Chem. Senses 24: 473–480, 1999

Pheromone-triggered Orientation Flight of Male Moths can be Disrupted by Trifluoromethyl Ketones

J. Bau, D. Martínez, M. Renou[1] and A. Guerrero

Department of Biological Organic Chemistry, Institute of Chemical and Environmental Research of Barcelona (CSIC), Jordi Girona 18–26, E-08034 Barcelona, Spain and [1]INRA Unité de Phytopharmacie et Médiateurs Chimiques, Route de St Cyr, F-78026 Versailles Cédex, France

Correspondence to be sent to: A. Guerrero, Department of Biological Organic Chemistry, Institute of Chemical and Environmental Research of Barcelona (CSIC), Jordi Girona 18–26, E-08034 Barcelona, Spain. e-mail: agpqob@cid.csic.es

Abstract

In a wind tunnel trifluoromethyl ketones (TFMKs) have been found to disrupt the orientation flight of male moths to pheromone sources (virgin females or synthetic pheromone). This is demonstrated by comparison of the flight parameters of the Egyptian armyworm *Spodoptera littoralis* and the Mediterranean corn borer *Sesamia nonagrioides*, which had been topically treated with TFMKs, with those calculated for untreated insects. Inhibition occurred in all types of behavior and that of the source contact has been quantified and found to be dose-dependent. The same effect has also been noticed in Mediterranean corn borer males flying to an attraction source consisting of mixtures of (*Z*)-11-hexadecenyl trifluoromethyl ketone (8), a closely related analogue of the major component of the pheromone, and the natural pheromone blend. The most active TFMKs are those closest in structure to the natural pheromone, along with those chemicals which easily hydrate in solution, such as the β-thiosubstituted derivatives. Along with the previously reported reduction of catches in the field, our results suggest the possible application of these chemicals in future new pest control strategies.

Introduction

Insect behavior can be controlled by simple odor compounds or by blends of several components of the sex pheromone. Therefore, many behavior-modifying chemicals can be visualized as potentially useful agents for pest control (Ridgway *et al.*, 1990). Pheromone-mediated upwind flight orientation to the source constitutes a precise system to establish how semiochemicals can elicit complex behavioral responses in moths (Vickers and Baker, 1992; Mafra-Neto and Cardé, 1994; Kaissling, 1997); key factors in governing these responses are the quality and composition of the odor and the temporal pattern of odor exposure (Kennedy *et al.*, 1980; Murlis and Jones, 1981; Baker and Kuenen, 1982; Baker *et al.*, 1985).

Trifluoromethyl ketones (TFMKs) are potent inhibitors of a number of serine esterases and proteases, such as acetylcholinesterase, chymotrypsin or human liver carboxylesterases (Gelb *et al.*, 1985; Ashour and Hammock, 1987). In insects, TFMKs reversibly inhibit the antennal esterases responsible for the catabolism of pheromone molecules in male olfactory tissues (Vogt *et al.*, 1985; Prestwich and Streinz, 1988; Durán *et al.*, 1993). Activity of these chemicals arises from the unique features induced by fluorine, which closely mimics the steric volume of hydrogen at the enzyme receptor sites. The strong electronegativity of the halogen induces fluorinated ketones to form stable hydrates in aqueous solutions, forming an adduct, probably a hemiacetal of tetrahedral geometry, with the active site of the enzyme (Linderman *et al.*, 1988; Rosell *et al.*, 1996). In the field, some TFMKs have been noted to reduce catches of males when mixed with the pheromone in several ratios (Parrilla and Guerrero, 1994; Riba *et al.*, 1994). We present evidence for the first time that in a wind tunnel TFMKs can disrupt the chemical communication system of two flying moths, the Egyptian armyworm *Spodoptera littoralis* (Boisd.) and the Mediterranean corn borer *Sesamia nonagrioides* (Lef.), which had been either topically treated with several doses of these chemicals or attracted to a source containing mixtures of the pheromone and the fluorinated compounds. The effect was noticed when the attraction source was either virgin females or synthetic pheromone.

Materials and methods

Insects

The Egyptian armyworm and the Mediterranean corn borer were reared in the laboratory on slightly modified artificial diets (Poitout *et al.*, 1972; 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 16:8 light:dark regime at 25 ± 1ºC and 65 ± 10% relative humidity until emergence. Adults were provided with 10% sucrose solution, separated daily by age and kept on filter paper in plastic containers.

Chemicals

Compounds **1**–**5** are β-thiosubstituted TFMKs of molecular structure $RSCH_2COCF_3$ (**1**, R=C_6H_{13}; **2** (OTFP), R=C_8H_{17}; **3**, R=$C_{10}H_{21}$; **4**, R=$C_{12}H_{25}$; **5**, R=$C_{15}H_{31}$), compounds **6**–**10** and **14** are unsaturated chemicals of the type $RCOCF_3$ (**6**, R=Z11-$C_{14}H_{27}$; **7**, R=Z9,E11-$C_{14}H_{25}$; **8**, R=Z11-$C_{16}H_{31}$; **9**, R=Z9-$C_{18}H_{35}$; **10**, R=Z9,Z12-$C_{18}H_{33;}$ **14**, R=Z12-$C_{17}H_{33}$), compound **11** is a *bis*-TFMK ($CF_3COC_{10}H_{20}COCF_3$) and **12** (OP) is the non-fluorinated derivative $C_8H_{17}SCH_2COCH_3$. Abbreviations and chemical names (in parenthesis) of the compounds used are as follows: **1**, HTFP (hexylthiotrifluoropropan-2-one); **2**, OTFP (octylthiotrifluoropropan-2-one); **3**, DTFP (decylthiotrifluoropropan-2-one); **4**, DoTFP (dodecylthiotrifluoropropan-2-one); **5**, PTFP (pentadecylthiotrifluoropropan-2-one); **6**, Z11-14TFMK [(*Z*)-11-tetradecenyl trifluoromethyl ketone]; **7**, Z9E11-14TFMK [(*Z*,*E*)-9,11-tetradecadienyl trifluoromethyl ketone]; **8**, Z11-16TFMK [(*Z*)-11-hexadecenyl trifluoromethyl ketone]; **9**, Z9-18TFMK [(*Z*)-9-octadecenyl trifluoromethyl ketone]; **10**, Z9Z12-18TFMK [(*Z*,*Z*)-9,12-octadecadienyl trifluoromethyl ketone]; **11**, 10BTFMK (10-trifluoroacetyldecyl trifluoromethyl ketone); **12**, OP (octylthiopropan-2-one); **13**, Z9E11-14Ac [(*Z*,*E*)-9,11-tetradecadienyl acetate]; **14**, Z12-17TFMK [(*Z*)-12-heptadecenyl trifluoromethyl ketone]; **15**, Z11-16Ac [(*Z*)-11-hexadecenyl acetate]. The major component of the sex pheromone of the Egyptian armyworm (**13**) and of the Mediterranean corn borer (**15**), were obtained from commercial sources. All fluorinated compounds as well as pheromone components had already been synthesized in our laboratory (Parrilla *et al.*, 1994; Villuendas *et al.*, 1994) and their purity was >98%.

Behavioral tests

The experiments were performed on insects of scotophases 2 and 3, in *S. littoralis* during hours 5 and 6, and in *S. nonagrioides* between hours 3 and 7. Males were anaesthetized with CO_2 and carefully handled to apply on the antennae different doses of the compounds, which had been dissolved in hexane in the required concentration so that 0.1 µl of the solution contained the desired dose for the test. In preliminary assays, this amount of solvent did not exert a significant diminution of any behavioral response in moths relative to control. Males were allowed to recover for 30 min, introduced into the tunnel and individually tested. Inhibition percentage was determined by the relative decrease in number of contacts with the source displayed by treated males in comparison to solvent-applied insects. The attraction source was either 10 µg of **13**, the major component of the sex pheromone of the Egyptian armyworm (Nesbitt *et al.*, 1973), or 1 µg of the pheromone blend of the corn borer [a mixture of **15**, (*Z*)-11-hexadecenol, (*Z*)-11-hexadecenal and dodecyl acetate in 77:8:10:5 ratio] (Sans *et al.*, 1997), or virgin females. Just prior to the experiments, the required amount of attractant was dissolved in 10 µl of nanograde hexane and applied to dispensers: a brown female-shaped piece of cardboard for *S. nonagrioides* or a 5 × 2 cm piece of filter paper for *S. littoralis*. The solvent was allowed to evaporate and the dispensers suspended at 18 cm from the top and 40 cm from the upwind end of the tunnel. When virgin females were used as attractants, four individuals (10–35 h old) were placed in 6.5 × 4 × 3 cm stainless steel cages of 0.2 × 0.2 cm mesh.

All types of behavior [wing fanning and taking flight (TF), arrival to the middle of the tunnel, close approach to the lure and contact with the source (SC)] were recorded and compared with control and hexane-treated insects. Experiments were conducted in blocks including two or three treatments and control or hexane-treated insects, and statistical analysis was performed within every block. On each day of experimentation, three groups of 8–10 treated males were compared with 10–14 control males until completion of the block.

Assays were conducted in a 180 × 55 × 50 cm glass tunnel, as described previously (Quero *et al.*, 1995). The active space was visualized with the aid of a SO_3 smoke dispenser (Drägerwerk, Germany). Illumination (2–5 lx) was obtained through a dimmed fluorescent red light. A video camera (Pulnix B/W TM50), linked to a JVC SR306E video recorder and a Panasonic TC-14S1RC monitor, was placed 135 cm above the tunnel and in a perpendicular position to minimize optical distortion of the flight. The camera allowed recording of a flight path within a 130 cm long and 45 cm wide section of the tunnel, and the tracks were traced onto millimetrically scaled acetate sheets and laid over the monitor screen. Positions were displayed frame-by-frame and the successive moth positions, separated 0.2 s, marked on the acetate sheet. Locations were arbitrarily converted into X, Y coordinates with regard to the initial male position ($X = 0$, $Y = 0$). Plume limits were recorded and traced with a set of points that were adjusted to two polynomial regression equations ($r^2 = 0.98$), which established the upper ($Y > 0$) and lower edge ($Y < 0$) of the plume. Insect positions the coordinates of which fell between those delimited by the equations were considered to be inside the plume. The estimated crosswind dimensions for *S. littoralis* were 16 and 40 cm wide at 50 and 100 cm, respectively, from the source. For *S. nonagrioides* the active space was 12 and 31 cm, respectively, at the same distances from the lure. These values result from the optimum wind speed found for each species: 15 cm/s for *S. littoralis* and 22 cm/s for *S. nonagrioides*. Flight parameters were determined on uninterrupted flights from the platform to the source and only those males arresting at the source for a minimum period of 5 s were recorded as SC. For out-of-plume parameters, only males

which spent at least 20% of their total flight time outside the plume boundaries were considered.

For each flight track the following parameters were recorded: flight distance, flight duration, ground speed, turning frequency, number of intersections with plume, track leg length, track width, track angle, course angle and drift angle (Marsh *et al.*, 1978) and analyzed for significance (LSD test, $P < 0.05$).

Results

In preliminary tests, the pheromone doses cited, i.e. 10 μg of the major component of the pheromone (**13**), of the Egyptian armyworm and 1 μg of the pheromone blend of the Mediterranean corn borer, had induced the highest number of contacts, fully comparable with those induced by virgin females (unpublished data).

Compounds **2** (OTFP) and **6**, which had been found *in vitro* to be good inhibitors of antennal esterases of the Egyptian armyworm (Rosell *et al.*, 1996), significantly induced inhibition of SC behavior (77 and 58% for $n = 37$ and 44 respectively), as did other β-thioderivatives, such as compounds **3** and **5** (66 and 44% inhibition for $n = 39$ and 33 respectively; Figure 1). Compound **9**, with a double bond at the same location as in the pheromone structure, also elicited a remarkable inhibitory effect (49%, $n = 33$). The best inhibitor, however, was compound **7**, the most closely related analogue of the major component of the pheromone (**13**), which exerted an inhibition of 83% ($n = 40$) at 500 ng and 63% ($n = 32$) at 50 ng. These values were only slightly lower than those obtained with the actual pheromone component **13** (100% inhibition at 500 ng, 79% at 50 ng and 41% at 25 ng/antenna; Figure 1).

By contrast, compound **12** (OP), the non-fluorinated analogue of **2**, did not significantly decrease the number of males contacting the source (55% with regard to 60% of control insects, i.e. only 8% inhibition), which confirms the key role played by fluorine in the inhibitory action of these molecules. This finding was also corroborated when virgin females were used as lures. In this experiment, only 30% of males were able to contact the cage when treated with 100 ng of OTFP (**2**) vs 73% of solvent-applied males (59% inhibition), while 62% of insects reached the source upon treatment with 500 ng of OP (**12**) (15% inhibition).

Flight tracks of moths, previously treated with OTFP (**2**), showed profound differences compared with those of hexane-applied insects. Males treated with the inhibitor frequently exhibited erratic progress towards the plume, flying across the wind with high numbers of intersections with plume boundaries (Figure 2). Males treated with OTFP took significantly longer to contact the source and flew longer distances than control, hexane-treated and OP-treated insects (Table 1). Likewise, the number of intersections with the plume edges was also more than twice the mean value observed for control, hexane-treated or OP-treated males

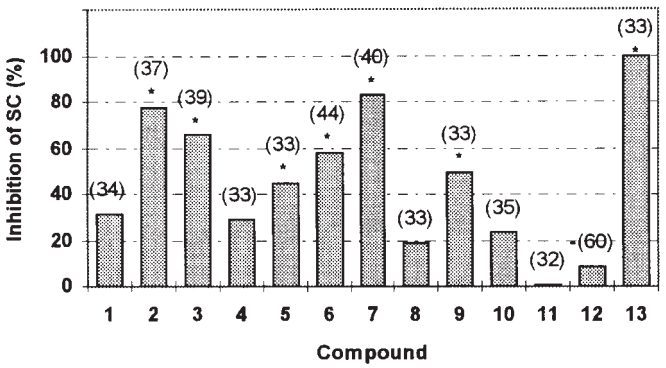

Figure 1 Inhibition of contacts with the source induced on *S. littoralis* males by topical application of 500 ng of TFMKs **1–12** (**1**, HTFP; **2**, OTFP; **3**, DTFP; **4**, DoTFP; **5**, PTFP; **6**, Z11-14TFMK; **7**, Z9E11-14TFMK; **8**, Z11-16TFMK; **9**, Z9-18TFMK; **10**, Z9Z12-18TFMK; **11**, 10BTFMK; **12**, OP) on the antenna in comparison with the major component of the pheromone (**13**). Asterisks indicate a significant level of inhibition ($P < 0.05$, χ^2 homogeneity test). The attraction source was 10 μg of (*Z*,*E*)-9,11-tetradecadienyl acetate (**13**). Number of insects tested is shown in parenthesis.

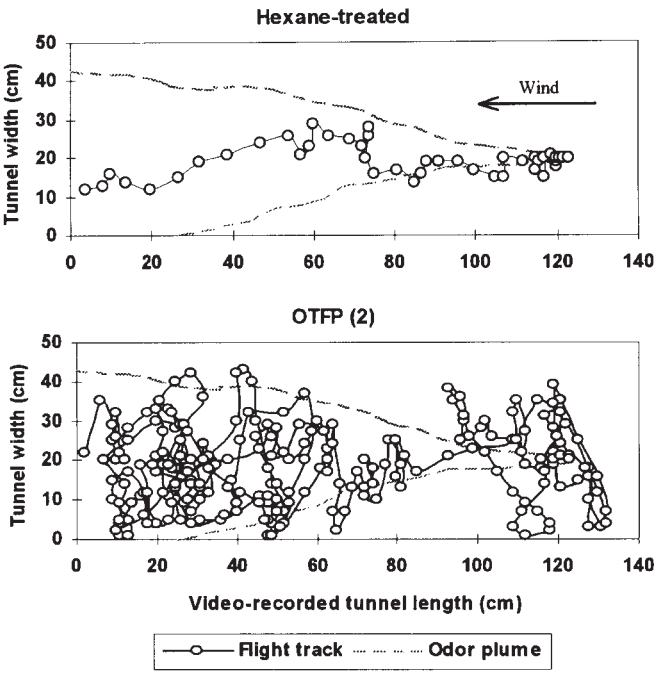

Figure 2 Flight track of one hexane- and one OTFP-treated *S. littoralis* male. Insects moved upwind from left to right towards the source located at a distance of 120 cm. Open circles denote positions of moths at 0.5 s intervals. Discontinuous line represents boundaries of the time-averaged pheromone plume.

(22.3 ± 5.6 vs 9.6 ± 1.3, 8.6 ± 1.2 and 6.8 ± 0.9, respectively). Ground speed of males flying inside the plume was not modified by the treatment; however, it was significantly higher in OTFP-treated males flying outside the plume than in control insects (Table 2). Interestingly, the turning frequency was practically identical (2.3–2.5 turns/s) in all

Table 1 Parameters for the whole flight of *S. littoralis* males which had been subjected to the action of OTFP (**2**) and OP (**12**) (500 ng/antenna each) by topical application to the antennae

	Control (n = 23)	Hexane-treated (n = 22)	OTFP (n = 19)	OP (n = 21)
Total flight distance (cm)	348.6^{b} (34.3)	301.4^{b} (30.3)	793.5^{a} (148.5)	236.4^{b} (18.4)
Total flight duration (s)	13.0^{b} (1.3)	9.7^{b} (1.1)	24.2^{a} (3.6)	9.7^{b} (0.9)
No. of intersections with plume	9.6^{b} (1.3)	8.6^{b} (1.2)	22.3^{a} (5.6)	6.8^{b} (0.9)

The pheromone source was 10 μg of **13**. Means (± SE) in the same row followed by superscript letters are significantly different ($P < 0.05$, LSD test). n = number of insects tested.

Table 2 Selected parameters from tracks of *S. littoralis* males flying upwind inside and outside the plume, which had been subjected to the action of OTFP (**2**) and OP (**12**) (500 ng/antenna each) by topical application to the antennae

Parameter	Insects flying inside the plume				Insects flying outside the plume[1]	
	Control (n = 23)	Hexane-treated (n = 22)	OTFP-treated (n = 19)	OP-treated (n = 21)	Control (n = 16)	OTFP-treated (n = 16)
Ground speed (cm/s)	$29.3^{c,d}$ (1.8)	$33.3^{a,b}$ (1.9)	$33.0^{a,b,c,d}$ (2.4)	27.7^{d} (2.4)	$28.8^{b,c,d}$	37.8^{a} (1.1)
Turning frequency (turns/s)	$2.3^{a,b}$ (0.1)	2.4^{a} (0.1)	$2.3^{a,b}$ (0.1)	2.5^{a} (0.1)	2.1^{b} (0.1)	2.1^{b} (0.2)
Track leg length (cm)	$12.4^{b,c}$ (0.6)	$13.7^{b,c}$ (0.7)	14.2^{b} (1.1)	11.3^{c} (1.0)	14.8^{b} (1.1)	20.7^{a} (1.4)
Track width (cm)	7.1^{c} (0.4)	7.3^{c} (0.4)	9.6^{b} (0.6)	5.8^{d} (0.4)	11.2^{b} (0.9)	14.5^{a} (1.1)
Track angle (degrees)	50.6^{b} (1.9)	50.4^{b} (3.1)	69.1^{a} (2.2)	44.8^{b} (2.2)	68.6^{a} (3.4)	71.3^{a} (5.3)
Course angle (degrees)	$33.2^{b,c}$ (1.8)	35.2^{b} (2.5)	48.3^{a} (2.3)	27.1^{c} (1.3)	45.6^{a} (3.5)	52.3^{a} (4.3)
Drift angle (degrees)	$17.3^{a,b}$ (1.3)	15.2^{b} (1.2)	20.8^{a} (1.5)	$17.6^{a,b}$ (1.3)	22.9^{a} (1.0)	$19.0^{a,b}$ (1.8)

The pheromone source was 10 μg of **13**. Means (± SE) in the same row followed by superscript letters are significantly different ($P < 0.05$, LSD test). n = number of insects tested.

[1]Only males which spent at least 20% of their total flight time outside the plume boundaries were considered.

insects flying upwind into the plume and also similar in control and OTFP-treated males flying out of the plume (2.1 turns/s). Track leg length was significantly higher in OTFP-treated males out of the plume than in any other control or treated males. Also, OTFP significantly increased the mean track width in flights inside and outside the plume. Likewise, OTFP-treated moths steered significantly larger track and course angles inside the plume than untreated or OP-applied males, but they were comparable to those displayed by control insects out of the plume.

A similar experiment was performed on *S. nonagrioides* males but, in this case, and on the basis of the previous results, only the steric mimics Z11-16:TFMK (**8**) and Z12-17:TFMK (**14**) of the sex pheromone were considered as well as OTFP (**2**). Previously, application of 500 ng of the pheromone blend to the antenna resulted in a remarkable inhibition of response from the first steps of behavior, i.e. wing fanning and taking flight with only 48% (n = 29) and 10% (n = 29) of males, respectively, responding to the stimulus in comparison to hexane-treated insects (n = 34). This represents an inhibition of these behavioral steps of 51 and 89%, respectively. None of the males treated and flying upwind contacted the source (100% inhibition; Figure 3). Topical application of 1–50 μg of OTFP (**2**) and 1–10 μg of **14** also resulted in a reduction in the number of males showing the entire sequence of the pheromone-mediated behavior. Both TFMKs evoked a significant inhibition of response at 5 μg dose, the inhibition amounting up to ~53–56% in the number of SC relative to control (Figure 3). Again, the most closely related analogue **8** of the pheromone proved to be the best inhibitor, inducing a significant reduction in the number of males contacting the source at 1 ng/antenna (42% inhibition). The inhibition was dose-dependent (Figure 4).

The TFMK effect was also tested in a different experiment by recording the behavior of untreated corn borer males flying to an attraction source consisting of mixtures of **2**, **8** and **14** and the natural pheromone in 1:1 and 10:1 ratios. In this case, whereas **2** showed no inhibitory effect at any of the doses tested, the presence of **14** in the lure significantly reduced the number of SC at the highest ratio. Thus, only 8 out of 47 (17%) males tested contacted the source, while 24 out of 40 (60%) insects were attracted to the pheromone alone. Compound **8** was again found to be the most active: when mixed with the pheromone in a 1:1 ratio it significantly decreased the number of males

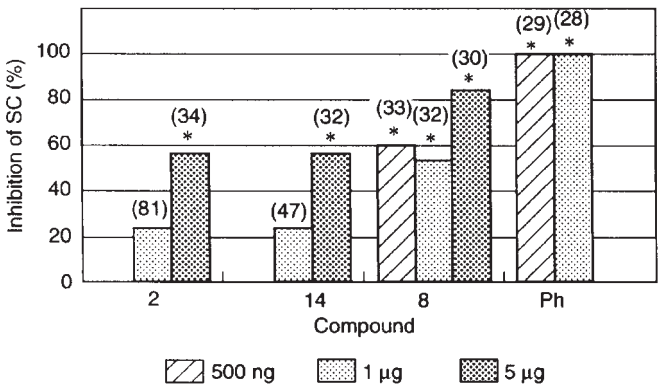

Figure 3 Inhibition of source contacts of *S. nonagrioides* males elicited by topical application on the antenna of several amounts (0.5–5 µg) of TFMKs **2**, **8** and **14** in comparison to that obtained with the pheromone blend (0.5–1 µg). Compounds **2** and **14** have not been tested at 0.5 µg and the pheromone at the 5 µg dose. Asterisks denote a significant level of inhibition ($P < 0.05$, χ^2 homogeneity test). The attraction source was 1 µg of the pheromone blend. Number of insects tested is given in parenthesis.

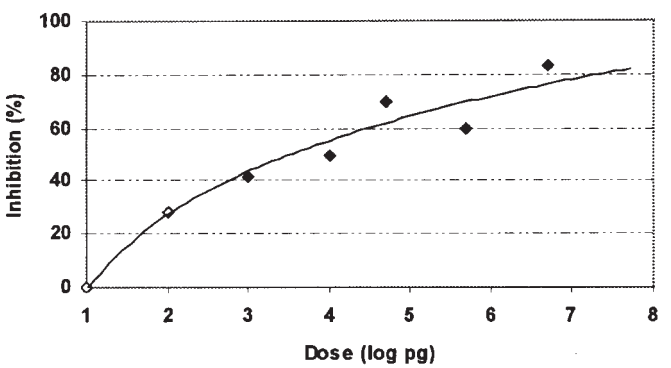

Figure 4 Dose–response curve of inhibition of SC induced by topical application of several amounts (10 pg–5 µg) of TFMK **8** ($r^2 = 0.947$). The attraction source was 1 µg of the pheromone blend. Filled dots represent statistically significant inhibition values ($P < 0.05$, χ^2 homogeneity test). Number of insects tested ranged from 30 to 40.

displaying all types of behavior. The number of contacts was also reduced by a significant 20.6% ($n = 91$, $P < 0.05$, χ^2 homogeneity test). The effect was also dose-dependent (Figure 5).

A number of Mediterranean corn borer flight tracks were also video-recorded after males were treated with 1 ng/antenna of compound **8** applied topically (Figure 6). As in the Egyptian armyworm, males displayed significantly higher track widths and track leg lengths than untreated males, with frequent inter-turn crosswind and downwind reversals and little progress towards the source. As a result, treated males flew significantly longer than those untreated but, since their ground speed was also comparatively higher, they did not take longer to reach the target (Table 3). In

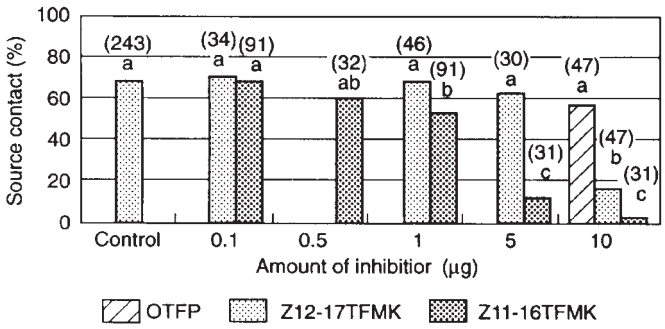

Figure 5 Source contact behavior of *S. nonagrioides* males attracted to a dispenser loaded with a mixture of 1 µg of pheromone complex and different amounts of inhibitor. OTFP (**2**) was only tested at 10 µg, while Z12-17TFMK (**14**) was not assayed at 0.5 µg. Different letters on treatments with the same amounts of inhibitor are statistically significant ($P < 0.05$, χ^2 homogeneity test). Number of insects tested is given in parenthesis. Level of SC of control insects is the average of the values obtained in all experimental blocks, *n* being the total number of control males tested.

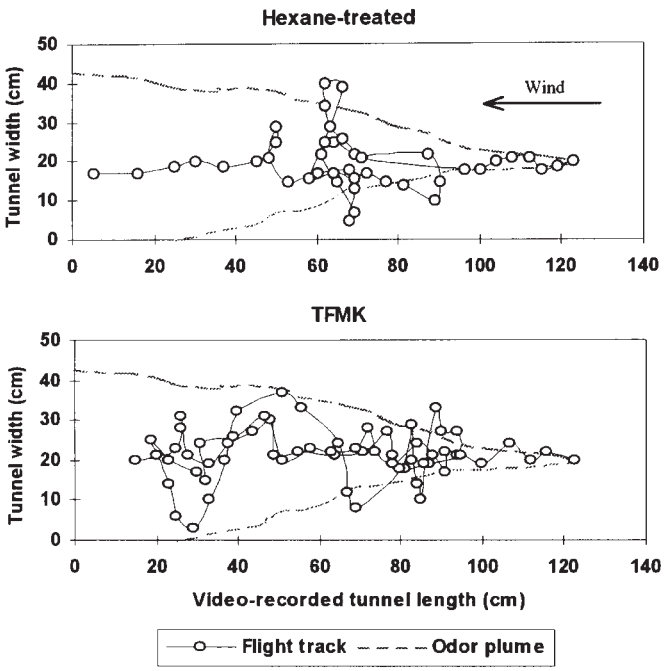

Figure 6 Flight track of one hexane- and one Z11-16TFMK (**8**)-treated *S. nonagrioides* male. Insects moved upwind from left to right towards the source located at a distance of 120 cm. Open circles denote positions of moths at 0.5 s intervals. Discontinuous line represents boundaries of the time-averaged pheromone plume.

addition to flying faster, moths steered larger course and track angles than control or hexane-treated males, and therefore headed less into the wind. The drift angles were similar in all flying moths. The turning frequency was also relatively constant, as in the Egyptian armyworm. In contrast, treated insects did not lose the active space in

Table 3 Selected parameters from flight tracks of *S. nonagrioides* males flying upwind to a pheromone source which had been subjected to the action of TFMK **8** (1 ng/antenna) by topical application to the antennae

Parameter	Control (n = 21)	Hexane-treated (n = 15)	TFMK **8** (n = 20)
Total flight distance (cm)	213^{b} (15.2)	207^{b} (16.4)	308^{a} (30.5)
Total flight duration (s)	9.2^{a} (0.6)	7.9^{a} (0.7)	9.2^{a} (0.7)
No. of intersections with plume	10.1^{a} (1.5)	10.7^{a} (1.7)	10.2^{a} (1.2)
Ground speed (cm/s)	23.4^{b} (1.0)	26.9^{b} (1.6)	33.4^{a} (1.5)
Turning frequency (turns/s)	2.9^{a} (0.1)	2.8^{a,b} (0.1)	2.6^{b} (0.1)
Track leg length (cm)	8.4^{b} (0.5)	9.9^{b} (0.6)	12.9^{a} (0.5)
Track width (cm)	4.2^{b} (0.3)	4.7^{b} (0.3)	7.7^{a} (0.4)
Track angle (degrees)	42.8^{b} (2.0)	40.8^{b} (2.2)	51.5^{a} (2.4)
Course angle (degrees)	21.7^{b} (1.5)	22.2^{b} (1.6)	31.2^{a} (4.7)
Drift angle (degrees)	21.1^{a} (0.8)	18.5^{a} (1.3)	20.3^{a} (0.9)

The attraction source was 1 μg of the pheromone blend. Means (± SE) in the same row followed by superscript letters are significantly different ($P < 0.05$, LSD test). n = number of insects tested.

higher numbers than untreated males, as shown by the similar number of intersections with the plume boundaries (Figure 6).

Discussion

Our results show that for the Egyptian armyworm, in addition to the highly hydrated β-thiosubstituted TFMKs, whose degree of hydration is an important factor for esterase inhibition (Linderman *et al.*, 1991), the most potent inhibitors were those whose structures closely mimic the natural pheromone. It is particularly noteworthy that the inhibition promoted by compounds **7**, the most closely related analogue of the major component of the pheromone **13**, as well as by **6**, the analogue containing only one double bond at position 11, one of the unsaturation locations of the parent pheromone. Even compound **9**, with one unsaturation at C-9 as compound **13**, did induce a significant inhibitory effect in spite of its chain being four carbon atoms longer than the natural attractant. These results correlate well with the inhibitory activity of antennal esterases of this insect induced by these TFMKs and β-thioderivatives in *in vitro* assays (Rosell *et al.*, 1996). Similar results were obtained for the Mediterranean corn borer in which, again, the most closely related analogue **8** of the pheromone proved to be the best inhibitor, the diminution of response being three orders of magnitude higher than that induced by OTFP (**2**). The inhibition was remarkable from the first steps of behavior and the effect was dose-dependent, in a similar manner to that observed after pre-exposure of *Cadra cautella* (Mafra-Neto and Baker, 1996) and *Epiphyas postvittana* (Bartell and Lawrence, 1976) to their respective pheromones.

Most flight parameters of insects treated with the inhibitors were significantly different in comparison with those of control or solvent-applied males, the exception being the turning frequency which remained constant before and after treatments. In *Lymantria dispar* the turning frequency was also consistent across a 100-fold range of pheromone concentration (Charlton *et al.*, 1993), which suggests that in *S. littoralis* and *S. nonagrioides* the turns are also regulated by an internal self-steered counterturning program (Kennedy, 1986).

In *S. littoralis*, all flight parameters displayed by OTFP (**2**)-applied insects flying inside the plume were fully comparable to those shown by control insects flying outside the plume. This suggests that treated males may not perceive subtle changes in pheromone concentration or that perception of individual pheromone filaments within the plume is being 'suppressed' by the treatments, thus inducing insects to behave as if they had lost the plume. This compound therefore induced a large number of casting tracks, with frequent lateral crosswind excursions, in some cases with a slight regression downwind. In *S. nonagrioides*, TFMK **8** elicits its disruptive effect by strongly modifying the orientation pattern inside the plume, but without inducing males to lose the active space.

It is well known that antennal enzymes are responsible for the catabolism of the sex pheromone in insects (Kasang, 1971, 1973; Ferkovich, 1982). Inhibition of these enzymatic systems has been considered as a potential new approach for pest control (Prestwich, 1986). The effects of TFMKs as inhibitors of esterases (Vogt *et al.*, 1985; Prestwich and Streinz, 1988; Durán *et al.*, 1993; Rosell *et al.*, 1996), on pheromone catabolism (Quero, 1996), as well as in the electrophysiological responses to synthetic pheromone components have been reported (Renou *et al.*, 1997). In the same regard and in a preliminary report, pre-exposure of males to vapors of some TFMKs for 4 h decreased the number of SC (Renou *et al.*, 1997). The *in vivo* mode of action of TFMKs cannot be explained by the antiesterase effect alone, since responses to pheromones with an alcohol or aldehyde function can also be affected (Renou *et al.*, 1997). Similar results were obtained by Pophof (Pophof,

1998), who proposed that the inhibition of the electrophysiological responses promoted by the chemicals might be due to an antagonistic action at the pheromone binding sites, either at the receptor molecules or at the pheromone binding protein. In addition, and as shown by us on the processionary moth *Thaumetopoea pityocampa* (Feixas *et al.*, 1995), aliphatic TFMKs may be bound to the pheromone binding proteins and transported through the sensillum lymph, thus facilitating interaction with the enzymes responsible for pheromone catabolism. The inhibitory effect of pheromone application is possibly due to overstimulation and adaptation of the receptor cells, in contrast to the inhibitory effect of the TFMKs which may be due to competitive action of the inhibitors on the pheromone binding protein and/or the receptor molecules. In any case, the data presented here show that TFMKs effectively disrupt the orientation flight of the Egyptian armyworm and the Mediterranean corn borer males, resulting in a significant decrease in the number of insects reaching the pheromone source (synthetic pheromone or virgin females). These results, along with the previously reported reduction of male catches in the field promoted by mixtures of some TFMKs with the pheromone (Parrilla and Guerrero, 1994; Riba *et al.*, 1994), suggest the possible application of these chemicals in future new pest control strategies.

Acknowledgements

We gratefully acknowledge CICYT (AGF 97-1217-C0201), EC (FAIR CT96-1302), and Comissionat per a Universitats i Recerca (1997SGR 00021) for financial support. We are also indebted to X. Bellés and W.L. Roelofs for useful comments on the manuscript and to SEDQ, SA for providing free samples of *S. nonagrioides* pheromone.

References

Ashour, M.-B.A. and **Hammock, B.D.** (1987) *Substituted trifluoroketones as potent selective inhibitors of mammalian carboxylesterases.* Biochem. Pharm., 36, 1869–1879.

Baker, T., Willis, M.A., Haynes, K.F. and **Phelan, P.L.** (1985) *A pulsed cloud of pheromone elicits upwind flights in male moths.* Physiol. Entomol., 10, 257–265.

Baker, T.C. and **Kuenen, L.P.S.** (1982) *Pheromone source location by flying moths: a supplementary non-anemotactic mechanism.* Science, 216, 424–427.

Bartell, R.J. and **Lawrence, L.A.** (1976) *Reduction in sexual responsiveness of male light brown apple moth following previous brief pheromone exposure is concentration dependent.* J. Aust. Entomol. Soc., 15, 236.

Charlton, R.E., Kanno, H., Collins, R.D. and **Cardé, R.T.** (1993) *Influence of pheromone concentration and ambient temperature on flight of the gypsy moth,* Lymantria dispar *(L.), in a sustained-flight wind tunnel.* Physiol. Entomol., 18, 349–362.

Durán, I., Parrilla, A., Feixas, J. and **Guerrero, A.** (1993) *Inhibition of antennal esterases of the Egyptian armyworm* Spodoptera littoralis *by trifluoromethyl ketones.* Bioorg. Med. Chem. Lett., 3, 2593–2598.

Feixas, J., Prestwich, G.D. and **Guerrero, A.** (1995) *Ligand specificity of pheromone-binding proteins of the processionary moth.* Eur. J. Biochem., 234, 521–526.

Ferkovich, S.M. (1982) *Enzymatic alteration of insect pheromones.* In Norris, D.M. (eds), Perception of Behavioral Chemicals. Elsevier/North Holland, Amsterdam, pp. 165–185.

Gelb, M.H., Svaren, J.P. and **Abeles, R.H.** (1985) *Fluoroketone inhibitors of hydrolytic enzymes.* Biochemistry, 24, 1813–1817.

Kaissling, K.-E. (ed.) (1997) *Pheromone-controlled anemotaxis in moths.* Birkhäuser Verlag, Basel.

Kasang, G. (1971) *Bombykol reception and metabolism on the antennae of the silkworm* Bombyx mori. In Ohloff, G. and Thomas, A.F. (eds), Gustation and Olfaction. Academic Press, New York, pp. 245–250.

Kasang, G. (1973) *Physikochemische Vorgänge beim Riechen des Seidenspinners.* Naturwissenschaften, 60, 95–101.

Kennedy, J.S. (1986) *Some current issues in orientation to odour sources.* In Payne, T.L., Birch, M.C. and Kennedy, C.E.J. (eds), Mechanisms in Insect Olfaction. NSF-NATO Symposium on Insect Olfaction. Clarendon Press, Oxford, pp. 11–25.

Kennedy, J.S., Ludlow, A.R. and **Sanders, C.J.** (1980) *Guidance system used in moth sex attraction.* Nature, 288, 475–477.

Linderman, R.J., Jamois, E.A. and **Roe, R.M.** (1991) *Correlation of equilibrium hydration constant and inhibitory potency for trifluoromethyl ketone inhibitors of insect juvenile hormone esterase.* Rev. Pest. Toxicol., 1, 261–270.

Linderman, R.J., Leazer, J., Roe, R.M., Venkatesh, K., Selinsky, B.S. and **London, R.E.** (1988) *^{19}F NMR spectral evidence that 3-octylthio-1,1,1-trifluoropropan-2-one, a potent inhibitor of insect juvenile hormone esterase, functions as a transition state analog inhibitor of acetylcholinesterase.* Pest. Biochem. Physiol., 31, 187–194.

Mafra-Neto, A. and **Baker, T.C.** (1996) *Elevation of pheromone response threshold in almond moth males pre-exposed to pheromone spray.* Physiol. Entomol., 21, 217–222.

Mafra-Neto, A. and **Cardé, R.T.** (1994) *Fine-scale structure of pheromone plumes modulates upwind orientation of flying moths.* Nature, 369, 142–144.

Marsh, D., Kennedy, J.S. and **Ludlow, A.R.** (1978) *An analysis of anemotactic zigzagging flight in male moths stimulated by pheromone.* Physiol. Entomol., 3, 221–240.

Murlis, J. and **Jones, C.D.** (1981) *Fine scale structure of odor plumes in relation to insect orientation to distant pheromone and other attractant sources.* Physiol. Entomol., 6, 71–86.

Nesbitt, B.F., Beevor, P.S., Hall, D.R., Lester, R. and **Poppi, R.G.** (1973) *Sex pheromones of two noctuid moths.* Nature New Biol., 244, 208–209.

Parrilla, A. and **Guerrero, A.** (1994) *Trifluoromethyl ketones as inhibitors of the processionary moth sex pheromone.* Chem. Senses, 19, 1–10.

Parrilla, A., Villuendas, I. and **Guerrero, A.** (1994) *Synthesis of trifluoromethyl ketones as inhibitors of antennal esterases of insects.* Bioorg. Med. Chem., 2, 243–252.

Poitout, S. and **Bues, R.** (1974) *Élevage des chenilles de vingt-huit espèces de lepidoptères Noctuidae et deux espèces d'Arctiidae sur milieu artificiel simple. Particularités de l'élevage selon les espèces.* Ann. Zool. Ecol. Anim., 6, 431–441.

Poitout, S., Bues, R. and **Le Rumeur, C.** (1972) *Élevage sur milieu artificiel simple de deux noctuelles parasites du coton* Earias insulana *et* Spodoptera littoralis. Ent. Exp. Appl., 15, 341–350.

Pophof, B. (1998) *Inhibitors of sensillar esterase reversibly block the responses of moth pheromone receptor cells.* J. Comp. Physiol. A, 183, 153–164.

Prestwich, G.D. (1986) *Fluorinated sterols, hormones and pheromones: enzyme-targeted disruptants in insects.* Pest. Sci., 37, 430–440.

Prestwich, G.D. and **Streinz, L.** (1988) *Haloacetate analogs of pheromones: effects on catabolism and electrophysiology in* Plutella xylostella. J. Chem. Ecol., 14, 1003–1021.

Quero, C. (1996) Estudios sobre el proceso de percepción, inhibición y catabolismo de feromonas sexuales de Lepidópteros. PhD thesis, University of Barcelona.

Quero, C., Camps, F. and **Guerrero, A.** (1995) *Behavior of processionary males (*Thaumetopoea pityocampa*) induced by sex pheromone and analogs in a wind tunnel.* J. Chem. Ecol., 21, 1957–1969.

Renou, M., Lucas, P., Malo, E., Quero, C. and **Guerrero, A.** (1997) *Effects of trifluoromethyl ketones and related compounds on the EAG and behavioural responses to pheromones in male moths.* Chem. Senses, 22, 407–416.

Riba, M., Eizaguirre, M., Sans, A., Quero, C. and **Guerrero, A.** (1994) *Inhibition of pheromone action in* Sesamia nonagrioides *by haloacetate analogues.* Pest. Sci., 41, 97–103.

Ridgway, R.L., Silverstein, R.M. and **Inscoe, M.N. (eds)** (1990) Behavior-modifying Chemicals for Insect Management. Marcel Dekker, Inc., New York.

Rosell, G., Herrero, S. and **Guerrero, A.** (1996) *New trifluoromethyl ketones as potent inhibitors of esterases: ^{19}F NMR spectroscopy of transition state analog complexes and structure–activity relationships.* Biochem. Biophys. Res. Commun., 226, 287–292.

Sans, A., Riba, M., Eizaguirre, M. and **Lopez, C.** (1997) *Electroantennogram, wind tunnel and field responses of male Mediterranean corn borer,* Sesamia nonagrioides*, to several blends of its sex pheromone components.* Ent. Exp. Appl., 82, 121–127.

Vickers, N.J. and **Baker, T.C.** (1992) *Male* Heliothis virescens *maintain upwind flight in response to experimentally pulsed filaments of their sex pheromone.* J. Insect Behav., 5, 669–687.

Villuendas, I., Parrilla, A. and **Guerrero, A.** (1994) *An efficient and expeditious synthesis of functionalized trifluoromethyl ketones through lithium–iodine exchange reaction.* Tetrahedron, 50, 12673–12684.

Vogt, R.G., Riddiford, L.M. and **Prestwich, G.D.** (1985) *Kinetic properties of a pheromone-degrading enzyme: the sensillar esterase of* Antheraea polyphemus. Proc. Natl Acad. Sci. USA, 82, 8827–8831.

Accepted May 18, 1999