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Alcoholism in cockchafers: orientation of male *Melolontha melolontha* towards green leaf alcohols

Received: 11 July 2001 / Accepted: 30 January 2002 / Published online: 13 March 2002
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Abstract Chemical orientation of the European cockchafer, *Melolontha melolontha* L., a serious pest in agriculture and horticulture, was investigated by field tests and electrophysiological experiments using plant volatiles. In total, 16 typical plant volatiles were shown to elicit electrophysiological responses in male cockchafers. Funnel trap field bioassays revealed that green leaf alcohols (i.e. (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol and 1-hexanol) attracted males, whereas the corresponding aldehydes and acetates were behaviourally inactive. Furthermore, male cockchafers were attracted by volatiles from mechanically damaged leaves of *Fagus sylvatica* L., *Quercus robur* L. and *Carpinus betulus* L. However, volatiles emitted by damaged leaves of *F. sylvatica* attracted significantly more males than those from the other host plants. Odour from intact *F. sylvatica* leaves was not attractive to *M. melolontha* males. Females were not attracted by any of the tested volatile sources. The results suggest that plant volatiles play a similar role as a sexual kairomone in mate finding of *M. melolontha*, as has been shown for the forest cockchafer, *Melolontha hippocastani* F. Nevertheless, both species show remarkable differences in their reaction to green leaf alcohols.

Introduction

The European cockchafer (May beetle), *Melolontha melolontha* L. (Coleoptera: Scarabaeidae), is a polyphagous insect that shows mass outbreaks every 30 to 40 years (Keller 1986). Adults may defoliate infested host trees, but even severe losses in leaf biomass are widely com-

pensated by secondary sprouting (Schwerdtfeger 1970). In contrast, the root-feeding larvae cause important economic losses in agriculture, horticulture and viticulture (Hurpin 1962; Keller 1986). During the past decade, massive outbreaks in central Europe have promoted the demand for sustainable control strategies which make use of kairomones and pheromones modifying the cockchafer's behaviour.

Adults of *M. melolontha* occur from mid-April until the end of May. An intensive swarming flight around host treetops can be observed at dusk of warm and dry days throughout the flight season. During these swarming flights, mating activity increases. Both males and females mate several times (Krell 1996). At the beginning of the flight season, emerging beetles perform a spectacular, optically guided flight from the areas of larval development (e.g. orchards, vineyards) to nearby forest edges (Schneider 1952).

Little is known about olfactory orientation in *M. melolontha*. In contrast, increasing knowledge has become available concerning pheromones and plant volatiles in the orientation of many other scarab beetles (Leal 1998). For example, feeding-damage-induced plant volatiles function as aggregation kairomones in the Japanese beetle, *Popillia japonica* Newman (Loughrin et al. 1995, 1998).

Green leaf volatiles (GLV) are released by damaged plant tissue (Hatanaka et al. 1995). They consist of a series of saturated and monounsaturated (Z)-3- or (E)-2-configured six-carbon aldehydes, alcohols, and esters thereof (Visser and Avé 1978; Ruther 2000). In some insects they are known to synergistically enhance the response to pheromones (Landolt and Phillips 1997). Males of the forest cockchafer, *Melolontha hippocastani* F. locate their mates by olfactory orientation towards the sex pheromone 1,4-benzoquinone and GLV induced by feeding females. Orientation towards both pheromone and plant kairomones allows males to discriminate sites where females feed from sites with unspecific leaf damage (Ruther et al. 2000, 2001). In experiments addressing the role of individual GLV, only the leaf alcohol (Z)-3-hexen-1-ol was attractive to *M. hippocastani*.

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Since (Z)-3-hexen-1-ol plays a key role in mate location of the forest cockchafer, it has been referred to as a sexual kairomone (Ruther et al. 2002).

The present study aimed to elucidate the role of plant volatiles for orientation of *M. melolontha*. The physiological response was investigated by means of gas chromatography with coupled electroantennographic detection (GC-EAD). Furthermore, we examined the behavioural response of swarming beetles to volatiles emitted by host plant leaves and synthetic GLV in the field.

Materials and methods

GC-EAD experiments

A series of GC-EAD runs was performed using male and female antennae. The experimental details were described by Ruther et al. (2000). Synthetic mixtures containing typical plant volatiles (e.g. Takabayashi et al. 1991; Mattiacci et al. 1994; Loughrin et al. 1997; Ruther 2000; Table 1) at concentrations of 1 mg ml⁻¹ each were injected onto the column of a gas chromatograph, and EAD responses to individual compounds were recorded. Each mixture was tested on at least three different antennae of both sexes.

Funnel trap experiments

Experiments were performed between 29 April and 13 May 2001 at the edge of forests close to Obergrombach, Baden-Württemberg, in southern Germany.

Funnel traps used for the experiments were the same as those described by Ruther et al. (2000). Synthetic lures were applied 1.5 h before the swarming period on balls of cotton wool, which were placed into round plastic boxes (ID × H: 36×29 mm) and fixed at the top of the traps. All single chemicals and mixtures were applied in 500 µl dichloromethane. Natural baits were placed into the bottles and volatiles were allowed to evaporate through the funnel. When testing the attractiveness of volatiles from intact versus damaged leaves (Expt 1), access of trapped beetles to the leaf material was prevented by separating the plastic bottles into two chambers of equal volume with round wire meshes (5 mm mesh).

Sets of funnel traps were arranged in a complete randomised block design. Each set was placed at least 30 min before the swarming period at equivalent positions (3–5 m above ground) of an infested host tree (*Fagus sylvatica*, *Quercus robur*, *Quercus petraea*, *Acer pseudoplatanus* or *Carpinus betulus*) as described by Ruther et al. (2000) with a minimum distance of 1.5 m between traps. Catches were sexed and counted the next morning. Numbers of beetles trapped with each treatment were analysed by a Friedman-ANOVA and sequential Bonferroni-corrected Wilcoxon matched pairs test using Statistica 4.5 scientific software (StatSoft, Hamburg, Germany).

Natural odorant sources

Experiment 1: Attractiveness of volatiles from intact vs mechanically damaged leaves. Each block (three traps) consisted of the following treatments: (1) 5 g intact leaves of *F. sylvatica*, and (2) 5 g damaged leaves of *F. sylvatica* as volatile sources, plus (3) an empty control trap. For mechanical damage, leaves were torn into several pieces, while intact leaves were picked from the twig and carefully placed into the bait chamber to avoid mechanical damage. Two hours before the swarming period, traps were baited with freshly picked or damaged leaves (*n*=23 replications).

Experiment 2: Attractiveness of volatiles from mechanically damaged leaves of different host plants. Each block (four traps) consist-

ed of the following treatments: damaged leaves of either (1) *F. sylvatica*, (2) *C. betulus*, or (3) *Q. robur* as volatile sources and (4) an empty control. The amount of leaves per trap and handling of leaves were the same as described in Expt 1 (*n*=20 replications).

Synthetic odorants

Experiment 3: Attractiveness of (Z)-3-configured GLV. Each block (four traps) consisted of the following treatments: (1) 5 mg of (Z)-3-hexenal, (2) 5 mg of (Z)-3-hexen-1-ol, (3) 5 mg of (Z)-3-hexenyl acetate, and (4) 500 µl dichloromethane (solvent control) (*n*=19 replications).

Experiment 4: Attractiveness of (E)-2-configured GLV. Each block (five traps) consisted of the following treatments: (1) 5 mg of (E)-2-hexenal, (2) 5 mg of (E)-2-hexen-1-ol, (3) 5 mg of (E)-2-hexenyl acetate, (4) 5 mg of a GLV mixture composed of (Z)-3-hexenyl acetate (3.2 mg), (Z)-3-hexen-1-ol (1.5 mg), (E)-2-hexenal (0.2 mg) and (Z)-3-hexenal (0.1 mg), and (5) 500 µl dichloromethane. The GLV mixture was developed to mimic the bouquet of mechanically damaged host leaves and has been shown to be highly attractive to *M. hippocastani* (Ruther et al. 2002) (*n*=22 replications).

Experiment 5: Attractiveness of saturated GLV. Each block (five traps) consisted of the following treatments: (1) 5 mg of hexanal, (2) 5 mg of 1-hexanol, (3) 5 mg of hexyl acetate, (4) 5 mg of the GLV mixture as described in Expt 4, and (5) 500 µl dichloromethane (*n*=17 replications).

Results

GC-EAD experiments

The results of the GC-EAD experiments are shown in Table 1. In total 23 compounds were tested for electrophysiological activity using antennae from male *M. melolontha*. Sixteen compounds elicited responses up to 4 mV. With female antennae, 12 out of 18 tested compounds triggered a physiological response. A chromatogram showing the response of a male antenna to (Z)-3-hexen-1-ol and some other typical plant volatiles is shown in Fig. 1.

Funnel trap experiments

Around sunset on warm and dry days, numerous cockchafers started to fly and hover around twigs. On cold and wet days, almost no flight activity could be observed. Therefore, catch numbers varied widely from day to day depending on weather conditions. A total of 3,667 males and 126 females were caught within a period of 14 days. A preference of female cockchafers for any treatment could not be detected. Many females were observed to stay feeding on the host trees during the swarming period.

Experiment 1: Attractiveness of intact vs mechanically damaged leaves

Traps baited with mechanically damaged *F. sylvatica* leaves caught significantly more male beetles (144) than

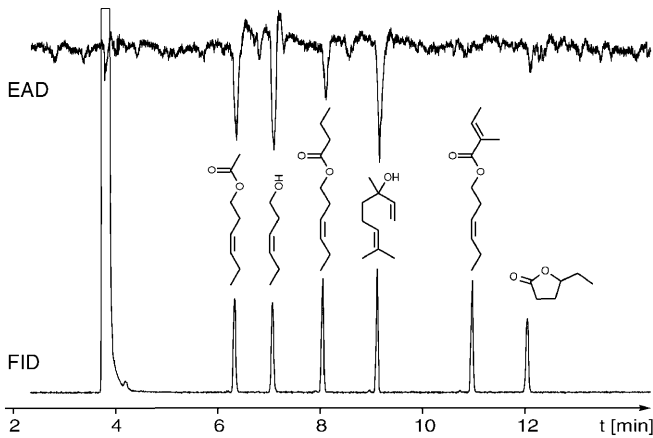


Fig. 1 Gas chromatogram with simultaneous flame ionisation detection (FID) and electroantennographic detection (EAD) obtained by injection of 1 μ l of a standard solution containing some typical plant volatiles at concentrations of 1 μ g μ l⁻¹ each. Compounds are in order of appearance: (Z)-3-hexenyl acetate, (Z)-3-hexen-1-ol, (Z)-3-hexenyl butyrate, linalool, (Z)-3-hexenyl tiglate, γ -hexalactone

Table 1 GC-EAD activities of selected synthetic plant volatiles on male (m) and female (f) antennae of *Melolontha melolontha*. + Clear response (0.5–4 mV), (+) weak response (<0.5 mV), 0 no response, n.t. not tested

Compound	EAD activity	
	Male	Female
(Z)-3-hexenyl acetate	+	+
(Z)-3-hexen-1-ol	+	+
(Z)-3-hexenyl butyrate	+	+
Linalool	+	+
cis-Linalool oxide	0	0
trans-Linalool oxide	0	0
(Z)-3-hexenyl tiglate	0	+
γ -hexalactone	(+)	0
(Z)-3-hexenyl benzoate	0	0
(E)-2-hexenyl acetate	(+)	n.t.
6-methyl-5-hepten-2-one	+	n.t.
(E)-2-hexen-1-ol	+	+
Benzaldehyde	(+)-+	n.t.
β -caryophyllen	0	0-(+)
Ethyl benzoate	+	n.t.
Methyl jasmonate	0	n.t.
Hexyl acetate	+	+
1-hexanol	+	+
Hexyl hexanoate	0	0-(+)
Methyl salicylate	+	+
Ethyl salicylate	+	+
Benzyl alcohol	+	+
2-phenylethanol	+	+

traps with intact leaves (40) or empty control traps (36) ($P < 0.001$; Fig. 2a).

Experiment 2: Attractiveness of volatiles from mechanically damaged leaves of different host plants

Traps baited with damaged *F. sylvatica* leaves caught significantly more males (792) than traps baited with

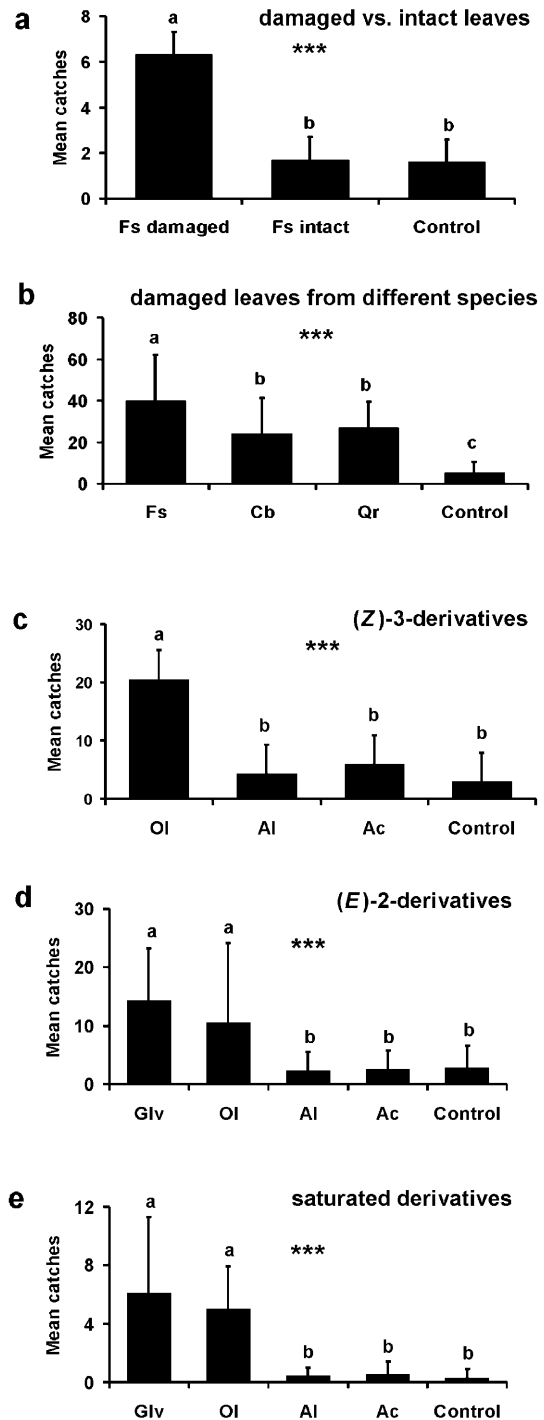


Fig. 2 Mean number of males (\pm SD) caught in funnel trap experiments with natural (a, b) and synthetic odorant sources (c–e). Blocks of traps were tested simultaneously. a–e correspond to Expts 1–5. *Fs* *F. sylvatica*, *Cb* *C. betulus*, *Qr* *Q. robur*, *GLV* *GLV* mixture as described for Expts 4 and 5, *OI* C_6 -alcohol, *Al* C_6 -aldehyde, *Ac* C_6 -acetate, *Control* empty control trap in a–b and solvent control trap in c–e. *** indicates significant with $\chi^2=22.35$, $df=2$, $n=23$, $P < 0.00001$ (a); $\chi^2=40.60$, $df=3$, $n=20$, $P < 0.00001$ (b); $\chi^2=38.48$, $df=3$, $n=19$, $P < 0.00001$ (c); $\chi^2=36.93$, $df=4$, $n=22$, $P < 0.00001$ (d); $\chi^2=41.16$, $df=4$, $n=17$, $P < 0.00001$ (e) (Friedman ANOVA). Means with different letters are significantly different at $P < 0.001$ (a, c), $P < 0.01$ (e), $P \leq 0.010$ (b), $P \leq 0.029$ (d) (sequential Bonferroni-corrected Wilcoxon matched pairs test)

C. betulus (480) or *Q. robur* (536) ($P=0.010$). Volatiles from mechanically damaged leaves of any tested host tree species caught significantly more male *M. melolontha* than empty control traps (103) ($P<0.001$; Fig. 2b).

Experiment 3: Attractiveness of (Z)-3-configured GLV

Traps baited with (Z)-3-hexen-1-ol caught significantly more male cockchafer (389) than (Z)-3-hexenal (82), (Z)-3-hexenyl acetate (114) or solvent control traps (56) ($P<0.001$). Traps baited with (Z)-3-hexenyl acetate caught twice as many beetles as solvent control traps. Statistical analysis showed that this difference was not significant at the 0.05 level ($P=0.069$, Fig. 2c).

Experiment 4: Attractiveness of (E)-2-configured GLV

Traps baited with (E)-2-hexen-1-ol (230) and the GLV mixture (315) caught significantly more cockchafer than (E)-2-hexenal (53), (E)-2-hexenyl acetate (67) or solvent control traps (61) ($P<0.01$ except for (E)-2-hexen-1-ol vs control $P=0.026$ and (E)-2-hexen-1-ol vs (E)-2-hexenal $P=0.029$; Fig. 2d).

Experiment 5: Attractiveness of saturated GLV

Traps baited with 1-hexanol (72) and the GLV mixture (87) caught significantly more cockchafer than hexanal (7), hexyl acetate (9) or solvent control traps (4) ($P<0.01$; Fig. 2e).

Discussion

The results of this study demonstrate that during the swarming period at dusk only males of the European cockchafer are attracted by volatiles emitted by mechanically damaged host plant leaves and by synthetic green leaf volatiles occurring in the bouquet of mechanically damaged host plant leaves. This suggests that in *M. melolontha* green leaf volatiles play a similar role as a sexual kairomone as in the forest cockchafer, *M. hippocastani*, enabling males to locate sites of mechanical damage caused by feeding females (Ruther et al. 2000, 2001, 2002). In the latter species a sexual dimorphism in the flight behaviour has been demonstrated. While male *M. hippocastani* perform the swarming flight at dusk, females remain feeding on the leaves of the host trees (Ruther et al. 2001). During this study, 97% of the beetles trapped were males. Therefore we assume that *M. melolontha* shows the same sexual dimorphism in flight behaviour as the closely related species.

However, there is a remarkable difference between the two *Melolontha* species regarding the role of individual GLV. *M. hippocastani* males are attracted only by (Z)-3-hexen-1-ol, while (E)-2-hexen-1-ol and 1-hexanol

were behaviourally inactive (Ruther et al. 2002). Thus, forest cockchafer males discriminate between leaf alcohols regarding the presence, the position, and/or the configuration of a double bond in the candidate compounds. In contrast, *M. melolontha* males are attracted by any of the tested green leaf alcohols.

In both species, *M. melolontha* (the present study) and *M. hippocastani* (Ruther et al. 2002), males were not attracted to *n*-hexyl-, (E)-2-hexenyl- or (Z)-3-hexenyl acetates and aldehydes. However, at least the acetates elicited EAD signals similar to the behaviourally active alcohols.

Olfactory receptor neurons (ORNs) specific for the detection of single GLV compounds have been identified in the scarab beetle *Phyllopertha diversa* Waterhouse (Hansson et al. 1999). The response threshold to (Z)-3-hexenyl acetate was two orders of magnitude below the sensitivity of the pheromone-detecting ORNs in this species (Nikonov et al. 2001). The discussed ecological function of ubiquitous green leaf alcohols as sexual kairomones in *M. hippocastani* (Ruther et al. 2002) and *M. melolontha* may help to explain, why ORNs with a high sensitivity to GLV compounds are found in scarab beetles.

In the present study, the odorants emanating from damaged leaves of *F. sylvatica* attracted significantly more *M. melolontha* males than volatiles from leaves of the other host plants *C. betulus* and *Q. robur*. The question whether damaged *F. sylvatica* leaves release higher amounts of attractive green leaf alcohols than damaged leaves from the other host plants, or whether species specificity of the volatile pattern is responsible for their higher attractiveness, needs further investigation.

Acknowledgements We thank Manfred Fröschle, Landesanstalt für Pflanzenschutz Stuttgart, and Robert Weiland, mayor of Obergrombach, for logistical support; Ute Braun from our laboratory for technical assistance; Maya Ulbricht, Berlin, for assistance in the field; and Dr. Stefan Sieben for his never ending enthusiasm and help in the field. Part of this work has been funded by the Deutsche Forschungsgemeinschaft (DFG, Hi 416/13-1).

References

- Hansson BS, Larsson MC, Leal WS (1999) Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. *Physiol Entomol* 24:121–126
- Hatanaka A, Kajiwara T, Matsui K (1995) The biogenesis of green odour by green leaves and its physiological functions – past, present and future. *Z Naturforsch* 50:476–472
- Hurpin B (1962) Famille des Scarabaeides. In: Balachowsky AS (ed) *Entomologie appliquée à l'agriculture*. Masson, Paris, pp 24–203
- Keller S (1986) Biologie und Populationsdynamik. In: *Neuere Erkenntnisse über den Maikäfer*. Beih Mitt Thurgau Naturforsch Ges 1:12–39
- Krell FT (1996) The copulatory organs of the cockchafer, *Melolontha melolontha* (Insecta: Coleoptera: Scarabaeidae). A contribution to comparative and functional anatomy of ectodermal genitalia of the Coleoptera. *Stuttgarter Beitr Naturkd Ser A* no 537
- Landolt PJ, Phillips TW (1997) Host plant influence on sex pheromone behavior of phytophagous insects. *Annu Rev Entomol* 42:371–391

- Leal WS (1998) Chemical ecology of phytophagous scarab beetles. *Annu Rev Entomol* 43:39–61
- Loughrin JH, Potter DA, Hamilton-Kemp TR (1995) Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese beetle (*Popillia japonica* Newman). *J Chem Ecol* 21:1457–1467
- Loughrin JH, Potter DA, Hamilton-Kemp TR, Byers ME (1997) Response of Japanese beetles (Coleoptera: Scarabaeidae) to leaf volatiles of susceptible and resistant maple species. *Environ Entomol* 26:334–342
- Loughrin JH, Potter DA, Hamilton-Kemp TR (1998) Attraction of Japanese beetles (Coleoptera: Scarabaeidae) to host plant volatiles in field trapping experiments. *Environ Entomol* 27:395–400
- Mattiacci L, Dicke M, Posthumus MA (1994) Induction of parasitoid attracting synomone in Brussels sprout plants by feeding of *Pieris brassicae* larvae: role of mechanical damage and herbivore elicitor. *J Chem Ecol* 20:2229–2247
- Nikonov AA, Valiyaveetil JT, Leal WS (2001) A photoaffinity-labeled green leaf volatile compound ‘tricks’ highly selective and sensitive insect olfactory receptor neurons. *Chem Senses* 26:49–54
- Ruther J (2000) Retention index database for identification of general green leaf volatiles in plants by coupled capillary gas chromatography – mass spectrometry. *J Chromatogr A* 890:313–319
- Ruther J, Reinecke A, Thiemann K, Tolasch T, Francke W, Hilker M (2000) Mate finding in the forest cockchafer, *Melolontha hippocastani*, mediated by volatiles from plants and females. *Physiol Entomol* 25:172–179
- Ruther J, Reinecke A, Tolasch T, Hilker M (2001) Make love not war: a common arthropod defense compound as sex pheromone in the forest cockchafer *Melolontha hippocastani*. *Oecologia* 128:44–47
- Ruther J, Reinecke A, Hilker M (2002) Plant volatiles in the sexual communication of *Melolontha hippocastani*: response towards time-dependent bouquets and novel function of (Z)-3-hexen-1-ol as a sexual kairomone. *Ecol Entomol* 27
- Schneider F (1952) Untersuchungen über die optische Orientierung der Maikäfer (*Melolontha vulgaris* F. und *M. hippocastani* F.) sowie über die Entstehung von Schwärmbahnen und Befallskonzentrationen. *Mitt Schweiz Entomol Ges* 25:269–340
- Schwerdtfeger F (1970) Die Waldkrankheiten. Parey, Berlin, pp 160–163
- Takabayashi J, Dicke M, Posthumus MA (1991) Variation of predator-attracting allelochemicals emitted by herbivore-infested plants: relative influence of plant and herbivore. *Chemoecology* 2:1–6
- Visser JH, Avé DA (1978) General green leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata*. *J Chem Ecol* 24:538–549