the manner of infection of *Bufo* tadpoles with *N. carpocapsae*, it is probably similar to the results presented here, with death resulting from bacteria that entered the host's coelom through penetration holes made by the nematodes. These nematodes are not being used or recommended to control aquatic insects at this time; however, if they are so in the future, then their effect on young tadpoles should be taken into consideration.

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Identification and electroantennographic activity of sex-specific geranyl esters in an abdominal gland of female *Agriotes obscurus* (L.) and *A. lineatus* (L.) (Coleoptera, Elateridae)[1]

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Summary. Both geranyl hexanoate and geranyl octanoate were identified by GC/MS as the major volatiles in a hitherto uncharacterized abdominal gland in females in *Agriotes obscurus*. Only geranyl octanoate was found in *A. lineatus*. In EAG tests performed on *A. obscurus* males, geranyl butanoate and geranyl hexanoate elicited the strongest antennal responses.
Key words. Electroantennogram; GC/MS; geraniol; geranyl hexanoate; geranyl octanoate; opalescent gland; nerol.

The click beetles *Agriotes lineatus* and *A. obscurus* are widely distributed in Europe[4], with large populations typically in fallow fields. During the larval stage, which may extend over five years, these species are saprophagous[4,5], or feed on roots; as adults, they are thought to feed on rye and wheat, particularly the pollen[5]. Click beetle larvae (commonly called wireworms) can reach numbers high enough to cause economic damage, and many species, including *A. lineatus* and *A. obscurus*, are important pest insects. Control of elaterids has received considerable attention[6], and one biological control approach consists of capturing the males by luring them to the odor of live females[7]. The odor signals of elaterids are not well known, although sex attractants have been reported for several species (see review by Jacobson[8]). In *Agriotes*, evidence suggests that male attractants are produced in the female abdomen[9–11], but the precise site has not been established. To determine both the source and chemical identity of these volatile substances released by female *A. lineatus* and *A. obscurus*, extracts of isolated abdominal glands and intact abdomina were compared by GC/MS. The physiological response of males and females to individual components was evaluated by electroantennography (EAG)[12].

Material and methods. Chemical analyses. Seven *Agriotes lineatus* (Linnaeus, 1767) and 21 *A. obscurus* (Linnaeus, 1758) females were collected in May–June, at the peak of their activity season[13], at the Ecological Research Station on Öland, Sweden. Intact abdomina, isolated abdominal accessory glands[14] and hitherto undescribed (see Results) abdominal opalescent glands from single insects were extracted separately in redestilled hexane (Merck; 0.1 ml for glands, 1 ml for abdomina). For comparison, intact abdomina of 5 *A. lineatus* and 12 *A. obscurus* males were similarly extracted. Each extract was analyzed by GC or GC/MS.

Separations of volatile constituents were carried out on an HP 5880 gas chromatograph using a Superox FA 30 m fused-silica capillary column (i.d. 0.25 mm), temperature programmed at 50 °C for 4 min, followed by 4 °C/min to 200–220 °C. Identifications of components were made using an LKB 2091 mass spectrometer combined with a PYE gas chromatograph, equipped with a WG11 50-m glass capillary column (i.d. 0.25 mm); mass spectra and retention times were compared with those of authentic samples and references. Mass spectra and voucher beetle specimens are deposited at the Ecological Research Station.

Electrophysiological studies. EAG tests were performed on 57 *A. obscurus* and 2 *A. lineatus* males, 26 *A. obscurus* and 1 *A. lineatus* females. Two procedures were followed in the preparation of test samples: 1) most substances were diluted in redistilled hexane; 10 µl samples were then pipetted onto pieces (size found not to be critical) of Whatman No. 1 filter paper, which were placed in vials within disposable plastic syringes; and 2) a few substances were diluted in paraffin (Merck) and put directly into vials within syringes. After equilibrating the gas/liquid phases for a minimum of 5 min, 7.3 ml were injected with a hydraulic piston for 1.0 s into a steady air flow (4.0 l/min) aimed towards the antennae 10 mm away. Seventeen different substances (fig. 3) were tested at 1-min intervals, and in duplicate on the majority of the insects. EAGs were amplified with a purpose-built amplifier (Murphy Developments, Hilversum, The Netherlands, type CD 83-1 b), which also displayed the initial fast DC deflections and was equipped with an automatic base-line drift compensator.

Depolarization data, expressed in mV, were treated according to van der Pers[15]. Several error factors were minimized by a step-wise procedure. These factors include: 1) the decreasing sensitivity of the antenna (accounted for by presenting a standard as every third stimulus and evaluating responses to test substances in relation to the standard); 2) small differences between test runs, i.e. setup and animal conditions (accounted for by expressing each relative value above as a fraction of the sum of relative values for each animal). Evaporation rates (molecules/s) of each test substance were computed volumetrically according to a method to be described in detail elsewhere[16].

Significant differences in responses were determined using the Wilcoxon rank sum test ($p < 0.05$).

Results and discussion. Volatiles in *A. obscurus* and *A. lineatus* were detected in extracts of intact female abdomina and isolated opalescent glands, but not in the accessory glands. Similarities in volatile composition of the former pair of extracts point to the opalescent gland as the source of these abdominal substances. This gland is sacciform, ca 0.2×1.0 mm, and located in the 7th abdominal segment. It discharges posteriorly into the outer part of the oviduct.

The volatile profiles of the female extracts were on the whole similar in both species (figs 1 and 2, table 2), with the notable exception that geranyl octanoate (G 8) was the single dominant constituent in *A. lineatus,* but both G8 and geranyl hexanoate (G6) in *A. obscurus*. The ratios of G 6 to G 8 were significantly different between the two species (*A. obscurus:* $x = 1.12$, s.d. $= 0.63$, $n = 14$; *A. lineatus:* $x = 0.0200$, s.d. $= 0.0267$, $n = 7$). Minor constituents included geranyl decanoate, geraniol, nerol, corresponding aldehydes, small amounts of aliphatic methyl esters, and hydrocarbons. Contrary to earlier investigations [17], no traces of farnesyl acetate could be found. Intra- and interspecific variation, however, was generally high, perhaps in part reflecting differing ages of beetles. Unsurprisingly, none of these volatiles were detected in extracts of male abdomina.

Antennae of female and male *A. obscurus* differed in their electrophysiological responses to female abdominal volatiles shown in figure 3 for substances tested at the same syringe-loaded concentration (w/v) (without accounting for differences in vapor pressure). Differences between the sexes were statistically significant at the $p < 0.05$ level only for the reference (octanol) and geranyl butanoate (G4); for G 6 it fell just below ($p = 0.0502$). The responses of females and males did not differ for either G 8 (the main component of the abdominal extract) or the female extracts. Female responses were overall very weak, the only substances eliciting significant depolarization above noise level (i.e., above control) being citral, geranyl proprionate (G 3), and the reference. In contrast, males demonstrated strong responses to female extracts, G4, G6, G 3 (tested on only one male), and somewhat weaker responses to geraniol, geranyl acetate, and the reference; all were significantly different from the control. Nei-

Table 1. Female body parts and chemicals reported in literature as attractants for male Elateridae*

Species	Attractant
Agriotes ferrugineipennis	Female abdomen [21]
A. gurgistanus	Females [9]
	Geranyl butanoate [17, 22, 23]
	-3-methyl butanoate [17]
A. lineatus	Female abdomen [9, 10, 11]
	E, E-farnesyl acetate [17]
	Geranyl butanoate [23]
A. litigiosus	Female abdomen [7, 9, 24]
	Geranyl butanoate [17, 23]
	-isopentanoate [25]
	-3-methyl butanoate [17]
A. ponticus	Geranyl butanoate [26]
A. reiteri	Females [9]
A. sparsus	Geranyl butanoate [27]
	-isopentanoate [27]
	pentanoate [27]
A. sputator	Geranyl butanoate [28]
	-propanoate [28]
	E, E-farnesyl hexanoate [28]
Athous lecontei	Geraniol + derivatives [27]
Ctenicera destructor	Females [29]
	Female abdomen [21]
C. sylvatica	Female abdomen [21]
Hemicrepidius decoloratus	Females [30]
H. morio	Females [31]
Limonius californicus	Lactic acid [32]
	Butanoic acid [32]
	Pentanoic acid [32–35]
	Hexanoic acid [32]
	Female abdomen [21, 33, 36]
L. canus	Lactic acid [32]
	Butanoic acid [32]
	Pentanoic acid [32]
	Hexanoic acid [32, 37]
	A carboxylic acid [38]
	Unsaturated carboxylic acid, c:a 10 carbons [37]
Melanotus depressus	(Z)-11-tetradecen-1-ol [39]
	(E)-11-tetradecen-1-ol [39, 40]
	(E)-11-tetradecenyl acetate [39, 40]
Selatosomus latus	*Valerianella dentata* [41] (flowers)

* Chemical nomenclature adapted according to IUPAC rules.

Table 2. Volatile substances* identified by GC/MS in intact abdomina and abdominal opalescent glands of *Agriotes lineatus* and *A. obscurus* females

Substances	Mw.	*A. lineatus* ($n = 7$)	*A. obscurus* ($n = 21$)
Neral	152	X	X
Geranial	152	X	X
Nerol	154	X	X
Geraniol	154	X	X
Geranoic acid	170	X	trace
Geranyl hexanoate (G6)	252	X	XXX
Geranyl octanoate (G8)	280	XXX	XXX
Geranyl decanoate	308	X	X
Methyl hexadecanoate	270	X	X
Methyl octadecenoate**	296	X	X
Methyl octadecanoate	298	X	X

* Expressed in relative amounts of total area, X < 15%, XX = 15%–25%, XXX > 25%. Absolute amount of G8 per individual (both species), 2–4 µg. ** Position of double bond not estimated.

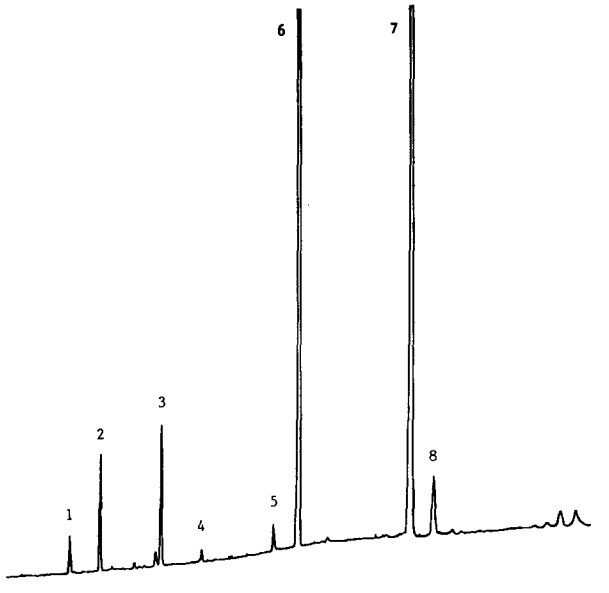

Figure 1. Capillary gas chromatogram of abdominal extract of *A. obscurus* female. 1 = neral; 2 = geranial; 3 = nerol; 4 = geraniol; 5 = eicosane; 6 = geranyl hexanoate; 7 = geranyl octanoate; 8 = methyl octadecanoate.

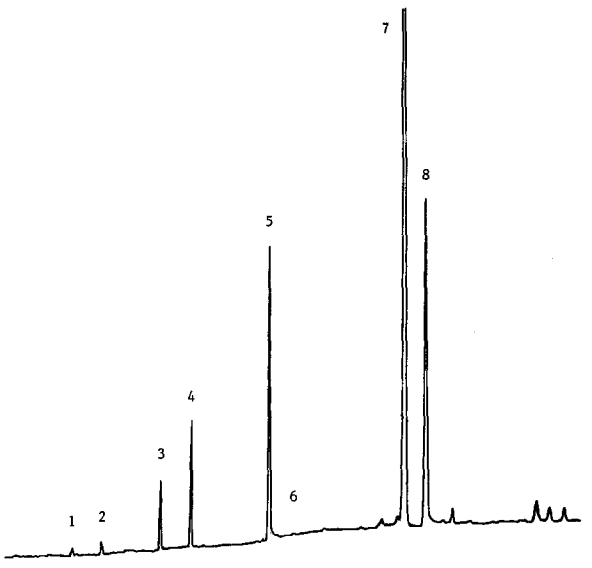

Figure 2. Capillary gas chromatogram of abdominal extract of *A. lineatus* female. Number symbols as in fig. 1, geranyl hexanoate (G6) present in trace quantities.

ther the *A. obscurus* nor the few tested *A. lineatus* males reacted to farnesyl acetate, which is reported to be a major component in the pheromone of *A. lineatus*[17]. The recording, exclusively in males, of strong depolarizations to some of the substances found in the female abdominal glands suggests that these compounds carry a sex-related function. Clarification of this question requires behavioral evidence.

It should be noted that vapor pressure differences between the compounds tested can influence EAG results. Indeed, under constant syringe conditions (loaded concentration and temperature), the number of molecules entering the gaseous phase will vary with the carbon chain length of the compound tested. Actual concentrations (molecules/ml in stimulus puff) of tested substances were obtained by determining their evaporation rates at 2 or 3 syringe-loaded concentrations. Preliminary dose-response plots (EAG recordings to each substance) based on these actual concentrations[16] indicate the following: 1) When at the same actual concentration (same number of molecules offered as stimulus), G3, G4, and G6 elicit EAG recordings at least ten times stronger than does geranyl acetate, thus pointing further to these compounds' greater depolarization capacity; 2) The relatively low responses recorded in this study for G8 might in fact result from its lower vapor pressure (fewer molecules in the stimulus puff); 3) The relatively weak responses recorded here to the non-geranylic compounds are reinforced by their having *actual* concentrations that are magnitudes higher in comparison with the geranyl esters. In order to reliably interpret electroantennogram data, and recordings of olfactory stimulations in general, it is crucial that more care be taken to base data on the actual concentrations of tested substances.

Available information on elaterid sex attractants, compiled in table 1, points to the female abdomen as the source of chemicals attracting males. Evidence presented here from our own chemical analyses on *A. obscurus* and *A. lineatus* and EAG tests on *A. obscurus* not only verify these findings, but furthermore identify the production site of the attractants as a small gland in the 7th abdominal segment.

From a taxonomic point of view, our results support previous indications (table 1) that elaterid sex attractants fall into chemical categories corresponding to taxonomic groups within Elateridae. We have defined these emerging categories as comprising 1) geranyl- and/or farnesyl esters, found in *Agriotes* (and *Athous*?), 2) aliphatic acids in *Limonius*, and 3) tetradecenols and derivatives in *Melanotus*.

Males of *Agriotes*[18] and *Limonius*[19] have been observed to visit and pollinate the orchid *Ophrys litigiosa*, upon which

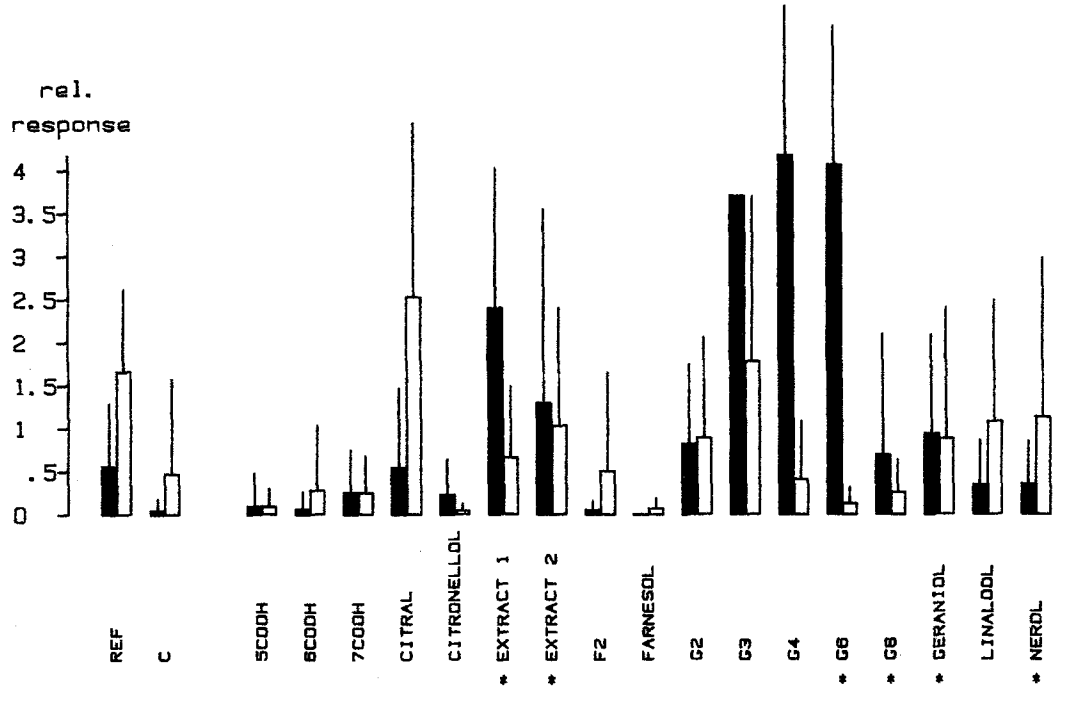

Figure 3. EAG response spectra of *A. obscurus* males and females to all tested compounds. Chemicals detected by GC/MS in female abdomina indicated by *. Black bars, males; white bars, females. Lines on top of bars represent positive standard deviation. REF = reference (octanol); C = control (hexane or paraffin oil); 5COOH = pentanoic acid; 6COOH = hexanoic acid; 7COOH = heptanoic acid; F2 = farnesyl acetate; G2 = geranyl acetate; G3 = geranyl proprionate; G4 = geranyl butanoate; G6 = geranyl hexanoate; G8 = geranyl octanoate. All test substances diluted 1/100 (w/v).

they behave as if stimulated sexually by the flower labellum. Analyses of volatiles from *O. litigiosa* flowers [20] indicate the presence of citronellyl and farnesyl esters. These are biochemically closely related to geranyl esters and thus might be responsible for the attraction of male elaterid beetles to various *Ophrys* flowers.

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Accumulation of phenylpropanoids in the rectal glands of males of the Oriental fruit fly, *Dacus dorsalis*

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Summary. Two phenylpropanoid compounds, 2-allyl-4,5-dimethoxyphenol(II) and coniferyl alcohol(III), were characterized from body tissue of wild males of the Oriental fruit fly, *Dacus dorsalis*. These compounds accumulated in the rectal glands only when laboratory-reared males were fed with methyl eugenol. Compound II was released into the air during dusk, which coincides with the fly courtship period. Pheromonal and allomonal effects of the phenylpropanoids were examined.
Key words. Oriental fruit fly; *Dacus dorsalis;* methyl eugenol; 2-allyl-4,5-dimethoxyphenol; coniferyl alcohol; phenylpropanoid; pheromone; allomone; sequestration.

Methyl eugenol(I) is a highly potent attractant for the Oriental fruit fly, *Dacus dorsalis*, and several other species in the family Tephritidae (Diptera). It has been successfully used as a trapping agent in eradication programs for these pests in many countries [1]; and in capturing native males for population estimation [2]. However, the biological significance of such specific attraction of the lure for the male tephritid flies has not yet been clarified. We have found two phenylpropanoid compounds (II and III), which are closely related to methyl eugenol, in the body tissue of wild *D. dorsalis* males. We describe here the identification and possible ecological functions of these compounds.

Adult *D. dorsalis* males were collected at various field sites in West Malaysia, and immediately used for extraction with ethanol. As shown in figure B, the capillary gas-liquid chromatogram exhibited two major volatile substances, II and III, in unusually large quantities. In contrast, ethanolic extracts of the sexually mature males of a laboratory-reared culture (raised from star-fruits, *Averrhoa carambola,* and fed with water and a honey-yeast mixture during the adult stage) entirely lacked these compounds (fig. A).

Compounds II and III were isolated from wild males by means of silica gel column chromatography (Wako gel C-200) followed by high performance liquid chromatography (Nucleosil 100-5, 300 mm × 8 mm i.d., eluted with a mixture of 20–42% ethyl acetate in hexane, yield: 12 µg and 10 µg per male, respectively). Compound II was identified as 2-allyl-4,5-dimethoxyphenol from its mass (MS), proton and carbon-13 nuclear magnetic resonance (PMR and CMR) spectra: MS (70 eV) m/z (%) 194 (M^+, 100), 179 (68), 151 (13), 123 (22), 69 (20). PMR ($CDCl_3$): δ 6.62 (1H, singlet), 6.46 (1H, singlet), 5.98 (1H, multiplet, J = 17.5, 8.0