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Antagonism of Pheromone Response of *Ostrinia nubilalis* Males and Implications on Behavior in the Laboratory and in the Field

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The antagonistic effect on the pheromone response and catabolism of male European corn borers, *Ostrinia nubilalis*, by several trifluoromethyl ketones is reported. (*Z*)-11-Tetradecenyl trifluoromethyl ketone (Z11-14:TFMK), the most closely related analogue of the main component of the pheromone, elicits a remarkable disruptive effect on close approach and source contact of males flying to a source baited with mixtures of the pheromone and the antagonist in 5:1 and 10:1 ratios. In this experiment, the male displayed an erratic flight track with frequent counter turns and intersections with the plume. In the field, the TFMK significantly lowered the number of males caught when mixed with the pheromone in a 10:1 ratio in comparison with the natural attractant. The compound was also a good inhibitor of the antennal esterase of the insect with a IC_{50} value of 0.28 μM. The homologous (*Z*)-10-tridecenyl trifluoromethyl ketone, with one carbon less in the chain, also elicited an antagonistic effect in the wind tunnel, but in the field, the results were not conclusive. The effect induced was lower than the one displayed by Z11-14:TFMK including the activity as the esterase inhibitor (IC_{50} value of 7.55 μM). The saturated tetradecyl trifluoromethyl ketone, tetradecyltrifluoropyruvamide, and (*Z*)-11-2-thiatetradecenyl trifluoromethyl ketone resulted completely inactive. The results obtained in conjunction to the previously shown low toxicity to mice by related trifluoromethyl ketones provide new important data for the putative utilization of these chemicals as new pest control agents.

KEYWORDS: Pheromone antagonism; esterase inhibition; *Ostrinia nubilalis*; trifluoromethyl ketones; European corn borer; wind tunnel; field tests

INTRODUCTION

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), is a major pest of maize and other crops, such as potato, green pepper, and winter wheat, in Europe, North America, North of Africa, the Philippines, and Japan (*1*). Control of this species is particularly difficult because insecticide sprays are only effective during the short period that elapses between egg hatching and young larvae boring into the stems. The species displays polymorphism in the pheromone communication system, i.e., different populations utilize different compounds or different proportions of the same compound. Despite the two different populations, ECB shows a successful species isolation, and in areas where both strains are present, there is enough genetic compatibility between them to produce fertile hybrids (*2*). Hybridization also takes place readily in the laboratory, and a great effort has been devoted to study the genetic basis of pheromone production, perception, and response (*2*−*5*). The Z strain uses a blend of (*Z*)-11-tetradecenyl acetate (Z11-14:Ac) and (*E*)-11-tetradecenyl acetate (E11-14:Ac) in a 97:3 ratio (*6*), whereas the E strain utilizes the same compounds in blends ranging from 1:99 to 4:96 ratios (*7*, *8*).

Trifluoromethyl ketones (TFMKs) are known to inhibit a number of esterases and proteases, such as acetylcholinesterase, chymotrypsin, or human liver carboxylesterases (*9*, *10*), or particularly the antennal esterases present in insect olfactory tissues (*9*, *11*−*13*). These are key enzymes for a rapid degradation of pheromone esters, thus maintaining a low stimulus noise level in sensory hairs (*14*, *15*). The mode of action of TFMKs has been explained in terms of the formation of a stable hemiacetal of tetrahedral geometry with the serine residue of the enzyme (*16*, *17*). These chemicals have elicited significant reduction of the EAG pheromone responses on

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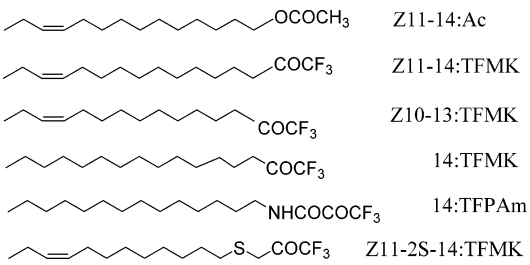
Chart 1. List of Chemicals Tested as Putative Inhibitors of the Pheromone Response and Catabolism of *O. nubilalis*



Spodoptera littoralis (SL), *Mamestra brassicae*, and *Heliothis zea* (*18*) and of single sensillum responses to the pheromone of SL (*18*) and *Antheraea polyphemus* (*19*). In a wind tunnel, TFMKs have been found to disrupt the orientation flight of SL and *Sesamia nonagrioides* (SN) males to pheromone sources (*20*). In the field, Z11-16:TFMK, a closely related analogue of the pheromone, elicited on SN males a significant decrease in the number of catches in traps baited with mixtures of the inhibitor and the pheromone in comparison with the pheromone alone (*21, 22*). To investigate the effect of this type of chemical on other economically important pests, we present herein the activity of (*Z*)-11-tetradecenyl trifluoromethyl ketone (Z11-14:TFMK), (*Z*)-10-tridecenyl trifluoromethyl ketone (Z10-13:TFMK), tetradecyl trifluoromethyl ketone (14:TFMK), tetradecyltrifluoropyruvamide (14:TFPAm), and (*Z*)-11-2-thiatetradecenyl trifluoromethyl ketone (Z11-2S-14:TFMK) (**Chart 1**) in wind tunnel bioassays and in the field, as well as on the antennal esterases present in extracts of the Z strain of the insect.

MATERIALS AND METHODS

Chemicals. Z11-14:Ac was obtained by acetylation of (*Z*)-11-tetradecenol (Aldrich Chemical Co., 95% purity), and E11-14:Ac (97% purity) was purchased from-Sigma Chemicals Ltd. and used directly as received. The solvents (trace analysis quality) were from Fluka-Riedel-de Haën.

Z11-14:TFMK. This compound was obtained by reaction of the corresponding iodide (*23*) with *tert*-butyllithium and ethyl trifluoroacetate, as previously described by us (*24*). IR (film): υ 3010, 2932, 1771, 1541, 1203, 1142 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.34 (m, 2H), 2.70 (t, J = 7.2 Hz, 2H), 2.03 (m, 4H), 1.67 (m, 2H), 1.27 (br, 14H), 0.95 (t, J = 7.5 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.65 (q, J = 35 Hz), 131.53, 129.28, 115.57 (q, J = 290 Hz), 36.36, 29.74, 29.48, 29.33, 29.24, 29.16, 28.72, 27.07, 22.36, 20.50, 14.39 ppm. ^{19}F NMR ($CDCl_3$): δ −79.91 (s) ppm. MS *m*/*z* (%): 292 (M^+, 18), 223 (20), 97 (60), 83 (71), 69 (92), 55 (100).

14:TFMK. This compound was also obtained as previously described (*24*). IR (film): υ 2928, 1762, 1210, 1154 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.70 (t, J = 7.2 Hz, 2H), 1.67 (m, 2H), 1.25 (br, 22H), 0.87 (t, J = 8.7 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.68 (q, J = 34 Hz), 115.58 (q, J = 291 Hz), 36.36, 31.91, 29.66, 29.63, 29.60, 29.52, 29.34, 29.16, 28.73, 22.68, 22.37, 14.10 ppm. ^{19}F NMR ($CDCl_3$): δ −79.87 (s) ppm. MS *m*/*z* (%): 294 (M^+, 0.4), 225 (24), 97 (65), 83 (70), 57 (100), 69 (80).

(*Z*)-10-Tridecenyl Trifluoromethyl Ketone. This compound was obtained starting from (*Z*)-11-tetradecenol after oxidation to the corresponding carboxylic acid.

(Z)-11-Tetradecenoic Acid. In a round-bottomed flask containing (*Z*)-11-tetradecenol (400 mg, 1.88 mmol), pyridinium dichromate (4.96 g, 13.2 mmol) in anhydrous dimethyl formamide (30 mL) was added at 0 °C. The mixture was magnetically stirred overnight at room temperature, cooled, and quenched by the addition of water (75 mL). The organic material was extracted with ether (5 × 30 mL), the organic phase was washed with water (6 × 50 mL) and dried ($MgSO_4$), and the solvent was stripped off. The residue was purified by column chromatography on silica gel eluting with hexane−ether mixtures to provide the corresponding carboxylic acid (369 mg, 86% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.32 (m, 2H), 2.3 (t, J = 7.2 Hz, 2H), 2.0 (m, 4H), 1.63 (m, 4H), 1.26 (s, 10H), 0.93 (t, J = 7.4 Hz, 3H) ppm.

(Z)-10-Tridecenyl Trifluoromethyl Ketone. To a solution of (*Z*)-11-tetradecenoic acid (0.36 g, 1.61 mmol) in anhydrous CH_2Cl_2 (5.5 mL), cooled to 0 °C, a 2 M solution of oxalyl chloride in CH_2Cl_2 (2.42 mL, 4.84 mmol) (*25*) was added under Ar. The mixture was stirred at room temperature for 3 h. The solvent was stripped off under anhydrous conditions, the acid chloride was taken up in anhydrous ether (5 mL), and the solution was cooled again to 0 °C. Then, trifluoroacetic acid (1.34 mL, 9.67 mmol) and anhydrous pyridine (1.04 mL, 12.9 mmol) were added. The reaction mixture was stirred at room temperature for 1.5 h and cooled to 0 °C, and water (30 mL) was slowly added so that the temperature was kept below 10 °C. The organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The organic phase was washed with water, dried ($MgSO_4$), and concentrated under vacuum to leave a residue, which was purified by flash column chromatography on SiO_2 to afford the expected ketone (234 mg, 52% yield). IR (film): ν 3005, 1764 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.27 (m, 2H), 2.64 (t, J = 7.2 Hz, 2H), 1.94 (m, 4H), 1.61 (m, 2H), 1.22 (s, 14H), 0.89 (t, J = 7.5 Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.6 (q, J = 35 Hz), 131.5, 129.2, 115.6 (q, J = 290 Hz), 36.3, 29.7, 29.4, 29.3, 29.2, 29.1, 28.7, 14.3 ppm. ^{19}F NMR (282 MHz, $CDCl_3$): δ −79.87 (s) ppm. MS *m*/*z* (%): 278 (M^+, 13), 209 (10), 97 (34), 69 (100).

N-Tetradecyltrifluoropyruvamide. This compound was prepared in a two-step process from N-tetradecylamine (*26*).

N-Tetradecylisonitrile. To a solution of NaOH (0.726 g) in water (0.9 mL) was slowly added a solution of tetraethylammonium bromide (0.042 g, 0.2 mmol) and N-tetradecylamine (0.92 g, 2.343 mmol) in chloroform (10 mL). The reaction was stirred at room temperature overnight. Water was then added, the organic phase was decanted, and the aqueous layer was extracted with chloroform (5 × 15 mL). The combined organic phases were washed with water, dried ($MgSO_4$), and concentrated to leave a residue, which was chromatographed in neutral alumina (act. II) eluting with hexane:ethyl acetate 97:3. The expected isonitrile was obtained in pure form (0.247 g, 47% yield). IR (film): ν 2925, 2854, 2147, 1463, 1376, 1353 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.37 (tt, J_1 = 6.6 Hz, J_2 = 1.9 Hz, 2H), 1.63 (m, 2H), 1.25 (br, 22H), 0.87 (t, J = 6.5 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$): δ 155.85, 41.54, 31.88, 29.63, 29.60, 29.55, 29.46, 29.32, 29.07, 28.66, 26.27, 22.64, 14.06 ppm. Elemental analysis: calcd for $C_{15}H_{29}N$: C, 80.65; H, 13.08; N, 6.27. Found: C, 80.70; H, 13.15; N, 6.27.

N-Tetradecyltrifluoropyruvamide. To a solution of N-tetradecylisonitrile (100 mg, 0.44 mmol) in CH_2Cl_2 (1.5 mL) was added, under Ar at −78 °C, trifluoroacetic anhydride (74 μL, 0.53 mmol) freshly distilled. The mixture was stirred at this temperature for 90 min, water was added (5 mL), and the mixture was left to warm to room temperature. The organic phase was decanted, and the aqueous layer was extracted with CH_2Cl_2 (4 × 10 mL). The combined organic phases were washed with water, dried ($MgSO_4$), and concentrated to leave a residue, which was purified by recrystallization in hexane to give the expected product as a white solid. The compound was a mixture of the keto and hydrate forms in 4:96 ratio; mp 105−108 °C. IR (film): ν 3313, 2920, 2850, 1681, 1644, 1557, 1475, 1204, 1182 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.10 (br, 3H), 3.33 (dd, J_1 = 13.1 Hz, J_2 = 7.0 Hz, 2H), 1.55 (m, 2H), 1.25 (br, 22H), 0.88 (t, J = 6.8 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$): δ 165.65, 121.69 (q, J_{C-F} = 285.7 Hz), 90.49 (q, J_{C-F} = 33.1 Hz), 40.69, 31.92, 29.64, 29.52, 29.44, 29.34, 29.12, 29.06, 26.60, 22.67, 14.06 ppm. ^{19}F NMR ($CDCl_3$): δ −76.9 (s), −84.5 (s) ppm. MS *m*/*z* (%): 318 (23), 240 (31), 155 (18), 85 (65), 71 (85), 57 (100). Exact mass: calcd for $C_{19}H_{34}F_3NO_2$, 337.2199; found, 337.2229.

Z11-2S-14:TFMK. This compound was prepared from 9-dodecyn-1-thiol in a three-step process.

9-Dodecyn-1-thiol. This compound was obtained following a similar procedure to that previously described (*27*). Thus, a solution of 9-dodecyn-1-ol (0.941 g, 5.16 mmol), tosyl chloride (2.947 g, 15.5 mmol), and anhydrous pyridine (12 mL) was placed in a round-bottomed flask and left in the refrigerator for 12 h. The solution was acidified with 0.1 N HCl and extracted with hexane. The combined organic phases were washed with brine and dried ($MgSO_4$), and the

solvent was removed to afford the corresponding tosylate (1.562 g, 90% yield), which was used directly in the next step without further purification. The tosylate (0.828 g, 2.46 mmol) was added to a solution of potassium ethyl xanthogenate (0.610 g, 3.69 mmol) in acetone (9 mL), and the mixture was refluxed for 30 min. The solution was then allowed to cool to room temperature, the precipitated potassium salt was filtered out, and the solvent was evaporated. The residue was taken up in chloroform, and the organic phase was washed with brine, dried ($MgSO_4$), and evaporated at reduced pressure. The crude ester was decomposed at room temperature to the corresponding thiol by stirring for 30 min in the presence of ethylenediamine (4 mL). The solution was acidified with 0.1 N HCl and extracted with hexane. The combined organic phases were washed with brine and dried ($MgSO_4$), and the residue was chromatographed on silica gel eluting with hexane to obtain pure thiol (0.371 g, 76% yield). IR (film): υ 3421, 2929, 1457, 1217 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.50 (q, $J = 7.4$ Hz, 2H), 2.13 (m, 4H), 1.59 (m, 2H), 1.29 (br, 10H), 1.09 (t, $J = 7.4$ Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 81.59, 79.45, 33.98, 29.06, 28.98, 28.91, 28.72, 28.28, 24.60, 18.68, 14.36, 12.38 ppm. MS *m*/*z* (%): 81 (36), 68 (84), 67 (100).

2-Thiatetradec-11-ynyl Trifluoromethyl Ketone. To a solution of 9-dodecyn-1-thiol (0.200 g, 1.01 mmol) in anhydrous CH_2Cl_2 (10 mL) was added diisopropylethylamine (0.17 mL, 1.01 mmol) and 3-bromo-1,1,1-trifluoropropan-2-one (0.955 g, 5.04 mmol) (*13*). The mixture was stirred at room temperature for 4 h. The solvent was eliminated at reduced pressure, and the residue was directly purified by column chromatography on silica gel eluting with hexane:ether 90:10 to afford the expected acetylenic trifluoromethyl ketone (0.239 g, 77% yield) as a mixture of ketone and hydrate in a 30:70 ratio. IR (film): υ 3438, 2933, 1746, 1185 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 4.13 (s, 2H), 3.46 (s, 2H), 2.87 (s, 2H), 2.69 (t, $J = 7.5$ Hz, 2H), 2.49 (t, $J = 7.5$ Hz, 2H), 2.12 (m, 4H), 1.57 (m, 2H), 1.35 (br, 10H), 1.09 (t, $J = 7.5$ Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.03 (q, $J = 34$ Hz), 122.9 (q, $J = 285$ Hz), 115.49 (q, $J = 291$ Hz), 92.33 (q, $J = 32$ Hz), 81.65, 79.46, 36.41, 34.73, 33.54, 31.89, 29.25, 29.00, 28.94, 28.91, 28.67, 28.53, 28.50, 28.47, 18.63, 14.30, 12.35 ppm. ^{19}F NMR ($CDCl_3$): δ −76.27 (s), −85.92 (s) ppm. MS (CI, NH_3) *m*/*z* (%): 326 [$(M + 18)^+$, 100], 309 [$(M + 1)^+$, 4], 197 (49).

(Z)-11-2-Thiatetradecenyl Trifluoromethyl Ketone. In a pressure flask was placed a suspension of the previous acetylenic ketone (0.200 g, 0.65 mmol) and PtO_2 (20 mg, 0.08 mmol) in ethanol (5 mL). The mixture was stirred at room temperature under a 2.5 bar pressure of hydrogen for 4 h. The catalyst was then filtered over Celite and washed thoroughly with hexane, and the solvent was evaporated under vacuum to give, after purification by column chromatography on silica gel eluting with hexane:ether 90:10, the trifluoromethyl ketone (176 mg, 87% yield) as a mixture of ketone and hydrate in a 45:55 ratio. IR (film): υ 3423, 3005, 2928, 2854, 1746, 1182 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 5.34 (m, 2H), 3.87 (s, 2H), 3.48 (s, 2H), 2.90 (s, 2H), 2.71 (t, $J = 7.2$ Hz, 2H), 2.51 (t, $J = 7.5$ Hz, 2H), 2.03 (m, 4H), 1.59 (m, 2H), 1.29 (m, 10H), 0.95 (t, $J = 7.5$ Hz, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 185.06 (q, $J = 34$ Hz); 131.58, 129.21, 122.90 (q, $J = 284$ Hz), 115.52 (q, $J = 290$ Hz), 92.36 (q, $J = 32$ Hz), 36.39, 34.77, 33.60, 31.94, 29.69, 29.33, 29.30, 29.15, 29.08, 29.04, 28.61, 28.57, 28.52, 27.03, 20.48, 14.36 ppm. ^{19}F NMR ($CDCl_3$): δ −76.26 (s), −85.96 (s) ppm. MS *m*/*z* (%): 241 (1.8), 199 (79), 69 (71), 55 (100). Elemental analysis: calcd for $C_{15}H_{25}F_3OS$: C, 58.04; H, 8.12; F, 18.36; S, 10.33. Found: C, 58.11; H, 8.15; F, 18.34; S, 10.46.

Selection and Rearing of a Z Strain in the Laboratory. Because preliminary analytical studies of gland extracts of wild females pointed out the possible presence of hybrids, to establish a "pure" reliable Z race in the laboratory, 12 wild mated females were allowed to hatch in the laboratory. The resulting progenies were isolated, and between 6 and 12 females of each progeny were chosen to study the chemical composition of the pheromone. Gland analysis was carried out individually on 3 day old virgin females between the 3rd and 4th h into the scotophase. The ovipositor was cut and transferred into a conical glass vial containing 10 μL of hexane and 10 ng of dodecyl acetate as internal standard. The vial was sealed, and after extraction for 30 min at room temperature, the solid tissue was removed and the extract was concentrated under nitrogen to a 2−5 μL volume for gas chromatography (GC) analysis. Analyses were carried out in a Thermo Quest GC Trace 2000 gas chromatograph, fitted with a splitless sample injector, a flame ionization detector (FID), and a SP-2300 (60 m × 0.25 mm i.d.) fused silica capillary column (Supelco, Sigma-Aldrich Co.). The chromatographic conditions were as follows: column flow, 0.5 mL He/min; column temperature, 80 °C for 2 min followed by a program of 5 °C/min up to 180 °C, which was maintained for 10 min. These conditions led to a full resolution of the Z and E isomers (retention times 18.4 and 18.9 min for E11-14:Ac and Z11-14:Ac, respectively). Out of 120 females analyzed, 118 belonged to the Z strain, one to the E strain, and one appeared to be hybrid. The progenies of these two females were discarded.

The pure Z strain was reared following a typical artificial diet for Noctuidae (*28*) to which the following chemicals were added, nipagin (methyl-4-hydroxybenzoate) (Fluka Biochemika) (0.12%), flumidil (potassium o-oxyquinolinsulfonate + sulfonylamidotiazol) (Kessler Ibérica, S. L.) (0.12%), and aureomicin (chlortetracycline hydrochloride) (Fluka Biochemika) (0.04%). Pupae were sexed and placed in cylindrical boxes (12 cm height × 17 cm diameter) in a climatic chamber with a 16:8 L:D photoperiod at 25 ± 1 °C and 65 ± 10% relative humidity until emergence. Adults were provided with a 10% sucrose solution soaked on a cotton pad, separated daily by age, and kept on a filter paper in plastic containers until use. For mating, couples were transferred to mating cages (17 cm diameter × 11.5 cm height) containing a waxed paper around the walls to allow females to lay eggs.

Esterase Inhibition Assays. Inhibition bioassays were carried out according to the methodology already described by us (*17*). Two day old males *O. nubilalis* were anesthetized with CO_2, and their antennae were removed. The antennae were immediately frozen in liquid nitrogen and kept at −80 °C until use. Crude antennal esterase preparations were obtained by homogenizing batches of frozen antennae in 100 mM phosphate buffer solution (pH 7.4) on a variable speed mixer (Heidolph ZZR-2000) at 680 rpm for 5 min in an ice bath. The contents of the tube were transferred to an Eppendorf tube, sonicated at 40 W for 10 s, and centrifuged at 3000 rpm for 5 min at 6 °C to remove the cuticular debris. In borosilicate tubes, previously treated with a saturated solution of 1-decanol in ethanol for 24 h and washed with distilled water (3×), were placed 100 μL of the extract (equivalent to three antennae) and the corresponding amount of inhibitor (2 μL of a 1.5 μM to 5 mM solution in ethanol). The solution was vortexed for 30 s and preincubated in a thermostatized bath at 28 °C for 10 min. Then, 2 μL of an ethanol solution (1 μg/μL) of Z11-14:Ac was added and incubation was continued for 1 h more under the same conditions. Previous studies had shown that this incubation period was required for a good level of hydrolysis (>75%) of Z11-14:Ac. Incubation was stopped by addition of 180 μL of hexane, the mixture was shaken, and the organic phase was separated and concentrated to 20−30 μL for GC analysis. No inhibitor was added in control experiments. Chromatographic analyses were carried out by injection of 1 μL of the above solution into a Thermo Quest Trace 2000 gas chromatograph, equipped with a split-splitless injector system, FID, and a HP-5 (25 m × 0.2 mm i.d.) fused silica capillary column. The chromatographic conditions used were as follows: injection at 80 °C for 2 min and program of 10 °C/min up to 280 °C, which was maintained for 5 min. The carrier gas was helium at a flow of 1 mL/min. Under these conditions, Z11-14:Ac and Z11-14:OH showed retention times of 15.00 and 13.57 min, respectively. The inhibition degree of the chemicals was calculated as the percentage of the relative decrease of hydrolysis in the presence of inhibitor in relation to the mean values of hydrolysis in control experiments, according to the formula:

$$\%\ \text{inhibition} = \left(1 - \frac{\%\ \text{hydrolysis with inhibitor}}{\%\ \text{hydrolysis in control}}\right) \times 100$$

The IC_{50} of each compound was calculated by least squares regression analyses in duplicate experiments considering the following doses of inhibitor: 10, 100, 500, 1000, and 2000 ng for Z11-14:TFMK and 100, 1000, 2000, and 5000 ng for Z10-13:TFMK.

Wind Tunnel Experiments. Assays were conducted in a glass tunnel of 180 cm × 50 cm × 50 cm as previously described (*29*). The wind

was pushed through the tunnel by a 30 cm diameter fan at 30 cm/s. The active space of the pheromone was viewed with the aid of a $TiCl_4$ smoke dispenser to ensure that most of the insect flight took place within the plume boundaries. The tunnel was illuminated with two red light fluorescent tubes dimmed to 0.75 lux. The temperature was maintained at 23 ± 1 °C, and the relative humidity was 65 ± 5%. A video camera Pulnix B/W TM50 was installed at 135 cm above the tunnel and in perpendicular position to the floor to minimize optical distortion of the flight track. The camera covered a 130 cm × 45 cm section of the tunnel, and flight tracks were recorded with a JVC-SR306E video recorder and converted to computer files at a rate of 25 frames/s. With the aid of in-house computer software, insect positions were arbitrarily converted to *X*,*Y* coordinates.

O. nubilalis males were acclimatized to the experimental conditions of the tunnel for 30 min and individually released into the tunnel between the 3rd and 4th h of the 2nd or 3rd scotophase. Before the tests, individual insects were introduced into a wire mesh cylinder (3 cm diameter, 8 cm height) and placed on a holder at 20 cm high and 125 cm distance from the emission source. After a further 20−30 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 four types of behavior were recorded: TF, taking flight; OF, oriented flight (upwind flight onto the pheromone plume and arrival to the middle of the tunnel); CA, close approach (arrival to the proximity of the lure); and SC, source contact (landing, contact with the source, and copulation attempts). In each treatment, 40 males were used and each insect was tested only once.

As control, the attractant source consisted of a rubber septum loaded with 30 μg of the pheromone blend (mixture of Z11-14:Ac and E11-14:Ac in a 97/3 ratio), whereas for the antagonism experiments the required amount of the antagonist was also added to the lure. Experiments were conducted in blocks including exposed and control insects, and statistical analyses (χ^2 homogeneity test, $P < 0.05$) were performed within every block.

Field Tests. *Heliothis* traps (Scentry, Ecogen Inc.) were deployed in maize fields of the Lleida province (Catalonia, northeast Spain) from July to October 2001−2003 to cover the most damaging 2nd and 3rd generations of the pest. Baits were prepared by dissolving 100 μg of the pheromone and the appropriate amount of the disruptant in 100 μL of hexane to obtain the required pheromone:antagonist ratio and transferring the blends to rubber septa (Sigma-Aldrich Co). The solvent was allowed to evaporate, and septa containing pheromone alone (100 μg) were used as control. The field was divided into four independent blocks, equally spaced around the field and separated ca. 50 m from each other. In every block, the traps were hung at a height of ca. 1.5 m and spaced 25 m from each other. The traps were set up in a randomized block design and revised and rotated every week. All of the data were transformed ($\sqrt{x} + 0.5$) and analyzed for significance (Student's *t*-test, $P < 0.05$). The disruptant effect of the compounds was calculated by the decrease in catches obtained with a specific formulation relative to those obtained with pheromone alone.

RESULTS

Esterase Inhibition. Two representative compounds, Z11-14:TFMK and Z10-13:TFMK, were chosen as putative esterase inhibitors. Plots of inhibition percentage vs logarithm of dose of each compound gave a straight line ($r^2 = 0.97$ for Z11-14:TFMK, $r^2 = 0.89$ for Z10-13:TFMK) from which the IC_{50} values were determined. Z11-14:TFMK exhibited an IC_{50} value of 0.28 μM, and Z10-13:TFMK displayed an IC_{50} value of 7.55 μM. For Z11-14:TFMK, the most similar analogue of the major component of the pheromone, incubation of 1 ng of this chemical on an extract equivalent to three male antennae was sufficient to inhibit the total esterasic activity of the extract by 40%.

Wind Tunnel. In previous studies, we found that 3−4 day old males were the most active and that the optimum peak of activity was during the 2nd and 3rd h into the scotophase. When males were attracted to mixtures of pheromone and Z11-14:TFMK in 1:0.05, 1:0.1, 1:1, 1:5, 1:10, and 1:20 ratios, no apparent effect on the number of TF was observed, and about 75−80% of insects were able to orient their flights to the source (**Table 1**). Nevertheless, CA and SC of males attracted to a mixture of pheromone:disruptant 1:5 and higher were significantly lower than the corresponding values for control males approaching the pheromone alone ($P < 0.05$). Thus, only 15−30% of males closely approached the source and 7−25% successfully contacted the lure. The disruptive effect was dose-dependent. The pheromone itself (control) induced activation and oriented flights to 77% of males, attracted 65% to the proximity of the lure, and led 60% of males to land (**Table 1**).

Table 1. Percentage of Behavioral Responses of *O. nubilalis* Males Flying toward a Source Baited with Mixtures of Pheromone and Z11-14:TFMK in Several Ratios in a Wind Tunnel ($N = 40$)[a,b]

	pheromone (%)	pheromone + Z11-14:TFMK ratio (%)					
		1:0.05	1:0.1	1:1	1:5	1:10	1:20
TF	77 a	80 a	82 a	82 a	85 a	75 a	82 a
OF	77 a	77 a	75 a	62 a	67 a	50 a	50 a
CA	65 a	57 ab	47 ab	45 ab	30 bc	22 bc	15 c
SC	60 a	57 ab	42 ab	37 ab	25 bc	12 c	7 c

[a] Means within a file followed by the same letter are not significantly different (2×2 χ^2 homogeneity test, $P < 0.05$). [b] Behavioral responses are as follows: TF, taking flight; OF, oriented flight; CA, close approach; and SC, source contact.

Table 2. Percentage of Behavioral Responses of *O. nubilalis* Males Flying toward a Source Baited with Mixtures of Pheromone and Z10-13:TFMK in Several Ratios in a Wind Tunnel ($N = 40$)[a,b]

	pheromone (%)	pheromone + Z10-13:TFMK ratio (%)				
		1:0.1	1:1	1:5	1:10	1:20
TF	77 a	80 a	77 a	80 a	85 a	80 a
OF	77 a	77 a	60 a	55 a	45 a	37 a
CA	65 a	60 a	40 ab	37 ab	17 bc	5 c
SC	60 a	52 a	35 a	30 a	7 b	5 b

[a] Means within a file followed by the same letter are not significantly different (2×2 χ^2 homogeneity test, $P < 0.05$). [b] Behavioral responses are as follows: TF, taking flight; OF, oriented flight; CA, close approach; and SC, source contact.

The same type of experiment was conducted with the one-carbon shorter analogue Z10-13:TFMK (**Table 2**). This chemical displayed a similar effect than the parent homologue, although significant results were now obtained when Z10-13:TFMK was mixed with the pheromone in 10:1 and 20:1 ratios. In the first case, only 17% of males, out of the 85% that had initially taken flight, were able to approach the lure and only 7% contacted with the source, whereas in the second case the percentage of males displaying both behaviors was only 5%. Here, also, the effect was dose-dependent (**Table 2**).

The antagonistic effect of Z11-14:TFMK became evident when a flight track of a moth flying to a source containing a 1:2 mixture of pheromone and antagonist was videorecorded. As shown, the track showed profound differences as compared with the one displayed when males were flying toward pheromone alone (**Figure 1**). In the presence of antagonist, males frequently exhibited erratic progress toward the plume, flying across the wind and with multiple intersections with plume boundaries. In addition, males took longer to contact with the source flying longer distances than control insects (**Figure 1**).

Field Tests. The activity of the antagonists in the field was evaluated by comparing the number of males caught with mixtures of the chemicals and the pheromone relative to those

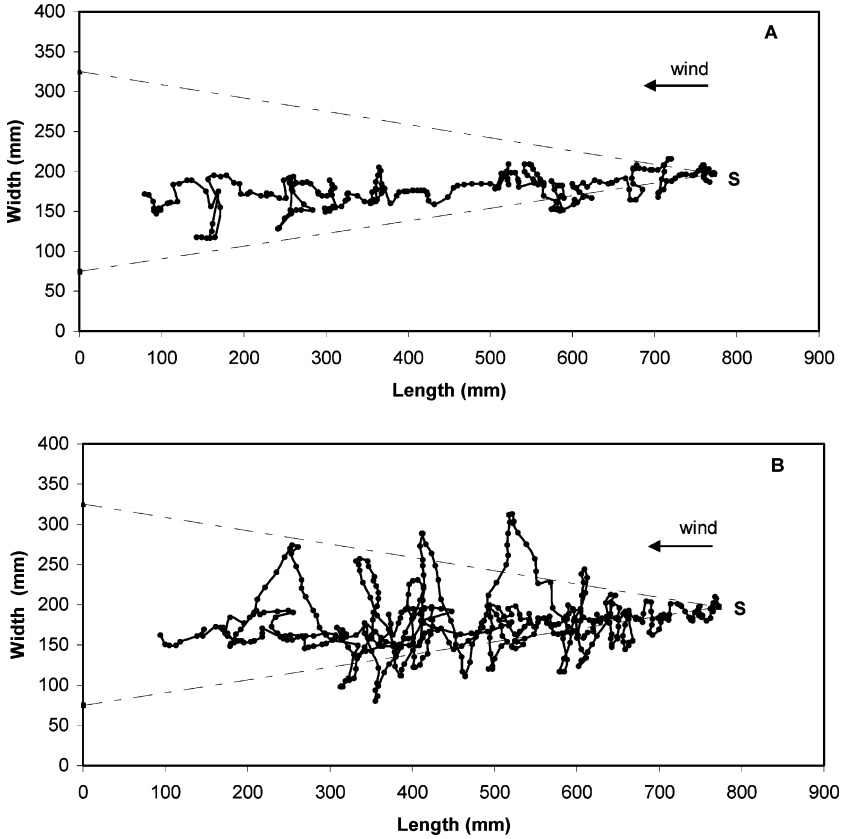


Figure 1. Representative flight track of *O. nubilalis* males flying upwind toward a dispenser containing a 1:2 mixture of pheromone blend and Z11-14:TFMK (**B**) relative to control (**A**). Black dots represent insect positions at 0.04 s intervals.

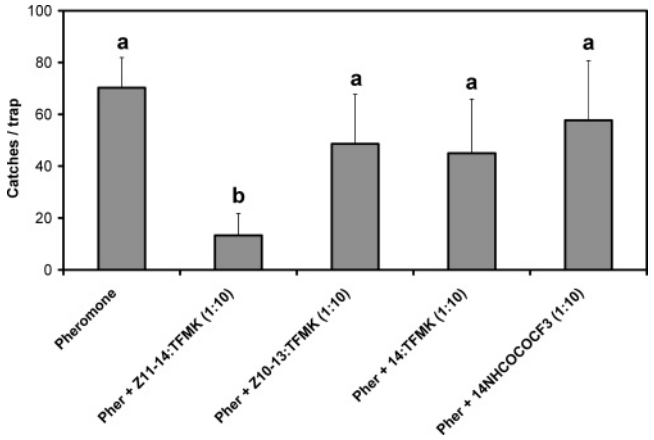


Figure 2. Number of catches of *O. nubilalis* males in traps baited with mixtures of the trifluoromethyl ketones Z11-14:TFMK, Z10-13:TFMK, 14:TFMK, and 14:NHCOCOCF$_3$ and pheromone in a 10:1 ratio in comparison with catches in traps containing pheromone alone. Rubber septa were used as dispensers. The amount of pheromone in each trap was 0.1 mg. Bars with the same letter are not significantly different (Student's *t*-test, $P < 0.05$). Tests were carried out on infested maize fields from July to October 2001.

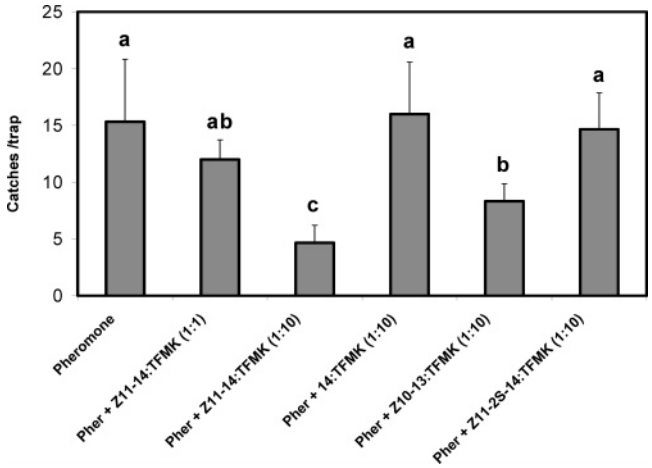


Figure 3. Number of catches of *O. nubilalis* males in traps baited with mixtures of the trifluoromethyl ketones Z11-14:TFMK, Z10-13:TFMK, 14:TFMK, and Z11-2S-14:TFMK and pheromone in 1:1 and 10:1 ratio in comparison with catches in traps containing pheromone alone. Rubber septa were used as dispensers. The amount of pheromone in each trap was 0.1 mg. Bars with the same letter are not significantly different (Student's *t*-test, $P < 0.05$). Tests were carried out on infested maize fields from July to October 2003.

trapped with the pheromone alone. In tests carried out in 2001, Z11-14:TFMK, Z10-13:TFMK, 14:TFMK, and 14:NHCOCOCF$_3$ were tested in 10:1 blends with the pheromone but only Z11-14:TFMK induced a significant reduction of catches (13.3 ± 8.4) as compared to the number of males caught with the pheromone (70.3 ± 11.6) ($P < 0.05$) (**Figure 2**). The remaining compounds did not elicit any significant effect although baits containing blends of Z10-13:TFMK and 14:TFMK with the pheromone appeared to catch lower number of males (48.7 ± 19.1 and 45.0 ± 20.9, respectively) than the reference attractant alone.

New experiments were implemented in 2003 including blends of Z11-14:TFMK:pheromone (1:1) and Z11-2S-14:TFMK:pheromone (10:1) (**Figure 3**). In this case and although a relatively low infestation was detected, Z11-14:TFMK again

displayed a good disruptant activity, particularly in a 10:1 mixture with the pheromone (4.7 ± 1.5 vs 15.3 ± 5.5 males/trap with the pheromone). Z10-13:TFMK also reduced the number of males caught but to a lower extent than the homologue (8.3 ± 1.5). Neither 14:TFMK nor the 2-thia analogue elicited any disruptant effect (**Figure 3**).

DISCUSSION

Following a series of putative esterase inhibitors (*12*, *17*, *21*, *30*), we have selected the fluorinated compounds shown in **Chart 1** in base to the following. Z11-14:TFMK and Z10-13:TFMK were considered because of the close structural analogy with the major component of the pheromone Z11-14:Ac. 14:TFMK was chosen to establish the role of the olefinic bond upon the activity. 14:TFPAm was selected in base to the enhanced electrophilic character of the carbonyl adjacent to the CF_3 group and, therefore, as the putative acceptor for nucleophilic residues, such as the serine hydroxyl or the cysteine thiol of the enzyme. In addition, because this compound is highly hydrated, it could provide a good model to relate the antagonistic potency with the extent of hydration as already pointed out (*30*, *31*). Z11-2S-14:TFMK was also considered by the enhanced stability of the hydrate form due to the presence of a hydrogen bond between the free electron pair of sulfur with an OH group of the hydrate (*32*, *33*).

The inhibitory potency of Z11-14:TFMK and Z10-13:TFMK showed that both compounds were remarkable antennal esterase inhibitors of ECB; Z11-14:TFMK, the most similar analogue of the major component of the pheromone, was 10-fold more active than the one-carbon shorter analogue. In addition and in EAG studies, we have found that topical application on the antennae of 10 pg of Z11-14:TFMK resulted in a significant 78% lower depolarization amplitude than the pheromone, whereas Z10-13:TFMK required a 100 pg dose per antenna to elicit a significant 60% lower EAG signal (Riba et al. Unpublished results). The saturated 14:TFMK needed 10 ng, i.e., a dose 3 orders of magnitude higher than the unsaturated analogue, to exert a similar effect. The inhibitory potency results confirm others previously obtained by us in that a stringent structural similarity of the analogue is required for an optimum level of inhibition (*17*, *30*). The Z and E isomers of a one-carbon longer TFMK analogue of the ECB pheromone (Z12-15:TFMK and E12-15:TFMK) had been found by Klun et al. (*34*) to be moderate inhibitors of the esterase activity in both pheromone strains.

When ECB males were allowed to fly toward a source baited with mixtures of the pheromone and Z11-14:TFMK or Z10-13:TFMK, both compounds elicited a disruptive effect only of the CA and SC behaviors. Z11-14:TFMK exerted a significant effect when mixed with the pheromone in a 5:1 ratio wherein only 30% of the insects were able to approach to the vicinity of the lure. Z10-13:TFMK, in turn, needed a double dose in the lure to elicit a significant reduction in the number of approaching males. In SC, the effect was similar with regard to the minimum dose of chemical required to disrupt this behavior. When either TFMK was present in the lure, males displayed a highly erratic flight to the source in contrast to the much more oriented flight onto the plume shown by control insects. Therefore and again, a TFMK structurally similar to the pheromone elicits a remarkable disruption of flight, as previously described for SL and SN (*20*, *21*). This effect was also noted on SL males flying to cage-containing females after their antennae were topically treated with some TFMKs (*20*). Z12-15:TFMK and E12-15:TFMK did not disrupt upwind flight behavior when coevaporated with the pheromone in a flight tunnel, but the authors did not provide data on other key behaviors, such as CA and SC (*34*).

In two different experiments in the field, Z11-14:TFMK resulted in an effective antagonist of the pheromone action when mixed with the natural attractant in a 10:1 ratio, the effect being dose-dependent. The presence of the double bond with the right location and stereochemistry as in the pheromone structure is a determinant for the recognition and transduction processes (*35*). It should be noted that the initial pheromone:antagonist ratio present in the bait may not be the real active formulation because the TFMKs considered in this study, with the exception of the thia analogue Z11-2S-14:TFMK and pyruvamide 14:TFPAm, are more volatile than the corresponding major component of the pheromone Z11-14:Ac (*21*). In addition, the different diffusion rate of the antagonist and the natural attractant, provided they do not interact with the support, should also lead to a different release rate of the compounds into the air. With regard to Z10-13:TFMK, dissimilar results were obtained since the 10:1 blend with the pheromone was active in only one of the two experiments, which indicates that further experimentation is needed to clarify the effect of this chemical in the field. The 2-thia analogue Z11-2S-14:TFMK, resulting from replacement of a methylene group by sulfur at position 2, was disappointingly inactive. Presumably, the exceedingly high content in hydrate form of the chemical, although desirable in in vitro tests in which aqueous solutions are the reaction media, may not be advisable in vivo where a sustained release from the support is required. In this context, the general widely known esterase inhibitor 2-octylthiotrifluoropropan-2-one (OTFP), a β-thio-substituted TFMK similar to Z11-2S-14:TFMK but of much shorter chain length, was also ineffective in the field (*21*). This compound had previously displayed a remarkable antiesterase activity in vitro with an IC_{50} value of 5.9 μM in SL and 16.3 μM in SN (*30*), decreased the EAG amplitude and increased repolarization time in SL, and reduced the responses of males to the pheromone after preexposure to vapors of the chemical in a wind tunnel (*18*). The same assumption could be made for the lack of activity of the trifluoropyruvamide 14:TFPAm (keto:hydrate form 4:96). With regard to the lack of activity of the saturated 14:TFMK, the structural deficiency of the analogue makes it probably unable to interact properly with the receptor site and to trigger adequate receptor cell responses to provoke a successful behavioral output.

From the above results, it is inferred that in the ECB only TFMKs very closely structurally related to the major component of the natural attractant can be effective antennal esterase inhibitors in vitro, and at the same time remarkable behavioral antagonists in vivo of the male pheromone responses in the laboratory and in the field. As far as the mechanism of action of these TFMKs is concerned, whereas in in vitro assays the effect of these chemicals can be attributed in principle to the inhibition of the esterases present in the antenna, in vivo, this may not be the only mechanism of action. In fact, TFMKs have also elicited decreased responses to pheromones containing an alcohol or aldehyde function in electrophysiological tests (*18*, *36*). Moreover, TFMKs may be bound to the pheromone binding proteins and transported to the sensillum lymph in competition with pheromone molecules, facilitating interaction with the pheromone catabolic enzymes (*19*, *37*). However and because of the structural similarity to the pheromone, these compounds may also produce an overstimulation and adaptation of the pheromone receptor cells (*20*).

In summary, a new antennal esterase inhibitor, structurally related to the major component of the pheromone, has been found as a promising disruptant of the pheromone response of the Z strain of ECB males. This provides new valuable data to add to our previous reports (*21*, *22*, *30*) for the potential utilization of TFMKs in future pest control studies, for instance, in disruption experiments. The low toxicity to mice displayed by these compounds is an added value in this possible goal (*21*).

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