

Development of an Attractant-Baited Trap for *Oxythyrea funesta* Poda (Coleoptera: Scarabaeidae, Cetoniinae)

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In electroantennographic tests isosafrol, methyl salicylate, ($\pm$)-lavandulol, geraniol, (*E*)-anethol, and β -ionone evoked the largest responses from antennae of female or male *Oxythyrea funesta* (Coleoptera: Scarabaeidae, Cetoniinae) adult beetles. In field trapping tests in Hungary the 1:1 blend of ($\pm$)-lavandulol and 2-phenylethanol attracted significantly more adult *O. funesta* than the single compounds. The addition of (*E*)-anethol, a previously described attractant for the species, was without effect. There was no difference in the responses of male or female beetles. The binary 2-phenylethanol/($\pm$)-lavandulol bait described in this study is recommended for the use in traps of *O. funesta* for agricultural purposes.

Key words: ($\pm$)-Lavandulol, 2-Phenylethanol, *Oxythyrea funesta*

Introduction

Adults of *Oxythyrea funesta* Poda (Coleoptera: Scarabaeidae, Cetoniinae) damage the reproductive parts of flowers of several ornamental plants and orchard trees. They are active during daytime; during the night they hide in the soil (Hurpin, 1962). Control of this species is difficult, since most insecticides cannot be applied during flowering without affecting honeybees or other beneficials. Mass trapping of adult beetles can be an alternative control method provided that an efficient trapping system is available.

The objective of the present research was to study the electrophysiological and field activity of a range of synthetic floral compounds in the hope of developing an attractant-baited trap for *O. funesta*.

First, we screened floral compounds in electroantennogram (EAG) assays on the antennae of female and male *O. funesta*. It was thought that high EAG responses show that the antenna is sensitive for the given compound, and chances to find attractants are higher among compounds eliciting high EAG activity. Second, in the course of our studies on floral attractants for other beetle species, sizeable numbers of *O. funesta* were caught in some tests. These captures were evaluated and components of the baits showing chance activity for *O. funesta* were further tested in optimization trials aimed for *O. funesta*.

Also, since (*E*)-anethol was previously found to be attractive with low activity towards the adults of *O. funesta* (Tóth *et al.*, 2003), this compound was also included into the combinations tested.

Materials and Methods

Baits

For preparing the bait dispensers, a 1 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany) was placed into a tight polythene bag made of 0.02 mm linear polyethylene foil. The dimensions of the polyethylene sachets were ca. 1.5 cm $\times$ 1.5 cm. The dispenser was attached to a plastic strip (8 cm $\times$ 1 cm) for easy handling when assembling the traps. For making up the baits 100 mg of each compound were administered onto the dental roll and the opening of the polythene bag was heat-sealed. Earlier experience showed that the bait did not loose from its activity during several weeks of field exposure; hence we decided that it was safe to renew the lures at 2- to 3-week-intervals.

Dispensers were wrapped singly in pieces of aluminum foil and were stored at -30°C until use. Synthetic compounds were obtained from Sigma-Aldrich Kft. (Budapest, Hungary). All compounds were $> 95\%$ pure as stated by the supplier.

Traps

In the tests, CSALOMON® VARb3 modified funnel traps (produced by Plant Protection Institute, HAS, Budapest, Hungary) were used, which proved to be excellent for the capture of related scarabs (Imrei *et al.*, 2001; Schmera *et al.*, 2004). Photos of VARb3 traps can be viewed at: www.julia-nki.hu/traps.

Field tests

Experiments were conducted at several sites near Budapest, Hungary. Traps were set up in a randomized complete block design. The distance between traps was 10–15 m. Traps were set up in sunny places, attached to poles or hung from the vegetation. Traps were inspected twice weekly; when captured insects were recorded, they were removed and sexed.

Tests originally aimed at other beetles, showing sizeable *O. funesta* catches

Experiment 1. This test was originally aimed at *Plagionotus floralis* Pallas (Coleoptera: Cerambycidae). Site: Tárnok (Pest county, Hungary). Biotope: alfalfa field. Period: May 22–July 19, 2004. Number of blocks: 4. Traps with a fluorescent yellow upper funnel were placed at 30–40 cm height above ground.

Experiments 2, 3. These tests were originally aimed at *Cetonia a. aurata* L. and *Potosia cuprea* Fabr. (Coleoptera: Scarabaeidae, Cetoniinae). Site: Telki (Pest county, Hungary). Biotope: edge of an oak forest with mostly *Rosa canina* L. Period: experiment 2: July 3–August 3, 2006; experiment 3: May 24–July 10, 2007. Number of blocks: experiment 2, 10; experiment 3, 5. Traps with a transparent upper funnel were placed on bushes at 1.5 m height above ground.

Test aimed at optimizing baits for *O. funesta*

Experiment 4. Site: Julianna major (Budapest, Hungary). Biotope: edge of alfalfa field. Period: June 12–July 9, 2007. Number of blocks: 5. Traps with a fluorescent yellow upper funnel were placed at 30–40 cm height above ground.

Capture data were transformed to $(x + 0.5)^{1/2}$ and percentage data were transformed to $\arctan(x)$. Transformed data were analyzed by ANOVA. Treatment means were separated by Student-

Newmann-Keuls test. All statistical procedures were conducted using the software packages Stat-View® v4.01 and SuperANOVA® v1.11 (Abacus Concepts Inc., Berkeley, CA, USA).

Electroantennograms (EAGs)

For recording EAGs a stainless steel tube (teflon-coated inside) with a constant humidified airflow of ca. 0.7 l/min was set up. An antenna freshly amputated at the base from a live beetle was mounted between two glass capillaries containing 0.1 M KCl solution, and 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). Test compounds (10 µg each) were administered in hexane solution to a 10 mm × 10 mm piece of filter paper inside a Pasteur pipette. 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 means of responses to 1-phenylethanol (eliciting medium high responses from antennae), which was tested before and after the test compounds. Stimuli were administered at ca. 20–30 s intervals. Experimental insects were collected from the edge of an oak forest at Julianna major (Pest county, Hungary).

In analysis of EAG responses we used Fisher's Protected LSD for significance levels.

Results

Electroantennographic measurements

When screening synthetic flower volatiles on the antennae of both sexes of *O. funesta*, among the three compounds found to be active in the field in previous experiments, (*E*)-anethol and (±)-lavanulol evoked significantly higher responses than the 1-phenylethanol control in both females and males. 2-Phenylethanol gave higher responses than the 1-phenylethanol control in males, although in females the difference was not significant. Besides these compounds several others evoked high responses from the antennae (Figs. 1, 2).

Field experiments

Experiment 1. Significantly more *O. funesta* were recorded in traps with 2-phenylethanol or a

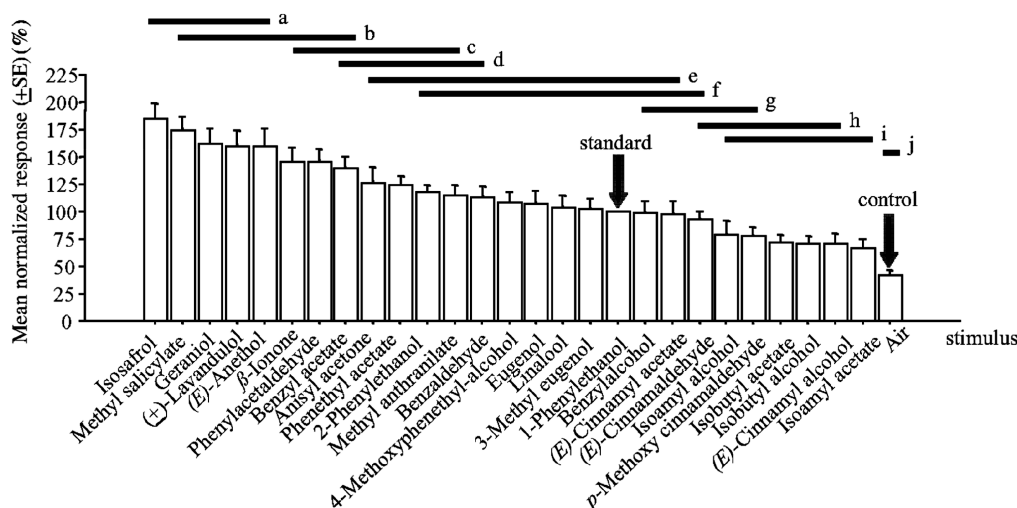


Fig. 1. EAG responses of antennae of *O. funesta* females to synthetic compounds. Responses were normalized to response evoked by the standard 1-phenylethanol. Columns show means of responses from 15 insects. Mean columns not significantly different at $P = 5\%$ (ANOVA, Fisher's PLSD) are labelled with the same group line (drawn above the columns). Group lines with different letters are significantly different at $P = 5\%$ (ANOVA, Fisher's PLSD).

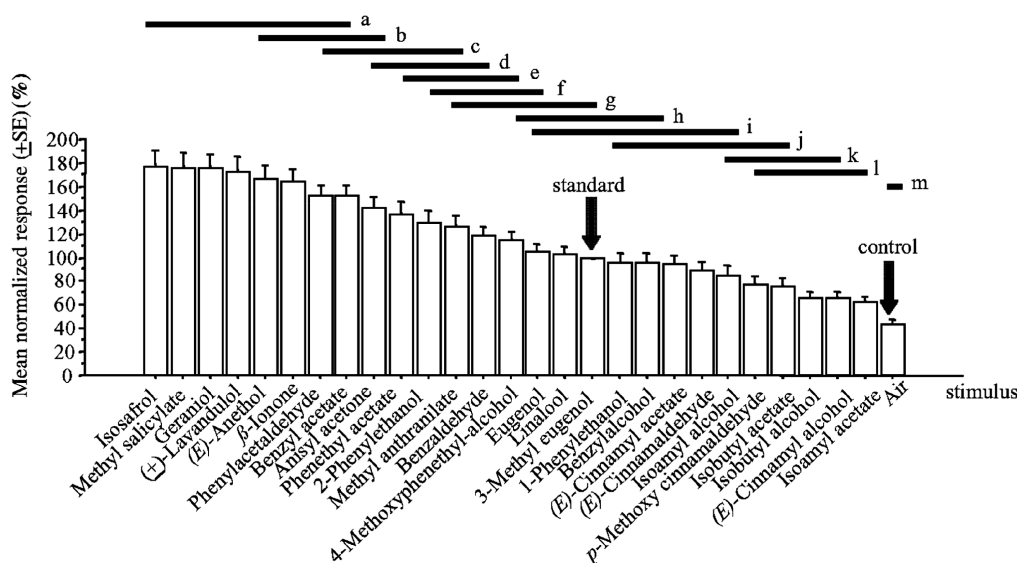


Fig. 2. EAG responses of antennae of *O. funesta* males to synthetic compounds. For details and significance see Fig. 1.

ternary mixture of this compound with (*E*)-anethol and 3-methyl eugenol than in unbaited traps (Fig. 3). These two baits did not differ from each other.

Experiment 2. Traps baited with (*±*)-lavandulol, 3-methyl eugenol, 1-phenylethanol and (*E*)-anethol caught significantly more *O. funesta* than

combinations lacking (*±*)-lavandulol or unbaited traps (Fig. 4A).

Experiment 3. Traps baited with multicomponent mixtures containing (*±*)-lavandulol caught significantly more *O. funesta* than baits lacking this compound (Fig. 4B). Capture data on other species in experiments 1–3 will be published in detail elsewhere.

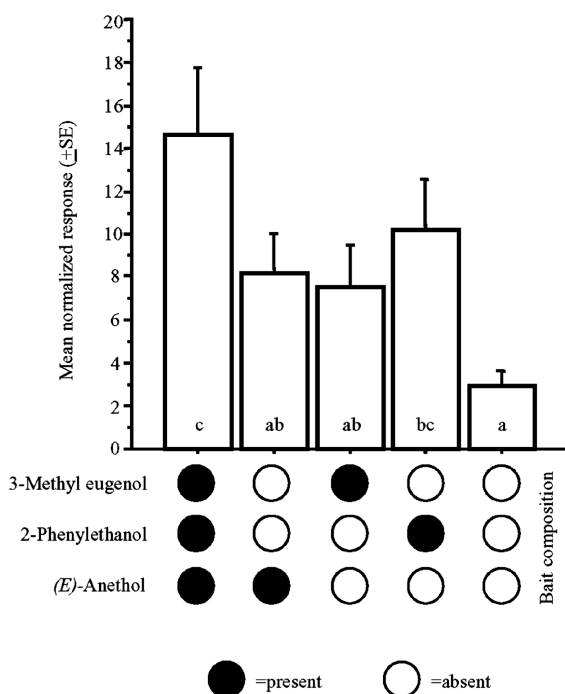


Fig. 3. Mean catches of *O. funesta* in a test originally aimed at catching *Plagionotus floralis* Pallas (Coleoptera: Cerambycidae) (experiment 1; total catch: 2939 beetles). Columns with the same letter are not significantly different at $P = 5\%$ (ANOVA, Student-Newman-Keuls test).

Experiment 4. Catches in traps containing (*E*)-anethol alone did not differ statistically from those in unbaited traps (Fig. 5). Traps baited with 2-phenylethanol or ($\pm$)-lavandulol caught significantly more *O. funesta* than unbaited traps. Among binary combinations only the mixture of 2-phenylethanol and ($\pm$)-lavandulol caught significantly more beetles than the single compounds. No significant differences between the catches of traps containing 2-phenylethanol and ($\pm$)-lavandulol and those baited with the blend of all three compounds was found. Female or male beetles showed the same response to the combinations tested.

In experiment 4, traps baited with the ternary mixture or the binary combination of 2-phenylethanol and (*E*)-anethol caught significantly more of the closely related *C. a. aurata* and *P. cuprea* than traps with the blend of 2-phenylethanol and ($\pm$)-lavandulol or the single compounds or unbaited traps. The mixture of (*E*)-anethol and ($\pm$)-lavandulol tended to attract more *C. a. aurata* and *P. cuprea* than the binary combination of 2-phenyl-

ethanol and ($\pm$)-lavandulol, but the difference was not significant (Fig. 6).

Discussion

The present results clearly show the synergistic attraction of *O. funesta* to baits containing 2-phenylethanol plus ($\pm$)-lavandulol. Catches with this blend were higher than catches with the previously described attractant (*E*)-anethol (Tóth *et al.*, 2003), and the addition of this compound to the 2-phenylethanol/($\pm$)-lavandulol lure was also without effect. Consequently, to date this binary bait appears to be the best for attracting *O. funesta* and can be suggested for the use in traps of *O. funesta* for agricultural purposes.

($\pm$)-Lavandulol was among the compounds eliciting highest responses from antennae, while 2-phenylethanol evoked medium responses. In further tests aimed at increasing the activity of the binary lure, the influence of the addition of other compounds yielding high EAG responses (*i.e.* isosafrol, methyl salicylate, geraniol, β -ionone) should be studied. These compounds presented on their own have not attracted *O. funesta* in field tests (Tóth *et al.*, 2003 and unpublished data).

As for specificity of the baits, blends containing (*E*)-anethol attracted high amounts of *C. a. aurata* and *P. cuprea* which are closely related to *O. funesta*. The binary combination of 2-phenylethanol and ($\pm$)-lavandulol, which was the most effective in attracting *O. funesta*, caught negligible numbers of *C. a. aurata* and *P. cuprea*, thus it seems to be not only the most powerful, but also the most selective mixture for *O. funesta* as compared with the other blends.

2-Phenylethanol is a very widespread floral scent constituent, and has been found in the plant families of Actinidiaceae, Linnaeaceae, Orchidaceae, Theaceae, Solanaceae, Ranunculaceae, Asteraceae, Cucurbitaceae, Rosaceae, Hyacinthaceae, Liliaceae, Fabaceae, Cactaceae, Salicaceae, Oleaceae and Nymphaeaceae (Knudsen *et al.*, 1993). ($\pm$)-Lavandulol is present in several plant families, *i.e.* Orchidaceae (Kaiser, 1993), Asteraceae, Valerianaceae (Brunke *et al.*, 1993), Polemoniaceae, Scrophulariaceae and Thymelaeaceae (Andersson *et al.*, 2002).

2-Phenylethanol is exploited as a chemical stimulus in the chemical communication of many insects. It has been described as an attractant for the

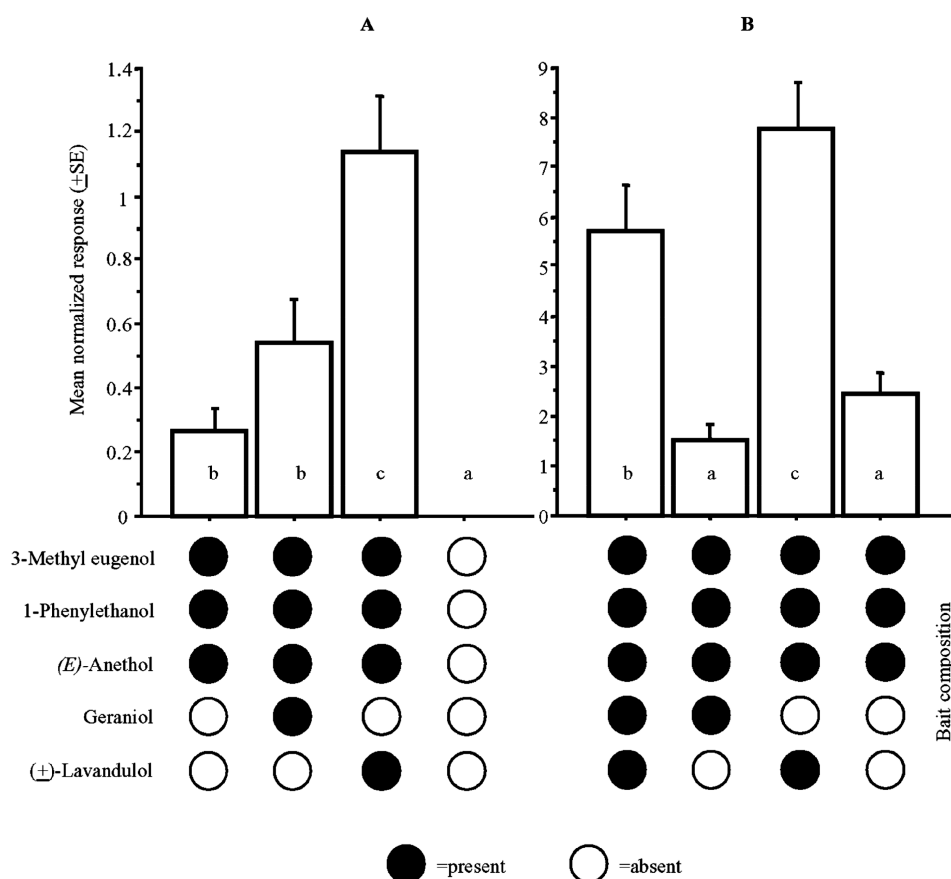


Fig. 4. Mean catches of *O. funesta* in tests originally aimed at catching *Cetonia a. aurata* L. and *Potosia cuprea* Fabr. (Coleoptera: Scarabaeidae: Cetoniinae). (A) Experiment 2; total catch: 167 beetles; (B) experiment 3; total catch: 1091 beetles. For significance see Fig. 3.

green lacewing *Chrysoperla carnea* Say (Neuroptera: Chrysopidae) (Zhu *et al.*, 2005), several beetles (Dickens, 2006, Zhu *et al.*, 1999), flies (Chapman *et al.*, 1998; Ishikawa *et al.*, 1983) and moths (Haynes *et al.*, 1991). Among scarabs, it attracts *Hoplia communis* Waterhouse (Coleoptera: Scarabaeidae, Hoplinae) (Imai *et al.*, 1998). The compound may prove to be a pheromone component also, as it was found in collections as a male specific compound in several bark beetles, cerambycids and hemipterans (Kohnle *et al.*, 1992; Hall *et al.*, 2006; James *et al.*, 1994).

In *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae) 2-phenylethanol proved to be an aggregation inhibitor (Sullivan *et al.*, 2007). In *Synanthedon exitiosa* Say (Lepidoptera: Sesiidae) it functioned as an oviposition stimulator (Derk-

sen *et al.*, 2007). *Apis mellifera* L. (Hymenoptera: Apidae) queens emit 2-phenylethanol as a queen-specific compound (Gilley *et al.*, 2006). Finally, 2-phenylethanol was also found in male hairpencils of *Mamestra brassicae* L. (Lepidoptera: Noctuidae), presumably attractive to females of conspecifics (Jacquin *et al.*, 1991).

The second component of the *O. funesta* attractant in the present study, (±)-lavandulol, is the pheromone component of *Anthonomus rubi* Herbst (Coleoptera: Curculionidae) (Innocenzi *et al.*, 2001) and *Planococcus ficus* Signoret (Homoptera: Pseudococcidae) (Hinkens *et al.*, 2001).

To our knowledge the synergistic attractant combination of 2-phenylethanol and (±)-lavandulol has not yet been described in any other insects apart from *O. funesta* in the present study.

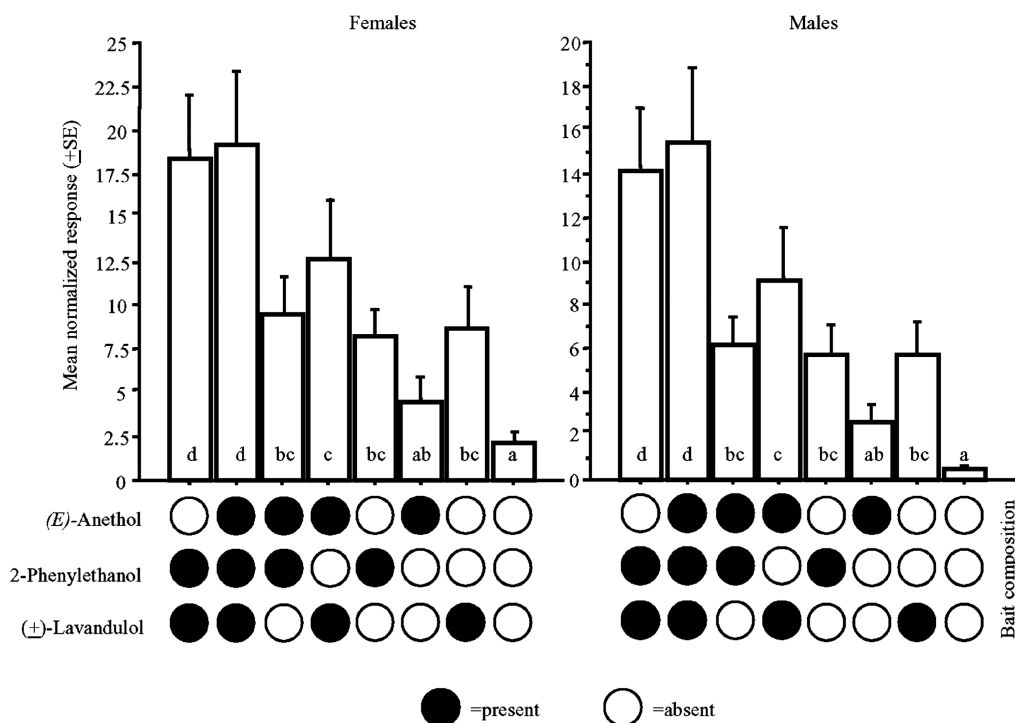


Fig. 5. Mean catches of *O. funesta* in traps baited with (*E*)-anethol, 2-phenylethanol and (±)-lavandulol and their binary and ternary combinations (experiment 4; total female catch: 3325 beetles; total male catch: 2377 beetles). For significance see Fig. 3.

In tests aimed at the capture of *O. funesta* in this study we used traps with fluorescent yellow funnels. *O. funesta* has been found to be highly sensitive to the visual cue of fluorescent yellow (Tóth *et al.*, 2005). In the present research the effect of visual stimuli was not studied, but it may be advantageous to combine this optimal visual cue together with the chemical attractant for best performance in a trap for practical purposes. Possible interactions between visual and chemical cues in

O. funesta are under study and will be published in detail elsewhere.

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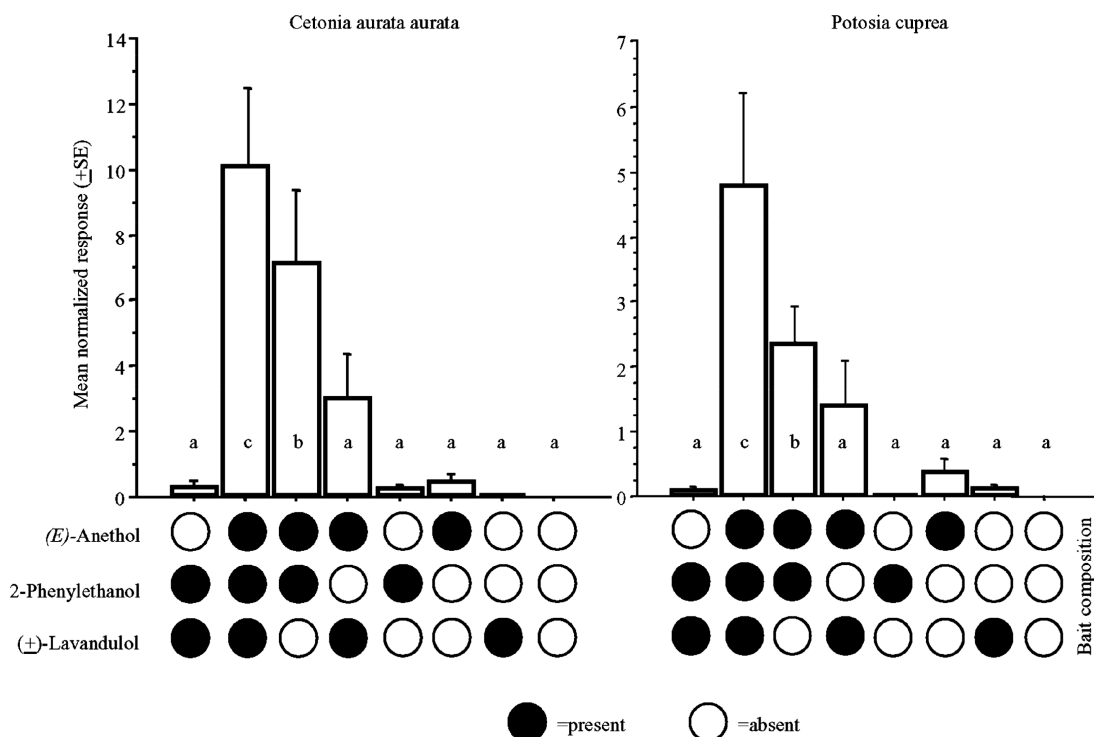


Fig. 6. Mean catches of *C. a. aurata* and *P. cuprea* in traps baited with (*E*)-anethol, 2-phenylethanol and (±)-lavandulol and their binary and ternary combinations (experiment 4; total *C. a. aurata* catch: 854 beetles; total *P. cuprea* catch: 367 beetles). For significance see Fig. 3.

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