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Sex- and maturity-dependent antennal detection of host plant volatiles in the cabbage root fly, *Delia radicum*

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Highlights

- Dose-dependent electroantennogram responses were obtained for all tested compounds
- Mature insect antennae respond more to several host-derived compounds
- Immature insect antennae respond more to a behaviourally attractive flower volatile
- Male and female antennae respond with different sensitivity to certain compounds

Abstract

Adult insect behaviour in response to plant-emitted volatile compounds varies between the sexes and as a function of maturity. These differences in behavioural responses can be due to modulation in the peripheral or central nervous system. In the cabbage root fly, *Delia radicum*, behavioural effects of certain host plant volatiles on mature female behaviour have been evaluated, and a large number of compounds emitted by brassicaceous host plants have been identified. We recorded here dose-dependent electroantennogram responses to all tested compounds and investigated if the antennal detection of individual volatile compounds emitted by intact and damaged host plants differs between male and female, as well as immature and mature flies. Our results showed dose-dependent responses in mature and immature males and females. Mean response amplitudes varied significantly between sexes for three compounds, and between maturity states for six compounds. For some additional compounds significant differences occurred only for high stimulus doses (interaction between dose and sex and/or dose and maturity status). Multivariate analysis revealed a significant global effect of maturity on electroantennogram response amplitudes and for one experimental session also a significant global

effect of the sex. Interestingly, allyl isothiocyanate, a compound stimulating oviposition behaviour, elicited stronger responses in mature than in immature flies, whereas ethylacetophenone, an attractive flower volatile, elicited stronger responses in immature than in mature flies, which correlates with the behavioural role of these compounds. Several host-derived compounds elicited stronger responses in females than in males and, at least at high doses, stronger responses in mature than in immature flies, indicating differential antennal sensitivity to behaviourally active compounds. Six compounds did not cause any significant differences in responses between the different groups of flies. Our results thus confirm peripheral plasticity in plant volatile detection in the cabbage root fly and provide a basis for future behavioural investigations on the function of individual plant compounds.

Keywords: cabbage root fly (*Delia radicum*), olfactory plasticity, host plant volatiles, antennal sensitivity, electroantennogram

Introduction

Insect olfactory-guided behaviour is known to differ between males and females and to show strong plasticity as a function of adult maturity (age), and mating state (Gadenne et al., 2016). This plasticity helps insects to make optimal decisions when responding to sex pheromones, host-, food-, or oviposition site odours. In several moth species, recently mated male moths do not respond to female-emitted sex pheromones, because they would not be able to mate successfully (Gadenne et al., 2001). Blood-feeding insects such as mosquitoes and hematophagous bugs also adapt their responses to host odours as a function of age and mating state, thus avoiding taking unnecessary risks during the approach and attack of a host animal ((Gadenne et al., 2016) and references therein).

More recently, differences in behavioural responses to plant volatiles as a function of sex, maturity and mating state have been investigated in various phytophagous insects. In the fruit fly *Bactrocera tyroni* (Froggatt) (Diptera: Tephritidae), mated females were more attracted to their host fruit volatiles than unmated females (Devescovi et al., 2021). In the tomato fruit fly, *Neoceratitis cyanescens* (Bezzi) (Diptera: Tephritidae), both mated and unmated females responded to ripe tomato fruits, whereas males were rather attracted by odours from unripe fruits (Brevault and Quilici, 2010). In the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera, Noctuidae), females moth change their behavioural preferences from flower odours to odours emitted by plants used for oviposition after mating (Saveer et al., 2012). As a last example, in several species, male moths are only rarely attracted by host plant volatiles, but host plant volatiles can synergize responses to the female-emitted sex pheromone (Masante-Roca et al., 2007; Ochieng et al., 2002; Reddy and Guerrero, 2004; Varela et al., 2011; Yang et al., 2004).

The physiological mechanisms underlying behavioural plasticity have been investigated in detail specifically in male moths responding to sex pheromones. Here, depending on the species, either changes in the peripheral or central olfactory system have been described ((Gadenne et al., 2016) and references therein). In the noctuid moth *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), male responses to the sex pheromone increase with maturity, but not responses to plant volatiles (Greiner, 2002). However central, but not peripheral neuron responses to a flower volatile increased after mating in *A. ipsilon*, possibly facilitating a switch from searching for a mate to feeding behaviour (Barrozo et al., 2011). In some moth species, on the contrary, both peripheral and central nervous responses to host plant volatiles have been found to vary between sexes and as a function of maturity and mating state, in line with behavioural changes. For example in the grapevine moth *Lobesia botrana* (Denis and Schiffmüller) (Lepidoptera: Tortricidae), antennal lobe neurons in unmated males and in mated females responded more often to selected plant compounds than in mated males and unmated females

(Masante-Roca et al., 2007). Antennal responses of *Spodoptera littoralis* females to plant odours, on the other hand, were generally stronger in unmated females as compared to mated females (Martel et al., 2009). Interestingly, glomerular responses to a flower odour measured by calcium imaging activity representing essentially receptor neuron responses in the same species decreased after mating, whereas glomerular responses to host plant volatiles (cotton) increased after mating (Saveer et al., 2012). Finally, differential expression of antennal olfactory genes has been shown in different insects, such as *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) and *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) after mating (Crava et al., 2019; Siciliano et al., 2014; Jin et al., 2017).

Adults of the cabbage root fly, *Delia radicum* L. (Diptera: Anthomyiidae), feed from the nectar of a large diversity of plant species, whereas females are specialists of Brassicaceae plants for oviposition (Finch, 1989; Rännbäck, 2008). The cabbage root fly represents an interesting model to study sex differences and physiological plasticity in olfaction, because adults need several days to reach maturity and males and females might potentially respond differently to host-derived compounds, due to a differential role of host plants between the sexes. In addition to visual cues, females have been shown to use certain host plant-emitted odours, such as allyl isothiocyanate (AITC), to localize oviposition sites in the field (Tuttle et al., 1988). Also *cis*-3-hexenyl acetate, a volatile emitted by damaged host plants has been shown to be attractive for females (Kergunteuil et al., 2012). Other compounds such as dimethyl disulfide (DMDS) strongly decrease oviposition (Ferry et al., 2009; Kergunteuil et al., 2012; Lamy et al., 2017). Upon emergence and for the first few days of their life, unmated females and males search first for food sources and seem not to respond to host plant odours (Hawkes, 1975; Nottingham, 1988). Females usually start oviposition after a maturation phase that lasts about five days after emergence (Hawkes, 1975; Nottingham, 1988), but it is unknown whether this maturation, in addition to mating, contributes to changes in plant odour responses. Much less is known about how *D. radicum* males find their mating partners and if they use odour cues for orientation (Finch and Skinner, 1982). In particular, no sex pheromone has been identified so far. Males show some behavioural responses to AITC, but lower than females. This response could potentially help them to find a mating partner as this compound is only emitted by brassicaceous plants (Finch and Skinner, 1982) and mating usually takes place near the emergence site and therefore near the host plants (Coaker and Finch, 1971 in Nottingham, 1988). The main olfactory organ of *D. radicum*, the funiculus of the antenna, carries large numbers of four types of olfactory sensilla: trichoid, basiconic, grooved and clavate (Ross and Anderson, 1991, 1987). Interestingly, female antennae carry about 40 % more sensilla than male antennae and lower sensillum numbers in males were observed for all types, which correlates with behavioural differences between sexes (Ross and Anderson, 1987).

As a basis for future behavioural and physiological studies, we examine here for the first time antennal responses of *D. radicum* to a selection of host-derived compounds using electroantennographic recordings. We reveal dose-dependent antennal responses for all tested compounds, showing that the tested compounds are all detected by the cabbage root fly antenna. Our secondary aim was to identify volatile organic compounds, previously identified from intact and damaged host plants, which might be detected differentially between the sexes or as a function of maturity, in order to choose compounds to investigate variation in behavioural responses in the future. To detect such potential differences, we compared dose-response curves and mean response amplitudes between sexes, and between recently hatched immature, and mature mated insects.

Materials and Methods

Experimental insects

Delia radicum originated from a laboratory rearing initially constituted from a field population collected in the field in 2019 (Pleumeur-Gautier, Brittany, France). Flies were reared on rutabagas in a climate chamber at 20 ± 2 °C, 16L:8D, and 60 ± 10 % RH and fed with a milk powder: yeast: sugar (1:1:1) mixture as described in Neveu Bernard-Griffiths (1998). For EAG experiments, pupae were transferred to small cages, which were changed every two days to a new cage, controlling thus for age of emerged insects. Males and females were kept together to obtain mated mature males and females, which were fed *ad libitum* as under rearing conditions. Mature, assumedly mated insects were used at five to seven days of age. In favourable condition such as these, mating is known to take place on the 4th day after emergence (Coaker and Finch, 1971 in Nottingham, 1988). Immature, unmated insects were obtained by transferring individual pupae into Eppendorf cups with a small amount of food and a moist piece of cotton. Emerging insects were checked every day and insects were used within 24 h after emergence. Assuming that all immature insects were unmated, and all mature insects were mated, we use only the terms “immature” for immature unmated flies and “mature” for mature mated flies in the remaining text.

EAG recording

Whole insects were used for EAG recordings, with flies slipped into a plastic disposable pipette tip with the head and antennae protruding, secured with dental wax. Glass pipettes filled with Beadle Ephrussi Ringer were used as electrodes. The reference electrode was inserted into a small opening in the cuticle of the eye and the recording electrode was placed tightly in the centre of the undamaged funiculus similarly to EAG recordings in other flies (e.g. Hieu et al., 2014). The electrodes were connected to an amplifier (axoclamp 2B, Molecular Devices, San Jose, CA, USA), and an IDAC-4 device to digitize signals (Syntech, Kirchzarten, Germany). EAG Pro software (Syntech, Kirchzarten, Germany) was used to record the antennal responses.

Stimulation procedure

The antennae were superfused by a constant air-stream (0.3 m.s^{-1} , 17 mL.s^{-1}) through a glass tube. Stimuli were applied onto a piece of filter paper inserted into a Pasteur Pipette and the pipette was introduced into the glass tube through a hole in the sidewall. A stimulus controller (CS 55, Syntech, Kirchzarten, Germany) was used to apply a 500 ms air pulse through the stimulation pipette. During the stimulation, the airflow was switched from the continuous to the stimulus flow, which allowed the mechanoreceptive component to be minimized. Inter-stimulus intervals lasted at least 1 s. For each stimulus, increasing doses in decadic steps from 0.01 to 1000 μg were applied to obtain dose-response curves and the solvent, mineral oil, was applied before and after each stimulus series. The mean response to mineral oil before and after a stimulus dose series was subtracted from each amplitude value (= net amplitude) to compensate for amplitude differences between insects and over time. Recordings from fly antennae were, however, extremely stable over several hours, so that we did not need to further normalize data as a function of time. For each stimulus, sex, and maturity state 10 individuals were tested per session (see below).

Volatile organic compounds used for antennal stimulation

All compounds tested were previously identified in odour blends emitted by host plants of *D. radicum* (Supplementary Table A). Fifteen compounds were retained, which were taken as representatives for a variety of chemical classes having demonstrated effects on phytophagous insects in general and specialists of Brassicaceae in particular: sulfur compounds (AITC, DMDS), green leaf volatiles (*cis*-3-hexenyl acetate), defense hormones (methyl salicylate), aromatic compounds (ethylacetophenone), sesquiterpenes (α -farnesene and β -caryophyllene) and monoterpenes (α -pinene, α -thujene, eucalyptol,

limonene, linalool, myrcene, ocimene and sabinene). Whereas differences in behavioural responses between the sexes and between immature and mature flies have not been studied so far, some of these compounds have been identified as having effects on mature *D. radicum* females. Indeed, AITC and cis-3-hexenyl acetate have been shown to attract mature flies toward oviposition sites (Finch and Skinner, 1982; Kergunteuil et al., 2012; Nottingham, 1988; Tuttle et al., 1988) and to stimulate oviposition in field (Kergunteuil et al., 2012; Lamy et al., 2018) and laboratory conditions (Traynier, 1967, 1965). DMS (Ferry et al., 2009; Kergunteuil et al., 2012; Lamy et al., 2017, 2018), eucalyptol (Lamy et al., 2017) and limonene (Kergunteuil, 2013), **when applied in dispensers close to the host plants, were found to reduce oviposition**. Moreover, ethylacetophenone is emitted by flowers of many species including *Lobularia maritima*, a brassicaceous species whose flowers are particularly attractive to *D. radicum* females (Kergunteuil et al., 2012; Rännbäck, 2008), and methyl salicylate is a herbivore-induced plant volatile emitted by damaged brassicaceous plants in particular (Pierre et al., 2011). All other tested compounds are found in the volatile organic compounds (VOC) profiles of brassicaceous plants, but have not been tested for their effect on behavioural responses of *D. radicum* adults yet (Kergunteuil et al., 2015; Pierre et al., 2011; Tollsten and Bergstrom, 1988). Three of the fifteen compounds (namely ethylacetophenone, α -thujene and sabinene) were not available at the beginning of the experiments. Therefore, compounds were tested in two experimental sessions separated by a few weeks, with different individuals used in each session. Nine compounds were tested in the first session only, three compounds were tested in the second session only, and three compounds were tested in both sessions to ensure consistency in the interpretation of the results of the two sessions (Table 1).

Data processing and statistical analyses

All analyses were performed using the R software version 4.1.3 (R Core Team 2022). EAG amplitude responses were analyzed both univariately and multivariately. First, for each compound, the mean amplitude response was analyzed using Wald tests applied to Linear Mixed Models in which the sex, maturity status and dose (log-transformed), as well as all interactions between these variables, were considered as independent variables and the individual was considered as a random factor (R packages 'car' (Fox and Weisberg, 2019), 'lme4' (Bates et al., 2015), 'RVAideMemoire' (Hervé, 2022)). Responses were log-transformed to ensure model fit and, since some negative responses were obtained, normalized by computing $x + |\min(x)| + 0.01$ to avoid zeroes that would cause infinite logs. Pairwise comparisons of Estimated Marginal Means (EMMeans) were computed with p-values adjusted using the False Discovery Rate correction (Benjamini and Hochberg, 1995) (R package 'emmeans' (Lenth, 2022)). The multivariate approach consisted in two Redundancy Analyses (RDAs, R package 'vegan' (Oksanen et al., 2022), i.e. one per session. Responses were the overall mean EAG amplitude per individual and per compound (i.e. all doses confounded), which were normalized as above for consistency with the univariate approach and autoscaled. Independent variables were the sex, the maturity status and the interaction between these two factors. The effect of these factors was tested using a permutation F test with 9 999 permutations (Legendre and Legendre, 2012). Pairwise comparisons were performed also using permutation F-tests and p-values corrected with the False Discovery Rate method.

Results

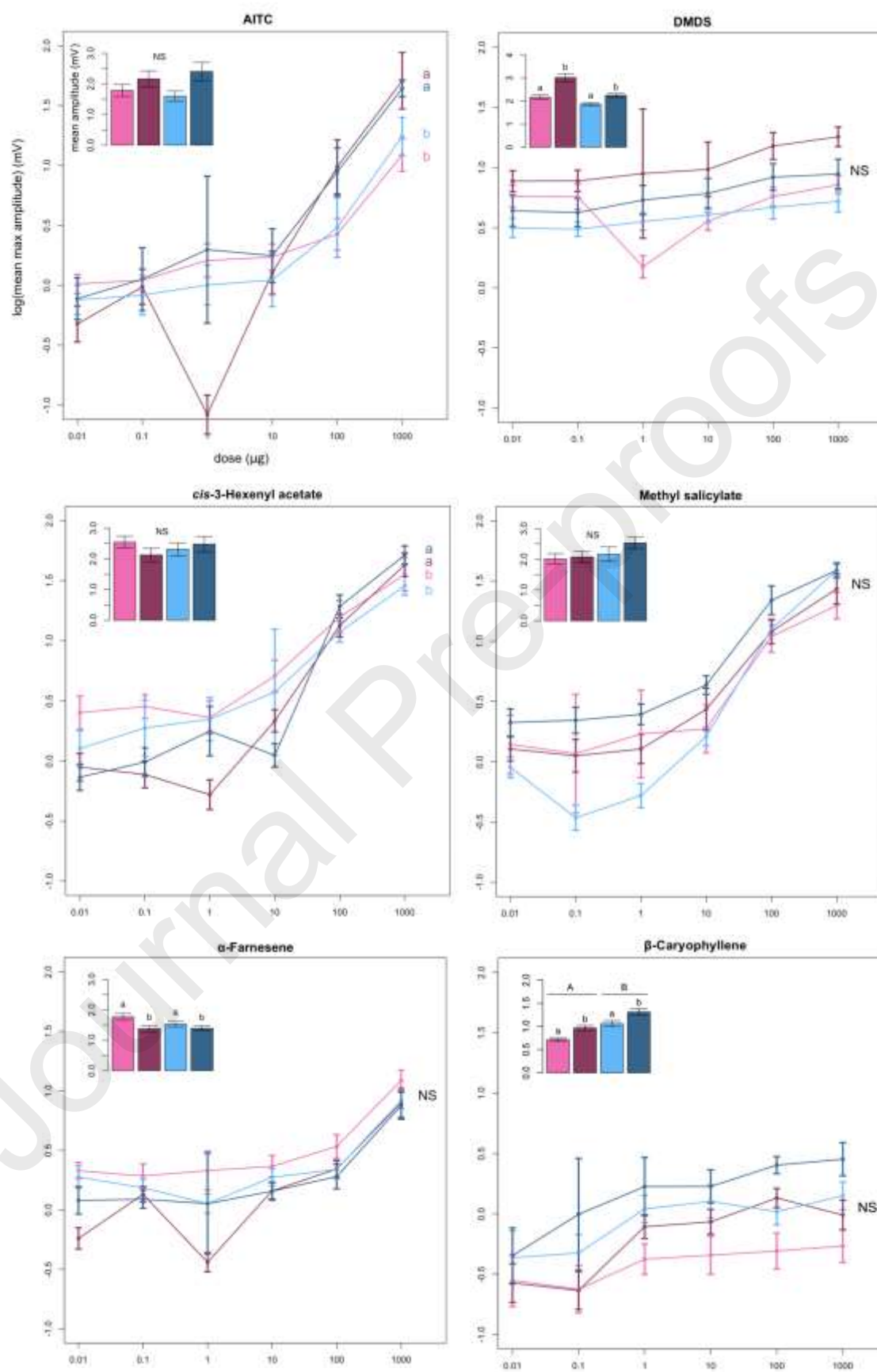
EAG response characteristics and dose-response curves vary between tested compounds

A significant dose effect was observed for all compounds showing that antennae of *D. radicum* responded in a dose-dependent manner to the fifteen volatiles tested (Table 1, Fig. 1, Fig. 2) The EAG amplitudes of responses varied between individual insects and reached maximum net amplitudes of up

1 to 10 mV, for the highest tested dose for certain compounds in some insects. Dose-response curves with
2 different thresholds and slopes were found for different compounds (Fig. 1, Fig. 2). Generally, antennae
3 responded already at the lowest tested dose to ocimene, β -caryophyllene and DMDS, but responses
4 increased only slightly when increasing the tested doses (Fig. 3). For other compounds such as
5 eucalyptol and α -pinene, responses only started at a dose of 100 μ g and increased at a dose of 1 000 μ g
6 (Fig. 3). For still other compounds such as linalool and AITC, responses started at 1 or 10 μ g and
7 increased steeply at high doses (Fig. 3).

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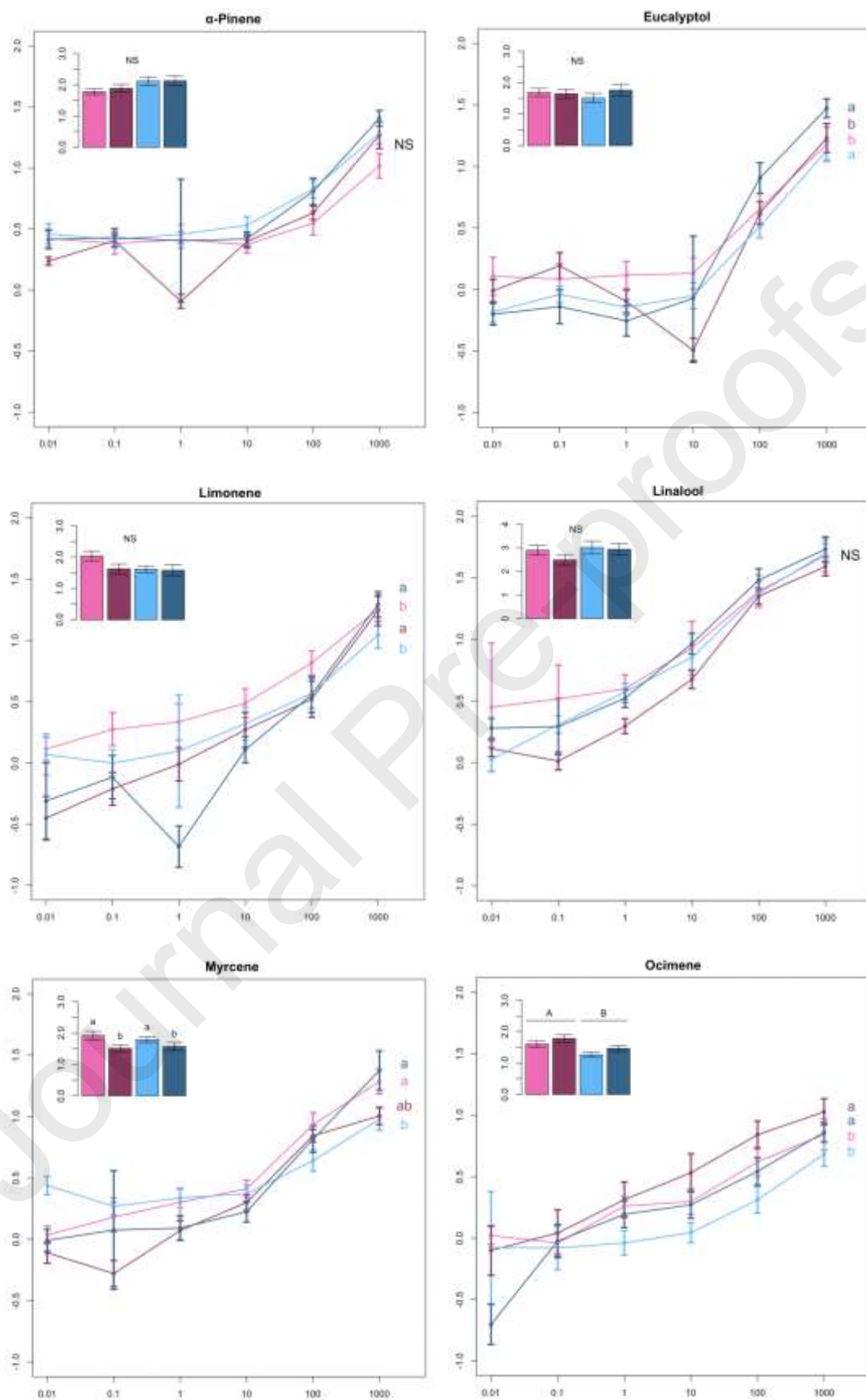


Fig. 1 Dose-response curves (mean \pm SEM) of *D. radicum* EAG amplitudes in response to the different VOCs tested in session 1. Colored lowercase letters indicate significant difference in dose-response slopes. The inserts represent the mean amplitude (\pm SE) (i.e., all doses confounded) in response to the different VOCs. Note that in the inserts, different uppercase letters indicate significant differences between sexes whereas different lowercase letters indicate significant differences between maturity statuses. Light pink = immature females, dark pink = mature females, light blue = immature males, dark blue = mature males. AITC: allyl isothiocyanate; DMDS: dimethyl disulfide

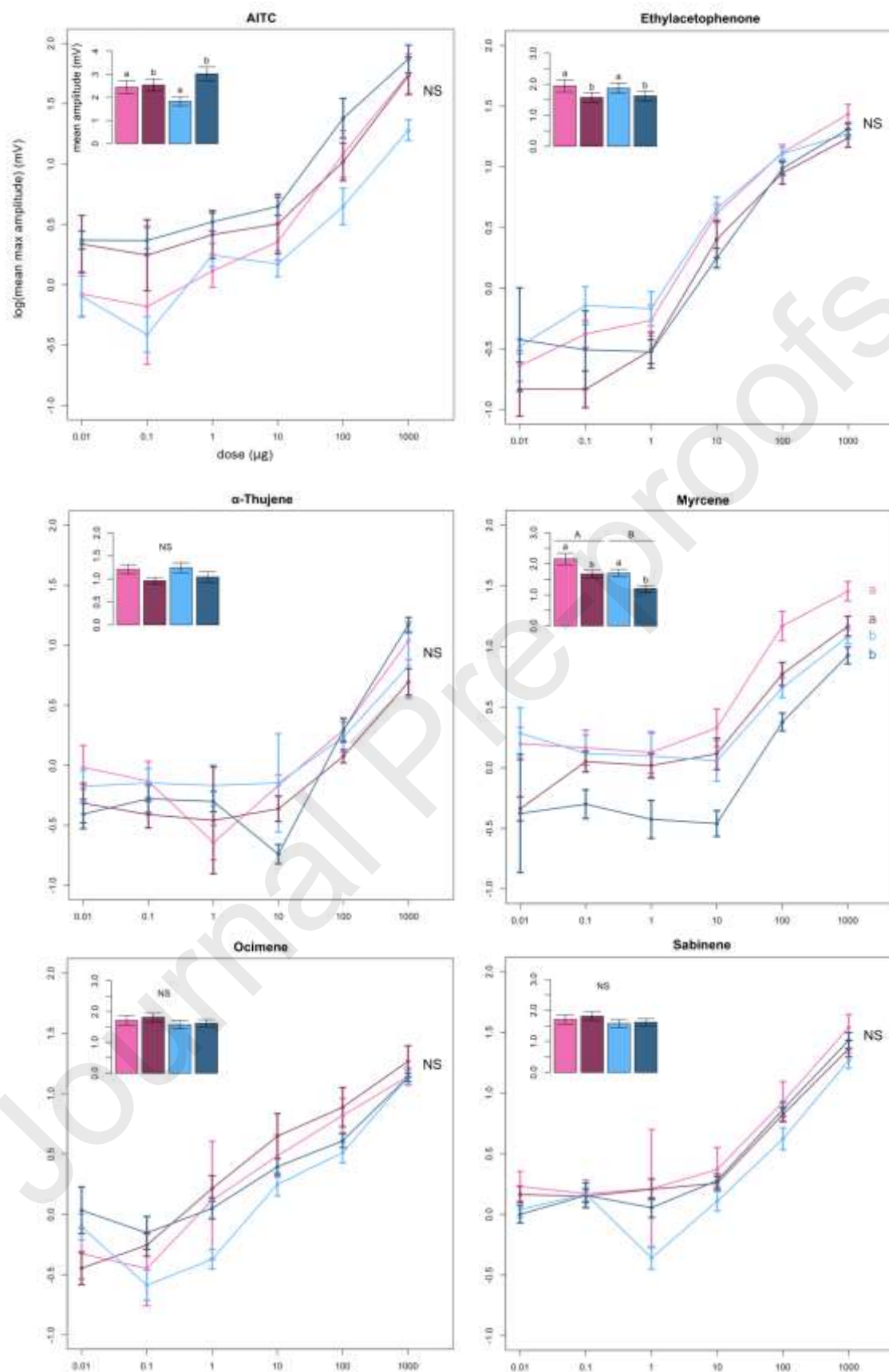


Fig. 2 Dose-response curves (mean \pm SEM) of *D. radicum* EAG amplitudes in response to the different VOCs tested in session 2. Colored lowercase letters indicate significant difference in dose-response slopes. The inserts represent the mean amplitude (\pm SE) (i.e., all doses confounded) in response to the different VOCs. Note that in the inserts, different uppercase letters indicate significant differences between sexes whereas different lowercase letters indicate significant differences between maturity statuses. Light pink = immature females, dark pink = mature females, light blue = immature males, dark blue = mature males. AITC: allyl isothiocyanate

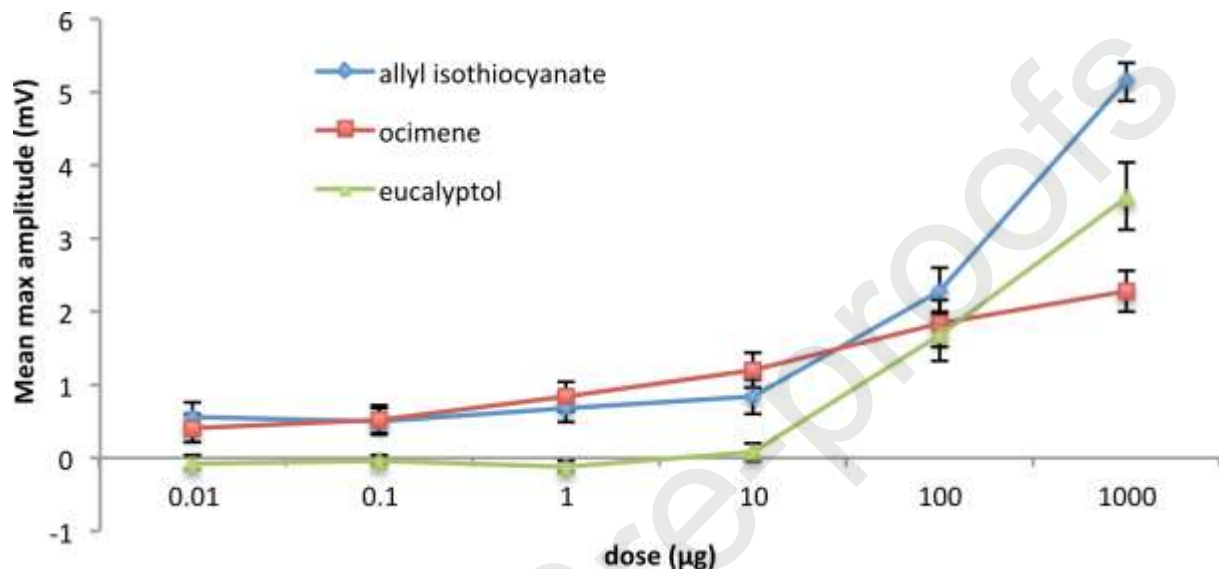


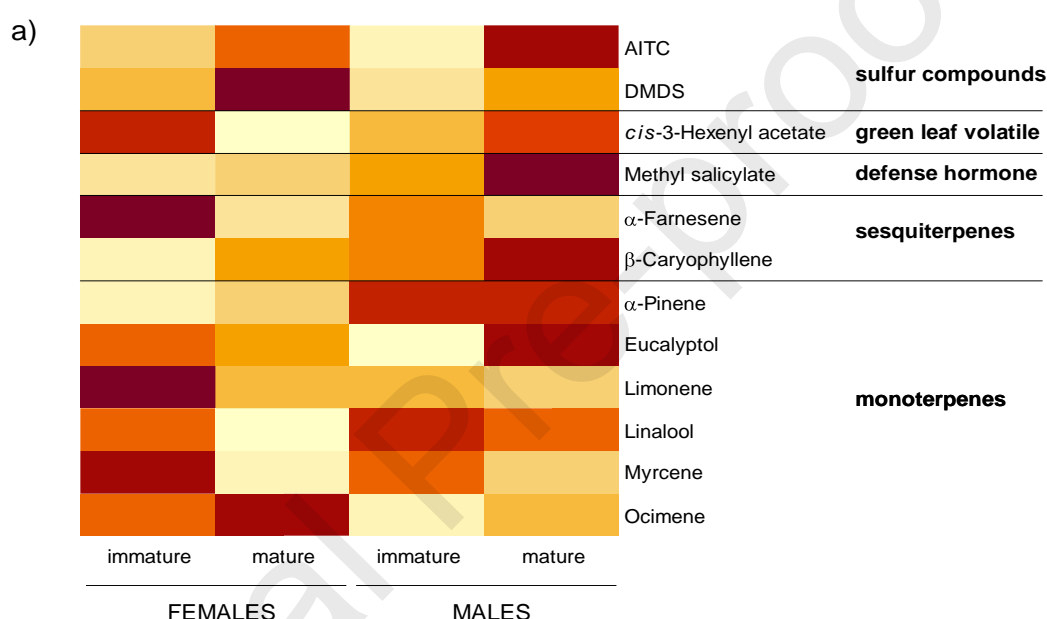
Fig. 3 Examples for dose-dependent EAG responses to different plant compounds in mature *Delia radicum* females. Examples for dose-response curves (mean amplitude \pm SE, n=10) for stimulation with allyl isothiocyanate, ocimene and eucalyptol. Note the differences in thresholds and slopes as a function of the applied stimuli.

EAG responses to a majority of compounds are sex- and maturity-dependent

Mean amplitudes of responses, as well as slopes of the dose-response curves, varied significantly between sexes, maturity status or both for 10 of the 15 compounds tested (Table 1). These effects were very similar between the two experimental sessions and are therefore described together.

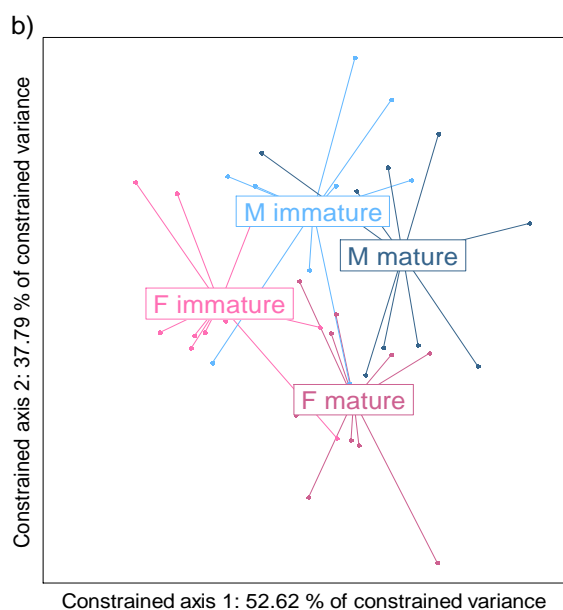
A significant, independent effect of the sex was observed for two compounds (Table 1, Fig. 4a, Fig. 1). In males larger responses were found for β -caryophyllene than in females, whereas female antennae exhibited larger responses for ocimene than male antennae. An interaction of dose and sex was only significant for two of the 15 compounds tested (Table 1). In males larger responses were found at high stimulus doses for eucalyptol than in females (Fig. 1). In females, on the other hand, larger responses were found at high stimulus doses for myrcene than in males (Fig. 2). A significant, sex-independent effect of maturity was observed for four compounds (Table 1, Fig. 4a,d, Fig. 1, Fig. 2). Antennae of immature flies exhibited larger responses to α -farnesene and ethylacetophenone, while larger responses were found in mature flies for DMDS and β -caryophyllene. An interaction of dose and maturity was significant for five compounds (Table 1). Responses at high doses of AITC, *cis*-3-hexenyl acetate, limonene, myrcene and ocimene exhibited larger amplitudes in mature compared to immature flies (Fig. 1).

When focusing on the mean amplitude of response per compound, both the univariate and multivariate approaches gave a consistent picture (Table 1, Fig. 4). In the first test session, a significant global effect of the sex was observed ($F = 2.57$, $p = 0.019$, Fig. 4b). Male antennae respond more intensely to methyl salicylate, β -caryophyllene and α -pinene (Fig. 4a,c). An independent, non-interacting ($F = 0.72$, $p = 0.703$), effect of the maturity status was observed ($F = 2.86$, $p = 0.008$, Fig. 4b). Antennae of immature individuals of both sexes responded more strongly to α -farnesene, limonene, and myrcene, in contrast to mature individuals who responded more strongly to AITC and DMDS (Fig. 4a,c). In the second session, only a significant effect of the maturity status was observed ($F = 4.48$, $p = 0.004$, Fig. 4e), with a stronger antennal response of immature individuals to ethylacetophenone and α -thujene, while mature individuals responded more strongly to AITC (Fig. 4d,f). No significant effect of the sex or the interaction between the sex and the maturity status was observed in the second session (respectively $F = 0.22$, $p = 0.165$; $F = 1.01$, $p = 0.395$) (Fig. 4d,f).

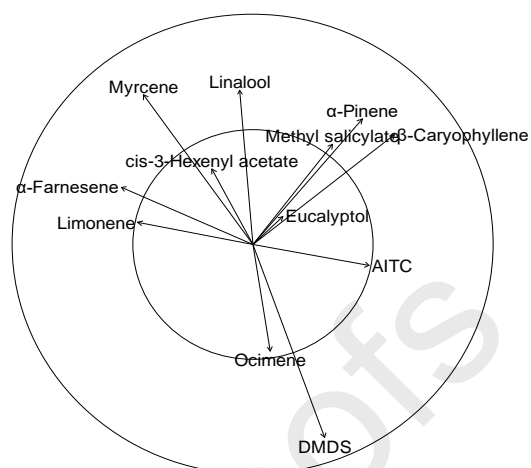


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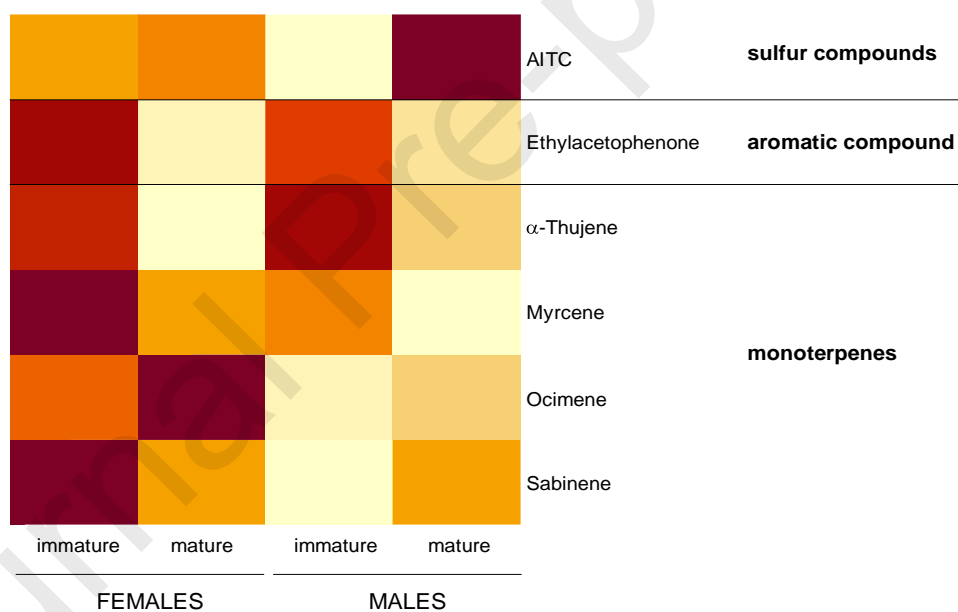
2



c)



d)



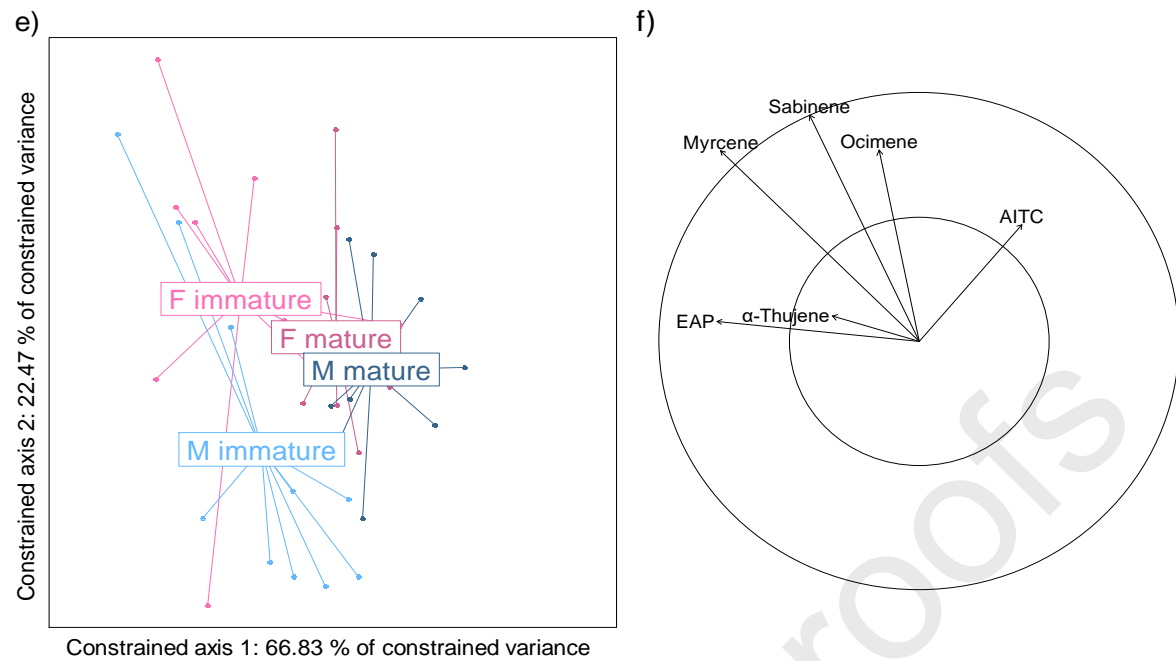


Fig. 4 Sex and maturity-dependent EAG responses to plant volatile compounds. Heatmaps of mean EAG amplitudes (i.e., all doses confounded) in response to the tested compounds according to sex and maturity of *Delia radicum* individuals, for session 1 (a) and session 2 (d). Mean amplitudes are pictured independently for each compound and represented by a color gradient from light yellow (lowest level) to dark red (highest level). Note that results are not comparable between lines but only between columns on a given line, due to compound-specific statistical standardisation of mean response amplitudes. Score plots and associated correlation circles of the redundancy analyses performed on mean EAG response amplitudes for each individual, for session 1 (b)(c) and session 2 (e)(f). AITC: allyl isothiocyanate, EAP: ethylacetophenone.

1

2 **Table 1: Wald tests on the effect of sex, maturity status, dose as well as all interactions between these variables on the mean EAG amplitude of *Delia***
3 ***radicum* for the compounds tested according to sessions. Significant p-values are indicated in bold**

	Compound	Dose			Sex			Maturity			Dose:Sex			Dose:Maturity			Sex:Maturity			Dose:Sex:Maturity		
		χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P
Session 1	AITC	128,53	1	< 0,001	0,42	1	0,519	0,05	1	0,824	0,05	1	0,815	8,22	1	0,004	2,16	1	0,142	1,85	1	0,174
	DMDS	14,76	1	< 0,001	1,68	1	0,195	7,78	1	0,005	0,07	1	0,797	1,74	1	0,187	0,94	1	0,333	0,31	1	0,579
	<i>cis</i> -3-Hexenyl acetate	254,43	1	< 0,001	0,01	1	0,918	2,21	1	0,137	0,23	1	0,630	8,63	1	0,003	0,86	1	0,354	0,11	1	0,743
	Methyl salicylate	162,23	1	< 0,001	1,08	1	0,298	3,39	1	0,065	0,94	1	0,331	0,06	1	0,805	0,74	1	0,391	2,33	1	0,127
	α -Farnesene	77,08	1	< 0,001	0,08	1	0,775	6,19	1	0,013	1,53	1	0,216	1,85	1	0,174	2,23	1	0,135	0,51	1	0,477
	β -Caryophyllene	48,25	1	< 0,001	9,98	1	0,002	6,92	1	0,009	0,46	1	0,498	2,25	1	0,133	0,02	1	0,879	0,18	1	0,674
	α -Pinene	99,68	1	< 0,001	3,64	1	0,056	0,09	1	0,767	0,74	1	0,389	2,74	1	0,098	0,03	1	0,870	1,08	1	0,299
	Eucayptol	159,40	1	< 0,001	0,92	1	0,336	0,08	1	0,777	4,34	1	0,037	1,65	1	0,198	1,09	1	0,296	1,52	1	0,217
	Limonene	140,33	1	< 0,001	1,96	1	0,162	3,48	1	0,062	0,12	1	0,726	5,84	1	0,016	0,37	1	0,541	0,02	1	0,882

	Linalool	379,59	1	< 0,001	0,69	1	0,406	0,28	1	0,598	0,95	1	0,329	0,28	1	0,597	0,83	1	0,364	1,19	1	0,276
	Myrcene	144,22	1	< 0,001	0,06	1	0,799	5,42	1	0,020	10,31	1	0,001	6,15	1	0,013	0,74	1	0,389	1,63	1	0,202
	Ocimene	124,60	1	< 0,001	4,69	1	0,030	2,05	1	0,152	0,10	1	0,752	4,35	1	0,037	0,01	1	0,928	0,64	1	0,423
Session 2	AITC	214,44	1	< 0,001	0,38	1	0,537	7,46	1	0,006	0,04	1	0,848	1,10	1	0,294	2,22	1	0,136	2,16	1	0,142
	Ethylacetophenone	386,01	1	< 0,001	0,13	1	0,714	6,44	1	0,011	2,31	1	0,128	0,21	1	0,647	0,08	1	0,782	0,01	1	0,934
	α -Thujene	47,81	1	< 0,001	0,07	1	0,789	2,16	1	0,141	0,01	1	0,939	0,06	1	0,804	0,10	1	0,758	0,20	1	0,651
	Myrcene	158,87	1	< 0,001	14,05	1	< 0,001	6,11	1	0,013	6,09	1	0,014	1,82	1	0,177	0,02	1	0,883	0,85	1	0,357
	Ocimene	215,26	1	< 0,001	3,44	1	0,064	0,80	1	0,372	3,58	1	0,059	0,28	1	0,597	0,05	1	0,828	0,85	1	0,356
	Sabinene	216,50	1	< 0,001	2,59	1	0,107	0,07	1	0,790	0,03	1	0,855	0,10	1	0,746	2,11	1	0,146	1,08	1	0,300

Discussion

Our results show that all tested plant-derived compounds are detected by *D. radicum* antennae in a dose-dependent manner and reveal highly variable responses to these compounds, as a function of sex and maturity. Interestingly, the differences in antennal responses between mature and immature flies, as well as between males and females, depend strongly on the tested compound. Behavioural significance of the tested compounds is known only for a few of them and almost exclusively in mature females so far. Therefore, we can here only speculate on the behavioural significance of the differences in antennal detection, which we observed between the sexes and immature and mature flies. Two of the three compounds, known to reduce oviposition in *D. radicum* females, DMDS and limonene, elicited stronger responses in mature than in immature flies of both sexes, at least at high doses. The observed reduced oviposition rate of female flies exposed to these compounds (Lamy et al., 2017; Liu et al., 2018) might thus be due to a high sensitivity to these compounds, mediating a potential repulsive effect. However, behavioural assays with immature insects need to confirm whether the sensitivity difference is indeed responsible for such an effect only in mature insects. Allyl isothiocyanate and *cis*-3-hexenyl acetate, compounds known to attract flies towards oviposition sites (Traynier, 1965, 1967; Städler, 1978), elicited increased EAG responses, at least at high doses, in mature compared to immature adults. Whereas increased sensitivity in mature females correlates with the stimulation of oviposition, it would now be interesting to evaluate behavioural effects of these compounds on mature males. A compound emitted by flowers used for nectar feeding in *D. radicum* (Rännbäck, 2008), ethylacetophenone, elicited much stronger EAG responses in immature insects than in mature insects. This could be related to the importance to find food sources in immature flies. Interestingly many of the compounds shown to be emitted in various host plants of *D. radicum* elicited stronger responses in mature than in immature insects (at least at higher doses) and some compounds elicited stronger responses in females than in males at higher doses. This increased sensitivity could indicate that the concerned compounds might play a role in host finding behaviour. However, the occasionally observed higher sensitivity in males needs to be further investigated.

Our results on the plasticity of antennal responses to host plant odours in this species provide a complex dataset, allowing to choose compounds of interest for future behavioural experiments as a function of sex and maturity of this insect. However, even though the compounds not eliciting any differences in antennal responses between sexes and maturity might be potentially of less importance for adult flies, one should not exclude these compounds from further investigations, because modulation of plant odour sensitivity might well occur at the central nervous level, as previously observed in different moth species (Barrozo et al., 2011; Masante-Roca et al., 2007).

Differences in antennal olfactory sensitivity to plant-derived volatiles between mature and immature insects and between the sexes have rarely been investigated systematically. In other studies, single doses of different components have been used as stimuli (Martel et al., 2009), but this limits the conclusions that can be drawn. Indeed, as shown by our results, dose-response curves with different thresholds and different slopes can occur and thus differences can be hidden when testing only a single dose. Nevertheless significant differences in responses to a panel of plant-derived compounds have been found in females of the noctuid moth *Spodoptera littoralis* as a function of mating and adult age (Martel et al., 2009). Also in the Mediterranean fruit fly, *Ceratitis capitata*, EAG responses to plant headspace of different host plants varied between the sexes, but also in both males and females as a function of mating state and the origin of the flies (laboratory-reared or wildtype) (Sollai et al., 2020). EAG responses of female blowflies to attractive and repellent compounds similarly depended on the maturity: response amplitudes increased with age (Crnjar et al., 1990). Our results on the cabbage root fly confirm that indeed differences in plant volatile responses between the sexes and maturity of insects exist already at the detection level.

Whereas modulatory mechanisms of central olfactory sensitivity have been described in different insects, among others as a function of maturity (Gadenne et al., 2016; Ignell et al., 2001; Jarriault et al., 2009), the mechanisms underlying peripheral modulation of olfactory sensitivity are not well understood. Up- and down- regulation of the expression of olfaction-related antennal genes, such as

olfactory receptors and odorant binding proteins, for example, seem to correlate with antennal sensitivity. In *C. capitata* for example, odorant binding protein expression levels varied as a function of sex, sexual maturity and mating state, correlated with recently found EAG differences in response to volatile organic compounds (Siciliano et al., 2014; Sollai et al., 2020). In the oriental fruit fly *Bactrocera dorsalis*, mating leads to changes in the expression of various olfactory receptor genes, including ORs, GRs and IRs, but the corresponding ligands are not known (Jin et al., 2017). In the same species, changes of EAG sensitivity to a compound present in host plants as a function of starvation depended on the presence of short neuropeptide F and the expression of its receptor (Jiang et al., 2017). With the recently sequenced genome of *D. radicum* available (Sontowski et al., 2022), it will be possible in the future to identify olfactory genes involved in the differences in antennal sensitivity between the sexes and as a function of maturity. Another potential mechanism is modulation of peripheral olfactory sensitivity by modulatory feedback neurons from the brain and reaching the antennae, as it has been shown for serotonergic neurons in mosquitoes (Siju et al., 2008). Immunostaining of *D. radicum* antennae would be necessary to potentially reveal such a mechanism.

CRediT author contribution statement

Kathleen Menacer: Conceptualization, Formal analysis, Writing. **Maxime R. Hervé:** Conceptualization, Formal analysis, Writing, Supervision, Project administration, Funding acquisition. **Anne Marie Cortesero:** Conceptualization, Writing, supervision, Funding acquisition. **Tom Aujames:** Investigation. **Sylvia Anton:** Conceptualization, Methodology, Investigation, Formal analysis, Writing-original draft, Supervision.

Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that would have appeared to influence the work reported in this paper.

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1 **Supplementary Table A** Compounds used for stimulation in EAG experiments.

Compound	Chemical class	Purity	Purchased from*	Ecological relevance	References
Allyl isothiocyanate	sulfur compound	≥ 95%	Aldrich	Attractive to gravid females Found in some Brassica volatile organic compounds (VOC) profiles Stimulates oviposition	Wallbank & Wheatley, 1976; Finch, 1978; Finch & Skinner, 1982; Nottingham, 1988; Tuttle et al., 1988; Liu et al., 2018
Dimethyl disulfide	sulfur compound	≥ 99%	Aldrich	Reduces oviposition Found in some Brassica VOC profiles	Wallbank & Wheatley, 1976; Pierre et al., 2011; Kergunteuil et al., 2012; Lamy et al., 2017; Liu et al., 2018
<i>cis</i> -3-Hexenyl acetate	green leaf volatile	≥ 98%	Sigma-Aldrich	Main compound of attractive Chinese cabbage Found in other Brassica species	Wallbank & Wheatley, 1976; Tollsten & Bergstrom, 1988; Pierre et al., 2011; Kergunteuil et al., 2012; Liu et al., 2018
Methyl salicylate	defense hormone	≥ 99%	Sigma-Aldrich	Herbivore-induced plant volatile and attracting insects in the field in different crops, including Brassica	Orre et al., 2010; Simpson et al., 2011; Pierre et al., 2011; Kergunteuil et al., 2012
Ethylacetophenone	aromatic compound	97%	Sigma Aldrich	Flower-derived compound Attractive as food signal	Rännbäck, 2008
α-Farnesene	sesquiterpene	≥ 90%	Bedoukian Bio	Found in the volatile profiles of different <i>D. radicum</i> Brassica host species Predominant in the VOC profile of broccoli	Tollsten & Bergstrom, 1988; Pierre et al., 2011; Kergunteuil et al., 2015

β -Caryophyllene	sesquiterpene	$\geq 98.5\%$	Sigma	Found in the volatile profiles of different <i>D. radicum</i> Brassica hosts species Predominant in the VOC profile of oil seed rape	Tollsten & Bergstrom, 1988; Pierre et al., 2011; Kergunteuil et al., 2015
α -Pinene	monoterpene	98%	Aldrich	Found in the VOC profiles of different <i>D. radicum</i> Brassica hosts species	Tollsten & Bergstrom, 1988; Pierre et al., 2011; Kergunteuil et al., 2015
α -Thujene	monoterpene	95 %	Toronto Research Chemicals	Found in the VOC profiles of different <i>D. radicum</i> Brassica hosts species,	Kergunteuil et al., 2015
Eucalyptol	monoterpene	99%	Aldrich	Reduces oviposition Found in some Brassica VOC profiles	Lamy et al., 2017; Liu et al., 2018
Limonene	monoterpene	97%	Sigma Aldrich	Found in volatile profiles of different <i>D. radicum</i> Brassica hosts species Reduces plant colonization	Pierre et al., 2011; Kergunteuil, 2013; Kergunteuil et al., 2015; Liu et al., 2018
Linalool	monoterpene	97%	Aldrich	Predominant in the VOC profile of Chinese cabbage	Kergunteuil et al., 2015
Myrcene	monoterpene	Technical grade	Aldrich	Found in the volatile profiles of different <i>D. radicum</i> Brassica host species	Tollsten & Bergstrom, 1988; Kergunteuil et al., 2015
Ocimene (mixture of isomers)	monoterpene	$\geq 90\%$	Sigma-Aldrich	Found in the volatile profiles of different <i>D. radicum</i> Brassica hosts species	Tollsten & Bergstrom, 1988
Sabinene	monoterpene	98%	abcr chemicals	Found in the volatile profiles of different <i>D. radicum</i> Brassica hosts species	Tollsten & Bergstrom, 1988

1 * suppliers: Bedoukian, Danbury, CT, United States; Sigma-Aldrich, Saint Quentin Fallavier, France; Toronto Research Chemicals, Toronto, Canada; abcr chemicals, Karlsruhe,
2 Germany

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