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Effects of Trifluoromethyl Ketones and Related Compounds on the EAG and Behavioural Responses to Pheromones in Male Moths

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Abstract

Trifluoromethyl ketones (TFMKs) and analogues affect pheromone detection and behaviour in male moths. 3-Octylthio-1,1,1-trifluoropropan-2-one (OTFP), one of the most effective antiesterase agents, decreased the EAG amplitude and increased the repolarization time in *Spodoptera littoralis*. It also modified EAG responses of *Mamestra brassicae* and *Heliothis zea* to their pheromones and analogues, containing an acetate, an alcohol or an aldehyde function. In addition, OTFP also reduced the amplitude of the EAG response to linalool, a monoterpenic alcohol, but not its kinetics. The responses of male *S. littoralis* to the pheromone in the wind tunnel were significantly reduced after pre-exposure to vapours of OTFP. Comparison of the activity of other TFMKs and analogues with that of OTFP revealed a good agreement on EAG and behaviour. The effects of TFMKs on the EAG kinetics are discussed considering the hypothesis of an inhibition of the pheromone deactivation in the antenna. **Chem. Senses 22: 407–416, 1997.**

Introduction

Fluorinated compounds have been the subject of great interest due to their activity as enzyme inhibitors (Begué and Bonnet-Delpont, 1991). This particular property arises from unique physical features induced by fluorine, which closely mimics the steric requirement of hydrogen at the enzyme acceptor site. The strong electron-withdrawing character of the fluorine atom induces that trifluoromethylketones (TFMKs) form stable hydrates in aqueous solutions (Liang and Abeles, 1987). In addition, their tetrahedral geometry resembles the transition state involved in the enzymatic hydrolysis of esters or peptides. Therefore, TFMKs inhibit the action of a variety of serine esterases, such as acetylcholinesterase (Gelb *et al.*, 1985), juvenile hormone esterase (Székács *et al.*, 1988) or mammalian carboxyl esterases (Abdel-Aal and Hammock, 1986).

In insects, TFMKs also reversibly inhibit the antennal esterases that are responsible for the catabolism of the pheromone molecules in the olfactory tissues of male moths. Various TFMKs, structurally related to natural pheromones, have been prepared and their inhibitory activity established in different moth species (Vogt *et al.*, 1985; Prestwich and Streinz, 1988; Durán *et al.*, 1993;

Parrilla and Guerrero, 1994). Inhibition of enzymatic catabolism of odorant molecules has been considered as a potential approach for the disruption of pheromone reception in the search for new strategies for pest control (Prestwich, 1986). In this context, some effort has been devoted to evaluate the effects of TFMKs on pheromone detection in male behaviour of different moth species. These chemicals generally showed very low intrinsic electroantennographic (EAG) activity in *Spodoptera littoralis* (Boisd.) (Malo, unpub. data), as in other species (Prestwich and Streinz, 1988; Riba *et al.*, 1994). A variety of TFMKs were used to investigate whether they inhibit the reception process of the sex pheromone by male *Thaumetopoea pityocampa* (Denis and Schiff.) (Parrilla and Guerrero, 1994). After presaturation of the receptors, an EAG inhibition ranging from 20 to 90% was observed, depending on the chemical used. In turn, presaturation of the olfactory organs of *Sesamia nonagrioides* (Lef.) with (Z)-1,1,1-trifluoro-14-nonadecen-2-one, a pheromone analogue, resulted in a weak inhibition of the EAG (Riba *et al.*, 1994). Effects of TFMKs on male behaviour have also been tested both in wind tunnel and in the field. (Z)-1,1,1-Trifluoro-14-heptadecen-2-one, a pheromone analogue for *Ostrinia nubilalis*, did not inhibit male attraction when it was co-evaporated with pheromone in a wind tunnel assay (Klun *et al.*, 1991). However, this fluorinated compound is only a moderate inhibitor of the *in vivo* esterase activity. With regard to the field tests, some aliphatic and aromatic TFMKs displayed potent inhibitory effects on *T. pityocampa* male catches when mixed with the pheromone (Parrilla and Guerrero, 1994). Unexpectedly, catches of male *S. nonagrioides* were increased when (Z)-1,1,1-trifluoro-14-nonadecen-2-one was added to the pheromone blend (Riba *et al.*, 1994). At the same time, catches of males of two sympatric moth species were significantly reduced. The complex set of data gathered from these studies indicates that the link between the anti-esterase activity of TFMKs and their effects on pheromone communication is not straightforward. Further physiological and behavioural work is needed to determine to which extent effects on male behaviour are related to anti-esterase activity.

Following a series of papers dealing with the synthesis (Parrilla *et al.*, 1994; Villuendas *et al.*, 1994) and biochemical evaluation of the inhibition of pheromone catabolism (Durán *et al.* 1993; Rosell *et al.*, 1996), in the present work we report the effects of several TFMKs on the electrophysiological responses of male moths to synthetic pheromone components. Since responses of the pheromone receptor cells can be recorded as a summated depolarization of the antenna (electroantennogram; EAG), our working hypothesis was based on the assumption that TFMKs should alter EAG kinetics according to their antiesterase effect.

Thus, three parameters—amplitude, depolarization and repolarization times—were measured on the EAG responses to pheromone components, with different functional groups. The values of these parameters, obtained before and during treatment, were compared to quantify the effects of TFMKs on the amplitude and kinetics of the olfactory responses.

Furthermore, since modification of olfactory detection should affect behaviour, the response of males to synthetic pheromone was studied in a wind tunnel after presaturation of their receptors with TFMKs.

Material and methods

Insects

The Egyptian armyworm *Spodoptera littoralis* (Boisd.), the cabbage armyworm, *Mamestra brassicae* (L.) and the corn earworm *Heliothis zea* (Bod.) were reared in the laboratory on artificial diets slightly modified after Poitout *et al.* (1972) and Poitout and Bues (1974). Pupae were sexed, placed in groups of 20–25 into 20 × 20 cm plastic boxes and maintained in a climatic chamber on a 16 h light:8 h dark regime at 25 ± 1°C with 60–70% relative humidity until emergence. Adults were provided with 10% sucrose solution, separated daily by age and kept on filter paper in plastic containers.

Compounds

Natural stimuli were pheromone components, chosen for their high EAG activity, including two acetates [(Z,E)-9,11-tetradecadienyl acetate, Z9,E11-14:Ac; and (Z)-11-hexadecenyl acetate, Z11-16:Ac], two alcohols [(Z)-9-tetradecenol, Z9-14:OH; and (Z)-11-hexadecenol, Z11-16:OH] and one aldehyde [(Z)-11-hexadecenal, Z11-16:Ald]. A plant volatile, linalool, was also used, since it has a very different chemical structure to that of most moth pheromones. All pheromone compounds had been synthesized in the laboratory and their purity was at least 98%.

The TFMKs and other analogues included three saturated fluorinated ketones [nonyl-1,1,1-trifluoromethyl

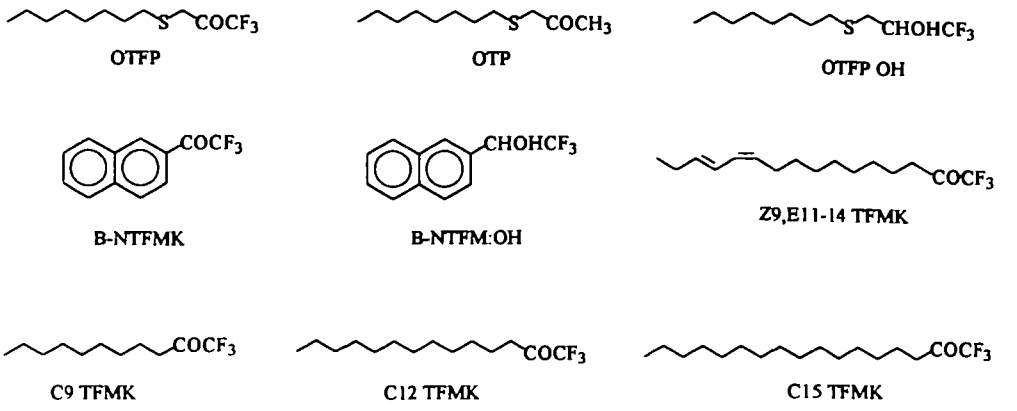

Figure 1 Formulae of the trifluoromethylketones and their analogues prepared to evaluate their biological activity

ketone, C9:TFMK; dodecyl-1,1,1-trifluoromethyl ketone, C12:TFMK; and pentadecyl-1,1,1-trifluoromethyl ketone, C15:TFMK], a dienic fluorinated ketone [(Z,E)-9,11-tetradecadienyl-1,1,1-trifluoromethyl ketone, Z9,E11-14:TFMK], two aromatic compounds [β-naphthyltrifluoromethyl ketone, β-NTFMK; and β-naphthyltrifluoromethyl carbinol, β-NTFM:OH], and three β-thio derivatives [3-octylthio-propan-2-one, OTP; 3-octylthio-1,1,1-trifluoropropan-2-one, OTFP; and 3-octylthio-1,1,1-trifluoropropan-2-ol (OTFP:OH)] (Figure 1). C9:TFMK, C12:TFMK, C15:TFMK and β-NTFMK were synthesized previously by us (Parrilla *et al.*, 1994; Gaspar and Guerrero, 1995). Z9,E11-14:TFMK was obtained by metallation of the required iodide with one equivalent of *tert*-butyllithium followed by reaction with ethyl trifluoroacetate, as described by Villuendas *et al.* (1994). OTFP was obtained by alkylation of the corresponding thiol with 3-bromo-1,1,1-trifluoropropan-2-one (Parrilla *et al.*, 1994). β-NTFM:OH and OTFP:OH were prepared by sodium borohydride reduction of the corresponding ketones. For bioassays compounds were dissolved in hexane to achieve the required concentrations.

Electrophysiology

Modifications of the shape and amplitude of EAG in air loaded with vapours of the chemicals were monitored on living insects. A male moth was anaesthetized by CO_2 and restricted in a Styrofoam block. The reference electrode was introduced in its neck. One antenna was immobilized, its last subsegments were excised and it was inserted into the recording electrode. The EAG signal was amplified (×1000) and filtered (DC to 1 kHz). Single sensillum recordings (SSRs) were performed according to the tip recording technique (Kaissling and Thorson, 1980). The recording glass electrode was filled with sensillar saline and slipped over the cut tip of a trichoid sensillum. The reference electrode was filled with haemolymph saline and implanted in the insect neck. The SSR responses were filtered (100 Hz–10 kHz) and amplified (×1000).

A flow of humidified pure air (1.5 l/min) was constantly directed onto the antenna through the main branch of a glass tube 10 mm in diameter. Pheromone test stimulation was performed by a puff of air (1 s, 0.5 l/min) through a Pasteur pipette connected to a lateral branch and containing a filter paper loaded with 0.5 μg of one of the pheromone compounds. Stimuli were given at 2 min intervals; when two different compounds were used, the two stimulations were alternated. TFMKs were applied by turning on a flux of air (0.1 l/min) through another Pasteur pipette containing a filter paper loaded with 50 μg of compound and connected to a second lateral branch of the glass tube. Three EAG responses to the pheromone and to its alcohol analogue were measured at 2 min intervals in pure air. This gave the EAG response level before treatment. Then the TFMK flux was turned on and, after 2 min, a series of six EAGs were recorded (during treatment). The treatment was turned off after 15 min. After a further 5 min, three test stimulations were performed (after treatment). A similar procedure was followed for SSRs.

The EAG and SSR recordings were digitized and stored on a PC-AT microcomputer via a DASH 16 analogue-to-digital conversion board. Control of the acquisition board and analysis of EAGs and SSRs were performed by programs we developed in Asyst (McMillan Software Co.). Fast display and plotting of data for their visual inspection were performed with AWAVE, a Microsoft Windows hosted

application developed in visual C++ (Marion-Poll, 1995). EAG signals were analysed to determine their maximum amplitude, the depolarization time at 4/5 of maximum amplitude (4/5DT; Figure 2) and the repolarization time at 2/3 of baseline (2/3RT). The differences of responses before, during and after treatments were analysed by a Wilcoxon matched-pairs signed-ranks test. The Mann–Whitney *U* test was used to compare independent samples (Siegel, 1956). To compare the activity of the TFMKs, we calculated the ratios before/during-treatment of the EAG amplitude, 4/5DT and 2/3RT.

Behaviour

The tests were performed in a wind tunnel made of glass of 180 cm long, 55 cm wide and 50 cm high (Quero *et al.*, 1996). The air was pushed through by a centrifugal fan and pulled out of the building with the aid of an exhaust blower. The airstream was cleansed through a 2 cm thick glass wool bed and conducted through two consecutive nylon screens to smooth the airflow and get a laminar regime through the tunnel. A 58 W red fluorescent light located 16 cm above the tunnel provided the required illumination. Light intensity was adjusted to 3–10 lux. A white board containing irregular-shaped, dark-brown spots was placed on the floor of the tunnel. The airspeed was 28 cm/s, the temperature of the experiment room was 22 ± 1°C and the relative humidity 60 ± 5%.

Male *S. littoralis* aged 32–52 h were placed in 12 cm Petri dishes containing a piece of filter paper (2 × 2 cm) on which 100 µg of one of the TFMKs had been deposited. Care was taken to prevent the insects coming into contact with the chemicals. At the onset of the scotophase, the moths were exposed to vapours of the compounds for 4 h in the dark, then transferred to a clean container. Control males were exposed to a piece of filter paper on which the solvent only had been deposited. Males were then allowed to acclimate to the tunnel conditions for a few minutes and utilized only once. The males were carefully placed over a filter paper on a stainless steel jack at 130 cm downwind from the source and allowed to respond for 5 min. A cotton wick containing 500 µg of Z9,E11-14:Ac was hung 18 cm from the top and 40 cm from the upwind end of the tunnel. The experiments were carried out 3–6 h into the scotophase. The number of males landing onto the source was scored. The results were analysed for significance using a 2 × 2 chi-square test (Sokal and Rohlf, 1969).

Results

EAG responses to pheromone in air

The EAGs recorded in response to the synthetic pheromone from male *S. littoralis* showed a steep decline to the peak amplitude, followed by a fast return to a plateau which was stationary for the stimulus time and a slower return to the baseline (Figure 2). Depending on the individuals, the EAG peak amplitude varied from 1.7 to 3.4 mV in response to 0.5 µg of the main component of the pheromone (Z9,E11-14:Ac). The depolarization time (4/5DT) was 56.4 ± 1.3 ms on average (SEM, n = 225). The repolarization

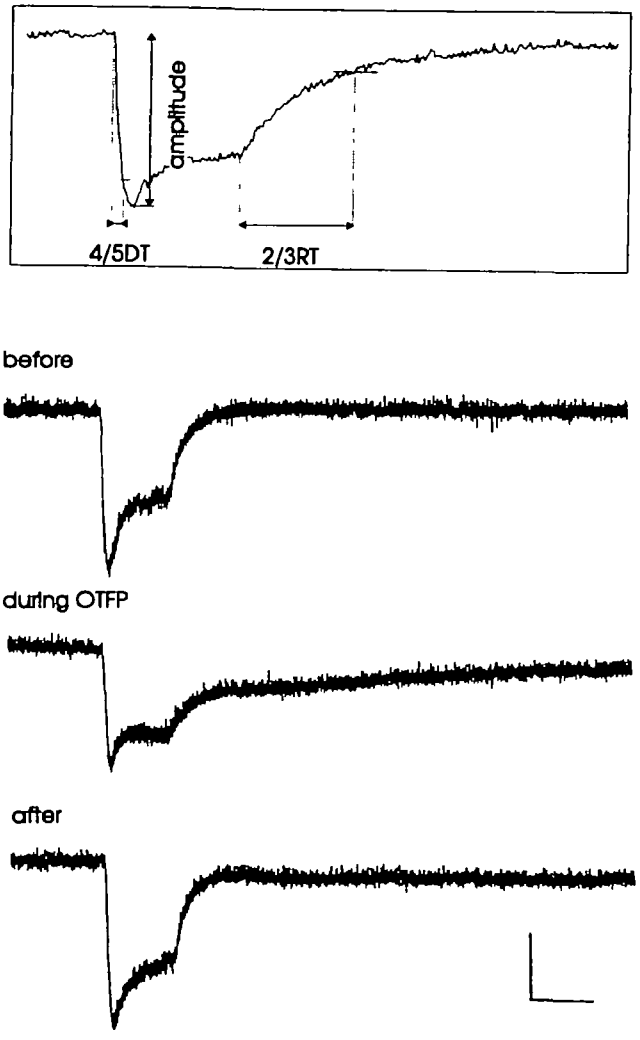

Figure 2 Samples of EAGs recorded in *S. littoralis* in response to 0.5 µg Z9,E11-14:Ac, before (upper record), during (middle record) and after (lower record) exposure of the antenna to OTFP loaded air. Vertical scale bar = 1 mV, horizontal scale bar = 1 s. Inset: general shape of the EAG recorded in male *S. littoralis* in response to Z9,E11-14:Ac, with the three parameters measured in this study: EAG amplitude, time to reach 4/5 of the peak depolarization (4/5DT) and time to reach 2/3 of repolarization (2/3RT).

time (2/3RT) was longer and it averaged 318.6 ± 6.7 ms (SEM, n = 225). Values for the 4/5DT and 2/3RT were relatively independent from the EAG amplitude (Figure 3). Thus, it was not necessary to correct the time-parameter values as a function of the amplitude.

In addition to receptor cells tuned to Z9,E11-14:Ac, *S. littoralis* possesses specific cells for the monoenic alcohol Z9-14:OH (Ljungberg *et al.*, 1993; Quero *et al.*, 1996).

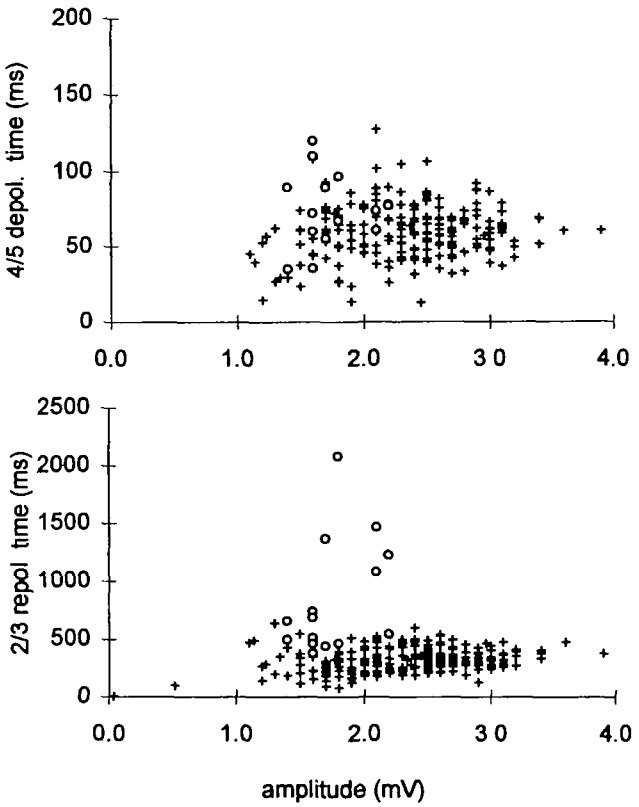

Figure 3 Relation between the EAG amplitude and 4/5 depolarization time (4/5DT, upper diagram) and the 2/3 repolarization time (2/3RT, lower diagram) in *S littoralis* measured in air (+) and in OTFP (o) in response to the synthetic pheromone, Z9,E11-14 Ac.

However, this alcohol was less active than Z9,E11-14:Ac and a ten times stronger dose, 5 µg, was routinely used to stimulate the antenna of *S. littoralis*.

Effects of OTFP on EAG

During exposure of the antenna to air loaded with OTFP (50 µg), the amplitude of the EAG in response to 0.5µg of Z9,E11-14:Ac decreased from 2.5 ± 0.2 mV to 1.8 ± 0.10 mV (n = 15; Table 1). The 4/5DT slightly increased from 60.2 ± 12.2 ms to 74.7 ± 9.9 ms, but this difference was not statistically significant. The main effect of OTFP was on the 2/3RT which drastically increased from 326.5 ± 20.4 ms in pure air to 839.0 ± 203.1 ms in its presence (Table 1, Figure 4). Similar decrease of the EAG amplitude associated to a slight but significant increase in the 4/5DT and a strong increase in the 2/3RT were observed in responses to Z9-14:OH (Table 1). The effects on EAG amplitude and kinetics were fully reversible for both compounds. When the flux of OTFP was turned off, the average amplitude, 4/5DT and 2/3RT fall, within the experimental error, to their level before exposure to OTFP (Table 1).

Effects on the EAG kinetics increased when the dose of OTFP was raised to 100 µg (Table 2). In this case, the repolarization was very slow after a Z9,E11-14:Ac stimulus, 2/3RT being 4.3 times longer in OTFP than in pure air. The amplitude of the EAG was decreased, the ratio of the amplitude during treatment over its value before treatment was 0.8 ± 0.1. The ratio for 4/5DT was 1.4 ± 0.2.

When the antenna was stimulated with a sequence of nine short pulses of Z9,E11-14:Ac of 0.1 s duration, at 1 s intervals (Figure 4), each pulse elicited a well defined EAG peak, although the response amplitude decreased according to the pulse rank in the sequence. OTFP amplified this effect, the amplitude showing a stronger rank-dependent decrease.

Table 1 Average amplitude, 4/5 depolarization (4/5DT) and 2/3 repolarization (2/3RT) times of the EAG measured in male *S littoralis* before, during and after treatment of the antenna with vapours of OTFP (50 µg on a filter paper)

		Before	During OTFP	After
Z9,E11-14:Ac (0.5 µg)	amplitude (mV)	2 5 ± 0.2	1.8 ± 0.1*	2.3 ± 0.1
	4/5DT (ms)	60.2 ± 12.2	74.7 ± 9.9	56 7 ± 7.2
	2/3RT (ms)	326.5 ± 20.4	839.0 ± 203.1*	345.4 ± 43.4
Z9-14·OH (5 µg)	amplitude (mV)	1.1 ± 0.1	0.8 ± 0 1*	0.9 ± 0.1
	4/5DT (ms)	104.0 ± 13 6	94.7 ± 8 7	95 2 ± 12 9
	2/3RT (ms)	257.9 ± 25.7	524.5 ± 113.6*	291 6 ± 37 4

Mean values ± SEM of 15 replicates, from five insects * indicates a significant difference ($P < 0.05$) with the values measured before the treatment

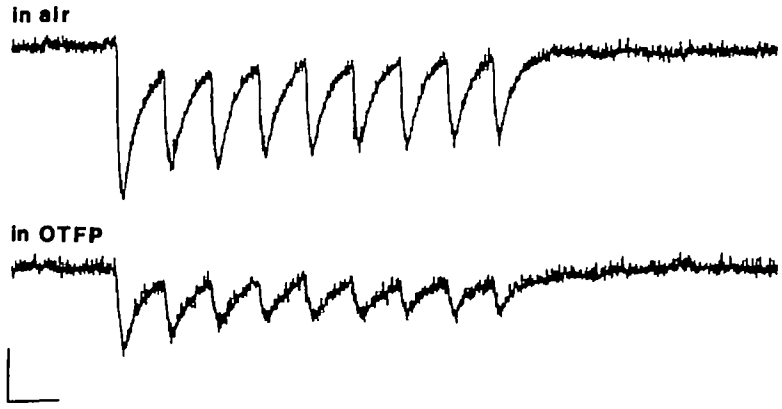

Figure 4 Typical EAG response of the antenna of male *S littoralis* to a sequence of nine short pulses of 0 2 s duration of Z9,E11-14 Ac at 1 s intervals in pure air (upper record) or in OTFP (lower record). Vertical scale bar = 1 mV, horizontal scale bar = 1 s

Effects of OTFP on spike firing

Spike firing by the Z9,E11-14:Ac receptor cell in the trichoid sensilla of *S. littoralis* in response to this pheromone was 30.0 ± 12.3 spikes/s at 100 µg OTFP (mean of n = 12 sensilla ± SEM), a 53% lower response in comparison with neuron response in air (45.9 spikes/s ± 16.2; $P < 0.05$). Besides this effect, the pattern of the firing was not modified during a 2 s post stimulus time, the period where most of the effects were observed on the EAG. Treatment of the antenna with a high dose of OTFP (500 µg) resulted in a complete inhibition of the responses of the Z9,E11-14:Ac receptor neuron (Figure 5). The firing response of the receptor neuron slowly recovered after the treatment.

Effects of OTFP on other insect species

Specificity of OTFP was studied on two other moth species with pheromone main components possessing different functional groups. Trichoid hairs of *M. brassicae* house receptor cells tuned either to the main pheromone component, Z11-16:Ac, or to an attraction inhibitor, Z11-16:OH (Renou and Lucas, 1994). In this species, OTFP significantly diminished the amplitude of the EAG responses to Z11-16:Ac and Z11-16:OH (Table 2). The repolarization time was slightly increased, but the difference was not significant. The depolarization time was increased by a factor of 2.9 for the acetate and by 1.8 for the alcohol. In turn, EAG responses of *M. brassicae* to 5 µg of linalool, a monoterpenic plant volatile, showed a decreased amplitude but no significant changes either in 4/5DT or in 2/3RT (Table 2).

In *H. zea* a receptor cell type is tuned to Z11-16:Ald, the main component of the pheromone blend (Almaas *et al.*, 1991). Here again, the amplitude of the EAG responses to Z11-16:Ald was decreased in the presence of OTFP. During treatment, 4/5RT and 2/3DT were both significantly increased (Table 2).

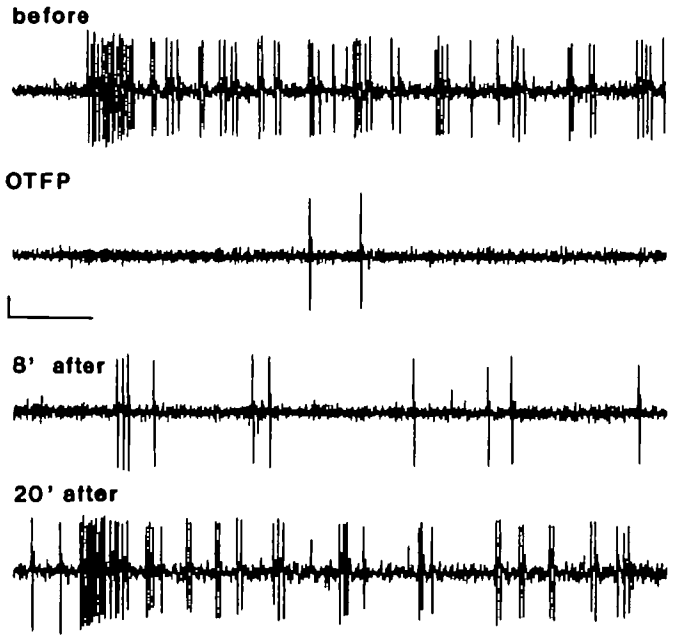

Figure 5 Reversible inhibition of the firing of a pheromone receptor cell by OTFP Inhibition has been achieved by ten puffs from a cartridge containing 500 µg of OTFP Responses to 0 5 µg Z9,E11-14 Ac, before treatment (upper record), immediately after treatment (OTFP), 8 (8') and 20 min (20') after treatment Vertical scale bar = 0 5 mV, horizontal scale bar = 0 2 s

Effects of other compounds on EAG

In addition to OTFP, other TFMKs and analogues were tested on *S. littoralis*. To make comparison of their activities easier, we calculated the ratios of the EAG parameter values obtained during treatment over before treatment. The

Table 2 Effects of OTFP (at 100 µg) on the EAG responses from three insect species to pheromone compounds with different functional group and the plant volatile linalool

	EAG amplitude	4/5RT	2/3DT
S littoralis			
Z9,E11-14.Ac	0 80 ± 0 10*	1 40 ± 0.24(ns)	4 30 ± 1.68*
Z9-14 OH	0.70 ± 0.16*	1.30 ± 0.19*	2 80 ± 0 43*
M brassicae			
Z11-16:Ac	0 87 ± 0 07*	1 30 ± 0 13(ns)	2.88 ± 0.30*
Z11-16:OH	0 72 ± 0 09*	1 14 ± 0.15(ns)	1 75 ± 0.39*
Linalool	0 74 ± 0 07*	0 90 ± 0 06(ns)	0 97 ± 0 17(ns)
H zea			
Z11-16:Ald	0 84 ± 0 04*	1 38 ± 0 14*	2 56 ± 0 14*

Ratios of the amplitude, 4/5 depolarization, and 2/3 repolarization times are during/before Mean values ± SEM n = 8 males for *S littoralis*, n = 4 for the other species * indicates a significant difference between the before- and during-treatment values ($P < 0.05$)

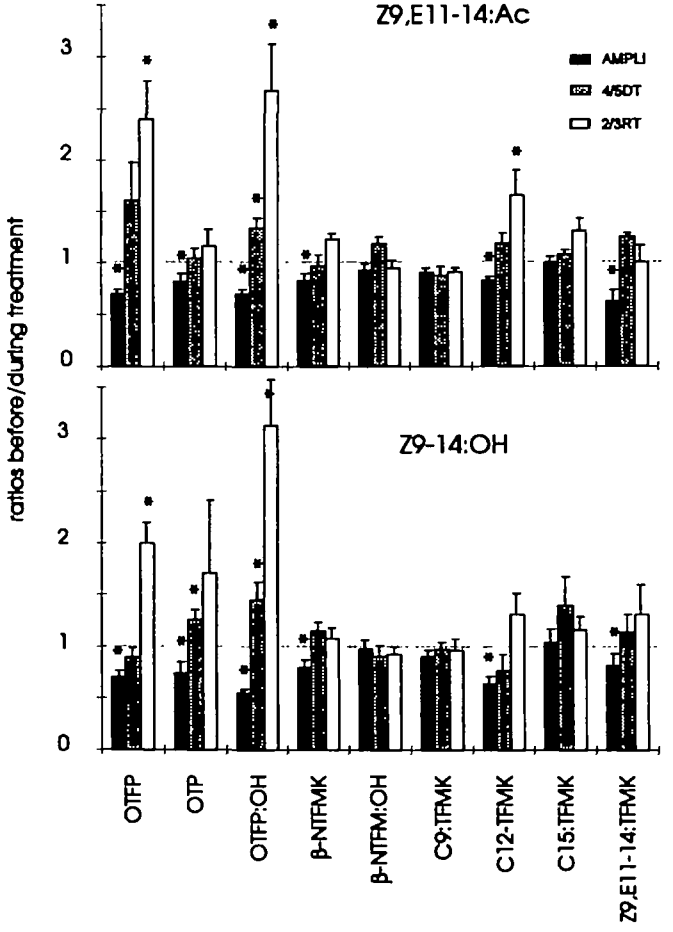

Figure 6 Comparison of the activity of the different TFMKs and analogues measured as the ratios during/before of amplitude, 4/5DT and 2/3RT of the EAG responses of *S.littoralis* to Z9,E11-14·Ac or Z9-14 OH * indicates a significant difference with values before the treatment ($P < 0.05$).

Table 3 Percentage of male *S littoralis* landing at the source (Z9,E11-14 Ac, 500µg) in a wind tunnel after being exposed to 100 µg of Z9,E11-14 Ac, TFMKs or analogues for 4 h

	% of males		
	taking flight	reaching the middle of the tunnel	landing at the source
Control	98	93	72
Z9,E11-14·Ac	100	72	40*
OTFP	91	80	47*
OTP	100	97	47*
OTFP·OH	87	73	43*
β-NTFMK	100	87	40*
β-NTFM·OH	92	88	80
C9·TFMK	100	93	67
C12 TFMK	100	90	37*
C15·TFMK	100	90	57
Z9,E11-14·TFMK	97	87	70

Percentage values followed by an * are significantly different from the control (2×2 chi-square test, $P < 0.05$)

compounds fall into three groups according to their effects on the amplitude and the kinetics of the EAG:

1. OTFP, OTFP:OH, and C12:TFMK decreased the EAG amplitude and increased the 4/5DT and the 2/3RT, being OTFP and OTFP:OH the most active (Figure 6). The effects were reversible.
2. OTP, β-NTFMK, and Z9,E11-14:TFMK also reversibly decreased the EAG amplitude, but did not modify significantly 4/5DT and 2/3RT.
3. β-NTFM:OH, C9-TFMK, and C15-TFMK did not alter any of the 3 EAG parameters (Figure 6).

Effects on male behaviour

In the wind tunnel almost all control males took flight and reached the middle of the tunnel in response to Z9,E11-14:Ac. The percentage of individuals landing at the source was 72% (Table 3). Pre-exposure of *S. littoralis* males for 4 h to the pheromone, TFMKs, and analogues did not significantly decrease the percentage of insects taking flight nor that of individuals reaching the middle of the tunnel. In turn, the number of males landing at the pheromone source was significantly lower than in control insects when males were previously exposed to vapours of their pheromone, β-NTFMK, OTFP, OTFP:OH, C12:TFMK, C15:TFMK or OTP. On the contrary, pre-exposure to β-NTFM:OH, C9:TFMK or Z9,E11-14:TFMK did not modify the percentage of males landing at the source (Table 3).

Discussion and conclusions

The EAG response to an odorant at a given concentration depends upon a variety of physical and chemical factors. The kinetics and amplitude of EAGs or single sensillum potentials are affected by temperature (Bestmann and Dippold, 1989; Kodadova, 1993; Kodadova and Kaissling 1996). Dependence of the time constants of EAGs and sensillum potentials on stimulus concentration and type of compound has been reported by Kaissling (1977). In field EAG experiments performed on *Epiphyas postvittana* (Walker), Rumbo *et al.* (1995) observed that the EAGs measured in the presence of volatiles from the environment showed smaller amplitudes and delayed recovery times compared with responses in clean air. They concluded that environmental volatiles and pheromone interact at the receptor level. However, in comparison with these volatiles, TFMKs showed low EAG intrinsic activity in *S. littoralis* (E.Malo, unpublished data), so that such interactions are unlikely in our case. Furthermore, measures of EAGs in the presence of four different insect repellents failed to show any effect on the amplitude and the depolarization and repolarization times on *M. brassicae* (M.Renou, unpublished data). Zack (1979) observed that in *Antheraea polyphemus* the sensillum potential showed a decrease in amplitude and repolarization time and an increase in depolarization time after cross- or self-adaptation. Variations of opposite sign were observed for the three parameters when the stimulus concentration was increased. A similar dependence of the time constants of the EAG upon the stimulus concentration was reported by Alcorta (1991) in *Drosophila melanogaster*, wherein EAG responses to ethyl acetate showed shorter depolarization time and longer repolarization time when the concentration of the stimulus increased. In our work, the two main effects of OTFP on the EAG responses of *S. littoralis, M. brassicae* and *H. zea* to pheromones were a decrease in the EAG amplitude and an increase in the repolarization time. Thus, the effect of OTFP appears to be different from those produced by adaptation and stimulus increase.

There is a good agreement between the activity of the TFMKs on the EAG and on male behaviour in the wind tunnel. All the compounds which decreased the EAG amplitude reduced the percentage of insects landing at the source. However, the lack of behavioural effect of Z9,E11-14:TFMK is unexpected, since it decreases significantly the amplitude of the EAG response. Similarly, (Z)-1,1,1-trifluoro-14-heptadecen-2-one failed to inhibit the responses of male *O. nubilalis* to their pheromone in the wind tunnel (Klun *et al.*, 1991). However, these authors used low concentrations of TFMK, which could account for the lack of inhibition.

Most of the TFMKs which displayed good antiesterase activity (IC_{50} < 10 μM), measured *in vitro* from antennal extracts of *S. littoralis*, like OTFP and the diene analogue Z9,E11-14:TFMK (Durán *et al.*, 1993; Rosell *et al*, 1996), significantly decreased EAG amplitude. However, the corresponding alcohol OTFP:OH, which displayed low antiesterase activity *in vitro* (Rosell *et al.*, 1996) was very active on EAG. In this regard, it should be noted that OTFP:OH is a potent inhibitor of juvenile hormone esterase in spite of its lack of activity on acetylcholine esterase (Linderman *et al*, 1993). The non-fluorinated ketone OTP, with poor antiesterase activity (IC_{50} = 73; G. Rosell and A.Guerrero, unpublished data) also reduced amplitude but had no effect on 4/5DT and 2/3RT. In this context it should be noted that effects on amplitude and on kinetics are not fully correlated. First, β-NTFMK, Z9,E11-14:TFMK and OTP significantly decreased EAG amplitude but failed to modify EAG kinetics. Second, OTFP also decreased the response to linalool, a monoterpenic tertiary alcohol, without altering its kinetics.

If all models of olfactory transduction include an inactivating system, the mechanism of pheromone deactivation is a matter of controversy. Evidence for pheromone catabolism in moth antennae have been accumulated for a long time (reviewed by Vogt, 1987). However, there is still no direct proof for its involvement in the transductory processes. Taking into account that the half-life of pheromone, measured in freshly excised antennae, is in the range of a few min (e.g. Kasang *et al*, 1988, 1989), it has been considered that the enzymatic conversion is not fast enough to be compatible with the time course of the neuronal response (Kaissling, 1986). Furthermore, Maida *et al.* (1995) reported that esterase activity was highly variable between individuals of *Antheraea polyphemus* and showed no correlation with the time course of the sensillum potential. On the contrary, Vogt *et al.* (1985) characterized the kinetic properties of the purified sensillar esterase and estimated the pheromone half-life in the sensory hair below 15 ms, a time compatible with fast stimulus deactivation. In our case, the *in vivo* effects on EAG repolarization time of OTFP and other TFMKs are in agreement with the

hypothesis of a decrease of pheromone deactivation. In the presence of TFMKs, pheromone deactivation could become a rate-limiting step in the reception process, consequently to the inhibition of the esterase. The decrease in EAG amplitude might be an indirect effect due to a displacement of the kinetic equilibrium (Vogt, 1987) between pheromone binding proteins and receptor proteins. Alternatively, it could result from a more direct effect of OTFP, interacting with an unidentified molecular step of olfactory transduction unrelated to pheromone catabolism. Some of our observations are not fully compatible with an antiesterase effect only. First, the responses to pheromones with an alcohol or aldehyde function are also affected. In this case, other enzymes (dehydrogenases, oxidases, reviewed by Prestwich, 1987) are involved in the catabolism of the pheromone molecules and they might also be affected by TFMKs, whose activity spectrum, besides esterases, is poorly known. Second, although the correlation between biological activity and antiesterase effect is good for most of the TFMKs tested, this is not the case when fluorinated alcohol (OTFP:OH) or non-fluorinated ketones (OTP), both of which have no antiesterase activity, are considered. Thus, further work is necessary to fully understand the biological mode of action of TFMKs.

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