DOI:10.1021/jf901979k

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Development and Biological Activity of a New Antagonist of the Pheromone of the Codling Moth *Cydia pomonella*

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A new pheromone antagonist of the codling moth *Cydia pomonella* is reported. Presaturation of the antennae of the insects with vapors of the antagonist (*E*,*E*)-8,10-dodecadienyl trifluoromethyl ketone, analogue of the main component of the pheromone (codlemone), resulted in lower electrophysiological responses to the pheromone relative to untreated insects. In the wind tunnel, the compound elicited a remarkable disruptive effect on all types of behavior of males flying toward a source baited with a pheromone/antagonist blend in 1:1, 1:5, and 1:10 ratios. The insects displayed erratic flights in the presence of the antagonist, as shown by their flight parameters in comparison to insects attracted to the pheromone alone. In the field, traps baited with mixtures of 1:10 codlemone/antagonist attracted considerably lower numbers of males than the natural attractant. The antagonist, however, did not inhibit the pheromone-degrading enzymes present in male antennae, suggesting that trifluoromethyl ketones are not sufficiently electrophilic to produce a stable intermediate adduct with a cysteine residue of the enzyme, a mechanism previously proposed for oxidase inhibition in insects. Overall and taking into account our previous reports and, particularly, the reduction in damage induced in maize fields by a trifluoromethyl ketone analogue of the pheromone of *Sesamia nonagrioides* (Solé, J.; Sans, A.; Riba, M.; Rosa, E.; Bosch, M. P.; Barrot, M.; Palencia, J.; Castellà, J.; Guerrero, A. Reduction of damage by the Mediterranean corn borer, *Sesamia nonagrioides*, and the European corn borer, *Ostrinia nubilalis*, in maize fields by a trifluoromethyl ketone pheromone analog. *Entomol. Exp. Appl.* **2008**, *126*, 28−39), the antagonist might be a new candidate to consider in future strategies to control the codling moth.

KEYWORDS: Pheromone antagonist; codling moth; *Cydia pomonella*; trifluoromethyl ketones; electrophysiology; wind tunnel; field tests

INTRODUCTION

The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is one of the most important pests of apples, pears, and walnuts worldwide. It is widely distributed across the pome fruit-producing regions and can cause a high level of damage if it is not properly controlled (*2*). Broad-spectrum pesticides have been widely used to control the pest, but their secondary effects on human health, natural enemies, and the environment along with induced resistance to a variety of them preclude their use in the future. The main sex pheromone component of the codling moth was reported by Roelofs et al. (*3*) as (*E*,*E*)-8,10-dodecadienol (codlemone), and several minor components of the pheromone were later identified (*4*). The pheromone has been extensively used to monitor and control pest populations via mating disruption techniques (*5*). However, control by mating disruption alone is only effective at low population levels and where immigration of gravid females is prevented (*6*). Therefore, new approaches to control the codling moth are needed.

Pheromone disruptants of chemical communication are new biorational approaches for insect control (*7*). Trifluoromethyl ketones have been shown to be potent pheromone antagonists of *Spodoptera littoralis*, *Sesamia nonagrioides* (*8*), and *Ostrinia nubilalis* (*9*) when mixed with the pheromone in different ratios. In these insects, structurally similar trifluoromethyl ketones to the major component of the pheromone disrupted male orientation flights to pheromone sources in a wind tunnel. In the field, these chemicals elicited a significant decrease in the number of males caught in traps baited with mixtures of the pheromone and the antagonist compared to the pheromone alone. In addition, these compounds reduced the electrophysiological responses to the pheromone in *S. littoralis*, *Mamestra brassicae*, and *Heliothis zea* (*10*). Trifluoromethyl ketones are also able to inhibit a large number of esterases and proteases (*11*), including pheromone-degrading enzymes (PDEs) present in insect olfactory tissues (*8*, *10*, *12*). These are key enzymes for the rapid degradation

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 Published on Web 08/24/2009

of pheromone esters, thus maintaining a low stimulus noise level in sensory hairs. The high potency of trifluoromethyl ketones is due to the high polarization of the carbonyl by the trifluoromethyl group, thereby increasing the electrophilicity of the carbonyl carbon and making it more susceptible to nucleophilic attack by a catalytic residue in the enzyme active site.

We have reported that antennal esterase extracts of *S. littoralis* and *S. nonagrioides* were inhibited in a slow-binding manner by a number of trifluoromethyl ketones and other fluorinated derivatives (*12*, *13*). More recently, (*Z*)-11-tetradecenyl trifluoromethyl ketone and its homologue (*Z*)-10-tridecenyl trifluoromethyl ketone were found to be potent inhibitors of male antennal esterase preparations of *O. nubilalis*, with an IC_{50} value of 0.28 and 7.5 μM, respectively (*9*). As noticed, all insects considered thus far have an ester (acetate) as the main pheromone structural motif, and therefore, at this point, it would be important to know whether these fluorinated chemicals also act as pheromone antagonists of insects lacking the ester moiety in their pheromone composition and at the same time inhibit other enzymes (oxidases, dehydrogenases, etc.) that are responsible for pheromone degradation. In this study, we investigated for the first time the activity of (*E*,*E*)-8,10-dodecadienyl trifluoromethyl ketone (**Figure 1**) as a putative antagonist of the pheromone of *C. pomonella* in the laboratory and in the field and disclose its effect on male antennal degrading enzymes.

MATERIALS AND METHODS

Chemicals. Codlemone (99.5 and 99.8% chemical and isomeric purity, respectively) was purchased from Pherobank (The Netherlands). (*E*,*E*)-1-Iodo-8,10-dodecadiene was obtained from (*E*,*E*)-8,10-dodecadienol as previously described (*14*). The solvents were purchased from Fluka (Sigma-Aldrich, Schweiz) and dried according to established procedures. Other commercial products [*tert*-BuLi, *n*-decanol, nicotinamide adenine dinucleotide (NAD), and ethyl trifluoroacetate] were obtained from Sigma-Aldrich.

(*E*,*E*)-8,10-Dodecadienyl Trifluoromethyl Ketone. A solution of (*E*,*E*)-1-iodo-8,10-dodecadiene (196 mg, 0.67 mmol) in a mixture of anhydrous 3:2 pentane/ether (6 mL) was cooled to −78 °C under Ar. Then, a 1.7 M *tert*-BuLi solution in hexane (0.6 mL, 1 mmol) was added, and the mixture was stirred for 5 min. A solution of ethyl trifluoroacetate (0.47 mL, 4.02 mmol) was then added, and the mixture stirred for 1 h at −78 °C. The system was allowed to warm to room temperature and quenched with NH_4Cl saturated solution, and the organic phase was washed with brine. After drying with anhydrous $MgSO_4$ and evaporation of the solvent, the crude was purified by flash chromatography on silica gel, eluting with hexane to provide the expected trifluoromethyl ketone as an oil (146 mg, 81%). IR (film) ν: 3016, 2929, 2856, 1765, 1456, 1404, 1377, 1295, 1208, 1150, 1042 cm^{-1}. ^1H nuclear magnetic resonance (NMR) (300 MHz, $CDCl_3$) δ: 5.95−6.05 (m, 2H), 5.5−5.6 (m, 2H), 2.7 (t, J = 8 Hz, 2H), 2.03 (broad t, J = 8 Hz, 2H), 1.73 (d, J = 8 Hz, 3H), 1.60−1.68 (b, 2H), 1.25−1.4 (b, 8H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ: 191.9 (q, J = 35 Hz, CO), 132.16 (CH), 131.85 (CH), 130.57 (CH), 127.07 (CH), 115.79 (q, J = 292.8 Hz, CF_3), 36.58 (CH_2), 32.70 (CH_2), 29.50 (CH_2), 29.26 (CH_2), 29.08 (CH_2), 28.90 (CH_2), 22.57 (CH_2), 18.25 (CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$) δ: 79.87 (s) ppm. High-resolution mass spectrometry (HRMS) Calcd. for $C_{14}H_{20}F_3O$ (M^+), 261.1466; found, 261.1459.

Insects. Insects were collected from an infested apple orchard in Lleida (northeast Spain) and maintained on a semi-synthetic diet (*15*) under a 16:8 h light/dark photoperiod at 25 ± 5 °C. Insects were reared in small groups (3−4 individuals) in small cylindrical plastic boxes. The last instar larvae were sexed, and males were maintained on corrugated cardboard in plastic boxes (15 cm diameter × 7 cm high) at 23 ± 2 °C until emergence. Males of 2−3 days old were used for the experiments.

Electrophysiology Tests. The electroantennogram (EAG) apparatus was purchased from Syntech (Hilversum, The Netherlands). In brief, a flow of humidified pure air (1000 mL/min) was continuously directed over the male antenna through the main branch of a glass tube (7 cm long × 5 mm diameter). Test stimulations were carried out by giving puffs of air

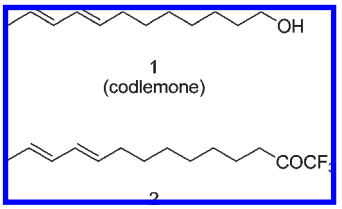

Figure 1. Chemical structures of codlemone (**1**) and the antagonist (*E*,*E*)-8,10-dodecadienyl trifluoromethyl ketone (**2**).

(300 mL/min) through a Pasteur pipet for 100 ms using a stimulus controller TC-05 (Syntech). The pipet contained a small piece of filter paper (2 × 1 cm) on which the stimulus compounds, diluted in hexane to the appropriate concentrations, had been deposited. The solvent was allowed to evaporate before the tests. For the intrinsic activity, five puffs over 100 ng of each compound were applied to one male antenna at 45 s intervals. Antennae of 12 insects were used for the experiments. Control puffs with a piece of paper containing only solvent (hexane) were also intercalated between two consecutive stimuli to determine the baseline depolarization of the antenna. The signals were amplified (100×) and filtered (DC to 1 kHz) with an ID-2 interface (Syntech), digitized on a PC, and analyzed with the EAG2000 program. Depolarization means were compared for significance using the least significant difference (LSD) test ($p < 0.05$).

For the inhibition assays, 2−3-day-old males (N = 20) were placed in Petri dishes (9 cm in diameter) containing a small piece of filter paper (2 × 2 cm) on which varying amounts of the antagonist (1, 10, 50, and 100 μg) dissolved in hexane had been deposited. The hexane was allowed to evaporate before the assays. Males were exposed for 2 h in the dark to vapors of the compound. Then, they were taken to another clean container, conditioned to light for 5 min, and their antennae were excised for EAG recordings. The same protocol as for the intrinsic activity assay was followed, and the depolarization means were compared for significance (LSD test, $p < 0.05$) to those obtained with control insects (N=20), which had been treated under the same conditions in the absence of the antagonist.

Wind Tunnel Experiments. Assays were conducted in a glass tunnel of 200 × 50 × 50 cm, as previously described (*8*). Illumination (10−20 lux, simulating crepuscular conditions) was obtained by two red light bulbs covered with a white sheet and located 2 m above the tunnel. The average temperature inside the tunnel was 23 ± 1 °C, and the relative humidity was 65 ± 5%. The air flow in the tunnel was regulated to 0.15 m/s. The odor source consisted of a rubber septum containing 10 μg of codlemone alone or mixed with varying amounts of antagonist (10, 50, and 100 μg). The septa were hung at 25 cm high and 30 cm distance of the far end of the tunnel. Before the tests, a single insect in the first hour of scotophase was placed into a glass cylinder (2.5 cm in diameter and 15 cm in height), and this was placed on a holder into the tunnel at 20 cm high and 125 cm from the emission source. After a 30 s acclimatization period, the cover of the cylinder was removed and the behavior of the male was recorded for 3 min. For each responding insect, the following types of behavior were considered: wing fanning (WF), upwind flight onto the pheromone plume until $^1/_3$ (F1), $^2/_3$ (F2), and $^3/_3$ (F3) of the total length of the tunnel, and source contact (SC). For each treatment, a minimum of 45 males were used and each insect was tested only once. The experiments were conducted in blocks with exposed and control insects, and statistical analysis was performed within each block (χ^2 homogeneity test, $p < 0.05$).

Tracks of flying insects to an attractant source containing pheromone (10 μg) and a 2:1 mixture of codlemone (10 μg) and the trifluoromethyl ketone (5 μg) were recorded with a video camera CCD 2400JB Presentco (Rister, Barcelona, Spain) equipped with a 12 mm wide-angle lens. The camera was mounted at a height of 140 cm above the tunnel in a perpendicular position to record the insects flight with minimal optical distortion. The camera covered a 130 × 45 cm section of the tunnel, and the recorded tracks were sent to a monitor for visualization. The tracks were converted into computer files at a rate of 25 frames/s with the aid of digital video software (Pinnacle Systems 5.1, Mountain View, CA). The successive insect positions were converted into XY coordinates using an in-house software, and only uninterrupted flights were considered. The following

flight parameters were recorded: flight duration (s), flight distance (cm), ground speed (cm/s), turning frequency (turns/s), track angle (degrees), drift angle (degrees), and course angle (degrees) (for the significance of these parameters, see Kuenen and Cardé (*16*)) and analyzed for significance (LSD test, $p < 0.05$).

PDE Inhibition. Antennae of 1−2-day-old males were excised and stored at −80 °C until use. To minimize individual variations in the extracts, a single large extract was prepared, from which the required aliquots were taken out for the assays. Several (3−4) Eppendorf tubes containing a total of ca. 600 antennae were immersed in liquid nitrogen and powdered with a plastic homogenizer. The tubes were then placed on ice, and to them, a total of 1.8 mL of 100 mM sodium phosphate buffer (pH 6) was added. The suspensions were combined, and the resulting extract was sonicated at 40 W in an ultrasonic bath at 4 °C for 10 s. The suspension was centrifuged at 12000*g* and 4 °C for 10 min. The supernatant was removed, distributed in several Eppendorf tubes, and stored at −80 °C until analysis. To each tube a solution of NAD in buffer (pH 6) was added, so that the final concentration in the tube was 1 mM. A total of 100 μL of these solutions (equivalent to 10 antennae) was placed in borosilicate tubes with the corresponding amount of the possible inhibitor (2 μL of a 250−1500 μM solution in DMSO or MeCN). The solution was vortexed for 30 s and preincubated in a thermostatic bath at 25 °C for 10, 30, and 60 min. Then, 2 μL of a 15 μM solution of codlemone in DMSO or MeCN was added, and incubation was continued for a further 60 or 90 min. Then, 180 μL of 9:1 hexane/ether containing *n*-decanol as the internal standard was added. The mixture was shaken, and the organic phase was carefully decanted and concentrated to 20−30 μL for gas chromatography (GC) analysis. No inhibitor was present in control experiments. Chromatographic analyses were carried out in a Trace 2000 gas chromatograph, equipped with a split−splitless injector system, flame ionization detector (FID), and a 25 m × 0.2 mm inner diameter × 0.25 μm HP-5 fused silica capillary column (Agilent Technologies, Madrid, Spain). The chromatographic conditions were as follows: injection at 80 °C for 1 min and program of 10 °C/min up to 150 °C, 3 °C/min up to 180 °C, and 5 °C/min up to 250 °C, which was maintained for 5 min more. The carrier gas was helium at a flow of 1 mL/min. Under these conditions, codlemone, (*E*,*E*)-8,10-dodecadienal, and (*E*,*E*)-8,10-dodecadienoic acid showed retention times of 14.8, 13.2, and 16.3 min, respectively. GC−mass spectrometry (MS) analyses were carried out on a Fisons MD 800 instrument under EI conditions using a 30 m × 0.25 mm inner diameter × 0.25 μm HP-5MS fused silica capillary column (Agilent Technologies). The analyses were performed in 2−3 replicates.

Field Tests. Delta traps were deployed in commercial apple orchards cv. Golden in the fruit-growing area of the Lleida province from May to August 2006−2008. Solutions of codlemone and (*E*,*E*)-8,10−12:trifluoromethyl ketone were prepared in hexane, and the required volume containing 100 μg of the pheromone and the corresponding amount of the antagonist was applied on 9−10 mm red rubber septa (Aldrich, Milwaukee, WI). The traps were hung in the canopy of apple trees at a height of 2.5 m and spaced a minimum of 20 m from each other. Four trials using blends of codlemone/trifluoromethyl ketone in 1:0, 1:0.5, 1:1 (this mixture in two different fields), and 1:10 ratios were carried out in 2006 with 3−6 replicates per formulation and no rotation of traps. New experiments were conducted in 2007 and 2008, with blends in 1:0, 0:1, 1:5, and 1:10 ratios (5 replicates per formulation). In these trials, a fully randomized block was considered and the traps were rotated once or twice a week according to the population density. Trials finished after two complete trap position rotations in 2007 and three complete rotations in 2008. The total number of catches per trap was transformed ($\sqrt{x} + 0.5$) prior to analysis. Data from the 2006 trials were analyzed by a Student's *t* test ($p < 0.05$). Data from the 2007 and 2008 trials were combined in a 2-way ANOVA, and the mean number of catches was compared by a Tukey's test ($p < 0.05$) because the interaction treatment−year was not significant. Analyses were carried out with GenSat 11th ed. for Windows. Details of the field trials are shown in the Supporting Information.

RESULTS AND DISCUSSION

Disruption of chemical communication in insects is a biorational approach to interfere location and mating of insects, and therefore, it may lead to development of new species-selective pest control agents (*7*, *17*). After having discovered new disruptants of pheromone communication in insect pests, such as *S. littoralis* (*8*, *10*), *S. nonagrioides* (*1*, *8*, *18*), and *O. nubilalis* (*1*, *9*), we were intrigued to know whether the same family of chemicals (trifluoromethyl ketones) could also affect the chemical communication of other important pests, such as the codling moth, whose major pheromone component does not contain an ester moiety. Trifluoromethyl ketones are known to inhibit the antennal esterase responsible for the catabolism of pheromone acetates (*12*, *13*, *19*), but there are no reports on the effect on other PDEs.

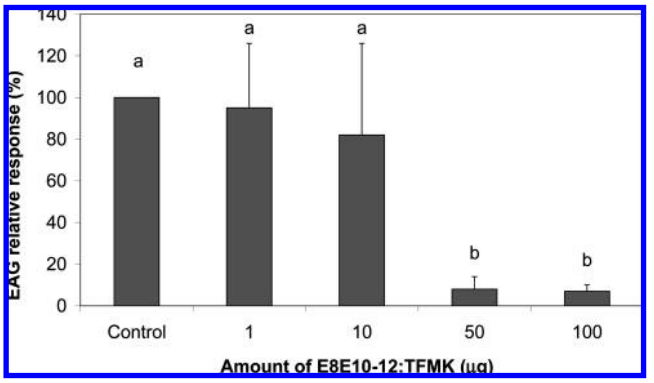

Figure 2. Mean [±standard deviation (SD)] EAG responses of *C. pomonella* male antennae ($N = 20$) to codlemone after 2 h of exposure in a Petri dish to vapors of different amounts of (*E*,*E*)-8,10-dodecadienyl trifluoromethyl ketone absorbed on a filter paper relative to the control ($N = 20$). Bars with the same letter are not significantly different (LSD test, $p < 0.05$).

Electrophysiology. (*E*,*E*)-8,10−12:Trifluoromethyl ketone elicited on male antennae an electrophysiological response of 42 ± 11% in comparison to codlemone. This is in line with the lower but significant activity displayed by other antagonists of the pheromone, such as codlemone acetate (*20*). In presaturation experiments using several amounts of the fluorinated ketone, antennae of treated males displayed lower EAG depolarization responses to the pheromone than those of untreated insects, with the effect being dose-dependent. Vapors of 1 and 10 μg of the antagonist induced only a small, non-significant inhibition of the pheromone response. However, doses of 50 and 100 μg of the chemical elicited a significant 92−93% lower EAG response to the pheromone (**Figure 2**). For comparison purposes, control insects that had been handled similarly to treated males in the absence of ketone displayed virtually identical EAG responses to regular insects (2.1−2.7 ± 0.6 versus 2.4 ± 0.6 mV, respectively). Similar results were previously reported by Renou et al. (*10*), who found that *S. littoralis* males that had been exposed to air loaded with 3-octylthiotrifluoropropan-2-one displayed a decreased EAG amplitude and an increased repolarization time in response to the pheromone. The authors also found that 3-octylthiotrifluoropropan-2-one reduced the amplitude of the EAG responses to (*Z*)-11-hexadecenol, a known inhibitor of the main pheromone compound of *M. brassicae*. In all cases, the effects were reversible.

Wind Tunnel. In the presence of codlemone, over 90% of males took flight and 61% of them contacted the source. However, when males were allowed to fly toward a lure containing mixtures of codlemone and the fluorinated ketone, they experienced a clear disruptive effect on all types of behavior. Thus, in the presence of a 1:1 mixture of codlemone/trifluoromethyl ketone only 53% of males displayed wing fanning, 45% flew $^1/_3$ of the length of the tunnel, and 20% contacted the source. This disruptive effect was directly correlated with the amount of antagonist present in the

lure, and thus, in the presence of 1:5 and 1:10 codlemone/antagonist mixtures, only 7−11% of males passed $^2/_3$ the length of the tunnel and 2% successfully contacted the source (**Figure 3**). A similar type of effect in a wind tunnel has also been induced by trifluoromethyl ketones on other insects, such as *S. littoralis* (*8*), *S. nonagrioides* (*8*, *18*), and *O. nubilalis* (*9*).

A number of flight tracks of males flying toward the pheromone and 2:1 pheromone/antagonist were also recorded. Tracks in the presence of the antagonist showed profound differences compared to those of males flying toward codlemone alone (**Table 1**). Track and course angles were remarkably higher in insects flying in plumes containing the antagonist than in codlemone alone (24.2 ± 6° versus 87.2 ± 4.6°, respectively, for the track angle and 8.2 ± 3.3° versus 32 ± 12°, respectively, for the course angle), indicating a much more directed flight toward the source in the absence of antagonist. The drift angle (difference between the course and the track angle) was also significantly higher. In the presence of antagonist, males flew 1.5-fold longer distances than control insects, although they did not take longer to reach the source. Consequently, insects increased their ground speed (121 ± 15 versus 97 ± 8 cm/s of control insects) to compensate for the casting movement induced by the antagonist (**Table 1**).

PDE Inhibition. In base to previous reports, we assumed that the most likely metabolic products of codlemone could be the aldehyde and the acid. Thus, Prestwich and co-workers (*21*, *22*) detected the corresponding carboxylic acid in metabolism studies of aldehyde pheromones of *Heliothis* sp. In addition, degradation of (*Z*)-11-tetradecenyl acetate, the major component of the pheromone of the *Z* strain of *O. nubilalis*, was reported to occur through hydrolysis to the corresponding alcohol followed by oxidation to the acid probably through the action of an alcohol oxidase (*23*).

To develop a reliable procedure for inhibition of the PDE, incubation of antennal extracts with the antagonist was tested under different experimental conditions (concentration of the substrate, time and temperature of incubation, co-solvent, type of buffer, and pH). The optimal conditions were use of MeCN as a co-solvent in amounts ≤4%, the presence of NAD and phosphate buffer at pH 6, preincubation of the ketone for 30 min, and incubation of the antennal extracts for 90 min. Degradation of codlemone against incubation time followed an exponential decaying curve, with the following relative amounts of pheromone remaining: 72.9% of pheromone at 0 min, 80.2% of pheromone at 10 min, 45.5% of pheromone at 30 min, 25.1% of pheromone at 60 min, 11.1% of pheromone at 90 min, and 2.2% of pheromone at 180 min (**Figure 4**). It is interesting to note that immediately after the addition of the substrate ca. 27% of pheromone was not extracted from the system, whereas in a blank experiment in the absence of enzyme extraction of the pheromone was almost complete. A fast binding of the substrate (bombykol) with the pheromone-binding protein (PBP) of *Bombyx mori* has been noted by Leal et al. (*24*), who determined the half-life for the uptake of pheromone *in vivo* as ca. 1 ms in base to the high concentration of PBP in the sensillar lymph (10 mM) (**Figure 4**). The presence of the possible catabolic products of the pheromone [(*E*,*E*)-8,10-dodecadienal and (*E*,*E*)-8,10-dodecadienoic acid; see above] was checked by GC and GC−MS in comparison to authentic samples. However, because these compounds were only detected in very minute amounts (ca. 1% relative to the nondegraded pheromone) at incubation times up to 180 min, the extent of the inhibition was determined in base to the amount of the

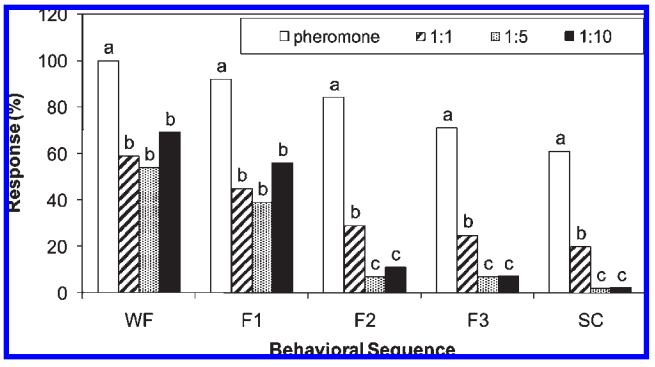

Figure 3. Behavioral responses of *C. pomonella* males flying toward a source baited with mixtures of codlemone and (*E*,*E*)-8,10-dodecadienyl trifluoromethyl ketone in 1:1 (*N* = 51), 1:5 (*N* = 46), and 1:10 (*N* = 45) ratios with regard to codlemone alone (*N* = 79) in the wind tunnel. Means within a specific behavior followed by the same letter are not significantly different (2×2 χ^2 homogeneity test, $p < 0.05$). Behavioral responses are wind fanning (WF), flying upwind over $^1/_3$ (F1), $^2/_3$ (F2), or $^3/_3$ (F3) the length of the tunnel, and source contact (SC).

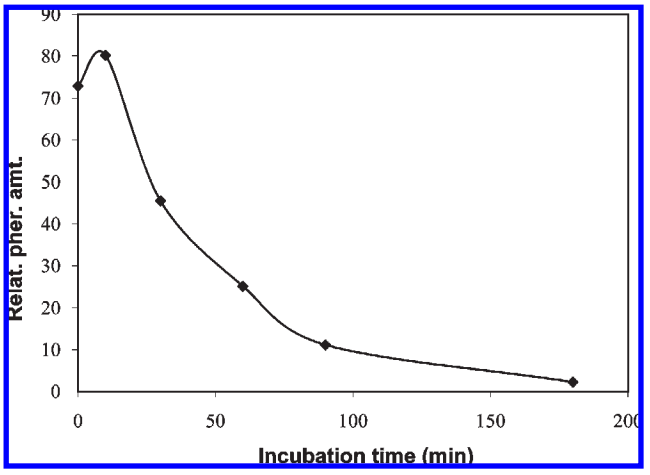

Figure 4. Effect of the incubation time on the pheromone catabolism of the codling moth *C. pomonella*.

Table 1. Selected Parameters from Tracks of *C. pomonella* Males Flying Upwind Toward an Attractant Source Containing a 2:1 Mixture of Codlemone and (*E*,*E*)-8,10-Dodecadienyl Trifluoromethyl Ketone Relative to Pheromone Alone[a]

parameter	codlemone (*N* = 5)	2:1 codlemone/trifluoromethyl ketone (*N* = 4)
total flight distance (cm)	227 (17.6) a	340.5 (63.5) b
total flight duration (s)	7.1 (0.4) a	8.05 (1.1) a
ground speed (cm/s)	97 (8) a	121 (15) b
turning frequency (turns/s)	11.6 (2.45) a	4.75 (0.5) b
track angle (degrees)	24.2 (6) a	87.2 (4.6) b
drift angle (degrees)	16 (4.5) a	55.2 (8.5) b
course angle (degrees)	8.2 (3.3) a	32 (12) b

[a] Means [±standard error of the mean (SEM)] within a row followed by different letters are significantly different (LSD test, $p < 0.05$).

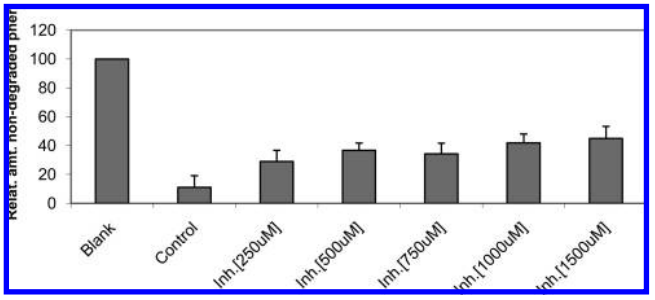

Figure 5. PDE inhibition by (*E*,*E*)-8,10-dodecadienyl trifluoromethyl ketone (average ± SD of 2−3 replicates) in acetonitrile at pH 6 and in the presence of NAD as a co-factor. Blank experiment, no enzyme; control, enzyme present but no antagonist. Preincubation period of the antagonist, 30 min; incubation period with the enzyme extract, 90 min.

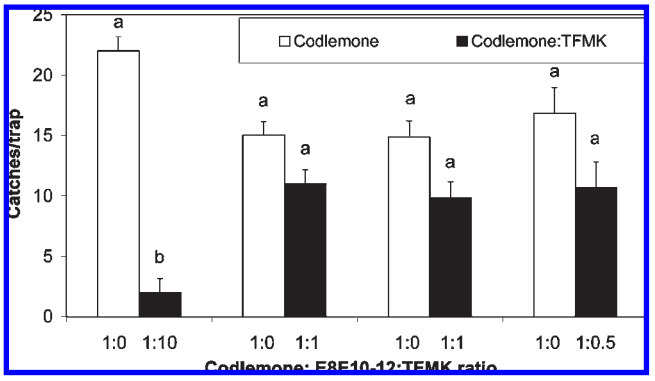

Figure 6. Mean (±SD) number of catches of *C. pomonella* males per trap baited with codlemone and mixtures of codlemone and (*E*,*E*)-8,10-dodecadienyl trifluoromethyl ketone in 1:10, 1:1, and 1:0.5 ratios in field trials conducted in 2006 (3−6 replicates per formulation). The amount of codlemone in each trap was 100 μg. Within the same trial, bars with the same letter are not significantly different (Student's *t* test, $p < 0.05$).

remaining nondegraded pheromone relative to the internal standard (*n*-decanol).

The potential antagonist was not a good inhibitor of the PDE because only 20.3% inhibition was recorded at 250 μM, 30.6% at 500 μM, etc. and only at exceedingly high concentrations (over 1 mM) did the amount of nondegraded pheromone approach 50% (**Figure 5**). These results suggest that oxidase activity in the antenna is not the only factor involved in the behavioral response of the male. The lack of *in vitro* activity of the chemical may be due to the fact that the trifluoromethyl ketone moiety is not sufficiently electrophilic to produce a stable tetrahedral adduct with a cysteine residue of the active enzyme. This mechanism has been postulated to occur with highly reactive cyclopropanones as inhibitors of the aldehyde dehydrogenase of *H. virescens* (*21*).

The metabolism of pheromone esters and pheromone aldehydes has been studied in a variety of insects (*7*, *25*). However, metabolism of pheromone alcohols has received little attention. Using [^{3}H]-labeled bombykol and homologous fatty alcohols applied over male antennae, Kasang (*26*) discovered that the major component of the silkworm moth pheromone was converted to long-chain fatty acids and fatty acid esters. The degradation was sensitive to temperature, suggesting catalysis by a dehydrogenase. Similarly, degradation of the tritiated pheromone of the European corn borer (*Z*)-11-tetradecenyl acetate occurred through hydrolysis to the alcohol followed by conversion to tetradecenoic acid, possibly by an alcohol oxidase, which in turn was degraded via β-oxidation to carbon dioxide and water (*23*). An interesting case was the catabolism of

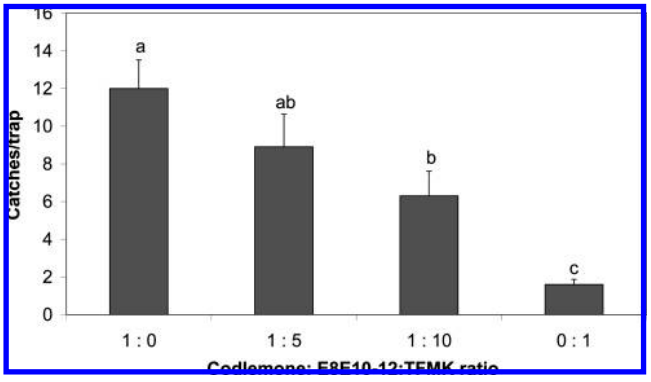

Figure 7. Mean (±SD) number of catches of *C. pomonella* males per trap baited with mixtures of codlemone and (*E*,*E*)-8,10-dodecadienyl trifluoromethyl ketone in 1:0, 1:5, 1:10, and 0:1 ratios in field trials conducted in 2007 and 2008 (5 replicates per formulation each year). The amount of codlemone in each trap was 100 μg. The results of the 2 years are combined because the interaction treatment−year was not significant. Bars with the same letter are not significantly different (Tukey's test, $p < 0.05$).

(*Z*,*Z*)-3,6-dodecadienol, the trail pheromone of *Reticulitermes flavipes*, which did not degrade to the corresponding carboxylic acid but underwent a ω-oxidation to the 1,12 diol, probably through the action of a monooxygenase (*27*).

Field Tests. The activity of the antagonist in the field was evaluated by comparing the number of insects caught with blends of codlemone/trifluoromethyl ketone relative to those trapped with codlemone alone. In 2006, although fewer catches than the pheromone were caught with 1:0.5 and 1:1 (this formulation tested in two different fields) blends, the difference was only significant when the baits contained the 1:10 pheromone/antagonist mixture (22.0 ± 0.4.6 insects/trap caught with the pheromone versus 2.0 ± 1.2 with the blend) (**Figure 6**). New experiments with 1:0, 1:5, 1:10, and 0:1 codlemone/antagonist formulations were also implemented in 2007 and 2008. In these trials, the number of catches were quite low, and because the interaction treatment−year was not significant ($F = 1.3$; df = 3.28; $p = 0.29$), the number of catches per trap and year were combined. Here, again, the 1:10 blend caught a significantly lower number of moths (6.3 ± 1.3) than codlemone alone (12.0 ± 1.5). The antagonist was not attractive to codling moth males when used alone (1.6 ± 0.3 insects/trap) (**Figure 7**).

Pheromone antagonists have been used to control pests in mating disruption experiments (*17*). In general, these chemicals are analogues of the natural pheromones that prevent heterospecific attraction, and in some cases, they have resulted in more potent communication disruptants than the pheromone itself (*28*). In previous papers, we have reported that trifluoromethyl ketones can be effective *in vivo* pheromone antagonists in the field for a number of insect pests (*1*, *9*, *18*). Particularly remarkable is the reduction in damage caused in maize fields by the Mediterranean corn borer and the European corn borer by (*Z*)-11-hexadecenyl trifluoromethyl ketone, an analogue of the major component of the Mediterranean corn borer pheromone (*1*). We have shown here that (*E*,*E*)-8,10-dodecadienyl trifluoromethyl ketone can be an effective pheromone antagonist of the codling moth in the laboratory and in the field and, therefore, a putative candidate in future strategies to control the pest. In this context, it is worth noting the low toxicity displayed by trifluoromethyl ketones. Administration of (*Z*)-11-hexadecenyl trifluoromethyl ketone and 3-octylthiotrifluoropropan-2-one, a general esterase inhibitor, on Swiss mice elicited a LD_{50} of 1 g/kg body weight after

6 days of treatment, whereas the parent pheromone (*Z*)-11-hexadecenyl acetate induced a LD_{50} of 5 g/kg body weight (*18*). These data agree with the report of Ashour and Hammock (*29*), in which doses of up to 250 mg/kg body weight of some fluorinated ketones caused no mortality on Swiss-Webster mice over a period of 3 months. In addition and in aquatic ecotoxicity studies, we have found that the toxicity of (*Z*)-11-hexadecenyl trifluoromethyl ketone was only moderate, with EC_{50} values ranging from 3.11 to 103.7 mg/L in algae growth and from 0.07 to 1.2 mg/L in *Daphnia* survival (*30*). The low toxicity of these chemicals is likely due to their reversible inhibition mechanism in contrast to other much more toxic irreversible inhibitors of carboxylesterases and proteases.

ACKNOWLEDGMENT

We gratefully acknowledge Catalina Perelló for preliminary inhibition assays and Dr. Jesús Avilla for technical advice.

Supporting Information Available: Table containing details of field trials conducted to test the antagonist effect of (*E*,*E*)-8,10-dodecadienyl trifluoromethyl ketone. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received June 10, 2009. Revised manuscript received July 27, 2009. Accepted July 29, 2009. We gratefully acknowledge CICYT (AGL2006-13489-C02-01 and AGL2007-62366) for financial support.