Phenol – Another Cockchafer Attractant Shared by *Melolontha hippocastani* Fabr. and *M. melolontha* L.

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Z. Naturforsch. **57 c**, 910–913 (2002); received June 18, 2002

Phenol, Attractant, *Melolontha* sp., Scarabaeidae

The response of the two most abundant cockchafer species in central Europe, *Melolontha hippocastani* and *M. melolontha*, towards phenol, mixtures of phenol with the leaf alcohol (*Z*)-3-hexen-1-ol and the known cockchafer pheromones, 1,4-benzoquinone (*M. hippocastani*) and toluquinone (*M. melolontha*), was investigated in the field. During the swarming period at dusk, phenol attracted males of both species, and enhanced the known attraction of cockchafer males towards (*Z*)-3-hexen-1-ol. A mixture of phenol plus (*Z*)-3-hexen-1-ol was less attractive for *M. hippocastani* males than a mixture of (*Z*)-3-hexen-1-ol plus 1,4-benzoquinone, whereas phenol plus (*Z*)-3-hexen-1-ol attracted as many *M. melolontha* males as a mixture of (*Z*)-3-hexen-1-ol plus toluquinone. In both species three component mixtures containing phenol, (*Z*)-3-hexen-1-ol, and the respective benzoquinone did not capture more males than two component mixtures consisting of only (*Z*)-3-hexen-1-ol and the benzoquinone. A possible role of phenol as another cockchafer sex pheromone component is discussed.

Introduction

Cockchafers of the genus *Melolontha* (may beetles) (Coleoptera: Scarabaeidae) can be severe pests in forestry, agriculture, and horticulture. Mass breeding of the two most important species, the forest cockchafer *M. hippocastani* Fabr. and the European cockchafer *M. melolontha* L., occurs currently in several parts of central Europe. The demand for environmentally sound control methods has initiated an intense research focusing on the chemical orientation of cockchafers. This research revealed an intriguing mechanism of chemically mediated mate finding for both species including orientation of males towards damage-induced plant volatiles and sex pheromones. Green leaf alcohols emitted by infested host leaves after feeding of the females attract swarming males (Ruther *et al.*, 2000, 2002; Reinecke *et al.*, 2002b). Beetle-derived benzoquinones (1,4-benzoquinone in *M. hippocastani* and toluquinone in *M. melolontha*) synergize the male response enabling discrimination between leaf damage caused by feeding females and unspecific leaf damage (Ruther *et al.*, 2001b; Reinecke *et al.*, 2002a).

In a recent study, we identified seven compounds in whole body extracts of *M. hippocastani* exhibiting physiological activity in experiments using gas chromatography with coupled electroantennographic detection (GC–EAD) (Ruther *et. al.*, 2001b). Among these compounds were the two mentioned benzoquinones and phenol, the first known scarab beetle sex pheromone identified in the grass grub beetle *Costelytra zealandica* (Henzell and Lowe, 1970). Hence, we suggested phenol as a pheromone candidate for *Melolontha* cockchafers. We present data showing that phenol attracts males of *M. hippocastani* and *M. melolontha* in the field.

Methods and Materials

Experimental sites

Both species were investigated in heavily infested deciduous forests in southwestern Germany. Field experiments with *M. hippocastani* were performed between 24 April and 13 May 2002 in a mixed woodland area near Bürstadt, in the state of Hesse. Predominant deciduous trees

were *Quercus rubra* L. and *Carpinus betulus* L. Experiments with *M. melolontha* were carried out between 25 April and 10 May 2002 in a mixed woodland near Endingen (Kaiserstuhl) in the state of Baden-Württemberg. Predominant trees in the *M. melolontha* test site were *Quercus* spp., *C. betulus*, and *Fagus sylvatica* L.

Funnel trap experiments

The funnel traps used in the field experiments were the same as described before (Ruther *et al.*, 2000). Test chemicals dissolved in 500 µl dichloromethane and solvent controls were applied on balls of cotton wool. Sets of four traps (3 treatments and 1 control) were randomized and arranged in a complete block design. At least 30 min before the swarming period, traps of each block were placed at equivalent positions (4–7 m above the ground) of infested host trees. Captures were sexed and counted the next morning. Only captures obtained in blocks of the same design were compared statistically. Numbers of beetles trapped with each treatment were analyzed by a Friedman ANOVA and consecutive multiple Wilcoxon matched pairs tests with sequential Bonferroni-correction. Statistica 4.5 scientific software was used for statistical analysis (StatSoft Inc., Hamburg, Germany).

Experiment 1. The response of *M. hippocastani* and *M. melolontha* towards the following treatments was compared: (1) 500 µl dichloromethane, (2) 5.0 mg phenol, (3) 5.0 mg (*Z*)-3-hexen-1-ol, (4) 5.0 mg each of phenol and (*Z*)-3-hexen-1-ol (n = 44 for *M. hippocastani* and n = 23 for *M. melolontha*).

Experiment 2. The response of *M. hippocastani* towards the following treatments was compared: (1) 500 µl dichloromethane, (2) 5.0 mg each of phenol and (*Z*)-3-hexen-1-ol, (3) 5.0 mg each of 1,4-benzoquinone and (*Z*)-3-hexen-1-ol, (4) 5.0 mg each of phenol, 1,4-benzoquinone, and (*Z*)-3-hexen-1-ol (n = 20).

Experiment 3. The response of *M. hippocastani* towards the following treatments was compared: (1) 500 µl dichloromethane, (2) 5.0 mg each of phenol and (*Z*)-3-hexen-1-ol, (3) 5.0 mg each of 1,4-benzoquinone and (*Z*)-3-hexen-1-ol, (4) 2.5 mg each of phenol and 1,4-benzoquinone, plus 5.0 mg of (*Z*)-3-hexen-1-ol (n = 20).

Experiment 4. The response of *M. melolontha* towards the following treatments was compared: (1) 500 µl dichloromethane, (2) 5.0 mg each of phenol and (*Z*)-3-hexen-1-ol, (3) 5.0 mg each of toluquinone and (*Z*)-3-hexen-1-ol, (4) 5.0 mg each of phenol, toluquinone, and (*Z*)-3-hexen-1-ol (n = 20).

Results

Field data are summarized in Table 1. Like in our previous cockchafer studies (Ruther *et al.*, 2000, 2001a,b, 2002; Reinecke *et al.*, 2002a,b), exclusively males were captured. Traps baited with phenol, (*Z*)-3-hexen-1-ol, or a mixture of both compounds caught significantly more males of both cockchafer species than non-baited control traps (experiment 1). For *M. hippocastani,* the attractiveness of phenol and (Z)-3-hexen-1-ol was statistically not distinguishable, in *M. melolontha* the leaf alcohol was more attractive than phenol. In both species a mixture of phenol + (Z)-3-hexen-1-ol was more attractive than the single compounds.

The mixture of 1,4-benzoquinone + (*Z*)-3-hexen-1-ol attracted significantly more *M. hippocastani* males than the mixture of phenol + (*Z*)-3-hexen-1-ol (experiment 2–3). A three component mixture consisting of phenol + 1,4-benzoquinone + (*Z*)-3-hexen-1-ol at 5 mg each did not lead to a further increase of male captures. On the contrary, when 5 mg each of phenol + 1,4-benzoquinone was applied (experiment 2), the number of captured males was decreased significantly compared to 1,4-benzoquinone + (Z)-3-hexen-1-ol and reached the level of phenol + (*Z*)-3-hexen-1-ol. When the dose of phenol and 1,4-benzoquinone in the three-component mixture was halved to 2.5 mg each (experiment 3), the same tendency was visible even if the difference was no longer significant (P = 0.053).

In *M. melolontha* the mixture of phenol + (*Z*)-3-hexen-1-ol captured as many males as the mixture of toluquinone + (*Z*)-3-hexen-1-ol (experiment 4). A three component lure containing phenol + toluquinone + (*Z*)-3-hexen-1-ol did not increase the number of captured males when com-

Table I. Mean captures of cockchafer males in differently baited funnel traps. Numbers represent mean catches ± standard deviation. Means with different lowercase letters indicate significant differences within columns for each experiment at $P < 0.05$ (multiple Wilcoxon matched pairs test after sequential Bonferroni correction).

	lure	*M. hippocastani*	*M. melolontha*
Exp. 1			
	control	0.39 ± 0.95 a	2.96 ± 4.18 a
	5.0 mg phenol	2.52 ± 5.65 b	8.17 ± 10.67 b
	5.0 mg (*Z*)-3-hexen-1-ol	6.43 ± 14.34 b	11.70 ± 9.95 c
	5.0 mg each of phenol + (Z)-3-hexen-1-ol	10.66 ± 15.97 c	22.70 ± 24.11 d
	Friedman ANOVA	$Chi^2 = 46.86$; df = 3 $P < 0.001$; $n = 44$	$Chi^2 = 43.15$; df = 3 $P < 0.001$; $n = 23$
Exp. 2			
	control	1.65 ± 1.87 a	
	5.0 mg each of phenol + (*Z*)-3-hexen-1-ol	67.90 ± 82.44 b	
	5.0 mg each of 1,4-benzoquinone + (*Z*)-3-hexen-1-ol	117.20 ± 80.97 c	
	5.0 mg each of 1,4-benzoquinone + phenol + (*Z*)-3-hexen-1-ol	65.05 ± 51.41 b	
	Friedman ANOVA	$Chi^2 = 44.34$; df = 3 $P < 0.001$; $n = 20$	
Exp. 3			
	control	1.60 ± 1.88 a	
	5.0 mg each of phenol + (*Z*)-3-hexen-1-ol	40.80 ± 25.31 b	
	5.0 mg each of 1,4-benzoquinone + (*Z*)-3-hexen-1-ol	69.55 ± 25.44 c	
	2.5 mg each of 1,4-benzoquinone + phenol + 5 mg (*Z*)-3-hexen-1-ol	53.40 ± 27.03 bc	
	Friedman ANOVA	$Chi^2 = 42.66$; df = 3 $P < 0.001$; $n = 20$	
Exp. 4			
	control		1.65 ± 1.76 a
	5.0 mg each of phenol + (*Z*)-3-hexen-1-ol		22.00 ± 12.49 b
	5.0 mg each of toluquinone + (*Z*)-3-hexen-1-ol		23.70 ± 15.56 b
	5.0 mg each of toluquinone + phenol + (*Z*)-3-hexen-1-ol		23.20 ± 17.88 b
	Friedman ANOVA		$Chi^2 = 35.24$; df = 3 $P < 0.001$; $n = 20$

pared to mixtures of phenol + (*Z*)-3-hexen-1-ol and toluquinone + (*Z*)-3-hexen-1-ol, respectively.

Discussion

Our results demonstrate that phenol exclusively attracts males of both *M. hippocastani* and *M. melolontha.* Since phenol was identified from female whole body extracts of *M. hippocastani* (Ruther *et al.*, 2001b) and *M. melolontha* (unpublished data), this compound could be considered as another sex pheromone component shared by both species. Like the sex pheromones reported before in cockchafers, i. e. 1,4-benzoquinone in *M. hippocastani* (Ruther *et al.*, 2001b) and toluquinone in *M. melolontha* (Reinecke *et al.*, 2002a), phenol enhances the male response towards damage-induced plant volatiles. However, phenol did not further increase male captures in both species if added to lures consisting of only (*Z*)-3-hexen-1-ol and the respective benzoquinone. On the contrary, in *M. hippocastani* the number of males was de-

creased significantly after addition of phenol (experiment 2). A possible explanation for this phenomenon might be suboptimal ratios of the three components in the lure. It is known from Lepidoptera that species specificity in closely related sympatric species could be achieved by different blends of shared pheromone components (Cardé and Baker, 1984). Since our results of experiments 2–4 at present do not allow to postulate the role of phenol as another cockchafer sex pheromone unambiguously, future studies will be necessary to investigate this aspect more thoroughly.

Phenol has been identified before as the sex pheromone of the grass grub beetle *C. zealandica.* (Henzell and Lowe, 1970) and without behavior modifying properties in another melolonthine scarab beetle, *Holotrichia consanguinea* (Leal *et al.*, 1996). Like the other sex pheromones reported in melolonthine species (Leal, 1997; Ruther *et al.*, 2001a), phenol is a potent antimicrobial agent. Hence, phenol as a sex pheromone in *Melolontha* cockchafers would support the secondary function hypothesis on the evolution of melolonthine sex pheromones from defensive compounds (Leal, 1997).

Acknowledgements

The authors thank J. Collatz, G. Dröge, N. Hesler, S. Hülse and K. Schrank for their help during the fieldwork. Dr. H. Gossenauer-Marohn, Dr. J. Gonschorrek (Hessen-Forst, Hann.-Münden) and M. Fröschle (Landesanstalt für Pflanzenschutz Stuttgart) gave logistic support. This research was financially supported by the Hessian state forest administration and the Deutsche Forschungsgemeinschaft (Hi 416/13–1).

Cardé R. T. and Baker T. C. (1984), Sexual communication with pheromones. In: Chemical Ecology of Insects (Bell W. J., Cardé R. T., eds.). Chapman & Hall, New York, pp. 355–386.

Henzell R. F. and Lowe M. D. (1970), Sex attractant of the grass grub beetle. Science **168**, 1005–1006.

Leal W. S. (1997), Evolution of sex pheromone communication in plant feeding scarab beetles. In: Insect Pheromone Research: New Directions (Cardé R. T., Minks A. K., eds.). Chapman & Hall, New York, pp. 505–513.

Leal W. S., Yadava C. P. S. and Vijayvergia J. N. (1996), Aggregation of the scarab beetle *Holotrichia consanguinea* in response to female-released pheromone suggests secondary function hypothesis for semiochemical. J. Chem. Ecol. **22**,1557–1566.

Reinecke A., Ruther J. and Hilker M. (2002a), The scent of food and defence: Green leaf volatiles and toluquinone as sex attractant mediate mate finding in the European cockchafer, *Melolontha melolontha*. Ecol. Lett. **5**, 257–263.

Reinecke A., Ruther J., Tolasch T., Francke W. and Hilker M. (2002b), Alcoholism in cockchafers: Orientation of male *Melolontha melolontha* towards green leaf alcohols. Naturwissenschaften **89**, 265–269.

Ruther J., Reinecke A., Thiemann K., Tolasch T., Francke W. and 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., Podsiadlowski L. and Hilker M. (2001a), Quinones in cockchafers: additional function of a sex attractant as an antimicrobial agent. Chemoecology **11**, 225–229.

Ruther J., Reinecke A., Tolasch T. and Hilker M. (2001b), Make love not war: A common arthropod defence compound as sex pheromone in the forest cockchafer, *Melolontha hippocastani*. Oecologia **128**, 44–47.

Ruther J., Reinecke A. and 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**, 76–83.