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Olfactory antennal responses of the vine weevil *Otiorhynchus sulcatus* to plant volatiles

R. W. H. M. van Tol[1] & J. H. Visser[2]
[1]*Applied Plant Research, Nursery Stock Research Unit, P.O. Box 118, 2770 AC Boskoop, The Netherlands;* [2]*Plant Research International, Wageningen-UR, P.O. Box 16, 6700 AA Wageningen, The Netherlands*

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Abstract

Electroantennograms (EAGs) were recorded from the vine weevil, *Otiorhynchus sulcatus* F. (Coleoptera: Curculionidae) to a broad range of volatile plant compounds. The response profile is restricted to a small number of volatiles that evoke substantial EAGs. Large EAG responses were particularly found among green leaf volatiles (GLV) such as (*E*)-2-hexenol-1, (*Z*)-3-hexenol-1, hexanol-1, hexanal, and heptanal. Other plant volatiles eliciting responses in the weevils' antenna are 2,5-dimethylpyrazine, hexylamine, benzylalcohol, 1,2-dimethoxybenzene, *o*-cresol, myrtenol, 3-methylcyclohexanol, γ-hexalactone, and γ-heptalactone. EAG responses to terpenes were generally weak. Many of the monoterpenes are characteristic for the odour of conifers, a group of plants which tend to be avoided by adult vine weevils. The EAG response to several GLV and benzene derivatives in three geographically distinct populations of the vine weevil differed, suggesting between-population variation in receptor sensitivities for several compounds under test. The GLV-composition of the odour profile of potential food plants may be an important criterion for a polyphagous insect like the vine weevil to be used in host-plant selection, since compounds in this odour group dominate so strongly the EAG response profile. In multiple food-choice situations the weevils are known to prefer certain plant species and we hypothesize that they combine GLV information with that of more specific plant volatiles, thereby promoting attraction or avoidance.

Introduction

The vine weevil (*Otiorhynchus sulcatus* F.) (Coleoptera: Curculionidae) is a serious pest in a number of hardy ornamental plants and some fruit crops. The genus *Otiorhynchus* is of European origin (Feytaud, 1918; Wilcox et al., 1934) and the species *O. sulcatus* is endemic to the temperate zone in Europe. Since the 1930's the vine weevil is causing increasing economic damage to the nursery industry and small fruits production (e.g., cranberry, strawberry, and hops) worldwide (Moorhouse et al., 1992).

The weevils are parthenogenetic and mainly active during the night, hiding in the soil or other dark places during daylight. This nocturnal activity makes it difficult for growers to observe the presence of weevils. For effective control programmes, monitoring of this weevil is therefore essential. Good monitoring methods, making use of attractants, need to be developed to improve the timing of control measures. Moreover, such monitoring methods may be developed into trapping methods to control the vine weevil.

Although the vine weevil is polyphagous, it prefers plant species in the families Rosaceae, Ericaceae, and Taxaceae (Evenhuis, 1978; Masaki et al., 1984; Smith, 1932). Remarkable is its preference for ornamental plant species in the genus *Taxus, Rhododendron*, and *Euonymus*. These plant species are very toxic for most vertebrates and only few (mostly sucking) insects specialised on these plant species can deal with the associated toxins (van Genderen et al., 1996). The vine weevil is the sole insect that prefers to eat from the green parts of these toxic plants (Doss, 1983; Hanula, 1988; van Tol & Visser, 1998). *Euonymus* belongs to the family of Celastraceae and not to one of the three plant families mentioned before. First studies (van

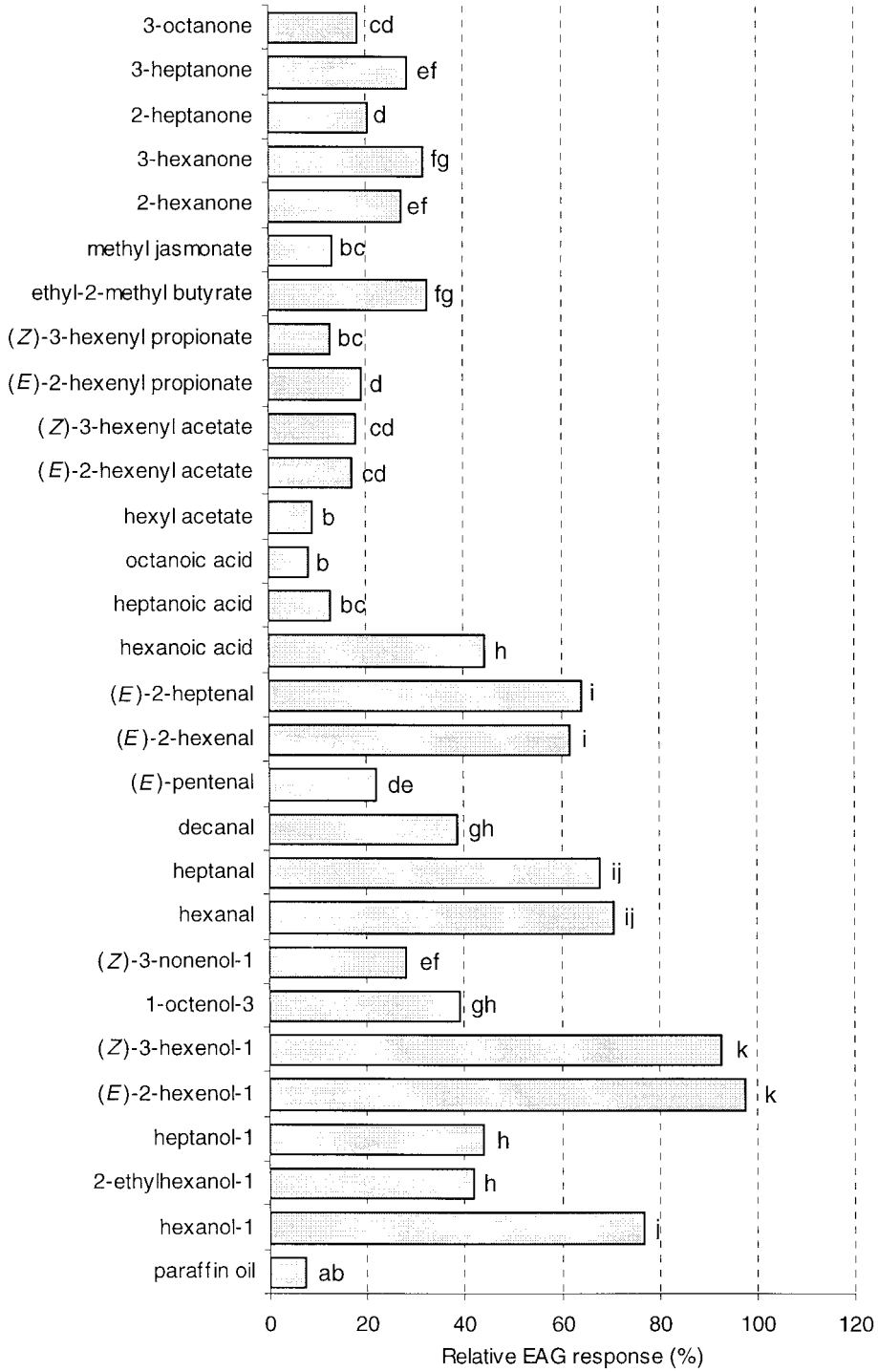

Figure 1. EAG response profile of *Otiorhynchus sulcatus* to plant volatiles from the fatty acid derivatives group (in log 2 dilution at the source). EAG peak responses are expressed relative to the standard (*Z*)-3-hexenol-1 (log 2 dilution). Data were analysed with ANOVA after square root transformation of the data. Bars marked by a different letter indicate statistically different EAG responses at the 5% level ($n = 12$).

Tol, unpubl.) on taxonomic relation between preferred plant species and suitability of these plant species for survival and reproduction of the vine weevil show that their main host plants are in the subclass of the Rosidae to which both families of the Rosaceae and Celastraceae belong.

Euonymus fortunei (Turcz.) Hand.-Mazz. is an ornamental ground cover grown commercially in Holland. It is used by several growers as a monitoring tool due to its attractive properties for the adult weevils. The characteristic notching pattern of the weevils on the leaves of this shrub is easy to recognize for the growers. IPM growers spray their crops with chemicals after spotting the first damage on these plants (van Tol, 1996; van der Horst & van Tol, 1995). Although useful, it is a very laborious way of monitoring the presence of the adult weevils. The grower has to plant the shrubs on regular distances in the field and damaged leaves have to be removed regularly to avoid double monitoring later on. The indirect monitoring of the weevils via plant damage in early summer also increases the risk of observing the first weevil damage too late, in that weevils may have started laying eggs before spraying. Using host-plant odour to monitor the first weevils directly in traps would overcome most of the problems mentioned. However, the attractive plant odours have been identified only for more specialised weevil species (Landolt & Phillips, 1997), but not for *O. sulcatus*. Since the vine weevil is a generalist, it is not wise to focus exclusively on odours from *E. fortunei*. Here, we present the results of a comprehensive analysis using electroantennogram (EAG) tests to record the sensory response of vine weevils to a broad range of volatiles known to occur in plant odours. There are no data available about plant preferences for this weevil species. Therefore we did not select certain volatiles but chose a range of common as well as more specific plant compounds to study the response profile of the vine weevil. As such, our study is an extension of earlier work carried out by Pickett et al. (1996). In addition, we assessed whether there are differences between three geographically distinct populations of vine weevils. We selected weevil populations from different host plants for this comparison because in previous research we found that weevils from our test population were attracted to the odour of certain plant species and not to others (van Tol et al., 2000).

Materials and methods

Insects. Three populations of *O. sulcatus* from different origins were kept at 22 °C in a climate room under long-day conditions (L16:D8). The populations were collected from geographically distinct areas and all three populations have been reproducing for an unknown number of generations on different host plants:

BNL : Boskoop, the Netherlands. Weevils collected in June 1997 on the Research Station in a field with yew (*Taxus baccata* L.) and spindle tree. This population was originally collected in the Boskoop area on nurseries growing ornamental shrubs and released on a small peninsula with *Taxus, Rhododendron*, and *Euonymus* at the Research Station for Nursery Stock in 1993.

CUS : East Windsor, Connecticut, USA. Weevils collected in July 1997 in a strawberry field.

WUS : Aberdeen, Washington State, USA. Weevils collected in July 1997 in a cranberry field.

All populations were fed with a mixture of yew and spindle tree (*E. fortunei* cv. 'Dart's Blanket') after collection from the different areas. A Dutch population collected near Boskoop was used in the EAG screening tests. For a subset of volatiles we recorded the EAGs of the three collected populations to enable comparison between these geographically distinct populations. Weevils (BNL) were collected shortly after emergence from the field and pots in the period April to July 1997 and tested in EAG screening in the period June to September 1997. Populations from the USA (CUS and WUS) were collected in July 1997 and tested in September 1997.

Electroantennogram recordings. For EAG recordings the weevils' antenna was amputated at the base of the flagellum and the tip of the last antennal segment was slightly damaged to create an open connection with the internal fluid of the antenna. The base of the antenna was placed in one glass electrode and the top of the antenna only touched the open end of the other glass electrode. Both glass electrodes were filled with a 0.1 M KCl-solution. AgCl-coated-silver wires were inserted into the electrodes and connected to a Grass P16D amplifier via an HIP16A input probe. Signals were amplified using a Philips PM3302 storage oscilloscope and recorded with a Krenz TRC 4010 transient-recorder (12 bits ADC) connected to a computer (Visser & Piron, 1995).

Table 1. Volatiles tested in EAG screening of *Otiorhynchus sulcatus*

Chemical[a]	Source	Purity (%)	Chemical[a]	Source	Purity (%)
Fatty acid derivatives			2-hydroxy-5-methylacetophenone	Aldrich	98
hexanol-1	Fluka	99	*o*-cresol	Fluka	99
2-ethylhexanol-1	Fluka	99	*m*-cresol	Fluka	98
heptanol-1	Fluka	99	*p*-cresol	Fluka	99
(*E*)-2-hexenol-1	Roth	97	methyl salicylate	Fluka	99
(*Z*)-3-hexenol-1	Roth	97	2-phenylethyl acetate	Roth	97
1-octenol-3	Fluka	98	hexylbenzene	Fluka	97
(*Z*)-3-nonenol-1	Aldrich	95	**Terpenes and derivatives**		
hexanal	Fluka	98	β-ocimene	Fluka	97
heptanal	Merck	97	myrcene	Roth	91
decanal	Fluka	97	citral	Roth	99
(*E*)-pentenal	Fluka	95	citronellal	Roth	98
(*E*)-2-hexenal	Roth	98	(+)-citronellol	Roth	97
(*E*)-2-heptenal	Aldrich	97	nerol	Aldrich	97
hexanoic acid	Fluka	99	geraniol	Fluka	99
heptanoic acid	Fluka	99	linalool	Fluka	97
octanoic acid	Fluka	99	α-terpinene	Roth	90
hexyl acetate	Fluka	99	γ-terpinene	Roth	94
(*E*)-2-hexenyl acetate	ICN/K&K	99	(−)-(*R*)-α-phellandrene	Fluka	99
(*Z*)-3-hexenyl acetate	Roth	99	terpinolene	Roth	96
(*E*)-2-hexenyl propionate	ICN/K&K	99	(+)-limonene	Roth	99
(*Z*)-3-hexenyl propionate	ICN/K&K	99	α-terpineol	Roth	98
ethyl-2-methyl butyrate	Fluka	95	terpinyl acetate	Roth	94
methyl jasmonate	Aldrich	95	(+)-(*S*)-carvone	Roth	98
2-hexanone	Fluka	98	(−)-(*R*)-carvone	Aldrich	98
3-hexanone	Fluka	96	carvacrol	Fluka	96
2-heptanone	Aldrich	98	carvacrol methylether	Fluka	98
3-heptanone	Aldrich	98	sabinene	Roth	96
3-octanone	Aldrich	99	thujylalcohol	Roth	95
N- and/or S-containing volatiles			α-thujone	Roth	95
hexylamine	Fluka	99	δ-3-carene	Roth	98
ethanolamine	Fluka	99	(+)-(1*R*)-α-pinene	Fluka	99
hexanonitrile	Aldrich	98	(−)-(1*S*)-β-pinene	Fluka	99
heptanonitrile	ICN/K&K	92	(−)-(1*S*)-α-pinene	Fluka	99
4-methoxyphenylacetonitrile	Fluka	97	myrtenol	Fluka	99
pyridine	Aldrich	99	myrtenal	Fluka	97
ethyl nicotinate	Fluka	98	1,4-cineole	Roth	93
ethyl nipecotate	Fluka	97	1,8-cineole	Roth	99
2,5-dimethylpyrazine	Fluka	93	(−)-fenchone	Fluka	98
1-hexanethiol	Fluka	97	(+)-fenchone	Fluka	98
1,6-hexanedithiol	Fluka	97	(*E*, *E*)-farnesol	Aldrich	96
butyl isothiocyanate	Aldrich	97	(*E*, *E*)-farnesyl acetate	Aldrich	96
tert-butyl isothiocyanate	Aldrich	99	(*E*, *E*)-α-farnesene	TNO	95
allyl isothiocyanate	Aldrich	95	(*E*)-β-farnesene	TNO	98
3-butenyl isothiocyanate	Rothamsted	98	α-ionone	Roth	92
4-pentenyl isothiocyanate	Rothamsted	98	β-ionone	Fluka	95
benzyl isothiocyanate	Fluka	97	(−)-α-bisabolol	Roth	96
2-phenylethyl isothiocyanate	Fluka	97	α-humulene	Fluka	98
Benzene derivatives			germacrone	Fluka	99
benzylalcohol	Aldrich	99	(−)-(*E*)-caryophyllene	Fluka	99

Table 1. Continued

Chemical[a]	Source	Purity (%)	Chemical[a]	Source	Purity (%)
2-phenylethylalcohol	Fluka	99	**Cyclic alcohols, ketones**		
benzaldehyde	Roth	98	1-methylcyclopentanol	Aldrich	99
3-phenylpropionaldehyde	Fluka	95	2-methylcyclohexanol	Fluka	98
(*E*)-cinnamic aldehyde	Roth	99	3-methylcyclohexanol	Fluka	98
2-methoxybenzaldehyde	Aldrich	98	4-methylcyclohexanol	Fluka	98
3-methoxybenzaldehyde	Aldrich	99	2-methylcyclohexanone	Fluka	99
4-methoxybenzaldehyde	Aldrich	98	3-methylcyclohexanone	Fluka	97
2-hydroxybenzaldehyde	Roth	99	4-methylcyclohexanone	Fluka	97
4-isopropylbenzaldehyde	Roth	97	**Lactones and lactols**		
2-hydroxy-4-methoxybenzaldehyde	Fluka	98	γ-hexalactone	Roth	98
2-hydroxy-5-methoxybenzaldehyde	Fluka	98	γ-heptalactone	Roth	98
1,2-dimethoxybenzene	Aldrich	99	γ-octalactone	Roth	97
1,2-dimethoxy-4-propenylbenzene	Aldrich	99	γ-nonalactone	Roth	99
α-asarone	Roth	93	δ-heptalactone	Roth	87
β-asarone	Roth	98	(+)-(4a*S*,7*S*,7a*R*)-nepetalactone	Rothamsted	98
eugenol	Roth	99	(−)-(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol	Rothamsted	98

[a]Chemicals are diluted 100 fold in paraffin oil.

The recordings were digitized and stored for 20.5 s, starting 4 s prior to the 2-s odour stimulus injection. The data were analysed by software developed in Asyst 3.1 (Visser & Piron, 1995). The digitized signals were subjected to the smooth function of Asyst (cut-off frequency at 0.1) and corrected for DC drift by subtraction (Visser & Piron, 1995). Several parameters were assessed, but here we only present the results of the peak response, being the largest negative potential recorded upon stimulus injection. The absolute EAG responses were normalized and expressed as a percentage response relative to the responses of adjacent standard stimuli using 1% (v/v) (*Z*)-3-hexenol-1 in paraffin oil.

Protocol for odour stimuli. Table 1 lists all volatiles tested. The volatiles were diluted to 1% (v/v) in paraffin oil (Merck, Uvasol). At this dilution several volatiles, evoked significantly different EAG peak amplitudes. At higher concentrations overstimulation and insufficient recovery of the antenna resulted in large variability of the EAGs (see results dose-response curves). Twenty-five μl of each solution was applied on filter papers (6×0.8 cm), which were then placed in Pasteur pipettes. The weevils' antenna was stimulated for 2 s by air passing at a rate of 1 ml s^{-1} through the Pasteur pipette into a tube with a continuous air stream of 40 cm s^{-1} (flow-rate: 30 ml s^{-1}) over the antennal preparation. The maximum deflection of the signal (absolute EAG response) was recorded.

The EAG response to the volatiles listed in Table 1 was repeated 12 times with weevils of the Dutch population (BNL). For each of the five subgroups of volatiles tested (subgroups are groups of volatiles as presented in Figures 1–5) order of volatiles tested per weevil antenna was randomly determined. Paraffin oil without volatiles served as a control for the antennal response in the series of volatiles tested. Twenty-two plant volatiles (Table 2), viz., those showing substantial peaks in the EAG recordings with the BNL population, were selected for comparison with the populations of Connecticut (CUS) and Washington State (WUS). The volatiles were tested on the antennae of 12 weevils from each population. EAG data for the volatiles tested on the BNL-population were analysed with ANOVA after square root transformation of the data. Analysis for population differences of EAG peak responses to 22 volatiles was performed by Inorthogonal Analysis of Variance after square root transformation of the data with the Genstat 4.2 computer program.

Dose-response curves. Serial dilutions from a limited number of green leaf volatiles (fatty acid derivatives) were prepared and used for recording EAGs. This was done in order to investigate whether the rank order of EAG responses changes with the dosage

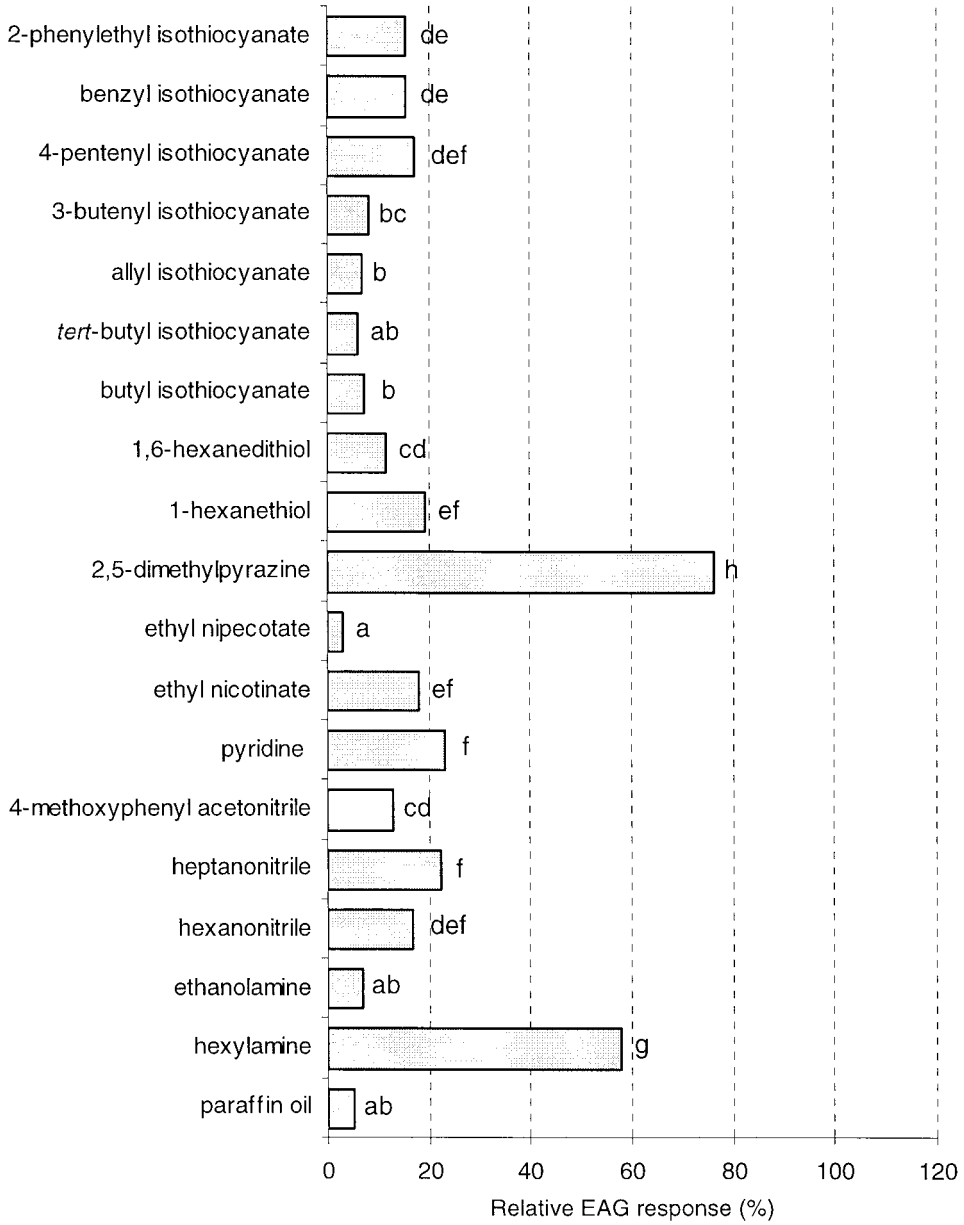

Figure 2. EAG response profile of *Otiorhynchus sulcatus* to plant volatiles with N- and/or S-containing molecules (in log 2 dilution at the source). EAG peak responses are expressed relative to the standard (*Z*)-3-hexenol-1 (log 2 dilution). Data were analysed with ANOVA after square root transformation of the data. Bars marked by a different letter indicate statistically different EAG responses at the 5% level ($n = 12$).

and to determine at what dosage differences in EAG peak amplitude between the tested volatiles occur. Four volatiles with high absolute peak responses, i.e., (*E*)-2-hexenal, (*E*)-2-hexenol-1, (*Z*)-3-hexenol-1, and (*E*)-2-heptenal, were tested at five dilutions (v/v) in paraffin oil. (*Z*)-3-hexenol-1 at 1% (v/v) dilution in paraffin oil was used as a standard.

Results

Response profile

Fatty acid derivatives (Figure 1). The absolute EAG response of the vine weevil to the standard (*Z*)-3-hexenol-1, diluted 100-fold in paraffin oil, was 1.3 ± 0.3 mV (mean ± 95% c.i.). Volatiles in the group of general green leaf volatiles evoking substantial

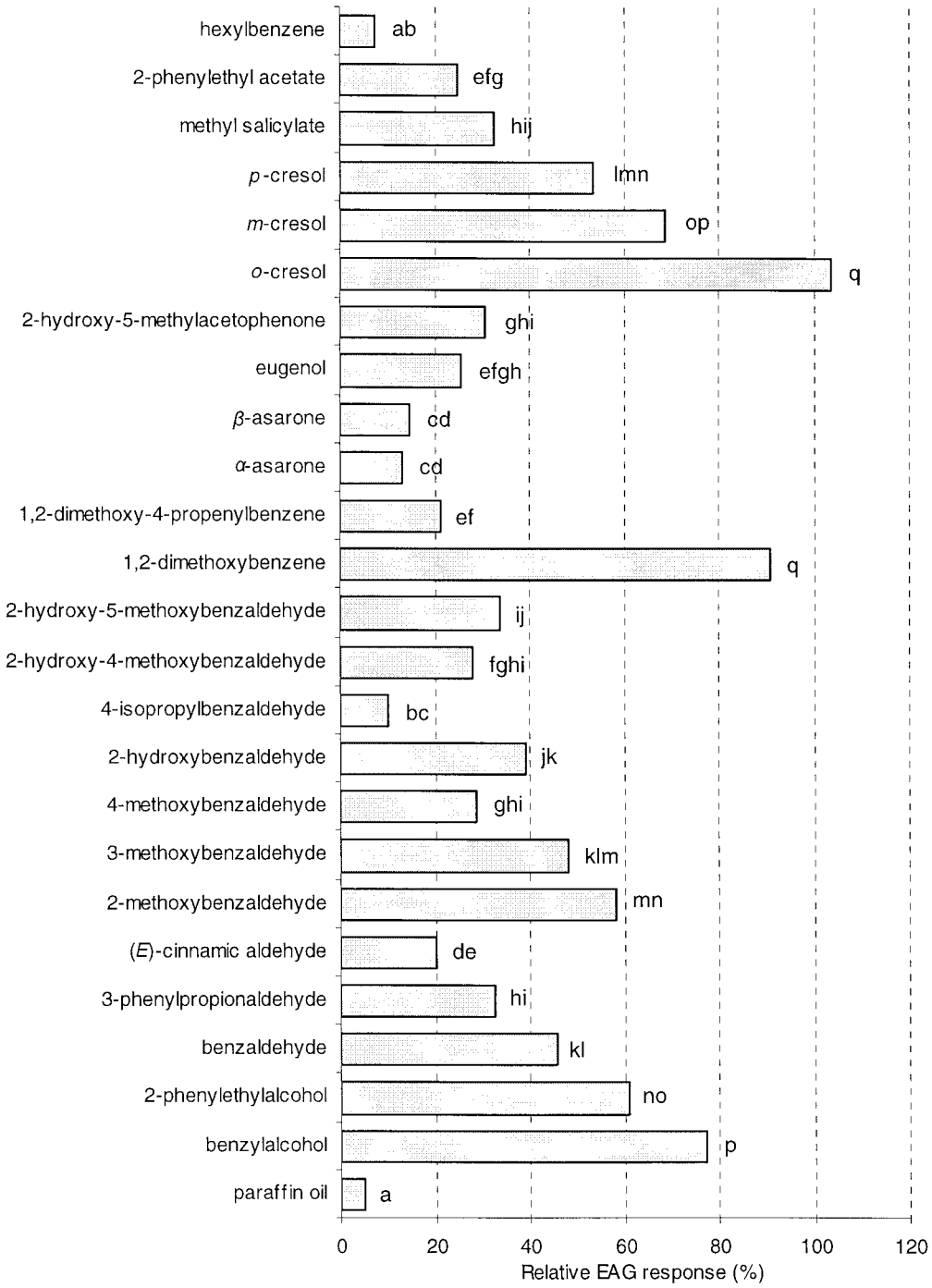

Figure 3. EAG response profile of *Otiorhynchus sulcatus* to plant volatiles from the benzene derivatives group (in log 2 dilution at the source). EAG peak responses are expressed relative to the standard (*Z*)-3-hexenol-1 (log 2 dilution). Data were analysed with ANOVA after square root transformation of the data. Bars marked by a different letter indicate statistically different EAG responses at the 5% level ($n = 12$).

Table 2. Comparison of mean normalised EAG peak responses ($n = 12$) to 22 plant volatiles (in log 2 dilution at the source) for three geographically isolated populations of *Otiorhynchus sulcatus*

Chemical[a]	EAG peak responses[c]		
	CUS population[b]	WUS population[b]	BNL population[b]
(*E*)-2-hexenal	62 a	57 ab	54 b
(*E*)-2-hexenol-1	94	93	91
(*Z*)-3-hexenol-1	103 a	86 b	88 b
benzylalcohol	62	64	61
2,5-dimethylpyrazine	63	58	59
hexylamine	22 b	19 b	29 a
benzaldehyde	51 a	38 b	48 a
2-methoxybenzaldehyde	58 ab	56 b	61 a
3-methoxybenzaldehyde	53 a	45 b	50 a
4-methoxybenzaldehyde	24 a	20 b	20 b
2-hydroxybenzaldehyde	41 a	37 ab	35 b
1,2-dimethoxybenzene	96	103	98
o-cresol	107 b	127 a	123 a
m-cresol	46 b	54 a	59 a
p-cresol	51 b	46 b	59 a
linalool	53	52	56
α-terpineol	42	43	47
(+)-(*S*)-carvone	33	31	34
(−)-(*R*)-carvone	29	29	28
myrtenol	58	61	56
myrtenal	44 a	38 b	42 a
γ-hexalactone	54 a	48 b	55 a

[a]Chemicals are diluted 100 fold in paraffin oil.
[b]BNL = weevil population collected from a field with yew and spindle tree in Boskoop, The Netherlands; CUS = weevil population collected from a strawberry field in East Windsor, Connecticut, USA; WUS = weevil population collected from a cranberry field in Aberdeen, Washington State, USA.
[c]Values are predicted values from the statistic analysis. Inorthogonal Analysis of Variance was performed on square root transformed data (corrected for response to mineral oil alone) with the Genstat 4.2 computer program. Values followed by a different letter in the same row indicate statistically significant different EAG responses between the populations for a particular odour at the 5% level. Odours with no different EAGs are not followed by a letter.

responses, in order of decreasing values, were (*E*)-2-hexenol-1, (*Z*)-3-hexenol-1, hexanol-1, hexanal, heptanal, (*E*)-2-heptenal, and (*E*)-2-hexenal. Except for hexanoic acid, acids elicited very low EAG responses. The ketones as well as the tested acetates and propionates also elicited weak EAG responses.

Several saturated and unsaturated aliphatic alcohols and aldehydes within this group elicited distinct EAG peaks. Comparing the properties of these volatiles revealed the potential of particular molecular structures to stimulate the weevil's sensors. The unsaturated C6-alcohols elicited larger EAGs than the saturated homologue molecule or the saturated and unsaturated C6-aldehydes ((*Z*)-3-hexenol-1 = (*E*)-2-hexenol-1 > hexanol-1 = (*E*)-2-hexenal = hexanal). For the saturated C7-alcohol the response was lower than for the saturated and unsaturated C7-aldehydes (heptanol-1 < heptanal = (*E*)-2-heptenal). The number of C-atoms in alcohols and aldehydes also affected the response. For saturated and unsaturated alcohols the response decreased with longer carbon chains ((*Z*)-3-hexenol-1 = (*E*)-2-hexenol-1 > 1-octenol-3 > (*Z*)-3-nonenol-1 and hexanol-1 > heptanol-1). In the aldehyde group there was no increase in response comparing the C6-chain with the C7-chain. Only in the saturated aldehyde group we tested a volatile with a longer carbon chain (C10) that evoked a smaller response than the C6- and C7-

homologue (hexanal = heptanal > decanal). In the unsaturated aldehyde group we also examined a C5-chain that elicited a lower response than the C6- and C7-carbon chains indicating that C6- and C7-carbon chains are the most active molecules in the group of general green leaf volatiles ((*E*)-pentenal < (*E*)-2-hexenal = (*E*)-2-heptenal). Among the acids, the C6-carbon chain also was the most active one (hexanoic acid > heptanoic acid = octanoic acid). The ester ethyl-2-methyl butyrate gave results comparable with hexanoic acid. Within the group of green leaf volatiles the ketones, acetates, and propionates gave generally low responses with no structure-related differences in activity.

N- and/or S-containing volatiles (Figure 2). Except for 2,5-dimethylpyrazine and hexylamine, only low response amplitudes to volatiles from this group were recorded. Hexylamine gave rise to large variation in the antennal response of the weevil. Since shape (rise and decay of EAGs in time) of the responses did not vary and speed of decay was not abnormal when compared to other volatiles tested we suspect that the variability in peak response is caused by the variable damage to the club of the antenna. The club of the antenna is artificially damaged prior to recording to make EAG measurements possible.

Benzene derivatives (Figure 3). In this group *o*-cresol, 1,2-dimethoxybenzene, and benzylalcohol were eliciting high EAG responses. Other volatiles eliciting a clear response were *m*-cresol, *p*-cresol, and 2-phenylethylalcohol. Benzaldehyde gave a relatively low EAG response compared to the alcohol equivalent, benzylalcohol. Further, 2-methoxybenzaldehyde and 3-methoxybenzaldehyde evoked intermediate EAG responses.

As for the green leaf volatiles there was a higher EAG response to the benzene ring with an alcohol group compared to an aldehyde group (benzylalcohol > benzaldehyde). The addition of a methoxy-group to the benzaldehyde molecule clearly affected the EAG response: larger EAGs were recorded for 2-methoxybenzaldehyde than benzaldehyde, but 4-methoxybenzaldehyde was less active than 2-methoxybenzaldehyde (2-methoxybenzaldehyde > benzaldehyde = 3-methoxybenzaldehyde > 4-methoxybenzaldehyde). Replacing the methoxy-group with a hydroxy-group reduced the EAG response (2-methoxybenzaldehyde > 2-hydroxybenzaldehyde). Adding a hydroxy-group or a combination of hydroxy- and a methoxy-group at the benzaldehyde molecule did not increase the response (benzaldehyde = 4-hydroxybenzaldehyde < 2-hydroxy-4-methoxybenzaldehyde = 2-hydroxy-5-methoxybenzaldehyde).

Clear structure-related EAGs were found for the cresols. Cresol (methylphenol) with the methyl-group placed at the 2-position was superior in eliciting EAG responses to the 3- or 4-positioned methyl-group. Veratrole (1,2-dimethoxybenzene) has two methoxy-groups adhering at the benzene ring whereas *o*-cresol has a hydroxy- and a methyl-group at these places instead. Both volatiles gave similarly high response-peaks (> 90%). Volatiles like 1,2-dimethoxy-4-propenylbenzene, α-asarone, and β-asarone differing from 1,2-dimethoxybenzene by one or two extra adhering groups, evoke only small responses. Finally, the comparison of 3-phenylpropionaldehyde structurally related to 2-phenylethylalcohol and 2-phenylethylacetate indicated that the antennal response decreased from alcohol to aldehyde to acetate.

Terpenes and derivatives (Figure 4). We tested 42 terpenes and observed only low EAG responses to most of the volatiles tested. Myrtenol elicited the highest EAG response of all tested terpenoids. The alcohol structure was more active than the aldehyde structure (myrtenol > myrtenal). Similar ring-structured molecules as myrtenol and myrtenal, but without an aldehyde or alcohol group elicited only low EAG responses (myrtenol > myrtenal > pinenes). Comparing related alcohols within the group of terpenoids revealed the importance of the configuration of the molecule for the EAG response (linalool > nerol > geraniol = citronellol).

Cyclic alcohols and ketones (Figure 5). Several of the cyclic hexanols evoke comparable, large EAG responses as the non-cyclic hexanol-1. The response to the cyclic hexanones is lower than to the equivalent alcohols.

For methylcyclohexanols it was the 3-position of the methyl-group that elicited larger EAG responses than the 2-position or the 4-position. The results with the methylcyclohexanols and the structurally related methylcyclohexanones indicated the higher impact of the alcohol group over the ketone group.

Lactones and lactols (Figure 5). γ-Hexalactone and γ-heptalactone evoked high responses from the weevils' antennae (70–80%). Response to other lac-

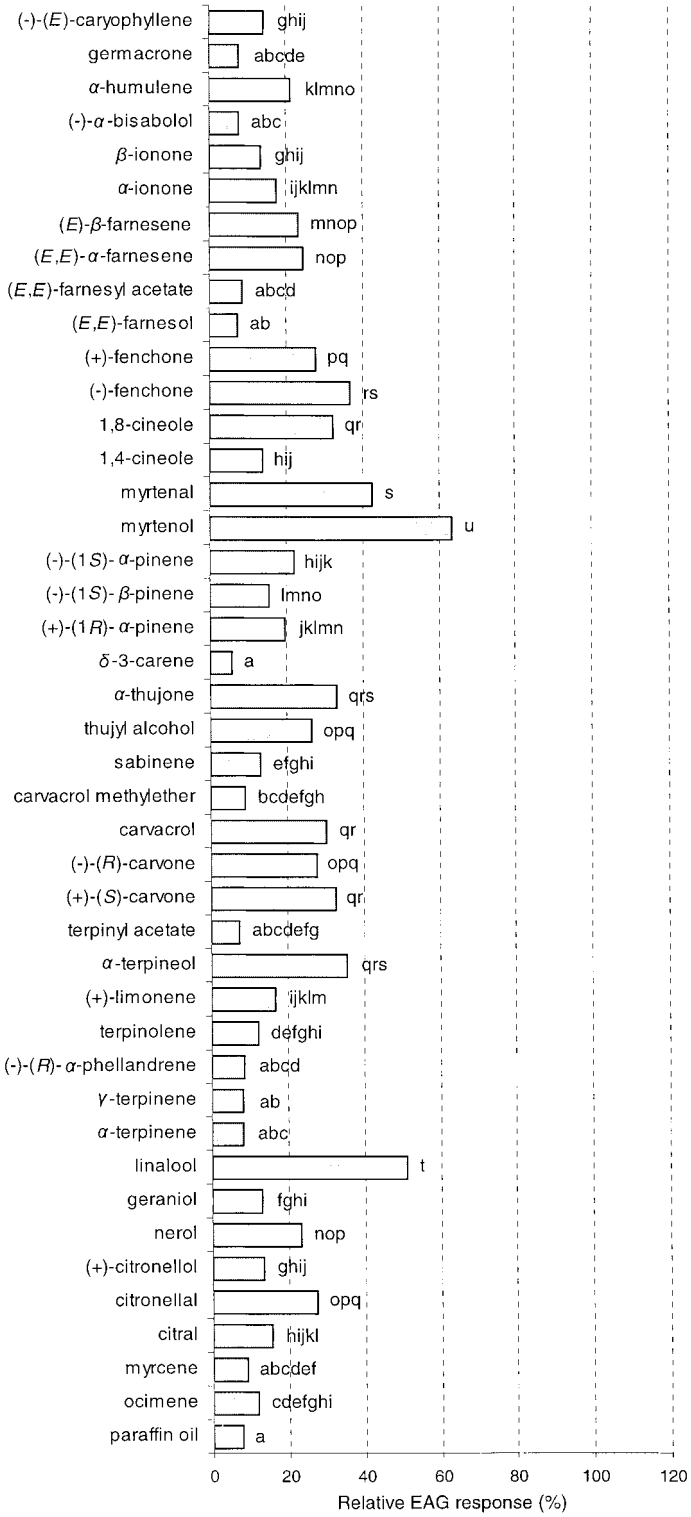

Figure 4. EAG response profile of *Otiorhynchus sulcatus* to plant volatiles from the terpenes and derivatives group (in log 2 dilution at the source). EAG peak responses are expressed relative to the standard (*Z*)-3-hexenol-1 (log 2 dilution). Data were analysed with ANOVA after square root transformation of the data. Bars marked by a different letter indicate statistically different EAG responses at the 5% level ($n = 12$).

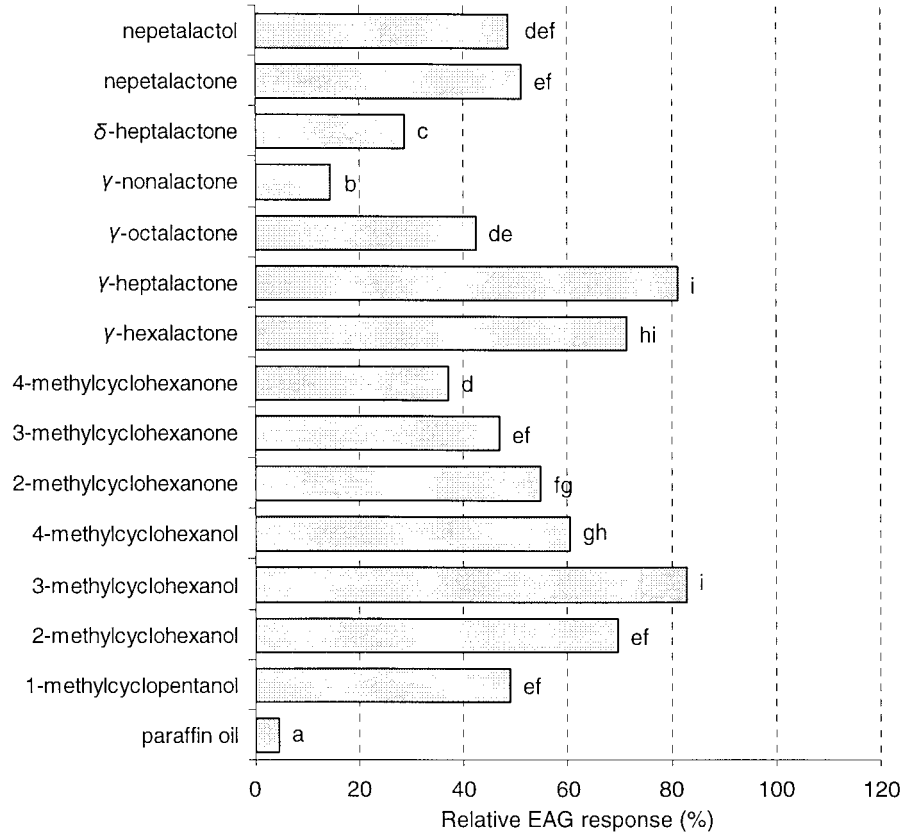

Figure 5. EAG response profile of *Otiorhynchus sulcatus* to plant volatiles from the group with cyclic alcohols, cyclic ketones, lactones, and lactols (in log 2 dilution at the source). EAG peak responses are expressed relative to the standard (*Z*)-3-hexenol-1 (log 2 dilution). Data were analysed with ANOVA after square root transformation of the data. Bars marked by a different letter indicate statistically different EAG responses at the 5% level ($n = 12$).

tones were low. A moderate response to the aphid sex pheromone components nepetalactone (52%) and nepetalactol (49%) was recorded.

The EAG response to the lactones depended on the carbon chain length. The C6- and the C7-chain gave equally large EAG responses as the C8-chain. The ring structure had also a large effect on the response. δ-Heptalactone gave compared to γ-hexalactone only a very weak EAG response. The ring structure and not chain length was causing this lower response to δ-heptalactone, because γ-heptalactone (a five-ring lactone) evoked a larger response on the antennae than δ-heptalactone.

Dose-response relationships

The relative EAG responses (Figure 6) to (*Z*)-3-hexenol-1 and (*E*)-2-hexenol-1 were logarithmically increasing with the dosis from log 5 dilution to log 1. In contrast (*E*)-2-hexenal and (*E*)-2-heptenal increased linearly. At the low dosages (log 3 to log 5) the response to the alcohols could not be distinguished from the response to the aldehydes. At log 5 dilution the EAG responses to the leaf aldehydes and alcohols did not differ from the response to pure paraffin oil.

Variation between populations

The EAG response profiles of the three weevil populations from the Netherlands and The USA (Table 2) differed significantly ($P = 0.04$). Volatiles with high relative EAG peak responses (80 to 130%) that differ significantly were (*Z*)-3-hexenol-1 and *o*-cresol. The EAG response to (*Z*)-3-hexenol-1 was on average 20% higher and to *o*-cresol 20% lower for the North-American CUS-population (strawberry field) than for the other two populations. The other GLV tested ((*E*)-2-hexenal) as well as the two cresols structurally related to *o*-cresol gave similar differences in response for the CUS-population when compared to the BNL-

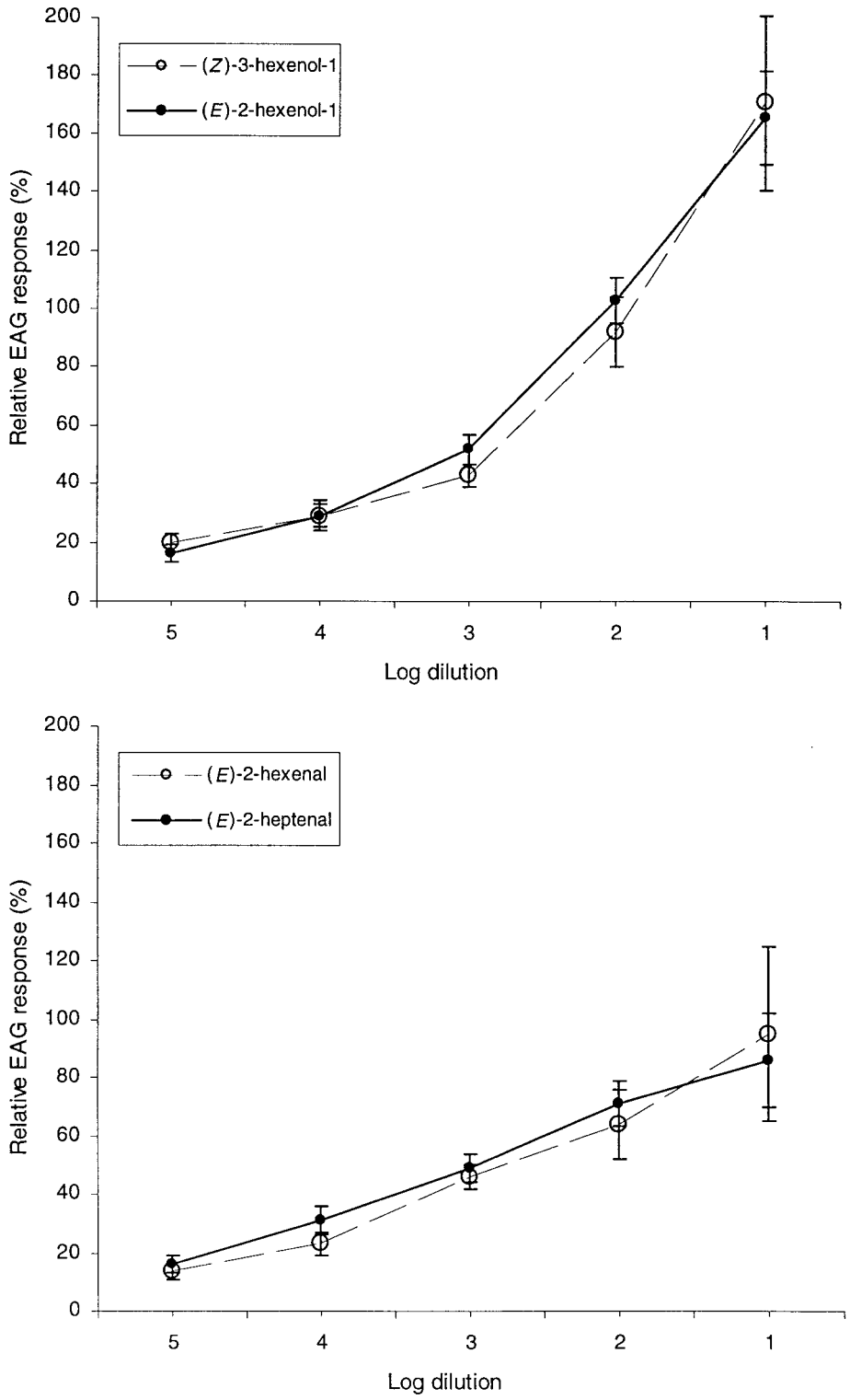

Figure 6. Dose-response relationships for EAG peak responses of *Otiorhynchus sulcatus* to the green leaf volatiles (*Z*)-3-hexenol-1, (*E*)-2-hexenol-1, (*E*)-2-hexenal, and (*E*)-2-heptenal. Means ± 95% c.i. ($n = 12$).

population. Other volatiles evoking lower EAG values (<65%) that differed significantly between populations were several benzaldehydes, myrtenal, and γ-hexalactone. All these volatiles evoked similar or higher EAG responses on the antenna of the CUS-population than on the antenna of one or both of the other populations tested. The absolute EAG response of the vine weevil to the standard (*Z*)-3-hexenol-1, diluted 100-fold in paraffin oil, was 1.2 ± 0.2 mV (mean $\pm$ 95% c.i.) for the BNL-population, 1.3 ± 0.2 mV (mean $\pm$ 95% c.i.) for the CUS-population, and 1.4 ± 0.2 mV (mean $\pm$ 95% c.i.) for the WUS-population.

Discussion

The EAGs recorded from a wide spectrum of plant volatiles revealed only a limited number of odours that evoked substantial responses on the vine weevils' antenna. Large EAG responses were more numerous in the group of fatty acid derivatives: a group with components containing the so-called green leaf volatiles (GLV). These GLV are common odour compounds that all green plant parts release and only the proportion and composition of the components vary between different plant species (Visser et al., 1979). General characteristic of the weevils' response profile was the large EAG response to C6-molecules with an alcohol group and the virtual absence of sensitivity to odours in the terpene group and the group containing N- or S-atoms which contain many plant specific odours, used by other weevil species to find their preferred host-plants. Pickett et al. (1996) presented for the vine weevil EAG results of 14 volatiles. The results of their work are in accordance with our results.

In our trials, release rates of different plant volatiles, at the same dilution in paraffin oil, may vary and cause dose-response related effects in the EAG response on the insects' antenna. However, the comparison of EAG responses to a range of volatiles is still feasible as the variation in responses between volatiles depends mainly on the sensitivities of the receptor system under study. Visser et al. (1996) showed this by comparing the EAG response of four aphid species to 35 volatiles.

Variability in peak response is relatively high for hexylamine. The variation is not due to the properties of the volatile since EAG shapes are identical. We therefore suspect that the artificial damage to the club of the antenna, provided for EAG measurements, is an important source of variation in the EAG recordings. Most receptor neurons for weevil species are located on the club of the antenna. If only a small number of neurons respond to certain volatiles it is likely that this source of variation is more important. For the boll weevil, *Anthonomus grandis* Boh., Dickens (1990) showed that a variable number of neurons on the club of the antenna respond to different plant volatiles. A large number of the tested neurons responded to GLV and for example only a few to plant volatiles like benzaldehyde, *trans*-β-ocimene and linalool.

Green leaf volatiles. For a polyphagous insect like the vine weevil the green leaf volatiles may be important for orientation to host plants. Visser (1986) was the first to stress the importance of green leaf volatiles for host plant location by insects. Recently Hansson et al. (1999) reported the existence of specific neurones for the detection of a number of green leaf volatiles, which supports Visser's hypothesis. Several coleopteran insects have been shown to use GLV in host plant finding. The Colorado potato beetle, *Leptinotarsa decemlineata* Say, uses the specific, green leaf odour composition of its preferred host plant as an orientation cue (Visser & Avé, 1978). For the boll weevil, *A. grandis*, Dickens (1990) showed with single neuron recordings that a relatively large number of neurons respond to GLV and that most of these neurons were primarily responsive to (*E*)-3-hexenol-1. Both (*E*)-3-hexenol-1 and hexanol-1 enhance behavioural response of the boll weevils to their aggregation pheromone, grandlure (Dickens, 1989). This shows that interactions exist between green leaf volatiles and pheromones in the attraction of insects. In choice situations, vine weevils also prefer certain plant species (van Tol & Visser, 1998) thus green leaf odour composition, possibly in combination with other plant volatiles, may also be involved in the orientation to their preferred host plants.

Host plant volatile composition. It is possible to relate our EAG results to information about plant volatiles available in some of the preferred plant species. Hamilton-Kemp et al. (1988) detected 15 volatiles in the headspace from undamaged strawberry leaves, an important host plant of the vine weevil. The main components were (*Z*)-3-hexenol-1, (*Z*)-3-hexenyl acetate, hexanol-1, linalool, benzylalcohol, and 2-phenylethylalcohol. All these volatiles, except for hexenyl acetate, evoked intermediate to high responses in the antenna of the vine weevil, as shown in this paper. Analysis of the essential oils of strawberry

leaves by Khanizadeh & Bélanger (1993) yielded several compounds absent from headspace volatiles composition. They found linalool and nonanal as predominant components and further compounds like (*Z*)-3-hexenol-1, (*E*)-2-hexenal and heptanol in larger quantities. These volatiles detected by headspace analyses contain the potential attractants for the vine weevils. Essential oil analysis reveals often more compounds that are not, or in trace amounts, released by undamaged plants. Herbivory-damaged plants may lead to the emission of relatively large amounts of plant volatiles that are not emitted – or only in trace amounts – by mechanically damaged plants or undamaged plants (Dicke et al., 1990; Turlings et al., 1990). Thus, essential oil analysis may give additional information about potentially attractive volatiles. *Taxus canadensis* Marsh.' essential oil does not contain monoterpenes in substantial amounts in contrast to other conifers. This is why this plant species is less aromatic and resinous than other conifers (Jean et al., 1993). The lack of monoterpenes in the essential oils is probably also the case in most other *Taxus* species. Vine weevil was found to be not attracted to conifers except for *Taxus* spec. and thus many specific monoterpenes emitted in large amounts by conifers may not play an important role in attraction of this weevil. We even suspect that the terpenes in conifers have deterrent effects on the feeding vine weevil, explaining the aversion for conifers and acceptance of *Taxus* as a food source (van Tol, unpubl.).

Defensive plant compounds and lignins. Many of the food plants of the vine weevil are poisonous to vertebrates and insects. Several of these defensive secondary plant compounds are released or formed when plants are damaged. These compounds could be involved in attraction of the vine weevils. Except for the green leaf volatiles, the compounds eliciting a high EAG response are benzene structures and some lactones. Several plant species in the families of the Rosaceae and Taxaceae produce cyanogenic glucosides (Seigler, 1991): In *Taxus baccata* taxifylline, dhurrine, triglochinine, and isotriglochinine (Seigler, 1991; Khan & Parveen, 1987). When plants are damaged these compounds are enzymatically broken down and benzaldehyde related molecules are released (Seigler, 1991; van Genderen et al., 1996). Several of these evoke strong antennal responses in the vine weevil. Seigler (1991) reviewed reports in which several specialist insects were documented to use cyanide and cyanogenic compounds that are released after enzymatic breakdown of these glycosides, as kairomones or phagostimulant. Vine weevils heavily attack both *Euonymus* and *Sedum*. These plants, belonging to two different plant families (Celastraceae and Crassulaceae, respectively), both produce the so-called butenolides in their leaves (Fung et al., 1988, 1990; Menken et al., 1992). The main butenolide produced is sifonodine. Sifonodine represents an α, β-unsaturated γ-lactone. Interestingly two closely related γ-lactones elicit large EAG responses in the vine weevil.

Lignins in woody plants, preferred by the vine weevil, are another large reservoir, containing potential volatiles. It is beyond the scope of this paper to list all possible compounds that have lignin as a precursor. However, typical structures that are present in lignin of *Taxus* spp. (Khan & Parveen, 1987) are closely related to EAG-active substances like *o*-cresol and 1,2-dimethoxybenzene.

Variation between populations. The EAG response profiles to a group of volatiles in three geographically distinct populations of weevils suggest between-population variation in receptor sensitivity. Both populations from the USA are restricted to single plant species (strawberry for the Connecticut population and cranberry for the Washington State population) and have been isolated from the European population (from a field with a mixture of *Taxus* and *Euonymus*) for an unknown period. The variation in response to the group of volatiles tested is mainly found for the CUS-population when compared to the other two populations. When drawing conclusions from these results one should keep in mind that the results are based on a relative small selection of weevils from the populations. Further, the condition of the weevils from the different populations may vary and influence the effects found between the three tested groups of weevils. Two compounds evoking high EAGs on the antenna differ in peak response between populations. The North-American population of the vine weevil (CUS), collected from strawberry fields, is giving a generally 20% lower EAG response to cresols and a 20% higher response to the green leaf volatile (*Z*)-3-hexenol-1 when compared with the populations from cranberry and the population from the Netherlands (collected from a field with *Taxus* and *Euonymus*). Cresol is a phenolic compound. Phenolic compounds are commonly found in woody plants but are not detected by headspace and essential oil analysis in strawberry leaves (Hamilton-Kemp et al., 1988; Khanizadeh & Bélanger, 1993). For (*Z*)-3-hexenol-1,

Hamilton-Kemp (1988) showed this to be the dominant volatile released by the undamaged leaves (more than 50% of the total amount of volatiles released). Unfortunately, we have no data about the relative release rate of this GLV from undamaged leaves of cranberry and *Taxus*. Other differences between the populations were found for several volatiles evoking only moderate EAGs on the weevils' antenna. In general the EAGs for the CUS-population to these volatiles were higher than for the other two populations. Small differences in EAG responses can still be associated with different host-plant preferences as van der Pers (1981) showed for several *Yponomeuta* species. Behavioural tests with these volatiles should be performed to see whether these EAG differences found have led to a significant different behaviour in host-plant searching for the different weevil populations. Most likely, however, the composition of the odour blend determines the insects' preference and not the EAG response to the single compounds, stating the importance of performing behavioural tests.

Many of the volatiles reported from food plants of the vine weevil occur in other plant species as well. Therefore the EAG response profile can give only limited indications about the possible attractiveness of the volatiles. The weevils prefer certain plant species in choice situations and we suspect that they combine the information of the GLV-odours with the information of some more specific plant volatiles to select their host plant in the field. The strong attraction, and absence of preference, of the vine weevil to mechanically and weevil-damaged *Euonymus* (van Tol, unpubl.) combined with the results of our EAG study indicate an important role for the volatile green leaf compounds in attraction. With GC-EADs and behavioural assays of the attractive plants and plant odour components, we hope to elucidate the attractant and develop an effective lure to monitor the incidence of vine weevils.

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References

Dicke, M., T. A. van Beek, M. A. Posthumus, N. Ben Dom, H. van Bokhoven & A. E. de Groot, 1990. Isolation and identification of volatile kairomone that effects acarine predator-prey interactions: Involvement of host plant in its production. Journal of Chemical Ecology 16: 381–396.

Dickens, J. C., 1989. Green leaf volatiles enhance aggregation pheromone of boll weevil, *Anthonomus grandis*. Entomologia Experimentalis et Applicata 52: 191–203.

Dickens, J. C., 1990. Specialized receptor neurons for pheromone and host plant odors in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae). Chemical Senses 15: 311–331.

Doss, R. P., 1983. Root weevil feeding on *Rhododendron*: A review. Journal of Environmental Horticulture 1: 67–71.

Evenhuis, H. H., 1978. Bionomics and control of the black vine weevil *Otiorhynchus sulcatus*. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 43: 607–611.

Feytaud, J., 1918. Etude sur l'otiorhynque sillonné (*Otiorhynchus sulcatus* Fabr.). Annales du Service des Epiphyties 5: 145–192.

Fung, S. Y., W. M. Herrebout, R. Verpoorte & F. C. Fischer, 1988. Butenolides in small ermine moths, *Yponomeuta* spp. (Lepidoptera: Yponomeutidae), and spindle-tree, *Euonymus europaeus* (Celastraceae). Journal of Chemical Ecology 14: 1099–1111.

Fung, S. Y., J. Schripsema & R. Verpoorte, 1990. α, β-unsaturated γ-lactones from *Sedum telephium* roots. Phytochemistry 29: 517–519.

Genderen, H. van, L. M. Schoonhoven & A. Fuchs, 1996. Chemisch-ecologische flora van Nederland en België: een inleiding over aard en ecologische betekenis van secundaire plantestoffen, KNNV Uitgeverij, Utrecht, 299 pp.

Hamilton-Kemp, T. R., R. A. Andersen, J. G. Rodriguez, J. H. Loughrin & C. G. Patterson, 1988. Strawberry foliage headspace vapor components at periods of susceptibility and resistance to *Tetranychus urticae* Koch. Journal of Chemical Ecology 14: 789–796.

Hansson, B. S., M. C. Larsson & W. S. Leal, 1999. Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. Physiological Entomology 24: 121–126.

Hanula, J. L., 1988. Oviposition preference and host recognition by the black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Environmental Entomology 17: 694–698.

Horst van der, M. J. & R. W. H. M. van Tol, 1995. Integrated pest management in nursery stock in the Netherlands. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 60: 759–762.

Jean, F.-I., F.-X. Garneau, G. J. Collin & M. Bouhajib, 1993. The essential oil and glycosidically bound volatile compounds of *Taxus canadensis* Marsh. Journal of Essential Oil Research 5: 7–11.

Khan, N. U.-D. & N. Parveen, 1987. The constituents of the genus *Taxus*. Journal of Scientific and Industrial Research 46: 512–516.

Khanizadeh, S. & A. Bélanger, 1993. Analysis of the essential oil of the leaves of *Fragaria* × *ananassa* Duch. Journal of Essential Oil Research 5: 109–111.

Landolt, P. J. & T. W. Phillips, 1997. Host plant influences on sex pheromone behavior of phytophagous insects. Annual Review of Entomology 42: 371–391.

Masaki, M., K. Ohmura & F. Ichinohe, 1984. Host range studies of the black vine weevil *Otiorhynchus sulcatus* (Fabricius) (Coleoptera: Curculionidae). Applied Entomology and Zoology 19: 95-106.

Menken, S. B. J., W. M. Herrebout & J. T. Wiebes, 1992. Small ermine moths (*Yponomeuta*): their host relations and evolution. Annual Review of Entomology 37: 41–66.

Moorhouse, E. R., A. K. Charnley & A. T. Gillespie, 1992. A review of the biology and control of the vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae). Annals of Applied Biology 121: 431–454.

Pers van der, J. N. C., 1981. Comparison of electroantennogram response spectra to plant volatiles in seven species of *Yponomeuta* and in the tortricid *Adoxophyes orana*. Entomologia Experimentalis et Applicata 30: 181–192.

Pickett, J. A., E. Bartlet, J. H. Buxton, L. J. Wadhams & C. M. Woodcock, 1996. Chemical ecology of adult vine weevil. Mitteilungen an der Biologischen Bundesanstalt 316: 41–45.

Seigler, D. S., 1991. Cyanide and cyanogenic glycosides. In: G. A. Rosenthal & M. R. Beerenbaum (eds), Herbivores. Their interactions with secondary plant metabolites. Academic Press, San Diego, pp. 35–77.

Smith, F. F., 1932. Biology and control of the black vine weevil. USDA Technical Bulletin 325.

Tol, R. W. H. M. van, 1996. Prospects for biological control of black vine weevil in nursery stock. Mitteilungen an der Biologischen Bundesanstalt 316: 69–75.

Tol, R. W. H. M. van & J. H. Visser, 1998. Host-plant preference and antennal responses of the black vine weevil (*Otiorhynchus sulcatus*) to plant volatiles. Proceedings of the section Experimental and Applied Entomology, N.E.V. Amsterdam 9: 35–40.

Tol, R. W. H. M. van, J. H. Visser & M. W. Sabelis, 2000. Responses of the black vine weevil (*Otiorhynchus sulcatus*) to weevil and host-plant odours. Proceedings of the section Experimental and Applied Entomology, N.E.V. Amsterdam 11: 109–114.

Turlings, T. C. J., J. H. Tumlinson & W. J. Lewis, 1990. Exploitation of herbivore-induced plant odours by host-seeking wasps. Science 250: 1251–1253.

Visser, J. H., 1986. Host odor perception in phytophagous insects. Annual Review of Entomology 31: 121–144.

Visser, J. H. & D. A. Avé, 1978. General green leaf volatiles in the olfactory orientation of the Colorado beetle, *Leptinotarsa decemlineata*. Entomologia Experimentalis et Applicata 24: 738–749.

Visser, J. H. & P. G. M. Piron, 1995. Olfactory antennal responses to plant volatiles in apterous virginoparae of the vetch aphid *Megoura viciae*. Entomologia Experimentalis et Applicata 77: 37–46.

Visser, J. H., S. van Straten & H. Maarse, 1979. Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the colorado beetle, Leptinotarsa decemlineata. Journal of Chemical Ecology 1: 13–25.

Visser, J. H., P. G. M. Piron & J. Hardie, 1996. The aphids' peripheral perception of plant volatiles. Entomologia Experimentalis et Applicata 80: 35–38.

Wilcox, J., D. C. Mote & L. Childs, 1934. The root weevil injurious to strawberries in Oregon. Oregon Agriculture Experimental Station Bulletin 330.