brelih *et al*., 2006; Nappini and Bracalini, 2008; Faggi *et al*., 2010; Cihan *et al*., 2013). In Bulgaria, *P. livida* is widely distributed (Angelov, 1995; Migliaccio *et al*., 2007). Nikolova (1968) reports that the adults damage flowers of the oil-bearing Damask rose, *Rosa damascena* Mill., and the "dog rose" *Rosa canina* L. in Bulgaria, but there are no recent records for such damage. Recently, Leontyeva (2013) reported *P. livida* as part of the fauna of an alfalfa crop, *Medicago sativa* L. (Fabaceae), in Tatarstan (Russia).

In a field trial, testing combinations of synthetic floral compounds and visual stimuli (colours) for the Lucerne longicorn, *Plagionotus floralis* (Pallas), in an alfalfa crop in Sofia, Bulgaria, apart from the target species, relatively high catches of *P. livida* adults were registered (Toshova *et al*., 2010). To date, the sensory and behavioural ecology of adults of this species have not been studied. The objectives of the present studies were: 1) to measure the antennal responses of adults to a range of synthetic floral volatiles by electroatennography (EAG), 2) to assess the visual responses of *P. livida* to differently coloured traps, and 3) to test the behavioural responses of *P. livida* to the most active EAG compounds in field trapping trials.

Materials and methods

Test compounds

Synthetic compounds were purchased from commercial sources (Sigma-Aldrich Kft, Budapest, Hungary) and were over 95% pure as stated by the supplier. A total of 42 compounds from different chemical classes (table 1) were used for EAG recordings, the majority commonly found in flowers (Knudsen *et al*., 2006). Green leaf volatiles that are produced by flowers (Robertson *et al*., 1995; Bialecki and Smadja, 2014) were also included.

Electroantennography (EAG)

P. livida adults were hand-collected on flowers of randomly chosen specimens of *Matricaria trichophylla* (Boiss.) Boiss and *Achilea* sp. in fields belonging to Training and Experimental Field Station (University of Forestry) in Vrazhdebna suburb of Sofia, on 16 and 22 June 2009.

EAG responses from six male and two female *P. livida* antennae (one antenna per specimen) to 42 synthetic compounds were recorded using a Syntech (Kirchzarten, Germany, www.syntech.nl) equipment comprising of micromanipulators, a CS-05 stimulus air controller and an IDAC signal connection box for data acquisition. EAG signals and data were analysed using a customized software package (EAG for Windows, 1999, Syntech). The antennae were excised and mounted between Ag-AgCl glass electrodes filled with Ringer solution (Roelofs, 1984).

Each compound was diluted in hexane to give a 1 µg/1 µl solution. Test solutions (10 µl) were applied onto pieces of folded filter paper (1.5 × 1.5 cm), which were then inserted into glass Pasteur pipettes (Brand) after complete evaporation of the solvent and used as stimulus cartridges. The cartridges were only used on the same day for recordings from one or two antennae and fresh cartridges were prepared thereafter.

The stimuli were delivered in a random order into a constant 2 l/min airstream and applied (2 sec duration) at 30-40 s intervals. The hexane control (10 µl) was applied before and after stimulation with each test chemical. Nonanal can cause obvious EAG responses on *P. livida* and it is a compound with medium molecular weight and volatility (table 1) (Raguso *et al*., 1996), so it was chosen as a standard stimulus and presented at the beginning and end of the replicate, as well as in between each group of 9-11 test compounds.

Field experiments

Field tests were carried out in Training and Experimental Field Station (University of Forestry) in Vrazhdebna, Sofia in 2009-2011, using CSALOMON® VARb3 modified funnel traps produced by the Plant Protection Institute (CAR HAS, Budapest, Hungary, www.csalomontraps.com). A small piece (1 cm × 1 cm) of insecticidal strip (Vaportape II, Hercon®, Emigsville, USA; active ingredient 10% dichlorvos) was placed into the catch container as a killing agent for captured insects.

Traps were set up in blocks and each block comprised one of each treatment. The distance between traps within a block was 7-10 m and the distance between blocks was 100-200 m. Traps were attached to wooden poles and set up at ground level in sunny places. The crops at the test site were alfalfa *M. sativa,* grape *Vitis vinifera* L. and wheat *Triticum aestivum* L. There were also meadows of mixed plant species.

For preparing the bait dispensers, a 1 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany) was placed into a polythene bag made of 0.02 mm linear polyethylene foil. The polyethylene sachets were ca. 1.5 × 1.5 cm. The dispenser was attached to a plastic strip (8 × 1 cm) for easy handling when assembling the traps. For making the baits, the required amount of each synthetic compound was loaded on the dental roll, and the opening of the polythene bag was heat-sealed. Each lure was wrapped individually in a piece of aluminum foil and stored at −10 °C before use. For experiments 2 and 3, lures were replaced with fresh ones after three weeks.

Experiment 1 tested the colour preference of *P. livida*, using traps without chemical lures, from 19 May to 3 August 2009. The treatments were funnel traps with transparent, white, blue, yellow or fluorescent yellow upper parts. Treatments were set up in five blocks. The light reflectance of the coloured sheets was recorded by an Ocean Optics USB 2000+ portable spectrophotometer using R200-7-UV-VIS reflection probe, PX-2 pulsed Xenon lamp and WS-1 diffuse reflectance white standard. Light reflectance spectra were recorded from 275 nm to 800 nm at 0.2 nm intervals and data were processed by the software SpectraSuite. Raw data were imported and saved in *.xls format and the means were calculated in Excel for five measurements, which were

performed at different spots of each sheet and presented in figure 1.

Experiment 2 was carried out from 2 June to 29 July, 2010. Compounds that showed the highest EAG activity on *P. livida* antennae, that is, methyl anthranilate, methyl salicylate and 2-phenylehtyl alcohol, were selected for this experiment. Lures containing the single compounds (100 mg of each), their binary combinations in a ratio of 1:1 (200 mg total load) and ternary combination in a ratio 1:1:1 (300 mg total load) were tested. Transparent traps were used from 2 June to 11 June 2010 and no catches of *P. livida* were recorded during this period, although adults of this species were observed in the field in the close vicinity (< 10 m) of the traps on two sam-

Table 1. Volatile compounds used for EAG recordings and their chemical properties [a].

Chemical class compound	Molecular formula	Molecular weight	Boiling point, °C at 760 mmHg
Aliphatic compounds			
2-methyl-1-propanol	$C_4H_{10}O$	74	108
isoamyl alcohol	$C_5H_{12}O$	88	132
(*E*)-2-hexenal	$C_6H_{10}O$	98	–
hexanal	$C_6H_{12}O$	100	130
(*E*)-2-hexen-1-ol	$C_6H_{12}O$	100	–
(*Z*)-3-hexen-1-ol	$C_6H_{12}O$	100	156-157
1-hexanol	$C_6H_{14}O$	102	157
isobutyl acetate	$C_6H_{12}O_2$	116	117
6-methyl-5-hepten-2-one	$C_8H_{14}O$	126	173
octanal	$C_8H_{16}O$	128	171
isoamyl acetate	$C_7H_{14}O_2$	130	142
(*Z*)-3-hexenyl acetate	$C_8H_{14}O_2$	142	–
nonanal	$C_9H_{18}O$	142	195
decanal	$C_{10}H_{20}O$	156	212
(*Z*)-3-hexenyl butanoate	$C_{10}H_{18}O_2$	170	–
Aromatic compounds			
benzaldehyde	C_7H_6O	106	179
benzyl alcohol	C_7H_8O	108	205
phenylacetaldehyde	C_8H_8O	120	195
acetophenone	C_8H_8O	120	202
1-phenylethyl alcohol	$C_8H_{10}O$	122	204
2-phenylethyl alcohol	$C_8H_{10}O$	122	218
(*E*)-cinnamaldehyde	C_9H_8O	132	253
(*E*)-cinnamyl alcohol	$C_9H_{10}O$	134	250
methyl benzoate	$C_8H_8O_2$	136	198-200
(*E*)-anethole	$C_{10}H_{12}O$	148	234
benzyl acetate	$C_9H_{10}O_2$	150	213
methyl anthranylate	$C_8H_9NO_2$	151	256
methyl salicylate	$C_8H_8O_3$	152	220-224
4-methoxyphenethyl alcohol	$C_9H_{12}O_2$	152	–
isosafrole	$C_{10}H_{10}O_2$	162	253
4-methoxy cinnamaldehyde	$C_{10}H_{10}O_2$	162	–
eugenol	$C_{10}H_{12}O_2$	164	225
phenethyl acetate	$C_{10}H_{12}O_2$	164	–
(*E*)-cinnamyl acetate	$C_{11}H_{12}O_2$	176	–
methyl eugenol	$C_{11}H_{14}O_2$	178	255
anisyl acetone	$C_{11}H_{14}O_2$	178	–
Terpenoids			
myrcene	$C_{10}H_{16}$	136	167
limonene	$C_{10}H_{16}$	136	177
linalool	$C_{10}H_{18}O$	154	198
(±)-lavandulol	$C_{10}H_{18}O$	154	229-230
geraniol	$C_{10}H_{18}O$	154	230
β-ionone	$C_{13}H_{20}O$	192	271
caryophyllene	$C_{15}H_{24}$	204	–

[a] Data from National Center for Biotechnology Information, U.S. National Library of Medicine. PubChem Compound: https://www.ncbi.nlm.nih.gov/pccompound (accessed May, 2016).

– Boiling point data not available.

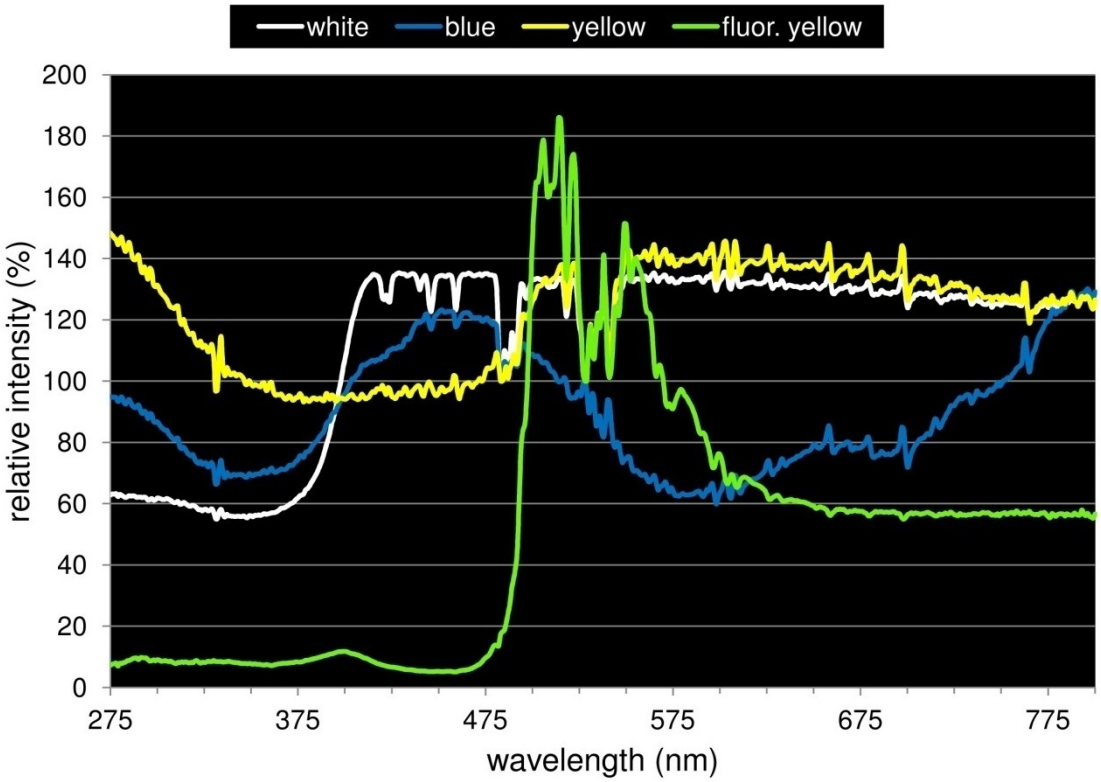

Figure 1. Reflectance spectra of the four trap colour tested.

pling dates, 7 June and 11 June 2010. On 11 June 2010 we replaced the transparent upper parts of the traps with fluorescent yellow parts and they operated until 29 July 2010. Transparent and fluorescent yellow traps without lures were used as control traps in both trapping periods, respectively. Treatments were set in four blocks.

Experiment 3 was carried out from 7 June to 8 August 2011. The following ratios of methyl anthranilate and 2-phenylethyl alcohol, respectively, were tested: 1) 10:1 (110 mg total load), 2) 1:1 (200 mg total load) and 3) 1:10 (110 mg total load). For all treatments, fluorescent yellow traps were used. Treatments were set up in four blocks.

Traps were checked every 2-7 day. All cerambycid species captured during this study were identified to species according to Angelov (1995). Captured *P. livida* beetles were sexed using a character on the metathoracic sternite and the shape of the terminal abdominal sternite (Angelov, 1995).

Statistics

EAG test

Student *t* test was used to compare the EAG responses of males and females to the compounds tested, and those elicited by the nonanal standard and hexane control. Taking into account the differences of volatility of the compounds tested, and to reduce variability in responses (millivolts) among preparations, EAG responses were normalized with respect to the standard by subtracting the value of the hexane control and then expressing the corrected mean EAG values (mV) as a percentage of the standard. In this normalization procedure, the responses to the standard were defined as 100%.

The EAG data were analyzed using a linear mixed model containing compound class (aliphatic compounds, aromatic compounds and terpenoids) as fixed effects and compound as random effects. The EAG values (in % of standard) were transformed using $Y = \text{sign}(EAG) \times \sqrt{|EAG|}$ to reduce skewness in the response variable. The transformed value is sign(EAG) times the square-root of the absolute EAG value, where sign(EAG) equals −1, 0 or 1 if EAG < 0, = 0 or > 0 respectively. Examination of residual plots for the fitted model supported the data transformation used.

For all chemical class × compound combinations, the predicted means were subsequently ranked by using Fisher's Least Significant Difference (LSD) ranking for all compound class × compound combinations. A package *asreml* (Butler, 2009) under R (R Core Team, 2015) was used to fit this model.

Experiment 1

To investigate the relationship between the numbers of beetles caught and trap colour, a Poisson log linear analysis with effects for date and colour, allowing for possible over-dispersion, was used. The colour effects, with four degrees of freedom after adjusting for a baseline effect, were further separated into effects BTW vs FY (a contrast for blue, transparent and white versus fluorescent yellow and yellow colours), F vs Y (a contrast for flourescent yellow versus yellow) and Within BTW (comparisons across blue, transparent and white on two degrees of freedom). Over-dispersion is included to allow for variation in results greater than expected for usual Poisson variation (e.g. variation attributable to date × colour interactions).

Experiments 2 and 3

To assess the effect of lure composition, i.e. treatment (Trt), on the numbers of beetles caught (females, males and totals separately), Poisson log linear regression analyses were used, allowing for possible over-dispersion. Model effects were date and Trt. For each count (females, males and totals) Analysis of Deviance methods were used Trt effects after adjusting for date.

The Relative effects (Rel. Effects), and associate standard errors (Std. Err.) are estimated for each lure composition, where Rel. Effect corresponds to the ratio for the expected numbers caught on a given date for a given lure and the control (Rel. Effect for control equals 1). Rel. Effect and Std. Err estimates were obtained using Monte Carlo methods by repeated sampling (10^6 repeats) of the log-linear model parameter from their estimated distribution. A LSD ranking across treatments for each sex × experiment were also obtained.

The numbers of females to males within traps having caught at least one beetle were compared by fitting logistic regression model, assuming a binomial distribution with possible over-dispersion. Effects in the model were date and Trt.

For the field experiments, the models were fitted using the *glm* function in R (R Core Team, 2015).

Results

EAG test

EAG responses of *P. livida* antennae ranged from −0.03 to −7.49 mV. Mean EAG responses to hexane and the nonanal standard (10 µg) were −0.25 ± 0.01 mV and −0.67 ± 0.04 mV, respectively. The mean response of the antennae to the standard stimulus was significantly higher than to the hexane control ($P < 0.001$, Student *t* test). There were no significant differences in responses by males and females to the compounds tested, therefore the data were pooled. To make comparisons between groups of different compound classes, EAG responses to individual compounds belonging to a particular chemical class were pooled and averaged.

In general, there were highly significant differences in response between compound classes (Wald F-statistic on the transformed data, $F_{2, 39} = 8.938$, $P < 0.001$). Aromatic compounds elicited highest EAG responses as a compound class and the differences with terpenoids and aliphatic compounds were significant. Among the aromatic compounds tested, methyl anthranilate, methyl salicylate and 2-phenylethyl alcohol elicited the strongest EAG responses from *P. livida* (figure 2A). Mean EAG responses to the remaining aromatic compounds were identified as moderate to low. Among terpenoids, the greatest EAG responses were evoked by (±)-lavandulol and geraniol (figure 2B). Among aliphatic compounds, (*Z*)-3-hexenyl butanoate, decanal and hexanal showed the highest EAG responses, although there was no significant difference with the responses to (*E*)-2-hexen-1-ol and isoamyl alcohol (figure 2C).

Field experiments

Experiment 1

The numbers of *P. livida* adults trapped over the five trap dates were 59, 10, 2, 1 and 0 for fluorescent yellow, yellow, transparent, white and blue traps, respectively.

The analysis of deviance for the fitted model showed that: 1) there were no significant ($P > 0.05$) differences in *P. livida* catches between blue, transparent and white traps on a given date; 2) there was a significant ($P < 0.001$) difference between fluorescent yellow and yellow traps on a given date, and 3) there were significant ($P < 0.001$) differences in average catches on blue, transparent and white compared with average catches on fluorescent yellow and yellow traps (table 2).

It should be noted that there was one highly influential observation, that being for a fluorescent yellow trap on 22 June, 2009, which reportedly caught 45 beetles (all other observations were ≤ 3). If this result is replaced with a more realistic value, for example five beetles, and the results re-analyzed, similar conclusions were drawn to those above except that there was no longer a significant ($P > 0.05$) difference between catches in fluorescent yellow and yellow traps on a given date.

Nevertheless, the results suggest that fluorescent yellow and yellow traps catch more *P. livida* adults than either of the other colour traps.

Experiment 2

As it was already mentioned, no catches of *P. livida* were recorded in the period of 2-11 June 2010 (traps inspected on 7 and 11 June 2010) when traps with transparent upper parts were used, although adults of this species were observed in the field during this period. When, however, all treatments were tested using traps with fluorescent yellow upper parts, the highest mean catches of *P. livida* adults were observed in traps baited with a combination of methyl anthranilate and 2-phenylethyl alcohol and it was 12-fold more than mean catches in the control traps (without lures) (figure 3).

Based on Wald F-statistics, treatment had an effect on female ($F_{7, 212} = 6.367$, $P < 0.001$), male ($F_{7, 212} = 3.685$, $P < 0.001$) and total ($F_{7, 212} = 6.103$, $P < 0.001$) catches.

Table 2. Effect of trap colour on *P. livida* catches (analysis of deviance for fitted model); Vrazhdebna, Sofia, 26 May - 30 June, 2009, n = 5. For abbreviations, see materials and methods, Experiment 1.

	df	Deviance	Residual df	Residual deviance	F	*P*
NULL	-	-	119	455.34	-	-
Date	4	110.14	115	345.20	9.60	< 0.001
BTW vs FY	1	120.09	114	225.11	41.86	< 0.001
F vs Y	1	37.81	113	187.30	13.18	< 0.001
Within BTW	2	2.77	111	184.53	0.48	0.618

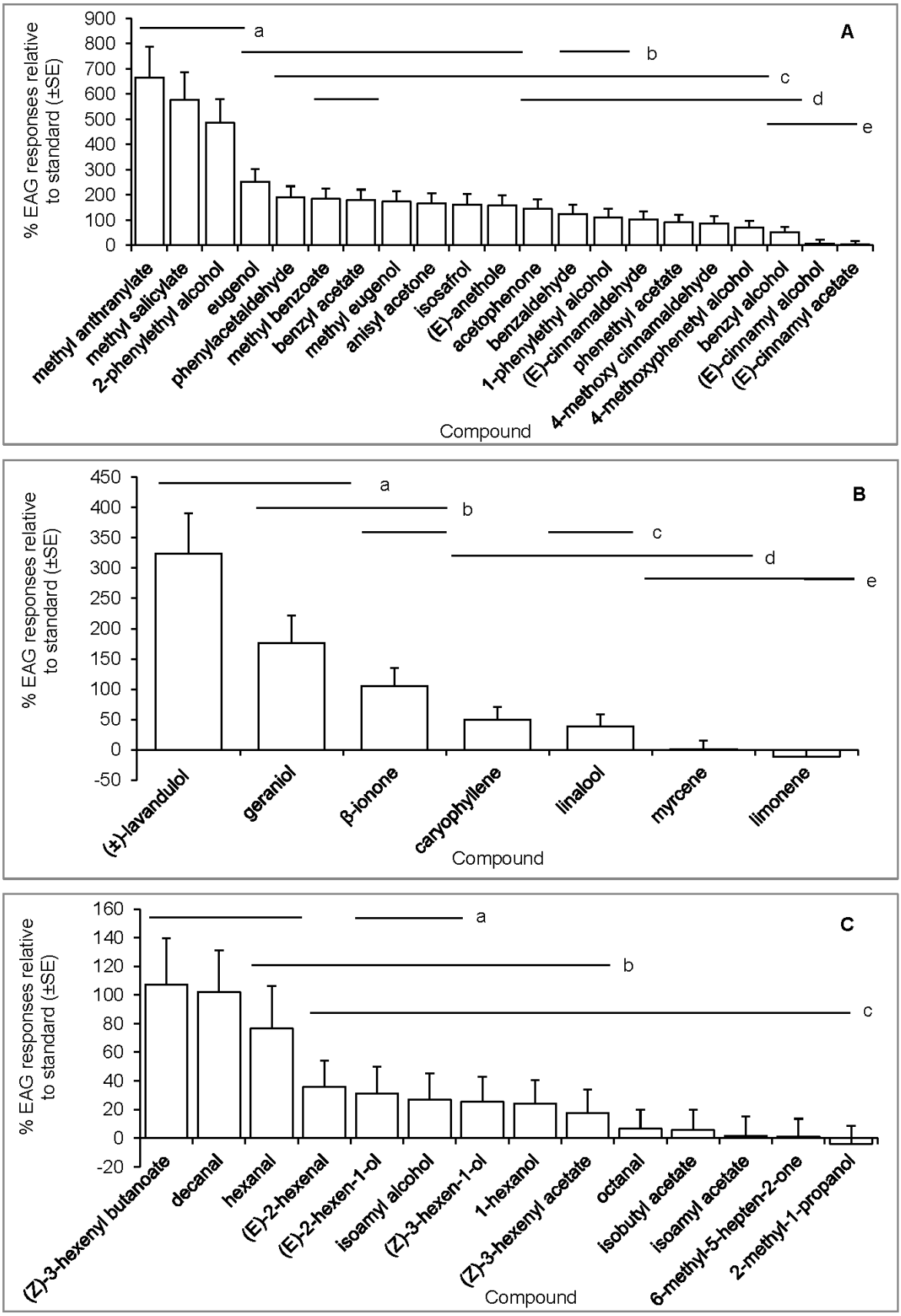

Figure 2. Relative EAG responses of *P. livida* adults (mean ± SE) to 10 µg doses of synthetic compounds of different chemical classes. A) aromatic compounds, B) terpenoids, C) aliphatic compounds; n = 5-8. Responses were normalized to the standard (10 µg of nonanal). Response bars, which are not significantly different ($P > 0.05$; LSD test) are marked with the same group line and letter.

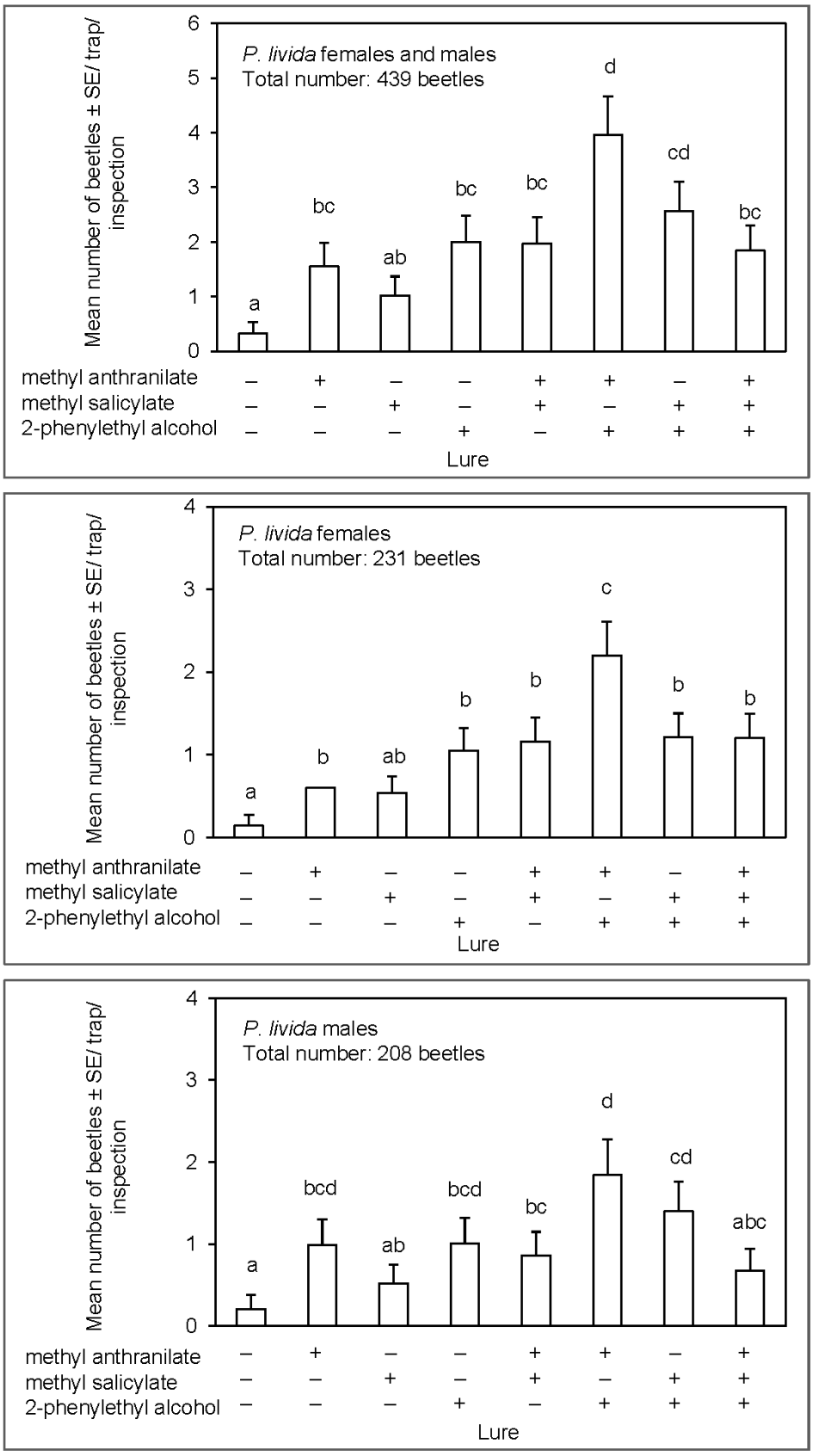

Figure 3. Mean catches of *P. livida* in fluorescent yellow traps without lure and in traps with methyl anthranilate, methyl salicylate and 2-phenylethyl alcohol and their blends. Vrazhdebna, Sofia, 11 June – 9 July, 2010, n = 4. Plus (+) and minus (-) below the bars indicate presence and absence of chemical compounds in the treatment, respectively. Bars marked with the same letter are not significantly different ($P < 0.05$, LSD test).

With the exception of the trap with a lure of methyl salicylate only, all baited traps captured significantly higher number of *P. livida* adults than unbaited traps (figure 3). Catches of females in traps baited with methyl anthranilate and 2-phenylethyl alcohol were significantly higher than those in the other baited traps. Catches of males in traps baited with both these compounds were not significantly different from those baited with either compound alone, or the combination of methyl salicylate and 2-phenylethyl alcohol.

The ratio of females to males caught within traps was not significantly different across dates ignoring treatment ($F_{7, 99}$ = 1.896, P = 0.07), nor significantly different across treatments adjusted for date ($F_{7, 92}$ = 1.513, P = 0.17).

E x p e r i m e n t 3

Comparing three different ratios of methyl anthranilate and 2-phenylethyl alcohol in fluorescent yellow traps, the highest number of *P. livida* adults was recorded in traps with a ratio of 1:1, followed by traps with the 10:1 ratio (figure 4). The treatment had significant effects, after adjusting for date effects, on female ($F_{2, 97}$ = 6.208, P = 0.003), male ($F_{2, 97}$ = 10.218, P < 0.001) and total ($F_{2, 97}$ = 9.798, P < 0.001) catches.

In this experiment, there was a significant treatment effect, after adjusting for date effects, on the proportion of females caught ($F_{2, 27}$ = 3.763, P = 0.036). This significant difference results from the significantly fewer females than males caught with methyl anthranilate and 2-phenylethyl alcohol in ratio of 1:1. For methyl anthranilate and 2-phenylethyl alcohol in ratio of 1:10 or 10:1, the numbers of females to males are not significantly different.

The seasonal flight of *P. livida* in Sofia, as registered by trap catches in 2009-2011, took place between the end of May - second part of July. The earliest catches were registered between 26 May - 3 June 2009, whereas the latest catches between 15-22 July 2010. The peak of the flight was in June.

Several other cerambycid species were also caught during the study. The most abundant was *P. floralis* with a total of 530 specimens in 2010 and 94 specimens in 2011. For this species, there were no significant differences between catches in traps with different treatments (Experiment 2: $F_{7, 310}$ = 1.587; P = 0.139 and Experiment 3: $F_{2, 108}$ = 0.628; P = 0.536). The earliest catches of *P. floralis* were recorded in the middle of June and the latest at the beginning of August 2011. Other species recorded were: *Chlorophorus sartor* (Muller) (two specimens, 21-28.06.2010 and two specimens, 11-18.07.2011), *C. varius* (Muller) (one specimen, 8-11.07.2011 and one specimen, 11-18.07.2011), *Clytus rhamni* Germar (21 specimens 11.06.-10.07.2010 and nine specimens, 13.06.-01.08.2011) (Cerambycinae), *Leptura quadrifasciata* L., (one specimen, 28.06.-01.07.2010), *Stenurella bifasciata* (Muller) (one specimen, 25-28.06.2010) and *Stenurella melanura* (L.) (one specimen, 7-10.06.2011 and one specimen, 1-8.08.2011) (Lepturinae).

Discussion

This study is the first to document the EAG and behavioural responses of *P. livida* adults to floral volatile compounds, as well as the effect of their interaction with visual stimuli (colour) on trap catches. *P. livida* antennae responded strongly to compounds associated with flowers (methyl anthranilate, methyl salicylate, 2-phenylethyl alcohol, eugenol) rather than to compounds usually emitted from foliage [green leaf volatiles, e.g., (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol]. The benzenoids, methyl salicylate and 2-phenylethyl alcohol, are among the most common compounds in the floral scent of many plant species in more than 40 plant families (Knudsen *et al*., 2006). 2-Phenylethyl alcohol is the dominant floral scent compound in the oil-bearing Damask rose blossoms (Oka *et al.*, 1999; Sakai *et al*., 2007; Baldermann *et al*., 2009; Rusanov *et al*., 2011a; 2011b). There are previous records that this compound is attractive to other insects, including scarabs (*Hoplia communis* Waterhouse and *Oxythyrea* spp., Scarabaeidae) (Imai *et al*., 1998; Vuts *et al*., 2008; 2012), dipterans (*Hylemya antiqua* Meigen and *Hylemya platura* Meigen, Anthomyiidae) (Ishikawa *et al*., 1983) and lepidopterans [*Trichoplusia ni* (Hubner) and *Pieris rapae* (L.)] (Haynes *et al*., 1991; Honda *et al*., 1998). In Cerambycidae, 2-phenylethyl alcohol has been identified in the aggregation pheromone of *Megacyllene caryae* (Gahan) males (Lacey *et al*., 2008) and in male sex pheromone of the coffee white stemborer, *Xylotrechus quadripes* Chevrolat (Hall *et al*., 2006). The same compound is an aggregation pheromone for females, males and 5th-instar nymphs of the Western boxelder bug, *Boisea rubrolineata* (Barber) (Heteroptera Rhopalidae), and it serves as a sex attractant pheromone for males (Schwarz and Gries, 2010).

Imai *et al*. (1997) reported methyl anthranilate to be an efficient kairomone attractant for *Anomala rufocuprea* Motschulsky (Scarabaeidae) and when applied in combination with the female sex pheromone, it increased traps catches. This compound attracted several species of flower thrips (Murai *et al*., 2000; Imai *et al*., 2001) and it is utilized in the chemical communications of ants (El-Sayed, 2016). In addition, methyl anthranilate is known as a repellent for insects and some vertebrates (Pankiw, 2009).

The importance of visual cues in insect host location has been well documented (Prokopy and Owens, 1983; Reeves, 2011), colour being an important attractant cue for many flower-visiting insects (Vrdoljak and Samways, 2011). In our study, trap colour had significant effect on *P. livida* catches in unbaited traps. We established that the fluorescent yellow colour of the upper part of the trap was highly attractive to *P. livida* adults, followed by the yellow colour. Spectral analysis showed that the fluorescent yellow colour reflected light in a similar pattern to yellow colour from 500-560 nm with two peaks of reflectance, but the former colour was of higher intensity and this might be an explanation for higher catch in fluorescent yellow traps. Similar preference for fluorescent yellow has been documented for the alfalfa longhorn beetle, *P. floralis* (Imrei *et al*., 2014), two *Oxythyrea* species (Coleoptera Cetoniidae) (Vuts *et*

al., 2008; 2012), the pollen beetle *Meligethes aeneus* F. (Coleoptera Nitidulidae) (Döring *et al*., 2012), the European cherry fruit fly, *Rhagoletis cerasi* (L.) and the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera Tephritidae) (Economopoulos, 1977; Tóth *et al*., 2004), psyllid species (*Ctenarytaina eucalypti* Maskell and *Ctenarytaina spatulata* Taylor) (Hemiptera Psyllidae) (Brennan and Weinbaum, 2001), and thrips (Thysanoptera) (Al-Ayedh and Al-Doghairi, 2004; Jenser *et al*., 2010). When studying diversity of beetles by coloured traps,

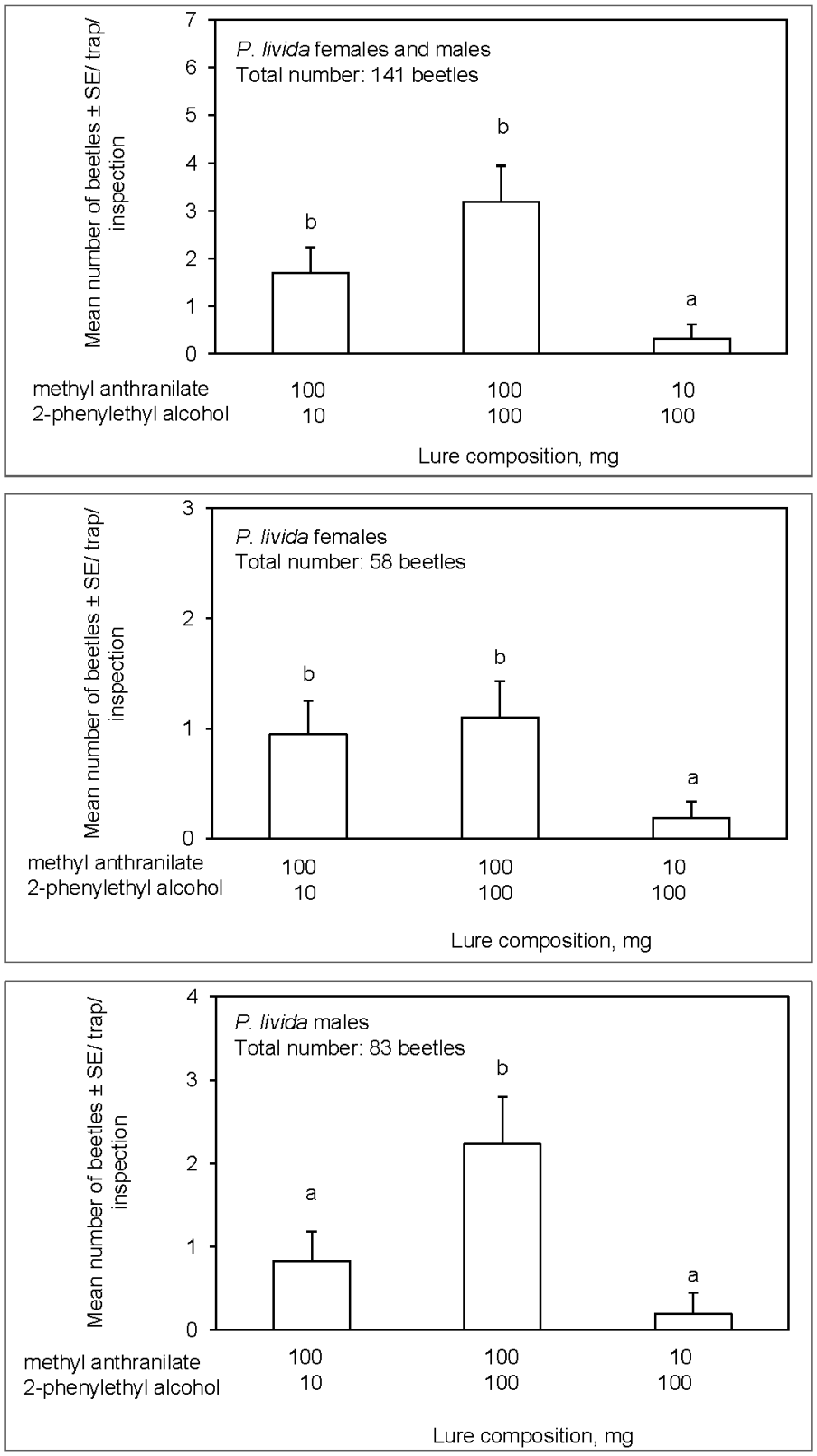

Figure 4. Mean catches of *P. livida* in fluorescent yellow traps with different ratios of methyl anthranilate and 2-phenylethyl alcohol. Vrazhdebna, Sofia, 7 June - 8 July 2011, n = 4. For further details, see figure 3.

Sakalian *et al.* (1993) and Hilszczański and Plewa (2009) reported on more catches of *P. livida* in yellow traps than in traps with other colours, but they have not tested fluorescent yellow colour.

Several studies on flower-visiting insects have shown a different role of olfactory and visual cues in attraction and feeding, and they also demonstrated their integration in many cases. When studying the behaviour of two sphingid species in the wind tunnel, Balkenius *et al.* (2006) have shown that the behaviour of the diurnal species *Macroglossum stellatarum* (L.) is strongly affected by visual cues, whereas that of the nocturnal *Deilephila elpenor* (L.) by olfactory stimuli. Olfactory and visual cues attract the nocturnal hawkmoth *Manduca sexta* (L.) within the 5 m range of a flower and integration of both types of stimulus is critical in the feeding behaviour (Raguso and Willis, 2002). Hirota *et al.* (2012) demonstrated that swallowtail butterflies and hawkmoths primarily use colour as a cue to find flowers, with contrasting preferences toward reddish and yellowish hues, respectively. The floral scent produced by the inflorescences of *Lysichiton americanum* Hulten et St John (Araceae) induced searching behavior in *Pelecomalium testaceum* (Mannerheim) (Staphylinidae) for yellow objects (Pellmyr and Patt, 1986).

In our study, the visual stimulus (colour) had a primary role in the attraction of *P. livida* beetles to the traps. However, adding of chemical stimuli, methyl anthranilate and 2-phenylethyl alcohol, increased catches significantly. Similarly, Imrei *et al.* (2014) demonstrated that the visual stimulus, i.e., fluorescent yellow colour, has a dominant role in the attraction of *P. floralis* adults, but the presence of a synthetic chemical lure comprising (*E*)-anethol, 1-phenylethyl alcohol and 3-methyl-eugenol increases the effect of the visual stimulus. Recently, Lyu *et al.* (2015) reported that the combination of visual and olfactory cues of host plants attracted more *Anoplophora glabripennis* (Motschulsky) (Cerambycidae) adults than either cue alone. In the pest control practice, visual stimuli are used most frequently in combination with chemical stimuli, thereby enhancing the efficacy of the application of a behavioural manipulation method (e.g., attractant traps) in insect pest management over the use of either stimulus type alone (Foster and Harris, 1997).

In Bulgaria, according to Angelov (1995), *P. livida* is active in June and July. Our results show that adults appear at the end of May and can be found in the field until the end of July, with the peak flight period in June. According to Şabanoğlu (2013), adults of this species are active from May to September in Turkey. In Sicily (Italy), *P. livida* adults appear from May to June (La Mantia *et al.*, 2010). In Germany, *P. livida* fly from the beginning of June to the beginning of August (Feldmann, 2001).

In conclusion, our results offer an insight into the sensory biology of *P. livida* adults, indicating that both trap colour and lure composition are important for field attraction. From a practical point of view, combination of methyl anthranilate and 2-phenylehtyl alcohol in a ratio of 1:1 in fluorescent yellow traps is recommended for the detection and monitoring of local population outbreaks of *P. livida*. In addition to this, De Cáceres *et al.* (2010) reported *P. livida* among the group of indicator species that indicate habitat alteration. The potential of this species as a single indicator for habitat quality need to be assessed.

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References

AL-AYEDH H., AL-DOGHAIRI M., 2004.- Trapping efficiency of various colored traps for insects in cucumber crop under greenhouse conditions in Riyadh, Saudi Arabia.- *Pakistan Journal of Biological Sciences*, 7: 1213-1216.

ALLISON J. D., BORDEN J. H., SEYBOLD S. J., 2004.- A review of the chemical ecology of the Cerambycidae (Coleoptera).- *Chemoecology*, 14 (3-4): 123-150.

ALLISON J. D., BHANDARI B. D., MCKENNEY J. L., MILLAR J. G., 2014.- Design factors that influence the performance of flight intercept traps for the capture of longhorned beetles (Coleoptera: Cerambycidae) from the subfamilies Lamiinae and Cerambycinae.- *PLoS ONE*, 9 (3): e93203.

ANGELOV P. A., 1995.- Coleoptera, Cerambycidae, Part I. Prioninae, Lepturinae, Necydalinae, Aseminae, Cerambycinae. In: *Fauna Bulgarica*. Vol. 24 (GOLEMANSKY V., Ed.).- Academie Scientiarium Bulgaricae, Sofia, Bulgaria (In Bulgarian).

BALDERMANN S., YANG Z., SAKAI M., FLEISCHMANN P., WATANABE N., 2009.- Volatile constituents in the scent of roses.- *Floriculture and Ornamental Biotechnology*, 3: 89-97.

BALKENIUS A., ROSÉN W., KELBER A., 2006.- Nocturnal and diurnal hawkmoths give different weight to visual and olfactory flower cues.- *Journal of Comparative Physiology A*, 192 (4): 431-437.

BIALECKI A., SMADJA J., 2014.- Identification of volatile compounds from flowers and aromatic plants: how and why?, pp. 15-39. In: *chemistry: the key to our sustainable future* (BHOWON M. G., JHAUMEER-LAULLOO S., WAH H. L. K., RAMASAMI P., Eds).- Springer, Netherlands.

BRELIH S., DROVENIK B., PIRNAT A., 2006.- Gradivo za favno hroscev (Coleoptera) Slovenije : 2. prispevek : Polyphaga: Chrysomeloidea (= Phytophaga): Cerambycidae. [Material for the beetle fauna (Coleoptera) of Slovenia : 2nd contribution: Polyphaga: Chrysomeloidea (= Phytophaga): Cerambycidae].- *Scopolia*, 58: 1-442.

BRENNAN E. B., WEINBAUM S. A., 2001.- Psyllid responses to colored sticky traps and the colors of juvenile and adult leaves of the heteroblastic host plant *Eucaluptus globulus*.- *Environmental Entomology*, 30 (2): 365-370.

BURAKOWSKI B., 1979.- Immature stages and bionomics of *Vadonia livida* (F.).- *Annales Zoologici*, 35 (2): 25-42.

BUTLER D., 2009.- asreml: asreml() fits the linear mixed model. R package version 3.0. www.vsni.co.uk.

CIHAN N., ÖZDIKMEN H., AYTAR F., 2013.- Contributions of the longhorned beetles knowledge of Turkey by the subfamilies Prioninae, Lepturinae, Dorcadioninae and Lamiinae (Coleoptera: Cerambycidae).- *Munis Entomology & Zoology*, 8: 883-894.

DE CÁCERES M., LEGENDRE P., MORETTI M., 2010.- Improving indicator species analysis by combining groups of sites.- *Oikos*, 119: 1674-1684.

DÖRING T. F., SKELLERN M. P., WATTS N. P., COOK S. M., 2012.- Colour choice behaviour in the pollen beetle, *Meligethes aeneus* (Coleoptera: Nitidulidae).- *Physiological Entomology*, 37 (4): 360-378.

ECONOMOPOULOS A. P., 1977.- Controlling *Dacus oleae* by fluorescent yellow traps.- *Entomologia Experimentalis et Applicata*, 22 (2): 183-190.

EL-SAYED A. M., 2016.- *The pherobase: database of pheromones and semiochemicals*.- http://www.pherobase.com (accessed May 2016).

FAGGI M., NAPPINI S., BISCACCIANTI A. B., 2010.- Studies on longhorn beetles (Coleoptera Cerambycidae) of the Monte Rufeno Nature Reserve and Bosco del Sasseto Natural Monument (Lazio, central Italy).- *Redia*, 93: 31-45.

FELDMANN R., 2001.- Bestand und Ausbreitung des Bockkaefers *Pseudovadonia livida* Fabricius, 1776 im suedwestfaelischen Bergland Col., Cerambycidae.- *Entomologische Nachrichten und Berichte*, 45 (3-4): 189-192.

FILIMONOV R. V., UDALOV S. G., 2002.- *Longhorn beetles of Leningrad region*.- Petroglif, S. Peterburg, Russia (in Russian).

FOSTER S. P., HARRIS M. O., 1997.- Behavioural manipulation methods for insect pest-management.- *Annual Review of Entomology*, 42: 123-46.

FRANCKE W., DETTNER K., 2005.- Chemical signaling in beetles.- *Topics in Current Chemistry*, 240: 85-166.

HALL D. R., CORK A., PHYTHIAN S. J., CHITTAMURU S., JAYARAMA B. K., VENKATESHA M. G., SREEDHARAN K., VINOD KUMAR P. K., SEETHARAN H. G., NAIDU R., 2006.- Identification of components of male-produced pheromone of coffee white stemborer, *Xylotrechus quadripes*.- *Journal of Chemical Ecology*, 32 (1): 195-219.

HANDLEY K., HOUGH-GOLDSTEIN J., HANKS L. M., MILLAR J. G., D'AMICO V., 2015.- Species richness and phenology of cerambycid beetles in urban forest fragments of northern Delaware.- *Annals of the Entomological Society of America*, 108 (3): 251-262.

HAYNES K. F., ZHAO J. Z., LATIF A., 1991.- Identification of floral compounds from *Abelia grandiflora* that stimulate upwind flight in cabbage looper moths.- *Journal of Chemical Ecology*, 17 (3): 637-646.

HILSZCZAŃSKI J., PLEWA R., 2009.- Longhorn beetles (Coleoptera, Cerambycidae) collected in Moericke traps in oak canopies of Krotoszyn forests.- *Forest Research Papers*, 70 (4): 395-401 (In Polish, English abstract).

HIROTA S. K., NITTA K., KIM Y., KATO A., KAWAKUBO N., YASUMOTO A. A., YAHARA T., 2012.- Relative role of flower color and scent on pollinator attraction: experimental tests using F1 and F2 hybrids of daylily and nightlily.- *PLoS ONE*, 7 (6): e39010.

HONDA K., ÔMURA H., HAYASHI N., 1998.- Identification of floral volatiles from *Ligustrum japonicum* that stimulate flower-visiting by cabbage butterfly, *Pieris rapae*.- *Journal of Chemical Ecology*, 24 (12): 2167-2180.

HOOVER K., KEENA M. A., NEHME M. E., WANG S., MENG P., ZHANG A., 2014.- Sex-specific trail pheromone mediates complex mate finding behavior in *Anoplophora glabripennis*.- *Journal of Chemical Ecology*, 40 (2): 169-180.

IMAI T., TSUCHIYA S., MAEKAWA M., FUJIMORI T., LEAL W. S., 1997.- Methyl anthranilate, a novel attractant for the soybean beetle, *Anomala rufocuprea* Motschulsky (Coleoptera: Scarabaeidae).- *Applied Entomology and Zoology*, 32 (1): 45-48.

IMAI T., MAEKAWA M., TSUCHIYA S., FUJIMORI T., 1998.- Field attraction of *Hoplia communis* to 2-phenylehtanol, a major component from host flower, *Rosa* spp.- *Journal of Chemical Ecology*, 24 (9): 1491-1497.

IMAI T., MAEKAWA M., MURAI T., 2001.- Attractiveness of methyl anthranilate and its related compounds to the flower thrips, *Thrips hawaiiensis* (Morgan), *T. coloratus* Schmutz, *T. flavus* Schrank and *Megalurothrips distalis* (Karny) (Thysanoptera: Thripidae).- *Applied Entomology and Zoology*, 36 (4): 475-478.

IMREI Z., KOVÁTS Z., TOSHOVA T. B., SUBCHEV M., HARMINCZ K., SZARUKÁN I., DOMINGUE M. J., TÓTH M., 2014.- Development of a trap combining visual and chemical cues for the alfalfa longhorn beetle, *Plagionotus floralis*.- *Bulletin of Insectology*, 67 (2): 161-166.

ISHIKAWA Y., IKESHOJI T., MATSUMOTO Y., TSUTSUMI M., MITSUI Y., 1983.- 2-phenylethanol: an attractant for the onion and seed-corn flies, *Hylemya antiqua* and *H. platura* (Diptera: Anthomyiidae).- *Applied Entomology and Zoology*, 18 (2): 270-277.

JENSER G., SZITA É., SZÉNÁSI Á., VÖRÖS G., TÓTH M., 2010.- Monitoring the population of vine thrips (*Drepanothrips reuteri* Uzel) (Thysanoptera: Thripidae) by using fluorescent yellow sticky traps.- *Acta Phytopathologica et Entomologica Hungarica*, 45 (2): 329-335.

KNUDSEN J. T., ERIKSSON R., GERSHENZON J., STÅHL B., 2006.- Diversity and distribution of floral scent.- *The Botanical Review*, 72 (1): 1-120.

LACEY E. S., MOREIRA J. A., MILLAR J. G., HANKS L. M., 2008.- A male-produced aggregation pheromone blend consisting of alkanediols, terpenoids, and an aromatic alcohol from the cerambycid beetle *Megacyllene caryae*.- *Journal of Chemical Ecology*, 34 (3): 408-417.

LA MANTIA T., BELLAVISTA M., GIARDINA G., SPARACIO I., 2010.- Longhorn beetles of the Ficuzza woods (W Sicily, Italy) and their relationship with plant diversity (Coleoptera, Cerambycidae).- *Biodiversity Journal*, 1 (1-4): 15-44.

LEONTYEVA I. A., 2013.- The trophic structure of the Coleoptera's fauna in the alfalfa crops. pp. 64-73. In: *Collection of scientific works SWorld. Proceedings of the international scientific-practical conference "Modern trends in theoretical and applied research 2013"*, Vol. 1, Tom 14 (KUPRIENKO S.V., Ed.) Odessa, Russia, 1-12 October, 2013. Kuprinenko, Odessa, Russia (In Russian, English abstract).

LYU F., HAI X., WANG Z., YAN A., LIU B., BI Y., 2015.- Correction: Integration of visual and olfactory cues in host plant identification by the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae).- *PLoS ONE*, 10 (12): e0146209.

MARTINOV V. V., PISARENKO T. A., 2004.- A review of the fauna and ecology of the long-horned beetles (Coleoptera: Cerambycidae) of Southeast Ukraine.- *The Kharkov Entomological Society Gazette*, 11 (2003): 44-69. (In Russian, English abstract).

MIGLIACCIO E., GEORGIEV G., GASHTAROV V., 2007.- An annotated list of Bulgarian Cerambycids with special view on the rarest species and endemics (Coleoptera: Cerambycidae).- *Lambillionea*, 107 (1) supplement 1: 1-79.

MURAI T., IMAI T., MAEKAWA M., 2000.- Methyl anthranilate as an attractant for two thrips species and the thrips parasitoid *Ceranisus menes*.- *Journal of Chemical Ecology*, 26 (11): 2557-2565.

NAPPINI S., BRACALINI M., 2008.- Xylophagous beetles in the forest complex of "Bandite di scarlino' (Southern Tuscany).- *Atti del Museo di Storia Naturale della Maremma*, 22: 73-104.

NIKOLOVA V., 1968.- Entomotsenologichni i biologichni prouchvaniya v nasazhdeniya s maslodajna roza. II. Coleoptera.- *Izvestiya na zoologicheskiya institut s muzej*, 26: 119-155 (in Bulgarian, Russian abstract).

OKA N., OHISHI H., HATANO T., HORNBERGER M., SAKATA K., WATANABE N., 1999.- Aroma evolution during flower opening in *Rosa damascena* Mill.- *Zeitschrift für Naturforschung C*, 54 (11): 889-895.

ÖZDIKMEN H., TURGUT S., 2009.- A review on the genera *Pseudovadonia* Lobanov *et al*., 1981 and *Vadonia* Mulsant, 1863 (Coleoptera: Cerambycidae: Lepturinae).- *Munis Entomology & Zoology*, 4 (1): 29-52.

PAJARES J. A., ALVAREZ G., IBEAS F., GALLEGO D., HALL D. R., FARMAN D. I., 2010.- Identification and field activity of a male-produced aggregation pheromone in the pine sawyer beetle, *Monochamus galloprovincialis*.- *Journal of Chemical Ecology*, 36 (6): 570-583.

PANKIW T., 2009.- Reducing honey bee defensive responses and social wasp colonization with methyl anthranilate.- *Journal of Medical Entomology*, 46 (4): 782-788.

PELLMYR O., PATT J. M., 1986.- Function of olfactory and visual stimuli in pollination of *Lysichiton americanum* (Araceae) by a staphylinid beetle.- *Madroño*, 33: 47-54.

PETANIDOU T., 2003.- Introducing plants for bee-keeping at any cost? Assessment of *Phacelia tanacetifolia* as nectar source plant under xeric Mediterranean conditions.- *Plant Systematics and Evolution*, 238 (1-4): 155-168.

PROKOPY R. J., OWENS E. D., 1983.- Visual detection of plants by herbivorous insects.- *Annual Review of Entomology*, 28: 337-364.

RAGUSO R. A., WILLIS M. A., 2002.- Synergy between visual and olfactory cues in nectar feeding by naïve hawkmoths, *Manduca sexta*.- *Animal Behaviour*, 64 (5): 685-695.

RAGUSO R. A., LIGHT D. M., PICHERSKY E., 1996.- Electroantennogram responses of *Hyles lineata* (Sphingidae: Lepidoptera) to floral volatile compounds from *Clarkia breweri* (Onagraceae) and other moth-pollinated flowers.- *Journal of Chemical Ecology*, 22 (10): 1735-1766.

RAY A. M., ZUNIC A., ALTEN R. L., MCELFRESH J. S., HANKS L. M., MILLAR J. G., 2011.- Cis-vaccenyl acetate, a female produced sex pheromone component of *Ortholeptura valida*, a longhorned beetle in the subfamily Lepturinae.- *Journal of Chemical Ecology*, 37 (2): 173-178.

RAY A. M., SWIFT I. P., MCELFRESH J. S., ALTEN R. L., MILLAR J. G., 2012.- (*R*)-desmolactone, a female-produced sex pheromone component of the cerambycid beetle *Desmocerus californicus californicus* (subfamily Lepturinae).- *Journal of Chemical Ecology*, 38 (2): 157-167.

RAY A. M., ARNOLD R. A., SWIFT I., SCHAPKER P. A., MCCANN S., MARSHALL C. J., MCELFRESH J. S., MILLAR J. G., 2014.- (*R*)-Desmolactone is a sex pheromone or sex attractant for the endangered valley elderberry longhorn beetle *Desmocerus californicus dimorphus* and several congeners (Cerambycidae: Lepturinae).- *PLoS ONE*, 9 (12): e115498.

R CORE TEAM 2015.- R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

REEVES J. L., 2011.- Vision should not be overlooked as an important sensory modality for finding host plants.- *Environmental Entomology*, 40 (4): 855-863.

ROBERTSON G. W., GRIFFITHS D. W., WOODFORD J. A. T., BIRCH A. N. E., 1995.- Changes in the chemical composition of volatiles released by the flowers and fruits of the red raspberry (*Rubus idaeus*) cultivar glen prosen.- *Phytochemistry*, 38 (5): 1175-1179.

ROELOFS W. L., 1984.- Electroantennogram assays: Rapid and convenient screening procedures for pheromones, pp. 131-159. In: *Techniques in pheromone research* (HUMMEL H., MILLER T. A., Eds).- Springer Verlag, New York, USA.

RUSANOV K. E., KOVACHEVA N. M., ATANASSOV I. I., 2011a.- Comparative GC/MS analysis of rose flower and distilled oil volatiles of the oil bearing rose *Rosa damascena*.- *Biotechnology & Biotechnological Equipment*, 25 (1): 2210-2216.

RUSANOV K., KOVACHEVA N., RUSANOVA M., ATANASSOV I. 2011b.- Traditional *Rosa damascena* flower harvesting practices evaluated through GC/MS metabolite profiling of flower volatiles.- *Food Chemistry*, 129 (4): 1851-1859.

ŞABANOĞLU B., 2013.- Systematic studies on the family Cerambycidae (Coleoptera) of Central Anatolian region. 386 pp. *PhD thesis*, Hacettepe University, Turkey (in Turkish, English abstract).

SAKAI M., HIRATA H., SAYAMA H., SEKIGUCHI K., ITANO H., ASAI T., DOHRA H., HARA M., WATANABE N., 2007.- Production of 2-phenylethanol in roses as the dominant floral scent compound from L-phenylalanine by two key enzymes, a PLP-dependent decarboxylase and a phenylacetaldehyde reductase.- *Bioscience, Biotechnology, and Biochemistry*, 71 (10): 2408-2419.

SAKAKIBARA Y., KIKUMA A., IWATA R., YAMANE A., 1998.- Performances of four chemicals with floral scents as attractants for longicorn beetles (Coleoptera: Cerambycidae) in a broadleaved forest.- *Journal of Forest Research*, 3 (4): 221-224.

SAKALIAN V., DIMOVA V., DAMIANOV G., 1993.- Utilization of colour traps for faunistic investigation on beetles (Insecta: Coleoptera), pp. 47-52. In: *Proceedings of the second national scientific conference of entomology* (TSANKOV G., Ed.), Sofia, Bulgaria, October, 25-27. Union of Bulgarian Scientists, Sofia, Bulgaria.

SCHWARZ J. J., GRIES G., 2010.- 2-phenylethanol: context-specific aggregation or sex-attractant pheromone of *Boisea rubrolineata* (Heteroptera: Rhopalidae).- *The Canadian Entomologist*, 142 (5): 489-500.

SHAPOVALOV A. M., 2012.- Longhorned beetles (Coleoptera, Cerambycidae) of Orenburg region: fauna, distribution, bionomics.- *Arhives of the Orenburg Branch of the Russian Entomological Society*, Issue 3, Orenburg (in Russian).

SWEENEY J. D., SILK P. J., GREBENNIKOV V., 2014.- Efficacy of semiochemical-baited traps for detection of longhorn beetles (Coleoptera: Cerambycidae) in the Russian Far East.- *European Journal of Entomology*, 111 (3): 397-406.

TEALE S. A., WICKHAM J. D., ZHANG F., SU J., CHEN Y., XIAO W., HANKS L. M., MILLAR J. G., 2011.- A male-produced aggregation pheromone of *Monochamus alternatus* (Coleoptera: Cerambycidae), a major vector of pine wood nematode.- *Journal of Economic Entomology*, 104 (5): 1592-1598.

TOSHOVA T. B., ATANASOVA D. I., TÓTH M., SUBCHEV M. A., 2010.- Seasonal activity of *Plagionotus (Echinocerus) floralis* (Pallas) (Coleoptera: Cerambycidae, Cerambycinae) adults in Bulgaria established by attractant baited fluorescent yellow funnel traps.- *Acta Phytopathologica et Entomologica Hungarica*, 45 (2): 391-399.

TÓTH M., SZARUKÁN I., VOIGT E., KOZÁR F., 2004.- Importance of visual and chemical stimuli in the development of an efficient trap for the European cherry fruit fly (*Rhagoletis cerasi* L.) (Diptera, Tephritidae).- *Növényvédelem*, 40: 229-236 (in Hungarian, English abstract).

VRDOLJAK S. M., SAMWAYS M. J., 2011.- Optimising coloured pan traps to survey flower visiting insects.- *Journal of Insect Conservation*, 16 (3): 345-354.

VUTS J., IMREI Z., TÓTH M., 2008.- Development of an attractant-baited trap for *Oxythyrea funesta* Poda (Coleoptera: Scarabaeidae, Cetoniinae).- *Zeitschrift für Naturforschung C*, 63 (9-10): 761-768.

VUTS J., KAYDAN B., YARIMBATMAN A., TÓTH M., 2012.- Field catches of *Oxythyrea cinctella* using visual and olfactory cues.- *Physiological Entomology*, 37 (1): 92-96.

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