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Electroantennogram responses of a parasitic wasp, *Microplitis croceipes*, to host-related volatile and anthropogenic compounds

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Abstract. The parasitic wasp *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) showed its own characteristic electroantennogram (EAG) response profiles to 13 host-related (*cis*-3-hexenol, α-pinene (*R*)-(+)-limonene (*S*)-(–)-limonene, *trans*-β-ocimene (±)-linalool, (–)-*trans*-caryophyllene, α-humulene, nerolidol, *trans*-nerolidol, *cis*-nerolidol, methyl jasmonate and indole) and four anthropogenic (2-diisopropylaminoethanol, 2,2′-thiodiethanol, 2-methyl-5-nitroaniline and cyclohexanone) volatile compounds. These profiles were similar between males and females except for 2-diisopropylaminoethanol, which elicited significantly larger EAG responses in males. Among the compounds tested, *cis*-3-hexenol, linalool and cyclohexanone elicited the largest EAG responses. EAG responses were not influenced by the age of wasps between 1 and 13 days after emergence. EAG responses were dose-dependent, and highly EAG-active compounds elicited significant EAG responses with less than 10 μg of the compounds at source. Quantification of compounds released from an odour cartridge indicates that release rate is highly dependent on the chemical nature of stimuli, showing up to 10 000-fold differences in the amount released between different compounds when the same amount was loaded in the odour cartridge. Wasps having undergone a behavioural training regime to be attracted to either cyclohexanone or methyl jasmonate did not show any differences in EAG responses from those of untrained wasps.

Key words. *cis*-3-hexenol, cyclohexanone, electroantennogram, linalool, *Microplitis croceipes*, training, wasp.

Introduction

Olfactory information is an important cue for host location by *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid specific to the larvae of *Heliothis* and *Helicoverpa* (Turlings *et al.*, 1990; Lewis *et al.*, 1991; Cortesero *et al.*, 1997; De Moraes *et al.*, 1998; Röse *et al.*, 1998). This wasp exhibits flexibility in the use of chemical cues for host location and demonstrates chemically mediated associative learning (Lewis & Takasu, 1990; Lewis *et al.*, 1991; Zanen & Cardé, 1991; Takasu & Lewis, 1996). Behavioural responses of *M. croceipes* to olfactory stimuli can be modulated by training with specific odours such as the faeces of the host larvae (Lewis *et al.*, 1991), plant odour (Zanen & Cardé, 1991) and single compounds (Takasu & Lewis, 1996). The effect of learning can last up to 48 h (Takasu & Lewis, 1996), indicating the involvement of the central nervous system in learning. However, it is unclear whether the learning process might also influence the sensitivity of antennal receptor neurones. Examples of antennal receptor neurones being modulated by changes in the physiological state of an insect are rare, although a few studies have suggested a change in sensitivity of receptor neurones after olfactory learning in two hymenopteran species, *Apis mellifera ligustica* (De Jong & Pham-Delègue, 1991) and *Leptopilina heterotoma* (Vet *et al.*, 1990).

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The electroantennogram (EAG) has been a useful tool for studying the chemical ecology of insects. An EAG response profile is thought to represent the sensitivity and relative abundance of olfactory receptor neurones on the antennae that are tuned to the compounds tested. Such profiles help identify behaviourally active compounds, although EAG activity does not by itself necessarily indicate behavioural activity. Most studies have shown that EAG responses are not influenced by nutritional status, hormones or time of day. However, EAG amplitudes are known to decline with the age of the insect, and also with the elapsed time following initial set-up of the recording preparation. A robust insect preparation is needed for long-lasting stable EAG recordings. A recent study showed that whole-body insect preparations can considerably improve the lifetime and stability of EAG (Park & Hardie, 1998).

In many EAG studies, two types of information have been used for discriminating highly olfactory active compounds among several candidate compounds. One is a comparison of EAG amplitudes elicited by the same amount of compound in an odour cartridge, and the other is a comparison of EAG dose–response profiles among different compounds. In any of these assessments, however, the amount of odourant blowing onto the antenna has rarely been quantified. The amount of stimulus released from an odour cartridge can vary greatly among compounds, especially due to differences in volatility. Although EAG dose–response studies have been useful, they can be improved by knowing the actual release rates of the compounds from odour cartridges.

There has been recent interest in using the learning ability of *M. croceipes* for detecting the presence, and finding sources, odours of interest to the U.S. Department of Defense, such as unexploded military ordinance or toxic agents. To use the wasps' olfactory abilities for these purposes, the wasps must be able to respond behaviourally to odours from chemicals of anthropogenic origin (designed and synthesized by humans that do not exist in nature). It also might be possible to use isolated receptor systems or entire antennae as biosensors, but only if such sensors can be shown to respond to anthropogenic volatile compounds. Therefore, a major goal of this study was to determine to what degree *M. croceipes* antennae would respond to volatiles of anthropogenic origin compared with those of host-related natural sources.

Materials and Methods

Insects

Pupae of *M. croceipes* were provided by Dr W. J. Lewis (USDA-ARS Tifton, Georgia, U.S.A.) and were kept in a cage under room condition (23 C, 50~70% RH, LD 16 : 8 h). Emerging adults were collected daily, sexed and kept in separate cages for each sex until used for the experiments. Distilled water and 10% sucrose solution were provided. One- to three-day-old adult wasps were used for the EAG experiments unless otherwise specified.

Test compounds

Seventeen compounds (*cis*-3-hexenol, α-pinene (*R*)-(+)-limonene, (*S*)-(−)-limonene, *trans*-β-ocimene, (±)-linalool, (−)-*trans*-caryophyllene, α-humulene, nerolidol (50 : 50 mixture of *trans*- and *cis*-nerolidol), *trans*-nerolidol, *cis*-nerolidol, methyl jasmonate, indole, 2-diisopropylaminoethanol, 2,2′-thiodiethanol, 2-methyl-5-nitroaniline and cyclohexanone) (chemical and isomeric purity >99%) were provided by Dr J. H. Tumlinson (USDA-ARS Gainsville, Florida, U.S.A., originally purchased from Sigma®). Each compound was diluted in hexane, acetone, or dichloromethane to give 100 μg/μL solutions. For dose–response studies, further dilutions were made to give 0.01, 0.1, 1, 10 and 100 μg/μL solutions. The solutions were kept in a freezer at <−20°C until used.

Antennal preparation and EAG recording

A glass capillary (0.86 mm I.D., A-M Systems Inc., Washington, U.S.A.) filled with electroconductive gel (Spectra® 360, Parker Laboratory Inc., Orange, New Jersey, U.S.A.) was connected to the neck of an isolated head of *M. croceipes* and used as a reference electrode. Another glass capillary connected to the antennal tip, after one or two terminal segments were cut off, served as a recording electrode. For whole-body preparations, a wasp was immobilized on a paraffin block with small U-shaped metal wires. A microglass electrode (tip diameter ≈ 1 μm) filled with 0.5 M KCl solution was introduced through the cuticle of the distal part of the antenna and served as a recording electrode; another microglass electrode penetrated into a compound eye to serve as a reference electrode. Silver–silver chloride junctions were used to maintain electrical contact between the electrodes and amplifier. The EAG signal recorded with the electrodes was first amplified with a high-input impedance ($>10^{12}\,\Omega$) head-stage preamplifier (Syntech®,Hilversum, The Netherlands), and further processed with a PC-based signal processing system (Syntech®).

Odour presentation

A piece of filter paper (6 × 40 mm, Whatman® no. 1) impregnated with 10 μL aliquot of test solution was inserted into a glass Pasteur pipette (110 mm in length, Fisher Scientific, Pittsburgh, Pennsylvania, U.S.A.) and used for odour presentation. The narrow end of the Pasteur pipette was inserted through a small hole in the wall of an L-shaped glass tube (15 cm from the hole to the outlet of the L-shaped tube). Antennae were positioned approximately 3 cm from the outlet of the L-shaped tube. For stimulation, 2 mL of purified, charcoal-filtered air was introduced through the Pasteur pipette cartridge for 0.2 s into the L-shaped glass tube with continuous humidified main air stream (1000 mL/min). An electrically controlled airflow controller (SFC-2, Syntech®) was used for the stimulation. At least 30 s was allowed between successive

stimulations, and the stimulations with different odourants were made in random order. For dose–response experiments, exposure proceeded from lowest to highest concentration to minimize the effect of olfactory adaptation by strong stimulation. One hundred micrograms of *cis*-3-hexenol was used as a standard stimulus. A test series of odourants or dosages was always preceded by, and followed with, the standard stimulus, and the standard stimulus also was applied after every four successive stimulations. Normalization was made by dividing the peak EAG amplitude of the test puff with the average EAG amplitude to the standard stimulus.

Quantification of stimuli released from odour cartridges

Quantitative analysis of stimuli released was made for five host-related compounds (*cis*-3-hexenol, caryophyllene, indole, methyl jasmonate and α-pinene) and two anthropogenic compounds (cyclohexanone and 2-methyl-5-nitroaniline). Initially, odour trapping with adsorbent (Tenax TA, 35/60 mesh, Alltech®, Deerfield, Illinois, U.S.A.) was tried in an attempt to measure release rates from the odour cartridges. However, back-pressure due to packing material inside the collection tube prevented unimpeded release of odourant from the cartridge tip. To minimize this tailing effect, an open-end odour collection system was used (Fig. 1). A freshly loaded odour cartridge identical to that used for EAG experiments was used for the quantification. The narrow end of the odour cartridge was inserted into a 120 cm long glass collection tube (2 mm I.D.) and the other end connected to an electronic flow controller (SFC-2, Syntech®). Two millilitres of air was passed through the odour cartridge for 0.2 s and the compound released was trapped with the glass collection tube precooled with dry ice. The collection tube was then rinsed twice with 500 μL of hexane containing *cis*-8-tridecenyl acetate (1 ng/μL) as an internal standard. *Cis*-5-undecenyl acetate was used as an internal standard for methyl jasmonate. The extract was concentrated with nitrogen gas and quantified using gas chromatography (fused silica, 30 m × 0.25 mm, EC-5)-mass selective detector (HP-5972, Hewlett Packard®, U.S.A.) in selective ion mode.

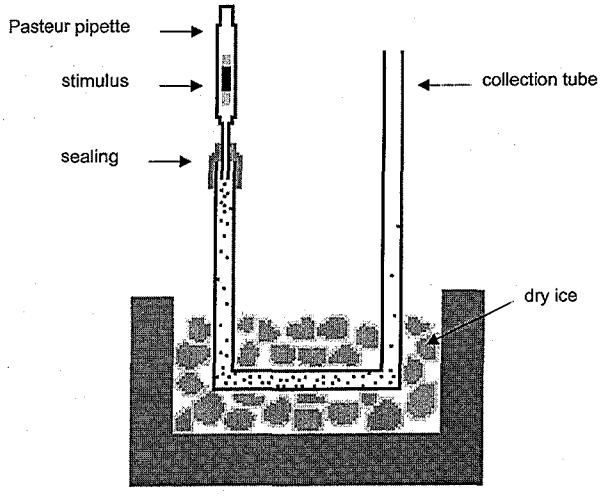

Fig. 1. An open-end collection system for the quantification of volatile compounds released from Pasteur pipette odour cartridges. The volatile molecules released from the odour cartridges are captured in a long U-shaped glass collection tube cooled with dry ice, rinsed with solvent, and then concentrated with nitrogen gas for GC-MS analysis.

Training associated with sugar bait

Female wasps were trained with cyclohexanone and methyl jasmonate and the EAG responses of trained wasps compared with those of untrained wasps. These two compounds were chosen because the wasps learned to respond to these compounds by flying upwind to them (W. J. Lewis, personal communication; Takasu & Lewis, 1996). Undiluted cyclohexanone or methyl jasmonate (>99.5%) was placed in a 200 μL capillary tube with one end sealed, and brought up to volume at 2 or 3 mm from the open end. The capillary tube was then positioned upright through a silicon septum of a glass T-tube (Wheaton®, Millville, New Jersey) in the training apparatus, where the air passed over the top of the capillary tube (300 mL/min), down to the Petri dish where a wasp was trained. A drop of sugar solution (10 μL, 50% in distilled water) was provided as an associative reward in conjunction with the stimulus. Wasps were trained in the Petri dish anywhere between 5 mm to 1 cm from the chemical airflow, and allowed to feed for 5 s at 30 s intervals, for three repetitions. Wasps subjected to the training were starved at least 24 h prior to the training. Wasps having undergone the training regime were tested for EAG response within 1 h after the training. Untrained naive wasps of the same age as trained ones were chosen and given sugar water under the same procedure as for trained wasps, but without introducing the odour stimulus. These wasps were used as controls.

Results

Microplitis croceipes showed typical rapid depolarizing EAG responses (Fig. 2). No hyperpolarizing EAG responses were elicited by the 17 compounds tested. The EAG amplitude in response to the standard stimulus (100 μg of *cis*-3-hexenol) was about 3–5 mV in whole-body preparations and < 0.5 mV in isolated head preparations. Freshly made whole-body EAG preparations showed the highest responsiveness, with gradual decreases over time. The whole-body preparation lasted much longer than the isolated head preparation (Fig. 3). EAG responsiveness of the isolated head preparation decreased rapidly, falling below 20% of initial EAG responses after 50 min. By contrast, the whole-body preparation retained 80% of its EAG responsiveness after 3 h. The age of the adult wasps did not influence EAG amplitudes (Fig. 4). Responses to 1 mg of *cis*-3-hexenol, linalool, diisopropylaminoethanol, and cyclohexanone were not significantly different between 1- and 13-day-old female *M. croceipes*.

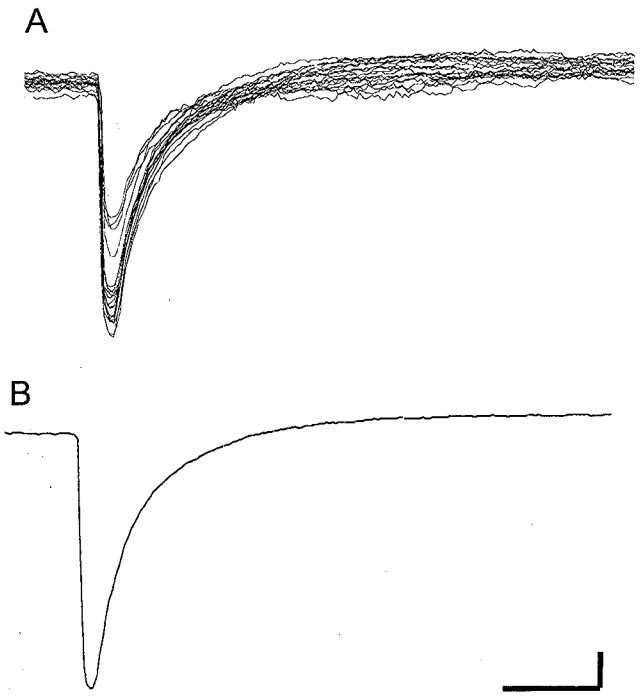

Fig. 2. EAG response waveforms of female *Microplitis croceipes*. (A) Individual traces of twenty EAG responses (from 10 females) to 1 mg of cyclohexanone, (B) superimposed EAG waveform of (A). Scales indicate 3 s (horizontal) and 1 mV (vertical).

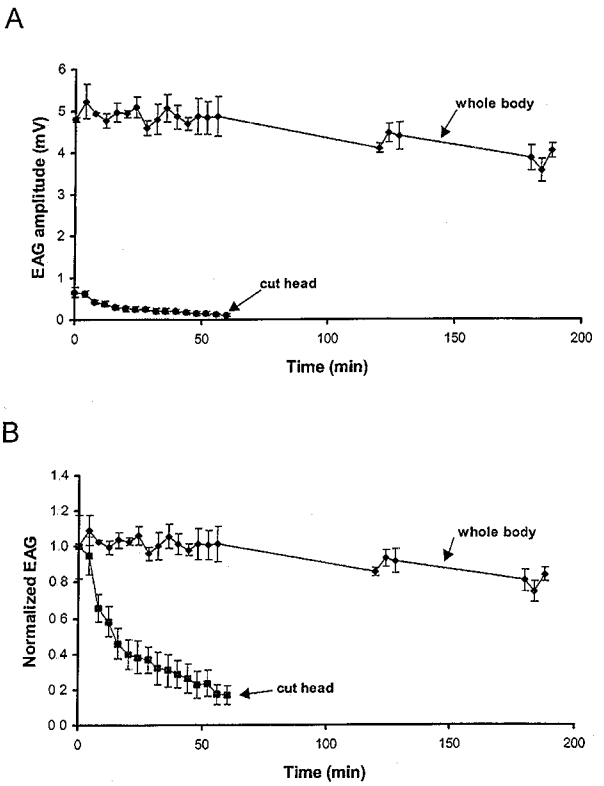

Fig. 3. Comparison of longevity of EAG responsiveness between whole-body preparations and typical cut head preparations in female *Microplitis croceipes*. Antennae were stimulated with 1 mg of *cis*-3-hexenol at 4 min intervals for 1 h and then at 1 h intervals for further recordings with whole-body preparation. (A) Absolute EAG amplitudes in –mV. (B) Relative EAG responses normalized to the average of the initial (zero min) EAG responses of each preparation (mean ± SEM, $n = 8$).

Microplitis croceipes showed characteristic EAG response profiles to 17 volatile compounds (Fig. 5). EAG response profiles were not significantly different between males and females, except for one non-host-related compound, 2-diisopropylaminoethanol (compound 14 in Fig. 5), which elicited significantly larger EAG responses in males. Two general plant volatiles, *cis*-3-hexenol and linalool, and one non-host-related compound, cyclohexanone, elicited the highest EAG responses both in male and female wasps.

When eight compounds that showed relatively large EAG responses (Fig. 5) were tested for their EAG dose–responses, most of these compounds elicited significant EAG responses at < 10 μg in the odour cartridge (Fig. 6). Two compounds, *cis*-3-hexenol and cyclohexanone, evoked the highest increases in EAG response at a cartridge dose of between 100 and 1000 μg. Another highly EAG active compound, linalool, evoked the highest increases between 10 and 100 μg. A less EAG-active compound, caryophyllene, reached maximum EAG responses at 100 μg. (*R*)-(+)-limonene evoked higher EAG amplitudes than its optical isomer (*S*)-(–)-limonene.

Considerable differences in the emission rates of compounds released from the odour cartridge were found between different compounds loaded at the same dose (Table 1). For example, three highly volatile compounds, *cis*-3-hexenol, α-pinene and cyclohexanone, loaded at 1000 μg in the cartridges were emitted from the cartridges at 33, 22 and 8 μg/puff, respectively. Methyl jasmonate and 2-methyl-5-nitroaniline, by contrast, were emitted at only 1.41 and 0.09 ng/puff, respectively, when loaded at 1000 μg. When the EAG responses to each compound were plotted against release rate the antenna was revealed to be more sensitive to less volatile compounds such as methyl jasmonate than to more volatile compounds such as cyclohexanone and *cis*-3-hexenol (Fig. 7A). Similarly, α-pinene, which elicited large EAG responses, evoked lower EAG responses at a given emission rate than some of the less volatile compounds such as caryophyllene (Fig. 7B).

EAG responses of wasps that had undergone a training programme to enhance upwind flight response selectively to cyclohexanone and methyl jasmonate were not significantly different from those of untrained wasps (Fig. 8).

Discussion

At first glance, *M. croceipes* antennae appear to display similar sensitivity to a variety of odourants as reflected in their EAG responses. This finding is perhaps not surprising due to the

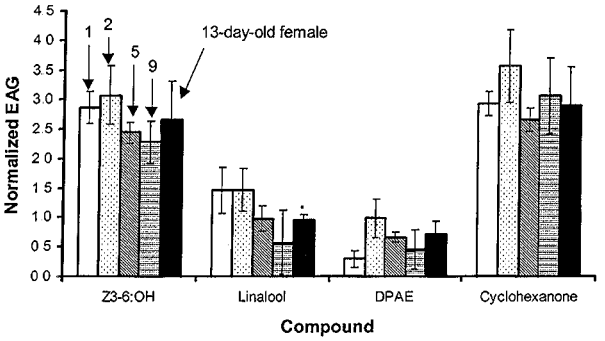

Fig. 4. Influence of age on EAG responsiveness in female *Microplitis croceipes*. Antennae of 1-, 2-, 5-, 9- and 13-day-old females were stimulated 1 mg of each compound loaded in the odour cartridge. DPAE: 2-diisopropylaminoethanol. EAG responses were normalized against mean EAG response to 100 µg of standard stimulus, *cis*-3-hexenol. (mean ± SEM, $n = 8$). No significant differences in EAG responses to a given stimulus were found among different ages (ANOVA, $P < 0.005$).

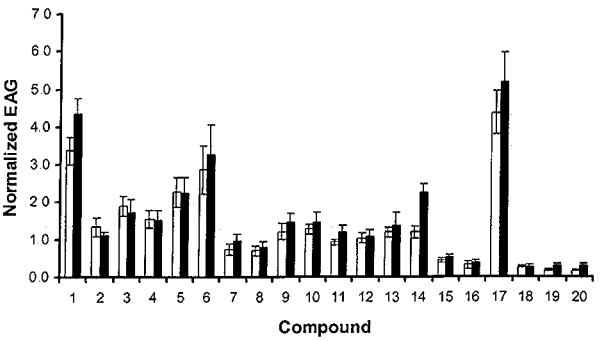

Fig. 5. EAG response profiles of 1–3-day-old male (solid bar) and female (open bar) *Microplitis croceipes* to 1 mg of a range of compounds. EAG responses were normalized against mean EAG response to 100 µg of standard stimulus, *cis*-3-hexenol (mean ± SEM, $n = 12$ for each sex). Only compound 14 shows significant difference between sexes (student's t-test, $p < 0.005$). Code for each compound: 1. *cis*-3-hexenol, 2. α-pinene, 3. (*R*)-(+)-limonene, 4. (*S*)-(−)-limonene, 5. ocimene, 6. (±)-linalool, 7. (−)-*trans*-caryophyllene, 8. α-humulene, 9. nerolidol, 10. *trans*-nerolidol, 11. *cis*-nerolidol, 12. methyl jasmonate, 13. indole, 14. 2-diisopropylaminoethanol, 15. 2,2′-thiodiethanol, 16. 2-methyl-5-nitroaniline, 17. cyclohexanone, 18. hexane, 19. dichloromethane, 20. acetone.

wasps' wide lepidopterous larval host range, and their ability to learn to respond to odour blends emitted by caterpillar-damaged host plants that differ slightly in composition (Alborn *et al.*, 1997; Pare & Tumlinson, 1997; De Moraes *et al.*, 1998). The amplitudes of response to at least one green-leaf volatile (*cis*-3-hexenol), a terpenoid (linalool), and an anthropogenic compound found in landmine fields (cyclohexanone) were equivalently high. The dose–response curves in response to selected green-leaf volatiles, terpenes and an anthropogenic compound did not differ greatly in appearance according to the dose loaded on filter paper.

However, quantification of the actual amounts of some selected compounds emitted from the odour cartridges during puffing began to reveal differences in antennal sensitivity that were not apparent when only filter-paper loading was considered. For example, the amplitude of the EAG in response to a 1 ng puff of methyl jasmonate was as high as to a 500 ng puff of indole or caryophyllene, and a 5000 ng puff of α-pinene. Thus, the EAG as a detector was revealed to be more than two orders of magnitude more sensitive to methyl jasmonate than to indole or caryophyllene, and more than four orders of magnitude more sensitive to methyl jasmonate than to α-pinene. This difference is completely obscured when only filter-paper loading is considered. Additionally, the antennae were ≈ 10 times more sensitive to cyclohexanone than to *cis*-3-hexenol when emission rate from the pipette rather than pipette loading is considered. Thus, emission rate measurements must be conducted in sensory physiological studies involving judgements about the 'tuning' of receptor neurones or whole antennae to compounds varying greatly in volatility. Even in studies with sex pheromone components that are similar in molecular weight, knowing the amounts issuing from odour cartridges can result in a report of receptor tuning that is more accurate and informative (Cossé *et al.*, 1995) than using the dosage loaded into the cartridge (Almaas *et al.*, 1991).

We did not measure the release rates from the majority of the compounds used in this study, but representatives from each class prove the importance of doing so in the future. Among the compounds not measured in this way, it is clear that in addition to the high EAG amplitudes evoked from at least one green leaf volatile, one terpene and one anthropogenic compound, equivalently low-to-moderate EAG amplitudes also were evoked from compounds from each of these classes (for example, α-pinene, nerolidiol and 2-diisopropylaminoethanol). The disparate responses to compounds within each of these classes of compounds show at least some differential tuning of receptors on the antennae, or differences in numbers of differentially tuned receptors. These differences could possibly be used for constructing a biosensor for detecting or locating sources of selected compounds. Our interest in these particular anthropogenic compounds is related to the need to develop a biosensor to detect or help locate sources of unexploded military ordinance.

The whole-body preparation retained its EAG responsiveness much longer than the isolated head preparation, which would have a shorter supply of oxygen and other materials with which to supply the antennal neurones and their support cells. Loss of nutrients throughout the large cut area at the base of the head and the decreased haemolymph circulation also could significantly contribute to the relatively rapid decline of the isolated head preparation.

Although the sensitivity of insect chemosensilla is influenced by various factors including ageing (Angioy *et al.*, 1983a, b), the EAG responses of *M. croceipes* were not influenced by age up to 13 days old. Lack of influence of age on EAG responsiveness has been reported in only a few other insect species, such as *Pseudaletia unipunctata* (Fitzpatrick *et*

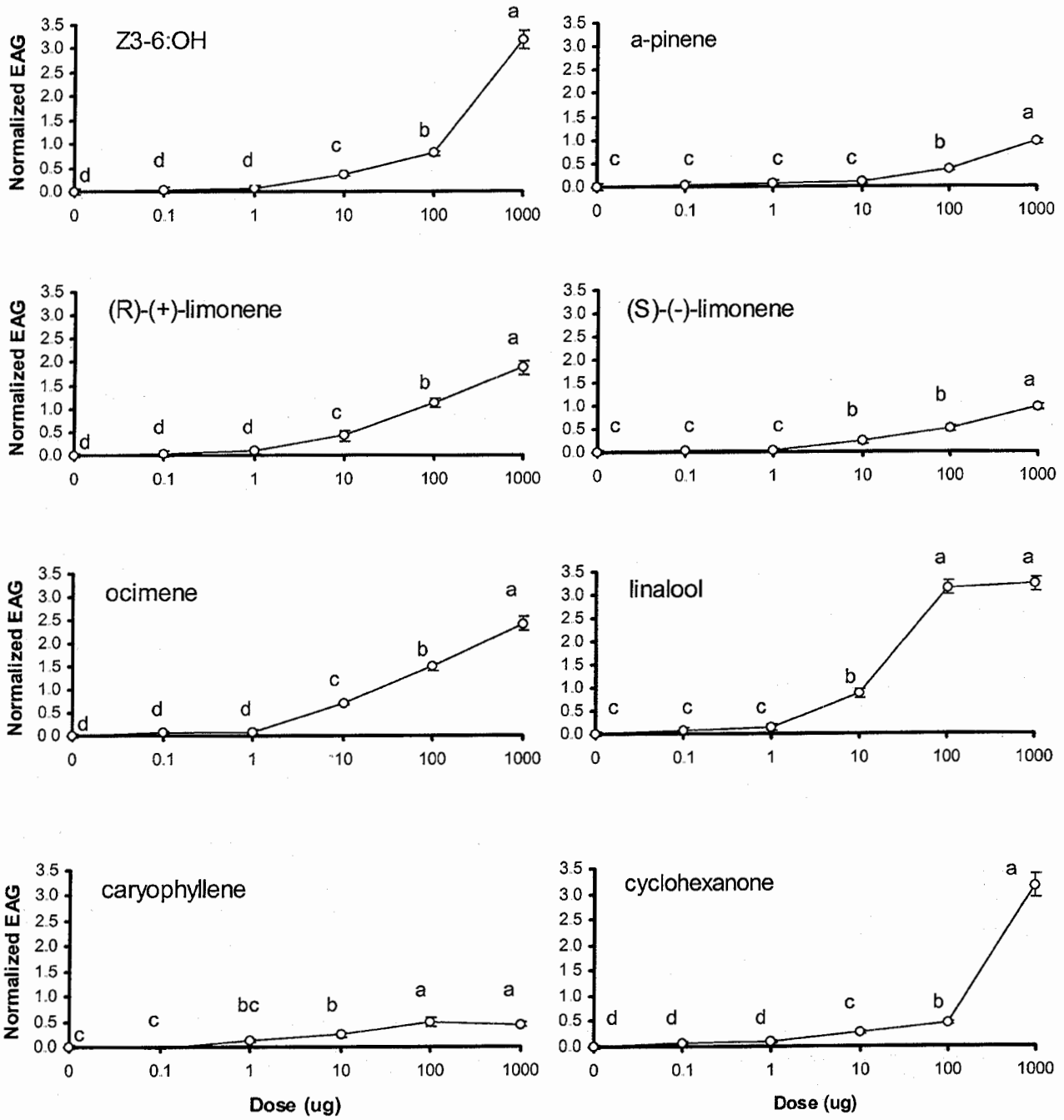

Fig. 6. EAG dose–responses of female *Microplitis croceipes* to different compounds (mean ± SEM, $n=8$). Dose indicates the amount of compound (μg) loaded onto a piece of filter paper in a Pasteur pipette odour cartridge. EAG responses were normalized against mean EAG response to 100 μg of standard stimulus, *cis*-3-hexenol. Different letters indicate significant differences by DMRT (Duncan's Multiple Range Test, $P<0.005$).

al., 1989). Most studies have shown an influence of age on EAG responses. For example, EAG responses decreased with age in *Argyrotaenia velutinana* (Roelofs & Comeau, 1971) and *Glossina morsitans morsitans* (Den Otter *et al.*, 1991). However, they increased in *Phormia regina* (Crnjar *et al.*, 1990), *Anthonomus grandis* (Dickens & Moorman, 1990) and worker bees (Allan *et al.*, 1987), or showed the highest responses at a specific age in *Ostrinia nubilalis* (Fescemyer & Hanson, 1990) and worker bees (Masson & Arnold, 1984). Although the factors involved in age-related EAG responsive-

Table 1. Amount of compounds (ng) released in a single puff from the glass odour cartridge for EAG experiments

	Dose (μg)[1]				
Compound	0.1	1	10	100	1000
cis-3-Hexenol	49.82 ± 8.64	125.31 ± 20.00	872.83 ± 139.24	25656.40 ± 9466.41	32510.40 ± 6036.00
α-Pinene	18.19 ± 4.24	150.32 ± 23.50	288.44 ± 13.31	11278.17 ± 428.36	21902.10 ± 2663.60
Cyclohexanone	24.05 ± 6.64	61.35 ± 18.57	397.42 ± 99.13	5534.37 ± 969.99	8001.65 ± 1733.86
Indole	–	4.37 ± 0.55	6.46 ± 0.92	396.10 ± 72.59	413.04 ± 67.54
Caryophyllene	0.92 ± 0.07	42.17 ± 23.66	65.14 ± 22.17	284.99 ± 80.76	327.03 ± 60.00
Methyl jasmonate	–	0.02 ± 0.00	0.12 ± 0.02	0.65 ± 0.19	1.41 ± 0.29
2-Methyl-5-nitroaniline	–	–	–	–	0.09 ± 0.02

[1] Amount of compound loaded onto a piece of filter paper in a Pasteur pipette odour cartridge.
$n = 3$ for each dose in each compound, mean ± SE.
–: not detectable.
† Mass selective ion for each compound: 67 (*cis*-3-hexenol), 68 (*cis*-5-undecenyl acetate), 91 (α-pinene), 96 (*cis*-8-tridecenyl acetate), 98 (cyclohexanone), 117 (indole), 133 (caryophyllene), 151 (methyl jasmonate) and 152 (2-methyl-5-nitroaniline).

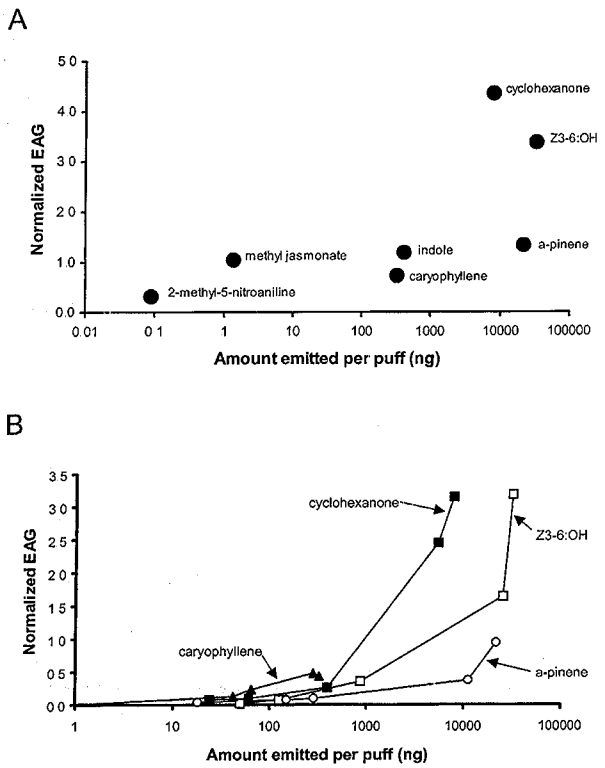

Fig. 7. Relationship between EAG responses and actual amount of compound released from the Pasteur pipette odour cartridge. EAG responses were normalized against mean EAG response to 100 μg of standard stimulus, *cis*-3-hexenol. (A) Mean EAG response to 1 mg of each compound in the odour cartridge in female *Microplitis croceipes* ($n = 12$). (B) EAG dose–responses of female *M. croceipes* to four compounds.

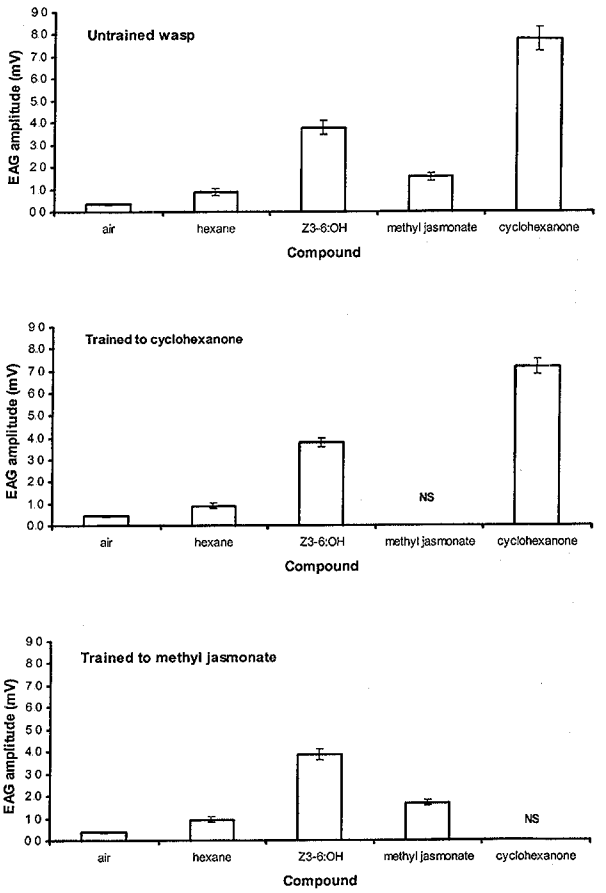

Fig. 8. Comparison of EAG responses between untrained and trained *M. croceipes*. (Mean ± SEM, $n = 33$, 45 and 52 with six female wasps for untrained, trained to methyl jasmonate and trained to cyclohexanone, respectively). No significant differences were found in EAG responses to each compound between trained and untrained wasps (student's t-test, $P < 0.005$). NS: not tested.

ness have not been identified, the maintenance of stable antennal neuronal sensitivity in ageing *M. croceipes* would provide more opportunity for successful host location during its entire adult life lasting > 20 days (Takasu & Lewis, 1996).

EAG amplitudes in whole-body preparations were much higher (a few mV) than in isolated head preparations, as in another study involving aphids (Park & Hardie, 1998). However, the difference in EAG amplitudes is not necessarily due to the inclusion of the abdomen and thorax. Indeed, when an EAG was recorded in whole-body wasp preparations with the glass capillary in contact with the cut antennal tip rather than distal antennal penetration, < 1 mV responses were recorded (personal observation). Therefore, the larger EAG amplitudes evoked from whole-body preparation could be related to differences in the location of the recording electrode along the antenna or to the connection made with a cut tip rather than by penetration the intact cuticle. In other studies, EAG amplitudes recorded with the glass recording microelectrode inserted into the antenna at a distal location were much larger than those recorded at the cut antennal tip (Den Otter, 1991; Cork & Park, 1996; Park & Hardie, 1998). The reasons for this difference are not clear. It would seem logical that less damage to the test insect should result in a longer-lasting EAG preparation. Rapid decrease of antennal responsiveness will make the EAG more difficult to interpret and less useful (Hardie *et al.*, 1994; Van Giessen *et al.*, 1994; Visser *et al.*, 1996). However, for *M. croceipes*, antennal cuticle penetration coupled with the use of a whole-body preparation seems to keep EAG responses from declining for several hours. Optimal EAG responses would need not only higher amplitude but also higher signal-to-noise ratio. It would not be useful if the noise level increased together with EAG amplitude. Our study indicates that the larger EAG amplitude in whole-body preparation is also associated with a better signal-to-noise ratio than in the cut antennal tip-isolated head preparation.

The lack of significant differences in EAG responses to 16 compounds between male and female *M. croceipes* is in concurrence with a previous study showing no sexual dimorphism in EAG responses of this species to 29 cotton compounds (Li *et al.*, 1992). Such similarity in EAG responses to host-related odours between both sexes has been reported for several phytophagous insect species (Fein *et al.*, 1982; Dickens, 1984; Wellso *et al.*, 1984; Light *et al.*, 1988; Fitzpatrick *et al.*, 1989; Hansson *et al.*, 1989; González *et al.*, 1994). A common explanation for the similarity is that both male and female insects live in the same habitat and may share the same chemical cues to locate host plants on which to feed and mate (Li *et al.*, 1992), and this would seem to be the case for *M. croceipes*. Currently, we cannot draw any conclusion about different EAG responses between sexes to the anthropogenic toxin-related compound, 2-diisopropylaminoethanol.

Microplitis croceipes is one of the better-known species for exhibiting chemically mediated associative learning (Lewis *et al.*, 1991; Takasu & Lewis, 1996). Our data show that there was no detectable peripheral enhancement of olfactory response after training. This corresponds with current knowledge about olfaction that learning is related to discrimination, not enhanced sensitivity to any one odourant. Interpretation of the results of the only two other studies that did in fact show changes in EAG following reward-based training (one a decrease in EAG response, Vet *et al.*, 1990; the other an increase, De Jong & Pham-Delègue, 1991) should be made with caution.

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