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Electrophysiological responses of *Cydia pomonella* to codlemone and pear ester ethyl (*E*,*Z*)-2,4-decadienoate: peripheral interactions in their perception and evidences for cells responding to both compounds

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Abstract

Electroantennography (EAG) recordings were made from both virgin and mated males and females of *Cydia pomonella* (L.) (Lepidoptera Tortricidae) (CM) on stimulation with the main component of its sex pheromone (*E*,*E*)-8,10-dodecadien-1-ol (*E*8*E*10-12:OH) and ethyl (*E*,*Z*)-2,4-decadienoate (Et-2*E*,4*Z*-DD), a ripe pear-derived volatile attractant. CMs of 7 Italian populations collected on different host-plants (3 apple, 3 walnut, 1 pear) were used. The dose-response curves to the volatile compounds (9 doses) were calculated and differences in olfactory sensitivity of virgin and mated males and females of the different populations are discussed. As expected, females were much less sensitive than males to *E*8*E*10-12:OH but generally showed a similar response to the higher doses of Et-2*E*,4*Z*-DD. EAG amplitudes were not significantly different among virgin and mated adults, as well as between two populations collected in a chemical treated apple orchard and an organic farm, respectively. The males of the walnut populations showed a lightly higher sensitivity to *E*8*E*10-12:OH.

Differential saturation experiments (DS-EAG) showed that the antennae of *C. pomonella* clearly reduce their response to Et-2*E*,4*Z*-DD when continuously stimulated with *E*8*E*10-12:OH. Recordings from single antennal olfactory cells SCR showed the presence of cells responding only to *E*8*E*10-12:OH or Et-2*E*,4*Z*-DD but also to the 2 compounds.

During field tests, all doses of Et-2*E*,4*Z*-DD showed an attractant activity on both sexes of CM. A synergistic effect on male attraction was not observed by adding Et-2*E*,4*Z*-DD to the pheromone; nevertheless, a clear interference on pheromone trap efficiency was detected.

These observations have to be highly considered when setting up mixtures of *E*8*E*10-12:OH and Et-2*E*,4*Z*-DD to monitor codling moth populations.

Key words: Sex pheromones, kairomones, EAG, DS-EAG, SCR, field test, codling moth.

Introduction

The codling moth (CM), *Cydia pomonella* (L.) (Lepidoptera Tortricidae), is the key insect pest of the apple orchard. Insecticides-based control strategies are still widely used. The CM insecticide resistance induced to change the traditional approach and, as a consequence, virus (CMGV) and semiochemical-based control methods (mating disruption) were introduced (Ioriatti and Bouvier, 2000).

New control strategies need of a more efficient monitoring lure able to provide information on both males and females field behaviour. Ethyl (*E*,*Z*)-2,4-decadienoate (Et-2*E*,4*Z*-DD), a ripe pear derived kairomone, is reported as highly attractive to the CM larvae (Knight and Light, 2001) and both male and female (virgin and mated) adults in walnut and apple orchards (Light *et al*., 2001; Coracini *et al*., 2004).

Host-plant volatiles can synergize the synthetic pheromone attractant power in CM and other insect species (Dickens *et al*., 1990; Light *et al*., 1993; Yang *et al*., 2004). In order to improve the efficiency of the traps and the Attract and Kill control method, the hypothesized synergistic effect of Et-2*E*,4*Z*-DD on (*E*,*E*)-8,10-dodecadien-1-ol (*E*8*E*10-12:OH, codlemone) attractancy needs to be investigated. It is also important to underline that traps baited with Et-2*E*,4*Z*-DD showed a different attractiveness according to the CM host-plant (Light *et al*., 2001).

In addition, insecticide resistance may limit the effectiveness and attractiveness of mixed formulations where resistance pleiotropic effects influence pheromone perception. It has already been shown that the dose-response relationships of males of susceptible and resistant strains of the CM to *E*8*E*10-12:OH does not differ significantly (Beslay *et al*., 2000). This occurrence could be different for the response to Et-2*E*,4*Z*-DD, as far as the enzymatic system mainly involved in insecticide detoxification in CM, like monoxygenases (Sauphanor *et al*., 1997), are also involved in the recognition of the host-plant (Feyresen, 1999).

In the present paper electrophysiological responses (electroantennography, EAG; differential saturation-electroantennography, DS-EAG; single cell recording, SCR) to *E*8*E*10-12:OH and Et-2*E*,4*Z*-DD of 7 CM populations living on different host plant (apple, pear, walnut) and under different cropping conditions (organic and chemical-based control methods) were re-

corded, focusing the attention on the possible influence of the kairomone on the perception of the sex pheromone main component. Moreover, field tests were carried out using both compounds and some blends.

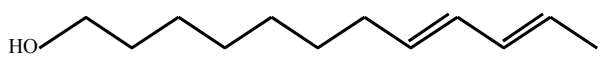
Materials and methods

Insects

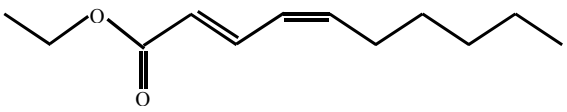
Overwintering larvae of CM were collected in October 2001 using cardboard bands in 3 walnut (Bleggio, TN; Rovigo, RO; Ceresi, MO), 3 apple (San Michele all'Adige, Gardolo, Borgo Valsugana, TN) and one pear (Maieutica, BO) orchards. S. Michele all'Adige population showed a reduced susceptibility to the insecticides (Ioriatti *et al*., 2003a), while the Borgo Valsugana one originated from an organic orchard. Larvae were kept in an outdoor insectary till March 2002, than the obtained pupae were isolated in plastic Petri dishes (i.d. 5 cm) and transferred to the laboratory at 16:8 L:D cycle, 23±2°C and 70±5% RH. Moths emerged from the middle of April to the beginning of June. Males and females were kept in different rearing rooms. Adults were fed with a 10% (W/V) sucrose aqueous solution absorbed on a cotton pad. During the experiments, 3-6 day old moths (virgin and mated) were used.

Stimuli

Stimuli were the main component of the CM sex pheromone (*E*8*E*10-12:OH; >99% pure) and the pear ester kairomone (Et-2*E*,4*Z*-DD; >97% pure) (figure 1). Gas cromatography (GC) and GC linked to mass spectrometry (GC-MS) analyses of hexane solution (100 ng/µl) of synthetic compounds did not detect the presence of related isomers or traces of *E*8*E*10-12:OH in Et-2*E*,4*Z*-DD. GC coupled to electroantennography detector (GC-EAD) analyses did not reveal any EAG activity at the *E*8*E*10-12:OH retention time. Attractants were supplied by Sigma-Aldrich (Germany). Aliquots of 10 µl of a mineral oil solution of a chemical were absorbed on a piece of filter paper (1 cm^2). For each compound, nine different stimuli containing from 10^{-6} to 10^2 µg were prepared. During EAG and DS-EAG tests all stimuli were applied; in SCRs, after preliminary assays, the more useful dose (1.0 µg) in discerning the responding cells to one or both compounds was adopted.



(*E*,*E*)-8,10-dodecadien-1-ol (*E*8*E*10-12:OH)



Ethyl (*E*,*Z*)-2,4-decadienoate (Et-2*E*,4*Z*-DD)

Figure 1. Compounds used during the electrophysiological studies: the main component of *C. pomonella* sex pheromone (*E*8*E*10-12:OH) and the ripe pear ester acting as kairomone (Et-2*E*,4*Z*-DD).

Electrophysiology

EAG responses (Rotundo and Tremblay, 1993; Den Otter *et al*., 1996; De Cristofaro *et al*., 2000) to *E*8*E*10-12:OH and Et-2*E*,4*Z*-DD of the 7 populations were recorded. The differential saturation-EAG (DS-EAG) (Baker and Roelofs, 1976; Miller *et al*., 1977; Nishino and Manabe, 1984) and single cell recordings (SCR, surface contact technique) (Den Otter *et al*., 1980; 1996) were performed on one population (Ceresi, MO).

The proximal end of an amputated antenna was inserted into a glass pipette indifferent electrode (i.d. tip 1 mm) filled with Beadle-Ephrussi saline containing 5 g/l polyvinylpyrrolidone K90 (Fluka Chemie, Switzerland). For EAG and DS-EAG recordings the different electrode was a similar pipette brought into contact with the distal end of the antenna from which 1-2 segments had been excised. The scale-less area of the antenna lodging, like in other tortricid moth, the highest number of olfactory hairs, was placed perpendicularly to the stimulus direction (De Cristofaro *et al*., 1997; 2000).

During SCR, spikes from individual cells were recorded by gently pressing the tip (i.d. < 3 µm) of the different glass electrode against the cuticle of the antenna; recordings were made from the rostral-ventral scale-less area of the antenna, at the distal end of a medial segment, were rows of hooked long olfactory sensilla trichoidea and sensilla auricillica are present (figure 2). Silver wires inserted in the pipettes were connected to a portable EAG/SCR equipment (Van der Pers and Minks, 1993). During the EAG and SCR recordings, the antenna was continuously flushed with charcoal-filtered, humidified air at room temperature (23°C). During the DS-EAG measurements the continuous air stream originated from a bottle containing a mineral oil solution (1 µg/µl) of a chemical (*E*8*E*10-12:OH or Et-2*E*,4*Z*-DD).

The air flowed at 50 cm^3/s through a stainless steel tube (i.d. 8 mm) the outlet of which was about 1 cm from the preparation. Through a hole (i.d. 4 mm), 9 cm from the outlet, stimuli were added to the air stream by injecting vapour from an odour cartridge. During injection, a stimulus controller (CS-01, Syntech, Hilversum, NL) was used to keep constant the flow over the antenna.

In EAG and DS-EAG recordings 2.5 cm^3 were injected during 0.1 s, in SCR experiments 12.5 cm^3 in 500 ms. Intervals between stimuli were 1 min. In EAG and DS-EAG tests, the doses of different chemicals were applied in ascending order; one minute before each series of stimulation with the same dose of chemicals, a reference stimulus (0.01 µg of *E*8*E*10-12:OH) was applied to correct for changes in EAG responsiveness. In order to verify the effect of saturation on the antennal sensitivity, during DS-EAG tests 2 plant volatile (heptanal, Z3-hexen-1-ol) stimuli (1.0 µg) were applied. EAGs were analysed using a proper programme (EAG 2.0, Syntech, Hilversum, NL). The absolute EAG amplitudes were calculated as described by Den Otter *et al*. (1988; 1991).

Action potentials were stored on tape and analysed using a suitable programme (Autospike 1.1., Syntech, Hilversum, NL); spikes were distinguished on the basis of shape, amplitude and frequency. Only SCRs showing

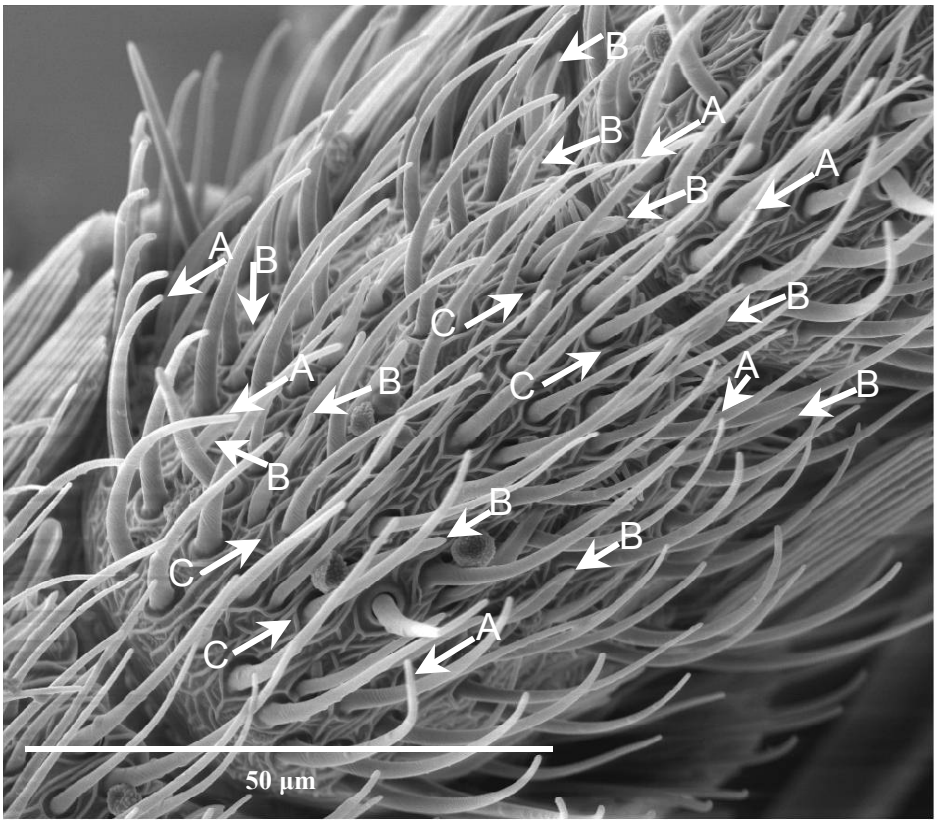


Figure 2. An antennal segment (33rd) of *C. pomonella* male showing the rostral-ventral scale-less area. The arrows indicate some sensilla trichoidea (long hooked olfactory hairs; i.e. A arrow) and auricillica (rabbit-ear shaped; i.e. B arrow) and where the recording electrode was placed (i.e. C arrow) during SCR recordings.

the activity of one sensitive cell, and during which it was possible to apply the different stimuli at least 3 times (complete recordings), were considered in the data analysis. The response frequency (spikes/s) were referred to the first 100 ms (spikes/100 ms x 10) and subsequently analyzed (LSD test).

Field tests

Trials have been carried out during two years (2003-04) in an organic orchard (Colletorto, CB, South Italy). Pherocon IIB (Trécé) traps were suspended from trees, 2-3 m above ground and spaced 15-20 meters apart.

Based on previous field studies (Ioriatti *et al*., 2003b), 2 doses of *E*8*E*10-12:OH (0.1, 1.0 mg), 5 doses of Et-2*E*,4*Z*-DD (0.1, 1.0; 3.0, 10.0, 40.0 mg), and 2 blends (0.1 + 0.1 mg and 1.0 + 0.1 mg, respectively) were evaluated. Rubber septa dispensers, placed at the centre of the trap bottom, were adopted. Baits were replaced every 3 weeks. Three replicates for each treatment as well as 3 unbaited traps (blank) were used.

Traps were checked weekly and bottoms of those containing insects were replaced; male and female catches were recorded.

Data were analysed by ANOVA and means separated by Duncan test (P = 0.05). Prior to the statistical analysis, data were transformed to $\sqrt{x+0.5}$ and submitted to Levene test for omogeneity of variance. For the same bait, differences among male and female catches were evaluated by Student's *t*-test.

Results

Laboratory

For each population, EAG responses to 1.0 µg of *E*8*E*10-12:OH or Et-2*E*,4*Z*-DD were not significantly different (*t*-test; P=0.05) between virgin and mated males or females (table 1). Comparing the populations on the basis of their host plant (apple, walnut, pear) no statistical differences (Duncan test; P=0.05) emerged (table 1).

EAG responses from virgin and mated males and females collected in a chemical treated orchard with a reduced sensitivity to insecticides (San Michele all'Adige, TN) were similar to those of individuals coming from an organic farm (Borgo Valsugana, TN) (table 2).

As expected, females were less sensitive than males to *E*8*E*10-12:OH (figure 3). This difference was not observed in respect to Et-2*E*,4*Z*-DD, particularly at higher doses (figure 4).

DS-EAG revealed that the antennae of the CM clearly reduce their response to *E*8*E*10-12:OH when continuously flushed with Et-2*E*,4*Z*-DD (figure 3) as well as their response to Et-2*E*,4*Z*-DD when saturated with *E*8*E*10-12:OH (figure 4). Responses of virgin adults (n = 10) to heptanal (male: 1.43±0.28 mV; female: 1.27±0.32 mV) and Z3-hexen-1-ol (male: 1.34±0.36 mV; female: 1.16±0.20 mV) were not significantly reduced by saturation with *E*8*E*10-12:OH (heptanal: male = 1.26±0.31 mV; female = 1.18±0.26 mV; Z3-hexen-1-ol: male = 1.21±0.34 mV; female = 0.96±0.25 mV) or

Table 1. Mean EAG responses of virgin and mated *C. pomonella* adults (n=10/category/population) from 7 Italian populations living on different host plant (3 apple; 3 walnut; 1 pear) to 1.0 µg dose of *E*8*E*10-12:OH or Et-2*E*,4*Z*-DD.

		EAG (mV) ± S.D.					
		*E*8*E*10-12:OH			Et-2*E*,4*Z*-DD		
		Apple[1]	Walnut[1]	Pear[1]	Apple[1]	Walnut[1]	Pear[1]
Females[1]	virgin	0.32±0.09 a	0.38±0.09 a	0.36±0.07 a	1.47±0.23 a	1.56±0.28 a	1.44±0.26 a
	mated	0.36±0.07 a	0.42±0.10 a	0.38±0.08 a	1.56±0.16 a	1.62±0.25 a	1.52±0.28 a
Males[1]	virgin	3.27±0.25 b	3.52±0.34 b	3.32±0.29 b	1.66±0.25 a	1.82±0.28 a	1.62±0.20 a
	mated	3.12±0.29 b	3.32±0.30 b	3.18±0.23 b	1.49±0.28 a	1.69±0.21 a	1.52±0.22 a

Different letters on the same column indicate significant differences between sexes (Duncan test; P=0.05).
[1] Between physiological status (virgin and mated) of both sexes and among populations from different host plant no significant differences were observed (Duncan test; P=0.05).

Table 2. Mean EAG responses of virgin and mated *C. pomonella* adults (n=10/category/population) from a population (I) with a reduced susceptibility to organophosphates (San Michele all'Adige, TN) and a population (II) from an organic orchard (Borgo Valsugana, TN) to 1.0 µg dose of *E*8*E*10-12:OH or Et-2*E*,4*Z*-DD.

		EAG (mV) ± S.D.			
		*E*8*E*10-12:OH		Et-2*E*,4*Z*-DD	
		I	II	I	II
Females	virgin	0.28±0.10 a	0.36±0.10 a	1.56±0.34 a	1.46±0.20 a
	mated	0.36±0.12 a	0.28±0.10 a	1.68±0.26 a	1.58±0.16 a
Males	virgin	3.12±0.24 b	3.22±0.38 a	1.74±0.18 a	1.72±0.30 a
	mated	2.96±0.30 b	3.02±0.34 a	1.72±0.20 a	1.60±0.24 a

Different letters on the same column indicate significant differences (Duncan test; P=0.05).

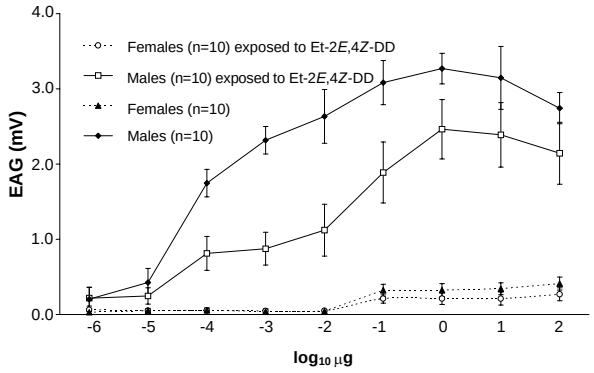


Figure 3. Mean EAG (mV) dose-response curves of *C. pomonella* (n=10) virgin adults (walnut population) to *E*8*E*10-12:OH alone and during saturation (DS-EAG) with Et-2*E*,4*Z*-DD. Vertical bars represent S.D. (two-sided).

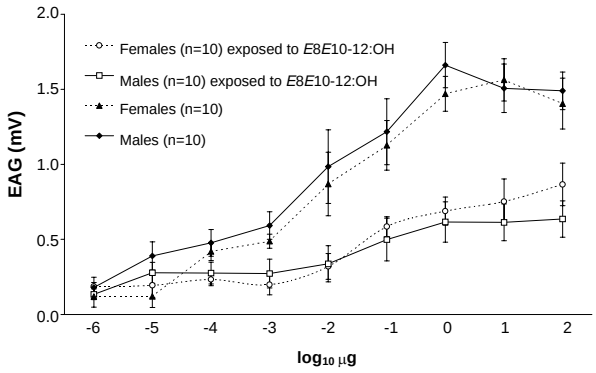


Figure 4. Mean EAG (mV) dose-response curves of *C. pomonella* (n=10) virgin adults (walnut population) to Et-2*E*,4*Z*-DD alone and during saturation (DS-EAG) with *E*8*E*10-12:OH. Vertical bars represent S.D. (two-sided).

when continuously exposed to Et-2*E*,4*Z*-DD (heptanal: male = 1.28±0.33 mV; female = 0.98±0.28 mV; Z3-hexen-1-ol: male = 1.11±0.38 mV; female = 1.06±0.22 mV).

Out of 123 attempts, complete recordings (n=20) from single antennal olfactory cells of males showed the presence of different cells (A, B, C) responding only to *E*8*E*10-12:OH (n=9) or Et-2*E*,4*Z*-DD (n=3) but also to the two compounds (n=8) (figure 5; table 3). In females, out of 115 attempts, complete recordings (n=10) revealed the presence of cells (D, E, F) responding to *E*8*E*10-12:OH (n=2), Et-2*E*,4*Z*-DD (n=4) and to both compounds (n=4) (table 3).

In all recordings, in response to the adequate stimulus (*E*8*E*10-12:OH, Et-2*E*,4*Z*-DD or both compounds separately applied) according to the cell type, spike frequencies significantly increased with respect to control stimulus (mineral oil) (table 3). Significant differences

between the response to the mineral oil and the cell resting activity were not detected.

Field

All doses of Et-2*E*,4*Z*-DD showed an attractant activity on both sexes of CM. During 2003, at the highest dose (40 mg) of Et-2*E*,4*Z*-DD male and female catches were significantly lower. In 2004, male and female catches did not vary according to the dose (table 4).

Adults of CM were attracted by Et-2*E*,4*Z*-DD (0.1, 1.0 mg) in a significant lower number than the corresponding doses of pheromone. A similar result was observed when only male catches were considered. During 2003, male attraction was significantly reduced by addition of Et-2*E*,4*Z*-DD (0.1 mg) to the highest dose (1.0 mg) of pheromone and slightly increased when added to the lowest one (0.1 mg).

On the contrary, females were significantly attracted by 0.1 and 1.0 mg doses of pear ester; catches decreased in the presence of pheromone. Some females were caught by pheromone traps in a not significant number than control (table 4).

During 2004, when the CM population density was low, significant differences among pheromone and binary blends did not emerge (table 4).

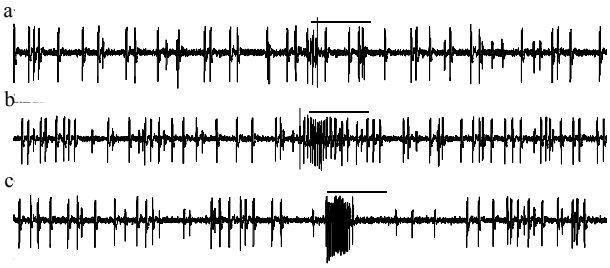


Figure 5. Spikes recorded on stimulation of male antennal olfactory cells of *C. pomonella* with solvent (a: mineral oil, 10 µl), Et-2*E*,4*Z*-DD (b: 1 µg) and *E*8*E*1-12:OH (c: 1 µg). Horizontal line above the recordings indicates the duration of stimulation (500 ms).

Table 3. Mean spike frequency (spikes/s±S.D.) recorded (surface contact technique) from *C. pomonella* male (A: n=9; B: n=3; C: n=8) and female (D: n=3; E: n=5; F: n=5) olfactory cells on stimulation with *E*8*E*10-12:OH and Et-2*E*,4*Z*-DD (1 µg) in mineral oil solution (10 µl). Mineral oil (10 µl) was used as control stimulus.

Cell	Spikes/s ± S.D.			
	Resting activity	Mineral oil	*E*8*E*10-12:OH	Et-2*E*,4*Z*-DD
Males				
A	7.89±1.45 a	8.56±1.33 a	47.78±5.07 b	9.22±1.64 a
B	9.33±1.53 a	8.67±1.15 a	9.67±1.53 a	28.67±2.52 b
C	8.13±1.25 a	8.75±1.04 a	43.88±3.72 c	31.25±3.06 b
Females				
D	6.67±1.53 a	7.67±1.53 a	23.33±4.04 b	8.67±2.08 a
E	9.20±1.43 a	8.60±1.14 a	10.20±1.64 a	32.80±4.38 b
F	7.40±1.14 a	9.60±2.30 a	26.60±4.34 b	28.60±4.22 b

Different letters on the same line indicate significant differences (LSD test; P=0.05).

Table 4. Field trapping of *C. pomonella* (no. adults/trap) in Colletorto (CB), Molise region, Italy (3 replicates; 21.V-20.IX.03; 20.V-25.VIII.04).

Compounds and blends	Males		Females		Total	
	2003	2004	2003	2004	2003	2004
*E*8*E*10-12:OH (0.1 mg)	67.0±5.0 c	40.0±2.0 a	1.3±1.2 d	0.3±0.6 c	68.3±6.0 c	40.3±1.5 a
*E*8*E*10-12:OH (1.0 mg)	110.7±6.4 a	27.7±7.4 b	2.3±0.6 cd	0.3±0.6 bc	113.0±6.1 a	28.0±7.9 b
*E*8*E*10-12:OH (0.1 mg) + Et-2*E*,4*Z*-DD (0.1 mg)	82.0±9.4 b	29.3±4.7 ab	7.3±1.2 b	2.3±2.5 abc	89.3±9.8 b	32.0±4.6 ab
*E*8*E*10-12:OH (1.0 mg) + Et-2*E*,4*Z*-DD (0.1 mg)	56.7±6.1 c	36.3±6.1 ab	4.7±2.5 bc	1.0±1.0 abc	61.3±8.6 c	37.3±7.1 ab
Et-2*E*,4*Z*-DD (0.1 mg)	21.3±5.1 e	2.7±1.2 d	15.7±3.1 a	1.3±2.3 abc	37.0±4.4 d	2.7±1.2 de
Et-2*E*,4*Z*-DD (1.0 mg)	25.3±5.1 de	1.3±1.5 de	13.7±5.0 a	1.3±1.5 abc	39.0±7.8 d	2.7±1.5 e
Et-2*E*,4*Z*-DD (3.0 mg)	27.7±0.6 de	10.3±8.1 c	13.3±6.1 a	2.7±1.2 ab	41.0±6.2 d	13.0±7.2 c
Et-2*E*,4*Z*-DD (10 mg)	30.0±5.2 d	5.0±2.6 cd	5.0±1.0 bc	3.3±2.5 a	35.0±6.1 d	8.3±3.5 cd
Et-2*E*,4*Z*-DD (40 mg)	11.0±3.0 f	3.0±1.0 d	4.7±0.6 bc	0.3±0.6 bc	15.7±2.5 e	3.3±1.5 e
Control	0.3±0.6 g	0.0±0.0 e	1.0±1.0 d	0.0±0.0 c	1.3±1.5 f	0.0±0.0 f

Means followed by the same letter are not significantly different at P=0.05 by Duncan test following ANOVA of √x+0.5 transformed data.

Discussion and conclusions

Sex, mating status and host-plant did not significantly influence the electrophysiological responses to pear ester, although mated females showed an higher response to this compound. Obviously, females were highly less sensitive to codlemone, confirming the results of previous studies and their ability in the autodetection of sex pheromone (De Cristofaro *et al*., 2002).

As a general tendency, adults of the walnut populations showed, on the average, a higher sensitivity to both compounds. The larval development on different host plants, affecting the female oviposition behaviour (Ansebo and Witzgall, 2002), could also be related to the perception of substances other than plant volatiles (Lombarkia and Derridj, 2002).

The reduced insecticides susceptibility of the tested population did not affect the electrophysiological responses both to the pear ester and codlemone, confirming the results related to the pheromone perception reported by Beslay *et al*. (2000).

SCR (directly) and DS-EAG (indirectly) results clearly showed the presence of cells responding both to codlemone and pear ester, at comparable doses, in CM male and female.

Olfactory cells sensitive only to a single pheromone component or to a plant volatile have been reported by several authors (i.e. Anderson *et al*., 1995). Receptor neurons responding to more than one pheromone compound, or to both synergists and antagonists, have been also found in different Lepidoptera species (i.e. Löfqvist *et al*., 1990; Renou and Lucas., 1994; Hansson *et al*., 1995; Monti *et al*., 1995; Wu *et al*., 1995; Cossè *et al*., 1998; Bäckman *et al*., 2000). Cells responding to more than one plant volatile are well known (i.e. Den Otter *et al*., 1996); most of them respond strongly to one compound and only weakly to a few other related constituents (i.e. Ulland *et al*., 2003).

Cells responding to pheromone components and to high doses of host-plant volatiles have been already found on male antennae of *Cydia fagiglandana* (Zeller) (Lepidoptera Tortricidae) (De Cristofaro, 1995; Den Otter *et al*., 1996; De Cristofaro *et al*., 1997), *Utetheisa ornatrix* (L.) (Lepidoptera Arctiidae) (Bogner *et al*., 1992), *Agrotis segetum* (Denis & Schiffermüller) (Hansson, 1995) and other insects (De Cristofaro *et al*., 2004). A male receptor neuron responding to a blend of the main pheromone component (*Z*11-hexadecenal) with either linalool or *Z*3-6:OH, but not to the plant compounds alone, has been reported in *Helicoverpa zea* (Boddie) (Ochieng *et al*., 2002). The biological meaning of these electrophysiological evidences is still not completely understood, since a separate peripheral perception mechanism and response processing of the two categories of volatile substances have been clearly shown (i.e. Mustaparta, 2002). Interestingly, our recordings allow to hypothesize that in CM the behavioural response to the plant volatile Et-2*E*,4*Z*-DD and to the main component of the sex pheromone *E*8*E*10-12:OH is partly (at least) mediated through a common sensory channel.

The occurrence of cells sensitive to the sex pheromone main component and pear ester in male and female CM (De Cristofaro *et al*., 2002; present paper), probably housed in sensilla trichoidea or auricillica, is particularly relevant. The absence of specificity of this receptor may be related to a similar olfactory affinity of the two molecules. In this sense pear ester acts as a codlemone mimic attracting CM and other tortricid moths sharing the same compound in the pheromone blend, like *C. fagiglandana*, *Cydia splendana* (Hübner), *H. nubiferana* (Haworth) (Schmidt *et al*., 2004). This finding could explain the pheromonal potency of Et-2*E*,4*Z*-DD (Light *et al*., 2001; Ioriatti *et al*., 2003b) and the peripheral interactions in the perception of the two compounds. A synergistic effect on male attraction was not observed by adding Et-2*E*,4*Z*-DD to the pheromone. On the other hand, the frequently observed codlemone male capture efficiency reduction when mixed to the pear ester, like in the first year of our field studies, could be justified by a possible competition of the two substances for the same receptor. When CM population density is low the combined effect of the two substances on field catches is hardly distinguishable.

CM males were more sensitive than females to Et-2*E*,4*Z*-DD. An EAG study on *Manduca sexta* (L.) suggested that female antennae have a higher number of olfactory receptor neurons sensitive to non-pheromone stimuli than males (Kalinová and Hansson, 2001). However, the higher number of pheromone olfactory sensilla together with the presence of cells responding to Et-2*E*,4*Z*-DD and pheromone components could explain the higher EAG response of CM males to this compound. A higher EAG response of males to plant volatiles has already been reported in other Lepidoptera (i.e. De Cristofaro *et al*., 1997; 1998: 1999; 2000; Vitagliano *et al*., 2004).

All these observations have to be highly considered when setting up mixtures of *E*8*E*10-12:OH and Et-2*E*,4*Z*-DD to monitor different CM populations. As a general consequence, it is necessary to pay more attention when setting up mixed blends of pheromone components and plant volatiles to monitor or control insect pest populations.

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