1,4-Benzoquinone Attracts Males of *Rhizotrogus vernus* Germ.

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Z. Naturforsch. **57c,** 177–181 (2002); received September 7/October 8, 2001

Rhizotrogus vernus, Field Trapping, Sex Attractant, Coleoptera: Scarabaeidae

Two candidate attractants, phenol and 1,4-benzoquinone, a synthetic mixture of typical compounds from green-leaf odours [(*Z*)-3-hexenyl acetate: (*Z*)-3-hexen-1-ol: benzaldehyde: (*E*)-2-hexen-1-ol: 1-hexanol; 100:20:10:1:1] and freshly damaged oak leaves were screened for field attractancy in funnel traps in Hungary. Males of two *Rhizotrogus* spp. (Coleoptera, Scarabaeidae, Melolonthinae), *R. aestivus* Ol. and *R. vernus* Germ. were caught in larger numbers. While *R. aestivus* catches were probably chance captures, male *R. vernus* was significantly attracted to the baits containing 1,4-benzoquinone. This compound represents a promising basis for the development of a monitoring trap for *R. vernus.*

Introduction

Chafers are among the most dangerous insect pests on agricultural plants. Effective chafer attractants can have a high value in an environmentally conscious plant protection. In the present project our aim was to define such attractants for chafers of the Hungarian fauna through field screening of synthetic candidate attractants. In these tests we concentrated on selected compounds described for species of Melolonthinae and Dynastinae subfamilies.

Test compounds included phenol, which has been known to be attractive towards males of *Costelytra zealandica* (White) (Coleoptera, Scarabaeidae, Melolonthinae) in New Zeland (Henzell and Lowe, 1970) and was identified from crude abdominal gland extracts of *Holotrichia consanguinea* Motschulsky (Leal *et al.*, 1996). Another test compound was 1,4-benzoquinone. Quinones have recently been described to attract adults of the rhinoceros beetle *Cyphonistes tuberculifrons* Quedenfeldt (Coleoptera, Scarabaeidae, Dynastinae) (Krell *et al.*, 1999). In the tests, also a synthetic mixture of typical compounds from green-leaf odours was included, as recently traps baited with such compounds or with damaged leaves of the host plant have been reported to attract males of the forest cockchafer *Melolontha hippocastani* F. (Coleoptera, Scarabaeidae, Melolonthinae) (Ruther *et al.*, 2000).

In the present paper we report on captures of *Rhizotrogus* spp. (Coleoptera, Scarabaeidae, Melolonthinae). Data on other chafers will be presented elsewhere.

Materials and Methods

Test site

All tests were done at Telki (Pest county, Hungary), ca. 20 km from Budapest, at the edge of a mixed oak forest. Tests were carried out between May 4 and June 6, 2001. Traps of a replicate were set out in a line by the edge of the forest, at a height of ca. 2 m, on branches of forest trees. Treatments were set out in a random order within the line. Traps within one replicate were ca. 10 m from each other; distances between replicates ranged from 50 m to 200 m. Traps were inspected on every second or third day when captured beetles were counted, their sex was determined by the size of the antennae (Endrödi, 1956) and removed from the traps.

Trap types

The VARb trap was the standard funnel trap used by the Budapest laboratory for catching scarab spp. (Tóth *et al.*, unpubl.). The trap consisted of a plastic funnel (top opening outer diameter: 13 cm, funnel hole diameter: 3 cm, height of funnel: 16 cm), under which a transparent plastic

0939–5075/2002/0100–0177 \$ 06.00

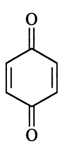
round catch container was attached by a rubber band. On top of the funnel a piece of plastic sheet (10×16 cm) was attached vertically reaching accross the top opening of the funnel. The dispenser was suspended from the vertical plastic sheet, and the bait hung in the middle of the funnel opening at ca. 1 cm higher than the level of the upper edge of the funnel.

VARb3 war a modification of the VARb trap. The main difference between the two trap designs was that in case of the VARb3 trap the top funnel opening was enlarged by attaching transparent plastic sheets to the trap body, so that the inner diameter of the top funnel opening became ca. 20 cm.

Chemicals

1,4-Benzoquinone was purchased from Sigma-Aldrich Kft (Budapest, Hungary) and phenol from REANAL Finomvegyszergyár Rt. (Budapest, Hungary).

The synthetic mixture of green leaf odours (GLmix) was a simplified version of a mixture described by Ruther *et al.* (2000). It contained (*Z*)-3-hexenyl acetate, (*Z*)-3-hexen-1-ol, benzaldehyde, (*E*)-2-hexen-1-ol and 1-hexanol in a proportion of 100:20:10:1:1 (v/v).



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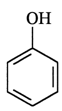

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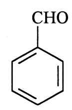

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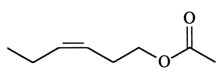

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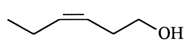

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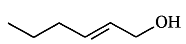

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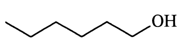

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Chemical structures of test compounds.

(*E*)-2-Hexen-1-ol and (*Z*)-3-hexenyl acetate were purchased from Bedoukian Inc. (Danbury, USA), while (*Z*)-3-hexen-1-ol, benzaldehyde, and 1-hexanol were from Sigma-Aldrich Kft. (Budapest, Hungary).

All compounds were >95% pure as stated by the suppliers.

Baits

Two types of bait dispensers were used in the tests:

Kartell: 0.7 ml polyethylene vials with lid (no. 730, Kartell Co., Italy) were used; the vial was kept closed when setting up the trap in the field.

PE bag: A 1 cm piece of dental roll (Celluron®, Paul Hartmann Ag. Heidenheim, Germany) was put into a tight bag made of 0.02 mm linear polyethylenefoil.

All types of baits were attached to 8×1 cm plastic handles for easy handling when assembling the traps.

For making up the baits, the required amounts of compounds were administered into (Kartell) or onto the dental roll (PE bag) dispensers, in dichloromethane solvent (quinone) or neat (phenol; GL mix). The lid of the PE vial dispensers was closed, and PE bag dispensers were heatsealed. Dispensers were wrapped singly in pieces of alufoil and were stored at −18 °C until use. Doses of compounds were 50 mg (Kartell) or 100 mg (PE bag), resp.

For traps baited with damaged leaves of the natural host plant, oak leaves (*Quercus sessiliflora* Salisb.) were torn up by hand to 1–2 cm^2 pieces. Freshly prepared leaves (ca. 40 g) were placed into the catch container of the traps when the traps were inspected.

Statistics

In statistical analyses, catches recorded at an inspection were regarded as replicates. Capture data were transformed to (x + 0.5)1/2, and differences between means were tested for significance by ANOVA followed by Games-Howell test. Differences of single treatments from zero catch (unbaited) were tested by one-sample *t* test. Statistical analyses were performed by the softwares StatView™ v.4.01 and SuperANOVA™ v1.11 (Abacus Concepts, Inc., Berkeley, USA).

Results and Discussion

Two *Rhizotrogus* species, *R. vernus* Germ. and *R. aestivus* Ol. were captured regularly during the first test.

In VARb traps, *R. vernus* males were caught in significantly higher numbers in all traps where the bait contained 1,4-benzoquinone, as compared to traps without bait or baited only with GLmix (Table I). No beetles were caught in the traps baited with damaged oak leaves. Among the VARb3 traps, again only the bait containing 1,4-benzoquinone attracted significantly more than the unbaited control or the trap with GLmix only.

Males of *R. aestivus* were captured in low numbers by several of the baits, but the catch was not significantly different from that of unbaited traps (Table I). No females of any of the above *Rhizotrogus* spp. were caught in this test.

In the second test, among the single compounds tested, traps with 1,4-benzoquinone captured males of *R. vernus* (Table II). Also the other two baits containing 1,4-benzoquinone in mixtures were attractive. There was no significant difference between the quinone-containing baits. GLmix or phenol alone or the unbaited traps caught negligible numbers. Apart from males, two *R. vernus* females were also caught in this test, one in the trap baited with GLmix plus phenol and another in that baited with GLmix, only. Again no preference of male *R. aestivus* towards any of the baits was noted, all treatments, even unbaited traps were catching low numbers (Table II). One female *R. aestivus* specimen was also caught in one of the GLmix-baited traps.

We conclude that *R. aestivus* catches were probably chance captures due to the physical characteristics of the traps, since no preference towards any of the baits could be detected in the present study.

On the other hand, the field attractivity of 1,4-benzoquinone towards males of *R. vernus* was clearly demonstrated in the above tests. Although the presence of quinones in defensive secretions of beetles is well documented in Carabidae (Eisner, 1958; Schildknecht and Holoubek, 1961), Staphylinidae (Dettner, 1991; Peschke *et al.*, 1996; Steidle and Dettner, 1995) and Tenebrionidae (Dettner, 1993; Hodges *et al.*, 1996; Tschinkel, 1975), reports on attractive activity of this group of compounds is scarce. An example of a scarab being attracted by quinones is that of the dynastin beetle *C. tuberculifrons* (Krell *et al.*, 1999), where quinones are thought to indicate food sources. Also, Krell *et al.* (1997, 1998) reported that necrophagous dung beetles of the genus *Onthophagus* (Scarabaeidae, Coprinae) use the quinones of

Table I. Captures of *Rhizotrogus* spp. in traps baited with mixtures of 1,4-benzoquinone, phenol and synthetic GLmix or damaged oak leaves in Hungary. For chemical structures see Figure. (May 14–18, 2001; from each trap/bait combination 4 repetitions were set up. In the test a total of 125 *R. vernus* and 27 *R. aestivus* male beetles were caught.) Descriptive statistics were calculated using captures of inspections per trap as single data. Means with same letter within one column do not differ significantly at P = 5% by ANOVA followed by Games-Howell test. Means with a # sign do not differ from zero catch at P = 5% (by one-sample *t* test).

	Baits (dispenser type)					Captures/trap/inspection	
	GLmix	Phenol		1,4-Benzoquinone		*R. vernus*	*R. aestivus*
Trap type	(PE bag)	(PE bag)	(Kartell)	(PE bag)	(Kartell)	mean (±S.E.)	
VARb	yes	no	no	no	no	0.00# (±0.00)	0.08a# (±0.08)
VARb	yes	yes	no	yes	no	1.75b (±0.48)	0.00# (±0.00)
VARb	yes	no	yes	no	yes	1.17ab (±0.44)	0.00# (±0.00)
VARb	no	yes	no	yes	no	2.42b (±0.63)	0.25a (±0.13)
VARb	no	no	yes	no	yes	3.08b (±0.80)	0.08a# (±0.08)
VARb	Unbaited					0.00# (±0.00)	0.08a# (±0.08)
VARb	Damaged oak leaves					0.00# (±0.00)	0.25a# (±0.18)
VARb3	yes	no	no	no	no	0.25a# (±0.18)	0.75a (±0.25)
VARb3	yes	yes	no	yes	no	1.75ab (±0.45)	0.25a# (±0.25)
VARb3	Unbaited					0.00# (±0.00)	0.50a (±0.29)

Table II. Captures of *Rhizotrogus* spp. in traps baited with 1,4-benzoquinone, phenol or synthetic GLmix alone or in binary and ternary combinations in Hungary. (May 21–June 6, 2001; VARb3 traps; from each trap/bait combination 4 repetitions were set up. In the test a total of 133 *R. vernus* and 39 *R. aestivus* male beetles were caught.) For statistics see Table I.

Baits (dispenser type)			Captures/trap/inspection	
GLmix	Phenol	1,4-Benzoquinone	*R. vernus*	*R. aestivus*
(PE bag)	(Kartell)	(Kartell)	mean (±S.E.)	
yes	no	no	0.00# (±0.00)	0.25a (±0.14)
yes	yes	yes	2.00b (±0.38)	0.38a (±0.22)
yes	yes	no	0.06a# (±0.06)	0.50a (±0.20)
yes	no	yes	2.00b (±0.57)	0.06a (±0.06)
no	yes	no	0.06a# (±0.06)	0.19a (±0.10)
no	no	yes	4.19b (±0.80)	0.44a (±0.16)
Unbaited			0.00# (±0.00)	0.56a (±0.22)

diplopod defensive secretions to locate fresh millipedecarcasses. Scarabs in the above examples belong to other subfamilies than *Rhizotrogus*.

Since in the present tests the quinone-containings baits caught exclusively males, it is possible that in the case of *R. vernus* quinone is part of the sex pheromonal signal. Very recently, 1,4-benzoquinone was described as one component of the pheromone of the forest cockchafer (*M. hippocastani*) (Ruther *et al.*, 2001). In this species, males orientate towards damage-induced green leaf volatiles allowing location of mechanically damaged foliage. In order to distinguish between unspecific leaf damage and damage caused by feeding females, male chafers take advantage of a female-produced pheromone (Ruther *et al.*, 2000). 1,4-Benzoquinone identified in female-derived extracts, and this compound synergistically increased the number of males caught in traps baited with the green leaf volatiles (Ruther *et al.*, 2001). The genus *Melolontha* belongs to the same subfamily as *Rhizotrogus*.

In contrast to *M. hippocastani* (Ruther *et al.*, 2000, 2001), no attractancy of the two *Rhizotrogus* spp. towards synthetic or natural leaf odours was observed in the present study. This is not surprising, as most authors agree that adult *Rhizotrogus* seldomly if ever feed during their adult life (Endrödi, 1956; Homonnay and Homonnayné, 1990; Hurpin, 1962).

The production of highly attractive sex pheromones by females has also been reported in the closely related *R. aequinoctialis* Herbst (Ivashchenko and Oleshchenko, 1972; Ivashchenko *et al.*, 1972; Zamotaylov, 1988), however, the chemical composition of the sex pheromone is yet unknown. *R. aequinoctialis* usually starts to fly very early (early April) in the season; probably this species already stopped flying at the test site by the time our tests were started.

R. vernus occurs in the major part of Southern Europe (Austria, Northern Italy, Hungary, the Balkans and Greece) and in the European parts of the former Soviet Union from the Black Sea to the Southern part of the Azov Sea in the south, while from the surroundings of Kiev, Poltava and Harkov in the north (Hurpin, 1962). Although this species is generally of secondary importance, it has been known to cause damage to sugarbeet and in nurseries (Hurpin, 1962). The male attractant 1,4-benzoquinone described in the present study provides a promising basis for the development of attractant traps for the use of detection and monitoring of this pest chafer.

Acknowledgements

The present paper is dedicated to Tibor Jermy on his 85th birthday. The present study was partially supported by OTKA grant T 029126 of the Hungarian Academy of Sciences.

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