Acta Phytopathologica et Entomologica Hungarica 54 (2), pp. 229–242 (2019)
DOI: 10.1556/038.54.2019.020

In Search of Co-attractants for Cetoniin Scarabs (Coleoptera: Scarabaeidae, Cetoniinae): Identification and Preliminary Field Evaluation of Volatiles from Fermenting Apple

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(Received: 7 February 2019; accepted: 13 March 2019)

When applied in funnel traps, the known three-component floral lure of *Cetonia a. aurata* and *Potosia cuprea* attracts large numbers of beetles. Further increasing the attractive power of these traps offers the opportunity to develop a more potent mass-trapping tool that directly reduces local scarab populations and, hence, fruit damage. The current study was initiated by the observation of adult beetles aggregating and feeding in large numbers on ripening fruit, accompanied by the presence of fermentation volatiles detectable by the human nose. Addition of apple pieces to the ternary *C. aurata aurata* / *P. cuprea* lure resulted in increased catches, but only in traps where the apple fermented as a result of beetle feeding. Volatile extracts collected from fermenting apple were subjected to GC-EAG, and bioactive peaks were identified as 1-hexanol, acetic acid, *n*-butyric acid, isovaleric acid, hexanoic acid and 3-methylphenol by GC-MS and GC peak enhancement. In preliminary field trials, a synthetic mixture of all identified compounds reduced activity of the ternary lure, indicating that some were inhibitory. As certain individual compounds or their particular combinations enhanced activity of the ternary lure only numerically, further experiments are discussed to optimize a synergistic blend of fruit fermentation and/or beetle-derived volatiles.

Keywords: Cetoniinae, attractant, mass-trapping, fermentation volatiles, scarab, fruit.

Adult *Cetonia a. aurata* L. and *Potosia cuprea* Fabr. (Coleoptera: Scarabaeidae, Cetoniinae) cause damage to fruit trees and ornamental plants, primarily in the Rosaceae, by chewing the reproductive parts of flowers and, later in the growing season, the fruit as an alternative food source (Hurpin, 1962). In addition, reports of significant damage to ripening fruit in Hungary and neighbouring countries has increased in frequency (Voigt et al., 2005; Razov et al., 2009), indicating a growing economic impact of these day-active scarabs. Their control is difficult, since most insecticides cannot be applied right before fruit harvest or during flowering without negatively affecting humans, pollinators or other beneficial insects.

As an alternative management method, a ternary chemical attractant, consisting of the ubiquitous flower scent compounds (Knudsen et al., 1993) 3-methyleugenol,

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(*RS*)-1-phenylethanol and *(E)*-anethol in a 1:1:1 ratio (Imrei, 2003; Tóth et al., 2005), is in use in funnel traps for monitoring and mass-trapping of both *C. aurata aurata* and *P. cuprea* in peach orchards (Voigt et al., 2005; Tóth et al., 2006; Razov et al., 2008). Further increasing the activity of the ternary floral lure by the addition of volatile compounds identified from new sources could enhance the mass-trapping potential of these traps.

When observing beetles feeding on ripening fruit of a range of species (e.g. peach, cherry, apple, grapes), it was revealed that fermentation processes were initiated, causing aggregations of both species on damaged fruit, also reported by Voigt et al. (2005). This was accompanied by the presence of characteristic volatiles detectable by the human nose (J. Vuts et al., personal observation). Such volatile compounds, induced primarily by yeast fermentation, have great promise in developing novel attractants that are capable of luring high numbers of both sexes into funnel traps (Gregg et al., 2018), because they are thought to provide an honest signal for food-searching beetles to locate ephemeral sources of sugar (Madden et al., 2018). We thus hypothesized that certain ubiquitous volatile components of fruit fermentation play an important role in food source localization by *C. aurata aurata* and *P. cuprea*. A series of field trapping trials and fruit headspace analysis experiments were conducted in search for compounds that may synergise the activity of the ternary synthetic bait for the development of a more efficient mass-trapping tool.

Materials and Methods

Collection of volatiles

Ten apple pieces of similar size, which attracted adults of *C. aurata aurata* and *P. cuprea* into funnel traps, were taken into the laboratory and individually placed into the glass tube of a closed-loop stripping apparatus (CLSA) (Boland et al., 1984), equipped with a DC12/16NK vacuum pump (Erich Fürgut GmbH, Tannheim, Germany) (5.0 L/min) and collection filter containing activated charcoal (1.5 mg) (Brechbühler AG, Schlieren, Switzerland). Collections were run for 1-2 hours. Trapped volatiles were eluted from the charcoal filter with dichloromethane (25 mL; Merck KGaA, Darmstadt, Germany). This way, ten headspace extracts were prepared.

Electrophysiology

The activity of headspace extracts collected from fermenting apple pieces was first evaluated by electroantennography (EAG). Experimental insects were collected from the edge of an oak forest at Telki (Pest county, Hungary). An antenna freshly amputated at the base from a live beetle was mounted between two glass capillaries containing 0.1 M KCl solution, then placed at ca. 3 mm distance from a stainless steel tube (teflon-coated inside) with a constant humidified airflow exiting at ca. 0.7 L/min (Fig. 1). The recording electrode was connected to a high-impedance DC amplifier (IDAC-232, Ockenfels Syntech GmbH, Kirchzarten, Germany). Three μL aliquots of the extracts were each administered onto a 1 cm^2 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.

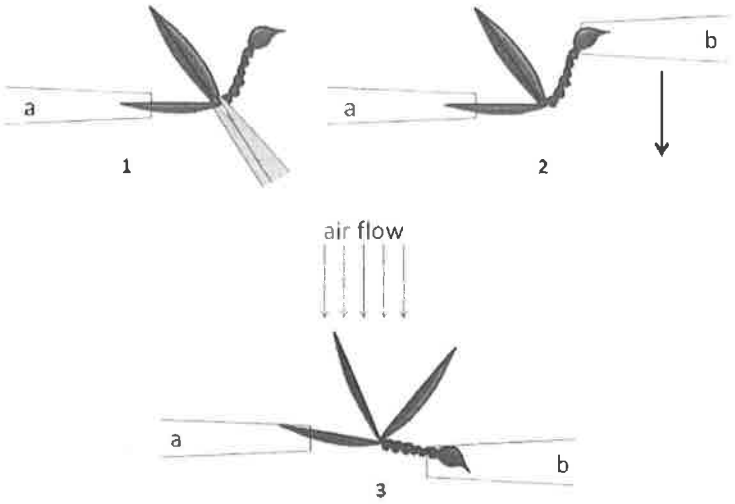

Fig. 1. Mounting of an antenna from a live *Cetonia a. aurata* or *Potosia cuprea* specimen between the recording (a) and ground electrode (b) for electroantennography (EAG) measurements

Three μL dichloromethane and blank air were used as control stimuli. Antennal responses were normalized against geraniol.

For analysis by coupled gas chromatography-electroantennography (GC-EAG) of the most EAG-active extract, the above apparatus was linked by an effluent conditioning assembly (Ockenfels Syntech GmbH, Kirchzarten, Germany) to a 6890 N GC (Agilent Technologies Inc., Santa Clara, USA) equipped with a DB-Wax column (30 m $\times$ 0.32 mm $\times$ 0.25 mm film thickness; J and W Scientific, Agilent Technologies, Santa Clara, USA). One μL injections were made in splitless mode (220°C). The oven temperature was held at 60°C for 1 min, then programmed at 10°C/min to 220°C and held for 10 min. The carrier gas was helium (4.0 L/min). Decyl acetate was used as internal standard.

A synthetic mixture of the identified compounds in ratios similar to those in the test extract was also tested in GC-EAG on the antennae of both *C. aurata aurata* and *P. cuprea*. Composition of the mixture (ng/μL): 1-hexanol : acetic acid : *n*-butyric acid : isovaleric acid : hexanoic acid : 3-methylphenol 17:350:15:35:35:1.

Identification of EAG-active peaks

Apple headspace extracts were analysed on an HP 6890 GC, equipped with a cool-on-column injector and FID, and fitted with a 30 m $\times$ 0.32 mm inner dia. $\times$ 0.5 μm film thickness polar DB-WAX column (J and W Scientific, Folsom, CA, USA). The oven temperature was maintained at 30°C for 2 min and then programmed at 10°C/min to 250°C. The carrier gas was hydrogen. For tentative identification of EAG-active peaks, GC-MS analysis was performed on a Micromass Autospec Ultima magnetic sector mass spectrometer (Waters, Milford, MA, USA), attached to an Agilent 6890 N GC (fitted with a 30 m $\times$ 0.32 mm inner dia. $\times$ 0.5 μm film thickness polar DB-WAX column, J and W Scientific, Folsom, CA, USA) and equipped with a cool-on-column injector. Ionization was

by electron impact (70 eV, 220°C). The GC oven temperature was maintained at 30°C for 5 min and then programmed at 5°C/min to 250°C. Tentative identifications were obtained by comparison of mass spectra with the NIST mass spectral database (2011), and were confirmed by comparison of KI values and GC peak enhancement with authentic standards. Quantification of compounds was achieved using the single-point external standard method with a series of alkanes.

Synthetic compounds for GC peak enhancement, GC-EAG and field trials were obtained from Sigma-Aldrich Kft. (Budapest, Hungary) and were >95% pure as stated by the supplier.

Field trapping experiments

CSALOMON® VARb3 modified funnel traps with transparent upper panels (produced by Plant Prot. Inst., CAR HAS, Budapest, Hungary) were used, since they efficiently capture both *C. aurata aurata* and *P. cuprea*, and other related scarabs (Imrei et al., 2001; Schmera et al., 2004; www.csalomontraps.com).

For preparing the bait dispensers, a 1 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany) was placed into a 1.25 cm^2 polythene bag made of 0.02 mm linear polyethylene foil. The dispenser was attached to a plastic strip (8 × 1 cm) for easy handling when assembling the traps. For making the baits, compounds were administered onto the dental roll and the opening of the polythene bag was heat-sealed. Earlier experience showed that the bait did not lose its activity during several weeks of field exposure; hence, we renewed the lures at 2–3-week intervals. For the ternary *Cetonia/Potosia* attractant (Tóth et al., 2005), 100 μL each of 3-methyleugenol (98.0 mg), (*RS*)-1-phenylethanol (101.2 mg) and (*E*)-anethol (99.8 mg) were loaded onto the same dental roll in a single dispenser.

Field trapping experiments were conducted at Telki (Pest county, Hungary) along the edge of an oak forest with mostly *Rosa canina* L. and *Crataegus* spp. (Rosaceae). Traps were set up in a randomized complete block design, with 10 m between traps within each block and 20 m between blocks. Traps were hung from the vegetation at 1.5 m height in sunny places and were inspected twice weekly, when captured beetles were removed, identified and recorded.

Experiment 1 was run between 19 July – 2 August 2004. The objective was to study the effect of the addition of apple pieces to the ternary floral attractant. Treatments: a) ternary attractant, b) apple pieces, c) a+b. One piece (ca. 10 cm^3) of fresh apple was placed into the catch container of each trap in treatment b-c at each inspection. Number of blocks: 5.

Experiment 2 was run between 15 – 19 June 2009. The objective was to study the effect of the addition of the synthetic blend of apple volatiles on the activity of the ternary floral attractant. Treatments included: a) the ternary attractant, b) synthetic apple blend, c) a+b, d) unbaited control traps. Number of blocks: 10. Composition of baits containing the synthetic blend of identified volatiles from fermenting apple pieces: 1-hexanol: 50 μL (40.7 mg), acetic acid: 200 μL (210 mg), *n*-butyric acid: 50 μL (48 mg), isovaleric acid: 50 μL (46.3 mg), hexanoic acid: 50 μL (46.5 mg), 3-methylphenol: 10 μL (10.3 mg). Compounds were loaded onto the same dental roll in a single dispenser.

Experiment 3 was run between 6 – 10 July 2009. The objective was to study the effect of the addition of individual components of the synthetic blend of apple volatiles

(100 μL each) to the ternary floral attractant. Treatments included: a) the ternary attractant, b) a + 1-hexanol, c) a + acetic acid, d) a + *n*-butyric acid, e) a + isovaleric acid, f) a + hexanoic acid, g) a + 3-methylphenol. Number of blocks: 6.

Experiment 4 was run between 20 – 27 July 2009. The objective was to study the effect of the addition of a subset of components of the synthetic blend of apple volatiles (100 μL each) to the ternary attractant (66 μL of each component). Treatments included: a) the ternary attractant, b) a + 1-hexanol + acetic acid + *n*-butyric acid + hexanoic acid, c) a + *n*-butyric acid. Number of blocks: 10.

Experiment 5 was run between 17 July – 17 August 2017. The objective was to study the effect of the addition of selected components of the synthetic blend of apple volatiles (1 mL each) to the ternary attractant (100 μL of each component). Treatments included: a) the ternary attractant, b) μ-butyric acid, c) μ-butyric acid + acetic acid + hexanoic acid, d) a + b, e) a + c, f) unbaited control traps. The baits in treatments b and c were loaded into polypropylene syringe dispensers. The dispenser consisted of a ca. 4 mL polypropylene tube, similar in shape to an injection syringe, and contained a 3 cm piece of dental roll (Celluron®, Paul Hartmann AG, Heidenheim, Germany). Compounds were administered onto the dental roll through the larger opening at the end of the syringe, which was then closed. The thin tube at the other end of the syringe was cut just before field deployment, so compounds could evaporate through the resulting 4 mm ID hole. Number of blocks: 6.

Statistics

For statistical analysis of EAG responses, ANOVA was performed, followed by Fisher's protected LSD for significance levels. Field catch data were summed over the duration of the experiment for each trap as they did not fulfil requirements for a parametric analysis, and were analysed by Kruskal–Wallis test. In case the Kruskal–Wallis test yielded significance ($P > 0.05$), paired comparisons of treatments were done by Mann–Whitney U test. All statistical procedures were conducted using the software package Genstat (18th edition; VSN International Ltd, Hemel Hempstead, UK).

Results

Addition of apple pieces to the ternary floral bait significantly increased catches of both *C. aurata aurata* and *P. cuprea* as compared to the ternary bait alone (Experiment 1; Table 1). The ternary lure attracted more *C. aurata aurata*, but not *P. cuprea*, than the apple pieces alone. In case of traps containing apple pieces only, it was observed that those in which the apple started to ferment had significantly higher numbers of both species (total *C. aurata aurata* catch: 584, total *P. cuprea* catch: 95) than those in which the apple became dry (total *C. aurata aurata* catch: 10, total *P. cuprea* catch: 8) (Mann-Whitney U test, $p < 0.001$ for both species, n(fermented) = 5, n(dry) = 10).

GC-EAG analysis of the highly EAG-active apple extract no. 10 (Fig. 2), using the antennae of *C. aurata aurata* and *P. cuprea*, located six peaks repeatedly evoking antennal responses in both species (Fig. 3). The active peaks were identified by GC-MS and GC peak enhancement with authentic standards as 1-hexanol, acetic acid, *n*-butyric acid,

Table 1

Catches of *Cetonia a. aurata* and *Potosia cuprea* in Experiment 1. Number of replicates/treatment = 5. *P* values at the bottom of the table are from Kruskal–Wallis one-way analysis of variance. *P* values of pairwise treatment comparisons derive from Mann–Whitney U test

Treatment	Treatment abbreviation	*Cetonia a. aurata*		*Potosia cuprea*	
		total catch	*p* values for pairwise comparisons	total catch	*p* values for pairwise comparisons
Ternary floral attractant	a	1893	a–b: < 0.001	131	a–b: 0.007
Ternary floral attractant + apple piece	b	4398	a–c: 0.002 b–c: < 0.001	611	a–c: ns b–c: 0.007
Apple piece	c	594		103	
		H(2) = 20.78, p < 0.001		H(2) = 13.69, p < 0.001	

ns = not significant (P = 0.05).

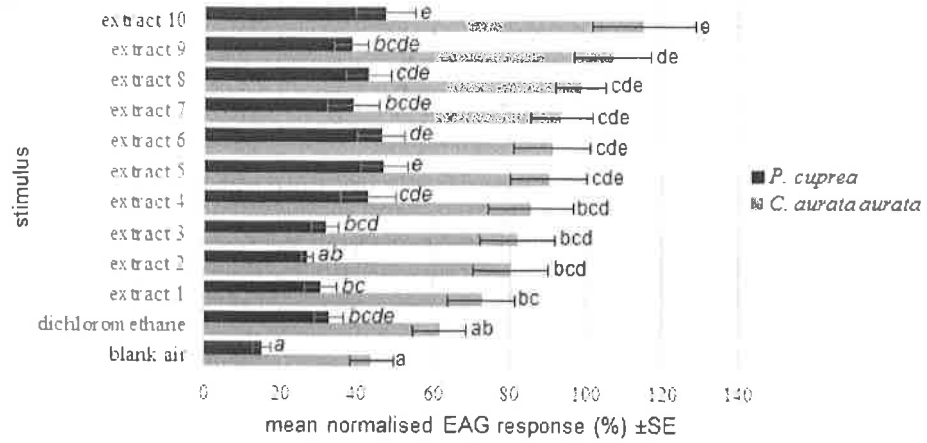

Fig. 2. Mean normalized EAG responses (± SE) of *Cetonia a. aurata* and *Potosia cuprea* antennae to 3 µL aliquots of headspace extracts collected from fermenting apple (n = 5/species). Bars with the same letter within one species are not significantly different (ANOVA, Fisher's LSD, P = 5%)

isovaleric acid, hexanoic acid and 3-methylphenol. Synthetic standards of the identified compounds also elicited EAG activity on the antennae of both species (Fig. 4).

The synthetic blend of volatiles, in approximately the same ratios as identified in headspace extracts from fermenting apple pieces, on its own attracted no beetles into the traps, catches not differing from those in unbaited traps. Addition of the synthetic blend to the ternary floral attractant significantly reduced catches of both species (Experiment 2; Table 2).

Addition of 1-hexanol, acetic acid, *n*-butyric acid and hexanoic acid individually to the ternary floral attractant caused numerical, but not significant, increase in *C. aurata aurata* catches, and 3-methylphenol reduced *P. cuprea* catches significantly, compared to the ternary lure (Experiment 3; Table 3).

Addition of the blend of 1-hexanol, acetic acid, *n*-butyric acid and hexanoic acid, as well as *n*-butric acid alone to the ternary lure reduced catches of both scarabs significantly (Experiment 4; Table 4).

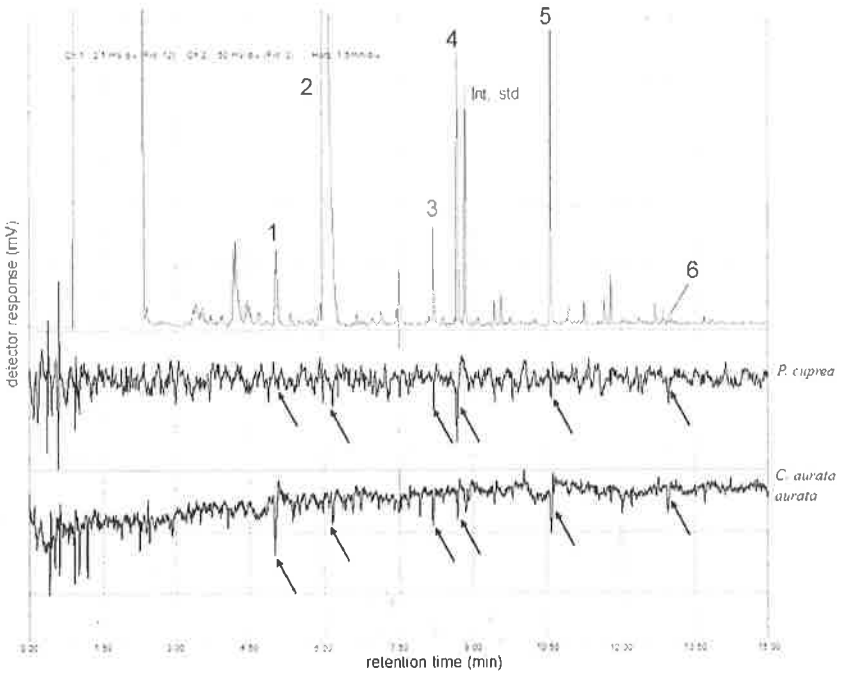

Fig. 3. Coupled gas chromatography-electroantennography (GC-EAG) analysis of a headspace extract from fermenting apple on antennae of *Cetonia a. aurata* and *Potosia cuprea* (1 µL injected). (1: 1-hexanol, 2: acetic acid, 3: *n*-butyric acid, 4: isovaleric acid, 5: hexanoic acid, 6: 3-methylphenol, Int. std.: decyl acetate). Arrows indicate EAG responses

Finally, neither *n*-butyric acid alone, nor the mixture of acetic acid, *n*-butyric acid and hexanoic acid increased the attractiveness of the ternary lure, and these baits alone caught no specimens of either species, similar to unbaited traps (Experiment 5; Table 5).

Discussion

Being present during a significant proportion of the fruit-growing period in Central Europe (May–August), *C. aurata aurata* and *P. cuprea* come across both the flowering and the fruit-bearing stages of members of the Rosaceae. As well as eating the generative parts of flowers, later generations readily feed on tree sap and fermenting fruit, similar to many cetoniin scarabs. Monitoring traps and mass-trapping tools now successfully exploit the olfaction-guided attraction of *C. aurata aurata* and *P. cuprea* to floral volatiles, and a similar scenario should exist for the beetles` behaviour towards fruit fermentation odours. The emission of these volatiles is induced by microorganisms; *Cotinis nitida* L. (Cetoniinae) beetles pick up their gut yeast flora from peach fruit (Johnson and Vishniac, 1991). After feeding for two days on ripening fruit, attraction of the *C. nitida* feeding complex to conspecifics increases due to the production of fermentation volatiles, which serve as aggregation kairomones. Johnson et al. (2009) identified several volatile compounds from such complexes and, indeed, found a synthetic mixture of these chemicals to be attractive to *C. nitida*.

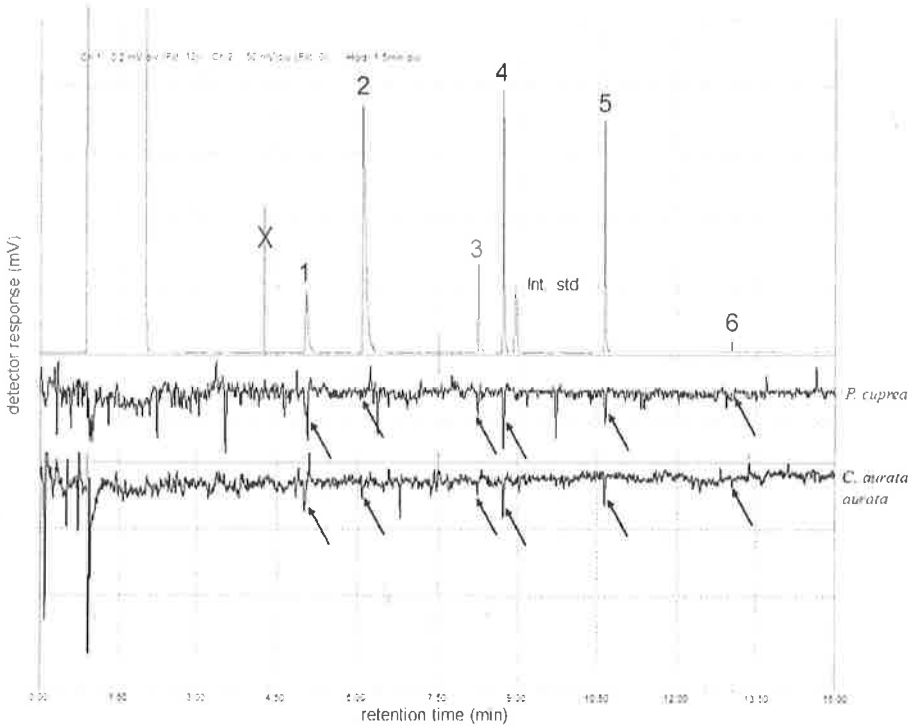

Fig. 4. Coupled gas chromatography-electroantennography (GC-EAG) analysis of a mixture of synthetic standards of volatiles, identified from headspace extracts of fermenting apple, on antennae of *Cetonia a. aurata* and *Potosia cuprea* (1 µL injected). (1: 1-hexanol, 2: acetic acid, 3: *n*-butyric acid, 4: isovaleric acid, 5: hexanoic acid, 6: 3-methylphenol, Int. std.: decyl acetate). Arrows indicate EAG responses

Both *C. aurata aurata* and *P. cuprea* are increasingly reported to attack ripening fruit, i.e. peach (Voigt et al., 2005; Razov et al., 2009), and native or acquired microflora can play a role in inducing fruit fermentation processes during feeding in these scarabs. In Experiment 1, traps baited with apple pieces on their own caught both species in numbers comparable to those with the ternary floral lure; however, beetles were mostly found in traps where the apple started to ferment. Similar to *C. nitida*, which is only weakly attracted to undamaged or mechanically damaged fruit (Johnson et al., 2009), it is likely that *C. aurata aurata* and *P. cuprea* are initially attracted in low numbers to traps baited with fresh pieces of apple. Antennae of *C. aurata aurata* can selectively detect headspace components of freshly cut apple (J. Vuts et al., unpublished), which may explain their field attraction to fruit in the traps. Apple pieces successfully located are then fed upon by the beetles and fruit fermentation initiated, accompanied by the emission of characteristic volatiles, whereas those pieces of apple that are not located early enough dry out and beetle attraction ceases. It is feasible to suppose that the feeding complex will continuously attract *C. aurata aurata* and *P. cuprea* specimens until the fruit material is completely consumed. This suggested scenario correlates well with the performance of traps baited with the ternary floral lure plus apple pieces, i.e. the floral lure as a potent attractant draws beetles into traps in high enough numbers for them to start feeding on the fruit, inducing the production of fermentation volatiles and subsequent beetle mass attraction.

GC-EAG experiments located six active peaks in headspace extracts from fermenting apple, which were identified by GC-MS and GC peak enhancement with authentic standards to be compounds often produced during microbial fermentation (Quinn et al., 2007; Johnson et al., 2009 and references therein; Zhang et al., 2009; Zareba et al., 2008;

Table 2

Catches of *Cetonia a. aurata* and *Potosia cuprea* in Experiment 2. Number of replicates/treatment = 10

Treatment	Treatment abbreviation	*Cetonia a. aurata*		*Potosia cuprea*	
		total catch	*p* values for pairwise comparisons	total catch	*p* values for pairwise comparisons
Ternary floral attractant	a	2480	a–b: < 0.001	547	a–b: < 0.001
Synthetic apple blend	b	0	a–c: 0.005	1	a–c: < 0.001
Ternary floral attractant + synthetic apple blend	c	809	a–d: < 0.001 b–c: < 0.001	81	a–d: < 0.001 b–c: < 0.001
Unbaited	d	0	b–d: ns c–d: < 0.001	1	b–d: ns c–d: < 0.001
		H(3) = 55.45, p < 0.001		H(3) = 57.23, p < 0.001	

For significance, refer to Table 1.

Table 3

Catches of *Cetonia a. aurata* and *Potosia cuprea* in Experiment 3. Number of replicates/treatment = 6

Treatment	Treatment abbreviation	*Cetonia a. aurata*		*Potosia cuprea*	
		total catch	*p* values for pairwise comparisons	total catch	*p* values for pairwise comparisons
Ternary floral attractant	a	706	a–b: ns	64	a–b: ns
Ternary floral attractant + acetic acid	b	707	a–c: ns a–d: ns	65	a–c: ns a–d: ns
Ternary floral attractant + n-butyric acid	c	1193	a–e: ns a–f: ns	54	a–e: ns a–f: ns
Ternary floral attractant + hexanoic acid	d	902	a–g: 0.029 b–c: ns	60	a–g: < 0.001 b–c: ns
Ternary floral attractant + 1-hexanol	e	848	b–d: ns b–e: ns	79	b–d: ns b–e: ns
Ternary floral attractant + isovaleric acid	f	605	b–f: ns b–g: 0.012	46	b–f: ns b–g: < 0.001
Ternary floral attractant + 3-methylphenol	g	295	c–d: ns c–e: ns c–f: ns c–g: 0.001 d–e: ns d–f: ns d–g: 0.014 e–f: ns e–g: 0.012 f–g: 0.014	13	c–d: ns c–e: ns c–f: ns c–g: 0.009 d–e: ns d–f: ns d–g: 0.008 e–f: ns e–g: < 0.001 f–g: 0.008
		H(6) = 12.93, p = 0.044		H(6) = 18.70, p = 0.004	

For significance, refer to Table 1.

Steinhaus and Schieberle, 2005), and which can function as insect semiochemicals (Davis et al., 2013; Vuts et al., 2014). Of these, 1-hexanol is an attractant of *Grapholita molesta* Busck (Lepidoptera: Tortricidae) (Pinero and Dorn, 2007), *Phyllopertha horticola* L. (Coleoptera: Scarabaeidae) (Ruther and Mayer, 2005) and *Melolontha melolontha* L. (Coleoptera: Scarabaeidae) (Imrei and Tóth, 2002). However, it can inhibit aggregation in bark beetles (Coleoptera: Scolytidae) (Deglow and Borden, 1998; Huber and Borden, 2001, 2003; Poland et al., 2004; Sullivan et al., 2007). Acetic acid attracts *Vespa* spp. (Hymenoptera: Vespidae) (Landolt et al., 1999, 2000), *Chrysoperla carnea* s.l. (Neuroptera: Chrysopidae) (Tóth et al., 2009) and moths (Lepidoptera: Noctuidae, Pyralidae and Phycitidae) (Landolt and Alfaro 2001; Tóth et al., 2002; Landolt et al., 2013). *n*-Butyric acid

Table 4

Catches of *Cetonia a. aurata* and *Potosia cuprea* in Experiment 4. Number of replicates/treatment = 10

Treatment	Treatment abbreviation	*Cetonia a. aurata*		*Potosia cuprea*	
		total catch	*p* values for pairwise comparisons	total catch	*p* values for pairwise comparisons
Ternary floral attractant	a	1425	a–b: 0.024	114	a–b: 0.015
Ternary floral attractant + n-butyric acid	b	1174	a–c: 0.007 b–c: 0.024	92	a–c: 0.004 b–c: 0.015
Ternary floral attractant + 4-component apple blend	c	912		59	
		H(2) = 8.395, p = 0.015		H(2) = 9.519, p = 0.008	

For significance, refer to Table 1.

Table 5

Catches of *Cetonia a. aurata* and *Potosia cuprea* in Experiment 5. Number of replicates/treatment = 6

Treatment	Treatment abbreviation	*Cetonia a. aurata*		*Potosia cuprea*	
		total catch	*p* values f or pairwise comparisons	total catch	*p* values for pairwise comparisons
Ternary floral attractant	a	57	a–b: 0.032	6	a–b: ns
Ternary floral attractant + μ-butyric acid	b	40	a–c: ns a–d: < 0.001	4	a–c: ns a–d: ns
Ternary floral attractant + acetic acid + μ-butyric acid + hexanoic acid	c	36	a–e: < 0.001 a–f: < 0.001 b–c: ns	6	a–e: ns a–f: ns b–c: ns
Acetic acid + μ-butyric Acid + hexanoic acid	d	0	b–d: 0.002 b–e: 0.002	0	b–d: ns b–e: ns
μ-butyric acid	e	0	b–f: 0.002 c–d: < 0.001	0	b–f: ns c–d: ns
Unbaited	f	0	c–e: < 0.001 c–f: < 0.001 d–e: ns d–f: ns e–f: ns	0	c–e: ns c–f: ns d–e: ns d–f: ns e–f: ns
		H(5) = 29.76, p = < 0.001		H(5) = 2.04, p = 0.015	

For significance, refer to Table 1.

is a pheromone component of *Riptortus serripes* Fabr. and *Mirperus scutellaris* Puton (Hemiptera: Alydidae and Coreidae, resp.) (Aldrich et al., 1993), and attracts *Anopheles gambiae* s.s. Giles mosquitoes (Constantini et al., 2001) and *Dacus tryoni* Froggat flies (Eisemann and Rice, 1992) (Diptera: Culicidae and Tephritidae, resp.). *n*-Butyric acid also acts as an allomone produced by *Alydus eurinus* Say (Hemiptera: Alydidae) (Aldrich et al., 2000). Isovaleric acid is an attractant of *A. gambiae* s.s. Giles (Diptera) (Constantini et al., 2001), as well as *Kaniska canace* L. and *Vanessa indica* Herbst (Lepidoptera: Nymphalidae) (Omura et al., 2000). Hexanoic acid attracts a number of beetles in the Scarabaeidae, Silvanidae and Laemophloeidae families (Poprawski and Yule, 1992; Williams et al., 2000; Collins et al., 2007), as well as *Lutzomyia* spp. (Andrade et al., 2008) and *Aedes aegypti* L. (Williams et al., 2006) (Diptera: Psychodidae and Culicidae, resp.). Finally, 3-methylphenol is a pheromone component of *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) (Heath et al., 1992).

Contrary to our expectation, field trapping experiments testing the synthetic blend of EAG-active volatiles revealed its inhibitory effect upon the ternary floral attractant. It is worth noting that if a compound elicits high EAG responses, it does not necessarily mean that it will also have behavioural activity, nor does it indicate what type of behaviour (attraction, repellence etc.) is to be expected (Roelofs, 1977). It is likely that the compounds identified as electrophysiologically active in the headspace extracts of fermenting apple fed upon by *C. aurata aurata* and *P. cuprea* chafers bear no attractiveness, but some of them rather have repellent activity, such as 3-methylphenol in Experiment 3, reducing beetle attraction to the ternary floral lure. Also, 1-hexanol in mixture with three acids in Experiment 4 was likely to be an inhibitory compound, because in Experiment 5, addition of the acids to the ternary floral lure did not reduce catches significantly. 1-Hexanol inhibited field attraction of a mirid bug in recent experiments (S. Koczor et al., unpublished). Formulation issues (dispenser type, composition, dose etc.) may have also played some part in the unsuccessful demonstration of the attractiveness of the newly identified compounds.

More importantly, we suggest new work to be done to isolate bioactive compounds connected to fruit fermentation. Rather than collecting from the headspace of fruit after being fed upon by the beetles, the feeding complex as a whole should be sampled, similar to the work by Hammons et al. (2009) on *C. nitida* and *Popillia japonica* Newman (Rutelinae) on grape berries. Such an approach might enable the identification of new fruit volatiles induced by beetle-mediated yeast contamination and fermentation, or those of beetle origin. To this end, feeding can induce pheromone production in many insects, such as bark beetles (Blomquist et al., 2010), and Foster (2009) showed that sucrose consumption increased sex pheromone titre in mated females of a moth. Also, pheromones and food-related volatiles often synergise each other`s activity (Reddy and Guerrero, 2004), thus, a combined lure of floral, fermentation and/or pheromone compounds may comprise a more potent lure for the mass-trapping of *C. aurata aurata* and *P. cuprea,* leading to the direct reduction of local populations.

Acknowledgements

This work was partially funded by the Hungarian Scientific Research Fund (OTKA) (grant K81494), and by the Research and Technology Innovation Fund (grant OMFB 00609/2010). The work at Rothamsted forms part of the Smart Crop Protection (SCP) strategic programme (BBS/OS/CP/000001) funded through Biotechnology and Biological Sciences Research Council's Industrial Strategy Challenge Fund. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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