



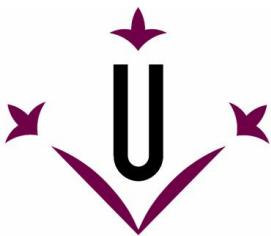
Universitat de Lleida

Comunicació química en els barrinadors del blat de moro *Ostrinia nubilalis* i *Sesamia nonagrioides*. Inhibició de l'atracció sexual per anàlegs de la feromona

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Universitat de Lleida

Departament de Química

Comunicació química en els barrinadors del blat de moro *Ostrinia nubilalis* i *Sesamia nonagrioides*. Inhibició de l'atracció sexual per anàlegs de la feromona

Memòria presentada per Joan Solé i Salla per optar al grau de Doctor per la
Universitat de Lleida

Treball realitzat al Departament de Protecció de Conreus del centre UdL-IRTA i
al Departament de Química de la Universitat de Lleida sota la direcció:

Dr. Magí Riba Viladot
Dr. Albert Sans Badia

Lleida 2009

A la Natàlia

...doncs bé, ja som als agraïments, la part més llegida de qualsevol tesi, serà qüestió d'esmerar-s'hi una mica...

La realització d'una tesi doctoral, ja sigui per la seva durada, pels coneixements o les vivències adquirides durant la realització de la mateixa, pels interessos, per les inquietuds o pel tracte diari amb els companys de feina, sol marcar la trajectòria vital d'aquella persona que la realitza; serveixen aquestes breus línies per a agrair a aquelles persones que m'han acompanyat durant aquest procés.

En primer lloc m'agradaria expressar el meu sincer agraïment als doctors Magí Riba i Albert Sans, els quals a més de supervisar i dirigir aquest treball, m'han ensenyat infinitat de conceptes, metodologies de recerca, estratègies, tècniques... Crec que sempre tindré un deute amb ells.

Al doctor Cèsar Gemenó, amb qui he compartit estones de discussió i de ciència pura, sobretot a l'hora d'entendre el significat biològic dels paràmetres de vol en túnel de vent.

L'ajuda dels membres del departament de Química Orgànica i Biològica del CSIC de Barcelona amb el doctor Àngel Guerrero al seu capdavant ha estat força important, a tots ells també el meu agraïment.

El fet de realitzar la tesi entre dos departaments fa que s'augmenti el camp visual en moltes situacions, actituds i tasques a realitzar no només en les vessants més científico-tècniques o pedagògiques, sinó també en aquelles vessants més pragmàtiques de la vida mateixa. Els coneixements adquirits en aquest sentit, per exemple en les estones dedicades a l'esmorzar o amb converses directes amb cadascun de vosaltres, no són menyspreables i en cap cas gens banals, per tant, el meu agraïment a tots els estudiants, professors, tècnics de laboratori, secretàries i algú més del departament de Química de la Universitat de Lleida i del departament de Protecció de Conreus del centre UdL-IRTA pels agradables moments compartits.

Als meus pares, Juan i Dolors, i al meu germà Albert, per tota una vida, per confiar en mi i per deixar-me fer. A la resta de la família, tant la de tota la vida, com la que un incorpora després del matrimoni, per estar al meu costat.

A tots ells i als qui porto en el cor, moltes gràcies.

En la present memòria de tesi doctoral es presenten els resultats de diferents estudis realitzats amb diferents compostos químics anàlegs de les feromones sexuals dels principals barrinadors del blat de moro (*S. nonagrioides* i *O. nubilalis*), totavaluant la seva activitat com a antagonistes de la resposta feromonal, o com a inhibidors del catabolisme de la feromona en diferent tipologia d'assaigs (tant de camp com de laboratori).

Els compostos de tipus trifluorometilcetona més similars al component majoritari de la feromona sexual d'*O. nubilalis* mostren un remarcable efecte inhibidor en túnel de vent. En assaigs de camp, disminueixen significativament el nombre de captures de masclles en comparació amb la feromona, quan són evaporats amb aquesta en una proporció d'1:10. A més, aquests compostos també són bons inhibidors d'esterases antenals.

En assaigs de confusió sexual en camp, utilitzant la trifluorometilcetona anàloga al component majoritari de la feromona sexual de *S. nonagrioides* s'ha observat una reducció en el nombre de plantes atacades i en el nombre de larves per planta, no només per aquesta espècie sinó també en el cas d'*O. nubilalis*.

Una altra tipologia de compostos anàlegs estudiada, les metilcetonas, també han mostrat una alta activitat, similar a les trifluorometilcetonas, en assaigs de túnel de vent, en assaigs d'electrofisiologia i en captures en trampes. En aquest cas, les metilcetonas, no són bons inhibidors d'esterases antenals en comparació amb les trifluorometilcetonas.

L'activitat d'aquests anàlegs feromonals (anàleg trifluorometilcetona i metilcetona) s'ha comparat amb un inhibidor natural, el (Z)-11-hexadecenal (Z11-16:Ald), tot analitzant les característiques i els paràmetres del vol de masclles d'*O. nubilalis* exposats a aquests productes en túnel de vent. Els resultats demostren que tot i que els tres inhibidors redueixen de forma similar el percentatge de contactes, aquesta reducció és deguda a mecanismes fisiològics d'inhibició diferents.

També s'han realitzat col·leccions de compostos volàtils de panís en dos períodes del dia diferents (matí i nit) i amb plantes estressades, tot identificant els compostos químics més abundants en les diferents condicions assajades, alhora que s'ha determinat l'activitat electrofisiològica d'aquests i altres compostos emesos per diferents plantes hoste d'*O. nubilalis*, així com en assaigs de doble elecció en olfactòmetre amb la idea de trobar compostos fisiològicament actius ja sigui com a atridents o bé com a repellents d'aquesta espècie.

En la presente memoria de tesis doctoral se presentan los resultados de distintos estudios realizados con diferentes compuestos químicos análogos de las feromonas sexuales de los principales taladros del maíz (*S. nonagrioides* y *O. nubilalis*), evaluando su actividad como antagonistas de la respuesta feromonal o bien como inhibidores del catabolismo de la feromona en distintos tipos de ensayos (tanto en ensayos de campo como de laboratorio).

Los compuestos de tipo trifluorometilcetona más similares al componente mayoritario de la feromona sexual de *O. nubilalis* muestran un notable efecto inhibidor en túnel de viento. En ensayos de campo, disminuyen significativamente el número de machos capturados en comparación con la feromona cuando son evaporados con ésta en una proporción de 1:10. Además, estos compuestos también son buenos inhibidores de esterasas antenales.

En ensayos de confusión sexual en campo utilizando la trifluorometilcetona análoga al componente mayoritario de la feromona sexual de *S. nonagrioides* se ha observado una reducción en el número de plantas atacadas y en el número de larvas por planta, no sólo para esta especie sino también en el caso de *O. nubilalis*.

Otra tipología de compuestos análogos estudiada, las metilcetonas, también han mostrado una alta actividad, similar a la mostrada por las trifluorometilcetonas, en ensayos de túnel de viento, en ensayos de electrofisiología y en capturas en trampas. En este caso, las metilcetonas, no son buenos inhibidores de esterasas antenales en comparación con las trifluorometilcetonas.

La actividad de estos análogos feromonales (análogo trifluorometilcetona y metilcetona) se ha comparado con la actividad de un inhibidor natural, el (Z)-11-hexadecenal (Z11-16:Ald), mediante el análisis de las características y los parámetros del vuelo de machos de *O. nubilalis* expuestos a estos productos en túnel de viento. Los resultados demuestran que aunque los tres inhibidores reducen de forma similar el porcentaje de contactos, esta reducción se debe a mecanismos fisiológicos de inhibición distintos.

También se han realizado colecciones de compuestos volátiles en plantas de maíz en dos períodos del día distintos (mañana y noche) y también con plantas estresadas, identificando los compuestos químicos más abundantes en las distintas condiciones ensayadas, a la vez que se ha determinado la actividad electrofisiológica de estos y otros compuestos emitidos por distintas plantas huésped de *O. nubilalis*, así como en ensayos de doble elección en olfactómetro con la idea de encontrar compuestos fisiológicamente activos ya sea como atrayentes o como repelentes de esta especie.

The present doctoral thesis report contains the results of different studies with different chemical analogues of the main corn borers (*S. nonagrioides* and *O. nubilalis*) sex pheromone compounds to assess their activity as pheromonal antagonists or as pheromone catabolism inhibitors in different types of tests (field and laboratory test).

The trifluoromethylketone compound-type more similar to the main sex pheromone compound of *O. nubilalis* has shown a remarkable inhibitory effect on wind tunnel. In field trials, these compounds have shown a significantly decreasing of the number of males caught in traps compared with the pheromone when they were co-evaporated with it in a ratio of 1:10. In addition, these compounds are also good antennal esterase inhibitors.

Using the trifluoromethylketone more similar to the main *S. nonagrioides* sex pheromone compound in field tests has been observed a reduction in the number of plants attacked and in the number of larvae per plant, not only for this species but also in *O. nubilalis*.

The methylketones, another of the studied analogue compounds, have also shown a high activity, similar to the results obtained with the trifluoromethylketones in wind tunnel, electrophysiological, and field tests. In this case, the methylketones are not good antennal esterase inhibitors compared with trifluoromethylketones compounds.

The activity of these pheromone analogues (trifluoromethylketones and methylketones) has been compared with the one showed by the natural inhibitor Z-11-hexadecenal (Z11-16:Ald) by analyzing flight parameters of *O. nubilalis* males exposed to these products. The results showed that although the three inhibitors reduced similarly the percentage of contacts in wind tunnel, this reduction is due to different physiological mechanisms of inhibition.

We have also made collections of volatile compounds from corn plants in two different periods of the day (morning and night) and also with stressed corn plants. We have identified the vast majority of the chemical compounds. Electrophysiological activity of these and other compounds emitted by different host plants of *O. nubilalis* has also been determined and dual-choice tests in olfactometer has been performed with the idea of finding physiologically active compounds either as attractants or repellents for this species.

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Introducció general

1. El cultiu del panís i la seva importància econòmica

El blat de moro és un cereal essencial per a l'alimentació humana, per a la fabricació de pinsos destinats a alimentació animal i per a diversos usos industrials (fabricació de plàstics biodegradables, fibres tèxtils, biocombustibles i altres matèries primes per a la indústria química).

La producció mundial de blat de moro segons l'*International Grains Council* (IGC) per al 2007/08 ha estat de 762 milions de tones, mentre que el consum mundial s'ha situat a un nivell record de 759 milions de tones (IGC, 2008).

Taula 1. Evolució de la producció, comerç, consum i existències mundials de blat de moro en el període 2003/2004 – 2007/2008 en milions de tones (International Grains Council, 2008).

	2003/2004	2004/2005	2005/2006	2006/2007	2007/2008
Producció	628	713	695	699	762
Comerç	80	76	79	87	93
Consum	647	686	700	720	759
Existències	105	132	127	106	110

La superfície espanyola cultivada és aproximadament 365.300 hectàrees, amb una producció de 3.646.100 tones a l'any 2007. La comunitat autònoma amb una major superfície dedicada a aquest cultiu és Castella i Lleó, seguida d'Aragó, Extremadura, Castella la Manxa, Andalusia, Catalunya... (M.A.P.A, 2007).

Taula 2. Evolució de les superfícies cultivades i produccions de gra espanyoles durant les últimes campanyes agrícoles (MAPA, 2007)

Campanya agrícola	Superfície (hectàrees)	Producció gra (tones)
2003	476.100	4.355.000
2004	479.800	4.831.100
2005	414.300	3.981.400
2006	353.600	3.460.800
2007	365.300	3.646.100

El cultiu de blat de moro a Catalunya (35.723 hectàrees de superfície; 344.100 tones de producció de gra l'any 2007) es centra principalment a la província de Lleida, concretament a les zones de regadiu de la vall del Segre (25.000 hectàrees de superfície; 250.000 tones de producció l'any 2007) (M.A.P.A, 2007).

La demanda espanyola de blat de moro supera les 6.000.000 de tones i per tant, aquest déficit s'ha de cobrir a través d'importacions, ja sigui de països de la Unió Europea (principalment de França) o de països eminentment exportadors de grans de cereals com Argentina, Brasil o USA.

2. Característiques d'*Ostrinia nubilalis*

El barrinador europeu del panís, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) és la plaga més important del blat de moro a nivell mundial essent també present, amb incidència variable, en altres cultius com patata, pebrot o cereal d'hivern a Europa, Amèrica del Nord, nord d'Àfrica, Filipines i Japó (Mason, et al., 1996). El control d'aquesta plaga és particularment difícil degut als hàbits endòfits de la mateixa, essent les aplicacions d'insecticides complicades d'executar i tan sols efectives en el curt espai de temps existent entre l'eclosió dels ous i l'entrada de les larves a la tija de la planta.

O. nubilalis mostra polimorfisme en el seu sistema de comunicació feromonal, així existeixen races que tot i utilitzar els mateixos components feromonals, aquests estan presents en proporció diferent. D'aquesta manera trobem l'acetat de tetradecenil (11-14:Ac) en dos formes isomèriques (cis i trans). En funció de la proporció d'aquests dos isòmers trobem poblacions de la raça Z quan la feromona està composta per una mescla de l'acetat de (Z)-11-tetradecenil (Z11-14:Ac) i de l'acetat de (E)-11-tetradecenil (E11-14:Ac) en una proporció 97:3 (Klun, et al., 1973) i de la raça E quan els mateixos components es troben en unes proporcions compreses entre 1:99 a 4:96 (Anglade i Stockel, 1984), (Peña et al., 1988).

Les diferents poblacions d'*O. nubilalis* mostren un fort comportament d'isolació en camp, però en les àrees en que coincideixen les dues races hi ha la suficient compatibilitat genètica per tal que les dues races es puguin aparellar i produir híbrids viables i fertils (Roelofs et al., 1987). Aquesta hibridació també és possible en poblacions de laboratori, quan aquest fet ocorre les femelles F₁ de l'encreuament de les dues races produueixen una feromona sexual amb una relació aproximada de 35Z:65E, de forma que els mascles híbrids responen més intensament a aquesta barreja, però apareixen diferents proporcions entre els dos isòmers a mesura que els híbrids s'aparellen entre ells (Glover et al., 1990).

La raça Z està àmpliament distribuïda per tot el món, mentre que la raça E tan sols es troba a Itàlia, Holanda, Suïssa i nord-est de Estats Units d'Amèrica (Klun et al., 1973). Segons els estudis realitzats per Sans (1995), sembla ser que el Nord-est de la Península Ibèrica la raça predominant és la Z (Sans, 1995).

3. Característiques de *Sesamia nonagrioides*

El barrinador mediterrani del panís *Sesamia nonagrioides* (Lefèbvре) (Lepidoptera: Noctuidae) està present en tota l'àrea mediterrània on predomina el cultiu del panís essent la plaga principal d'aquest cultiu en aquesta zona. A l'igual que *O. nubilalis*, *S. nonagrioides* presenta hàbits endòfits que juntament amb la fenologia pròpia del cultiu de blat de moro, fan particularment difícil el control d'aquesta plaga mitjançant aplicacions d'insecticides.

La composició feromonal de *S. nonagrioides* està composta per una barreja de l'acetat de (Z)-11-hexadecenil (Z11-16:Ac), (Z)-11-hexadecen-1-ol (Z11-16:OH), (Z)-11-hexadecenal (Z11-16:Ald) i acetat de dodecil (12:Ac) en proporció 69:8:8:15 en la zona de Grècia (Mazomenos, 1989) o 77:8:10:5 en la zona del nord-est de la península ibèrica (Sans et al., 1997).

4. Control de plagues en el panís

Per controlar les plagues d'*O. nubilalis* i *S. nonagrioides* s'ha utilitzat, clàssicament, insecticides químics de síntesis. Aquest tipus de control s'ha d'aplicar contra larves recent emergides durant la fase d'alimentació i de desplaçament d'aquestes sobre les parts externes de la planta, ja que un cop les larves s'han introduït a l'interior de la planta, les larves es tornen invulnerables a l'acció insecticida. Les diferents generacions d'ambdues plagues i el llarg període de posta fan necessàries successives aplicacions d'insecticida per a que aquestes resultin efectives.

Per altra banda, la baixa efectivitat, els problemes toxicològics, l'aparició de resistències i la fenologia del panís que dificulta el pas amb maquinària per la finca ha provocat que es busquin altres alternatives per al control d'aquests insectes.

En l'aproximació al control integrat dels barrinadors del blat de moro s'han utilitzat diferents estratègies com l'elecció de la data de sembra, la rotació de cultius amb espècies poc sensibles, l'eliminació de les restes de la collita, etc.

També s'han realitzat experiències de control biològic d'*O. nubilalis* amb certa eficàcia. Bàsicament s'han utilitzat insectes que parasiten les larves, com *Lydella thompsoni* (Herting) (Diptera: Tachinidae), la qual parasita les larves hivernants, o amb l'entomòfag *Trichogramma maidis* (Pintureau i Voegeli) (Hymenoptera: Trichogrammatidae), la femella d'aquesta espècie realitza la posta sobre els ous d'*O. nubilalis* els quals deixen de ser viables. Els alts costos econòmics d'implementació d'aquest tipus d'estratègies fan inviable el seu ús en plantacions comercials de panís.

L'ús de plantes transgèniques que produeixen la endotoxina de la bactèria *Bacillus thuringiensis* s'han utilitzat en plantes de panís, obtenint un control eficaç dels barrinadors del blat de moro. L'aplicació d'aquest tipus de tècniques és molt generalitzada als Estats Units, mentre que en certs països d'Europa s'observa certa desconfiança en l'ús d'organismes modificats genèticament. Aquest no és el cas de les àrees productores de panís de la vall del riu Ebre amb una alta pressió de plaga, tant d'*O. nubilalis* com de *S. nonagrioides*.

També s'han estudiat altres mètodes de control de plagues contra els barrinadors del blat de moro com la disruptió de la comunicació química entre ambdós sexes de la plaga (confusió sexual). En el cas de *S. nonagrioides* s'ha utilitzat la barreja de Z11-16:Ac i Z11-16:OH en una relació 90:10 (Albajes et al., 2002). Aquest mètode, no presenta problemes de toxicitat inespecífica, acumulació de residus en el medi ambient, aparició de resistències, etc.

En el nostre grup d'investigació s'ha desenvolupat una nova aproximació al control biorracional de plagues per inhibició de la percepció olfactiva, utilitzant anàlegs estructurals de tipus trifluorometilcetona (TFMC), els quals tenen la propietat d'inhibir el catabolisme de la feromona, fet que provoca una acumulació d'aquesta en els receptors olfactius de l'insecte i la saturació d'aquests (Riba et al., 2001; Bau et al., 1999). Aquest fet ocasiona que el mascle sigui incapàc de localitzar la femella i, per tant, de copular amb aquesta.

En la present tesis, es descriuen els primers assaigs de confusió sexual utilitzant TFMCs per al control dels barrinadors del blat de moro.

5. La comunicació química en insectes

El sistema de comunicació química entre insectes es realitza mitjançant substàncies transmissores de missatges, que s'anomenen *semioquímics* (missatgers químics) (Harbonne, 1982). Alhora, el seu estudi constitueix una alternativa prometedora en la recerca de nous mètodes de control de plagues, evitant problemes de toxicitat, acumulació de residus, desenvolupament de resistències, etc. (Karlson i Luscher, 1959).

Si ens centrem en la comunicació interespecífica (o **al·leloquímica**), aquesta pot ser de tipologia diversa segons existeixi un benefici per a l'emissor del missatge (**al·lomones**), per al receptor del missatge (**kairomones**), o bé un benefici per ambdós (**sinomones**) (Birch i Haynes, 1990).

5.1. Les feromones sexuals dels insectes

Les feromones són compostos químics, que emesos per un organisme, provoquen una reacció específica de comportament en altres membres de la mateixa espècie (Karlson i Luscher, 1959). Aquestes substàncies són particularment importants en els insectes, on es distingeixen segons el tipus de resposta obtinguda, feromones d'atracció sexual, feromones d'alarma, de marcatge, d'agregació, etc. (Harbonne, 1982).

Generalment, les feromones sexuals són secrecions constituïdes per una o varíes substàncies volàtils (components feromonals), produïdes per les femelles, encara que en algunes espècies també poden ser produïdes pels mascles, per tal d'atreure a gran distància a la parella de la pròpia espècie.

Les feromones sexuals en lepidòpters, són biosintetitzades a partir d'àcids grisos per reaccions de β -oxidació, dessaturació, reducció i acetilació en glàndules exocrines (Fabriàs, 1988). Així els components de les feromones sexuals, són generalment compostos alifàtics de cadena lineal insaturada. La seva diversitat estructural queda reflectida en el nombre d'àtoms de carboni, el nombre i posició de les insaturacions i el seu grup funcional. Normalment són acetats, aldehids, alcohols, hidrocarburs i alguns epòxids i cetonas. El nombre de carbonis oscil·la entre 10 i 22, essent els més corrents els de 12, 14 i 16, mentre que les insaturacions són, en general, de tipus doble enllaç, amb una freqüència més alta en les posicions 7, 9 i 11 (Quero, 1996) i amb configuracions cis (Z) o trans (E) (Baker, 1989). Tot i això els components feromonals presenten una gran simplicitat estructural, ja que aquesta és necessària per afavorir la volatilitat d'aquests, i així poder difondre's a grans distàncies (Guerrero, 1988).

Les mescles de components feromonals d'una determinada espècie són mescles molt precises d'aquests compostos. Una mateixa substància pot intervenir, a diferents proporcions, en feromones de diferents espècies (Guerrero, 1988). En aquest sentit, si es modifica la relació entre els components feromonals s'altera el comportament i la resposta de l'insecte ja que aquesta relació és constant per a una determinada espècie (Quero, 1996). Es necessiten tots i cada un dels components feromonals i en les proporcions adequades per poder reproduir completament la resposta del mascle.

5.2. Ús de les feromones sexuals en el control de plagues

L'estudi dels compostos semioquímics ens ofereix la possibilitat d'interferir en els sistemes de comunicació de les espècies perjudicials amb una elevada especificitat, a més a més les característiques pròpies de les feromones sexuals (innocuitat i ús en petites quantitats) converteixen aquests compostos en una alternativa a l'ús d'insecticides convencionals per al control de plagues (Quero, 1996).

L'ús de feromones o atraients sexuals sintètics constitueix avui en dia un dels elements bàsics dins el que s'anomena control dirigit i control integrat de plagues. Aquesta tipologia de lluita contra organismes perjudicials preveu, segons la Organització Internacional de Lluita Biològica (OILB), la utilització de diferents mesures que responguin de forma simultània a les necessitats econòmiques, toxicològiques i ecològiques d'una zona determinada, tot prioritant els elements naturals de control i respectant els límits de tolerància (Steiner, 1977).

La utilització de feromones sexuals en el control de plagues de lepidòpters està força generalitzada avui en dia, i cada vegada hi ha un major nombre d'espècies de les que se'n disposa la seva feromona sexual a nivell comercial (Arn et al., 1992).

A nivell pràctic les feromones tenen diversos usos. La primera aplicació de les feromones sexuals ha estat el seguiment de poblacions (“**monitoring**”) utilitzant les feromones sexuals sintètiques per detectar la presència i grau d'infestació d'una plaga i al mateix temps, obtenir informació addicional sobre la biologia de l'espècie, com pot ser el nombre de generacions a l'any o les corbes de vol. D'aquesta manera l'aplicació de les mesures de control poden realitzar-se en coordinació amb el cicle biològic de l'insecte i en base al nivell de danys predictible per la plaga.

En la lluita directa contra la plaga una tècnica utilitzada és la captura massiva (“**mass trapping**”) en la que s'utilitza un atraient altament específic, distribuït en trampes per tal de caçar un nombre prou elevat d'individus per reduir la població de la generació següent fins a nivells econòmicament acceptables. Aquests nivells acceptables es situen per a plagues de lepidòpters entorn del 80-95 % de captures (Knipling i McGuire, 1996). Per aquest motiu tan sols s'han obtingut nivells de control acceptables i econòmicament rentables quan els nivells de població són baixos (Birch i Haynes, 1990).

Un altre sistema és el de millorar l'eficàcia dels cultiu trampa (“**trap cropping**”) mitjançant l'ús de feromones sexuals, feromones d'agregació o altres atraients. Aquesta tècnica consisteix en la concentració de la plaga, mitjançant aquests compostos, en una petita part de la parcel·la (5-10 %) tractada prèviament o posteriorment amb plaguicides. Eliminant els insectes es disminueix el creixement de les poblacions de la plaga durant el cicle posterior del cultiu. Aquesta tècnica té l'inconvenient que si tan sols s'usen feromones sexuals, només s'eliminen els mascles de l'espècie plaga.

Finalment la tècnica de la confusió sexual (“**mating disruption**”) també és utilitzada per al control de plagues. Consisteix en difondre en l'ambient grans quantitats de feromona sintètica, dificultant així la localització de les femelles per part dels mascles, reduint els encontres dels dos sexes i evitar la copula, de forma que es disminueix els nivells de població de generacions successives. Els mecanismes mitjançant els quals s'aconsegueix l'efecte de confusió poden ser per adaptació dels receptors antenals i habituació del sistema nerviós central, per competència amb la font natural de feromona, és a dir, les femelles, o per emmascarament d'aquesta impossibilitant l'orientació dels mascles a les mateixes. (Guerrero, 1988).

5.3. Semioquímics volàtils de plantes. L'olfactòmetre

Les plantes estan contínuament emetent a l'atmosfera compostos orgànics volàtils (Kesselmeier i Staudt, 1999). Aquests compostos volàtils són metabòlits secundaris, els quals estan produïts per les plantes, però no estan relacionats amb els processos bàsics de la fotosíntesi o la respiració d'aquestes (Theis i Lerdau, 2003). Els insectes poden utilitzar els compostos volàtils semioquímics emesos per les plantes en benefici propi. Aquestes senyals químiques poden ser aprofitades pels insectes per tal de trobar llocs on alimentar-se o bé llocs beneficiosos on realitzar la posta. (Visser, 1986). A més a més, els semioquímics emesos per les plantes, no només poden tenir influència en un sistema bitràfic (planta-insecte), sinó que també poden ser utilitzats per part de depredadors o enemics naturals per localitzar les seves possibles preses (sistemes tritròfics) (Dicke, 1999; James, 2003).

D'aquesta manera, en un agroecosistema l'ambient semioquímic a l'entorn d'un determinat cultivar, és decisiu en les interrelacions planta-insecte (Metcalf i Metcalf, 1992).

La utilització de volàtils de plantes en el control de plagues individualment o bé conjuntament amb les feromones ha afavorit l'evolució i la racionalització de les estratègies de control per a determinades plagues amb tècniques molt menys agressives des del punt de vista mediambiental.

Per tal de poder identificar i analitzar l'activitat d'aquests compostos volàtils emesos per diferents tipus de plantes es realitzen col·leccions d'aquests compostos utilitzant trampes de productes adsorbents utilitzant sistemes de "headspace" dinàmics. Aquests sistemes consisteixen en confinar la font productora de compostos volàtils (en el nostre cas la planta de panís) en un ambient tancat i realitzar una concentració dels compostos emesos a la trampa adsorbent col·locant una corrent d'entrada d'aire filtrat al recipient mitjançant una bomba impulsora i una altra corrent d'aire de sortida, mitjançant una bomba extractora, a la qual es col·loca la trampa amb el producte adsorbent on quedaran retinguts els compostos volàtils (figura 1).

Els olfactòmetres són muntatges que permeten l'estudi científic del comportament d'insectes sotmesos a diferents estímuls, incloent estímuls olfactius. N'existeixen de varíes formes i mides depenen de l'insecte que s'estigui estudiant i el tipus de comportament d'aquest. Els bioassais de comportament en olfactòmetre es solen utilitzar un cop s'han identificat mitjançant tècniques d'electroantenografia compostos químics actius, per tal de constatar la influència d'aquests en el comportament de l'insecte. Els olfactòmetres de doble elecció (figura 2) permeten l'estudi del comportament dels insectes sotmesos a dos estímuls, un normalment és el compost a assajar, mentre que l'altre actua com a control.

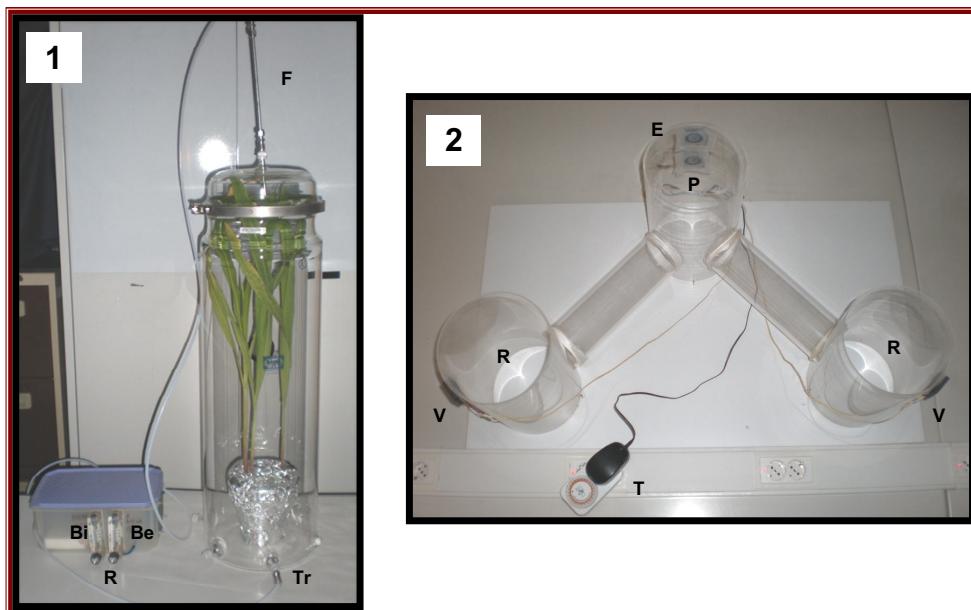


Figura 1. Sistema de col·lecció de compostos orgànics volàtils a partir de plantes senceres de panís confinades en un ambient tancat (**F**: Filtre de carbó actiu a l'entrada de l'aire, **Bi**: Bomba injectora d'aire, **Be**: Bomba extractora d'aire, **R**: Rotàmetres per al control dels fluxes d'aire, **Tr**: Trampa amb producte adsorbent).

Figura 2. Olfactòmetre de doble elecció (**V**: Ventiladors, **E**: Extractors, **P**: Braç principal, **R**: Braços receptors, **T**: Temporitzador).

6. Percepció i resposta a estímuls olfactius i la seva inhibició

6.1. Percepció de la feromona

En el cas dels lepidòpters, els òrgans de recepció dels estímuls olfactius es troben situats a les antenes, concretament en els pèls sensorials o *sensibles*. Aquestes estructures estan innervades per les dendrites d'una o més cèl·lules sensorials (Anderbrant i Hanson, 1995) que són les encarregades de transformar l'estímul químic en un corrent elèctric i transportar-lo, a través dels axons neuronals, fins al cervell de l'insecte (Figura 3). La paret cuticular de la *sensilla* està perforada per un nombre variable de porus d'un diàmetre que oscil·la entre 10 i 40 nm segons l'espècie i el tipus de *sensilla*, aquests porus permeten l'accés de molècules volàtils.

Una major superfície antenal correspon amb un major nombre de receptors i, per tant, un nivell superior de sensibilitat a les molècules de feromona i altres atraients (Quero, 1996). Tot i això la sensibilitat dels insectes dependrà del nombre de cèl·lules sensorials, del nombre de ramifications de cada dendrita i del nombre de llocs de recepció (Davies, 1991).

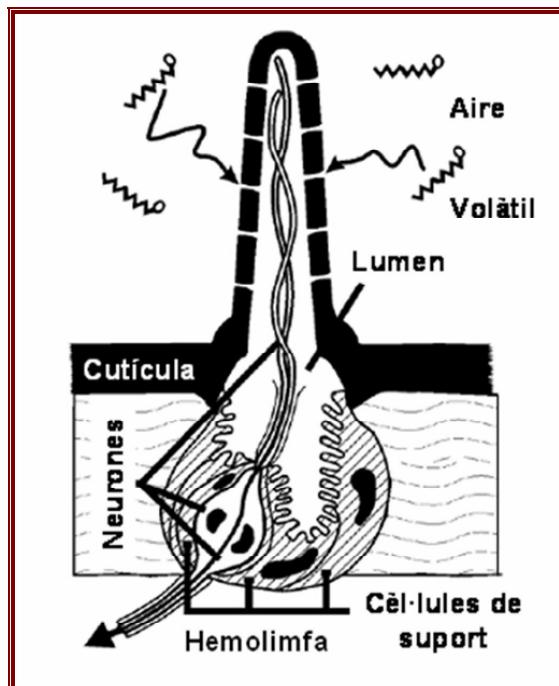


Figura 3. Secció longitudinal d'una sensilla olfactiva d'un insecte (modificat a partir de Vogt, 2005) en la que poden veure's les diferents parts de que consta aquest òrgan.

6.2. Mecanismes i fisiologia de la percepció feromonal

Les molècules de feromona absorbides a la cutícula penetren a l'interior de la paret sensilar a través dels porus cuticulars (Kanaujia i Kassling, 1985). En la limfa sensilar de les sensilla quimioreceptores dels muscles de lepidòpters nocturns no hi ha una solució iònica, sinó un medi proteic amb la presència de proteïnes solubles que s'enllacen amb les molècules de feromona (Vogt i Riddiford, 1981) i enzims que les degraden (Prestwich i Graham, 1989). D'aquests fets, sorgeix la idea que les proteïnes de la limfa sensilar poden controlar tant l'accés de les feromones als receptors presents a les dendrites com el temps de vida de les molècules de feromona (Figura 4).

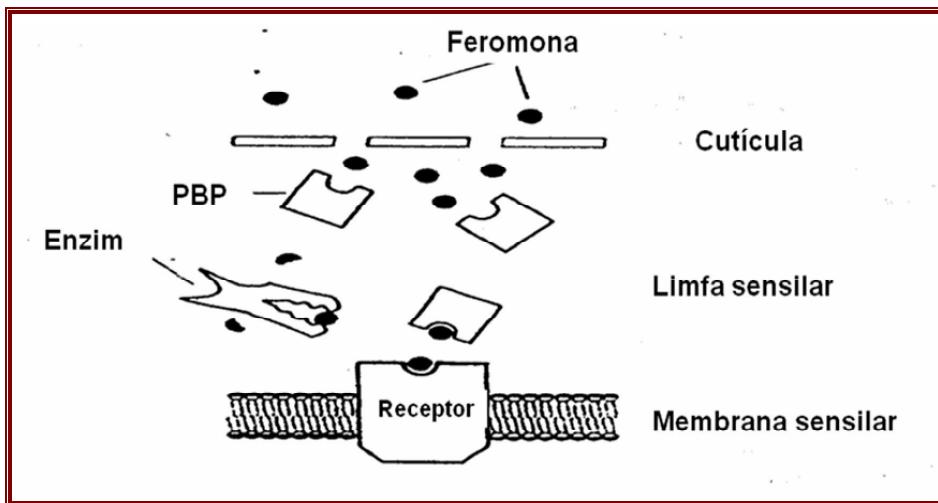


Figura 4. Model de com arriben les molècules de feromona a través de la limfa sensilar als receptors i com és regulat el temps d'aquestes en els receptors mitjançant l'acció de la proteïna transportadora (PBP). Uns enzims esterasa són els encarregats d'inactivar les molècules de feromona i així evitar l'acumulació en els receptors (modificat a partir de Cardé i Minks, 1997).

Segons aquesta idea Vogt i Riddiford (1981) proposen un mecanisme que permet la solubilització de les molècules de feromona en una limfa altament rica en proteïnes. En aquest model hi intervenen una proteïna enllaçant de la feromona ó PBP (*pheromone binding protein*) que, a una concentració elevada (20 mM) seria capaç de solubilitzar i transportar les molècules hidrofòbiques de feromona a través de la limfa sensilar, i una esterasa sensilar que la degradaria (Figura 5). Les interaccions entre la PBP, la feromona, l'esterasa i els receptors es trobarien en un equilibri cinètic, que asseguraria l'arribada de la major part de molècules de feromona als receptors a través de la limfa abans de ser degradades (Vogt i Riddiford, 1981).

Aquestes PBPs poden controlar l'accés de les feromones a les dendrites, així com el temps de durada de les molècules en la limfa sensilar, ja que en l'estructura primària de les PBPs existeixen dos dominis hidrofòbics dominants, els quals formarien la part que acompanya els components feromonals (Vogt, 2005).

A més de la funció de transport, s'ha proposat que les PBPs també participen activament en la presentació de la feromona als receptors situats en les membranes de les dendrites sensorials, de forma que les interaccions electrostàtiques i hidrofòbiques del conjunt de PBP i feromona serien necessaris i suficients per a l'activació del receptor (Prestwich i Du, 1997).

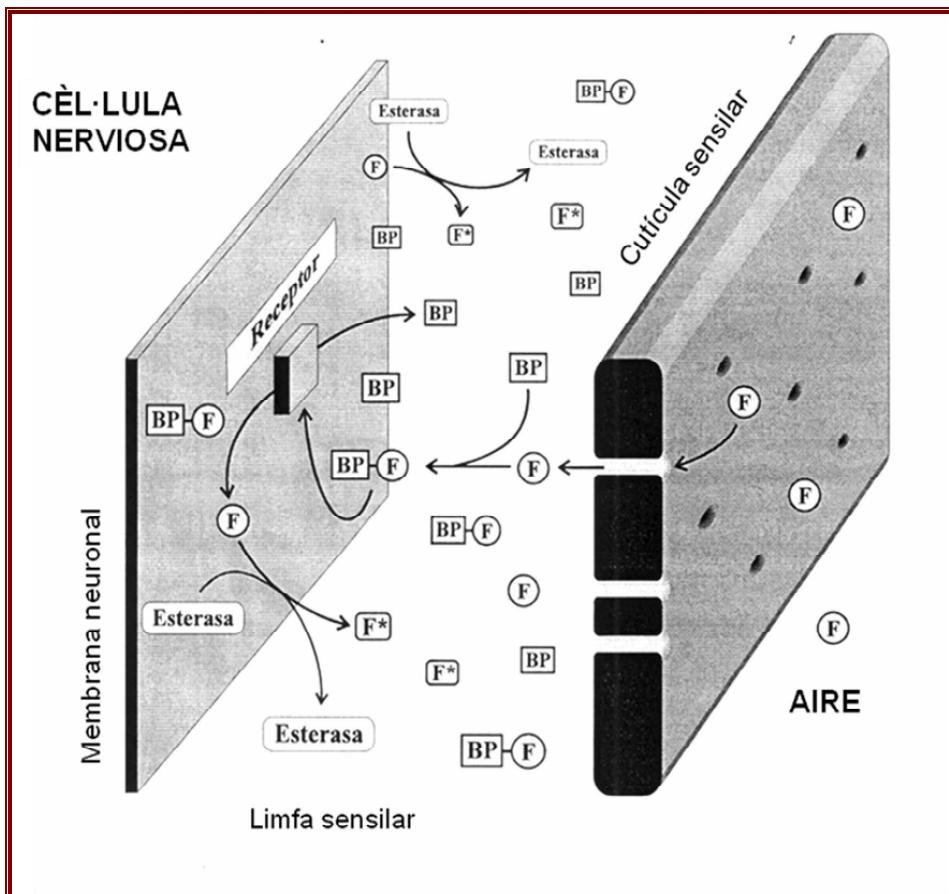


Figura 5. Model del procés de percepció de feromona a les sensibles de les antenes dels insectes lepidòpters segons Vogt et al., 2005). (*F*: molècula de feromona; *F**: molècula de feromona degradada; *BP*: proteïna enllaçant).

En diverses espècies de lepidòpters també s'ha pogut comprovar l'existència d'enzims sensilars que degraden les molècules de feromona i que la ruta catabòlica que es segueix és comuna en algunes d'elles (Prestwich, 1987; Prestwich et al., 1987; Klun et al., 1991, 1992, 1996; Klun i Schwarz, 1993; Quero, 1996).

El fet que un insecte volant en una ploma de feromona trobi canvis molt sobtats i freqüents en la concentració de molècules oloroses, fa que sigui necessària una ràpida inactivació de les molècules actives de feromona, per tal de mantenir la sensibilitat de l'antena a l'arribada de noves molècules. En aquest sentit sembla ser que les PBPs actuarien protegint les molècules de feromona enllaçades, de forma que únicament la fracció de feromona lliure seria degradada per les esterases (Figura 6) (Kaissling, 1996).

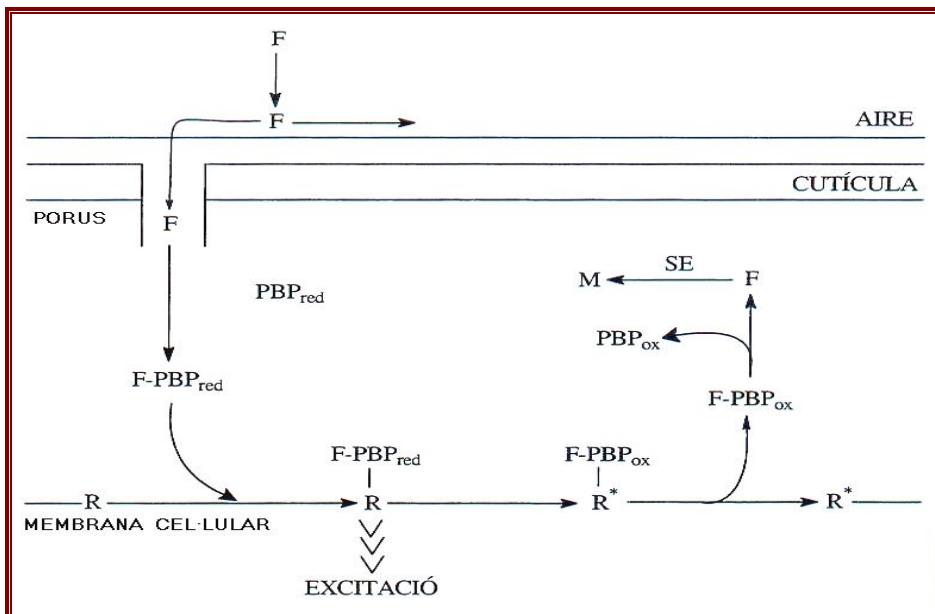


Figura 6. Model de Kaissling (1996) modificat. El mecanisme de desactivació ràpida de la feromona es basa en la existència de dues formes de la PBP, una oxidada (PBP_{ox}) i una altra forma parcialment reduïda (PBP_{red}). Aquesta última un cop unida a la feromona és capaç d'estimular els receptors de la feromona, mentre que la primera no. Quan el complex PBP -feromona estimula el receptor, la PBP passaria de la seva forma reduïda a la seva forma oxidada permetent una ràpida desactivació de la feromona. (F: molècula de feromona, PBP: proteïna enllaçant de la feromona, M: metabòlit inactiu, R: receptor, R^* : receptor excitat, SE: esterasa sensilar).

En el cas de la feromona sexual de femelles d'*O. nubilalis*, la degradació d'aquesta transcorre per transformació en alcohol i àcids corresponents. El model de catabolisme implica la hidròlisi de l'acetat, oxidació de l'alcohol en àcid gras i posterior degradació de l'àcid via β -oxidació (Klun et al., 1992, 1998).

6.3. Inhibició de la percepció olfactiva mitjançant anàlegs estructurals

La comunicació química dels insectes pot afectada significativament per compostos sintètics biològicament actius, relacionats o no amb la seva feromona sexual (Renou i Guerrero, 2000), conseqüentment, petites modificacions en compostos actius que juguen un paper important en el comportament de l'insecte, poden ser considerats com a potencials agents per a control de plagues (Ridgway et al., 1990; Renou i Guerrero, 2000). Així, anàlegs de la feromona són compostos estructuralment molt similars als components de la feromona sexual. Generalment aquests anàlegs resulten del desplaçament isostèric d'àtoms o grups funcionals sense produir pertorbacions substancials del medi electrònic i/o espaiial dels llocs clau de la molècula (Camps et al., 1990a).

Els anàlegs estructurals de les molècules de feromona es poden utilitzar per modificar les respostes de comportament i/o electrofisiològiques per diferents mecanismes d'acció: Així poden actuar com **antagonistes**, bloquejant l'acció de la feromona al receptor al saturar els quimioreceptors de la feromona, suprimint la interacció amb l'autèntica feromona i la resposta biològica usual. També poden actuar com **agonistes**, mimetitzant l'acció de la feromona, però produint una resposta modificada (Camps et al., 1990b).

Un anàleg estructural també pot ser actiu inhibint l'activitat catabòlica de les esterases de les *sensilla*. En aquest sentit, aquest tipus d'inhibidors han de ser compostos amb una estructura molt relacionada amb la de la feromona, per poder tenir capacitat d'accés i interacció amb proteïnes de la limfa sensilar (Fabriàs, 1988). L'activitat inhibidora es presenta en no poder donar-se el mecanisme de degradació posterior, de forma que el centre de recepció està completament i permanentment bloquejat, evitant l'orientació de l'insecte cap a la font de feromona (Prestwich, 1987). Aquests compostos presenten l'avantatge de no atreure insectes d'altres àrees infestades a les zones d'aplicació, al contrari del que ocorre amb la utilització de feromones.

La relació estructura-activitat és molt important, ja que únicament el canvi d'un o dos àtoms de carboni en la cadena carbonatada o l'eliminació d'un doble enllaç en aquesta provoca una notable disminució d'activitat biològica de les feromones sexuals de lepidòpters (Guerrero, 1988). També és important la estereoisomeria de la molècula, ja que afecta en gran mesura a l'activitat biològica dels components feromonals. En el cas d'isòmers geomètrics la presència de tan sols un 0.5 % de l'isòmer oposat pot reduir de manera significativa l'activitat de l'isòmer actiu (Guerrero, 1988).

Els anàlegs estructurals també poden comportar-se com a **sinèrgics** i millorants de la selectivitat, al augmentar l'especificitat respecte altres espècies properes (Riba et al., 1994). És pràcticament impossible *a priori* preveure quins compostos seran sinèrgics o inhibidors per a una determinada espècie de lepidòpters (Guerrero, 1988). Els sinèrgics sembla ser que actuen amb una afinitat menor amb els receptors antenals, però amb un efecte cooperatiu. Podrien originar pertorbacions en llocs adjacents de la proteïna, que la farien més receptiva i en conseqüència produiria una amplificació de les respostes (Fabriàs, 1988).

6.4. Importància del fluor en anàlegs estructurals. Trifluorometilcetonas

El fluor s'utilitza moltes vegades en la síntesi d'anàlegs per diferents raons, en primer lloc perquè el radi de Van der Waals de l'àtom de fluor (r_F 1.35A) és similar al de l'àtom d'hidrogen (r_F 1.10A), per tant la substitució d'un per l'altre pràcticament no induceix interaccions estèriques addicionals en la molècula fluorada. Tot i això, els dos elements tenen una reactivitat molt diferent, ja que l'àtom de fluor és molt electronegatiu i per tant pot influir substancialment la reactivitat de la molècula formada.

Els anàlegs haloacetats fluorats es presenten com uns dels més interessants, ja que han mostrat activitat inhibidora de la percepció feromonal (Prestwich i Streinz, 1988; Camps et al., 1990b; Riba et al., 1994, Sans, 1995).

Un altre tipus interessant d'anàleg estructural fluorat són les trifluorometilcetonas, que procedeixen de les substitucions isostèriques d'un àtom d'oxigen i un grup metil de l'acetat de la feromona per un grup metilè i un trifluorometil respectivament. El mecanisme d'acció consisteix en la unió de la trifluorometilcetona a un grup OH d'un residu de serina del centre actiu de l'esterasa sensilar, formant-se així un hemiacetal (Figura 7) (Gelb et al., 1985), que al poder dissociar-se en els productes inicials converteix les trifluorometilcetonas en inhibidors reversibles (Linderman et al., 1988).

La inactivació del procés catabòlic de les molècules de feromona es tradueix en una acumulació d'aquestes als receptors i, conseqüentment, en la impossibilitat de rebre noves molècules de l'atraient, afectant per tant l'electrofisiologia i el comportament de l'insecte. La substitució isostèrica del grup acetat ($\text{CH}_3\text{COO}-\text{R}$) de la molècula de feromona (grup habitual als components feromonals dels lepidòpters) per un grup trifluorometilcetona ($\text{CF}_3\text{COCH}_2-\text{R}$) transforma aquesta molècula en un anàleg potencialment inhibidor (Fabriás, 1988).

En aquest sentit s'ha vist que les trifluorometilcetonas inhibeixen amb eficàcia la esterasa de l'hormona juvenil d'insectes (Abdel Aal i Hammock, 1985), l'acetilcolinesterasa (Allen i Abeles, 1989) i les esterases sensilars dels insectes (Gelb et al., 1985; Duran et al., 1993; Quero, 1996; Rosell et al., 1996; Filizola et al., 1998; Quero et al., 2002).

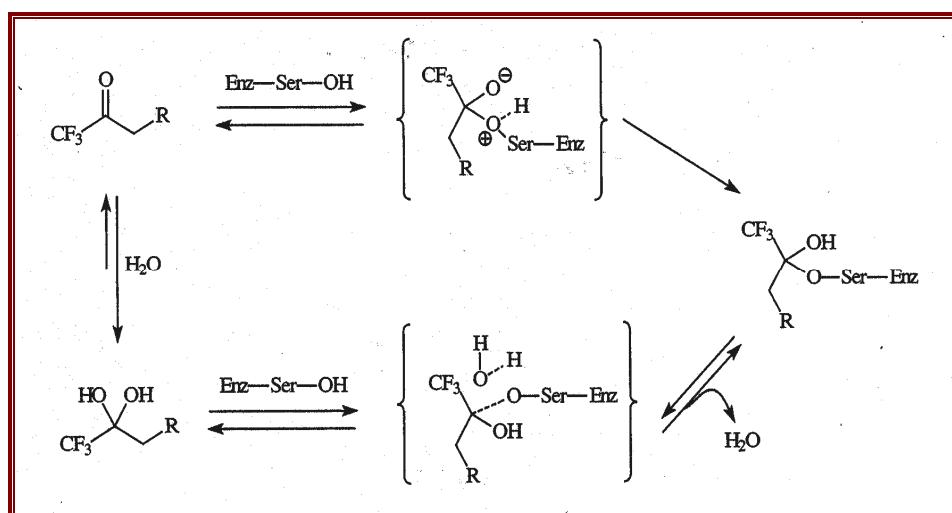


Figura 7. Esquema de la interacció de les trifluorometilcetonas amb les esterases sensilars formant hemiacetals estables en solicions aquoses.

Els efectes de les trifluorometilctones i altres derivats fluorats han estat i són estudiats tant en experiments de comportament com d'electrofisiologia en determinats insectes plaga tals com *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), *S. nonagrioides*, *Thaumetopoea pityocampa* (Denis i Schiffermüller) (Lepidoptera: Thaumetopoeidae) i *O. nubilalis* (Camps et al., 1990a, 1990b; Klun et al., 1991, 1994, 1997; Parrilla i Guerrero, 1994; Riba et al., 1994, 2001; Sans 1995; Warthen et al., 1995; Renou et al., 1997, 1999; Bau et al., 1999). Aquests treballs mostren que algunes trifluorometilctones inhibeixen d'una forma molt acusada l'activitat de la feromona sexual de lepidòpters, tant l'atracció de mascles com la resposta en electroantenograma, túnel de vent, en assaigs de camp o en anàlisis sobre l'activitat de les esterases sensilars.

6.5. Metilctones i altres anàlegs estructurals

Moltes espècies de lepidòpters són capaces de detectar compostos estructuralment relacionats amb els seus components feromonals. Aquests compostos, normalment són components feromonals d'altres espècies de lepidòpters que en molts casos poden actuar com un sistema d'isolació previ a l'aparellament en espècies que comparteixen un mateix habitat (Linn i Roelofs, 1989; Baker et al., 1998; Cardé i Haynes, 2004; Gemenó et al., 2006). Aquests anàlegs estructurals són anomenats antagonistes (Bengtsson et al., 1994; Karg et al., 1997; Baker et al., 1998; El-Sayed, 1999; Potting et al., 1999; Quero i Baker, 1999; Gemenó et al., 2006; Eizaguirre et al., 2007) i normalment inhibeixen la resposta dels mascles a la feromona, provocant un canvi en els patrons d'orientació cap a la font de feromona (Rumbo et al., 1993; Bengtsson et al., 1994; Fadamiro i Baker, 1997; El-Sayed, 1999; Potting et al., 1999; Quero i Baker, 1999; Gemenó et al., 2006).

Per altra banda, les anomenades metilctones (MCs), provenen de la substitució del grup acetat del component feromonal, per un grup metilcetona. S'intueix que poden ser també aplicades com a inhibidors de l'acció de la feromona amb un mecanisme d'acció relacionat amb una inhibició competitiva. En aquest cas les metilctones competirien amb els components de la feromona durant el procés de la percepció olfactiva en l'antena de l'insecte.

En aquest sentit, en el present treball s'avalua la possibilitat d'utilitzar metilctones anàlogues al component principal de la feromona sexual d'*O. nubilalis* com a inhibidors en el procés de percepció feromonal, alhora que es compara la seva acció amb trifluorometilctones, anàlogues al component principal de la feromona d'*O. nubilalis*), tant en assaigs de camp, com en assaigs electrofisiològics i de comportament en laboratori.

7. Referències

- ABDEL AAL, A. L.; HAMMOCK, B. D. (1985). 3-octylthio-1,1,1-trifluoro-2-propanone, a high affinity and slow binding of juvenile hormone esterase from *Trichoplusia ni* (Hübner). *Insect Biochem.* **15**, 111-122.
- ALBAJES R.; KONSTANTOPOULOU M.; ETCHEPARE O.; ELZAGUEIRRE M.; FRÉROT B.; SANS A.; KROKOS F.; AMELINE A.; MAZOMENOS B. (2002). Mating disruption of the corn borer *Sesamia nonagrioides* (lepidoptera: noctuidae) using spayable formulations of pheromones. *Crop prot.*, **21**, 217-225.
- ALLEN, K. N.; ABELES, R. H. (1989). Inhibition kinetics of acetylcolinesterase with fluoromethylketones. *Biochemistry* **28**, 8466-8473.
- ANDERBRANT, O.; HANSON, B. S. (1995). Electrophysiological and morphological characteristics of pheromone receptors in male pine sawflies *Diprion pini* (Hymenoptera: Diprionidae), and behavioural response to some compounds. *J. Insect Physiol.* **41**, 395-401.
- ANGLADE, P.; STOCKEL, J. (1984). Intraespecific sex-pheromone variability in the European Corn Borer, *Ostrinia nubilalis* Hbn. (Lepidoptera. Pyralidae). *Agronomie* **4**, 183-187.
- ARN, H.; TOTH, M.; PRIESNER, E. (1992). List of sex pheromones of Lepidoptera and related attractants, International Organization for Biological Control, West Palearctic Regional Section.
- BAKER, T.C. (1989). Sex pheromone communication in the Lepidoptera: New research progress. *Experientia* **45**, 248-262.
- BAKER, T. C.; FADAMIRO, H. Y.; COSSE A. A. (1998). Moth uses fine tuning for odour resolution. *Nature* **393**, 530.
- BAU, J.; MARTÍNEZ, D.; RENOU, M.; GUERRERO, A. (1999). Pheromone-triggered orientation flight of male moths can be disrupted by trifluoromethyl Ketones. *Chem. Senses* **24**, 473-480.
- BIRCH, M. V.; HAYNES, K. F. (1990). Feromonas de insectos. Oikos-tau S.A., Barcelona. 95 pp.
- BENGTSSON, M.; KARG, G.; KIRSCH, P. A.; LÖFQVIST, J.; SAUER, A.; WITZGALL, P. (1994). Mating disruption of pea moth *Cydia nigricana* F. (Lepidoptera: Tortricidae) by a repellent blend of sex pheromone and attraction inhibitors. *J. Chem. Ecol.* **20**, 871-887.
- CAMPS, F.; GASOL, V.; GUERRERO, A. (1990a). Inhibitory pheromonal activity promoted by sulfur analogs of the sex pheromone of the female processionary moth *Thaumetopoea pityocampa* (Denis and Schiff). *J. Chem. Ecol.* **16**, 1155-1172.
- CAMPS, F.; GASOL, V.; GUERRERO, A. (1990b). Inhibition of the processionary moth sex pheromone by some haloacetate analogues. *Pestic. Sci.* **29**, 123-134.
- CARDÉ, R. T.; HAYNES, K. F. (2004). Structure of the pheromone communication channel in moths. Advances in Insect Chemical Ecology. (ed. by Cardé, R. T. i Miller, J. G) 283-323. Cambridge University Press, Cambridge, UK.

- CARDÉ, R. T.; MINKS, A. K. (1997). Insect pheromone research, new directions. Chapman and Hall. New York. 684 pp.
- DAVIES, R. G. (1991). Introducción a la entomología. Mundi-Prensa. Madrid. 449 pp.
- DICKE, M. (1999). Are herbivore-induced plant volátiles reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entomol. Exp. Appl.* **91**, 131-142.
- DURÁN, I.; PARRILLA, A.; FEIXAS, J.; GUERRERO, A. (1993). Inhibition of antennal esterases of the Egyptian armyworm *Spodoptera littoralis* by trifluoromethyl ketones. *Bioorg. Med. Chem. Letters* **3**, 2593-2598.
- EL-SAYED, A. M. (1999). Behavioural effects of (E)-8(Z)-10-dodecadien-1-ol (E)-8(E)-10-dodecadienyl acetate on the orientation of male codling moth, *Cydia pomonella* to pheromone source. *Behaviour* **141**, 313-325.
- FABRÍAS, G. (1988). Antagonistas de feromonas sexuales de lepidópteros. A "Insecticidas biorracionales". X. Beller, Ed., CSIC. Madrid. pp. 297-314.
- FADAMIRO, H. Y.; BAKER, T. C. (1997). *Helicoverpa zea* males (Lepidoptera : Noctuidae) respond to the intermittent fine structure of their sex pheromone plume and an antagonist in a flight tunnel. *Physiol. Entomol.* **22**, 316-324.
- FILIZOLA, M.; ROSELL, G.; GUERRERO, A.; PÉREZ, J. J. (1998). Conformational requirements for inhibition of the pheromone catabolism in *Spodoptera littoralis*. *QSAR Comb. Sci.* **17**, 205-210.
- GELB, M. H.; SVAREN, J. P.; ABELES, R. H. (1985). Fluoro Ketone inhibitors of hydrolytic enzymes. *Biochemistry*, **24**, 1813-1817.
- GEMENO, C.; SANS, A.; LÓPEZ, C.; ALBAJES, R.; EIZAGUIRRE, M. (2006). Pheromone antagonism in the European corn borer moth *Ostrinia nubilalis*. *J. Chem. Ecol.* **32**, 1071-1084.
- GLOVER, T.; CAMPBELL, M.; ROBBINS, P.; ROELOFS, W. (1990). Sex-linked control of sex pheromone behavioural responses in European corn borer moths (*Ostrinia nubilalis*) confirmed with TPI marker gene. *Arch. Insect Biochem. Physiol.* **15**, 67-77.
- GUERRERO, A. (1988). Feromonas sexuales de insectos. A "Insecticidas biorracionales": X. Beller, Ed., CSIC. Madrid. pp. 297-314.
- GULLAN, P. S.; CRANSTON, P. S. (1996). The insects and outlines of entomology. Chapman and Hall. London. 491 pp.
- HARBONNE, J. B. (1982). Introduction to ecological biochemistry. 2nd Ed., Academic Press, London.
- INTERNATIONAL GRAINS COUNCIL. (2008). Grain Market Report nº 377 (April 2008).
- JAMES, D. G. (2003). Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing *Chrysopa nigricornis*. *J. Chem. Ecol.* **29**, 1601-1609.
- KAISSLING, K. E. (1996). Peripheral mechanisms of pheromone reception in moths. *Chem. Senses* **21**, 257-268.

- KANAUJIA, L.; KAISSLING, K. E. (1985). Interactions of pheromone with moth antennae: adsorption, desorption and transport. *J. Insect Physiol.* **31**, 71-81.
- KARG, G.; SUKLING, D. M.; BRADLEY, S. J. (1997). Defining interaction between electroantenogram responses of *Epiphyas postvittana* (Lepidoptera:Tortricidae) to pheromone and other volatiles. *J. Insect Physiol.* **33**, 179-187.
- KARLSON, P.; LUSCHER, M. (1959). "Pheromones" a new term for a class of biologically active substances. *Nature*, 153, pp 55-56.
- KESSELMEIER, J.; STAUDT, M. (1999). Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. *J. Atmos. Chem.* **33**, 23-88.
- KLUN, J. A.; SCHWARZ, M. (1993). Sex pheromone catabolism in the redbanded leafroller moth. *J. Chem. Ecol.* **19**, 751-762.
- KLUN, J. A.; CHAPMAN, O. L.; MAITES, K. C.; WODTKOWSKI, P. W.; BEROZA, M.; SONNET, P. E. (1973). Insect sex pheromones: minor amounts of opposite geometrical isomer critical to attraction. *Science* **181**, 661-663.
- KLUN, J. A.; KHRIMIAN, A. P.; OLIVER, J. E. (1998). Evidence of pheromone catabolism via beta-oxidation in the European corn borer (Lepidoptera: Crambidae). *J. Chem. Ecol.* **33**, 400-406.
- KLUN, J. A.; OLIVER, J. E.; KHRIMIAN, A. P.; DICKENS, J. C.; POTTS, W. J. E. (1997). Behavioral and electrophysiological activity of the racemate and enantiomers of a monofluorinated analog of European corn borer (Lepidoptera: Pyralidae) sex pheromone. *J. Entomol. Sci.* **32**, 37-49.
- KLUN, J. A.; POTTS, W. J. E.; OLIVER, J. E. (1996). Four species of noctuids moths degrade sex pheromone by a common antennal metabolic pathway. *J. Entomol. Sci.* **31**, 404-413.
- KLUN, J. A.; SCHWARZ, M.; UEBEL, E. C. (1992). Biological activity and "in vivo" degradation of tritiated female sex pheromone in the male European corn borer. *J. Chem. Ecol.* **18**, 283-298.
- KLUN, J. A.; SCHWARZ, M.; WAKABAYASHI, N.; WATERS, R. M. (1994). Moth responses to selectively fluorinated sex pheromone analogs. *J. Chem. Ecol.* **20**, 2705-2719.
- KNIPLING, E. F.; McGUIRE, J.U.Jr. (1966). Population models to test theoretical effects of sex attractants used for insect control. *U.S. Dep. Agric. Inf. Bull.* **308**, 20.
- LINDERMAN, R. J.; LEAZER, J.; ROE, R. M.; VENKATESH, K.; SELINSKY, B. S.; 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. *Pestic. Biochem. Physiol.* **31**, 187-194.
- LINN, C. E.; ROELOFS W. L. (1989). Response specificity of male moths to multicomponent pheromones. *Chem. Senses* **14**, 421-437.
- MASON, C. E.; RICEN, M. E.; CALVIN, D. D.; VAN DUYN, J. W.; HUTCHINSON, W. D.; WITKOWSKI, J. F.; HIGGINS, R. A.; ONSTAD, D. W.; DIVELY, G. P. (1996) *European*

- Corn Borer. Ecology and Management. North Central Regional Extension Publication **327**, 57. Iowa State University: Ames, Iowa. USA.
- MAZOMENOS, B. E. (1989). Sex pheromone components of corn stalk borer, *Sesamia nonagrioides* (Lef.). Isolation, identification and field tests. *J. Chem. Ecol.* **11**, 1241-1247.
 - METCALF, R. L.; METCALF, E. R. (1992). Plant Kairomones in Insect Ecology and Control. Chapman and Hall, New York (USA).
 - MINISTERIO AGRICULTURA PESCA Y ALIMENTACIÓN. (2007). Avances de Superficies y Producciones Agrícolas (noviembre 2007). Secretaría General Técnica. Madrid. Espanya.
 - PARRILLA, A.; GUERRERO, A. (1994). Trifluoromethyl ketones as inhibitors of the processionary moth sex pheromone. *Chem. Senses* **19**, 1-10.
 - PEÑA, A.; ARN, H.; BUSER, H. R.; RAUSCHER, S.; BIGLER, F.; BRUNETTI, R.; MAINI, S.; TOTH, M. (1988). Sex pheromone of European corn borer, *Ostrinia nubilalis*: Polymorphism in various laboratory and field strains. *J. Chem. Ecol.* **14**, 1359-1365.
 - POTTING, R. P. J.; LÖSEL, P. M.; SCHERKENBECK, J. (1999). Spatial discrimination of pheromones and behavioural antagonists by the tortricid moths *Cydia pomonella* and *Adoxophyes orana*. *J. Comp. Physiol. A* **185**, 419-425.
 - PRESTWICH, G. D. (1987). Chemistry of pheromone and hormone metabolism in insects. *Science* **237**, 999-1006.
 - PRESTWICH, G. D.; DU, G. (1997). Pheromone-Binding Proteins, pheromone recognition and signal transduction in moth olfaction. A "Insect pheromone research. New directions". R. T. Cardé and Minks, A. K. Eds. New York, Chapman & Hall pp. 131-143.
 - PRESTWICH, G. D.; GRAHAM, S. M. (1989). Enzymatic processing of pheromones and pheromones analogues. *Experientia* **45**, 263-270.
 - PRESTWICH, G. D.; STREINZ, L. (1988). Haloacetate analogs of pheromones: effects on catabolism and electrophysiology in *Plutella xylostella*. *J. Chem. Ecol.* **14**, 1003-1021.
 - PRESTWICH, G. D.; VOGT, R. G.; DING, Y. S. (1987). Chemical studies of pheromone catabolism and reception. A *Molecular Entomology* (Ed. J.H. Law), pp.57-66. UCLA Symposia, New York.
 - QUERO, C. (1996). Estudios sobre el proceso de percepción, inhibición y catabolismo de feromonas sexuales de lepidópteros. Tesi doctoral, Facultat de biología, Universitat de Barcelona. Barcelona. 228 pp.
 - QUERO, C.; BAKER, T. C. (1999). Antagonistic effect of (Z)-11-hexadecen-1-ol on the pheromone-mediated flight of *Helicoverpa zea* (Boddie) (Lepidoptera : Noctuidae). *J. Insect Behaviour* **12**, 701-710.
 - QUERO, C.; ROSELL, G.; JIMÉNEZ, O.; RODRÍGUEZ, S.; BOSCH, M. P.; GUERRERO, A. (2002). New fluorinated derivatives as esterase inhibitors. Synthesis, hydratation and crossed specificity studies. *Biorg. & Medi. Chem.* **11**, 1047-1055.
 - RENOU, M.; GUERRERO, A. (2000). Insect parapheromones in olfaction research and semiochemical-based pest control strategies. *Ann. Rev. Entomol.* **48**, 605-630.

- RENOU, M.; BERTHIER, A.; DESBARATS, L.; VAN DER PERS, J.; GUERRERO, A. (1999). Actographic analysis of the effect of an esterase inhibitor on male moth responses to sex pheromone. *Chem. Senses* **24**, 423-428.
- RENOU, M.; LUCAS, P.; MALO, E. A.; QUERO, C.; 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. (1994). Inhibition of pheromone action in *Sesamia nonagrioides* by haloacetate analogues. *Pest. Sci.* **41**, 97-103.
- RIBA, M.; SANS, A.; BAU, P.; GROLLEAU, G.; RENOU, M.; GUERRERO, A. (2001). Pheromone response inhibitors of the corn stalk borer *Sesamia nonagrioides*. Biological evaluation and toxicology. *J. Chem. Ecol.* **27**, 1879-1897.
- RIDGWAY, R. L.; SILVERSTEIN, R. M.; INSCOE, M. N. (1990). Behavior-Modifying Chemicals for Insect Management. New York.
- ROELOFS, W.; GLOVER, T.; TANG, X.-H.; SRENG, I.; ROBBINS, P.; ECKENRODE, C.; LÖFSTEDT, C.; HANSSON, B. S.; BENGTSSON, O. (1987). Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. *Proc. Natl. Acad. Sci. USA* **84**, 7585-7589.
- ROSELL, G.; HERRERO, S.; GUERRERO, A. (1996). New trifluoromethylketones as potent inhibitors of esterases. ¹⁹F NMR spectroscopy of transition state analog complexes and structure-activity relationships. *Biochem. Biophys. Res. Commun.* **226**, 287-292.
- RUMBO, E. R.; DEACON, S. M.; REGAN, L. P. (1993). Spatial discrimination between sources of pheromone and an inhibitor by the light-brown apple moth *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae). *J. Chem. Ecol.* **19**, 953-962.
- SANS, A. (1995). Les feromones sexuals en el control dels barrinadors del blat de moro *Ostrinia nubilalis* Hbn. i *Sesamia nonagrioides* Lef. Tesis Doctoral, ETSEA, Universitat de Lleida. Lleida. 185 pp.
- SANS, A.; RIBA, M.; EIZAGUIRRE, M.; LOPEZ, C. (1997). Electroantennogram, wind tunnel and field responses of male Mediterranean corn borer, *Sesamia nonagrioides*, to several blends of its sex pheromone components. *Entomol. Exp. Appl.* **82**, 121-127.
- STEINER, H. et al. (1977). Vers la production agricole intégrée par la lutte intégrée. Bull. OILB/SROP 1977/4; 153 pp. A BOLLER, E.F.; AVILLA, J.; GENDRIER, J.P.; JÖRG, E.; MALAVOLTA, C. (1988) "Integrated Production in Europe: 20 years after the declaration of Ovronnaz. Bull. OILB/SROP 21, 1988; 34pp.
- THEIS, N.; LERDAU, M. (2003). The evolution of function in plant secondary metabolites. *Int. J. Plant Sci.* **164**, S93-S102.
- VISSER, J.H. (1986). Host odor perception in phytophagous insects. *Ann. Rev. Entomol.* **31**, 121-144.
- VOGT, R. G. (2005). Molecular basis of pheromone detection in insects. In Comprehensive Insect Physiology, Biochemistry, Pharmacology and Molecular Biology. Volume 3. *Endocrinology*. 753-804. Elsevier, London.

- VOGT, R. G.; RIDDIFORD, L. M. (1981). Pheromone binding and inactivation by moth antennae. *Nature* **293**, 161-163.
- WARTHEN, J. D.; KLUN, J. A.; SCHWARZ, M.; WAKABAYASHI, N. (1995). Structure-activity relationship observations for European corn borer moth pheromone and fluoro analogs via computer molecular modelling. *J. Chem. Ecol.* **21**, 1921-1930.

Objectius

D'acord amb els antecedents presentats anteriorment i en el marc d'un projecte d'investigació centrat en “*nous estudis dirigits per a un control biorracional de plagues. Desenvolupament de nous inhibidors de la comunicació química intraespecífica i atridents d'oviposició*” i d'un projecte PETRI amb l'empresa privada SEDQ S.A. (Sociedad Española de Desarrollos Químicos) interessada en el desenvolupament i possible aplicació de les TFMCs com a nous productes per al control de plagues, els objectius que es plantegen en aquesta tesi s'engloben en els següents apartats:

- Efecte d'algunes TFMCs sobre l'inhibició de la percepció de la feromona en *O. nubilalis*.
 - Investigar l'activitat de Z11-14:TFMC, Z10-13:TFMC, 14:TFMC, 14:TFPAm i Z11-2S-14:TFMC com a possibles inhibidors de la percepció de la feromona sexual en assaigs de túnel de vent, assaigs de camp i d'inhibició d'esterases antenals en *O. nubilalis*.
- Utilització de la Z11-16:TFMC per al control dels barrinadors del blat de moro en parcel·les comercials de panís.
 - Determinar el potencial de la Z11-16:TFMC per al control de *S. nonagrioides* mitjançant assaigs de camp a escala comercial.
 - Comprovar l'efecte de la Z11-16: TFMC sobre altres espècies diferents a *S. nonagrioides*, però que comparteixen el mateix habitat, principalment l'efecte sobre *O. nubilalis*.
 - Determinar la cinètica d'alliberament de la Z11-16: TFMC en condicions de camp utilitzant els difusors “*inhibitor SN DCF*” de SEDQ.
- Altres tipus d'antagonistes feromonals (naturals i sintètics).
 - Comprovar l'efecte disruptiu del procés de percepció de la feromona sexual d'*O. Nubilalis* de la Z11-14:MC.
 - Determinar el mecanisme d'acció de la Z11-14:MC en comparació amb la Z11-14:TFMC.
 - Comparar els paràmetres de vol de mascles d'*O. nubilalis* sotmesos a la presència de Z11-14:TFMC, Z11-14:MC i Z11-16:Ald.

- Determinar a partir dels paràmetres de vol de mascles d'*O. nubilalis*, el mecanisme d'acció de Z11-14:TFMC, Z11-14:MC i Z11-16:Ald.
- Compostos orgànics volàtils provinents de plantes hoste d'*O. nubilalis*.
 - Identificar diferents compostos orgànics volàtils a partir "*headspace volatile collections*" provinents de plantes senceres de panís.
 - Comprovar si aquests compostos (o altres compostos relacionats amb aquests) tenen algun efecte d'atracció o repulsió en femelles adultes d'*O. nubilalis*.
 - Establir possibles relacions d'estructura-activitat amb els compostos identificats com a més actius.

Capítulo I

Behavioural and electrophysiological responses
of the European corn borer *Ostrinia nubilalis*
(Lepidoptera: Crambidae) to different host plant
volatiles

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 $0.28 \mu\text{M}$. 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 $7.55 \mu\text{M}$). 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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Pheromone Response of *Ostrinia nubilalis* MalesChart 1. List of Chemicals Tested as Putative Inhibitors of the Pheromone Response and Catabolism of *O. nubilalis*

	Z11-14:Ac
	Z11-14:TFMK
	Z10-13:TFMK
	14:TFMK
	14:TFPAm
	Z11-2S-14:TFMK

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 Haen.

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): ν 3010, 2932, 1771, 1541, 1203, 1142 cm^{-1} . ^1H 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): ν 2928, 1762, 1210, 1154 cm^{-1} . ^1H 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). ^1H 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} . ^1H 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} . ^1H 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 $\text{C}_{12}\text{H}_{29}\text{N}$: 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} . ^1H 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, 29.28, 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 $\text{C}_{19}\text{H}_{42}\text{FNO}_2$, 337.2199; found, 337.2229.

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

9-Dodecyl-1-thiol. This compound was obtained following a similar procedure to that previously described (27). Thus, a solution of 9-dodecyl-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): ν 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): ν 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): ν 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 $\text{C}_{13}\text{H}_{22}\text{F}_2\text{OS}$: 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 chromatog-

raphy (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 \times 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, napigargin (methyl-4-hydroxybenzoate) (Fluka Biochemika) (0.12%), flumidil (potassium o-oxiquinolininsulfonate + 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 \times 17 cm diameter) in a climatic chamber with a 16:8 LD 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 \times 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 CO₂, 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 thermostatted bath at 28 °C for 10 min. Then, 2 μL of an ethanol solution (1 $\mu\text{g}/\mu\text{L}$) 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 \times 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 IC₅₀ 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, 2000, 4000, and 5000 ng for Z10-13:TFMK.

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

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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 TiCl₄ 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 acclimated 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 acclimation 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 9/73 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 (Scentsy, 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 disruptive 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 IC₅₀ values were determined. Z11-14:TFMK exhibited an IC₅₀ value of 0.28 µM, and Z10-13:TFMK displayed an IC₅₀ 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:

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
OF	77 a	77 a	75 a	62 a	67 a	50 a
CA	65 a	57 ab	47 ab	45 ab	30 bc	22 bc
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 ($2 \times 2 \chi^2$ 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
OF	77 a	77 a	60 a	55 a	45 a
CA	65 a	60 a	40 ab	37 ab	17 bc
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 ($2 \times 2 \chi^2$ homogeneity test, $P < 0.05$). ^b Behavioral responses are as follows: TF, taking flight; OF, oriented flight; CA, close approach; and SC, source contact.

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).

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 video-recorded. 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

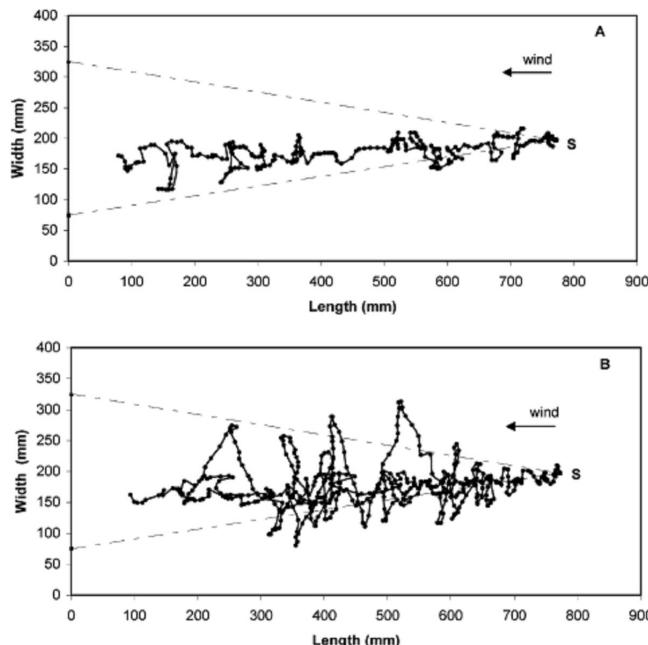


Figure 1. Representative flight track of *O. nubilalis* males flying upwind toward a dispenser containing a 1:2 mixture of pheromone blend and Z11-14:TFMK (**B**) relative to control (**A**). Black dots represent insect positions at 0.04 s intervals.

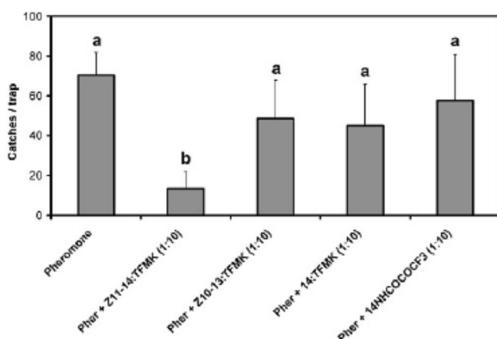


Figure 2. Number of catches of *O. nubilalis* males in traps baited with mixtures of the trifluoromethyl ketones Z11-14:TFMK, Z10-13:TFMK, 14:TFMK, and 14:NHCOCOCF₃ and pheromone in a 10:1 ratio in comparison with catches in traps containing pheromone alone. Rubber septa were used as dispensers. The 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.

trapped with the pheromone alone. In tests carried out in 2001, Z11-14:TFMK, Z10-13:TFMK, 14:TFMK, and 14:NHCOCOCF₃ 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

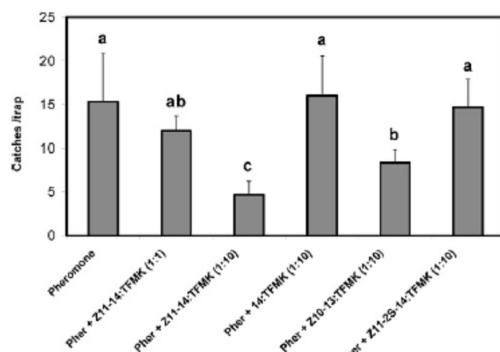


Figure 3. Number of catches of *O. nubilalis* males in traps baited with mixtures of the trifluoromethyl ketones Z11-14:TFMK, Z10-13:TFMK, 14:TFMK, and Z11-2S-14:TFMK and pheromone in 1:1 and 10:1 ratio in comparison with catches in traps containing pheromone alone. Rubber septa were used as dispensers. 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.

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

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displayed a good disruptive 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 disruptive 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 CF₃ 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 IC₅₀ value of $5.9 \mu\text{M}$ in SL and $16.3 \mu\text{M}$ 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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LITERATURE CITED

- Mason, C. E.; Rice, M. E.; Calvin, D. D.; Van Duyn, J. W.; Hutchinson, W. D.; Witkowski, J. F.; Higgins, R. A.; Onstad, D. W.; Dively, G. P. *European Corn Borer. Ecology and Management*; North Central Regional Extension Publication No. 327; Iowa State University: Ames, Iowa, 1996; p 57.
- Roelofs, W. L.; Glover, T.; Tang, X.-H.; Srung, I.; Robbins, P.; Eckenrode, C.; Löfstedt, C.; Hansson, B. O. Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. *Proc. Natl. Acad. Sci. U.S.A.* 1987, 84, 7585–7589.
- Glover, T. J.; Campbell, M. G.; Linn, C. R.; Roelofs, W. L. Unique sex chromosome mediated behavioral responses specificity of hybrid male European corn borer moths. *Experientia* 1991, 47, 980–984.
- Zhu, J. W.; Zhao, C. H.; Lu, F.; Bengtsson, M.; Löfstedt, C. Reductase specificity and the ratio regulation of E/Z isomers in pheromone biosynthesis of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Insect Biochem. Mol. Biol.* 1995, 26, 171–176.
- Ma, P. W. K.; Roelofs, W. L. Sites of synthesis and release of PBAN-like factor in the female European corn borer, *Ostrinia nubilalis*. *J. Insect Physiol.* 1995, 41, 339–350.
- Klun, J. A.; Chapman, O. L.; Mattes, K. C.; Wojtkowski, P. W.; Beroza, M.; Sonnet, P. E. Insect sex pheromones: Minor amount of opposite geometrical isomer critical to attraction. *Science* 1973, 181, 661–663.
- Anglade, P.; Stockel, J.; IGWO cooperators. Intraspecific sex-pheromone variability in the European corn borer, *Ostrinia nubilalis* Hbn. (Lepidoptera: Pyralidae). *Agronomie* 1984, 4, 183–187.
- Peña, A.; Arn, H.; Buser, H.-R.; Rauscher, S.; Bigler, F.; Brunetti, R.; Maini, S.; Tóth, M. Sex pheromone of European corn borer, *Ostrinia nubilalis*: Polymorphism in various laboratory and field strains. *J. Chem. Ecol.* 1988, 14, 1359–1366.
- Gelb, M. H.; Svaren, J. P.; Abeles, R. H. Fluoroketone inhibitors of hydrolytic enzymes. *Biochemistry* 1985, 24, 1813–1817.
- Ashour, M.-B. A.; Hammock, B. D. Substituted trifluoroketones as potent selective inhibitors of mammalian carboxylesterases. *Biochem. Pharmacol.* 1987, 36, 1869–1879.
- Prestwich, G. D. Chemical studies of pheromone receptors in insects. *Arch. Insect Biochem. Physiol.* 1993, 22, 75–86.
- Durán, I.; Parrilla, A.; Feixas, J.; Guerrero, A. Inhibition of antennal esterases of the Egyptian armyworm *Spodoptera littoralis* by trifluoromethyl ketones. *Bioorg. Med. Chem. Lett.* 1993, 3, 2593–2598.
- Parrilla, A.; Villuendas, I.; Guerrero, A. Synthesis of trifluoromethyl ketones as inhibitors of antennal esterases of insects. *Bioorg. Med. Chem.* 1994, 2, 243–252.
- Vogt, R. G.; Riddiford, L. M.; Prestwich, G. D. Kinetic properties of a pheromone-degrading enzyme: The sensillar esterase of *Antheraea polyphemus*. *Proc. Natl. Acad. Sci. U.S.A.* 1985, 82, 8827–8831.
- Prestwich, G. D.; Vogt, R. G.; Riddiford, L. M. Binding and hydrolysis of radiolabeled pheromone and several analogues by male-specific antennal proteins of the moth *Antheraea polyphemus*. *J. Chem. Ecol.* 1986, 12, 323–333.
- Linderman, R. J.; Leazer, J.; Roe, R. M.; Venkatesh, K.; Selinsky, B. S.; London, R. E. ¹⁹F 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 analogue inhibitor of acetylcholinesterase. *Pest. Biochem. Physiol.* 1988, 31, 187–194.
- Rosell, G.; Herrero, S.; Guerrero, A. New trifluoromethyl ketones as potent inhibitors of esterases: ¹⁹F NMR spectroscopy of transition state analogue complexes and structure–activity relationships. *Biochem. Biophys. Res. Commun.* 1996, 226, 2887–2892.
- Renou, M.; Lucas, P.; Malo, E.; Quero, C.; Guerrero, A. Effects of trifluoromethyl ketones and related compounds on the EAG and behavioural responses to pheromones in male moths. *Chem. Senses* 1997, 22, 407–416.
- Pophof, B.; Gebauer, T.; Ziegelberger, A. Decyl-thio-trifluoropropane, a competitive inhibitor of moth pheromone receptors. *J. Comp. Physiol. A* 2000, 186, 315–323.
- Bau, J.; Martínez, D.; Renou, M.; Guerrero, A. Pheromone-triggered orientation flight of male moths can be disrupted by trifluoromethyl ketones. *Chem. Senses* 1999, 24, 473–480.
- Riba, M.; Sans, A.; Bau, P.; Grolleau, G.; Renou, M.; Guerrero, A. Pheromone response inhibitors of the corn stalk borer *Sesamia nonagrioides*: Biological evaluation and toxicology. *J. Chem. Ecol.* 2001, 27, 1879–1897.
- Guerrero, A.; Bosch, M. P.; Rosell, G.; Riba, M.; Sans, A. New halomethyl ketones and use in traps for the biorational control of insect pests. Patent 200301667, Spain, 2003; 13 pp.
- Camps, F.; Gasol, V.; Guerrero, A. A new and efficient one-pot preparation of alkyl halides from alcohols. *Synthesis* 1987, 511–512.
- Villuendas, I.; Parrilla, A.; Guerrero, A. An efficient and expeditious synthesis of functionalized trifluoromethyl ketones through lithium–iodine exchange reaction. *Tetrahedron* 1994, 50, 12673–12684.
- Boivin, J.; El Kaim, L.; Zard, S. Z. A new and efficient synthesis of trifluoromethyl ketones from carboxylic acids. *Tetrahedron* 1995, 51, 2573–2584.
- El Kaim, L.; Pinot-Périgord, E. Trifluoropyruvamides from isocyanides and trifluoroacetic anhydride. *Tetrahedron* 1998, 54, 3799–3806.
- Beretta, E.; Cinquini, M.; Colonna, S.; Fornasier, R. A mild synthesis of optically active thiols. *Synthesis* 1974, 425–426.
- Poitout, S.; Bues, R. Élevage des chenilles de vingt-huit espèces de lépidoptères Noctuidae et deux espèces d'Arctiidæ sur milieu artificiel simple. Particularités de l'élevage selon les espèces. *Ann. Zool. Ecol. Anim.* 1974, 6, 431–441.
- Quero, C.; Camps, F.; Guerrero, A. Behavior of processionary males (*Thaumetopoea pityocampa*) induced by sex pheromone and analogues in a wind tunnel. *J. Chem. Ecol.* 1995, 21, 1957–1969.
- Quero, C.; Rosell, G.; Jiménez, O.; Rodriguez, S.; Bosch, M. P.; Guerrero, A. New fluorinated derivatives as esterase inhibitors. Synthesis, hydration and crossed specificity studies. *Bioorg. Med. Chem.* 2003, 11, 1047–1055.
- Linderman, R. J.; Jamois, E. A.; Roe, R. M. Correlation of equilibrium hydration constant and inhibitory potency for trifluoromethyl ketone inhibitors of insect juvenile hormone esterase. *Rev. Pest. Toxicol.* 1991, 1, 261–270.
- Olmstead, M. M.; Musker, W. K.; Hammock, B. D. Structure of the hydrate form of a β-thiotrifluoromethyl ketone, a potent esterase inhibitor. *Acta Crystallogr. C* 1987, 43, 1726–1728.

Pheromone Response of *Ostrinia nubilalis* Males*J. Agric. Food Chem.*, Vol. 53, No. 4, 2005 1165

- (33) Filizola, M.; Rosell, G.; Guerrero, A.; Pérez, J. J. Conformational requirements for inhibition of the pheromone catabolism in *Spodoptera littoralis*. *Quant. Struct.-Act. Relat.* **1998**, *17*, 205–210.
- (34) Klun, J. A.; Schwarz, M.; Uebel, E. C. European corn borer: Pheromonal catabolism and behavioral response to sex pheromone. *J. Chem. Ecol.* **1991**, *17*, 317–334.
- (35) Renou, M.; Guerrero, A. Insect parapheromones in olfaction research and semiochemical-based pest control strategies. *Annu. Rev. Entomol.* **2000**, *48*, 605–630.
- (36) Pophof, B. Inhibitors of sensillar esterase reversibly block the responses of moth pheromone receptor cells. *J. Comp. Physiol. A* **1998**, *183*, 153–164.
- (37) Feixas, J.; Prestwich, G. D.; Guerrero, A. Ligand specificity of pheromone-binding proteins of the processionary moth. *Eur. J. Biochem.* **1995**, *234*, 521–526.

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Capítulo II

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

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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

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Key words: pheromone antagonists, pest management, Lepidoptera, Noctuidae, Crambidae, *Zea mays*, Poaceae, mating disruption

Abstract

Large-scale field experiments on the Mediterranean corn borer, *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae), were carried out in 2004–2006 on maize [*Zea mays* L. (Poaceae)] fields using (*Z*)-11-hexadecenyl trifluoromethyl ketone, an antagonist analog of the pheromone of this species, to evaluate a possible reduction of damage caused by this pest. The effect of the treatments on the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), a sympatric species, was also determined. Evaluation of the success of the experiments was assessed by counting (i) the number of males caught by pheromone traps in treated and untreated fields, (ii) the number of plants attacked by both insects in both plots, and (iii) the number of larvae present in infested plants in both fields. Effectiveness of the treatment was high for the second generation of the Mediterranean corn borer, the most harmful to the crop (86–90% reduction in the number of plants attacked and 67–98% reduction in the number of larvae per plant in treated fields in comparison to untreated fields), and moderate for the third generation (reduction of 41–71% and 33–77%, respectively). Treatments were also effective for the second generation of the European corn borer (61–75% reduction in the number of plants attacked, 58–78% reduction in the number of larvae found per plant) as well as for the third generation (69–97% and 70–98% reduction, respectively). By plotting the amount of the antagonist remaining on the dispensers after 40–45 days of exposure with time, the mean release rate of the compound was calculated to be 2.2%/day in 2004, 1.95%/day in 2005, and 2.1%/day in 2006, with 26% of the initial compound remaining after 20 days of experimentation. The emission rate appears to cover the flight of the most damaging second generation of both insects. Prospects of using trifluoromethyl ketones as new potential agents for pest control are also outlined.

Introduction

The Mediterranean corn borer (also known as corn stalk borer), *Sesamia nonagrioides* Lefèbvre (Lepidoptera: Noctuidae), is an important pest of maize [*Zea mays* L. (Poaceae)] in the Mediterranean region (below 45°N parallel) and North Africa (Anonymous, 1979). A number

of broad spectrum insecticides have been used to control the pest but with little success owing to the endophytic habits of the species, high costs, and negative effects on secondary pests, such as aphids, mites, and leafhoppers. The sex pheromone of the moth was identified in 1985 as a mixture of (*Z*)-11-hexadecenyl acetate (*Z*11-16:Ac) and (*Z*)-11-hexadecenol (*Z*11-16:OH) (Seng et al., 1985). Later, Mazomenos (1989) found that the four-component blend of *Z*11-16:Ac, *Z*11-16:OH, (*Z*)-11-hexadecenal (*Z*11-16:Ald), and dodecyl acetate (12:Ac) in a 69:8:8:15 ratio considerably improved the efficiency of the pheromone.

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In 1997, a slightly different formulation based on a 77:8:10:5 mixture of the four compounds further improved trapping efficiency as well as the selectivity (Sans et al., 1997).

The European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), is not only a major maize pest, but it can also damage other crops, such as potato, green pepper, and winter wheat in Europe, North America, North Africa, the Philippines, and Japan (Mason et al., 1996). Control of this species is particularly difficult, because insecticide sprays are only effective during the short period between egg hatching and young larvae boring into the stems. The insect displays polymorphism in its pheromone communication but, despite having two different populations, in areas where both strains are present there is enough genetic compatibility to produce fertile hybrids (Roelofs et al., 1987; Ma & Roelofs, 1995; Zhu et al., 1995). The Z-race of *O. nubilalis* uses a 97:3 mixture of (*Z*)-11-tetradecenyl acetate (Z11-14:Ac) and (*E*)-11-tetradecenyl acetate (E11-14:Ac) (Klun et al., 1973), whereas the *E*-race uses the same compounds in 1:99 to 4:96 blends (Glover et al., 1987; Peña et al., 1988).

Mating disruption is an important biorational pest-management strategy to control insects relying on long-distance pheromones for mate finding (Sanders, 1997; Miller et al., 2006a, b). As one of the key benefits of using pheromone-based programs for mating disruption is their high selectivity, the vast majority of mating disruption experiments have been performed with the synthetic attractant (Silverstein, 1990; Shaver & Brown, 1993; Cardé & Minks, 1995; Shorey & Gerber, 1996) or in combination with an insecticide (Haynes et al., 1986; Trimble et al., 2001). Very few mating disruption experiments on the Mediterranean corn borer using pheromone formulations have been reported (Perdiguer et al., 1992; Frérot et al., 1997; Albajes et al., 2002; Eizaguirre et al., 2002) and only one on the European corn borer (Baker, 1999).

Little work in pest control, however, has been done using pheromone antagonists (Kaae et al., 1974; Beevor & Campion, 1979; Hathaway et al., 1985; Curtis et al., 1987; Bengtsson et al., 1994; Evenden et al., 1999). These compounds, which alter the behavior or physiology of the insect communication system (Renou & Guerrero, 2000), may be pheromone components of closely related species with a sufficiently similar structure to that of the natural pheromone to bind to the pheromone receptor sites, and therefore competing with the natural attractant (Sanders, 1997). In some other cases, the pheromones of other species are detected by receptor neurons different from the pheromone receptor cells and when activated they elicit an antagonistic response by the central nervous system (Glover et al., 1989).

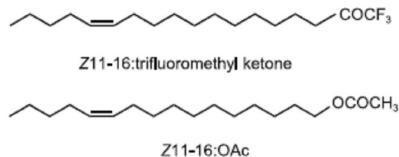


Figure 1 Structures of the antagonist Z11-16:trifluoromethyl ketone and Z11-16:OAc, the major pheromone component of the Mediterranean corn borer.

In this article and following previous work from our laboratories (Riba et al., 2001, 2005; Guerrero et al., 2003; Quero et al., 2003, 2004), we present the first large-scale experiments in the field on the effect of (*Z*)-11-hexadecenyl trifluoromethyl ketone (Z11-16:trifluoromethyl ketone, Figure 1), a non-natural antagonist of the major component of the Mediterranean corn borer pheromone (Z11-16:Ac, Figure 1). The main objective of this work was to determine the potential of the antagonist to control the Mediterranean corn borer by evaluation of three parameters: reduction of male catches by the pheromone, number of plants damaged, and number of larvae found per plant in treated and untreated fields. At the same time, we outlined the effect induced on the sympatric European corn borer.

Materials and methods

Chemicals

Sex pheromone components of the Mediterranean corn borer and European corn borer absorbed in 8-mm rubber septa (Aldrich, Milwaukee, WI, USA) were provided by Sociedad Española de Desarrollos Químicos, S.A. (Barberá del Valles, Barcelona, Spain). The pheromone was a mixture of Z11-14:Ac and E11-14:Ac in a 97:3 ratio for the European corn borer and Z11-16:Ac, Z11-16:OH, Z11-16:Ald, and 12:Ac in a 77:8:10:5 ratio for the Mediterranean corn borer. All compounds were ≥99% pure as determined by gas chromatographic analysis.

Preparation of Z11-16:trifluoromethyl ketone

This compound was prepared in the laboratory in a four-step process from Z11-16:OH. The method involved: (i) transformation of Z11-16:OH into the corresponding bromide, (ii) reaction of the bromide with sodium cyanide and aqueous tributylamine (Apparu et al., 1988), (iii) alkaline hydrolysis to the carboxylic acid, and (iv) reaction of the acid with oxalyl chloride followed by treatment with trifluoroacetic anhydride/pyridine (Boivin et al., 1995). The antagonist was obtained in 54% overall yield and the method was later optimized and adapted for a multigram scale at a pilot plant.

Table 1 Experimental fields and crop variety used to determine the effect of application of Z11:16trifluoromethyl ketone on reduction of damage induced by the Mediterranean corn borer and the European corn borer

Year	Location	UTM. coordinates ^a (utm 31 N)	Dose	Area (ha)	Irrigation system	Sowing date	Maize variety	Harvest date	Deployment dispensers	Damage control 1	Damage control 2
2004	Almàsselles	X:289361 Y:4621078	80 g ha ⁻¹	2.34	Spray	23-04-04	Eleonora Pioneer	20-10-04	08-09-04	11-10-04	
2005	Almàsselles	X:291007 Y:4623074	80 g ha ⁻¹	1.9	Flooding	25-04-05	PR34N43 Pioneer	06-10-05	12-09-05	03-10-05	
	Almàsselles	X:292616 Y:4625411	50 g ha ⁻¹	2.5	Flooding	20-04-05	PR34N43 Pioneer	03-10-05	09-09-05	03-10-05	
2006	Almàsselles	X:291007 Y:4623074	80 g ha ⁻¹	1.9	Flooding	27-04-06	PR34N43 Pioneer	15-10-06	12-07-06	15-09-06	05-10-06
	Juneda	X:319018 Y:4603643	50 g ha ⁻¹	2.35	Flooding	22-04-06	PR34N43 Pioneer	30-10-06	12-07-06	15-09-06	10-10-06

^aUniversal Transverse Mercator.

Field tests

The assays were performed in 1.9–2.5-ha maize fields located near Almàsselles and Juneda, two small towns in the province of Lleida in northeastern Spain for three consecutive years (2004–2006). A plot similar in size located between 300 and 1000 m from the experimental one was used as control. The dispensers ('inhibitor SN DCF'), a laminate with the antagonist absorbed between two layers containing 800 mg of Z11-16:trifluoromethyl ketone, were prepared at Sociedad Española de Desarrollos Químicos, S.A., tied to the top of the plant at ca. 1.5 m from the soil, and deployed every 10 m in parallel rows of plants along the field. The rows were spaced ca. 15 m apart. One hundred dispensers/ha were required for the experiments with 80 g ha⁻¹ of the antagonist and 62 dispensers/ha for the 50 g ha⁻¹ trials. The main features of the experimental fields are shown in Table 1.

To determine the effect of the treatments on the number of catches of European corn borer and Mediterranean corn borer males, three funnel traps containing rubber septa baited with 100 µg of the pheromone of both insects (see above) were deployed in each field. These traps were placed in the vertices of a triangle around the approximate center of the field and three more traps with similar baits were located along the borders of the plot. The traps were hung at a height of 1.2 m and were spaced in a minimum of 20 m apart. Trapped moths were counted and removed every week and the lures were renewed at the start of the third flight period (beginning of September). The mean number of males of both insects trapped along with that of *Valeria jaspidea* Villers (Lepidoptera: Noctuidae) and *Mythimna (Pseudaletia) unipuncta* Haworth (Lepidoptera: Noctuidae) caught in the treated plot was compared to those caught in the untreated plot (see below).

To evaluate plants damage, we randomly selected nine control points and at each one we examined 20 adjacent plants (180 plants in each plot). The control points were spaced ca. 50 m apart. The plants were dissected and carefully examined for the presence of larvae or pupae of the Mediterranean corn borer and the European corn borer at the end of the second generation. The number of plants attacked referred to the plants containing at least one larva of either insect. The galleries produced by both insects have not been considered for comparison as they are quite similar in size and it was difficult, and probably not reliable, to assign them to a specific insect. For the third generation, a new set of 90 plants (10 adjacent plants at nine control points spaced 50 m apart) was dissected and examined again for the presence of larvae or pupae of both insects. Percentage of plants damaged was calculated by counting the number of plants attacked with at least one larva or pupa in relation to the total number of sampled

plants in treated fields in comparison to untreated fields, according to the formula below:

$$\text{Percentage reduction} = \frac{\text{Plants infested in control plot} - \text{Plants infested in treated plot}}{\text{Plants infested in control plot}} \times 100$$

In the same manner, the number of larvae present in the attacked plants was related to the total number of plants dissected in both fields. Recognition of the larvae was done according to the literature (Anglade, 1972; Guennelon, 1972).

The release rate of Z11-16:trifluoromethyl ketone was determined from a new set of dispensers identical to those used in field experiments. The dispensers were deployed at the beginning of the second generation of the Mediterranean corn borer (from 20 July) and were hung close to the top of the maize plants (1.5 m from the soil) and at 2 m from the edge of the plot. They were spaced 0.5 m apart and left in the field for 0, 15, 30, 45, 60, 90, and 120 days in 2004 and 0, 10, 20, 30, and 40 days in 2005 and 2006. The average temperature of the region during the first 40 days of experimentation was 24.8 °C in 2004, 23.6 °C in 2005, and 24.1 °C in 2006. On each sampling day, three dispensers were collected, covered with aluminum foil, labeled, and sent to the laboratory, where they were kept at -20 °C until analysis.

Each group of dispensers was extracted in a Soxhlet using 200 ml of hexane (pesticide quality; Aldrich) at reflux for 12 h. Upon cooling, 5 ml of a 20 mg ml⁻¹ solution of dodecyl acetate in hexane as internal standard was added. The resulting solution was further diluted 10× and aliquots of 0.3 µl were injected onto GC for analysis. Quantification was carried out using an HP-1 33 m × 0.25 mm × 0.33 µm capillary column under the following chromatographic conditions: injection at 50 °C and program of 10 °C min⁻¹ up to 270 °C, which was maintained for 10 min. Plot of the antagonist amount remaining in each extract with exposure time in the field was fitted to an exponential parabolic curve from which the emission rate could be calculated according to the formula $V_{\text{em}} = -ae^{bx}$, V_{em} being the derivate of the loss of product y_{em} ($y_{\text{em}} = y_0 - y$, y_0 being the initial amount of the antagonist) with time of exposure (x) in the field, a is the intercept on the Y-axis, and b the slope.

Statistical analysis

Data of pheromone catches for the 3-year period were combined and analyzed for significance ($P < 0.05$) using a three-way analysis of variance (ANOVA) (year, location of traps, and plot). To normalize data, the values were transformed to $\sqrt{x+1}$ prior to analysis (Tables 1 and 2).

Table 2 Mean number of *Sesamia nonagrioides* (SN), *Valeria jaspidea*, *Mythimna unipuncta*, and *Ostrinia nubilalis* (ON) males caught per trap (\pm SE) by *S. nonagrioides* and *O. nubilalis* pheromone traps in fields treated with Z11-16:trifluoromethyl ketone (80 g ha⁻¹) vs. untreated fields in the period 2004–2006¹

	Dose of Z11-16:trifluoromethyl ketone (80 g ha ⁻¹)			
	With SN pheromone			With ON pheromone
	<i>S. nonagrioides</i>	<i>V. jaspidea</i>	<i>M. unipuncta</i>	
2004				
Control plot	8.16 ± 4.27	11.66 ± 1.93	32.50 ± 22.54	not determined
Treated plot	7.50 ± 4.04	9.66 ± 3.35	11.16 ± 7.95	not determined
Reduction (%)	8	17	66	not determined
2005				
Control plot	7.83 ± 2.49	6.33 ± 2.87	145.66 ± 72.55	2.00 ± 1.15
Treated plot	1.33 ± 0.61	27.13 ± 9.61	49.16 ± 21.51	4.66 ± 0.66
Reduction (%)	83	—	66	—
2006				
Control plot	4.66 ± 0.66	4.33 ± 0.33	55.66 ± 21.73	9.00 ± 1.15
Treated plot	2.00 ± 1.15	20.00 ± 6.24	11.33 ± 4.70	4.00 ± 3.05
Reduction (%)	57	—	79	55
2004–2006				
Control plot ²	7.33 ± 1.91a	8.06 ± 1.54a	82.40 ± 32.15a	5.50 ± 1.41a
Treated plot ²	3.93 ± 1.74b	18.73 ± 4.51b	26.80 ± 9.98b	4.33 ± 1.73a
Reduction (%)	46	—	67	21

¹Untransformed data.

²Three-way analysis of variance (ANOVA) (year, location of traps, and plot) was performed for statistical analysis. To normalize data, the values were transformed to $\sqrt{x+1}$ prior to analysis. Data followed by different letters within the same column are significantly different ($P < 0.05$).

Table 3 Mean number of *Sesamia nonagrioides* (SN), *Valeria jaspidea*, *Mythimna unipuncta*, and *Ostrinia nubilalis* (ON), males caught per trap (\pm SE) by *S. nonagrioides* and *O. nubilalis* pheromone traps in fields treated with Z11-16:trifluoromethyl ketone (50 g ha⁻¹) vs. untreated fields in the period 2004–2006¹

	Dose of Z11-16:trifluoromethyl ketone (50 g ha ⁻¹)			
	With SN pheromone		With ON pheromone	
	<i>S. nonagrioides</i>	<i>V. jaspidea</i>	<i>M. unipuncta</i>	<i>O. nubilalis</i>
2004				
Control plot	2.00 \pm 0.45	23.00 \pm 8.94	59.83 \pm 21.62	3.33 \pm 0.88
Treated plot	0.33 \pm 0.21	11.66 \pm 3.61	42.66 \pm 14.99	2.33 \pm 0.88
Reduction (%)	83	49	66	30
2005				
Control plot	21.00 \pm 7.64	8.00 \pm 1.15	102.00 \pm 16.19	6.33 \pm 4.84
Treated plot	11.33 \pm 4.91	20.67 \pm 10.49	12.67 \pm 5.24	4.00 \pm 1.73
Reduction (%)	46	—	88	37
2006				
Control plot ²	8.33 \pm 3.87a	18.00 \pm 6.30a	73.89 \pm 16.31a	4.83 \pm 2.30a
Treated plot ²	4.00 \pm 2.32b	14.67 \pm 4.11a	32.67 \pm 10.99b	3.16 \pm 0.94a
Reduction (%)	52	18	56	35

¹Untransformed data.

²Three-way analysis of variance (ANOVA) (year, location of traps, and plot) was performed for statistical analysis. To normalize data, the values were transformed to $\sqrt{x+1}$ prior to analysis. Data followed by different letters within the same column are significantly different ($P<0.05$).

Data of percentage of plants attacked and number of larvae per plant for the whole period were similarly combined and analyzed for significance ($P<0.05$) using a two-way ANOVA (year and plot). Prior to analysis, the percentage of plants was transformed to $\arcsin \sqrt{x}/100$ whereas the number of larvae per plant was converted to $\log(x+1)$ (Tables 3 and 4). The SAS system for Windows (version 9.0) (SAS Institute, 2003) software was used for analysis.

Results

Trap catch

In the 80 g ha⁻¹ treatment and over the 3-year experimentation, the mean number (\pm SE) of Mediterranean corn borer males caught per trap was low, 7.33 \pm 1.91 in control plots vs. 3.93 \pm 1.74 in treated plots along the period tested (ca. 20 July–2 October), but the difference was significant ($F = 6.10$, d.f. = 1, $P = 0.020$; Table 2). The mean reduction of catches was 46%. Concomitantly, 8.06 \pm 1.54 males/trap of a non-pest species, *V. jaspidea*, were caught in control plots vs. 18.73 \pm 4.51 males in treated plots, as well as *M. unipuncta* males (82.40 \pm 32.15 in control plots and 26.80 \pm 9.98 males in treated plots; Table 2). *Mythimna unipuncta* is a sympatric species whose pheromone complex (a mixture of Z11-16:Ac, 16:Ac, Z11-16:OH, and Z9-16:Ac in 77:12:10:1 ratio; McDonough et al., 1980)

closely resembles that of the Mediterranean corn borer pheromone. In the European corn borer pheromone traps, only conspecific males were caught, which is in agreement with earlier results that reported mutual pheromone antagonism between the two major species (Gemeno et al., 2006; Eizaguirre et al., 2007). The European corn borer population was also low, although it was only determined in 2005 and 2006 (5.50 \pm 1.41 of males/trap caught as average in control plots vs. 4.33 \pm 1.73 males caught in treated plots). The difference was not significant in this case ($F = 0.18$, d.f. = 1, $P = 0.678$; Table 2).

In the 50 g ha⁻¹ treatment and over the 2-year experimentation, the average level of catches of every insect was quite similar (Table 3). The Mediterranean corn borer pheromone caught an average of 8.33 \pm 3.87 conspecific males in control plots and 4.0 \pm 2.32 males in treated fields, the difference being significant ($F = 4.80$, d.f. = 1, $P = 0.046$). The traps also caught *V. jaspidea* males (18.0 \pm 6.30 vs. 14.67 \pm 4.11 in control and treated fields, respectively) and *M. unipuncta* (73.89 \pm 16.31 vs. 32.67 \pm 10.99 in control and treated fields, respectively). In this latter case, the difference was significant ($F = 8.05$, d.f. = 1, $P = 0.013$; Table 3). The European corn borer pheromone, in turn, caught 4.83 \pm 2.30 males/trap and 3.16 \pm 0.94 males/trap in control and treated plots, respectively, but the difference was not significant ($F = 0.32$, d.f. = 1, $P = 0.587$).

Table 4 Percentage reduction of number of plants attacked and number of larvae per plant (\pm SE) induced by the Mediterranean corn borer males in Z11-16:trifluoromethyl ketone treated (80 g ha^{-1}) and untreated fields in the period 2004–2006¹

	Mediterranean corn borer			
	Second generation		Third generation	
	Plants attacked ²	Larvae/plant ²	Plants attacked ²	Larvae/plant ²
2004				
Control plot	19.44 \pm 1.89	0.34 \pm 0.04	34.44 \pm 3.56	1.12 \pm 0.19
Treated plot	0.56 \pm 0.18	0.01 \pm 0.002	10.00 \pm 2.60	0.26 \pm 0.08
Reduction (%)	97	98	71	77
2005				
Control plot	20.00 \pm 2.33	0.28 \pm 0.04	18.90 \pm 2.46	0.23 \pm 0.03
Treated plot	2.77 \pm 0.92	0.09 \pm 0.03	5.55 \pm 0.98	0.10 \pm 0.02
Reduction (%)	86	68	71	57
2006				
Control plot	22.22 \pm 3.27	0.51 \pm 0.08	18.88 \pm 3.87	0.27 \pm 0.06
Treated plot	2.22 \pm 0.56	0.02 \pm 0.01	11.11 \pm 2.69	0.18 \pm 0.05
Reduction (%)	90	96	41	33
2004–2006				
Control plot ³	20.56 \pm 2.94a	0.37 \pm 0.04a	24.11 \pm 2.87a	0.54 \pm 0.08a
Treated plot ³	1.85 \pm 0.75b	0.04 \pm 0.018b	8.89 \pm 1.87b	0.18 \pm 0.04b
Reduction (%)	91	89	63	67

¹Untransformed data. Number of plants per year and per plot dissected for samplings was $n = 180$ for the second generation and $n = 90$ for the third generation.

²Two-way analysis of variance (ANOVA) (year and plot) was performed for statistical analysis. For attacked plants, the values were transformed to $\arcsin \sqrt{x/100}$ prior to analysis; for larvae/plant the data were transformed to $\log(x+1)$.

³Data within the same column followed by different letters are significantly different ($P < 0.05$).

Damage level

In the experiments with 80 g ha^{-1} of the antagonist, the number of plants attacked by the Mediterranean corn borer was significantly lower in treated plots than in control plots both for the second and the third generation of the pest [20.56 ± 2.94 males in untreated fields vs. 1.85 ± 0.75 males in treated fields for the second generation ($F = 22.15$, d.f. = 1, $P < 0.0001$), and 24.11 ± 2.87 males vs. 8.89 ± 1.87 males in control and treated plots, respectively, for the third generation ($F = 6.75$, d.f. = 1, $P = 0.0123$; Table 4)]. The calculated reduction percentage was 91% for the second flight of the insect and 63% for the third (Table 4). With regard to the number of larvae found per sampled plant, a significant reduction was also noticed after the 3-year experimentation for both generations of the pest. The mean values varied from 0.37 ± 0.04 to 0.54 ± 0.08 in control plots and from only 0.04 ± 0.018 to 0.18 ± 0.04 in treated plots. The reduction values were 89 and 67% for the second and third flight, respectively (Table 4).

Along with the effect on the Mediterranean corn borer, we found that the number of plants attacked by the European corn borer was also significantly decreased. Thus, for the second generation of this moth, the number of plants

affected was reduced by 61–75% in treated plots with an average value of 67% (Table 5). For the third generation, the results were more dramatic, particularly in 2004–2005 in which the reduction of infested plants rose to 84–97% (83% average over the 3-year period). With regard to the number of larvae per plant, the reduction observed in treated plots was also remarkable, particularly for the third generation in 2004–2005 (89–98%). Overall and considering all the data compiled for the 3 years and the two generations, the reduction in the number of larvae ranged from 71 to 87% (Table 5).

Additional experiments using 50 g ha^{-1} of the antagonist in 2005–2006 led to contradictory results. In the first year, the level of infestation was disappointingly low, as shown by the low number of plants attacked in both plots by either pest (Tables 6 and 7). However, for the second generation of both insects, the reduction of plants attacked and number of larvae/plant was quite remarkable (67 and 56%, respectively, for the Mediterranean corn borer and 80 and 81%, respectively, for the European corn borer). In the following year, however, a higher level of infestation resulted in anomalous results with the number of plants damaged and larvae found in the treated plot being similar

	European corn borer			
	Second generation		Third generation	
	Plants attacked ²	Larvae/plant ²	Plants attacked ²	Larvae/plant ²
2004				
Control plot	31.67 ± 1.92	0.44 ± 0.03	33.33 ± 2.22	0.48 ± 0.04
Treated plot	12.22 ± 1.69	0.19 ± 0.03	1.11 ± 0.37	0.01 ± 0.004
Reduction (%)	61	57	97	98
2005				
Control plot	35.55 ± 2.36	0.61 ± 0.04	56.66 ± 2.72	0.88 ± 0.05
Treated plot	13.33 ± 1.12	0.17 ± 0.02	8.88 ± 0.67	0.10 ± 0.01
Reduction (%)	63	72	84	89
2006				
Control plot	37.77 ± 2.17	0.60 ± 0.04	28.88 ± 0.87	0.33 ± 0.01
Treated plot	9.44 ± 1.16	0.13 ± 0.02	10.00 ± 1.24	0.10 ± 0.01
Reduction (%)	75	78	65	70
2004–2006				
Control plot ³	35.00 ± 2.55a	0.55 ± 0.04a	39.62 ± 2.11a	0.56 ± 0.05a
Treated plot ³	11.66 ± 1.62b	0.16 ± 0.02b	6.66 ± 0.80b	0.07 ± 0.016b
Reduction (%)	67	71	83	87

¹Untransformed data. Number of plants per year dissected for samplings was n = 180 for the second generation and n = 90 for the third generation.

²Two-way analysis of variance (ANOVA) (year and plot) was performed for statistical analysis. For attacked plants, the values were transformed to arcsin $\sqrt{x/100}$ prior to analysis; for larvae/plant the data were transformed to $\log(x + 1)$.

³Data within the same column followed by different letters are significantly different (P<0.05).

or higher than in the control plot. Overall, a small, non-significant, reduction in the number of plants attacked (13%) and larvae per plant (20%) was observed for the second generation of the Mediterranean corn borer (Tables 6 and 7).

Release rate

A plot of the amount of Z11-16:trifluoromethyl ketone remaining on the dispensers with time yielded a parabolic curve from which the release rate of the antagonist could be determined (Figure 2 shown for the experiment in 2006 only). The values obtained were 2.2%/day in 2004, 1.95%/day in 2005, and 2.1%/day in 2006, which means that ca. 26% of the initial compound remained after the first 20 days of treatment. This emission rate appears to cover entirely the flight duration of the most damaging second generation of the moths (2–3 weeks).

Discussion

Successful control of insect pests has been reported in almost all types of agricultural crops including orchards,

Table 5 Percentage reduction of number of plants attacked and number of larvae per plant (\pm SE) induced by the European corn borer males in Z11-16:trifluoromethyl ketone (80 g ha⁻¹) treated and untreated fields in the period 2004–2006¹

vineyards, annual vegetables, and fiber crops by mating disruption using pheromone formulations (Cardé & Minks, 1995). However, very few mating disruption experiments have been reported on the Mediterranean corn borer and the European corn borer (Perdiguer et al., 1992; Frérot et al., 1997; Baker, 1999; Albajes et al., 2002; Eizaguirre et al., 2002). Spraying pheromone formulations against the Mediterranean corn borer yielded inconsistent

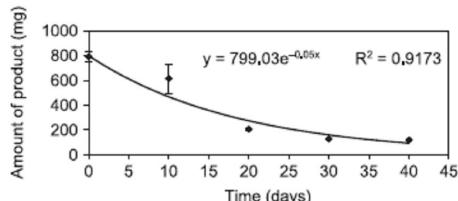


Figure 2 Amount of Z11-16:trifluoromethyl ketone remaining on the dispensers vs. time of exposure in the field used for calculation of the emission rate of the antagonist in the 2006 experiment. Values are the means of three replicates (\pm SE).

Table 6 Reduction of number of plants attacked and number of larvae per plant (\pm SE) induced by the Mediterranean corn borer males in Z11-16:trifluoromethyl ketone (50 g ha^{-1}) treated and untreated fields in the period 2005–2006¹

	Mediterranean corn borer			
	Second generation		Third generation	
	Plants attacked ² (%)	Larvae/plant ²	Plants attacked ² (%)	Larvae/plant ²
2005				
Control plot	1.66 \pm 0.55	0.05 \pm 0.029	not determined	not determined
Treated plot	0.55 \pm 1.67	0.022 \pm 0.022	not determined	not determined
Reduction (%)	67	56	–	–
2006				
Control plot	11.66 \pm 5.53	0.155 \pm 0.11	10.00 \pm 4.41	0.111 \pm 0.037
Treated plot	11.11 \pm 5.06	0.144 \pm 0.10	27.77 \pm 7.07	0.355 \pm 0.068
Reduction (%)	5	7	–	–
2005–2006				
Control plot ³	6.67 \pm 3.05a	0.10 \pm 0.023a	10.00 \pm 4.41a	0.111 \pm 0.037a
Treated plot ³	5.83 \pm 2.78a	0.08 \pm 0.02a	27.77 \pm 7.07a	0.355 \pm 0.068b
Reduction (%)	13	20	–	–

¹Untransformed data. Number of plants per year dissected for samplings was $n = 180$ for the second generation and $n = 90$ for the third generation.

²Two-way analysis of variance ANOVA (year and plot) was performed for statistical analysis. For plants attacked the values were transformed to arcsin $\sqrt{x/100}$ prior to analysis; for larvae/plant the data were transformed to $\log(x + 1)$.

³Data within the same column followed by different letters are significantly different ($P < 0.05$).

results in Spain and Greece, with reduction values from 0 to 90% in Spain and 0 to 69% in Greece, whereas in France the reduction was much more uniform, ranging from 66 to 82% in a 3-year experiment (Albajes et al., 2002). Larger disruption trials (25 ha) using 100 g ha^{-1} of the four-pheromone blend showed a reduction of 48% with a solid sprayable formulation, and 86% with a liquid formulation with respect to an insecticide-treated plot (Frérot et al., 1997). The effect of the disruption on the European corn borer populations was also noted when the pheromone was released from polyvinyl chloride dispensers, but not when the pheromone was applied as a liquid formulation (Eizaguirre et al., 2002). Regarding the European corn borer, the sex pheromone was also effectively used to significantly suppress female matings when released from two different dispenser types and in two deployment patterns (Baker, 1999).

Some work has been done on pest control using antagonists of pheromone action, for example, on the navel orangeworm, *Amyelois transitella* Walker (Lepidoptera: Pyralidae) (Curtis et al., 1987), the pea moth, *Cydia nigricana* Fabricius (Lepidoptera: Tortricidae) (Bengtsson et al., 1994), the oblique-banded leafroller, *Choristoneura rosaceana* Harris (Lepidoptera: Tortricidae) (Evenden et al., 1999), the Japanese giant looper, *Ascotis selenaria cretacea* Butler (Lepidoptera: Geometridae) (Ohtani et al.,

2001), the light-brown apple moth, *Epiphyas postvittana* Walker (Lepidoptera: Tortricidae) (Suckling et al., 1994), and the red bollworm, *Diparopsis castanea* Hmps. (Lepidoptera: Noctuidae) (Marks et al., 1981). The study presented herewith is the first large-scale attempt to evaluate the effect of an antagonist analog of the Mediterranean corn borer sex pheromone to reduce the damage caused by the insect in maize fields. The antagonist was chosen on the basis of its previously shown electrophysiological and behavioral activity (see below) (Riba et al., 2001, 2005; Guerrero et al., 2003; Quero et al., 2003, 2004).

Our results show that Z11-16:trifluoromethyl ketone, when applied in laminate dispensers at the dose of 80 g ha^{-1} , can be an effective new agent of control not only for the Mediterranean corn borer, from whose pheromone it structurally derives, but also for the sympatric European corn borer. Particularly worth of note is the high reduction in damaged plants and in the number of larvae/plant found after the second generation (the most damaging to the crop) of both pests. With regard to the unexpected effect on the European corn borer, it should be pointed out that the antagonist is an analog of (*Z*)-9-tetradecenyl acetate (*Z*9:14:Ac), sharing identical structural moieties at the hydrophobic end of the molecule. This latter compound is a strong antagonist of the European corn borer pheromone both in the laboratory (Glover et al., 1989) and in the field

Table 7 Reduction of number of plants attacked and number of larvae per plant (\pm SE) induced by the European corn borer males in Z11-16:trifluoromethyl ketone treated (50 g ha $^{-1}$) and untreated fields in the period 2005–2006¹

	European corn borer			
	Second generation		Third generation	
	Plants attacked ² (%)	Larvae/plant ²	Plants attacked ² (%)	Larvae/plant ²
2005				
Control plot	2.77 \pm 1.88	0.027 \pm 0.012	not determined	not determined
Treated plot	0.55 \pm 0.56	0.005 \pm 0.0055	not determined	not determined
Reduction (%)	80	81	—	—
2006				
Control plot	26.66 \pm 6.67	0.416 \pm 0.23	30.00 \pm 9.72	0.411 \pm 0.07
Treated plot	43.33 \pm 6.29	0.655 \pm 0.22	44.44 \pm 8.35	0.855 \pm 0.12
Reduction (%)	—	—	—	—
2005–2006				
Control plot ³	14.72 \pm 4.44a	0.22 \pm 0.037a	30.00 \pm 9.72a	0.411 \pm 0.07a
Treated plot ³	21.94 \pm 6.02a	0.33 \pm 0.039b	44.44 \pm 8.35a	0.855 \pm 0.12b
Reduction (%)	—	—	—	—

¹Untransformed data. Number of plants per year and per plot dissected for samplings was n = 180 for the second generation and n = 90 for the third generation.

²Two-way analysis of variance (ANOVA) (year and plot) was performed for statistical analysis. For plants attacked the values were transformed to arcsin $\sqrt{x/100}$ prior to analysis; for larvae/plant the data were transformed to log(x + 1).

³Data within the same column followed by different letters are significantly different (P < 0.05).

(Struble et al., 1987). In fact, a cell tuned to Z9:14:Ac has been found in the European corn borer male antenna in addition to two more receptor cells tuned to each pheromone component Z11-14:Ac and E11-14:Ac (Hansson et al., 1987). Therefore, the effect of the antagonist on the European corn borer may be explained by interaction of the fluorinated chemical with the antagonistic cell (or perhaps also with the pheromone cells).

The lower density of the Mediterranean corn borer larvae found in treated fields may be explained by an effect of the antagonist on female oviposition behavior. This is in line with our previously reported activity of 3-octylthio-1,1,1-trifluoropropan-2-one (OTFP), a well-known fluorinated ketone esterase inhibitor, as oviposition deterrent of the Mediterranean corn borer when the number of larvae emerged from OTFP-treated maize plants was found to be lower than that found in untreated plants (Reddy et al., 2002). A similar inhibitory effect on the European corn borer oviposition behavior might also have occurred, although in this case a specific oviposition deterrent experiment has not been developed yet to confirm this assumption.

The anomalous results obtained when 50 g ha $^{-1}$ of the antagonist were released into the field could have been due simply to a rate effect, to a possible immigration of mated females from untreated to treated plots, and/or to spatial

and temporal variation in the density of the pest (Cardé & Minks, 1995). In this regard, behavioral and light trap studies conducted by Eizaguirre and co-workers (2004) suggested that the Mediterranean corn borer females disperse only after mating, which can easily occur in the relatively small areas considered in this work.

Different mechanisms of action may explain the effects of trifluoromethyl ketones. They may act on receptor cells tuned to the pheromone components, for instance, Z11-16:trifluoromethyl ketone increased the firing activity of the alcohol and aldehyde cells in Mediterranean corn borer male sensilla and decreased the response of the pheromone receptor neurons to the respective pheromone compounds (Quero et al., 2004). Trifluoromethyl ketones can also be bound to the pheromone-binding proteins in competition with pheromone molecules (Feixas et al., 1995; Pophof et al., 2000), and thus Z11-16:trifluoromethyl ketone was able to displace the major component of the pheromone in binding experiments on antennal extracts of *Mamestra brassicae* L. (Lepidoptera: Noctuidae) (Campanacci et al., 1999). These effects are consistent with the disruption of male upwind flights induced by this compound on Mediterranean corn borer, European corn borer, and *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) males when attracted to virgin females or pheromone lures in a wind tunnel (Bau et al., 1999; Riba et al., 2001, 2005;

Guerrero et al., 2003). In the field, Z11-16:trifluoromethyl ketone and other similar fluorinated analogs induced significant reduction in male catches when lures were baited with mixtures of the inhibitor and the pheromone with respect to the pheromone alone (Riba et al., 2001, 2005).

In addition to the competitive mechanism for the pheromone receptors, the trifluoromethyl ketones are potent *in vitro* inhibitors of the olfactory esterases responsible for the catabolism of the pheromone (Vogt et al., 1985; Prestwich, 1987; Kasang et al., 1989; Durán et al., 1993; Rosell et al., 1996; Quero et al., 2003). Inhibition of these enzymes may lead to a decreased capability by the insect to detect new incoming pheromone molecules, which can be useful in pest control strategies. Trifluoromethyl ketone analogs of the pheromones of the Mediterranean corn borer and the European corn borer have displayed good antiesterase activity on antennal extracts of the respective males with an IC₅₀ of 123.7 µM (Quero et al., 2003) and 0.28 µM (Riba et al., 2005), respectively. The antiesterase activity of the chemicals has been attributed to the formation of a stable hemiacetal of tetrahedral geometry with a serine residue of the enzyme (Linderman et al., 1988; Durán et al., 1993; Rosell et al., 1996).

In summary, our study represents a step forward in the utilization of trifluoromethyl ketone analogs of insect sex pheromones in pest control. These compounds show a remarkable disruptive activity of the insect chemical communication, good stability in the field, and can be synthesized in high yield on multigram scale. However, additional work is needed (i) to determine whether these chemicals effectively disrupt matings in the field, and (ii) to assess their effectiveness in comparison to the parent pheromone.

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References

- Albajes R, Konstantopoulou M, Etchepare O, Eizaguirre M & Fréröt B et al. (2002) Mating disruption of the corn borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae) using sprayable formulations of pheromone. *Crop Protection* 21: 217–225.
- Anglade P (1972) Les Sésamia. Entomologie Appliquée à l'Agriculture, Tome II. Lépidoptères (ed. by AS Balachowsky), pp. 1389–1401. Masson et Cie, Paris, France.
- Anonymous (1979) Distribution Maps of Pests. Commonwealth Agricultural Bureau, London, UK.
- Apparu M, Comet M, Leo PM, Mathieu J & Du Moulinet A et al. (1988) Méthode de synthèse d'acides gras marqués substitués ou non en alpha et en beta. *Bulletin de la Société Chimique de France* 1: 118–124.
- Baker TC (1999) Sex Pheromone Mating Disruption: A 'Natural' for Integrating with Transgenic Crops. Des Moines, IA, USA.
- Bau J, Martínez D, Renou M & Guerrero A (1999) Pheromone-triggered orientation flight of male moths can be disrupted by trifluoromethyl ketones. *Chemical Senses* 24: 473–480.
- Beevor PS & Campion D (1979) The field use of 'inhibitory' components of lepidopterous sex pheromones and pheromone mimics. *Chemical Ecology: Odour Communication in Animals* (ed. by FJ Ritter), pp. 313–325. Elsevier/North Holland, Amsterdam, The Netherlands.
- Bengtsson M, Karg G, Kirsch PA, Löfqvist J, Sauer A & Witzgall P (1994) Mating disruption of pea moth *Cydia nigricana* F. (Lepidoptera: Tortricidae) by a repellent blend of sex pheromone and attraction inhibitors. *Journal of Chemical Ecology* 20: 871–887.
- Boivin J, El Kaim L & Zard SZ (1995) A new and efficient synthesis of trifluoromethyl ketones from carboxylic acids. Part I. *Tetrahedron* 51: 2573–2584.
- Campanacci V, Longhi S, Nagnan-Le Meillour P, Cambillau C & Tegoni M (1999) Recombinant pheromone binding protein 1 from *Mamestra brassicae* (MbraPBP1). Functional and structural characterization. *European Journal of Biochemistry* 264: 707–716.
- Cardé RT & Minks AK (1995) Control of moth pests by mating disruption: successes and constraints. *Annual Review of Entomology* 40: 559–585.
- Curtis CE, Clark JD, Carlson DA & Coffelt JA (1987) A pheromone mimic: disruption of mating communication in the navel orangeworm, *Amyelois transitella*, with Z,Z-1,12,14-heptadecatriene. *Entomologia Experimentalis et Applicata* 44: 249–255.
- Durán I, Parrilla A, Feixas J & Guerrero A (1993) Inhibition of antennal esterases of the Egyptian armyworm *Spodoptera littoralis* by trifluoromethyl ketones. *Bioorganic and Medicinal Chemistry Letters* 3: 2593–2598.
- Eizaguirre M, Albajes R, Lopez C, Sans A & Germen C (2007) Inhibition of pheromone response in *Sesamia nonagrioides* by the pheromone of the sympatric corn borer, *Ostrinia nubilalis*. *Pest Management Science* 63: 608–614.
- Eizaguirre M, Lopez C & Albajes R (2004) Dispersal capacity in the Mediterranean corn borer, *Sesamia nonagrioides*. *Entomologia Experimentalis et Applicata* 113: 25–34.
- Eizaguirre M, Sans A, López C & Albajes R (2002) Effects of mating disruption against the Mediterranean corn borer, *Sesamia nonagrioides*, on the European corn borer *Ostrinia nubilalis*. *IOBC/WPRS Bulletin* 25 (9): 59–68.
- Evenden MI, Judd GJR & Borden JH (1999) Simultaneous disruption of pheromone communication in *Choristoneura*

- rosaceana* and *Pandemis limitata* with pheromone and antagonist blends. *Journal of Chemical Ecology* 25: 501–517.
- Feixas J, Prestwich GD & Guerrero A (1995) Ligand specificity of pheromone-binding proteins of the processionary moth. *European Journal of Biochemistry* 234: 521–526.
- Frérot B, Guillou M, Bernard P, Madrennes L, Schepper B, Mathieu F & Coeur A (1997) Mating disruption of corn stalk borer, *Sesamia nonagrioides* Lef. (Lep. Noctuidae). IOBC/WPRS Bulletin 20 (1): 119–128.
- Gemenó C, Sans A, López C, Albajes R & Eizaguirre M (2006) Pheromone antagonism in the European corn borer moth *Ostrinia nubilalis*. *Journal of Chemical Ecology* 32: 1071–1084.
- Glover TJ, Perez N & Roelofs WL (1989) Comparative analysis of sex pheromone-response antagonists in three races of European corn borer. *Journal of Chemical Ecology* 15: 863–873.
- Glover TJ, Tang X-H & Roelofs WL (1987) Sex pheromone blend discrimination by male moths from E and Z strains of European corn borer. *Journal of Chemical Ecology* 13: 143–151.
- Guennelon G (1972) La pyrale du maïs. *Entomologie Appliquée à l'Agriculture, Tome II. Lépidoptères* (ed. by AS Balachowsky), pp. 1078–1130. Masson et Cie, Paris, France.
- Guerrero A, Bosch MP, Rosell G, Riba M & Sans A (2003) New halomethyl ketones and use in traps for the biorational control of insect pests. Spain Pat. 200301667, Madrid, Spain.
- Hansson BS, Löfstedt C & Roelofs WL (1987) Inheritance of olfactory response to sex pheromone components in *Ostrinia nubilalis*. *Naturwissenschaften* 74: 497–499.
- Hathaway DO, Moffitt HR & George DA (1985) Codling moth (Lepid.: Tortricidae): disruption of sexual communication with an antipheromone [E,E-8,10-dodecadien-1-ol acetate]. *Journal of Entomological Society British Columbia* 82: 18–22.
- Haynes KF, Li W-G & Baker TC (1986) Control of pink bollworm moth (Lepidoptera: Gelechiidae) with insecticides and pheromones (attracticide): lethal and sublethal effects. *Journal of Economic Entomology* 79: 1466–1471.
- Kaae RS, Shorey HH, Gaston LK & Hummel HH (1974) Sex pheromones of Lepidoptera: disruption of pheromone communication in *Trichoplusia ni* and *Pectinophora gossypiella* by permeation of the air with nonpheromone chemicals. *Environmental Entomology* 3: 87–89.
- Kasang G, Nicholls M & von Proff L (1989) Sex pheromone conversion and degradation in antennae of the silkworm moth *Bombyx mori* L. *Experientia* 45: 81–87.
- Klun JA, Chapman OL, Matthes KC, Wojtkowski PW, Beroza M & Sonnen PE (1973) Insect sex pheromones: Minor amount of opposite geometrical isomer critical to attraction. *Science* 181: 661–663.
- Linderman RJ, Leazer J, Roe RM, Venkatesh K, Selinsky BS & London RE (1988) ¹⁹F 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. *Pesticide Biochemistry and Physiology* 31: 187–194.
- Ma PWK & Roelofs WL (1995) Sites of synthesis and release of PBAN-like factor in the female European corn borer, *Ostrinia nubilalis*. *Journal of Insect Physiology* 41: 339–350.
- Marks RJ, Hall DR, Lester R, Nesbitt BF & Lambert MRK (1981) Further studies on mating disruption of the red bollworm, *Diparopsis castanea* Hampson (Lepidoptera: Noctuidae), with a microencapsulated mating inhibitor. *Bulletin of Entomological Research* 71: 403–418.
- Mason CE, Rice ME, Calvin DD, Van Duyn JW, Hutchinson WD et al. (1996) European Corn Borer Ecology and Management. North Central Regional Extension Publication No 327. Iowa State University, Ames, IA, USA.
- Mazomenos BE (1989) Sex pheromone components of corn stalk borer, *Sesamia nonagrioides* (Lef.). Isolation, identification and field tests. *Journal of Chemical Ecology* 15: 1241–1247.
- McDonough LM, Kamm JA & Bierl-Leonhardt BA (1980) Sex pheromone of the armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae). *Journal of Chemical Ecology* 6: 565–572.
- Miller JR, Gut LJ, de Lame FM & Stelinski LL (2006a) Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (Part 2): case studies. *Journal of Chemical Ecology* 32: 2115–2143.
- Miller JR, Gut LJ, de Lame FM & Stelinski LL (2006b) Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (Part 1): theory. *Journal of Chemical Ecology* 32: 2089–2114.
- Ohtani K, Witjaksono K, Fukumoto T, Mochizuki F, Yamamoto M & Ando T (2001) Mating disruption of the Japanese giant looper in tea gardens permeated with synthetic pheromone and related compounds. *Entomologia Experimentalis et Applicata* 100: 203–209.
- Peña A, Arn H, Buser H-R, Rauscher S & Bigler F et al. (1988) Sex pheromone of European corn borer, *Ostrinia nubilalis*: Polymorphism in various laboratory and field strains. *Journal of Chemical Ecology* 14: 1359–1366.
- Perdiguer A, Gimeno F, Aguilar L, Eizaguirre M, Riba M & Sans A (1992) Ensayos de confusión sexual en *Sesamia nonagrioides*. *Investigación Agraria: Producción y Protección Vegetales* 7: 253–260.
- Pophof B, Gebauer T & Ziegelberger A (2000) Decyl-thio-trifluoropropanone, a competitive inhibitor of moth pheromone receptors. *Journal of Comparative Physiology A, Sensory, neural, and behavioral physiology* 186: 315–323.
- Prestwich GD (1987) Chemical studies of pheromone reception and catabolism. *Pheromone Biochemistry* (ed. by GD Prestwich & GJ Blomquist), pp. 473–527. Academic Press, Inc., New York, NY, USA.
- Quero C, Bau J, Guerrero A & Renou M (2004) Responses of the olfactory receptor neurons of the corn stalk borer *Sesamia nonagrioides* to components of the pheromone blend and their inhibition by a trifluoromethyl ketone analogue of the main component. *Pest Management Science* 60: 719–726.
- Quero C, Rosell G, Jiménez O, Rodríguez S, Bosch MP & Guerrero A (2003) New fluorinated derivatives as esterase inhibitors. Synthesis, hydration and crossed specificity studies. *Bioorganic and Medicinal Chemistry* 11: 1047–1055.
- Reddy GVP, Quero C & Guerrero A (2002) Activity of octylthio-trifluoropropan-2-one, a potent esterase inhibitor, on growth,

- development, and intraspecific communication in *Spodoptera littoralis* and *Sesamia nonagrioides*. Journal of Agriculture and Food Chemistry 50: 7062–7068.
- Renou M & Guerrero A (2000) Insect parapheromones in olfaction research and semiochemical-based pest control strategies. Annual Review of Entomology 48: 605–630.
- Riba M, Sans A, Bau P, Grolleau G, Renou M & Guerrero A (2001) Pheromone response inhibitors of the corn stalk borer *Sesamia nonagrioides*. Biological evaluation and toxicology. Journal of Chemical Ecology 27: 1879–1897.
- Riba M, Sans A, Solà J, Muñoz L & Bosch MP et al. (2005) Antagonism of pheromone response of *Ostrinia nubilalis* males and implications on behavior in the laboratory and in the field. Journal of Agriculture and Food Chemistry 53: 1158–1165.
- Roelofs WL, Glover T, Tang X-H, Sreng I & Robbins P et al. (1987) Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. Proceedings of the National Academy of Sciences of the USA 84: 7585–7589.
- Rosell G, Herrero S & Guerrero A (1996) New trifluoromethyl ketones as potent inhibitors of esterases: ¹⁹FNMR Spectroscopy of transition state analog complexes and structure–activity relationships. Biochemical and Biophysical Research Communications 226: 2887–2292.
- Sanders CJ (1997) Mechanisms of mating disruption in moths. Insect Pheromone Research; New Directions (ed. by RT Cardé & AK Minks), pp. 333–346. Chapman & Hall, New York, NY, USA.
- Sans A, Riba M, Eizaguirre M & Lopez C (1997) Electroantennogram, wind tunnel and field responses of male Mediterranean corn borer, *Sesamia nonagrioides*, to several blends of its sex pheromone components. Entomologia Experimentalis et Applicata 82: 121–127.
- SAS Institute (2003) User's Manual. SAS Institute Inc., Cary, NC, USA.
- Shaver TN & Brown HE (1993) Evaluation of pheromone to disrupt mating of *Eoreuma loftini* (Lepidoptera: Pyralidae) in sugarcane. Journal of Economic Entomology 86: 377–381.
- Shorey HH & Gerber RG (1996) Use of puffers for disruption of sex pheromone communication of codling moths (Lepidoptera: Tortricidae) in walnut orchards. Environmental Entomology 25: 1398–1400.
- Silverstein RM (1990) Practical use of pheromones and other behavior-modifying compounds: overview. Behavior-Modifying Chemicals for Insect Management (ed. by RL Ridgway, RM Silverstein & MN Inscoe), pp. 1–8. Marcel Dekker, Inc., New York, NY, USA.
- Sreng I, Maume B & Frérot B (1985) Analyse de la sécrétion phéromonale produite par les femelles vierges de *Sesamia nonagrioides* (Lef.) (Lepidoptère, Noctuidae). Comptes Rendus de l'Academie des Sciences, Paris III 301: 439–442.
- Struble DL, Byers JR, McLeod DGR & Ayre GL (1987) Sex pheromone components of an Alberta population of European corn borer, *Ostrinia nubilalis* (Hbn.) (Lepidoptera: Pyralidae). Canadian Entomologist 119: 291–299.
- Suckling DM, Gerhard K, Bradley SJ & Howard CR (1994) Field electroantennogram and behavioral responses of *Epiphyas postvittana* (Lepidoptera: Tortricidae) under low pheromone and inhibitor concentrations. Journal of Economic Entomology 87: 1477–1487.
- Trimble RM, Pree DJ & Carter NJ (2001) Integrated control of Oriental fruit moth (Lepidoptera: Tortricidae) in peach orchards using insecticide and mating disruption. Journal of Economic Entomology 94: 476–485.
- Vogt RG, Riddiford LM & Prestwich GD (1985) Kinetic properties of a pheromone degrading enzyme: the sensillar esterase of *Antheraea polyphemus*. Proceedings of the National Academy of Sciences of the USA 82: 8827–8831.
- Zhu JW, Zhao CH, Lu F, Bengtsson M & Löfstedt C (1995) Reductase specificity and the ratio regulation of E/Z isomers in pheromone biosynthesis of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Insect Biochemistry and Molecular Biology 26: 171–176.

Capítulo III

Differential activity of non-fluorinated and
fluorinated analogues of the European corn
borer pheromone

Differential activity of non-fluorinated and fluorinated analogues of the European corn borer pheromone

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Summary. The differing antagonist activity of (*Z*)-13-hexadecen-2-one (*Z*11–14:MK, **1**) and its 1,1,1-trifluoro derivative (*Z*11–14:TFMK, **2**), two closely related analogues of the European corn borer pheromone *Ostrinia nubilalis* (*Z* strain), and their rationale is reported. Both chemicals exhibited some electrophysiological activity, and topical application of 10 pg of pheromone analogue on male antennae was sufficient to induce significantly lower depolarization responses to the pheromone versus untreated insects. In a wind tunnel, the number of European corn borer males attracted to sources containing mixtures of **1** + pheromone in ratios $\geq 1:1$ was significantly lower than the number attracted to a source containing pheromone alone. Source contact behaviour was dramatically impaired when the **1** + pheromone blend reached a ratio of 10:1, in which only 2% of males displayed source contact in the presence of antagonist. When compound **1** was present at the source, males usually flew upwind with occasional downwind reversals; when compound **2** was present at the lure, males performed wider crosswind reversals, with little progress toward the source. In the field, traps baited with mixtures of both compounds with the pheromone in ratios of 5:1 and 10:1 elicited a significantly decreased number of male catches. In esterase inhibition assays, compound **2** was a potent inhibitor ($IC_{50} = 70$ nM), whereas the non-fluorinated compound **1** was not. The different activity of both compounds is presumed to be due to different mechanisms of action; considerations for using methyl ketone analogues as new behavioural antagonists of the pheromone are outlined.

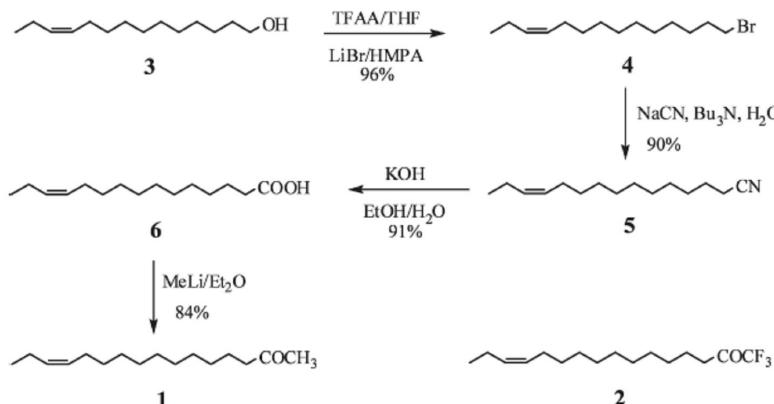
Key words: *Ostrinia nubilalis* – Pyralidae – methyl ketones – antagonist – trifluoromethyl ketones – esterase inhibitors

Introduction

The European corn borer (*ECB*), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), is a major pest of corn in Europe, North America, North Africa, Philippines, and Japan (Mason *et al.* 1996). The *ECB* may attack other crops as well, including potato, green pepper, winter wheat, mugwort, and hop (Pelozuelo *et al.* 2004). The species displays polymorphism in the pheromone communication system as different populations utilize different proportions of the same compounds. In most populations of Europe and North America, females release a blend of (*Z*)-11-tetradecenyl acetate (*Z*11–14:Ac) and (*E*)-11-tetradecenyl acetate (*E*11–14:Ac) in a 97:3 ratio (Klun *et al.* 1973) (*Z* strain). However, males from some populations in the same continents respond to blends ranging from 1:99 (*Z*11–14:Ac: *E*11–14:Ac) to 4:96 (*Z*11–14:Ac: *E*11–14:Ac) (Klun *et al.* 1973; Klun & cooperators 1975; Kochansky *et al.* 1975; Anglade *et al.* 1984; Peña *et al.* 1988; Pelozuelo *et al.* 2004) (*E* strain). Despite the two different populations, *ECB* shows strong behavioural isolation, and in areas of sympatry, there is enough genetic compatibility between the strains to produce viable and fertile hybrids (Roelofs *et al.* 1987; Linn Jr *et al.* 2003).

Control of this pest is particularly difficult because insecticide treatments are only effective during the short period between egg hatching and when young larvae bore into the stems. Therefore, substantial effort has been devoted to accurately monitoring the flight period of the moth (Bartels *et al.* 1997; Bartels & Hutchison 1998;

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Scheme 1. Synthesis of compound **1** and structure of the fluorinated counterpart **2**

Maini & Burgio 1999; Pelozuelo & Frerot 2006); however, only one paper reporting efforts to control the pest by mating disruption has been found in the literature (Baker 1999).

Trifluoromethyl ketones (TFMKs) are known to inhibit a number of esterases and proteases, such as acetylcholinesterase, chymotrypsin, and human liver carboxy-esterases (Gelb *et al.* 1985; Ashour & Hammock 1987). Of particular interest is their effect on antennal esterases present in insect olfactory tissues (Gelb *et al.* 1985; Durán *et al.* 1993; Prestwich 1993; Parrilla & Guerrero 1994). Antennal esterases are decisive in rapid degradation of pheromone esters, a necessary step for maintaining a low stimulus noise level in sensory hairs (Vogt *et al.* 1985; Prestwich *et al.* 1986). The mode of action of TFMKs has been explained in terms of formation of a stable hemiacetal with a serine residue of the enzyme (Linderman *et al.* 1988; Rosell *et al.* 1996). However, the variety of effects elicited by these compounds, such as reduction of the electrophysiological pheromone responses (Renou *et al.* 1997; Pophof *et al.* 2000; Quero *et al.* 2004), disruption of male flights to pheromone sources in a wind tunnel (Bau *et al.* 1999; Riba *et al.* 2001), and antagonist pheromone behaviour in the field (Bau *et al.* 1999; Riba *et al.* 2001; Guerrero *et al.* 2003; Riba *et al.* 2005), cannot be fully explained solely by inhibition of the serine esterases present in male antennae (Riba *et al.* 2001; Quero *et al.* 2004), and therefore other mechanisms of action must also exist. To tackle this problem, we have undertaken a series of experiments with two closely-related analogues of the Z strain ECB pheromone: the methyl ketone (*Z*)-13-hexadecen-2-one (**1**) and its fluorinated counterpart (*Z*)-1,1,1-trifluoro-13-hexadecen-2-one (**2**) (Riba *et al.* 2005) (Scheme 1). Specific aims were 1) to identify new pheromone antagonists and to establish their mode of action, and 2) to confirm the fundamental role played by the trifluoromethyl ketone moiety in these com-

pounds. Compounds **1** and **2** structurally derive from the major component of the pheromone by replacement of the acetoxy group of the latter by the acetyl and trifluoroacetyl moieties of compounds **1** and **2**, respectively. Although the importance of replacing hydrogen by fluorine has been demonstrated in many biological systems (Manabe *et al.* 1985; Dawson *et al.* 1990; Jeschke 2004; Ojima 2004; Guerrero & Rosell 2005), to our knowledge no such study on long chain, closely-related ketones potentially useful in pest control has been undertaken.

Materials and methods

Chemical:

Pheromone components. (*Z*)-11-tetradecenyl acetate was obtained by acetylation of (*Z*)-11-tetradecenol (**3**) (95%, Sigma-Aldrich Co.). (*E*)-11-tetradecenyl acetate (97%) was purchased from Sigma-Aldrich Co.

(Z)-1-Bromo-11-tetradecene (4). Trifluoroacetic anhydride (3.2 mL, 22.60 mmol) was added to a solution of *(Z)*-11-tetradecenol (3, 4.00 g, 18.83 mmol) in anh. THF (18 mL) and the mixture was stirred for 15 min at room temperature (Campbell *et al.* 1987). The solvent and the trifluoroacetic acid formed in the reaction were then evaporated off and the residue (taken up in anhydrous THF (18 mL)). Then, anh. hexamethylphosphoric triamide (18 mL) and anh. lithium bromide (8.18 g, 94.15 mmol), previously dried at 180°C/0.1 torr for 12 h, were added and the mixture was heated at reflux for 3 h. THF was removed under vacuum, water was added and the organic material extracted with hexane. The organic layer was washed with water and dried with MgSO₄. Evaporation of the solvent left a residue which was purified by column chromatography on silica gel 60 (35–70 µm, SDS, France). Elution with hexane gave 4.97 g (96 %) of pure product 4. ¹H NMR (300 MHz, CDCl₃, δ (ppm): 5.34 (m, 2H, CH=CH); 3.40 (t, J = 6.9 Hz, 2H, CH₂Br); 2.02 (m, 4H, allylic CH₂); 1.85 (m, 2H, CH₂CH₂Br); 1.36 (b, 14H, CH₂); 0.95 (t, J = 7.2 Hz, 3H, CH₃). ¹³C NMR (75 MHz, CDCl₃, δ (ppm): 131.5 (CH); 129.3 (CH); 34.0 (CH₂); 32.8 (CH₂); 29.7 (CH₂); 29.5 (CH₂); 29.4 (CH₂); 29.2 (CH₂); 28.7 (CH₂); 28.1 (CH₂); 27.1 (CH₂); 20.5 (CH₂); 14.4 (CH₃). IR (film, NaCl v: 3005, 2962, 2854, 1463, 1255, 1069, 721 cm⁻¹. MS (EI) m/z (%): 276 [(M+1)⁺], 71; [M]⁺, 71; 164 (20); 162 (21); 150 (40); 148 (41); 111 (34); 97 (89); 83 (84); 71 (100).

69 (100); 55 (94); 43 (43); 41 (93). *Elem. Anal.*: Calcd for $C_{14}H_{27}Br$ (275.27): C, 61.09; H, 9.89; Br, 29.03. Found: C, 61.14; H, 9.90; Br, 28.90.

(Z)-12-Pentadecenonitrile (5). A mixture of bromide 4 (4.00 g, 14.53 mmol), sodium cyanide (0.85 g, 17.44 mmol), *n*-tributylamine (0.25 mL), and water (5 mL) was heated at reflux for 4 h. The crude was diluted with brine and extracted with hexane. The combined organic layers were dried with $MgSO_4$, and the solvent was removed at reduced pressure. After purification by column chromatography (silica gel, hexane-ether 95 : 5), the nitrile 5 (2.90 g, 90%) was obtained as a colorless oil. 1H NMR (300 MHz, $CDCl_3$), δ (ppm): 5.33 (m, 2H, $CH=CH$); 2.32 (t, J = 6.9 Hz, 2H, CH_2CN); 2.02 (m, 4H, allylic CH_2); 1.64 (m, 2H, CH_2CH_2CN); 1.36 (b, 14H, CH_2); 0.94 (t, J = 7.5 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 131.5 (CH); 129.3 (CH); 119.8 (CN); 29.7 (CH₂); 29.4 (CH₂); 29.2 (CH₂); 29.2 (CH₂); 28.6 (CH₂); 27.0 (CH₂); 25.3 (CH₂); 20.4 (CH₂); 17.0 (CH₂); 14.3 (CH₃). IR (film, NaCl) v: 3005, 2927, 2855, 2246, 1463, 721 cm⁻¹. MS (EI) m/z (%): 221 (M⁺, 6); 206 (5); 192 (21); 178 (32); 164 (21); 150 (34); 136 (95); 122 (100); 108 (27); 97 (47); 83 (51); 69 (96); 55 (96); 41 (94). *Elem. Anal.*: Calcd for $C_{16}H_{28}N$ (221.38): C, 81.38; H, 12.29; N, 6.33. Found: C, 81.30; H, 12.31; N, 6.25.

(Z)-12-Pentadecenoic acid (6). To a solution of nitrile 5 (1.00 g, 4.52 mmol) in ethanol (15 mL) were added potassium hydroxide (1.01 g, 18.07 mmol) and water (2.5 mL). The resulting mixture was heated at reflux for 24 h, cooled to room temperature, acidified with 1N HCl and extracted with hexane. The combined organic layers were washed with $NaHCO_3$ sat. sol. and brine and dried ($MgSO_4$). Purification by column chromatography (silica gel/hexane) afforded the carboxylic acid 6 (0.99 g, 91%) as a yellow oil. 1H NMR (300 MHz, $CDCl_3$), δ (ppm): 5.34 (m, 2H, $CH=CH$); 2.35 (t, J = 7.2 Hz, 2H, CH_2CO); 2.02 (m, 4H, allylic CH_2); 1.63 (m, 2H, CH_2CH_2CO); 1.31 (b, 14H, CH_2); 0.95 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 180.4 (COOH); 131.5 (CH); 129.3 (CH); 34.1 (CH₂); 29.7 (CH₂); 29.5 (CH₂); 29.5 (CH₂); 29.4 (CH₂); 29.2 (CH₂); 29.2 (CH₂); 29.0 (CH₂); 27.1 (CH₂); 24.6 (CH₂); 20.4 (CH₂); 14.4 (CH₃). IR (film, NaCl) v: 3285, 3005, 2927, 2854, 1712, 1464, 1412, 1285, 939 cm⁻¹. MS (EI) m/z (%): 240 (M⁺, 1); 222 (8); 180 (5); 138 (8); 123 (10); 111 (16); 97 (35); 83 (42); 69 (78); 55 (100); 41 (71). *Elem. Anal.*: Calcd for $C_{16}H_{28}O_2$ (240.38): C, 74.95; H, 11.74. Found: C, 75.08; H, 11.79.

(Z)-13-Hexadecen-2-one (1). 1.6 M Methyl lithium solution (2.4 mL, 3.85 mmol) in hexane was added dropwise to a stirred solution of carboxylic acid 6 (0.42 g, 1.75 mmol) in anhydrous ether (5 mL) at room temperature. The mixture was refluxed for 30 min., cooled to room temperature and carefully quenched with water. The organic material was extracted with ether, washed with brine and dried ($MgSO_4$). Evaporation of the solvent left a residue which was purified by column chromatography (silica gel, hexane-ether 95 : 5) to obtain methyl ketone 1 (0.35 g, 84%) as a colorless oil. 1H NMR (300 MHz, $CDCl_3$), δ (ppm): 5.33 (m, 2H, $CH=CH$); 2.40 (t, J = 7.5 Hz, 2H, CH_2CO); 2.12 (s, 3H, $COCH_3$); 2.01 (m, 4H, allylic CH_2); 1.55 (m, 2H, CH_2CH_2CO); 1.29 (b, 14H, CH_2); 0.94 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 209.4 (CO); 131.5 (CH); 129.3 (CH); 43.8 (CH₂); 29.7 (CH₂CO); 29.5 (CH₂); 29.5 (CH₂); 29.4 (CH₂); 29.4 (CH₂); 29.2 (CH₂); 27.0 (CH₂); 23.8 (CH₂); 20.5 (CH₂); 14.4 (CH₃). IR (film, NaCl) v: 3005, 2926, 2854, 1719, 1460, 1357, 1164, 719 cm⁻¹. MS (EI) m/z (%): 238 (M⁺, 1); 125 (16); 111 (10); 96 (23); 82 (26); 71 (46); 58 (49); 55 (56); 43 (100); 41 (56). *Elem. Anal.*: Calcd for $C_{16}H_{30}O$ (238.41): C, 80.61; H, 12.68. Found: C, 80.71; H, 12.68.

(Z)-1,1,1-Trifluoro-13-hexadecen-2-one (2). This compound was prepared in 38% yield by reaction of (Z)-11-tetradecenyl iodide with *tert*-butyllithium and ethyl trifluoroacetate in pentane; ether 3:2 as previously described (Parrilla *et al.* 1994). Alternatively, compound 2 was also prepared in 57% yield from carboxylic acid 6, oxalyl chloride, trifluoroacetic anhydride and pyridine following the procedure of Boivin *et al.* (Boivin *et al.* 1995). 1H NMR (300 MHz, $CDCl_3$), δ (ppm): 5.34 (m, 2H, $CH=CH$); 2.70 (t, J = 7.2 Hz, 2H, CH_2CO); 2.03 (m, 4H, allylic CH_2); 1.67 (m, 2H, CH_2CH_2CO); 1.30 (b, 14H, CH_2); 0.95 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 191.6 (q, J = 35

Hz, $COCF_3$); 131.5 (CH); 129.3 (CH); 115.6 (q, J = 290 Hz, $COCF_3$); 36.4 (CH₂); 29.7 (CH₂); 29.5 (CH₂); 29.3 (CH₂); 29.2 (CH₂); 29.2 (CH₂); 28.7 (CH₂); 27.1 (CH₂); 22.4 (CH₂); 20.5 (CH₂); 14.4 (CH₃). ^{19}F NMR (282 MHz, $CDCl_3$), δ (ppm): -79.9 (s, $COCF_3$). IR (film, NaCl) v: 3005, 2932, 1771, 1541, 1203, 1142 cm⁻¹. MS (EI) m/z (%): 292 (M⁺, 18); 223 (20); 110 (26); 97 (60); 83 (71); 69 (92); 55 (91); 41 (100). Exact Mass: Calcd for $C_{16}H_{27}F_3O$: 292.201400; Found: 292.203050.

Insects Previous analyses of gland extracts from wild females collected at the Lleida province (N.E. of Spain) established the presence of the Z strain of the moth (Riba *et al.* 2005). Therefore, the pheromone applied in all assays was a 97 : 3 mixture of Z11-14 : Ac and E11-14 : Ac. For mating, wild couples were transferred to plastic cages (17 cm diameter x 11.5 cm height) containing waxed paper around the walls to allow females to lay eggs. After careful selection of the Z progeny, the "pure" Z strain was reared using a typical artificial diet for Noctuidae (Poiteau & Bues 1974), to which the following compounds were added: nipagin (methyl 4-hydroxybenzoate; 0.12%), flumidil (potassium o-oxyquinolinsulfonate + sulfonylamidotiazole; 0.12%) and aureomicin (chlorotetracycline hydrochloride; 0.04%). Pupae were sexed and placed at 25 ± 1 °C and $65 \pm 10\%$ relative humidity in cylindrical boxes (17 cm diameter, 12 cm high) with a 16 : 8 light:dark photoperiod until emergence. Adults were provided with a 10% sucrose solution soaked on a cotton pad, separated daily by age, and kept on filter paper in plastic containers until analysis.

Electrophysiology. 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 x 5 mm diameter). Test stimulations were performed by giving puffs of air (300 mL/min) for 100 ms with a stimulus controller TC-05 (Syntech) through a Pasteur pipette, which was inserted onto a lateral branch of the tube for the stimulations. The pipette contained a piece of filter paper on which the stimulus compounds, diluted in hexane to the desired concentrations, had been deposited. The solvent was allowed to evaporate before the tests. To check for the intrinsic activity of the compounds, 4 puffs over 10 µg of each compound intercalated with 4 puffs of 10 µg of pheromone (Z11-14 : Ac; E11-14 : Ac 97:3) were applied to one of the antennae of two to four-day old males (N = 12) at 45 second intervals. The other antenna of the insects was discarded. In addition, control puffs (filter paper with no compound) were intercalated between two consecutive stimuli to determine the baseline depolarization of the antennae. The signals were amplified (100x) and filtered (DC to 1 kHz) with an ID-02 interface (Syntech), digitized on a PC, and analyzed with the EAD 2.3 program. For the topical application experiments, males (N = 13) were previously anaesthetized with ice for 5–10 min, and different doses of the compounds (1 µg–1 µg) were carefully applied to one of the antennae by syringe. The test compounds had been dissolved in hexane at the concentration required to provide the desired test dose in a 0.15 µL aliquot. The other antenna of the insects was used as control and therefore treated with the same volume of hexane (0.15 µL). Insects were allowed to recover for 5 min, and their antennae excised for the EAG experiments. Eight puffs over 10 µg of pheromone were directed onto the antenna for each dose of the chemicals, and control puffs without solvent were intercalated between two consecutive stimuli, as above. The depolarization responses were normalized relative to controls.

Esterase inhibition assays. The antennae of a number of two-day-old male *O. nubilalis*, previously anaesthetized with CO_2 , were excised, immediately frozen in liquid nitrogen, and stored at -80 °C until analysis as previously described (Rosell *et al.* 1996). The esterase preparations were obtained by homogenizing batches of frozen antennae in 100 mM phosphate buffer solution (pH 7.4) in an ice bath using a variable speed homogenizer (Heidolph ZZR-2000) at 680 rpm for 5 min. The contents of the tubes were transferred to Eppendorf tubes, sonicated at 40 W for 10 s, and centrifuged at 3000 rpm for 5 min at 6 °C. Extracts (100 µL, equivalent to 3 antennae) were then transferred to a borosilicate tube that had been previously treated with a saturated solution of 1-decanol in ethanol for 24 h, followed by rinsing 3 x with

distilled water, along with the inhibitor (2 µL of a 0.01 µM–100 µM solution in ethanol). The extract was then vortexed for 30 s and pre-incubated in a thermostabilized bath at 28 °C for 10 min. Then, 2 µL of an ethanol solution (1 µg/µL) of the major component of the pheromone was added, and the incubation continued for 1 h. The extract was diluted with hexane (180 µL), vortexed for 30 s, and the organic phase separated and concentrated to 5–10 µL for GC analysis on a HP-FFAP capillary column (25 m × 0.25 mm × 0.25 µm). Control experiments contained no inhibitor. The inhibition potency of the chemicals was calculated as the percentage of the relative decrease of hydrolysis of the pheromone in the presence of inhibitor with regard to the mean values of hydrolysis obtained in control experiments. IC₅₀ values of the chemicals were calculated by square regression analysis in triplicate experiments (Rosell *et al.* 1996).

Wind tunnel tests. The assays were conducted in a 200 × 50 × 50 cm glass tunnel as previously described (Bau *et al.* 1999). Illumination (0.5–1.5 lux) was obtained through two red light bulbs covered with a white sheet and located 2 m above the tunnel. A video camera CCD 2400JB Presentco® equipped with a 12 mm CCTV lens was placed 135 cm above the tunnel in a perpendicular position to minimize optical distortion of the flight, allowing to record a flight path of a 130 cm long and 45 cm wide section of the tunnel. Before tests, three to five-day old males between the 3rd and 4th h of the scotophase were acclimated to the experimental conditions of the tunnel for 30 min and placed individually into a wire mesh cylinder cage (3 cm diameter, 8 cm high). The cage was introduced into the tunnel at 20 cm high and 25 cm from the upwind end. After 15–20 s, the cover of the cylinder was removed and the moth behaviour recorded. The following types of behaviour were recorded: TF = taking flight (activation, wind-beating, and beginning of flight), OF = oriented flight (upwind oriented flight to the source), CA = close approach (arrival to the proximity of the lure, which is less than 10% of the total flight distance), and SC = source contact (landing, contact with the source, and copulation attempts). For the SC responses, only males arrested at the source for a minimum of 2 s were recorded, and the number of insects completing the tracks was compared using the χ^2 homogeneity test ($P < 0.05$). The odor source was either 30 µg of the pheromone blend or 30 µg of pheromone to which variable amounts (3–600 µg) of the antagonist had been added. Just prior to the experiments, the required amount of the attractant and/or the antagonist was dissolved in 10–300 µL of nanograde hexane and applied to a standard 8-mm rubber septum (Aldrich Chem. Co.). The solvent was allowed to evaporate after which the dispensers were ready to use. At least 40 males were used for each treatment. The average temperature inside the tunnel was 23 ± 1 °C, and the relative humidity was 65 ± 5%. The airflow through the tunnel was regulated to 0.3 m/s.

For flight path recordings, images were sent to a VM70126 monitor for visualization and converted into computer files at 25 frames/s with the aid of digital video software (Pinnacle Systems v. 5.1). The successive positions of the insect were converted into X, Y coordinates with the aid of an in-house specific software (Track 2.3). For the flight tracks recorded ($N = 19$ for the pheromone, $N = 27$ for the pheromone + 2, and $N = 40$ for pheromone + 1) the following flight parameters were considered: total flight distance (cm), total flight time (s), air speed (cm/s), ground speed (cm/s), angular velocity (degrees/s), turning frequency (turns/s), length of track leg (cm), track width (cm), track angle (degrees), course angle (degrees), and drift angle (degrees). The meaning of these parameters has been reported (Marsh *et al.* 1978; Kuenen & Cardé 1994), and their mean values were compared for significance using the LSD test ($P < 0.05$).

Field tests. Field assays were performed in infested corn fields of the Lleida province in northeast Spain, from mid-July through October, 2004–2006. Blends of the antagonist and the pheromone in ratios of 1:1 to 10:1 were obtained by adding the required amount of the antagonist dissolved in 50 µL of hexane to 100 µg of the pheromone dissolved in 50 µL of hexane. The blends were transferred to rubber septa, the solvent allowed to evaporate, and the septa introduced into *Heliothis* traps (Scentsy, Ecogen, Inc.). Formulations containing only pheromone were used as control. The fields were divided into three independent blocks, spaced about 50 m from each other. Traps were hung at a height of

1.5 m and spaced a minimum of 25 m apart. They were set up in randomized blocks and revised and rotated once a week. Trapped moths were counted and transformed ($\sqrt{x} + 0.5$) and analyzed for significance using a one-way ANOVA followed by separation of the means by Duncan's multiple range test ($P < 0.05$). The antagonist effect of the chemicals was calculated by the relative decrease in catches by a specific blend in comparison to those obtained with the pheromone alone.

Results

Antiesterase activity. In the antennal esterase inhibition tests, a plot of the percentage of inhibition versus the logarithm of the inhibitor concentration (10, 50, 100 and 250 nM in EtOH) gave a straight line for compound 2 ($r^2 = 0.967$; data not shown) from which an IC₅₀ = 70 nM was determined. However, compound 1 displayed very low inhibition values (for 10, 50 and 100 µM solutions, only 5%, 15%, and 17% of inhibition, respectively, were obtained) so that the IC₅₀ was estimated to be >100 µM.

Electrophysiology. The intrinsic electrophysiological activity of compounds 1 and 2 was moderate in comparison to the pheromone (46.0 ± 10.8% for 1 and 37.4 ± 10.6% for 2; Fig. 1). Topical application of several amounts (1 pg – 1 µg) of the chemicals to male antennae induced lower depolarization responses to the pheromone relative to that displayed by control insects (Fig. 2). Application of 10 pg of either chemical was enough to produce a significant inhibitory effect (55% and 77% of the EAG relative response to the pheromone when compounds 1 and 2 were applied, respectively). The effect of both chemicals was dose-dependent. When 100 ng or more was applied, the electrophysiological response was ≤22% relative to that displayed by control males.

Wind tunnel. When males were attracted to mixtures of pheromone and compound 1 in various ratios, a dose-dependent decrease of the number of insects displaying all types of behaviour (TF, OF, CA and SC) was observed; the differences were significant at a pheromone: disruptant ratio ≥1:1 (Table 1). The number of SC was dramatically lowered when the source contained blends of pheromone and antagonist in ratios ≥1:10, decreasing from 62% to only 2% in the absence or presence of antagonist, respectively. With regard to compound 2, no inhibitory effect was apparent on the TF and OF behaviours, but CA and SC were significantly disrupted when mixtures of pheromone:inhibitor ≥1:5 were used as bait (Riba *et al.* 2005). Thus, depending on the dose, only 15–30% of males closely approached the source and 7–25% successfully contacted with the lure in comparison with 60–65% of males which displayed the same behaviours in the presence of pheromone alone (Riba *et al.* 2005). In this case, the effect was also dose-dependent (Table 1).

The disruptant effect exerted by the chemicals was evident when the flight tracks were video-recorded and analyzed (Fig. 3). As shown in Table 2, test males flew longer distances to reach the source than control insects,

Table 1. Percentage of behavioural responses of *O. nubilalis* males flying towards a source baited with mixtures of pheromone and Z11–14:MK (1) and Z11–14:TFMK (2) in several ratios in a wind tunnel^{a,b}.

	Ph	Ph + 1 / Ph + 2 (1:0.1)	Ph + 1 / Ph + 2 (1:1)	Ph + 1 / Ph + 2 (1:5)	Ph + 1 / Ph + 2 (1:10)	Ph + 1 / Ph + 2 (1:20)
TF	81 a/77 a	87 a/82 a	51 b/82 a	58 b/85 a	24 b/75 a	33 b/82 a
OF	76 a/77 a	77 a/75 a	35 b/62 a	43 b/67 a	12 c/50 a	21 c/50 a
CA	67 a/65 a	67 a/47 ab	24 b/45 ab	26 b/30 bc	2 c/22 bc	2 c/15 c
SC	62 a/60 a	67 a/42 ab	22 b/37 ab	26 b/25 bc	2 c/12 c	2 c/7 c

^a First values correspond to results obtained with Z11–14:MK (1) whereas second values correspond to those with Z11–14:TFMK (2). The latter (N = 40) have already been described (Riba *et al.* 2005) and are only cited here for comparison. For the experiments with compound 1 N = 40–43.

^b Means of a treatment within a file followed by the same letter are not significantly different ($2 \times 2 \chi^2$ homogeneity test, $P < 0.05$).

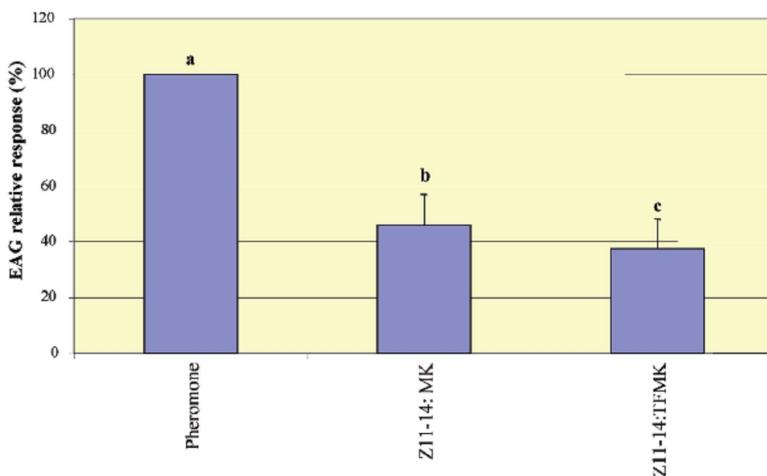


Fig. 1. Mean EAG relative response of *Ostrinia nubilalis* male antennae to the pheromone analogues Z11–14:TFMK (2) and Z11–14:MK (1). For each compound, four puffs of 10 µg of analogues alternated with 4 puffs of 10 µg of pheromone (Z11–14:Ac/E11–14:Ac 97:3) were applied to 12 antennae. Bars with the same letter are not significantly different (Duncan's multiple range test, $P < 0.05$).

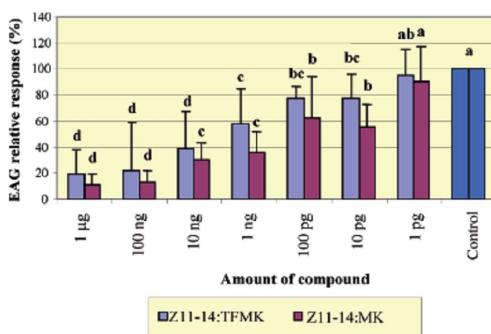


Fig. 2. Mean EAG relative response (± SD) of *O. nubilalis* male antennae to the pheromone after topical treatment with different amounts of Z11–14:MK (1) and Z11–14:TFMK (2) relative to control (no antagonist). For the same compound, bars with the same letter are not significantly different (Duncan multiple range test, $P < 0.05$).

although the effect was significant only when compound 2 was present in the lure ($P < 0.05$). As a result, both compounds induced insects to spend a longer time (two to three-times that of pheromone alone) to complete the flight. However, only compound 1 elicited a significant decrease in flight speeds, regardless of whether the parameter considered was ground speed or air speed. The number of turns was also higher in the presence of the chemicals (31.6 and 74.5 turns to the blends pheromone + 1 and pheromone + 2, respectively, vs 22.9 turns to the pheromone alone), although, as a consequence of the longer flight times, the turning frequency of the treated insects was either similar to or lower than that of control males (Table 2). In all cases, the turning frequency paralleled the track width, being similar for males flying to the pheromone and pheromone + 2 and lower for males attracted to pheromone + 1. Mean track angles were significantly higher among males flying towards pheromone + 2 than pheromone + 1, and males flying toward pheromone + 1 steered larger angles than insects at-

Table 2. Selected parameters from tracks of *O. nubilalis* males flying upwind towards a septum source containing a 1:10 mixture of pheromone (Z11–14:Ac:E11–14:Ac 97:3) and Z11–14:MK (1) or Z11–14:TFMK (2) relative to pheromone alone^a.

Parameter	Pheromone (N = 19)	Pheromone + 1 (N = 40)	Pheromone + 2 (N = 27)
Total flight distance (cm)	184.8 (17.9) a	248.9 (21.7) a	598.2 (71.7) b
Total flight time (s)	4.4 (0.1) a	8.1 (0.2) b	14.9 (0.3) c
Ground speed (cm/s)	41.3 (19.9) a	30.5 (12.0) b	40.0 (10.1) a
Turning frequency (turns/s)	5.2 (0.2) a	3.9 (0.1) b	5.0 (0.1) a
Length of track leg (cm)	20.3 (0.7) a	15.7 (0.4) c	17.9 (0.3) b
Track width (cm)	16.5 (0.8) a	12.4 (0.4) b	15.4 (0.3) a
Track angle (degrees)	67.5 (9.51) a	73.6 (8.79) b	80.0 (6.87) c
Course angle (degrees)	44.1 (8.41) ab	42.3 (8.53) a	48.4 (9.20) b
Drift angle (degrees)	23.4 (4.02) a	31.3 (14.62) b	31.6 (4.55) b
Air speed (cm/s)	50.9 (17.4) a	39.4 (11.0) b	46.5 (9.4) a
Angular velocity (degrees/s)	1002.8 (29.4) a	958.7 (22.9) a	885.2 (29.0) a

^a Means (\pm SEM) within a row followed by the same letter are not significantly different (LSD test, $P < 0.05$).

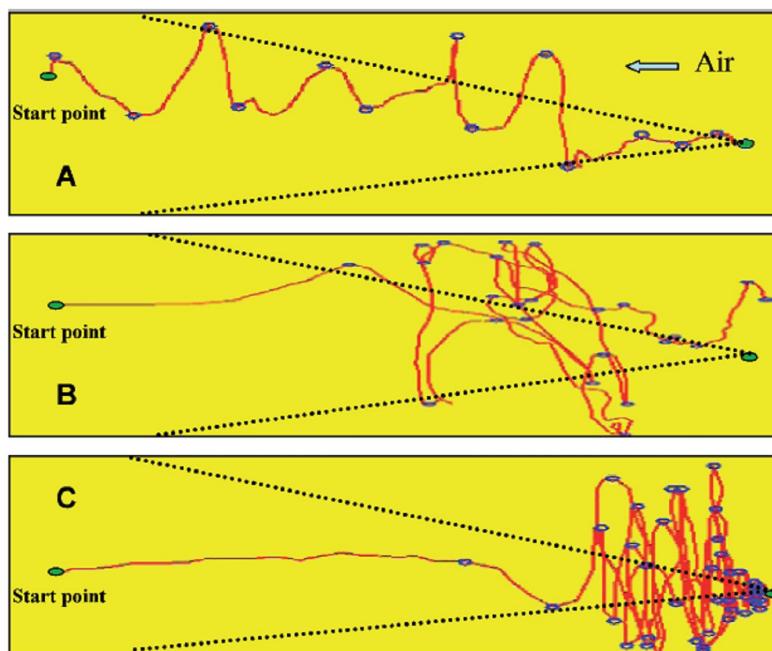


Fig. 3. Flight tracks of *O. nubilalis* males flying upwind towards a septum dispenser containing (A) pheromone, (B) 1:10 mixture of pheromone : Z11–14:MK (1) and (C) 1:10 mixture of pheromone : Z11–14:TFMK (2).

tracted to the pheromone alone. On the contrary, the mean course angles remained quite constant, so the resulting drift angles (the difference between the track angle and the course angles) were also higher in the presence of the chemicals (Table 2).

Most insects flying to the pheromone experienced typical upwind flights with occasional crosswind reversals that were more frequent in the second half of the flight (Fig. 3A). In the presence of compound 1, males usually

flew straight upwind up to the middle of the tunnel. Upon loss of the plume, they initiated casting flight with occasional downwind excursions but generally progressed upwind (Fig. 3B). Males attracted to pheromone + 2 also initially displayed straight flights inside the plume. However, when they reached about $\frac{3}{4}$ the distance to the source they began casting tracks with wide crosswind reversals and little progress to the source (Fig. 3C).

Field tests. In tests carried out in 2004, blends of compound **2** and the pheromone in 10:1 a ratio caught an average of 10.6 insects/trap as compared with 99.0 insects/trap using the pheromone (Fig. 4). In the same assays, a similar effect was noticed when **2** was replaced by the non-fluorinated **1** (19.6 insects/trap, $P < 0.05$). The trials were repeated in 2005, and again we detected a significant decrease in the number of males caught when the disrupting chemicals were present with the pheromone in a 10:1 ratio (124.3 and 145.3 catches/trap with blends containing compounds **2** and **1**, respectively, vs 689 males/trap with the pheromone). Similar results were obtained by blends of antagonist : pheromone in 5:1 ratio (127.0 and 56.0 males/trap caught by blends containing the fluorinated **2** and the non-fluorinated **1**, respectively) (Fig. 4). However, tests conducted in 2006 using 1:1 mixtures of the pheromone and the antagonists had no significant effect on the numbers of catches in comparison to the pheromone.

Discussion

The enzymatic systems present in male antennae are responsible for degradation of volatile organic compounds, particularly pheromones (Ferkovich 1982; Kasang *et al.* 1989). Inhibition of these enzymes may disrupt the chemical communication of moths; therefore, antennal enzyme inhibition has been investigated as a potential approach for pest control (Prestwich *et al.* 1986). This perspective has stimulated the search for volatile chemicals with specific activity as pheromone communication inhibitors (Prestwich 1987; Plettner 2002; Guerrero & Rosell 2005). As a result, a large variety of pheromone analogues, generically called *parapheromones*, have been developed as mimics, synergists, or antagonists of natural pheromones (Renou & Guerrero 2000). Replacement of the alcohol oxygen of an acetate pheromone by a methylene group produces a methyl ketone analogue that provokes various electrophysiological and behavioural consequences. For instance, Albans *et al.* (Albans *et al.* 1984) reported that (*Z*)-12-heptadecen-2-one, the methyl ketone analogue of the sex pheromone of *Heliothis virescens*, exerted a remarkable reversible inhibition of male behaviour responses (flight activation) to the pheromone. In contrast, Lilje fors *et al.* (Lilje fors *et al.* 1984) observed only modest electrophysiological activity of (*Z*)-10-pentadecen-2-one, the corresponding analogue of the pheromone of the turnip moth *Agrotis segetum*. In the same context, (*Z*)-16-nonadecen-14-yn-2-one, the analogue resulting from the same replacement on the pheromone structure of the processionary moth *Thaumetopoea pityocampa* displayed a modest EAG activity and in the field it behaved as a modest agonist of the pheromone action (Parrilla & Guerrero 1994). However, no synergy or inhibition was noticed when this compound was mixed with the pheromone in various ratios (Parrilla & Guerrero 1994).

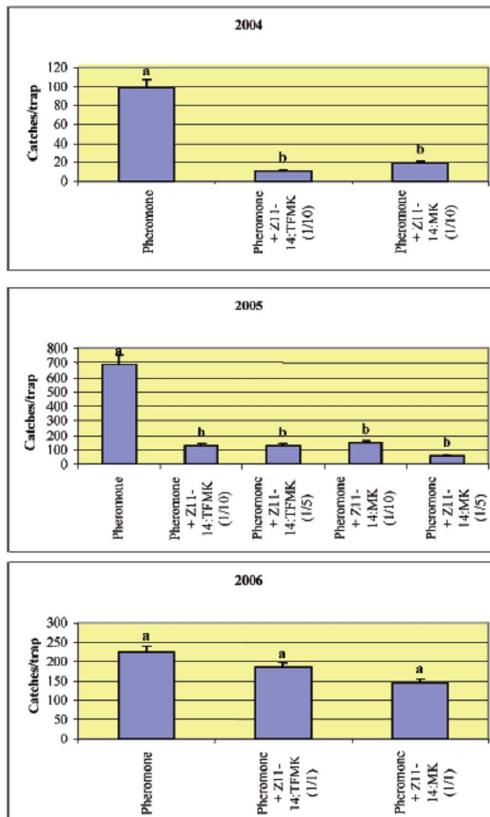


Fig. 4. Number (\pm SE) of *O. nubilalis* males caught in traps baited with mixtures of pheromone and Z11-14:TFMK (**2**) or Z11-14:MK (**1**) in several ratios. Rubber septa were used as dispensers. The amount of synthetic pheromone (Z11-14:Ac:11-14:Ac 97:3) in all baits was 0.1 mg. Bars with the same letter are not significantly different (Duncan's multiple range test, $P < 0.05$). Trials were performed in infested corn fields in the province of Lleida (Spain).

In the present work, we designed compound **1** and its fluorinated counterpart **2** as mimics of the major component of the ECB pheromone. In previous experiments, we showed that topical application of analogues to the antennae of *Spodoptera littoralis* induced disruption of males flight to pheromone sources in a wind tunnel (Bau *et al.* 1999), a similar type of effect induced by males previously exposed to vapours of the analogues (Renou *et al.* 1997). Taking into account these precedents, and since pre-exposure of the processionary moth males to the vapours of the methyl ketone analogue of the pheromone (see above) blocked pheromone detection in EAG (Parrilla & Guerrero 1994), we decided to test our analogues **1** and **2** topically on the antennae of *O. nubilalis*. Intrinsi-

cally, these chemicals displayed moderate EAG activity; the non-fluorinated analogue exhibited slightly but significantly higher activity than the fluorinated material. Topical application of both chemicals to the antenna inhibited the pheromone response; 10 pg was sufficient to promote a significant inhibition response. These results agree with other reports in which several TFMKs elicited a significant reduction of the EAG pheromone responses in *S. littoralis*, *Mamestra brassicae* and *Heliothis zea* (Renou *et al.* 1997), as well as of the single sensillum responses to the pheromone of *S. littoralis* (Renou *et al.* 1997) and *Antheraea polyphemus* (Popoff *et al.* 2000). Also, (Z)-11-hexadecenyl trifluoromethyl ketone (Z11–16 : TFMK), a closely-related analogue of the pheromone of the Mediterranean corn borer *Sesamia nonagrioides*, decreased the responses of the olfactory receptor neurons to the pheromone (Quero *et al.* 2004). Our results point to a putative competitive inhibition mechanism either for the pheromone binding proteins (PBP)s and/or for the pheromone receptors. In this regard, it should be mentioned that Z11–16 : TFMK almost completely displaced Z11–16 : OAc, the major component of the pheromone of *M. brassicae*, bound to a recombinant PBP1 when incubated before or after the pheromone component (Campanacci *et al.* 1999). Additionally, some aliphatic TFMKs may bind to a 15-KD PBP present in the sensory hairs of the processionary moth and be transported through the haemolymph, competing with pheromone molecules and inhibiting catabolic esterases (Feixas *et al.* 1995). In contrast to the TFMKs, however, no data are available on the mechanism of the methyl ketones at the receptor level. Nevertheless, because of their high structural similarity to the pheromone, the inhibitory effect of both compounds **1** and **2** is presumed to be due to overstimulation and adaptation of the receptor cells.

Behaviourally, compounds **1** and **2** elicited a disruptive effect on most types of behaviour, being particularly noticeable on CA and SC. Both chemicals induced erratic flights on males as they approach to the source, in a similar manner to the effect induced by topical application of Z11–16 : TFMK on *S. nonagrioides* male antennae, although in this case the effect was evident from the initial stages of the flight (Riba *et al.* 2001). Also, pre-exposure to vapours or topical application to the antenna of 3-octylthio-1,1,1-trifluoropropan-2-one (OTFP), an effective antiesterase agent, significantly reduced responses of male *S. littoralis* to the pheromone (Renou *et al.* 1997; Bau *et al.* 1999). In addition, OTPF behaved as an oviposition deterrent on *S. littoralis* and *S. nonagrioides* larvae, and diet-borne administration of OTPF to larvae inhibited male attraction to the pheromone or to virgin females as compared to untreated insects (Reddy *et al.* 2002). However, in contrast to these reports, Klun *et al.* (Klun *et al.* 1991) found that in *O. nubitalis* the *Z* and *E* isomers of 1,1,1-trifluoro-14-heptadecen-2-one mixed with pheromone in a physiologically excessive 30 : 1 ratio did not inhibit male upwind flight behaviour. The authors concluded that the chemical did not effectively compete for

putative behaviour-inducing pheromone receptor sites (Klun *et al.* (Klun *et al.* 1991). However, the exceedingly high amounts of the analogues tested suggest that these results should be taken with caution. Our results show that the fluorinated ketone **2** is a highly effective esterase inhibitor (IC_{50} of 70 nM), whereas the non-fluorinated ketone **1** is not. These results clearly differentiate the effects of both chemicals, and suggest that the methyl ketone, based on its structural similarity to the pheromone, may compete with the natural attractant for the pheromone receptors and elicit an antagonist response by the CNS (Glover *et al.* 1989). Our results also confirm the important role played by fluorine in the esterase inhibition of fluorinated ketones (Linderman *et al.* 1988; Durán *et al.* 1993; Rosell *et al.* 1996), as well as in their activity as competitors for pheromone receptor and PBP binding (Plettner 2002; Guerrero & Rosell 2005).

In the field, compounds **1** and **2** were effective antagonists of pheromone action when mixed with the natural attractant in 5 : 1 and 10 : 1 ratios, but not in 1 : 1 ratio. We have found few reports testing the activity of methyl ketone pheromone analogues in the field. We previously tested an analogue of the processionary moth pheromone blended with the natural attractant in 1 : 0.1, 1 : 1 and 1 : 10 ratios, but these mixtures did not exhibit any synergistic or inhibitory action in comparison to the pheromone (Parrilla & Guerrero 1994). This discrepancy with the present results is not unexpected considering that analogous modifications in different pheromone molecules are known to provoke dissimilar or even opposite effects. For instance, Prestwich and Streinz (Prestwich & Streinz 1988) reported that 1,1,1-trifluoro-(*Z*)-14-nonadecen-2-one was a poor inhibitor of esterase of *Plutella xylostella*, a result in opposition with the findings of Vogt *et al.* (Vogt *et al.* 1985) who reported that 1,1,1-trifluorotetradecan-2-one was a potent inhibitor of the sensillar esterase of *A. polyphemus*. In our hands the latter compound was a potent esterase inhibitor of *S. littoralis* ($IC_{50} = 1.16 \mu M$) (Durán *et al.* 1993), whereas the fluorinated ketone pheromone analogue 1,1,1-trifluoro-(*Z*)-13-octadecen-2-one was two orders of magnitude less active (Quero *et al.* 2003).

In summary, our results show that methyl ketone **1** is a new potent *in vivo* behavioural antagonist of the pheromone response of the ECB males. Its activity, easy preparation in the laboratory, and presumed stability under field conditions are important features to consider methyl ketone pheromone analogues in future pest control studies.

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References

- Albans KR, Baker R, Jones OT (1984) Inhibition of response of *Heliothis virescens* to its natural pheromone by antipheromones. Crop Prot 3(4): 501–506.
- Anglade P, Stockel J, and cooperators (1984) Intraspecific sex-pheromone variability in the European corn borer, *Ostrinia nubilalis* Hbn. (Lepidoptera: Pyralidae). Agronomie 4: 183–187.
- Ashour M-BA, Hammock BD (1987) Substituted trifluoroacetones as potent selective inhibitors of mammalian carboxylesterases. Biochem Pharmacol 36(12): 1869–1879.
- Baker TC. Sex pheromone mating disruption: A 'natural' for integrating with transgenic crops; 1999; Des Moines, Iowa.
- Bartels DW, Hutchison WD (1998) Comparison of pheromone trap designs for monitoring Z-strain European corn borer (Lepidoptera: Crambidae). J Econ Entomol 91: 1349–1354.
- Bartels DW, Hutchison WD, Udayagiri S (1997) Pheromone trap monitoring of Z-strain European corn borer (Lepidoptera: Pyralidae): optimum pheromone blend, comparison with blacklight traps, and trap number requirements. J Econ Entomol 90: 449–457.
- Bau J, Martinez D, Renou M, Guerrero A (1999) Pheromone-triggered orientation flight of male moths can be disrupted by trifluoromethyl ketones. Chem Senses 24: 473–480.
- Boivin J, El Kaim L, Zard SZ (1995) A new and efficient synthesis of trifluoromethyl ketones from carboxylic acids. Tetrahedron 51: 2573–2584.
- Campanacci V, Longhi S, Nagnan-Le Meillour P, Cambillau C, Tegoni M (1999) Recombinant pheromone binding protein 1 from *Mamestra brassicae* (MbraPBP1). Functional and structural characterization. Eur J Biochem 264: 707–716.
- Camps F, Gasol V, Guerrero A (1987) A new and efficient one-pot preparation of alkyl halides from alcohols. Synthesis: 511–512.
- Dawson GW, Mudd A, Pickett JA, Pile MM, Wadhams LJ (1990) Convenient synthesis of mosquito oviposition pheromone and a highly fluorinated analog retaining biological activity. J Chem Ecol 16(6): 1779–1790.
- Durán I, Parrilla A, Feixas J, Guerrero A (1993) Inhibition of antennal esterases of the Egyptian armyworm *Spodoptera littoralis* by trifluoromethyl ketones. Bioorg Med Chem Lett 3(12): 2593–2598.
- Feixas J, Prestwich GD, Guerrero A (1995) Ligand specificity of pheromone-binding proteins of the processionary moth. Eur J Biochem 234: 521–526.
- Ferkovich SM (1982) Enzymatic alteration of insect pheromones. Pp 165–185 in Norris DM (eds) Perception of Behavioral Chemicals. Amsterdam: Elsevier/North Holland.
- Gelb MH, Swaren JP, Abeles RH (1985) Fluoroketone inhibitors of hydrolytic enzymes. Biochemistry 24: 1813–1817.
- Glover TJ, Perez N, Roelofs WL (1989) Comparative analysis of sex pheromone-response antagonists in three races of European corn borer. J Chem Ecol 15: 863–873.
- Guerrero A, Bosch MP, Rosell G, Riba M, Sans A. (2003). Nuevas halometilcetonas y uso en la fabricación de trampas para el control biológico de plagas de insectos. Spain Patent 200301667.
- Guerrero A, Rosell G (2005) Bioregulatory approaches for insect control by enzymatic inhibition. Curr Med Chem 12: 461–469.
- Jeschke P (2004) The unique role of fluorine in the design of active ingredients for modern crop protection. ChemBioChem 5: 570–589.
- Kasang G, Nicholls M, von Proff L (1989) Sex pheromone conversion and degradation in antennae of the silkworm moth *Bombyx mori* L. Experientia 45: 81–87.
- Klun JA, and cooperators (1975) Insect sex pheromones: Intraspecific pheromonal variability of *Ostrinia nubilalis* in North America and Europe. Environ Entomol 4: 891–894.
- Klun JA, Chapman OL, Mattes KC, Wojtkowski PW, Beroza M, Sonnet PE (1973) Insect sex pheromones: Minor amount of opposite geometrical isomer critical to attraction. Science 181: 661–663.
- Klun JA, Schwarz M, Uebel EC (1991) European corn borer: Pheromonal catabolism and behavioral response to sex pheromone. J Chem Ecol 17(2): 317–334.
- Kochansky J, Cardé RT, Liebherr J, Roelofs WL (1975) Sex pheromone of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae) in New York. J Chem Ecol 1: 225–231.
- Kuene LPS, Cardé RT (1994) Strategies for recontacting a lost pheromone plume: casting and upwind flight in the male gypsy moth. Physiol Entomol 19: 15–29.
- Lilje fors T, Thelin B, Van Der Pers JNC (1984) Structure-activity relationships between stimulus molecule and response of a pheromone receptor cell in turnip moth, *Agrotis segetum*. Modification of the acetate group. J Chem Ecol 10: 1661–1675.
- Linderman RJ, Leazer J, Roe RM, Venkatesh K, Selinsky BS, London RE (1988) ¹⁹F 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.
- Linn Jr C, O'Connor M, Roelofs WL (2003) Silent genes and rare males: A fresh look at pheromone blend response specificity in the European corn borer, *Ostrinia nubilalis*. J Insect Sci 3:15: Available online: insectscience.org/3.15.
- Maini S, Burgio G (1999) *Ostrinia nubilalis* (Hb.) (Lep., Pyralidae) on sweet corn: Relationship between adults caught in multibaited traps and ear damages. J Appl Ent 123: 179–185.
- Manabe S, Nishino C, Matsushita K (1985) Studies on relationship between activity and electron density on carbonyl oxygen in sex pheromone mimics of the American cockroach. J Chem Ecol 11(9): 1275–1287.
- Marsh D, Kennedy JS, Ludlow AR (1978) An analysis of anemotactic zigzagging flight in male moths stimulated by pheromone. Physiol Entomol 3: 221–240.
- Mason CE, Rice ME, Calvin DD, Van Duyn JW, Hutchinson WD, Witkowski JF, Higgins RA, Onstad DW, Dively GP (1996) European Corn Borer. Ecology and Management. Pp. 57. Ames, Iowa: North Central Regional Extension Publication N° 327, Iowa State University.
- Ojima I (2004) Use of fluorine in the Medicinal Chemistry and chemical biology of bioactive compounds – A case study of fluorinated taxane anticancer agents. ChemBioChem 5: 628–635.
- Parrilla A, Guerrero A (1994) Trifluoromethyl ketones as inhibitors of the processionary moth sex pheromone. Chem Senses 19(1): 1–10.
- Parrilla A, Villuendas I, Guerrero A (1994) Synthesis of trifluoromethyl ketones as inhibitors of antennal esterases of insects. Bioorg Med Chem 2(4): 243–252.
- Pelozuelo L, Frérot B (2006) Behaviour of male European corn borer, *Ostrinia nubilalis* Hübner (Lep., Crambidae) towards pheromone-baited delta traps, bucket traps and wire mesh cone traps. J Appl Ent 130(4): 230–237.
- Pelozuelo L, Malosse C, Genestier G, Guenego H, Frérot B (2004) Host specialization in pheromone strains of the European corn borer *Ostrinia nubilalis* in France. J Chem Ecol 30(2): 335–352.
- Peña A, Arn H, Buser H-R, Rauscher S, Bigler F, Brunetti R, Maini S, Tóth M (1988) Sex pheromone of European corn borer, *Ostrinia nubilalis*: Polymorphism in various laboratory and field strains. J Chem Ecol 14: 1359–1366.
- Plettner E (2002) Insect pheromone olfaction: New targets for the design of species-selective pest control agents. Curr Med Chem 9: 1075–1085.
- Poitout S, Bues R (1974) Élevage des chenilles de vingt-huit espèces de lépidoptères Noctuidae et deux espèces d'Arctiidae sur milieu artificiel simple. Particularités de l'élevage selon les espèces. Ann Zool Ecol Anim 6: 431–441.
- Pophof B, Gebauer T, Ziegelberger A (2000) Decyl-thio-trifluoropropane, a competitive inhibitor of moth pheromone receptors. J Comp Physiol 186: 315–323.
- Prestwich GD (1987) Chemical studies of pheromone reception and catabolism. Pp 473–527 in Prestwich GD, Blomquist GJ (eds) Pheromone Biochemistry. New York: Academic Press, Inc.
- Prestwich GD (1993) Chemical studies of pheromone receptors in insects. Arch Insect Biochem Physiol 22: 75–86.
- Prestwich GD, Streinz L (1988) Haloacetate analogs of pheromones: Effects on catabolism and electrophysiology in *Plutella xylostella*. J Chem Ecol 14(13): 1003–1021.

- Prestwich GD, Vogt RG, Riddiford LM (1986) Binding and hydrolysis of radiolabelled pheromone and several analogs by male-specific antennal proteins of the moth *Antheraea polyphemus*. *J Chem Ecol* 12(2): 323–333.
- Quero C, Bau J, Guerrero A, Renou M (2004) Responses of the olfactory receptor neurons of the corn stalk borer *Sesamia nonagrioides* to components of the pheromone blend and their inhibition by a trifluoromethyl ketone analogue of the main component. *Pest Manag Sci* 60: 719–726.
- Quero C, Rosell G, Jiménez O, Rodriguez S, Bosch MP, Guerrero A (2003) New fluorinated derivatives as esterase inhibitors. Synthesis, hydration and crossed specificity studies. *Bioorg Med Chem* 11: 1047–1055.
- Reddy GVP, Quero C, Guerrero A (2002) Activity of octylthiotrifluoropropen-2-one, a potent esterase inhibitor, on growth, development and intraspecific communication in *Spodoptera littoralis* and *Sesamia nonagrioides*. *J Agric Food Chem* 50: 7062–7068.
- Renou M, Guerrero A (2000) Insect parapheromones in olfaction research and semiochemical-based pest control strategies. *Annu Rev Entomol* 48: 605–630.
- Renou M, Lucas P, Malo E, Quero C, 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, Sans A, Bau P, Grolleau G, Renou M, Guerrero A (2001) Pheromone response inhibitors of the corn stalk borer *Sesamia nonagrioides*. Biological evaluation and toxicology. *J Chem Ecol* 27: 1879–1897.
- Riba M, Sans A, Solé J, Muñoz L, Bosch MP, Rosell G, Guerrero A (2005) Antagonism of pheromone response of *Ostrinia nubilalis* males and implications on behavior in the laboratory and in the field. *J Agric Food Chem* 53: 1158–1165.
- Roelofs WL, Glover T, Tang X-H, Sreng I, Robbins P, Eckenrode C, Löfstedt C, Hansson BO (1987) Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. *Proc Natl Acad Sci USA* 84: 7585–7589.
- Rosell G, Herrero S, 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 Comm* 226: 2887–292.
- Vogt RG, Riddiford LM, Prestwich GD (1985) Kinetic properties of a pheromone-degrading enzyme: The sensillar esterase of *Antheraea polyphemus*. *Proc Natl Acad Sci USA* 82: 8827–8831.

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Capítulo IV

Comparison of peripheral and central
pheromone inhibition on the flight behaviour of
the European corn borer, *Ostrinia nubilalis*
(Lepidoptera: Crambidae)

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Comparison of peripheral and central pheromone inhibition on the flight behaviour of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae).

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Abstract.

Sex pheromone response of male moths can be interrupted by the presence of behavioural inhibitors in the pheromone blend. Some “natural” inhibitors are pheromone components of one species that inhibit the attraction of another species. Males of species inhibited by natural interspecific inhibitors have specialized receptor neurons that respond only to the inhibitor. In addition to these natural inhibitors there are anthropogenic compounds (parapheromones) that mimic the pheromone molecules and interfere with the normal pheromone perception mechanism causing inhibition. Natural inhibitors act directly on the central nervous system, via specialized receptor neurons that send a “good quality” signal to the brain upon their detection, provoking inhibition. In contrast, parapheromones alter normal pheromone perception of pheromone receptor cells, so that the brain receives a “distorted” pheromone signal from the pheromone receptors resulting in inhibition. Due to their different mode of action on the olfactory system, natural inhibitors and parapheromones may alter the insect flight behaviour in different ways. To explore this possibility we compared the flight performance parameters (triangle of velocities) of male *O. nubilalis* flying in a wind tunnel to sex pheromone alone or to mixtures of pheromone and pheromone inhibitors. We used Z11-16:Ald as the natural inhibitor and Z11-14 methyl-ketone (MK) and Z11-14 trifluoromethyl ketone (TFMK) as parapheromones. Males flying in a pheromone plume tainted with Z11-16:Ald had an intended flight direction (course angle) that was more direct upwind than to any other treatment. They also experienced reduced intended speed of flight (airspeed), and the largest drift from intended flight direction (drift angle), due to wind flow. The parapheromones did not affect the course angle but they resulted in higher drift and lower airspeeds than pheromone alone. Analysis of the flight track through time showed that Z11-16:Ald and MK affected flight parameters from the beginning of the flight, whereas TFKM did not have an effect until the insect was close to the pheromone source. These results demonstrate that although the three inhibitors reduce the percentage of pheromone response and location, they do so by altering different flight parameters, and suggest that this may be related to their different physiological mechanisms of inhibition.).

Keywords:

Behavioural inhibitors, Z11-14 methyl-ketone, Z11-14 trifluoromethyl ketone, Z11-16:Ald, European corn borer, *Ostrinia nubilalis*

Introduction

Pheromone response can be interrupted by inhibitory compounds, also called behavioural antagonists (Renou and Guerrero, 2000), some of which are of natural origin and others only produced in the laboratory. In several moth species the males have olfactory receptor neurons specialized in the detection of pheromone components which are produced by other species. Small quantities of these compounds, when mixed with the pheromone of their own species, reduce the probability of males responding to, or finding, their own pheromone (Lopez et al., 1990; Leal, 1996; Mustaparta, 1997; Potting et al., 1999; Eizaguirre et al., 2002; Cardé and Haynes, 2004; Gemenno et al., 2006; Eizaguirre et al., 2007). This is thought to be a mechanism to maintain reproductive isolation of closely related species (Löfstedt, 1993; Cardé and Haynes, 2004). In addition to these “*natural*” inhibitors, artificial compounds have been synthesized that mimic the pheromone molecules (parapheromones), reducing the probability of males responding or finding the pheromone source (Renou and Guerrero, 2000). Although the end result is the same, the mode that natural inhibitors and pheromones reduce male response is completely different.

The flight manoeuvres of male moths responding to female sex pheromone are composed of at least two mechanisms: optomotor anemotaxis (Kennedy and Marsh, 1974; Marsh et al., 1978) and self-steered counterturning (Kennedy, 1983). Optomotor anemotaxis integrates the mechanical and visual information that allows the insect to fly upwind, and self-steered counterturning refers to the characteristic zigzag shape of the male moth flight in response to a pheromone plume. High resolution flight analysis allows to realize that odour plumes are not homogeneous odour clouds but instead are formed of intermittent pockets, or filaments, of odour-bearing and clean air (Murlis and Jones, 1981), and the demonstration that this intermittency is essential for successful upwind flight, have lead to the discovery that the zigzag is composed of at least two elements (Vickers, 2006; Cardé and Willis, 2008). The first is a straight upwind surge which propels the insect directly upwind to the odour source when it encounters a filament of pheromone. Due to the intermittency of the odour plume the insect soon encounters a pocket of clean air which aborts the upwind component of the program and triggers its cross-wind, or zigzagging, component (Vickers, 2006). It is the combination of these two elements which results in the characteristic zigzag flight of moths, a most

useful mechanism to reencounter a turbulent pheromone plume generated several meters away that broke down by changes in wind direction.

Neurons specialized in detecting natural inhibitors respond maximally to them and send the nerve signal to glomeruli of the antennal lobe, the first centre of olfactory integration in the insect brain, which only receive input generated by these compounds (Lilljefors et al., 1984; Wu et al., 1993). This is exactly the same way by which pheromone compounds are perceived and integrated (Hansson et al., 1992), with the only difference that they stimulate pheromone receptor neurons that innervate antennal lobe (AL) glomeruli different from those innervated by the inhibitors. When the antennal lobe receives stimuli simultaneously in both pheromone and inhibitor glomeruli, the flight is altered (Vickers et al., 1998).

Synthetic pheromone analogues, on the other hand, do not have specialized receptors for their detection. Instead, they interfere with pheromone perception events in the pheromone sensillum itself. They either affect the activity of esterases and proteases, such as acetylcholinesterase, chymotrypsin, and human liver carboxylesterases (Gelb et al., 1985; Ashour and Hammock, 1987). Of particular interest is their effect on antennal esterases present in insect olfactory tissues (Gelb et al., 1985; Durán et al., 1993; Prestwich, 1993; Parrilla and Guerrero, 1994) which, by degrading the pheromone, prevent sensory adaptation, or compete with pheromone binding proteins or pheromone receptors (Riba et al., 2001). The response of the pheromone receptor neuron to pheromone is thus modified by the pheromone analogues resulting mainly in behavioural inhibition. These pheromone analogues also have been act as mimics, synergists or antagonists of natural pheromones (Renou and Guerrero, 2000).

Natural inhibitors send a “*good*” signal to the area of the brain that integrates inhibition signals, whereas pheromone analogues cause the pheromone receptor neuron to send a “*defective*” signal to the pheromone glomeruli. The result in both cases is decreased behavioural response to pheromone, but the mechanism that causes this disruption is different in each case. The natural inhibitor of *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae) males, (Z)-11-hexadecenal (Z11-16:Ald), is perceived by an olfactory receptor neuron (ORN) that also responds to another natural inhibitor, (Z)-9-tetradecenyl acetate (Z9-14:Ac) (Gemeno et al., 2006; Linn Jr. et al., 2007), and it causes a significant reduction in oriented flight and source contact at a ratio of 100:1 pheromone:inhibitor (Gemeno et al. 2006). The pheromone analogues are the methyl

ketone (*Z*)-13-hexadecen-2-one (MK) and its fluorinated counterpart (*Z*)-1,1,1-trifluoro-13-hexadecen-2-one (TFMK), which reduce oriented flight and source contact at ratios 1:1 and 1:10 pheromone:inhibitor (Solé et al., 2008).

Several studies have investigated the flight track of male moths in response to pheromone, and a few more to pheromone plus natural inhibitors or pheromone analogues, but no study has compared the effect of natural inhibitors and pheromone analogues in the same species. In this study we compare the flight track parameters of *O. nubilalis* males in response to their sex pheromone alone, or to mixtures of sex pheromone plus a natural inhibitor or one of two pheromone analogues.

Materials and Methods

Insects. Moths were selected from a colony maintained at the Institut de Recerca Agroalimentaria (IRTA, Lleida). The colony was started with mated females collected in different commercial maize fields from Lleida province (North-East Spain). Previous analyses of gland extracts of wild females from the area pointed out to the major presence of the *Z* strain of the moth (Riba et al. 2005). For mating, wild couples were transferred to plastic cages (17 cm diameter x 11.5 cm height) containing a waxed paper around the walls to allow females to lay eggs. After selection of the *Z* progenies, the *Z* strain was reared according to a typical artificial diet for Noctuidae (Poitout and Bues, 1974) to which the following antibiotics were added: nipagin (methyl-4-hydroxybenzoate) (0,12%), flumidil (potassium o-oxyquinolinsulfonate + sulfonylamidotiazol) (0,12%) and aureomicin (chlortetracycline hydrochloride) (0,04%). Pupae were sexed and placed in cylindrical boxes (17 cm diameter, 12 cm high) at 23 ± 1°C and 65 ± 10% relative humidity with a 16:8 L:D photoperiod. Upon emergence, adults were separated daily and kept on filter paper in plastic containers in the presence of a 10% sucrose solution.

Chemicals and solvents. n-Hexane (analytical purity >95% GC, Fluka, Riedel-de Haën, Buchs SG, Switzerland) was used as solvent of all synthetic compounds. (*Z*)-11-tetradecenyl acetate (Z11-14:Ac) and (*E*)-11-tetradecenyl acetate (E11-14:Ac) (components of *O. nubilalis* sex pheromone) were purchased from Sigma-Aldrich Química, SA. (Madrid, Spain). Z11-16:Ald was purchased from Sociedad Española de Desarrollos Químicos, S.A. (SEDQ, Barcelona, Spain). TFMK was obtained by reaction

of the corresponding iodide (Camps et al., 1987) using *tert*-butyllithium and ethyl trifluoroacetate, as previously described (Villuendas et al., 1994). And MK was obtained as described previously (Solé et al., 2008). All compounds were found to be 96–99% chemically pure and >99% isomerically pure by GC analysis. Chemical structures are shown in figure 1.

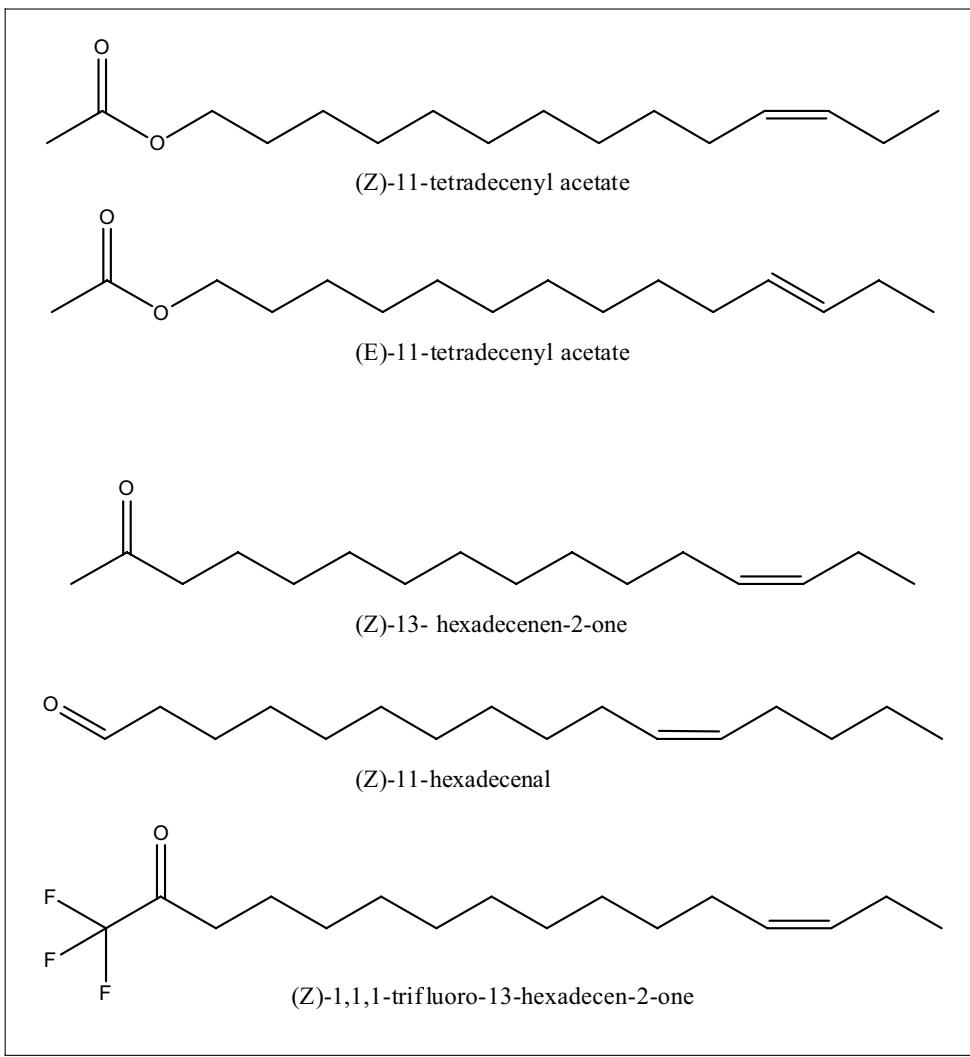


Figure 1. Structure of the pheromone components of *O. nubilalis* and the putative inhibitors of the pheromone response tested.

Treatments.

Pheromone. 30 µg of the pheromone blend consisting of Z11-14:Ac and E11-14:Ac at a 97:3 ratio. 30 µl of a 1 µg/µl hexane dilution were added to a 8-mm rubber septum (Sigma-Aldrich S.A., Madrid, Spain). The solvent was allowed to evaporate 30 min after which the dispensers were ready to use. N =19.

Pheromone plus MK and TFMK. 30 µg of pheromone plus 300 µg of either MK or TFMK. 300 µl of a 3 µg/µl hexane solution of inhibitor were added to the septum after the pheromone. Because the volume added was larger than the septum capacity, the inhibitor was added in turns following evaporation of the solvent. Proportion pheromone:inhibitor = 1:10. N = 24 (both).

Pheromone plus Z11-16Ald. 30 µg of pheromone plus 0.3 µg of Z11-16:Ald. 3 µl of a 0.1 µg/µl hexane solution of Z11-16:Ald were added to the septum after the pheromone. Proportion pheromone:inhibitor = 1:0.01. N = 25.

The pheromone: inhibitor ratios used (1:10, 1:10 and 1:0.01, for TFMK, MK and Z11-16:Ald, respectively) result in percentages of oriented flight (50, 12 and 18%, respectively), and source contact (12, 2 and 7%, respectively) that are, except for the oriented flight to TFMK, significantly lower than to those observed to pheromone alone (Riba et al., 2005; Gemenó et al., 2006; Solé et al., 2008)

Wind tunnel. The assays were conducted in a 200 x 50 x 50 cm glass tunnel as previously described (Gemenó et al., 2006). A fan blowing room air into the tunnel was placed in upwind end and an extractor was connected to the downwind end. Regulating the speed of fan and extractor created a laminar flow inside of the tunnel of 0.3 m/sec throughout. Air from the wind tunnel and wind tunnel room were exhausted outside of the building. Illumination (0.5-1.5 lux at bottom and top of tunnel, respectively) was provided by a 36-W red fluorescent light placed 1.4 m above the ceiling of the tunnel and covered with a white fabric sheet. The average temperature inside the tunnel was 23 ± 1°C and the relative humidity was 65 ± 5%.

Three to five-day old males were placed individually into galvanized-wire (1.5 mm openings, 0.25 mm wire diameter) cages (3 cm diameter, 8 cm high) covered with aluminium foil in one end, during the photophase previous to the experiment, and provided with 10 % sucrose water. Insects were taken to the wind tunnel room 30 min before the beginning of the test which was between the 3rd and 4th h of the scotophase.

The septum was placed at 20 cm from the upwind end and 30 cm high in the centre of the tunnel. The cage with the insect was introduced in the tunnel, the aluminium foil was removed, and the cage was placed over a 20-cm high platform covered with aluminium foil, located in the centre of the tunnel and 25 cm from its downwind end. When the insect started to become active (wing fanning, a few seconds after introduction in the pheromone plume) the cage was turned upside down, and the moth behaviour started to be recorded. If after 3 min the insect did not leave the cage, or did leave the cage but did not start to fly, or started to fly but not in a typical pheromone-response type of flight, the run was discarded. Runs in which insects that started a typical olfactory oriented flight but did not pass the middle of the tunnel were also discarded. Observations finalized when the insect contacted the pheromone source, or when the insect leaved the pheromone plume with clear indications of not being interested in pursuing odour-evoked upwind flight any longer. Each male was tested only once.

A black and white PAL camera (1/3 inch CCD, 0.02 lux light sensitivity, objective 5-50 mm, F 1.4) was placed 135 cm over the centre of the tunnel spanning a field of view of 135 cm of the length of the tunnel. The analogical signal was digitalized and stored at 25 frames/s in the computer by a video card (Pinnacle Systems v 5.1, Mountain View, California, USA). Each frame position of the insect was converted into X, Y coordinates with the aid of an in-house specific software (Track 2.3).

Flight parameters. The length of the insect displacement vector between two consecutive 1/25 sec. frames represents its real displacement with respect to the floor, or ground speed. The angle of the ground speed vector with the wind speed vector (i.e., the 0.3 cm/sec air flow in the tunnel) is the track angle. The track angle is the sum of the aimed insect flight angle with respect to the air flow direction, or course angle, and the drift produced by the wind displacement, or drift angle. The third vector of the triangle of velocities is the speed of the insect in the absence of drift, also known as air speed. Because ground speed, track angle and air speed are known, the rest of the parameters of the triangle of velocities can be calculated from them. Since angles can have both positive and negative values, their absolute value was used for analysis. Other parameters included are the number of turns (being a turn we need 2 frames in the same

direction before confirming a change of polarity as a real turn), turns per second, and total flight duration and distance.

For general analyses the triangle of velocities parameters for each successive pair of video frames was averaged in each individual. This individual mean constituted the experimental unit and was used to compare the four treatments. Observation of the flight tracks showed that the behaviour of the insect along the wind tunnel was not the same at the beginning of its flight, far away downwind, as it was further upwind, close to the pheromone source. To detect possible treatment effects that could have escaped in the global analysis, the flight of each insect was divided in three sections, each corresponding to a third of the wind tunnel track described by the insect. Triangle of velocities parameters were averaged for each section in each individual, and then compared across individuals and across sections.

Statistical data analysis. All analyses were performed with ANOVA (proc GLM of SAS) after determining the normality of data. Mean values were transformed to log ($x + 1$) when were necessary to fit normality and the means were compared by the Duncan test.

Results

Total track analysis. Flight tracks showed marked differences among treatments (Figure 2). Track angle, the direction of displacement of the insect over the ground, ranged between 69 and 83°. It was not affected by the addition of Z11-16:Ald to the pheromone, but it significantly increased, an average of 13°, when TFMK was added to the pheromone (Figure 3) and an average of 5° when we added MK (Figure 3). This corresponded with a high quantity of cross-wind flight in the flight track of this treatment, especially close to the pheromone source (Figure 2). The actual flight speed of the insect, the ground speed, was significantly reduced when MK or Z11-16:Ald were added to the pheromone, but it was not affected by TFMK (Figure 3).

Course angle, the intended direction of flight before drift, ranged between 29 and 52° (Figure 3). MK pheromone analogue not induced a change in the course angle when they were added to the pheromone, however Z11-16:Ald reduced it significantly, an average of 17°. The course angle of MK and pheromone was significantly lower than TFMK (an average of 10° and 7° respectively) and significantly higher than Z11-16:Ald.

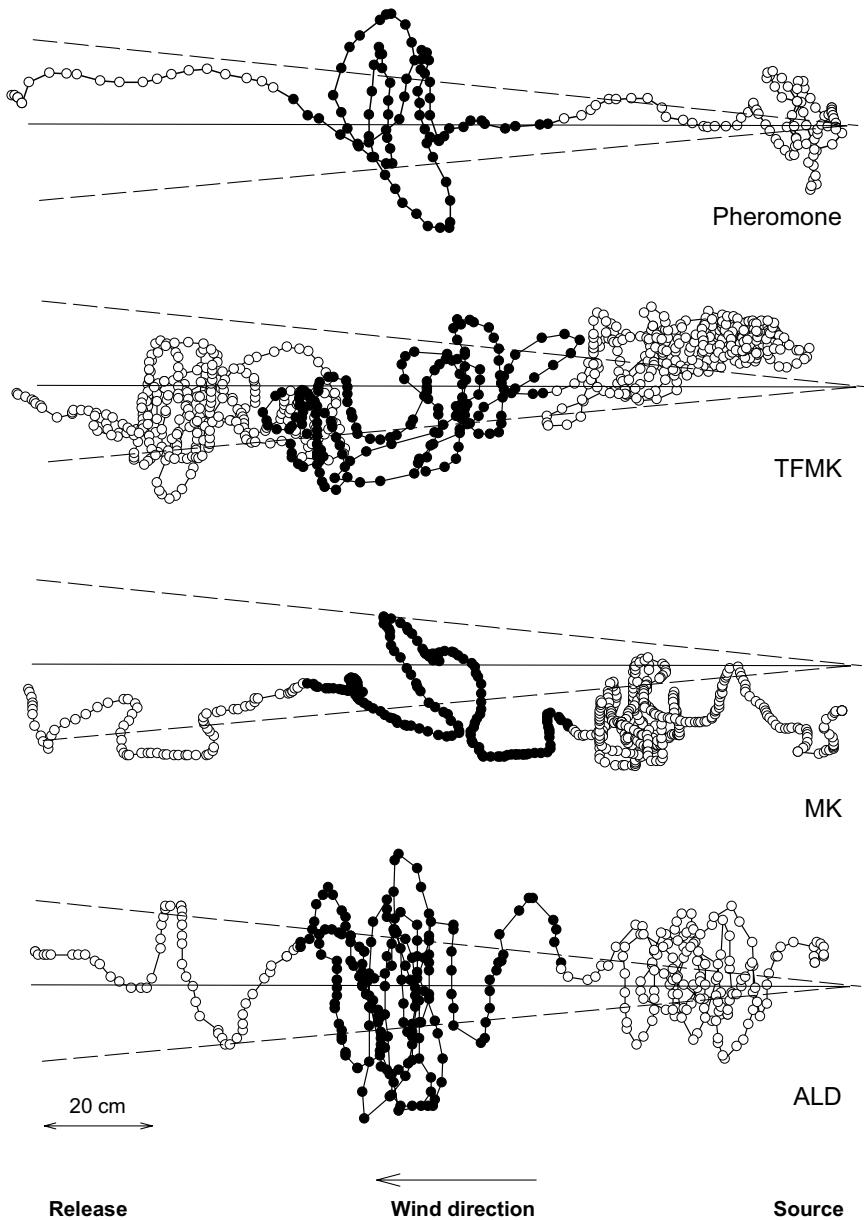


Figure 2. Representative flight tracks of *O. nubilalis* males flying to sex pheromone alone (a), and to blends of sex pheromone and the behavioural antagonists Z11-14TFMK (b), Z11-14:MK (c), and Z11-16:Ald (d). The sections in white represent the initial (left) and final (right) third sections of the flight and the section in black (middle) is the middle third flight section.

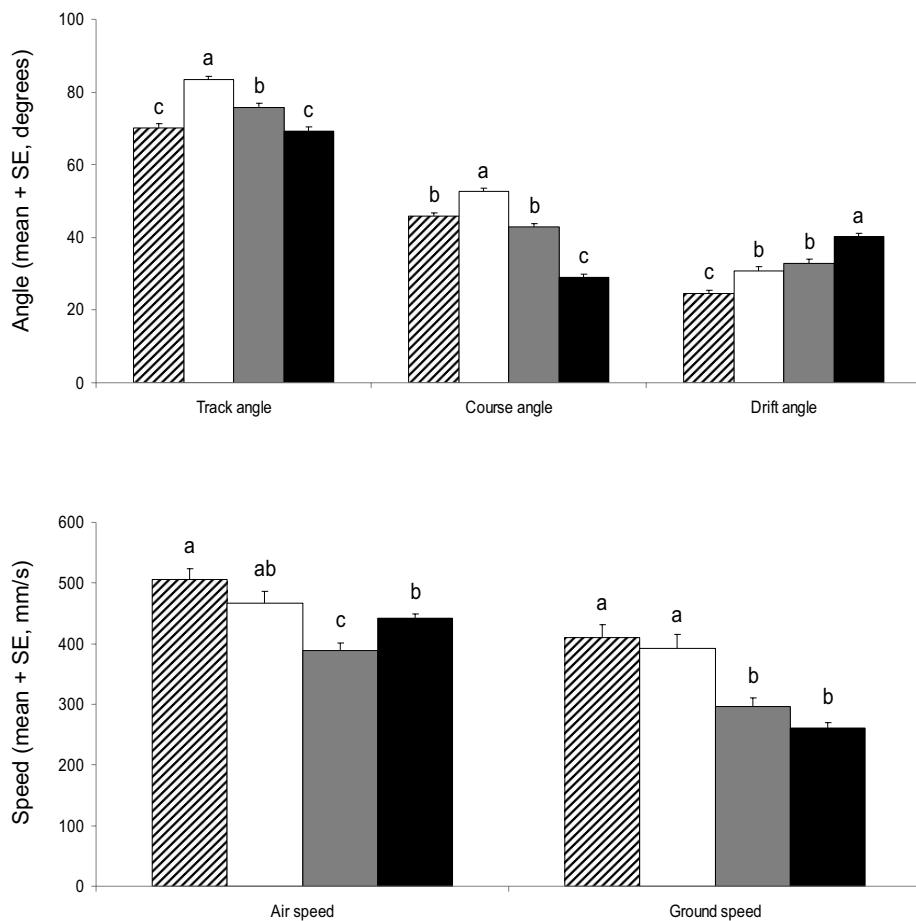


Figure 3. Triangle of velocities parameters (angles on top, velocities on bottom) of complete flights of *O. nubilalis* to their sex pheromone (strips) or blends of the sex pheromone and the behavioural antagonists Z11-14:TFMK (white), Z11-14:MK (grey) and Z11-16:Ald (black).

The deviation of the insect flight path by the wind, the drift angle, ranged between 24 and 40°, and was significantly different among treatments. Insects flying to the Z11-16:Ald were strongly deflected from their intended flight path, as shown by a significantly higher drift angle to this than to the other treatments, and almost double than the pheromone (Figure 3). By contrast, insects flying to pheromone alone showed the lowest drift angle. The drift angles to TFMK and MK lay between the other two treatments (Figure 3). The air speed, or intended speed of flight before drift, to pheromone was significantly reduced by MK and Z11-16:Ald, whereas TFMK did not affect this parameter (Figure 3).

Total number of turns are higher at TFMK treatment, respect to the other treatments (Figure 4), but this effect is due to in this treatment the insects fly a high total distance (Figure 4) and during more time (Figure 4). In contrast, if we calculated the turns per second they are no differences between the treatments were we added TFMK and Z11-16:Ald to the pheromone in comparison with the pheromone alone. But when we added MK to the pheromone, this compound induced a significantly turns per second reduction (Figure 4).

Partial track analysis. Partial track analysis provided temporal information about the time effect of each inhibitor. The track angle to TFMK and MK was larger than to any other treatment in section 3, but there were no differences among treatments in the middle section of the tunnel. In section 1 Track angle for TFMK treatment was larger than the other two inhibitors, but not to the pheromone alone (Figure 5). For all treatments, except the pheromone, the track angle increased as the insects approached the odour source, from sections 1 to 3 (Figure 5). The same occurred with the course angle but only with MK and Z11-16:Ald. Males flying to the pheromone alone, therefore, did not change either their aimed or their realized direction of flight while approaching the pheromone source (Figure 5). The course angle was smaller to Z11-16:Ald than to any other treatment in any section of the tunnel, showing that the strong effect of this treatment on the aimed orientation of the male was immediate and lasted throughout the entire flight. MK also reduced the course angle when added to the pheromone, and except for section 3 there were no differences between pheromone and TFMK (Figure 5). Drift angle increased as the insects approached the source in all treatments, as with the total track analysis, so Z11-16:Ac produced the highest drift, pheromone the lowest, and TFMK and MK stayed in between these two (Figure 5).

Ground speed and air speed were also very similar among sections and between total and partial track analysis. The pattern observed in the total track analysis was observed again in the partial track analysis, with only minor differences (Figure 6) and it seems that these two parameters were not altered during the flight and also indicate that ground speed and air speed are not affected by the time of exposition to the different inhibitors or by the distance to the pheromone or antagonist source.

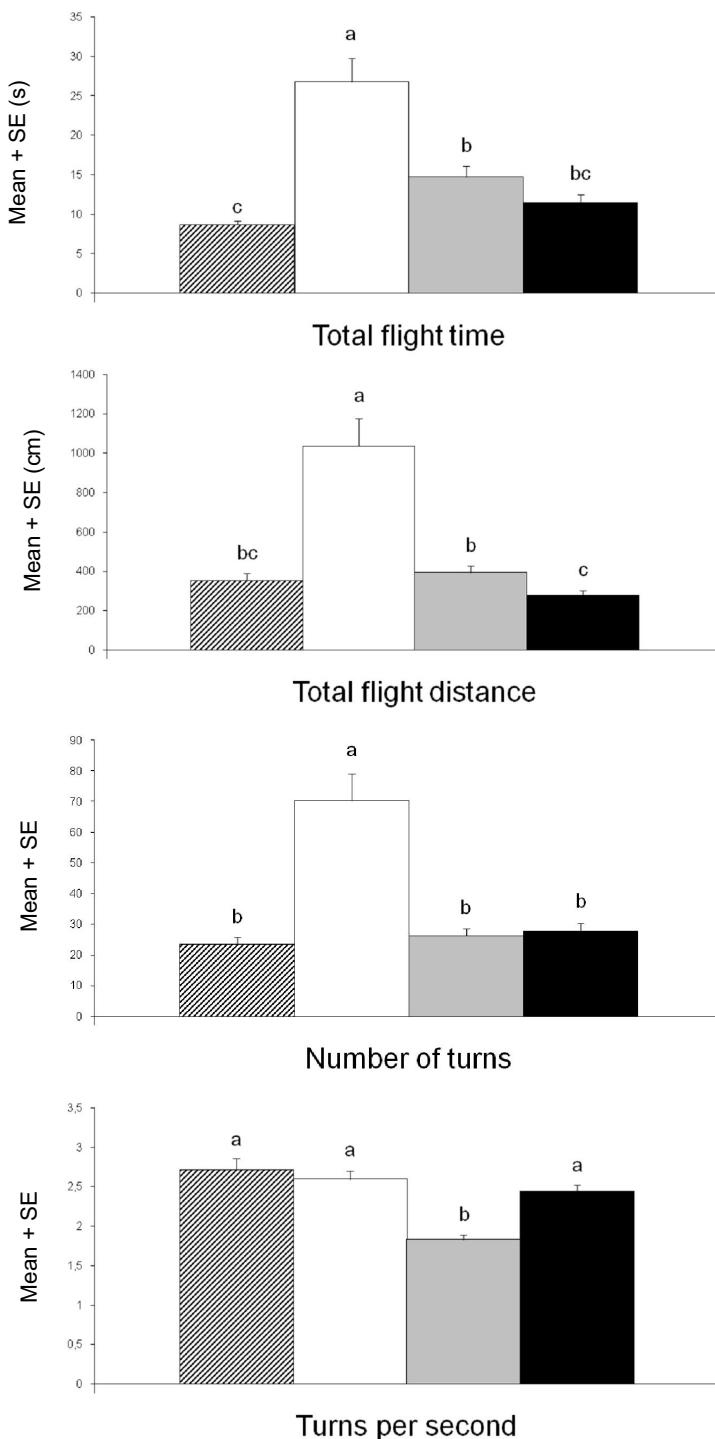


Figure 4. Total flight time, total flight distance, number of turns and turns per second parameters of complete flights of *O. nubilalis* to their sex pheromone (strips) or blends of the sex pheromone and the behavioural antagonists Z11-14:TFMK (white), Z11-14:MK (grey) and Z11-16:Ald (black).

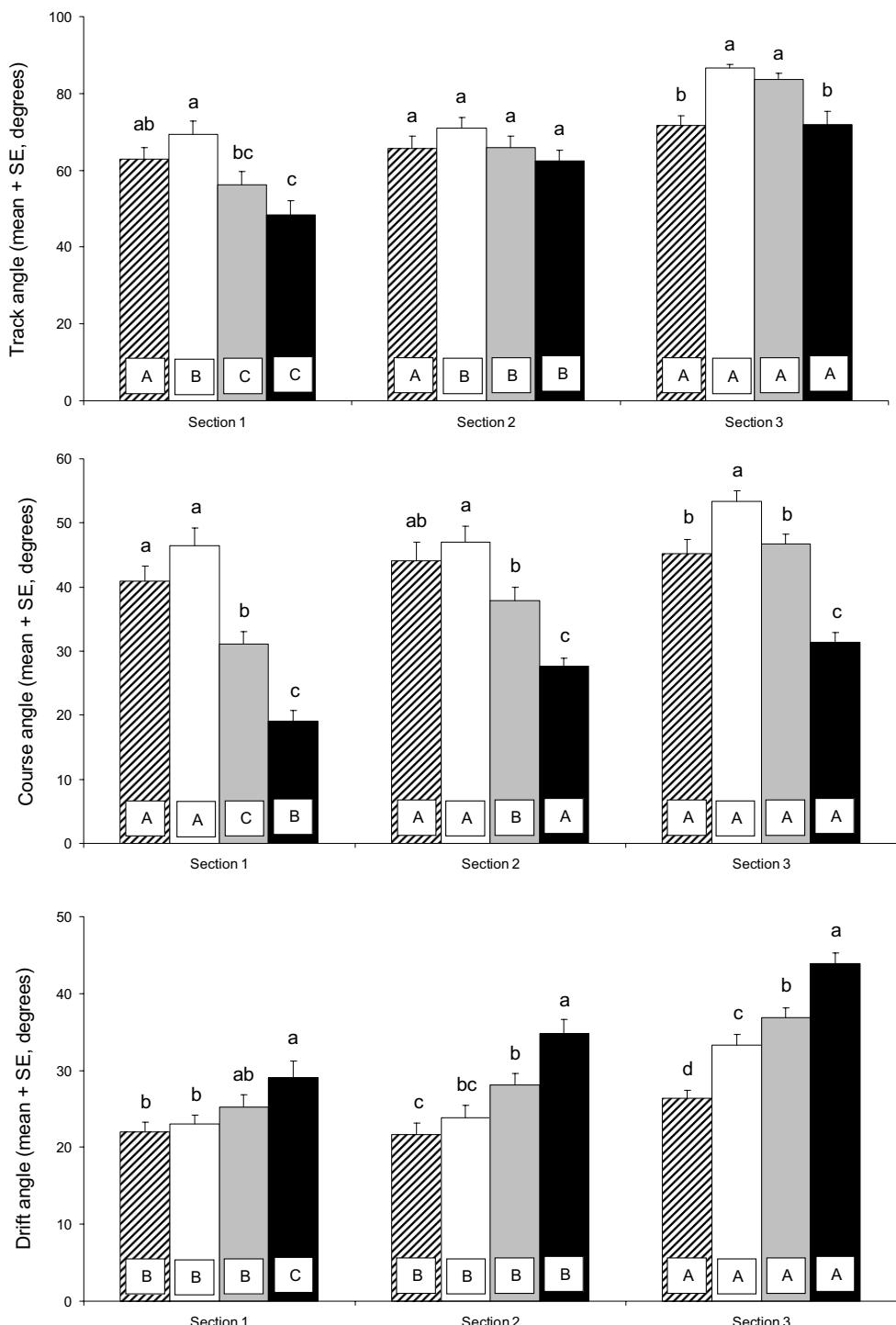


Figure 5. Angles of the triangle of velocities parameters of sectioned flights of *O. nubilalis* to their sex pheromone (strips) or blends of the sex pheromone and the behavioural antagonists Z11-14:TFMK (white), Z11-14:MK (grey) and Z11-16:Ald (black). Bars with the same capital letter indicate not significantly differences between sections.

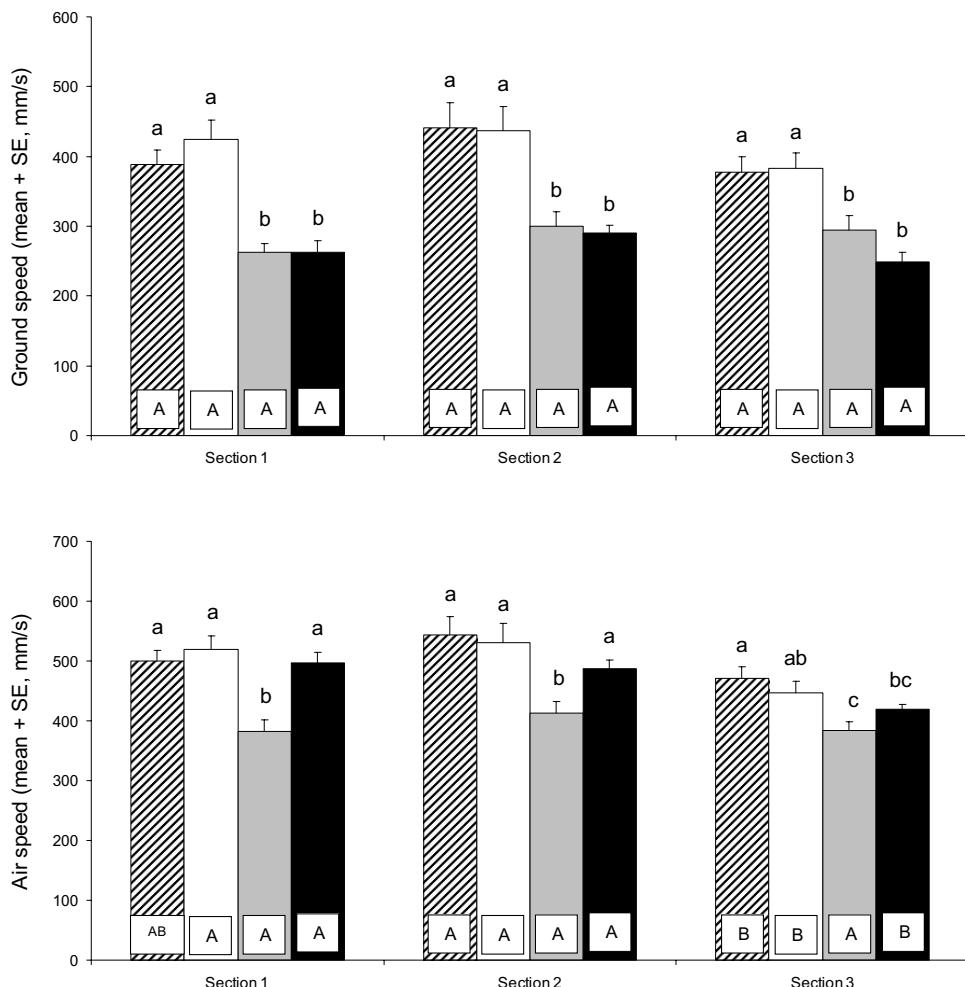


Figure 6. Velocities of the triangle of velocities parameters of sectioned flights of *O. nubilalis* to their sex pheromone (strips) or blends of the sex pheromone and the behavioural antagonists Z11-14:TFMK (white), Z11-14:MK (grey) and Z11-14:Ald (black). Bars with the same capital letter indicate not significantly differences between sections.

To compare flight distance among odour treatments we calculated the percentage of the total track distance in each section of the tunnel. Males flying to pheromone covered the same distance in each section of the tunnel, however males flying to any of the inhibitor treatments did a longer distance in the section of the tunnel closer to the pheromone source than in the previous sections (Figure 7). This is because with inhibitor the majority of males did not contact the odour source but stayed some time flying in the plume, in section 3, before they flew away, therefore increasing the percent of total distance in this section. This is especially prominent with TFMK (Figure 7).

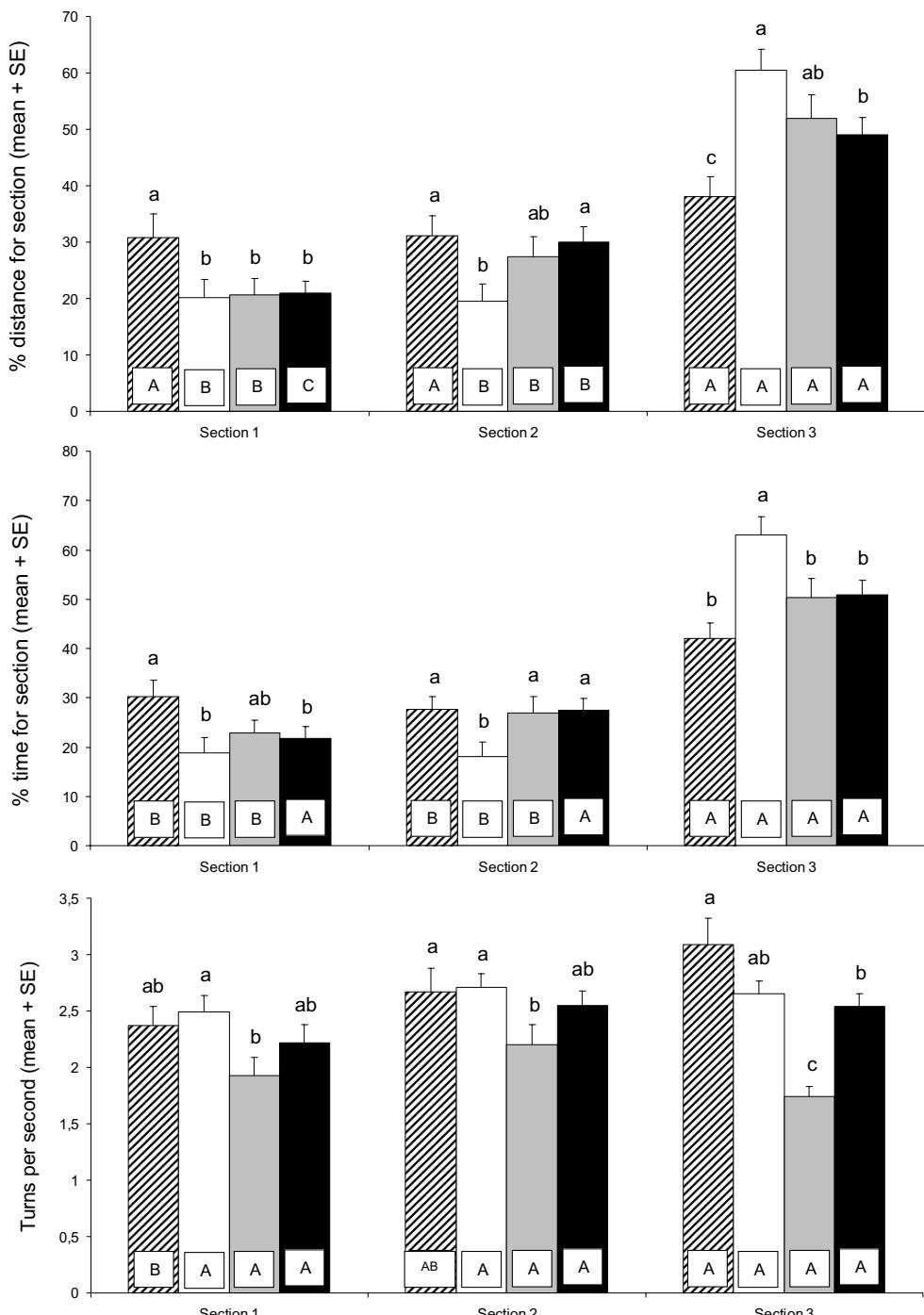


Figure 7. Percentage of distance, percentage of time and turns per second of sectioned flights of *O. nubilalis* to their sex pheromone (strips) or blends of the sex pheromone and the behavioural antagonists Z11-14:TFMK (white), Z11-14:MK (grey) and Z11-16:Ald (black). Bars with the same capital letter indicate not significantly differences between sections.

Turning frequency in pheromone treatment increased as the insects are closer to the source. In contrast, with all the inhibitors, turns per second were kept constant in all sections and followed the same pattern described in total flight analysis: A significantly reduction was observed when we added MK to pheromone in all sections of the wind tunnel. Also we observed that Z11-16:Ald induced a lower turn frequency, but only in the section near the source (Figure 7)

Discussion.

Ground speed and track angle, which correspond to the ground distance covered by the insect in its displacement and its angle with respect to the wind, respectively, were measured directly from the flight track to calculate the remaining elements of the triangle of velocities. These two parameters define the actual two-dimensional displacement of the insect in the tunnel, and if the inhibitors had an effect on flight performance we should expect it to be expressed as an alteration of these parameters (Marsh et al., 1978). However the effect of the inhibitors is less immediate on these parameters than on those that underlie them, such as the track angle which is the sum of the intended flight angle of the insect (course) and the displacement produced by the wind (drift). An insect with a relatively straight intended flight (low course), but that gets easily deflected by the wind (high drift), can produce the same track angle as an insect with higher course angle (less straight) and experiencing lower drift (Vickers and Baker, 1997). As an example, in our study, males responding to pheromone plus Z11-16:Ald intended a straight flight than those responding to pheromone alone, however with Z11-16:Ald males experienced almost twice as much drift than with pheromone so that the resulting track angle was not different between these two treatments. For the purpose of this study we focused more on the parameters that theoretically represent the motivational state of the insect (course and drift angles, and air speed) and less on the actual displacement of the insect in space (track angle and ground speed).

Previous studies have shown that the three inhibitors tested in this study reduce the percentage of males that contact the pheromone source (Riba et al., 2005; Gemenó et al., 2006; Linn Jr. et al., 2007; Solé et al., 2008). In this study we have shown that the behavioural steps that result in this outcome differ among inhibitors. Males flying to a pheromone plume tinged with Z11-16:Ald, the natural inhibitor, aimed for a straighter flight (low course angle) than if flying to pheromone alone, or to pheromone plus the

pheromone analogues (Figure 3). We also observed the highest drift (drift angle) of all treatments, and their aimed flight speed (air speed) was lower than to pheromone alone (Figure 3). Unlike Z11-16:Ald, the pheromone analogues did not change the intended direction of flight. However, as with the natural inhibitor, males exposed to the analogues were more susceptible to drift and had a lower intended fly speed than to pheromone alone. The effect of the analogues on drift angle was not as strong as the natural inhibitor, but MK had a stronger effect than Z11-16:Ald on intended flight speed (air speed) (Figure 3). The overall effect of Z11-16:Ald on males was an intended flight that was straight upwind, slow and weak, as it drifted easily with the wind. The pheromone analogues, on the other hand, did not affect the intended flight direction, but made males more susceptible to drift than pheromone, although not as much as Z11-16:Ald, and reduced their intended flight speed (air speed), in particular the MK (Figure 3). So, although the three inhibitors reduce the percentages to pheromone source contact, this final behavioural outcome is the result of a different effect of each inhibitor on the flight parameters of males responding to pheromone.

The effect of the inhibitors depended on the time elapsed from initiation of flight or distance to the source. The effect of Z11-16:Ald on course and drift angle was already showing at the beginning of the flight track, section 1, and was maintained through the end of the flight (Figure 5). The reduction in air speed, however, did not appear until the insects were closer to the pheromone source, in section 3 (Figure 6). MK reduced course angle and air speed from the beginning of the flight, but the increase in drift did not take place until the second section of the flight (Figure 5 and 6). The effects of TFMK on drift and course angle did not appear until the second and third sections of the flight track (Figure 5) and no differences in air speed were observed with the pheromone along the three sections (Figure 6). So it appears that TFMK it took a longer time than MK or Z11-16:Ac to affect or inhibit the flight of males. An alternative explanation is that the distance from the source or the exposition time to the antagonists was responsible for this effect. Our experiment was not designed to test time and distance effects separately. However in a parallel study (Gemeno et al., unpublished) we have shown that the natural inhibitor produce its inhibition a few fractions of a second earlier than the pheromone analogues.

The effect of anthropogenic parapheromones on behaviour is very variable, with some that have no effect at all (Parrilla and Guerrero, 1994) and others that cause

attraction (Renou and Guerrero, 2000) or inhibition (Bau et al., 1999; Riba et al., 2001; Riba et al., 2005). In some cases parapheromones are more effective attractants or synergists than the pheromone itself, even when differences in volatility are taken into account (Lucas et al., 1994). The mechanism of inhibition of antagonist is also very variable and in many cases not known. One difference between natural inhibitors and inhibitory parapheromones is that the former are very effective inhibitors at very low percentages with respect to the pheromone (Klun and Robinson, 1971; Struble et al., 1987; Glover et al., 1989; Klun et al., 1979; Gemenó et al. 2006; Eizaguirre et al., 2007), whereas inhibitory parapheromones usually require higher concentrations to affect male response (Riba et al., 2005; Solé et al., 2008). For example in this study the concentration of natural inhibitor needed to produce the same reduction in source contact as the parapheromones was 1000 times lower, a clear indication of the different mode of action of the two types of compounds. In *Agrotis segetum* (Denis & Schiff.) (Lepidoptera: Noctuidae) the trifluoromethyl analogue is 100-fold less active in single sensillum recording (SSR) than the natural pheromone compound Z5-10:Ac (Wu et al., 1993).

This strong inhibitory effect is due to their mechanisms of action. In different Lepidoptera species natural inhibitor receptor neurons innervate the macroglomerular complex, a male-specify glomerular compartment of the antennal lobe dedicated to pheromone perception, and there they arbores in specific glomeruli other than the pheromone glomeruli (Hansson et al. 1995). In *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) the pheromone olfactory receptor neurons converge on the same glomerulus and the inhibitor on a different glomerulus of the macroglomerular complex (Hansson et al., 1995). In *A. segetum* the inhibitor Z5-10:OH innervates the largest glomeulus of the macroglomerular complex (Hansson et al., 1992), whereas in *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) the olfactory receptor neuron sensitive to Z9-14:OH innervates one glomeruli of the macroglomerular complex (Ochieng et al., 1995). The ability of an olfactory receptor neuron to follow and transducer pulses of odour as they arrive at the antenna will clearly affect the response (Vickers and Baker, 1997) and if an specie have a specific olfactory receptor neuron specially tuned for antagonist, the inhibitory response of this compound is clear. This is the case of Z11-16:Ald.

In contrast, inhibitory mechanisms in TFMK antagonists have been explained for their activity as inhibitors of antennal esterase presents in insect olfactory tissues (Gelb et al., 1985; Durán et al., 1993; Prestwich 1993; Parrilla and Guerrero, 1994; Rosell et al., 1996) and due to their high structural similarity to the pheromone for a competitive inhibition mechanism either for the pheromone binding proteins and/or for the pheromone receptors (Feixas et al., 1995; Bau et al., 1999; Riba et al., 2001; Plettner, 2002; Solé et al., 2008). MK inhibitors are not strong esterase inhibitors (Sole et al., 2008) and base their action only in the structural similarity to the pheromone. This compound competes with the natural attractant for the pheromone receptors and elicits an antagonism response by the central nervous system (Glover et al., 1989).

These differences in the mode of action have had a real translation in flight parameters. TFMK molecules need to arrive to the olfactory tissues to produce their effect as an esterase inhibitor and they need to maintain their concentration to produce a noise level to disrupt the insect. For this reason the speed of response is low and the percentage of distance increase significantly in section 3 (Figure 7). Similarly effects on course and drift angle in TFMK treatment appeared in the last steps of the flight (Figure 5). It seems that insects in TFMK treatment trying to fly even in the presence of the antagonist (duration of flight higher) (Figure 4). In contrast, Z11-16:Ald has its own inhibition channel, there is an evolved mechanism to inhibit, directly connected with the brain of the insect, and the response to the antagonist is faster. More straight, slower flight and increased drift since the first section of the wind tunnel, are both departures from the ideal zigzagging flight in Z11-16:Ald treatments.

In summary, TFMK and MK did not change the intended flight direction (course angle), but Z11-16:Ald made insects aim more straight upwind than any other treatment. All the inhibitors made the insect flight tracks more susceptible to drift, but this effect was especially strong with Z11-16:Ald. When the inhibitors were present in the pheromone blend the insect aimed speed (air speed) was reduced, resulting in a lower actual speed (ground speed) to MK and Z11-16:Ald, but not to TFMK. In general the natural inhibitor Z11-16:Ald produced the strongest deviation to the flight parameters in comparison with pheromone alone. Male flight to Z11-16:Ald aimed more straight upwind and at a lower speed, and was deflected more strongly by the wind, than to pheromone alone. Males flying to TFMK and MK did not aim in a straighter upwind flight than to the pheromone, but their intended flight was slower and

they were more susceptible to drift by the air flow. All these differences are related to different mode of action of the compounds tested.

Pheromone inhibition should be considered in the context of pheromone perception, integration and response. Inhibition occurs when alien odorants are added to the pheromone blend. The end result is a reduced probability of stimulating flight or finding the pheromone source. Our results pointed out that different antagonist, with different mode of action, that produce similar final effects they provoke different flight parameters, and the study of these can be an interesting cue to identify new potential compounds that interfere in pheromone perception process.

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References

- ASHOUR, M. -B. A.; HAMMOCK, B. D. (1987). Substituted trifluoroketones as potent selective inhibitors of mammalian carboxylesterases. *Biochem. Pharm.* **36**, 1869-1879.
- BAU, J.; MARTÍNEZ, D.; RENOU, M.; GUERRERO, A. (1999). Pheromone-triggered orientation flight of male moths can be disrupted by trifluoromethyl Ketones. *Chem. Senses* **24**, 473-480.
- CAMPS, F.; GASOL, V.; GUERRERO, A. (1987). A new and efficient one-pot preparation of alkyl halides from alcohols. *Synthesis*, 511-512.
- CARDÉ, R. T.; HAYNES, K. F. (2004). Structure of the pheromone communication channel in moths. *Advances in Insect Chemical Ecology*. (ed. by Cardé, R. T. and Miller, J. G) 283-323. Cambridge University Press, Cambridge, UK.
- CARDÉ, R. T.; WILLIS, M. A. (2008). Navigational strategies used by insects to find distant, wind-borne sources of odor. *J. Chem. Ecol.* **34**, 854-866.
- DURÁN, I.; PARRILLA, A.; FEIXAS, J.; GUERRERO, A. (1993). Inhibition of antennal esterases of the Egyptian armyworm *Spodoptera littoralis* by trifluoromethyl ketones. *Bioorg. Med. Chem. Letters* **3**, 2593-2598.
- EIZAGUIRRE, M.; ALBAJES, R.; LÓPEZ, C.; SANS, A.; GEMENO, C. (2007). Inhibition of pheromone response in *Sesamia nonagrioides* by the pheromone of the sympatric corn borer, *Ostrinia nubilalis*. *Pest Manag. Sci.* **63**, 608-614.
- EIZAGUIRRE, M.; SANS, A.; LÓPEZ, C; ALBAJES, R. (2002). Effects of mating disruption against the Mediterranean corn borer, *Sesamia nonagrioides*, on the European corn borer *Ostrinia nubilalis*. *IOBC/WPRS Bull.* **25**, 59-68.
- FEIXAS, J.; PRESTWICH, G. D.; GUERRERO, A. (1995). Ligand specificity of pheromone-binding proteins of the processionary moth. *Eur. J. Biochem.* **234**, 521-526.
- GELB, M. H.; SVAREN, J. P.; ABELES, R. H. (1985). Fluoro Ketone inhibitors of hydrolytic enzymes. *Biochemistry*, **24**, 1813-1817.
- GEMENO, C.; SANS, A.; LÓPEZ, C.; ALBAJES, R.; EIZAGUIRRE., M. (2006). Pheromone antagonism in the European corn borer moth *Ostrinia nubilalis*. *J. Chem. Ecol.* **32**, 1071-1084.
- GLOVER, T. J.; PEREZ, N.; ROELOFS, W. L. (1989). Comparative analysis of sex pheromone-response antagonists in three races of European corn borer. *J. Chem. Ecol.* **15**, 863-873.
- HANSSON, B. S.; ALMAAS, T. J.; ANTON, S. (1995). Chemical communication in heliothine moths. V. Antennal lobe projection patterns of pheromone-detecting olfactory receptor neurons in male *Heliothis virescens* (Lepidoptera:Noctuidae). *J. Comp. Physiol. A* **177**, 535-543.
- HANSSON, B.S.; LJUNGBERG, H.; HALLBERG, F.; LÖFSTEDT, C. (1992). Functional specialization of olfactory glomeruli in a moth. *Science* **256**, 1313-1315.
- KENNEDY, J. S. (1983). Zigzagging and casting as a programmed response to wind-borne odour: a review. *Physiol. Entomol.* **8**, 109-120.
- KENNEDY, J. S.; MARSH, D. (1974). Pheromone-regulated anemotaxis in flying moths. *Science* **184**, 999-1001.
- KLUN, J. A.; ROBINSON, J. F. (1971). European corn borer moth: sex attractant and sex attractant inhibitors. *Ann. Entomol. Oc. Am.* **64**, 1083-1086.
- KLUN, J. A.; MAINI, S.; CHAPMAN, O. L.; LEPONE, G.; LEE, G. H. (1979). Suppression of male European corn borer sex attraction and precopulatory reactions with (E)-9-tetradecenyl acetate. *J. Chem. Ecol.* **5**, 345-352.
- LEAL, W. S. (1996). Chemical communication in scarab beetles: Reciprocal behavioral agonist-antagonist activities of chiral pheromones. *Proc. Natl. Acad. Sci. USA* **93**, 12112-12115.
- LILJEFORS, T.; THELIN, B.; VAN DER PERS, J. N. C. (1984). Structure-activity relationships between stimulus molecule and response of a pheromone receptor cell in turnip moth, *Agrotis segetum*. Modification of the acetate group. *J. Chem. Ecol.* **10**, 1661-1675.
- LINN, Jr. C. E.; MUSTO, C. J.; ROELOFS, W. L. (2007). More rare males in *Ostrinia*: response of Asian corn borer to the sex pheromone of the European corn borer. *J. Chem. Ecol.* **33**, 199-212.
- LÖFSTEDT, C. (1993). Moth pheromone genetics and evolution. *Philos. Trans. R. Soc. Lond. B* **340**, 167-177.
- LOPEZ, J. D.; SHAVER, T. N.; GOODENOUGH, J. L. (1990). Multispecies trapping of *Helicoverpa* (*Heliothis*) *zea*, *Spodoptera frugiperda*, *Pseudaletia unipuncta*, and *Agrotis ipsilon* (Lepidoptera: Noctuidae). *J. Chem. Ecol.* **12**, 3479-3491.
- LUCAS, P.; RENOU, M.; TELLIER, F.; HAMMOUD, A.; AUDEMARD, H.; DESCQINS, C. (1994). Electrophysiological and field activity of halogenated analogs of (E,E)-8,10-dodecadien-1-ol, the main pheromone component, in the codling moth. *J. Chem. Ecol.* **20**, 489-503.

- MARSH, D.; KENNEDY, J. S.; LUDLOW, A. R. (1978). An analysis of anemotactic zigzagging flight in male moths stimulated by pheromone. *Physiol. Entomol.* **3**, 221-240.
- MURLIS, J.; JONES, C. D. (1981). Fine-scale structure of odour plumes in relation to insect orientation to distant pheromone and other attractant source. *Physiol. Entomol.* **6**, 71-86.
- MUSTAPARTA, H. (1997). Olfactory coding mechanisms for pheromone and interspecific signal information in related species of moths. Advances in Insect Chemical Ecology. (ed. by Cardé, R. T. and Miller, J. G) 141-164. Chapman & Hall, New York. USA.
- OCHIENG, S. A.; ANDERSON, P.; HANSSON, B. S. (1995). Antennal lobe projections of pheromone specific receptor neurons in male and female *Spodoptera littoralis*. *Tissue & Cell* **27**, 221-232
- PARRILLA, A.; GUERRERO, A. (1994). Trifluoromethyl ketones as inhibitors of the processionary moth sex pheromone. *Chem. Senses* **19**, 1-10.
- PLETTNER, E. (2002). Insect pheromone olfaction: New targets for the design of species-selective pest control agents. *Curr. Med. Chem.* **9**, 1075-1085.
- POITOUT, S.; BUES, R. (1974). Élevage des Chenilles de vingt-huit espèces de lépidoptères Noctuidae et deux espèces d'Artidiidae sur milieu artificiel simple. Particularités de l'élevage selon les espèces. *Ann. Zool. Ecol. Anim.* **6**, 431-434.
- POTTING, R. P. J.; LÖSEL, P. M.; SCHERKENBECK, J. (1999). Spatial discrimination of pheromones and behavioural antagonists by the tortricid moths *Cydia pomonella* and *Adoxophyes orana*. *J. Comp. Physiol. A* **185**, 419-425.
- PRESTWICH, G. D. (1993). Chemical studies of pheromone receptors in insects. *Arch. Insect Biochem. Physiol.* **22**, 75 – 86.
- RENOU, M.; GUERRERO, A. (2000). Insect parapheromones in olfaction research and semiochemical-based pest control strategies. *Ann. Rev. Entomol.* **48**, 605-630.
- RIBA, M.; SANS, A.; BAU, P.; GROLLEAU, G.; RENOU, M.; GUERRERO, A. (2001). Pheromone response inhibitors of the corn stalk borer *Sesamia nonagrioides*. Biological evaluation and toxicology. *J. Chem. Ecol.* **27**, 1879-1897.
- RIBA, M.; SANS, A.; SOLÉ, J.; MUÑOZ, L.; BOSCH, M. P.; ROSELL, G.; GUERRERO, A. (2005). Antagonism of pheromone response of *Ostrinia nubilalis* males and implications on behavior in the laboratory and in the field. *J. Agric. Food Chem.* **53**, 1158-1165.
- ROSELL, G.; HERRERO, S.; GUERRERO, A. (1996). New trifluoromethylketones as potent inhibitors of esterases. ¹⁹F NMR Spectroscopy of transition state analog complexes and structure-activity relationships. *Biochem. Biophys. Res. Commun.* **226**, 287-292.
- SOLÉ, J.; SANS, A.; RIBA, M.; ROSELL, G.; ROSA, E.; MUÑOZ, L.; BOSCH, M. P.; GUERRERO, A. (2008). Differential activity of non-fluorinated analogues of the European corn borer pheromone. *Chemoecology* **18**, 99-108.
- STRUBLE, D. L.; BYERS, J. R.; McLEOD, D. G. R.; AYRE, G. L. (1987). Sex pheromone components of an Alberta population of European corn borer, *Ostrinia nubilalis* (Hbn.) (Lepidoptera: Pyralidae). *Can. Entomol.* **119**, 291-299.
- VICKERS, N. J. (2006). Winging it: moth flight behavior and responses of olfactory neurons are shaped by pheromone plume dynamics. *Chem. Senses* **31**, 155-166.
- VICKER, N. J.; BAKER, T. C. (1997). Chemical communication in heliothine moths: Correlation between diminished responses to point-source plumes and single filaments similarly tainted with a behavioral antagonist. *J. Comp. Physiol. A* **180**, 523-536.
- VICKERS, N. J.; CHRISTENSEN, T. A.; HILDEBRAND, J. G. (1998). Combinatorial odor discrimination in the brain: attractive and antagonist odor blends are represented in distinct combinations of uniquely identifiable glomeruli. *J. Comp. Neurol.* **400**, 35-36.
- VILLUENDAS, I.; PARRILLA, A.; GUERRERO, A. (1994). An efficient and expeditious synthesis of functionalized trifluoromethyl ketones through lithium-iodine exchange reaction. *Tetrahedron*, **50**, 12673-12684.
- WU, W. Q.; BENGTSSON, M.; HANSSON, B.S.; LILJEFORS, T.; LÖFSTEDT, C.; PRESTWICH, G. D.; SUN, W. C.; SVENSSON, M. (1993). Electrophysiological and behavioral responses of turnip moth males, *Agrotis segetum*, to fluorinated pheromone analogs. *J. Chem. Ecol.* **19**, 143-157.

Capítulo V

Behavioural and electrophysiological responses
of the European corn borer *Ostrinia nubilalis*
(Lepidoptera: Crambidae) to different host plant
volatiles

Behavioural and electrophysiological responses of the European corn borer *Ostrinia nubilalis* (Lepidoptera: Crambidae) to different host plant volatiles

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Abstract.

Headspace volatile compounds emitted by undamaged maize plants (*Zea mays* L.) in two different day periods (morning and night) and by stressed plants were collected and analyzed by their retention index and GC-MS. Forty two compounds were detected from undamaged plants, the most abundant being limonene, linalool, benzoic acid, indole, β -cariophyllene and acetophenone. Most of the compounds were obtained in similar amounts regardless the diurnal period except indole which was preferentially released in the morning conditions. In stressed plants, the most abundant chemicals were limonene, acetophenone, hexanoic acid, benzoic acid and indole. The most remarkable differences between undamaged and stressed plants were the higher release of 1,8-cineole in the former and hexanoic acid in the latter. Some of the compounds showed antennal activity on both sexes of the European corn borer (ECB) *Ostrinia nubilalis* being particularly remarkable the EAG responses elicited by pentadecane, tetradecane, tridecane and 2-ethylhexanol. Methyl salicylate displayed also a positive response in females antenna, but not on males, and significantly attracted females in dual choice tests suggesting that it can play a role in oviposition search and preference by ECB females. In behavioural tests tridecane and tetradecane, that elicited good electroantennographic responses in female antenna, appeared to be deterrents whereas 2-hexanol and nonanol, which displayed lower depolarization responses behaved as attractants. In contrast, 2-ethyl-hexanol and pentadecane that showed good depolarization responses in females were not behaviourally active. On the other hand, compounds eliciting low EAG responses were found to act as remarkable attractants (heptanal and β -pinene) or repellents (2-cyclopentylcyclopentanone).

Keywords:

Plant volatiles, electroantennogram, behavioural tests, maize plants, European corn borer, *Ostrinia nubilalis*

Introduction

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), is a worldwide maize pest particularly important in Europe, North America, North Africa, Philippines and Japan (Mason et al., 1996). Whereas maize is the preferred major host for the insect, other crops may be also attacked, such as potato, green pepper, winter wheat, sorghum, tobacco, etc. Control of this species is particularly troublesome since insecticide sprays are only effective during the short period between egg hatching and feeding by young larvae. There are two distinct populations of the ECB, each one displaying successful species isolation and in areas where both strains are present there is enough genetic compatibility between them to produce fertile hybrids (Ma and Roelofs, 1995; Roelofs et al., 1987; Zhu et al., 1995). The Z strain female emits a 97:3 mixture of (Z)-11-tetradecenyl acetate (Z11-14:Ac) and (E)-11-tetradecenyl acetate (E11-14:Ac) (Klun et al., 1973), whereas the reverse blend of both compounds in 1:99 to 4:96 ratio is used by the E race females (Glover et al., 1987; Peña et al., 1988).

Chemical studies based on the interaction between herbivores and plants have established that semiochemicals released by plants play a significant role for insects in the selection of suitable hosts for feeding or oviposition (Miller and Stricker, 1984; Feeny, 1992; Städler, 1992; Renwick and Chew, 1994; Konstantopoulou et al., 2004) (Honda, 1995). In this context, the decision by the female to oviposit or not, depends on the balance of positive and negative signals that determine if a plant is accepted or rejected (Huang and Renwick, 1993; Renwick and Chew, 1994).

At present, more than 1000 different organic compounds have been identified to be emitted from different plant species, including alkanes, alkenes, alcohols, ketones, aldehydes, ethers, esters and carboxylic acids (Dudareva et al., 2004; Ninemets et al., 2004). In the case of maize plants, a number of studies have been performed regarding volatile chemicals that can stimulate or deter oviposition in the ECB, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) and the Mediterranean corn borer (MCB) *Sesamia nonagrioides* (Lefèuvre) (Lepidoptera: Noctuidae) (Dittrick et al., 1983; Derridj et al., 1986; Lupoli et al., 1990; Udayagiri and Mason, 1995, 1997; Binder, 1999; Konstantopoulou et al., 2002, 2004; Varsnhey et al., 2003). In the vast majority of these studies, the chemicals were obtained by steam distillation or by solvent extraction of corn kernels, husks, silk, tassels and leaves. These methodologies imply removing the plant from its natural environment which can affect the natural composition (in quantity and/or in quality) of the volatile collection.

In the search for attractants or deterrents of ECB females we have collected and analyzed different headspace volatile collections from whole undamaged maize plants and tested their electrophysiological (EAG) and behavioural activity in a Y-tube olfactometer on ECB females. The effect of different volatile compounds in females behaviour is also discussed.

Materials and Methods

Insects. Moths were selected from a colony maintained at the Institut de Recerca Agroalimentaria (IRTA, Lleida). The colony was started with mated females collected in different commercial maize fields from Lleida province (North-East Spain). Previous analyses of gland extracts of wild females from the area pointed out to the major presence of the Z strain of the moth (Riba *et al.* 2005). For mating, wild couples were transferred to plastic cages (17 cm diameter x 11,5 cm height) containing a waxed paper around the walls to allow females to lay eggs. After selection of the Z progenies, the Z strain was reared according to a typical artificial diet for Noctuidae (Poitout and Bues, 1974) to which the following antibiotics were added: nipagin (methyl-4-hydroxybenzoate) (0,12%), flumidil (potassium o-oxyquinolinsulfonate + sulfonylamidotiazol) (0,12%) and aureomicin (chlortetracycline hydrochloride) (0,04%). Pupae were sexed and placed in cylindrical boxes (17 cm diameter, 12 cm high) at $25 \pm 1^\circ\text{C}$ and $65 \pm 10\%$ relative humidity with a 16:8 L:D photoperiod. Upon emergence, adults were separated daily and kept on filter paper in plastic containers in the presence of a 10% sucrose solution.

Maize seedlings. Maize seeds (*Zea mays* L.) cultivar Dracma®, (Syngenta. Seeds, Basel, Switzerland) were planted in regular potting soil (Klassman – Deilmann, Geeste, Germany) in 16 cm (diameter) x 14.5 cm (deep) ceramic pots. The plants were kept in a greenhouse at 25°C , 70% relative humidity, and 16:8 L:D regime (lights on at 6 a.m.). The light intensity at daytime was 25,000 lux. Fifteen-twenty days after planting, the seedlings were used for experiments when they had five leaves.

Table 1. Common name, chemical name, molecular formula, molecular weight, chemical suppliers, and purity of the volatile compounds used in EAG and/or olfactometer bioassays.

common name	chemical name	molecular formula	molecular weight	chemical supplier	purity (%)
cyclopentanone	cyclopentanone	C ₅ H ₈ O	84.1	Fluka ¹	≥ 99
methylcyclopentanone	2-methylcyclopentanone	C ₆ H ₁₀ O	98.1	Acros ²	99
(E)-2-hexenal	(E)-2-hexenal	C ₆ H ₁₀ O	98.1	Sigma ³	98
(Z)-3-hexenol	(Z)-3-hexen-1-ol	C ₆ H ₁₂ O	100.2	Fluka ¹	98
2-hexanol	2-hexanol	C ₆ H ₁₄ O	102.2	Fluka ¹	≥ 98
3-hexanol	hexan-3-ol	C ₆ H ₁₄ O	102.2	Acros ²	99
styrene	vinyl benzene	C ₈ H ₈ O	104.1	Acros ²	99
benzaldehyde	benzoic aldehyde	C ₇ H ₆ O	106.1	Probus ⁴	99
cycloheptanol	cycloheptanol	C ₇ H ₁₄ O	114.2	Acros ²	97
heptanal	heptaldehyde	C ₇ H ₁₄ O	114.2	Acros ²	95
6-methyl-5-hepten-2-one	6-methyl-5-hepten-2-one	C ₈ H ₁₄ O	126.2	Merck ⁵	≥ 95
octanal	octaldehyde	C ₈ H ₁₆ O	128.2	Acros ²	99
2-ethyl-hexanol	2-ethylhexan-1-ol	C ₈ H ₁₈ O	130.2	Fluka ¹	≥ 99
3-methylbutyl acetate	3-methylbutyl acetate	C ₇ H ₁₄ O ₂	130.2	Synthesis ⁶	> 95
methyl hexanoate	methyl hexanoate	C ₇ H ₁₄ O ₂	130.2	Acros ²	98
terpinolene	4-isopropylidene-1-methylcyclohexene	C ₁₀ H ₁₆	136.2	Fluka ¹	≥ 97
α-terpinene	1-isopropyl-4-methyl-1,3-cyclohexadiene	C ₁₀ H ₁₆	136.2	Fluka ¹	≥ 95
β-myrcene	7-methyl-3-methylene-1,6-octadiene	C ₁₀ H ₁₆	136.2	Fluka ¹	≈ 90
β-ocimene	3,7-dimethyl-1,3,6-octatriene	C ₁₀ H ₁₆	136.2	Fluka ¹	≈ 70
β-pinene	6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane	C ₁₀ H ₁₆	136.2	Fluka ¹	99
γ-terpinene	1-isopropyl-4-methyl-1,4-cyclohexadiene	C ₁₀ H ₁₆	136.2	Fluka ¹	97
(R)-(+)-limonene	(R)-4-(isopropenyl)-1-methyl-1-cyclohexene	C ₁₀ H ₁₆	136.2	Merck ⁵	> 97
nonanal	nonaldehyde	C ₉ H ₁₈ O	142.2	Acros ²	95
(E)-2-hexenyl acetate	(E)-2-hexenyl acetate	C ₈ H ₁₄ O ₂	142.2	Acros ²	98
butyl butanoate	n-butyl butanoate	C ₈ H ₁₆ O ₂	144.2	Synthesis ⁶	> 95
hexyl acetate	n-hexyl acetate	C ₈ H ₁₆ O ₂	144.2	Synthesis ⁶	> 95
nonanol	nonan-1-ol	C ₉ H ₂₀ O	144.3	Acros ²	97
methyl salicylate	methyl 2-hydroxybenzoate	C ₈ H ₈ O ₃	152.1	Fluka ¹	≥ 99
2-	(1,1'-Bicyclopentyl)-2-one	C ₁₀ H ₁₆ O	152.2	Sigma ³	> 95
Cyclopentylcyclopentanone	3,7-dimethyl-1,6-octadien-3-ol	C ₁₀ H ₁₈ O	154.2	Fluka ¹	97
(±)-linalool	decaldehyde	C ₁₀ H ₂₀ O	156.3	Acros ²	95
(Z)-jasmone	(cis)-3-methyl-(2)-(2-pentenyl)-2-cyclopenten-1-one	C ₁₁ H ₁₆ O	166.2	Chemos ⁷	≈ 90
(Z)-3-hexenyl butanoate	(Z)-3-hexenyl butanoate	C ₁₀ H ₁₈ O ₂	170.2	Sigma ³	98
dodecane	n-dodecane	C ₁₂ H ₂₆	170.3	Acros ²	99
butyl hexanoate	n-butyl hexanoate	C ₁₀ H ₂₀ O ₂	172.3	Synthesis ⁶	> 95
hexyl butanoate	n-hexyl butanoate	C ₁₀ H ₂₀ O ₂	172.3	Synthesis ⁶	> 95
methyl nonanoate	methyl nonanoate	C ₁₀ H ₂₀ O ₂	172.3	Fluka ¹	≥ 98
dimethyl adipate	dimethyl hexadenoate	C ₈ H ₁₄ O ₄	174.2	Acros ²	≥ 99
tridecane	n-tridecane	C ₁₃ H ₂₈	184.4	Acros ²	≥ 99
dimethyl pimelate	dimethyl heptadenoate	C ₉ H ₁₆ O ₄	188.2	Fluka ¹	97
tetradecane	n-tetradecane	C ₁₄ H ₃₀	198.4	Acros ²	99
hexyl hexanoate	n-hexyl hexanoate	C ₁₂ H ₂₄ O ₂	200.3	Synthesis ⁶	> 95
(Z)-3-hexenyl benzoate	(Z)-3-hexenyl benzoate	C ₁₃ H ₁₆ O ₂	204.3	Sigma ³	97
β-farnesene	7,11-dimethyl-3-methylene-1,6,10-dodecatriene	C ₁₅ H ₂₄	204.3	Sigma ³	≥ 90
α-farnesene	(trans,trans)-3,7,11,trimethyl-1,3,6,10-dodecatetraene	C ₁₅ H ₂₄	204.3	Chemos ⁷	95
(-)-(E)-β-caryophyllene	trans-(1R,9S)-8-methylene-4,11,11-trimethylbicyclo[7.2.0]undec-4-ene	C ₁₅ H ₂₄	204.4	Fluka ¹	99
pentadecane	n-pentadecane	C ₁₅ H ₃₂	212.4	Acros ²	99
methyl laurate	methyl dodecanoate	C ₁₃ H ₂₆ O ₂	214.3	Acros ²	96

¹ Fluka Riedel-de Haën (Buchs SG, Switzerland)

² Acros Organics (Geel, Belgium)

³ Sigma - Aldrich Química (Madrid, Spain)

⁴ Probus (Badalona, Spain)

⁵ MERCK - Schuchardt (Darmstadt, Germany)

⁶ This compound was synthesized (yield > 70% after distillation) following the method of Eras et al. (2002).

⁷ Chemos (Regenstauf, Germany)

Chemicals and solvents. n-Hexane (analytical purity >95% GC, Fluka, Riedel-de Haën, Buchs SG, Switzerland) was used as solvent of all synthetic compounds. These were chosen to cover most of the major volatiles released by the plants and identified in this work and others from different *O. nubilalis* host plants like different poaceous plant species (Chamberlain et al., 2006), other maize varieties and sorghum (Birkett et al., 2006), alfalfa (Blackmer et al., 2004) even bell pepper and tomato (Fraser et al., 2003), and tested in electrophysiology and olfactometer bioassays. The main features of the compounds (purity, molecular formula, molecular weight and chemical suppliers) are listed in Table 1.

Volatile collections. A dynamic headspace system similar to that described by Bäckman et al. (2001) was used. A 80 cm large x 23 cm diameter glass cylinder contained a pot with two five leaves maize plants whose volatiles were to be analyzed. To avoid collect volatiles from the soil, the pot where the plants were seeded was carefully wrapped up with aluminum foil and tightly closed with a glass lid equipped with a metallic clamp. To create a laminar flow, a vacuum pump pushed air at 0.5 l/min through a stainless steel tube containing 1.3 g of activated charcoal (20/40 mesh, SKC Limited, Dorset, U.K.), into the cylinder through a glass port located at the top of the cylinder. Around the base of the cylinder, six glass ports with screw caps and Teflon sealed O-rings served as ports to hold the collection cartridges. Only one port was used for the experiments; the others were sealed. The collection cartridges consisted of stainless steel tubes (6.4 mm diameter x 89 mm long), previously conditioned with oxygen-free nitrogen at a flow of 75 ml/min at 335°C during 30 min in a TC-20TM multi-tube conditioner unit (Markes Int. LtdTM, Pontyclun, U.K.). The cartridges (volatile traps hereafter), containing 200–204 mg of Tenax TA as adsorbent, were connected by Teflon tubing with a second vacuum pump that simultaneously extracted air from the cylinder at 0.45 l/min.

Headspace volatiles were collected at $25 \pm 4^\circ\text{C}$ for 4 h at two different sampling periods: morning (starting at 10:00 a.m. and night (starting 2 h after dusk). Another test with stressed plants (7 days without irrigation) was performed in the morning conditions.

Volatile analysis. At the end of the collection time, volatile traps were sealed with Difflock™ caps (Markes Int. Ltd™, Pontyclun, U.K.), and directly analyzed (without solvent) in a desorption system (Unity, Markes Int. Ltd™, Pontyclun, U.K.) connected to a gas chromatograph–mass spectrometer (GC–MS) 6890N (Agilent Technologies, Palo Alto, CA) with a network quadrupole MS 5973. The thermal desorption conditions were as follows. The volatile traps were placed into the desorption port for 5 min at 300°C and the volatiles collected into a cold (-10°C) trap (60 mm long x 2 mm diameter) from which 40 mm was filled with Tenax TA and 20 mm with Carbpak™ B (60–80 mesh). The cold trap was held at -10°C throughout the desorption process (3 min) and then heated at $60^\circ\text{C}/\text{min}$ to 300°C . Chromatographic separation of the volatiles was performed on a DB-Wax capillary column (30 m x 0.25 mm x 0.25 μm) (J&W Scientific, Folsom, CA) using a split ratio of 1:5. The GC conditions were injection at 50°C for 2 min, program at $5^\circ\text{C}/\text{min}$ to 150°C , hold for 5 min, program at $10^\circ\text{C}/\text{min}$ to 230°C , and kept at this temperature for 10 min. The carrier gas was helium at a constant flow rate of 1.5 ml/min. The MS operated under electron impact (EI) conditions at 70 eV, with a scan range from 40 to 400 m/z at 4 scan/s. Temperatures of transfer line and ionization source were 280 and 230°C , respectively.

The mass spectra of the different compounds were compared with those from commercially available MS libraries (NIST library 75K and Wiley 275) and whenever possible with commercial synthetic compounds. The retention indexes and mass spectra were also compared to those reported in the literature (Adams, 1995; Yu et al., 2004; Zheng et al., 2004; Njoroge, 2005; Casado, 2006). Four to eight volatile collections and at least one blank sample per day time and for stressed plant were analyzed. The amounts of volatile compounds not present in blanks were estimated with regard to a known amount of a standard (50 ng of n-tetradecane) which had been added to each sample.

Comparison of the plant (regular or stressed, i.e. 7 days without irrigation) volatiles at different diurnal periods was performed by analysis of variance (ANOVA). Data were transformed to $\log(x+1)$ followed by Duncan's multiple range test in case of significance. The results are shown in table 2.

Electrophysiology. The 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 x 5 mm diameter). Test stimulations were performed by puffing air (300 mL/min) for 100 ms with a stimulus controller TC-05 (Syntech) through a Pasteur pipette, which was inserted onto a lateral branch of the tube for stimulations. The signals were amplified (100x) and filtered (DC to 1 kHz) with a ID-02 interface (Syntech), digitized on a PC and analyzed with the EAD 2.3 program.

The stimulus compounds (0.2 µmol, between 16.8 and 45.7 µg) were dissolved in hexane, deposited in a filter paper (20 x 5 mm) and the solvent allowed to evaporate. Linalool (0.2 µmol, 31 µg,) was used as standard. Excised antennae of 2-3 day-old virgin males or females were stimulated with 12 puffs, separated 40-60 s, in the following order: air (empty pipette), standard, hexane, three randomized test compounds, standard, three other randomized chemicals, hexane, and standard. A given compound was never tested more than once over the same antenna, and 10-12 different antennae were considered per each compound and sex. The response to the closest blank stimulus was subtracted from the response of the test compounds, and the corrected EAG values were analyzed using ANOVA followed by Duncan's multiple range test (Table 3).

Dual choice bioassay. A Y-tube olfactometer was devised to test the olfactory responses of 1-3 day old females to synthetic volatile compounds and to odours emitted by undamaged maize plants. The olfactometer consisted of a three main plexiglas tubes (34 cm long x 20 cm diameter). One of the three tubes was used as a main arm and the other two as a reception arms. Two other plexiglas tubes (34 cm long x 10 cm diameter) joined the two reception arms with the main arm with an angle of 45°. These dimensions offer the insects enough room to freely fly inside the olfactometer. Two round holes (4 cm diameter) at 30 cm from the base of the main arm were connected with two small extractors (3 cm diameter) (Shen Zhen Jie Leng Ind. Develop.®, Shanghai, China). Another round hole (4 cm diameter) at 30 cm from the base of each reception arm was connected to a fan (flow rate 5 cm/sec) so that the complete system permitted a laminar air flow within the three arms of 5 cm/sec. The olfactometer was located in a climate chamber at $25 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ relative humidity under 16:8 (L:D) photoperiod and was illuminated by 6x36 w parallel tubes of white light which provide a uniform light intensity of 2200 lux. The attraction source was 0.2 µmol (between 30 and 46 µg) of the

compound dissolved in nanograde hexane that was applied to a standard 8-mm rubber septum (Sigma-Aldrich, Tres Cantos, Madrid). The solvent was allowed to evaporate and the dispensers were then ready to use. For comparison purposes, we used two undamaged maize plants (three leaves) cultivar Dracma®, (Syngenta Seeds, Basel, Switzerland) as attractant and different synthetic volatile compounds. Two dispensers were placed inside the reception arms in front of the fans, one with the test compound and the other containing hexane (30 µg) as control. For each replicate, the position of the odour dispenser in the reception arms was exchanged to avoid a positional bias, and the order of treatments was randomized. A timer was used to switch on and off the fans and extractors. The device was cleaned with hexane after each session. All bioassays were performed for 10 h, from 1 h before the onset of the scotophase to 1 h after the onset of the photophase. Groups of at least 15 females per replicate were released into the initial arm and allowed to choose one odour source. After 24 h, the moths captured in the reception arm with hexane were recorded as “detected” whereas moths in the reception arm with the tested compound or maize leaves were recorded as “attracted” (Figure 1). The remaining moths were removed and classified as non-responders. The assays were run until a minimum of 20 insects responded to each compound with a minimum of three replicates per compound. The percentage of responses was calculated as follows:

$$\% \text{ attracted} = (\text{number of insects recorded as "attracted"}/\text{number insects responding}) \times 100$$

$$\% \text{ deterred} = (\text{number of insects recorded as "detected"}/\text{number insects responding}) \times 100$$

Statistical analysis was performed with a binomial test (Zar, 1996). The null hypothesis that females showed no preference for either olfactometer arm (a response equal to 50:50) was analyzed with a Binomial test.

Results and Discussion

Emission of Volatiles from Whole Maize Plants. Forty two compounds were detected in the volatile collections from undamaged and stressed maize plants (Table 2). The most abundant compounds from undamaged plants detected in higher amounts than the standard (tetradecane, 50 ng) were limonene, linalool, benzoic acid, indole, β -caryophyllene and acetophenone. Most of them were obtained in similar amounts regardless the diurnal periods except indole and linalool whose presence in the morning conditions was significantly higher than in the night conditions. In stressed plants, the most abundant chemicals were limonene, acetophenone, hexanoic acid, benzoic acid and indole, with the more remarkable differences with the undamaged plants being the significantly lower release of 1,8-cineole and linalool and the higher of hexanoic acid with regard to the plants in morning conditions. In addition, the stressed plants lack (E)-4,8-dimethyl-1,3,7-nonatriene, 6-methyl-5-hepten-2-one, anisole and 1-carvone which were present in different amounts in undamaged plants in either condition. (E)-2-hexenol was undetected in undamaged plants under morning conditions, and n-hexadecanol, (E)-2-hexenal and 2,4-hexadienal were only present in stressed plants although in very minor amounts.

With regard to the total amount of volatiles released, no significant differences were found between plants in morning and night conditions ($df = 1$, $F = 0.32$, $p = 0.594$) but the volatiles detected from emission of stressed plants were remarkably lower than that obtained from undamaged plants in the morning conditions ($df = 1$, $F = 13.72$, $p = 0.014$) (Table 2). No significant difference was noted between amount of volatiles released by undamaged plants at night and by stressed plants ($df = 1$, $F = 3.42$, $p = 0.114$). These results agree with those of Gouinguéné and Turlings (2002) who found that changes in different abiotic factors can cause small but significant changes in the relative ratios among the different compounds identified in induced odor blends in maize plants. These authors studied the effect of soil humidity, light intensity, temperature and fertilization and found that emission of volatiles did not occur in the dark and increased steadily with an increase of the light intensity. In addition, the relative amounts of linalool, geranyl acetate, β -caryophyllene and nerolidol showed a significant increase in their relative proportion when the light was on, although in contrast β -myrcene and (Z)-3-hexenyl acetate decreased with increases in light intensity (Gouinguéné and Turlings, 2002). In our case, whereas these latter two chemicals were

not amongst the most abundant, β -myrcene was preferentially released under morning conditions, although not significantly, and (Z)-3-hexenyl acetate was completely absent in night conditions.

Gouinguéné and Turlings (2002) also found that soil humidity had an effect on the emissions of the volatiles when the plants were treated with caterpillar (*Spodoptera littoralis*) (Boisd.) (Lepidoptera: Noctuidae) regurgitant, these emissions were lower as higher was the soil humidity, but soil humidity had no effect with untreated plants. In our case, in stressed plants (seven days without irrigation) the emissions were lower compared with undamaged plants in the morning conditions. These differences can be explained by the different soil humidity availability, Gouinguéné and Turlings used plants not watered in 18 hours, and we use extremely dry soil conditions (plants not watered during 7 days).

In other plant species, water stress appears to act in different ways, in one hand studies in lima-bean (*Phaseolus lunatus* L.) reveal that water stress is directly related to the release of volatiles: With less water available for the plant, elevated levels of volatiles are released from infested individuals relative to non-water stressed controls (Takabayashi et al., 1994). On the other hand, In Mediterranean conditions of drought (associated with water stress) emissions of volatile compounds can be reduced considerably. (Bertin and Staudt, 1996; Llusià and Peñuelas 1998, 2000). This decline are associated with the stomatal closure that finally cause a decrease in the permeability of cuticles to gas exchange (Bertin and Staudt, 1996; Llusià and Peñuelas 1998, 1999).

Other reports have noticed variation in the emission profile or total emission of volatiles in other plants between light and dark periods. This is the case in apple trees (*Malus domestica* Borkh.) (Casado et al., 2006), sorghum (Chamberlain et al., 2006), *Pinus pinea* (Staudt et al., 1997, 2000) but not in the slash pine *Pinus elliottii* Engelm., in which light had not direct effect in monoterpene emissions (Tingey et al., 1980).

Table 2. List of volatile compounds detected in headspace collections from two whole undamaged maize plants maintained in two different day periods (morning and night) and two stressed plants maintained in morning conditions^{a,b}.

Compound	Retention time	morning conditions	night conditions	stressed plants
2,4-hexadienal	2.38	-	-	0.05 ± 0.03
α-pinene	3.15	0.56 ± 0.15 a	0.61 ± 0.09 a	0.43 ± 0.15 a
butyl acetate	3.81	0.22 ± 0.22 a	0.51 ± 0.18 a	0.32 ± 0.13 a
β-pinene	4.34	0.2 ± 0.16 a	0.35 ± 0.14 a	0.04 ± 0.04 a
δ-3-carene	5.10	0.21 ± 0.03 a	0.21 ± 0.03 a	0.15 ± 0.03 a
beta-mircene	5.41	0.94 ± 0.30 a	0.67 ± 0.25 a	0.30 ± 0.10 a
limonene	6.09	4.11 ± 0.37 a	4.23 ± 1.57 a	2.49 ± 0.55 a
1,8-cineole	6.26	0.79 ± 0.06 a	0.78 ± 0.12 a	0.46 ± 0.09 b
(E)-2-hexenal	6.53	-	-	0.05 ± 0.02
cyclohexanone	8.14	0.07 ± 0.04 a	0.13 ± 0.01 a	0.14 ± 0.02 a
octanal	8.19	0.17 ± 0.08 a	0.15 ± 0.02 a	0.11 ± 0.01 a
(E)-4,8-dimethyl-1,3,7 nonatriene	8.70	0.55 ± 0.38 a	0.24 ± 0.21 a	-
Z-3-hexenyl acetate	8.90	0.27 ± 0.15 a	-	0.12 ± 0.12 a
6-methyl-5-hepten-3-one	9.40	0.13 ± 0.07 a	0.11 ± 0.07 a	-
anisole	9.56	0.09 ± 0.09 a	0.37 ± 0.24 a	-
(Z)-3-hexenol	10.48	0.14 ± 0.07 a	0.16 ± 0.10 a	0.44 ± 0.27 a
nonanal	10.82	0.65 ± 0.17 a	0.60 ± 0.09 a	0.50 ± 0.06 a
(E)-2-hexenol	10.96	-	0.15 ± 0.09 a	0.17 ± 0.12 a
tetradecane	11.68	1	1	1
unknown 1	13.23	0.28 ± 0.20 a	0.17 ± 0.10 a	0.19 ± 0.11 a
α-copaene	13.43	0.28 ± 0.14 a	0.27 ± 0.03 a	0.09 ± 0.05 a
decanal	13.50	0.77 ± 0.32 a	0.57 ± 0.09 a	0.50 ± 0.06 a
camphor	13.72	0.27 ± 0.02 a	0.20 ± 0.07 a	0.27 ± 0.03 a
benzaldehyde	14.08	0.77 ± 0.04 a	0.82 ± 0.20 a	0.79 ± 0.09 a
linalool	14.71	3.14 ± 0.93 a	0.75 ± 0.09 b	0.81 ± 0.47 b
unknown 2	14.98	-	-	0.19 ± 0.13
junipene	15.22	0.28 ± 0.14 a	0.39 ± 0.08 a	0.21 ± 0.07 a
β-caryophyllene	15.89	2.65 ± 1.21 a	2.98 ± 2.11 a	0.58 ± 0.21 a
menthol	16.86	0.50 ± 0.18 a	0.36 ± 0.06 a	0.42 ± 0.04 a
acetophenone	17.18	2.26 ± 0.70 a	2.54 ± 0.71 a	2.02 ± 0.15 a
α-humulene	17.58	0.43 ± 0.13 a	0.27 ± 0.18 a	0.16 ± 0.10 a
unknown 3	18.74	0.65 ± 0.43 a	0.24 ± 0.24 a	0.01 ± 0.01 a
1-carvone	19.07	0.16 ± 0.16 a	0.21 ± 0.13 a	-
unknown 4	19.69	0.61 ± 0.15 a	0.35 ± 0.14 a	0.18 ± 0.10 a
methyl salicylate	20.05	0.99 ± 0.13 a	0.99 ± 0.23 a	0.69 ± 0.17 a
hexanoic acid	21.76	0.29 ± 0.29 a	0.94 ± 0.36 ab	3.03 ± 1.54 b
hexadecanol	24.72	-	-	0.20 ± 0.13
caryophyllene oxyde	24.93	0.27 ± 0.14 a	0.19 ± 0.11 a	0.09 ± 0.09 a
octanoic acid	27.93	0.16 ± 0.10 a	0.20 ± 0.07 a	0.23 ± 0.08 a
nonanoic acid	30.43	0.55 ± 0.32 a	0.45 ± 0.26 a	0.46 ± 0.26 a
benzoic acid	34.34	4.00 ± 1.59 a	2.23 ± 1.00 a	1.72 ± 0.15 a
indole	34.52	4.36 ± 1.54 a	1.16 ± 0.36 b	1.38 ± 0.36 b
Total emission	-	32.87 ± 4.64 a	29.35 ± 5.17 ab	19.99 ± 0.95 b

^aAmounts of compounds were estimated with regard to 50 ng of tetradecane.

^bAnalysis of variance (ANOVA) was performed for every single compound. Data were transformed to log ($x + 1$) followed by Duncan's multiple range test was performed in case of significance. Values within the same row followed by the same letters are not significantly different ($\alpha = 0.05$).

Electroantennogram recordings with synthetic compounds. Mean EAG responses of the ECB males and females antenna of different synthetic compounds relative to the standard stimulus (50 µg (\pm)-linalool) ranged from 0.68 (cyclopentanone) to 1.11 (α -farnesene) in females and from 0.54 (methyl salicylate) to 2.25 (tridecane) in males (Table 3). The compounds that generated higher responses than the standard stimulus in males were pentadecane, tetradecane, tridecane and 2-ethylhexanol. Methyl salicylate displayed a similar response than the standard in females antenna, but also the lowest response of all tested compounds in males antenna. (Table 3). Damaged and stressed plants by chilling (Ding et al., 2002), NaCl and osmotic pressure (Borsani et al., 2001), drought (Munné-Bosch and Peñuelas, 2003) and heat (Clarke et al., 2004) have been shown to increase the release ratio of methyl salicylate. In addition and in dual choice tests, the ECB females were significantly attracted by volatiles of this chemical (see below, Figure 1). Our data in conjunction with those of the literature suggest that methyl salicylate can play a role in oviposition search and preference by ECB females. α -Farnesene, another compound that displayed an interesting EAG response in female antenna, has also been described to induce a dose-dependent contradictory effect in *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) females: at low concentrations (63.4 and 634 ng loaded on Silicon/Teflon septum) it acts as an attractant and a repellent at high concentrations (12.7 µg loaded on Silicon/Teflon septum) (Hern and Dorn, 1999).

In all cases the responses in absolute value of the male antenna to the synthetic compounds tested were higher than those of the female antenna. In the latter, most of the tested compounds generated depolarization responses that did not differ significantly from those of the standard stimulus, being cyclopentanone the only compound eliciting smaller response than the standard. In males, tridecane, tetradecane, dodecane, nonanal, decanal and 2-ethylhexanol elicited significantly high electrophysiological responses, whereas β -myrcene, hexane, styrene, β -pinene, cyclopentanone, γ -terpinene and methyl salicylate induced the smallest responses (table 3).

Table 3. Mean relative responses of male and female *Ostrinia nubilalis* antennae to different synthetic compounds relative to the standard stimulus (50 µg of (\pm)-linalool). Ten-twelve antennal recordings were done for each compound and sex^a.

Males			Females		
Compound	Response ± SE	Duncan groups	Compound	Response ± SE	Duncan groups
tridecane	2,25 ± 0,23	a	α -farnesene	1,11 ± 0,18	a
tetradecane	2,17 ± 0,23	ab	pentadecane	1,10 ± 0,16	ab
dodecane	1,96 ± 0,27	ab	2-ethyl-hexanol	1,08 ± 0,12	abc
nonanal	1,88 ± 0,23	bc	methyl salicylate	1,03 ± 0,13	abcd
decanal	1,61 ± 0,28	cd	tetradecane	1,01 ± 0,11	abcde
2-ethyl-hexanol	1,35 ± 0,25	de	tridecane	1,01 ± 0,14	abcde
(Z)-3-hexenol	1,21 ± 0,11	ef	(\pm)-linalool	1	abcdef
3-hexanol	1,11 ± 0,13	efg	terpinolene	0,98 ± 0,12	abcdeg
heptanal	1,06 ± 0,13	efgh	(E)-2-hexenal	0,95 ± 0,08	abcdeg
6-methyl-5-hepten-2-one	1,05 ± 0,14	efgh	(E)-2-hexenyl acetate	0,95 ± 0,06	abcdeg
pentadecane	1,04 ± 0,09	efghi	β -farnesene	0,95 ± 0,13	abcdeg
(\pm)-linalool	1	fghij	nonanal	0,95 ± 0,10	abcdegh
octanal	0,95 ± 0,07	fghijk	nonanol	0,94 ± 0,05	abcdegh
butyl butanoate	0,87 ± 0,04	ghijkl	(R)-(+)-limonene	0,93 ± 0,08	abcdegh
nonanol	0,86 ± 0,08	ghijklm	dodecane	0,92 ± 0,10	abcdegh
2-hexanol	0,86 ± 0,11	ghijklm	decanal	0,90 ± 0,13	abcdeghi
(E)-2-hexenyl acetate	0,83 ± 0,04	ghijklm	octanal	0,90 ± 0,07	abcdeghi
(E)-2-hexenal	0,82 ± 0,06	ghijklm	2-hexanol	0,88 ± 0,07	abcdeghi
benzaldehyde	0,77 ± 0,05	ghijklm	α -terpinene	0,85 ± 0,08	abcdeghi
(-)-(E)- β -caryophyllene	0,73 ± 0,09	hijklm	butyl butanoate	0,85 ± 0,05	abcdeghi
α -terpinene	0,71 ± 0,06	hijklm	(-)(E)- β -caryophyllene	0,84 ± 0,08	bcd
terpinolene	0,71 ± 0,09	hijklm	3-methylbutyl acetate	0,83 ± 0,07	cdeghi
(R)-(+)-limonene	0,69 ± 0,07	ijklm	β -pinene	0,83 ± 0,10	cdeghi
3-methylbutyl acetate	0,65 ± 0,11	jklm	heptanal	0,83 ± 0,07	cdeghi
β -myrcene	0,63 ± 0,05	klm	(Z)-3-hexenol	0,82 ± 0,06	cdeghi
hexane	0,62 ± 0,06	klm	3-hexanol	0,81 ± 0,07	defghi
styrene	0,61 ± 0,06	klm	6-methyl-5-hepten-2-one	0,81 ± 0,05	defghi
β -pinene	0,61 ± 0,11	klm	β -myrcene	0,80 ± 0,06	defghi
cyclopentanone	0,57 ± 0,05	lm	benzaldehyde	0,76 ± 0,07	defghi
γ -terpinene	0,55 ± 0,09	lm	styrene	0,75 ± 0,06	efghi
methyl salicylate	0,54 ± 0,07	lm	γ -terpinene	0,73 ± 0,03	fghi
air	0,50 ± 0,07	m	hexane	0,72 ± 0,06	ghi
			cyclopentanone	0,68 ± 0,06	hi
			air	0,65 ± 0,08	i

^aEAG corrected values were analyzed using ANOVA followed by Duncan's multiple range means test. Values within the same column followed by the same letters are not significantly different ($\alpha = 0,05$).

Interestingly, in both sexes the maximum EAG responses were elicited by some alkanes structurally similar in chain length to the major pheromone compound (Z11-14:Ac), such as tetradecane, tridecane, pentadecane, and dodecane. This is also in line with other reports in which epicuticular n-alkanes have been reported to elicit oviposition responses to ECB (Udayagiri and Mason, 1995, 1997). In males some volatile aldehydes (nonanal, decanal, heptanal) and alcohols (2-ethylhexanol, 3-hexanol

and (Z)-3-hexenol) elicited remarkable depolarizations on the antennae in agreement with similar effect reported by some of these compounds in *C. pomonella* (Ansebo et al., 2004; Vallat and Dorn, 2005; Casado et al., 2006), *C. partellus* and *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) (Birkett et al., 2006), *Lobesia botrana* (Den. & Schiff.) (Lepidoptera: Tortricidae) (Tasin et al., 2004) and *Manduca sexta* (L.) (Lepidoptera: Sphingidae) (Fraser et al., 2003). These compounds, generally known as *green leaf volatiles* (GLVs) are emitted by many plants including maize and other ECB non-host plants (Langenheim, 1994; Fall, 1999; Kesselmeier and Staudt, 1999) suggesting that host finding more often involves mixtures of odorants that are common to many plants (Fraser et al., 2003) and female attraction to the host is regulated by the ratios of these common plant volatiles rather than by the presence of species specific chemicals (Visser, 1986).

Olfactometer bioassays. To study *in vivo* the effect (attractant or deterrent) of host plant volatiles on ECB females olfactory tests in a dual-choice olfactometer were implemented.

In preliminary assays we noticed that ECB females were highly attracted to whole maize plants ($P = 0.00009$) (figure 1). When tested individually some compounds displayed significant attractant activity, such as dimethyl adipate ($P = 0.00027$), heptanal ($P = 0.0041$), 2-hexanol ($P = 0.0041$), methyl salicylate ($P = 0.0047$), methyl laurate ($P = 0.014$), nonanol ($P = 0.021$), hexyl acetate ($P = 0.026$), (E)-2-hexenyl acetate ($P = 0.033$) and β -pinene ($P = 0.047$) (figure 1). In contrast, some other compounds were found to be deterrent to ECB females. These were tetradecane ($P = 0.00024$), methyl hexanoate ($P = 0.003$), (\pm)-linalool ($P = 0.0036$), methyl nonanoate ($P = 0.017$), (Z)-3-hexenyl benzoate ($P = 0.018$), tridecane ($P = 0.020$), 3-methylbutyl acetate ($P = 0.029$), 2-cyclopentylcyclopentanone ($P = 0.035$) and (Z)-3-hexenyl butanoate ($P = 0.04$) (figure 1).

Two hydrocarbons (tridecane and tetradecane) that elicited good electroantennographic responses in female antenna appeared to be deterrents whereas some alcohols (like 2-hexanol and nonanol) with lower depolarization responses behaved as attractants. Linalool, the reference standard in EAG, was also a deterrent to ECB females. In contrast, compounds like 2-ethylhexanol and pentadecane that showed also good depolarization responses in females were not behaviourally active. On the other hand, compounds eliciting low EAG responses were found to act as remarkable

attractants (heptanal and β -pinene) or repellents (2-cyclopentylcyclopentanone) (figure 1).

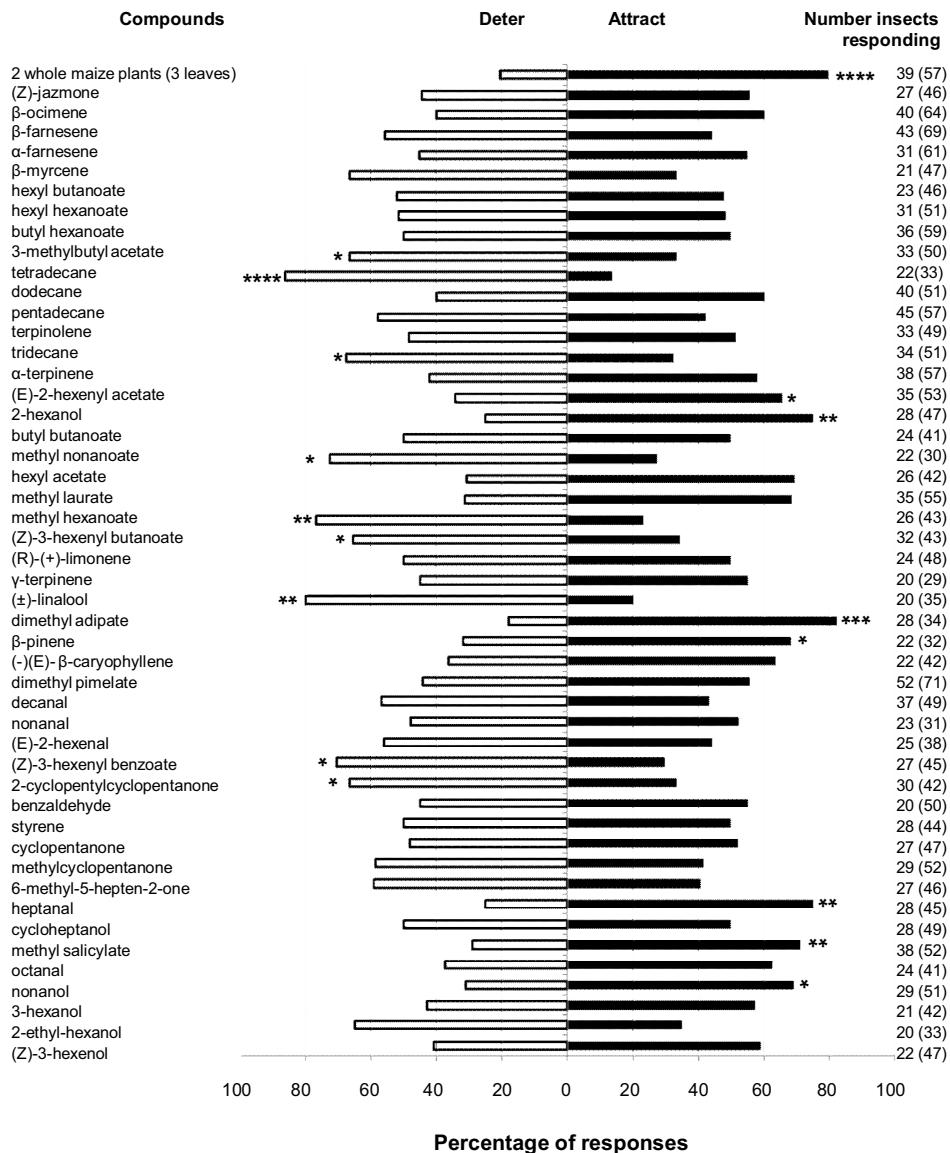


Figure 1. Percentage of responses of *Ostrinia nubilalis* females in dual choice tests to different synthetic volatile compounds (0.2 μ mol, 30–46 μ g) loaded in standard 8-mm rubber septa and to two undamaged maize plants (3 leaves) versus hexane (30 μ g). A binomial test was used for statistical analysis and asterisks by the bars indicate significant differences within a specific choice test (* $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$). In right column there are the number of insects responding and the total number of insects tested (in brackets).

From a structural point of view, no clear-cut conclusion could be drawn about a possible structure-activity relationship because whereas the attractants were mostly esters (5) followed by alcohols (2), aldehydes (1) and hydrocarbons (1), most of the repellents were also esters (5), followed by hydrocarbons (2), alcohols (1) and ketones (1).

Farnesene is a compound that has been cited to act as an attractant for the ECB females in oviposition tests (Binder et al., 1995; Binder and Robbins, 1997). In addition, α -farnesene, an alarm pheromone in aphids (Bowers et al., 1972), plays an important role as feeding stimulant on *C. pomonella* larvae and also as attractant of adults (Sutherland and Hutchins, 1973; Wearing and Hutchins, 1973; Coracini et al., 2004; Yang et al., 2005), and blended with E)-nerolidol and other maize odours also attracts the parasitic wasp *Cotesia marginiventris* (Hymenoptera: Braconidae) (Cresson) (Turlings et al., 1990). In our study, however, although α -farnesene displayed in females the highest EAG response among all the chemicals tested, it did not elicit (nor its β isomer) any effect in our behavioural assays.

Conclusions

Several compounds have been identified from the host and tested electrophysiologically and behaviourally on the ECB females. Some of them have shown an interesting attractant activity which may be important for host plant searching and oviposition preference. Although our work may represent a step forward in the identification of chemicals important for the orientation of the insect to the host, future work is needed to specifically determine whether these chemicals are real oviposition attractants and consequently their potential in future IPM programs.

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References

- ADAMS, R. P. 1995. Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publishing Corporation, Carol Stream. USA.
- ANSEBO, L., CORACINI, M. D. A., BENGTSSON, M., LIBLIKAS, I., RAMÍREZ, M., BORG-KARLSON, A. K., TASIN, M. and WITZGALL, P. 2004. Antennal and behavioral response of codling moth *Cydia pomonella* to plant volatiles. *J. Appl. Entomol.* 128: 488-493.
- BÄCKMAN, A. C., BENGTSSON, M., BORG-KARLSSON, A. K., LIBLIKAS, I. and WITZGALL, P. 2001. Volatiles from apple (*Malus domestica*) eliciting antennal responses in female codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae): effect of plant injury and sampling technique. *Z. Naturforsch. C*. 56:262-268.
- BERTIN, N. and STAUDT, M. 1996. Effect of water stress on monoterpenoid emissions from young potted holm oak (*Quercus ilex* L.) trees. *Oecologia* 107:456-462.
- BINDER, B. F. 1999. Chemical prospecting in maize for resistance to insect pests: The role of natural aldehydes in mediating oviposition of the European corn borer. *Recent Res. Dev. Agric. Food Chem.* 3:191-199.
- BINDER, B. F., ROBBINS, J. C. and WILSON, R. L. 1995. Chemically mediated ovipositional behaviors of the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *J. Chem. Ecol.* 21:1315-1327.
- BINDER, B. F. and ROBBINS, J. C. 1997. Effect of terpenoids and related compounds on the oviposition behavior of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *J. Agric. Food Chem.* 45: 980-984.
- BIRKETT, M. A., CHAMBERLAIN, K., KHAN, Z. R., PIKETT, J. A., TOSHOVA, T., WADHAMS, L. J. and WOODCOCK, C. M. 2006. Electrophysiological responses of the lepidopterous stemborers *Chillo partellus* and *Busseola fusca* to volatiles from wild and cultivated host plants. *J. Chem. Ecol.* 32:2475-2487.
- BLACKMER, J. L., RODRIGUEZ - SAONA, C., BYERS, J. A., SHOPE, K. L. and SMITH, J. P. 2004. Behavioral response of *Lygus hesperus* to conspecifics and headspace volatiles of alfalfa in a Y-tube olfactometer. *J. Chem. Ecol.* 30:1547-1564.
- BORSANI, O., VALPUESTA, V. and BOTELLA, M. A. 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiol.* 126:1024-1030.
- BOWERS, W. S., NAULT, L. R., WEBB, R. E. and DUTKY, S. R. 1972. Aphid alarm pheromone: isolation, identification, synthesis. *Science* 177:1121-1122.
- CASADO, D., GEMENO, C., AVILLA, J., and RIBA, M. 2006. Day-night and phenological variation of apple tree volatiles and electroantennogram responses in *Cydia pomonella* (Lepidoptera: Tortricidae). *Environ. Entomol.* 35: 258-267.
- CHAMBERLAIN, K., KHAN, Z. R., PICKETT, J. A., TOSHOVA, T. and WADHAMS, L. J. 2006. Diel periodicity in the production of green leaf volatiles by wild and cultivated host plants of stemborer moths, *Chillo partellus* and *Busseola fusca*. *J. Chem. Ecol.* 32:565-577.
- CLARKE, S. M., MUR, L. A . J., WOOD, J. E. and SCOTT, I. M. 2004. Salicylic acid dependent signaling promotes basal thermotolerance but is not essential for acquired thermotolerance in *Arabidopsis thaliana*. *Plant J.* 38:432-447.
- CORACINI, M. D. A., BENGTSSON, M. and WITZGALL, P. 2004. Attraction of codling moth males *Cydia pomonella* to apple volatiles. *Entomol. Exp. Appl.* 100: 1-10.
- DERRIDJ, S., FIALA, V. and JOLIVET, E. 1986. Increase of European corn borer (*Ostrinia nubilalis*) oviposition induced by a treatment of maize plants with maleic hydrazide: Role of leaf carbohydrate content. *Entomol. Exp. Appl.* 41: 305-310.
- DING, C. K., WANG, C. Y., CROSS, K. C. and SMITH D. L. 2002. Reduction of chilling injury and transcript accumulation of heat shock proteins in tomato fruit by methyl jasmonate and methyl salicylate. *Plant Sci.* 161:1153-1159.
- DITTRICK, L. E., JONES, R. L. and CHIANG, H. C. 1983. An oviposition deterrent for the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), extracted from larval frass. *J. Insect Physiol.* 29:119-121.
- DUDAREVA, N., PICHERSKY, E. and GERSHENZON, J. 2004. Biochemistry of plant volatiles. *Plant Physiol.* 135:1893-1902.
- ERAS, J. MÉNDEZ, J. J., BALCELLS, M. and CANELA, R. 2002. Chlorotrimethylsilane: a suitable reagent for synthesis of chlorhydrin esters. *J. Org. Chem.* 67:8631-8634.

- FALL, R. 1999. Biogenic emissions of volatile organic compounds from higher plants. In: Hewitt, C.N. (ed.): Reactive hydrocarbons in the atmosphere. Pp. 41-96. Academic Press, San Diego, California, USA.
- FEENY, P. 1992. The evolution of chemical ecology: Contribution from the study of herbivorous insects, pp. 1-44, in G. A. Rosenthal and M. R. Berenbaum (eds.). Ecological and Evolutionary Processes, Vol. II: Herbivores: Their Interaction with Secondary Metabolites. Academic Press, San Diego, CA.
- FRASER, A. M., MECHABER, W. L. and HILDEBRAND, J. G. 2003. Electroantennographic and behavioral responses of the sphinx moth *Manduca sexta* to host plant headspace volatiles. *J. Chem. Ecol.* 29:1813-1833.
- GLOVER, T. J., TANG, X.-H. and ROELOFS, W. L. 1987. Sex pheromone blend discrimination by male moths from E and Z strains of European corn borer. *J. Chem. Ecol.* 13:143-151.
- GOUINGUENÉ, S. P. and TURLINGS, T. 2002. The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiol.* 129: 1296 - 1307.
- HERN, A., and DORN, S. 1999. Sexual dimorphism in the olfactory orientation of adult *Cydia pomonella* in response to α - farnesene. *Entomol. Exp. Appl.* 92: 63-72.
- HONDA, K. 1995. Chemical basis of differential oviposition by lepidopterous insects. *Arch. Ins. Biochem. Physiol.* 30: 1 - 23.
- HUANG, X. and RENWICK, J. A. A. 1993. Differential selection of host plants by two *Pieris* species: The role of oviposition stimulants and deterrents. *Entomol. Exp. Appl.* 68: 59-69.
- HUBER, F. K., KAISER, R., SAUTER, F. and SCHIESTL, P. 2005. Floral scent emission and pollinator attraction in two species of *Gymnadenia* (Orchidaceae). *Oecologia* 142: 564-575.
- KESSELMEIER, J. and STAUDT, M. 1999 Biogenic Volatile Organic Compounds (VOC): An overview on emission, physiology and ecology. *J. Atmos. Chem.* 33: 23-88.
- KLUN, J. A., CHAPMAN, O. L., MATTES, K. C., WOJTKOWSKI, P. W., BEROZA, M. and SONNET, P. E. 1973. Insect sex pheromones: Minor amount of opposite geometrical isomer critical to attraction. *Science* 181:661-663.
- KONSTANTOPOULOU, M. A., KROKOS, F. D. and MAZOMENOS, B. E. 2002. Chemical stimuli from corn plants affect host selection and oviposition behavior of *Sesamia nonagrioides* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 95:1289-1293.
- KONSTANTOPOULOU, M. A., KROKOS, F. D. and MAZOMENOS, B. E. 2004. Chemical composition of corn leaf essential oils and their role in the oviposition behavior of *Sesamia nonagrioides* females. *J. Chem. Ecol.* 11:2243-2256.
- LANGENHEIM, J. H. 1994. Higher plant terpenoids: A phytocentric overview of their ecological roles. *J. Chem. Ecol.* 20:1223-1280.
- LLUSIÀ, J. and PEÑUELAS, J. 1998. Changes in terpene emission and content in potted Mediterranean woody plants under increasing drought. *Can. J. Bot.* 76:1366-1373.
- LLUSIÀ, J. and PEÑUELAS, J. 1999. *Pinus halepensis* and *Quercus ilex* terpene emission as affected by temperature and humidity. *Biol. Plant.* 42:317-320.
- LLUSIÀ, J. and PEÑUELAS, J. 2000. Seasonal patterns of terpene content and emission rate from seven Mediterranean woody species in field conditions. *Am. J. Bot.* 87:133-140.
- LUPOLI, R., MARION-POLL, F., PHAM-DELEGUE, M. H., and MASSON, C. 1990. Effect d'émissions volatiles de feuilles de maïs sur les préférences de ponte chez *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Comptes Rendus de l'Academie des Sciences (Serie 3) Sciences de la Vie* 311:225-230.
- MA, P. W. K. and ROELOFS, W. L. 1995. Sites of synthesis and release of PBAN-like factor in the female European corn borer, *Ostrinia nubilalis*. *J. Insect Physiol.* 41:339-350.
- MASON, C. E., RICE, M. E., CALVIN, D. D., VAN DUYN, J. W., HUTCHINSON, W. D., WITKOWSKI, J. F., HIGGINS, R. A., ONSTAD, D. W. and DIVELY, G. P. 1996. European corn borer ecology and management. Ames, Iowa: North Central Regional Extension Publication N° 327, Iowa State University. 57 p.
- MILLER, J. R. and STRICKLER, K. L. 1984. Finding and accepting hosts plants, pp. 127-157, in J. B. Willam and R. T. Cardé (eds.). Chemical Ecology of Insects. Chapman and Hall, New York.
- MUNNÉ-BOSCH, S. and PEÑUELAS, J. 2003. Photo- and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. *Planta* 217:758-766.
- NIELSEN, J. K., JAKOBSEN, H. B., FRIIS, P., HANSEN, K., MØLLER, J. and OLSEN, C. E. 1995. Asynchronous rhythms in the emission of volatiles from *Hesperis matronalis* flowers. *Phytochemistry* 38: 847-851.

- NIINEMETS, U., LORETO, F. and REICHSTEIN, M. 2004. Physiological and Physiochemical controls on foliar volatile organic compound emissions. *Trends Plant Sci.* 9:180-186.
- NJOROGE, S. M., KOAZE, H., KARANJA, P. N. and SAWAMURA, M. 2005. Essential oil constituents of three varieties of Kenyan sweet oranges (*Citrus sinensis*). *Flavour Frag. J.* 20: 80-85.
- PEÑA, A., ARN, H., BUSER, H.-R., RAUSCHER, S., BIGLER, F., BRUNETTI, R., MAINI, S. and TÓTH, M. 1988. Sex pheromone of European corn borer, *Ostrinia nubilalis*: Polymorphism in various laboratory and field strains. *J. Chem. Ecol.* 14:1359-1366.
- POITOUT S, BUÉS R (1974) Élevage des chenilles de vingt-huit espèces de lépidoptères Noctuidae et deux espèces d'Arctiidae sur milieu artificiel simple. Particularités de l'élevage selon les espèces. *Ann Zool Ecol Anim* 6:431-441.
- RENWICK, J. A. A. and CHEW, F. S. 1994. Oviposition behaviour in Lepidoptera. *Annu. Rev. Entomol.* 39:377-400.
- RIBA, M., SANS, A., SOLÉ, J., MUÑOZ, L., BOSCH, M. P., ROSELL, G. and GUERRERO, A. 2005. Antagonism of pheromone response of *Ostrinia nubilalis* males and implications on behavior in the laboratory and in the field. *J. Agric. Food Chem.* 53:1158-1165.
- ROELOFS, W. L., GLOVER, T., TANG, X.-H., SRENG, I., ROBBINS, P., ECKENRODE, C., LÖFSTEDT, C. and HANSSON, B. O. 1987. Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. *Proc. Natl. Acad. Sci. USA* 84:7585-7589.
- STAEDLER, E. 1992. Behavioral responses of insects to plant secondary compounds, pp. 45-48, in G. A. Rosenthal and M. R. Berenbaum (eds.). *Ecological and Evolutionary Processes*, Vol. II: Herbivores: Their Interaction with Secondary Metabolites. Academic Press, San Diego, CA.
- STAUDT, M., BERTIN, N., HANSEN, U., SEUFERT, G., CICCIOLI, P., FOSTER, P., FRENZEL, B. and FUGIT, L. 1997. Seasonal and diurnal patterns of monoterpene emissions from *Pinus pinea* (L.) under field conditions. *Atmos. Environ.* 31: 145-135.
- STAUDT, M., BERTIN, N., FRENZEL, B. and SEUFERT, G. 2000. Seasonal variation in amount and composition of monoterpenes emitted by young *Pinus pinea* trees. Implications for emission modeling. *J. Atmos. Chem.* 35: 77-99.
- SUTHERLAND, O. R. W. and HUTCHINS, R. F. N. 1973. Attraction of newly hatched codling moth larvae (*Laspeyresia pomonella*) to synthetic stereo-isomers of farnesene. *J. Insect Physiol.* 19:723-727.
- TAKABAYASHI, J., DICKE, M. and POSTHUMUS, M. A. 1994. Volatile herbivore-induced terpenoids in plant-mite interactions. Variation caused by biotic and abiotic factors. *J. Chem. Ecol.* 20:1329-1354.
- TASIN, M., ANFORA, G., IORATTI, C., CARLIN, S., CRISTOFARO, A., SCHMIDT, M. B., VERSINI, G. and WITZGALL, P. 2005. Antennal and behavioral responses of grapevine moth *Lobesia botrana* females to volatiles from grapevine. *J. Chem. Ecol.* 31:77-87.
- TINGEY, D.T., MANNING, M., GROTHAUS, L.C., BURNS, W.F. 1980. Influence of light and temperature on monoterpene emission rates from slash pine. *Plant Physiol.* 65:797-801.
- TURLINGS, T. C. J., TUMLINSON, J. H. and LEWIS, W. J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251-1253.
- UDAYAGIRI, S. and MASON, C. E. 1995. Host plant constituents as oviposition stimulants for a generalist herbivore: European corn borer. *Entomol. Exp. Appl.* 76: 59-65.
- UDAYAGIRI, S. and MASON, C. E. 1997. Epicuticular wax chemicals in *Zea mays* influence oviposition in *Ostrinia nubilalis*. *J. Chem. Ecol.* 23:1675-1687.
- VALLAT, A. and DORN, S. 2005. Changes in volatile emissions from apple trees and associated response of adult female codling moths over the fruit growing season. *J. Agric. Food Chem.* 53: 4083-4090.
- VALLAT, A., HAINAN, G., and DORN, S. 2005. How rainfall, relative humidity and temperature influence volatile emissions from apple trees in situ. *Phytochemistry* 66: 1540-1550.
- VARSHNEY, A. K., BADU, B. R., SINGH, A. K., AGARWAL, H. C. and JAIN, S. C. 2003. Ovipositional responses of *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) to natural products from leaves of two maize (*Zea mays* L.) cultivars. *J. Agric. Food Chem.* 51: 4008-4012.
- VISSER, J. H. 1986. Host odour perception in phytophagous insects. *Annu. Rev. Entomol.* 31:121-144.
- WEARING, C. H. and HUTCHINS, R. F. N. 1973. α -Farnesene, a naturally occurring oviposition stimulant for the codling moth, *Laspeyresia pomonella*. *J. Insect Physiol.* 19:1251-1256.
- YANG, Z., CASADO, D., IORATTI, C., BENGTSSON, M. and WITZGALL, P. 2005. Effect of pheromone pre-exposure and mating on attraction of codling moth *Cydia pomonella* to host volatiles. *Agric. Forest Entomol.* 7:1-6.

- YU, E. J., KIM, T. H., KIM, K. H. and LEE, H. J. 2004. Characterization of aroma-active compounds of *Abies nephrolepis* (Khinga fir) needles using aroma extract dilution analysis. *Flavour Frag. J.* 19: 74-79.
- ZAR, J. H. 1996. Biostatistical Analysis. Prentice Hall, New Jersey.
- ZHENG, C. H., KIM, T. H., KIM, K. H., LEEM Y. H. and LEE, H. J. 2004. Characterization of potent aroma compounds in *Chrysanthemum coronarium* L. (Garland) using aroma extract dilution analysis. *Flavour Frag. J.* 19: 401-405.
- ZHU, J. W., ZHAO, C. H., LU, F., BENGTSSON, M. and LÖFSTEDT, C. 1995. Reductase specificity and the ratio regulation of E/Z isomers in pheromone biosynthesis of the European corn borer, *Ostrinia nubilalis* (Lepidoptera. Pyralidae). *Insect Biochem. Mol. Biol.* 26:171-176.

Principals resultats i discussió general

1. Principals resultats i discussió general

La resposta d'insectes a l'acció de la feromona pot ser interrompuda o bé modificada per compostos inhibidors, també coneguts com a antagonistes. Alguns d'aquests compostos són d'origen natural (Leal, 1996), mentre que altres poden ser compostos sintètics (Renou i Guerrero, 2000).

Els compostos inhibidors d'origen natural normalment estan produïts per altres espècies d'insectes, relacionades o no amb les primeres i que poden compartir un mateix habitat (Lopez et al., 1990; Leal, 1996; Mustaparta, 1997; Potting et al., 1999; Eizaguirre et al., 2002; Cardé i Haynes, 2004; Gemenó et al., 2006; Eizaguirre et al., 2007). Petites quantitats d'aquests compostos, quan es troben en la mescla feromonal, redueixen la probabilitat que els mascles responguin a la feromona emesa per la/les femella/es de la seva pròpia espècie. Aquest efecte que es relaciona amb mecanismes d'isolació reproductiva (Löfsted, 1993), podria ésser usat en futures estratègies de control integrat de plagues.

Per altra banda, els inhibidors sintètics són compostos que mimetitzen l'estructura d'alguns dels components feromonals en els quals s'ha practicat substitucions en algun dels seus grups funcionals de forma que el compost resultant interfereix a nivell de receptor o inhibint els enzims del catabolisme de la feromona, fet que es tradueix en una interferència en el procés de percepció d'aquesta (Renou i Guerrero, 2000).

Tant amb els inhibidors d'origen natural com amb els sintètics, el resultat final sol ser força semblant: els mascles no percepren adequadament la feromona i tenen dificultats en localitzar les femelles de la seva pròpia espècie, per tant, els inhibidors sintètics també podrien ésser usats en estratègies de control integrat de plagues.

Capítol 1

Aprofitant l'ampli bagatge en l'estudi de diferents tipus d'inhibidors sintètics en diferents espècies de lepidòpters plaga que el nostre grup d'investigació atresora (Duran et al., 1993; Parrilla i Guerreiro, 1994; Riba et al., 1994; Rosell et al., 1996; Renou et al., 1997; Bau et al., 1999; Renou et al., 1999; Riba et al., 2001) i considerant els bons resultats obtinguts amb compostos inhibidors d'esterases (Durán et al., 1993; Rosell et al., 1996; Riba et al., 2001; Quero et al., 2003), es van considerar les TFMCs com a bons candidats per tal d'estudiar el seu comportament com a inhibidors de la resposta feromonal en mascles d'*O. nubilalis*.

En assaigs de túnel de vent, les TFMCs anàlogues al component majoritari de la feromona sexual d'*O. nubilalis* es comporten com a bons inhibidors en les últimes fases del vol quan són evaporades conjuntament amb la feromona a partir d'una concentració feromona:inhibidor de 1:5 en el cas de la Z11-14:TFMC i de 1:10 per la Z11-13:TFMC (taules 1 i 2, capítol 1). A més a més, aquest efecte disruptiu és dependent de la dosi.

Amb la idea de donar una major robustesa als resultats obtinguts en assaigs de túnel de vent i per tal de poder dilucidar el seu mecanisme d'acció, aquestes dues TFMCs (Z11-14:TFMC i Z10-13:TFMC) es van testar en assaigs d'inhibició d'esterases antenals. Els resultats obtinguts en aquests assaigs ($IC_{50} = 0.28 \mu M$ per a la Z11-14:TFMC i de $IC_{50} = 7.55 \mu M$ per a la Z10-13:TFMC) corroboren l'efecte inhibidor d'esterases d'aquests compostos (Linderman et al., 1988; Durán et al., 1993; Rosell et al., 1996; Riba et al., 2001).

El següent pas lògic per tal de considerar les TFMCs com a possibles compostos candidats per ser usats com a inhibidors de la resposta a la feromona fou assajar aquests compostos en condicions de camp. En aquests assaigs, el nombre de mascles capturats en trampes utilitzant mesclades d'aquests compostos amb la feromona, comparats amb les captures obtingudes en trampes únicament amb la feromona, mostren una reducció significativa d'aquestes en el cas de la Z11-14:TFMC (proporció feromona:inhibidor de 1:10) en assaigs realitzats l'any 2001 (figura 2, capítol 1). Altres TFMCs assajades no mostraren una reducció significativa en el nombre de captures en trampa (figura 2, capítol 1).

Altres assaigs realitzats l'any 2003 confirmen una reducció en el nombre de captures quan s'utilitza la Z11-14:TFMC a una proporció de feromona:inhibidor de 1:10. Si es disminueix la quantitat d'inhibidor fins a una proporció d'1:1 la disminució de captures deixa d'ésser estadísticament significativa (figura 3, capítol 1). A diferència dels assaigs en camp realitzats amb major pressió de plaga l'any 2001 (figura 2, capítol 1), en aquest cas, la Z10-13:TFMC també mostra una bona activitat inhibidora (figura 3, capítol 1).

En les tres tipologies d'assaigs realitzades s'evidencià que l'inhibidor estructuralment més semblant al component majoritari de la feromona sexual d'*O. nubilalis* exhibeix una major potència inhibitòria. En el cas d'assaigs d'inhibició d'esterases, aquesta diferència és de l'ordre de 10 cops superior quan es compara la Z11-14:TFMC amb la TFMC amb un carboni menys (Z10-13:TFMC). Aquests resultats estan en la línia de (Klun et al., 1991; Rosell et al., 1996; Quero et al., 2003) segons els quals es necessita una similitud estructural elevada entre l'anàleg i el component feromonal per tal d'obtenir un òptim grau d'inhibició.

La premissa de la similitud estructural entre anàleg i component feromonal també està suportada per altres estudis realitzats amb TFMCs en assaigs de túnel de vent en *S. littoralis* i en *S. nonagrioides* (Bau et al., 1999; Riba et al., 2001) i en assaigs de camp (Riba et al., 2001).

La presència del doble enllaç en la posició correcta i l'estequiometria de la molècula, similar a l'estructura de la feromona, són requisits indispensables per afectar al procés de reconeixement i transducció (Renou i Guerrero, 2000). En aquest sentit, el mecanisme d'acció d'aquestes TFMCs pot ésser atribuït no únicament a l'efecte inhibidor d'esterases presents en les antenes de mascles d'*O. nubilalis*, tal i com es demostra a partir dels resultats obtinguts, sinó que també aquesta similitud estructural amb la molècula de feromona produeix que aquests compostos siguin capaços d'unir-se a les *pheromone binding proteins* i ésser transportats a la limfa sensilar en competència amb les pròpies molècules de feromona tot interaccionant amb elsenzims que intervenen en el procés de catàlisis de la feromona (Feixas et al., 1995; Pophof et al., 2000) i inclús produir una sobreestimulació i una adaptació de les cèl·lules receptors de la feromona (Bau et al., 1999).

Aquest darrer extrem es veu suportat pel fet que diferents TFMCs també han mostrat una disminució de la resposta a la feromona en assaigs d'electrofisiologia en què aquesta (la feromona) és un alcohol o bé un aldehid (no pas un acetat com en el cas d'*O. nubilalis*) i que per tant les esterases de l'antena no tenen una gran importància en la seva degradació (Renou et al., 1997).

Capítol 2

En base a aquests bons resultats obtinguts amb les TFMCs en assaigs de laboratori i també en captures en trampes en assaigs de camp (capítol 1), es decideix fer un pas més en l'estudi de la possible aplicació de les TFMCs com a inhibidors de la percepció feromonal en estratègies de control integrat de plagues, i es dissenyen assaigs de camp a gran escala durant tres anys en parcel·les comercials de panís, utilitzant la Z11-16:TFMC (TFMC anàloga al component majoritari de la feromona sexual de *S. nonagrioides*) per tal d'estudiar l'efecte de l'inhibidor sobre aquesta plaga en condicions reals d'aplicació en camp.

En aquests experiments, utilitzant una dosis de 80 g/ha d'inhibidor, s'ha observat una disminució significativa en el nombre de plantes atacades i en el nombre de larves per planta en les parcel·les tractades en comparació amb les no tractades, per les generacions realment danyoses (segona i tercera) de *S. nonagrioides* (taula 4, capítol 2). A més a més d'aquest efecte sobre *S. nonagrioides*, també s'ha observat una important i sorprenent disminució en el nombre de plantes atacades i també en el nombre de larves per planta d'*O. nubilalis* (taula 5, capítol 2), l'altra lepidòpter plaga del cultiu del blat de moro.

En assaigs de les mateixes característiques, però usant una dosi d'inhibidor força més baixa (50 g/ha), els resultats obtinguts són força més irregulars i poc concloents. La baixa població de plaga existent a les parcel·les seleccionades, podria explicar l'anomalia dels resultats (taules 6 i 7, capítol 2).

La importància d'aquests assaigs i els resultats obtinguts rau en el fet en què es demostra la possibilitat d'usar les TFMCs anàlogues a la feromona sexual de *S. nonagrioides* per al control d'aquesta espècie i també per al control de l'espècie simpàtrica *O. nubilalis*, alhora que s'estableix una dosi i una metodologia d'aplicació vàlida per a aquesta finalitat en condicions de camp i aplicable per a parcel·les comercials de blat de moro.

A més, el fet que la Z11-16:TFMC no només actuï sobre *S. nonagrioides*, sinó que també ho faci sobre *O. nubilalis* dóna força per a la utilització comercial d'aquest compost, ja que precisament aquestes dues espècies són les dues plagues principals del panís a l'àrea mediterrània (Anglade, 1972; Mason et al., 1996; Albajes et al., 2002). Aquest efecte pot ser degut al fet de l'existència d'un antagonisme feromonal mutu entre aquestes dues espècies (Gemenó et al., 2006; Eizaguirre et al., 2007). A més a més, la Z11-16:TFMC és un anàleg del (Z)-9-tetradecenil acetat (Z9-14:Ac) compartint amb aquest compost una estructura idèntica en la part hidrofòbica de la molècula, essent aquest compost, un potent antagonista de la feromona d'*O. nubilalis* tant en assaigs de laboratori (Glover et al., 1989) com en assaigs de camp (Struble et al., 1987).

Si bé és cert que la metodologia utilitzada en les experiències de camp és molt similar a la que s'utilitza en estratègies de confusió sexual (substituint en aquest cas, l'antagonista per la pròpia feromona de l'insecte) i que s'han citat resultats acceptables de control d'*O. nubilalis* utilitzant aquesta tècnica (Baker, 1999), també és cert que les poques experiències de confusió sexual realitzades enfront *S. nonagrioides* han obtingut resultats poc consistents i força variables amb eficàcies de control que varien de 0 a 90 % a Espanya, de 0 a 69 % a Grècia i de 66 a 82 % a França (Albajes et al., 2002). En aquest sentit, els assaigs de camp realitzats a gran escala constitueixen la primera experiència d'utilització d'un antagonista feromonal per al control de *S. nonagrioides* i els bons resultats obtinguts no només sobre la plaga objectiu sinó també enfront *O. nubilalis*, representen un important pas endavant per tal de poder utilitzar les TFMCs en el control de plagues.

Capítol 3

Un cop completat tot aquest procés d'assaigs amb les TFMCs (capítols 1 i 2) ens plantejarem la possibilitat d'estudiar altres possibles antagonistes feromonals d'*O. nubilalis* i aprofundir en els seus mecanismes d'acció. Els diversos assaigs realitzats prèviament amb les TFMCs està clar que poden servir com a pal de paller on fer pivotar la nova recerca, i com a tal, l'experiència i el coneixement adquirits amb les TFMCs es pot utilitzar com a base comparativa per a una nova fornada d'assaigs amb un altre tipus d'antagonista feromonal: les metilcetones (MCs).

Aquest nou compost assajat (Z11-14:MC), amb el qual prèviament no s'havia realitzat cap mena d'estudi encaminat a la possible utilització d'aquesta tipologia de productes en estratègies de control de plagues, presenta, tot i tenir una similitud estructural molt elevada amb la feromona d'*O. nubilalis*, una activitat electrofisiològica intrínseca molt menor que la pròpia feromona, però major que la TFMC anàloga (Z11-14:TFMC) (figura 1, capítol 3).

Quan la Z11-14:MC és aplicada directament sobre l'antena induceix una menor resposta electroantenogràfica, que és dependent de la dosi aplicada i un efecte inhibidor a dosis iguals o majors a 10 pg. Similars resultats s'obtenen amb la Z11-14:TFMC (figura 2, capítol 3).

Quan el compost (Z11-14:MC) és assajat en túnel de vent (en evaporació conjuntament amb la feromona a diferents proporcions) s'obtenen un resultats en consonància amb els comentats anteriorment, així, s'observa una disminució dependent de la dosis aplicada en els diversos comportaments de l'insecte estudiats, essent aquesta disminució estadísticament significativa a partir d'una proporció major o igual a 1:1 (taula 1, capítol 3). Els mateixos assaigs realitzats amb la Z11-14:TFMC mostren uns resultats similars, l'efecte inhibidor és dependent de la dosi i estadísticament significatiu a partir d'una proporció feromona:inhibidor de 1:5, però aquest efecte no es manifesta en tots els comportaments estudiats, sinó únicament en les últimes fases del vol (taula 1, capítol 3). En canvi, si s'utilitza l'antagonista Z11-14:MC, la desorientació ja es manifesta des d'un principi i l'insecte es troba desorientat en totes les fases del vol (taula 1 capítol 3).

En assaigs de captures de mascles en trampes de camp realitzats l'any 2004 s'observa una clara disminució de captures quan tant la Z11-14:TFMC com la Z11-14:MC són mesclades amb la feromona en una proporció feromona:inhibidor de 1:10 (figura 4, capítol 3). L'any següent es repeteixen els assaigs i es baixa la dosi feromona inhibidor a 1:5, observant una clara disminució de les captures en el cas dels dos inhibidors i les dues proporcions (figura 4, capítol 3). Si es continua baixant la dosi fins a una proporció 1:1 (any 2006) la disminució en el nombre de captures deixa de ser estadísticament significativa (figura 4, capítol 3), establint-se d'aquesta manera una proporció mínima feromona:inhibidor a partir de la qual ambdós productes presenten una inhibició en el nombre de captures (en aquest cas, proporció 1:5).

Aquest paral·lelisme observat entre ambdós inhibidors (Z11-14:TFMC i Z11-14:MC) es treu però, quan es comprova la capacitat inhibidora d'esterases dels dos compostos, així amb la Z11-14:TFMC s'obté una IC₅₀ de 70 nM i per tant es considera un potent inhibidor d'esterases, essent aquest el mecanisme d'acció proposat per aquest compost, mentre que amb la Z11-14:MC s'obté una IC₅₀ major a 100 µM i, per tant, aquest producte presenta una baixa o nula potència inhibidora d'esterases, per aquest motiu es pot descartar aquesta possibilitat com a mecanisme d'acció per la Z11-14:MC (capítol 3). Aquesta actuació diferencial sembla estar relacionada amb les diferències observades en analitzar els paràmetres i les característiques de vol en túnel de vent. Diferents paràmetres específics (temps total de vol, l'angle de trajectòria, velocitats, distància total recorreguda, etc.) es veuen alterats de forma desigual ja sigui en presència d'un o altre inhibidor (taula 2, capítol 1).

Tot i l'existència d'alguns estudis realitzats amb metilcetones en espècies diferents d'*O. nubilalis* (Albans et al., 1984) (*Heliothis virescens*) (Liljefors et al., 1984) (*Agrotis segetum*) (Parrilla i Guerrero, 1994) (*Thaumetopoea pityocampa*), no existeixen dades sobre el possible mecanisme d'acció a nivell de receptor de la feromona. Tot i això, degut a l'alta similitud estructural entre els dos compostos assajats (Z11-14:MC i Z11-14:TFMC), s'intueix que ambdós compostos poden tenir un mecanisme d'acció per inhibició competitiva, ja sigui amb les *pheromone binding proteins* o bé amb els propis receptors de la feromona, d'aquesta manera els compostos serien capaços de produir una adaptació i sobreestimulació de les cèl·lules receptors.

A més a més, en el cas de les TFMCs, a part d'aquest possible mecanisme d'acció, se li hauria de sumar la seva capacitat inhibidora d'esterases àmpliament descrita prèviament (Linderman et al., 1988; Durán et al., 1993; Rosell et al., 1996; Riba et al., 2001), que seria el mecanisme d'acció prioritari.

Els resultats obtinguts ressalten el fet que els dos compostos tenen una capacitat inhibitòria final similar, però per tal d'arribar a aquests resultats, els dos compostos utilitzen camins o vies diferents: la Z11-14:TFMC seria bàsicament un efectiu inhibidor d'esterases (IC₅₀ de 70 nM), per tant el fet que la desorientació del mascle es produeixi en les últimes fases del vol de l'insecte en túnel de vent, s'explicaria pel fet que les molècules d'inhibidor necessitarien un cert temps per arribar a la limfa sensilar i desactivar elsenzims esterasa, espai de temps en que encara no es produeix la desorientació efectiva. Mentre que la Z11-14:MC basaria el seu mode d'acció amb la seva similitud estructural amb la feromona i competiria amb aquesta pels receptors de la feromona obtenint una resposta a nivell de sistema nerviós central (Glover et al., 1989). D'aquesta forma la Z11-14:MC pot definir-se com un nou i potent antagonista del comportament feromonal en mascles d'*O. nubilalis*. A més a més, degut a la seva bona activitat *in vivo* i a altres característiques interessants com la seva facilitat de preparació fan que s'hagi

de tenir en consideració aquest compost en futures estratègies de control d'aquesta plaga basades en la utilització d'antagonistes de la feromona.

Capítol 4

La sèrie de resultats obtinguda amb els diferents tipus de compostos inhibidors de l'acció de la feromona posa de manifest que el procés de percepció de la feromona és força complicat i en moltes fases no del tot ben elucidat fins al moment. Per aquest motiu, l'estudi comparatiu dels paràmetres i les característiques de vol de l'insecte sotmès a l'acció de diferents tipus d'inhibidors (naturals i sintètics) i que aquests actuïn amb diferent mecanisme d'acció pot ser de gran importància, ja l'estudi de les característiques de vol fa possible aprofundir en el mecanisme d'acció d'aquests antagonistes. Amb aquest objectiu es plantegen una sèrie d'assaigs en túnel de vent utilitzant dos inhibidors sintètics amb diferent mecanisme d'acció (Z11-14:TFMC i Z11-14:MC) i un inhibidor natural, el (Z)-11-hexadecenal (Z11-16:Ald), que és percutit per una neurona olfactiva receptora (ORN) que al seu torn també respon a un altre inhibidor natural (Z9-14:Ac) (Gemenò et al., 2006; Linn Jr. et al., 2007) en masclles d'*O. nubilalis*.

Tant la Z11-14:TFMC com la Z11-14:MC (els dos inhibidors sintètics utilitzats en els assaigs) no han produït un canvi en l'angle de curs (intenció de vol de l'insecte), però l'inhibidor natural (Z11-16:Ald) ha ocasionat que els insectes volessin més directament que la resta de tractaments (figura 3, capítol 4). Tots els tractaments amb els inhibidors han provocat que els insectes tinguessin una major susceptibilitat a la deriva, essent aquesta especialment marcada en el cas del Z11-16:Ald (figura 3, capítol 4).

Pel que fa referència a les velocitats, quan els inhibidors són mesclats amb la feromona, aquests produeixen una disminució de la velocitat respecte a l'aire (*airspeed*) (velocitat objectiu a la qual l'insecte desitja volar), que es tradueix en una reducció de la velocitat respecte el terra (*groundspeed*) (velocitat a la que acaba volant l'insecte després de considerar l'aire en direcció contrària), en el cas de la Z11-14:MC i Z11-16:Ald, però no pel que fa referència a la Z11-14:TFMC.

Així, el vol dels insectes amb la presència del Z11-16:Ald es tradueix amb vols més directes a una menor velocitat i fortament desviats de les trajectòries inicials per acció del vent en comparació amb la feromona, essent aquest tractament (Z11-16:Ald) el què produeix una desviació més severa dels diferents paràmetres de vol. En contraposició, els vols dels insectes en presència de la Z11-14:TFMC i la Z11-14:MC no tenen la intencionalitat de volar més directes que la feromona, però la seva intenció de vol és més lenta i aquests són molt més susceptibles d'ésser desviats en comparació amb la feromona. Aquesta sèrie de diferències estan íntimament relacionades amb el seu mecanisme d'acció:

Per una banda, les molècules de la Z11-14:TFMC necessiten arribar als teixits olfactius i produir el seu efecte com a inhibidor d'esterases. A més a més, necessiten mantenir una concentració elevada de molècules d'inhibidor per tal de seguir desenvolupant el seu efecte disruptiu. És per aquest motiu, que la velocitat de resposta de l'inhibidor és més baixa que en el cas de l'inhibidor natural (Z11-16:Ald) i és precisament en les últimes fases de vol on es noten més clarament els seus efectes inhibidors, per exemple en el cas del percentatge de distància recorreguda en cada tram (figura 7, capítol 4). D'igual manera, els efectes disruptius del producte en els diferents tipus d'angles també són més evidents en els trams finals del vol (figura 5, capítol 4). A més, tot i la presència de l'inhibidor i els seus efectes, els insectes persisteixen en el seu vol (figura 4, capítol 4), efectes que poden estar provocats per la competència existent pels receptors de la feromona entre les pròpies molècules de feromona i molècules de l'inhibidor.

En el cas del Z11-16:Ald, el fet de tenir una neurona olfactiva receptora específica per aquest compost (Gemenó et al., 2006; Linn Jr. et al., 2007) connectada directament amb el cervell de l'insecte, explica que la resposta a la presència de l'inhibidor sigui molt més ràpida i el fet que les diferències observades ja siguin evidents en les primeres fases del vol de l'insecte. A més a més, aquest fet també explicaria que amb aquest compost es necessita una quantitat de producte molt menor (X1000) per produir efectes inhibidors globals similars als altres inhibidors sintètics utilitzats (Z11-14:TFMC i Z11-14:MC) (Riba et al., 2005; Gemenó et al., 2006; Solé et al., 2008).

Pel que fa referència a la Z11-14:MC, els resultats obtinguts són generalment intermedis entre la Z11-14:TFMC i el Z11-16:Ald, aquest fet juntament amb la poca o nul·la activitat inhibidora d'esterases d'aquest compost, avalarien que el mecanisme d'acció de la Z11-14:MC estaria relacionat amb la seva similitud estructural amb la feromona, i per tant competiria amb aquesta pels receptors olfactius, alhora que tampoc és de descartar que aquest compost pugui provocar respostes a nivell de sistema nerviós central.

Els resultats obtinguts indiquen que diferents tipus d'antagonistes, amb diferent mecanisme d'acció i que provoquen resultats de comportament similar, poden ésser estudiats a partir de les diferències que presenten en els seus paràmetres de vol. A més a més, els paràmetres de vol ens aporten valiosa informació que ens serveix per poder relacionar l'acció d'aquest o altres tipus d'antagonistes amb el seu mecanisme d'acció, essent aquesta una eina vàlida en l'estudi de nous compostos que puguin interferir en el procés de la percepció de la feromona.

Capítol 5

Seguint amb l'estudi dels mecanismes de percepció olfactiva i en la recerca de possibles compostos que puguin tenir cert interès en el maneig de plagues, també s'ha enfocat aquest objectiu des d'una perspectiva totalment diferent a la descrita i discutida fins al moment (basada en l'estudi d'antagonistes de la percepció feromonal).

Aquesta nova aproximació, està sustentada en l'estudi de la possible acció o influència de compostos orgànics volàtils emesos per plantes de panís o per altres espècies hoste d'*O. nubilalis*. Alguns d'aquests compostos podrien ésser utilitzats com a atraients de femelles. Aquesta via és molt interessant ja que si bé en el cas de masclles ja es disposa de feromones sexuals per tal de realitzar el seguiment de les poblacions d'aquesta plaga, en el cas de femelles no es disposen d'atraients específics i viables. En aquesta línia s'han realitzat i analitzat col·leccions de compostos volàtils provinents de plantes senceres de panís, alhora que s'ha testat en assaigs d'electroantenografia i en olfactòmetre una sèrie de compostos orgànics volàtils amb la intenció d'identificar aquells compostos amb certa activitat, ja sigui com a atraients o bé com a repel·lents en femelles d'*O. nubilalis*.

En l'anàlisi per GC-MS de col·leccions de compostos volàtils emesos per plantes senceres de blat de moro mantingudes en condicions de dia i de nit, i per plantes amb estrès hídrig es van detectar un total de 42 compostos (taula 2, capítol 5). Entre aquests els compostos més abundants han estat: limonè, linalol, àcid benzoic, indol, β -cariofilè i acetofenona (taula 2, capítol 5). La majoria dels compostos són emesos en quantitats similars independentment del període de dia assajat, a excepció de l'indol, el qual és alliberat preferiblement en condicions diürnes (taula 2, capítol 5). En les plantes que patien estrès hídrig, els compostos majoritaris van ésser: limonè, acetofenona, àcid hexanoic, àcid benzoic i indol (taula 2, capítol 5). Les principals diferències observades entre les plantes amb estrès hídrig i les altres plantes, van ser un major alliberament d'àcid hexanoic en les plantes estressades i una major presència de 1,8 cineol en les plantes no estressades (taula 2, capítol 5). En aquest sentit, és conegut que les plantes sotmeses a diverses condicions d'estrès (danys físics, atac de depredadors, etc.) poden alliberar nous compostos, que des del punt de vista ecològic s'interpreten com una resposta de defensa per part de la planta (Paré i Tumlinson, 1997a,b)

Per altra banda, la quantitat total de volàtils emesa per les plantes amb estrès hídrig va ser estadísticament inferior a la emesa per plantes no estressades en condicions diürnes (taula 2, capítol 5). Aquesta reducció pot estar associada amb un tancament estomàtic, el qual causa una disminució en la permeabilitat de les cutícules a l'intercanvi gasós (Bertin i Staudt, 1996; Llusia i Peñuelas 1998, 1999).

En els assaigs d'electrofisiologia es pot observar que la majoria de compostos produeixen resposta, tant en l'antena de mascles com en la de femelles d'*O. nubilalis* (taula 3, capítol 5). Les respostes més altes es donen amb pentadecà, tetradeçà, trideçà i 2-etilhexanol. Un altre compost a destacar és el salicilat de metil, el qual produeix una elevada despolarització en l'antena de femelles, mentre que en antenes de mascles aquest compost presenta la menor activitat de tot els compostos assajats (taula 3, capítol 5).

Si bé és cert que existeix una multitud de compostos que responen positivament en assaigs d'electrofisiologia, també és cert que aquest tipus de resposta no és sinònim que aquest mateix compost desencadeni o presenti una activitat de comportament específica a l'insecte. És per aquest motiu, per completar la informació obtinguda en els assaigs d'electrofisiologia, que aquests es completen amb assaigs de comportament de doble elecció en olfactòmetre.

A partir d'aquests assaigs es pot comprovar que compostos com el trideçà i el tetradeçà que presenten una bona resposta electroantenogràfica (taula 3, capítol 5) es comporten com a repel·lents en olfactòmetre (figura 1, capítol 5), mentre que compostos com el 2-hexanol i el nonanol que presenten una baixa despolarització en electroantenografia, es poden considerar com a atraients en funció dels resultats obtinguts en olfactòmetre (figura 1, capítol 5). Per altra banda, altres compostos com el 2-etilhexanol i el pentadecà, amb bona activitat electroantenogràfica, no tenen cap mena de resposta comportamental (figura 1, capítol 5). El cas oposat també és possible, així compostos amb poca resposta en assaigs d'electroantenografia són considerats com a interessants atraients (heptanal i β -pinè) o com a repel·lents (2-ciclopentilciclopantanona) (figura 1, capítol 5).

Tot i que els compostos emesos per les plantes de blat de moro són bàsicament els mateixos en les diferents condicions assajades (taula 2, capítol 5), es corrobora que canvis en diferents factors abiotòpics poden causar petits, però significatius canvis en les proporcions relatives dels diferents compostos identificats (Gouinguéné i Turlings, 2002).

Altres compostos específics com el salicilat de metil han obtingut bones respostes electroantengràfiques en antenes de femelles, però no en mascles (taula 3, capítol 5). A més a més, el fet de que en assaigs de doble elecció en olfactòmetre aquest compost s'hagi comportat com un atraient (figura 1, capítol 5) pot indicar que el salicilat de metil jugui un paper important en el fet de buscar i seleccionar llocs de posta per part de les femelles d'*O. nubilalis*.

Des d'un punt de vista estructural, no es poden extreure conclusions clares sobre relacions d'estructura-activitat, ja que els compostos considerats atraients en assaigs d'olfactòmetre, van ser majoritàriament èsters (5), seguits per alcohols (2), aldehids (1) i hidrocarburs (1), però la majoria dels compostos considerats repellents també van ésser èsters (5), hidrocarburs (2), alcohols (1) i cetones (1) (figura 1, capítol 5). En tot cas, es pot afirmar que els compostos de tipus èster tenen una major incidència comportamental ja que la majoria d'aquest tipus de compostos assajats presenten activitat biològica, ja sigui com a atraients o bé com a repellents en femelles d'*O. nubilalis* (figura 1, capítol 5).

Tot i que s'han identificat diferents compostos provinents de plantes hoste d'*O. nubilalis*, s'han assajat electrofisiològicament i testat en assaigs de comportament amb interessant activitat ja sigui com a atraients o repellents per part d'alguns d'ells o inclús com a compostos que puguin jugar un paper important en l'orientació i determinació de llocs de posta per part de femelles d'*O. nubilalis*, sembla clar que són necessaris futurs treballs en aquesta direcció per tal de confirmar si aquests compostos són realment atraients o repellents de posta i en conseqüència avaluar el seu potencial en estratègies de control d'aquesta plaga.

2. Referències

- ALBAJES R.; KONSTANTOPOULOU M.; ETCHEPARE O.; ELZAGUEIRRE M.; FRÉROT B.; SANS A.; KROKOS F.; AMELINE A.; MAZOMENOS B. (2002). Mating disruption of the corn borer *Sesamia nonagrioides* (lepidoptera: noctuidae) using spayable formulations of pheromones. *Crop prot.* **21**, 217-225.
- ALBANS, K. R.; BAKER, R.; JONES, O. T. (1984). Inhibition of response of *Heliothis virescens* to its natural pheromone by antipheromones. *Crop. Prot.* **3**, 501-506.
- ANGLADE, P. (1972). Les Sésamia. Entomologie Appliquée à l'Agriculture, Tome II. Lepidoptères (ed. by AS Balachowsky), pp. 1389-1401. Masson et Cie, Paris, France.
- BAKER, T. C. (1999). Sex pheromone mating disruption: A 'natural' for integrating with transgenic crops. Annual Meeting North Central Branch ESA; Des Moines, Iowa. USA.
- BAU, J.; MARTÍNEZ, D.; RENOU, M.; GUERRERO, A. (1999). Pheromone-triggered orientation flight of male moths can be disrupted by trifluoromethyl Ketones. *Chem. Senses* **24**, 473-480.
- BERTIN, N.; STAUDT, M. (1996). Effect of water stress on monoterpene emissions from young potted holm oak (*Quercus ilex* L.) trees. *Oecologia* **107**, 456-462.
- CARDÉ, R. T.; HAYNES, K. F. (2004). Structure of the pheromone communication channel in moths. Advances in Insect Chemical Ecology. (ed. by Cardé, R. T. i Miller, J. G) 283-323. Cambridge University Press, Cambridge, UK.
- DURÁN, I.; PARRILLA, A.; FEIXAS, J.; GUERRERO, A. (1993). Inhibition of antennal esterases of the Egyptian armyworm *Spodoptera littoralis* by trifluoromethyl ketones. *Bioorg. Med. Chem. Letters* **3**, 2593-2598.
- EIZAGUIRRE, M.; ALBAJES, R.; LÓPEZ, C.; SANS, A.; GEMENO, C. (2007). Inhibition of pheromone response in *Sesamia nonagrioides* by the pheromone of the sympatric corn borer, *Ostrinia nubilalis*. *Pest Manag. Sci.* **63**, 608-614.
- EIZAGUIRRE, M.; SANS, A.; LÓPEZ, C; ALBAJES, R. (2002). Effects of mating disruption against the Mediterranean corn borer, *Sesamia nonagrioides*, on the European corn borer *Ostrinia nubilalis*. *IOBC/WPRS Bull.* **25**, 59-68.
- FEIXAS, J.; PRESTWICH, G. D.; GUERRERO, A. (1995). Ligand specificity of pheromone-binding proteins of the processionary moth. *Eur. J. Biochem.* **234**, 521-526.
- GEMENO, C., SANS, A., LÓPEZ, C., ALBAJES, R., EIZAGUIRRE., M. (2006). Pheromone antagonism in the European corn borer moth *Ostrinia nubilalis*. *J. Chem. Ecol.* **32**, 1071-1084.
- GLOVER, T. J.; PEREZ, N.; ROELOFS, W. L. (1989). Comparative analysis of sex pheromone-response antagonists in three races of European corn borer. *J. Chem. Ecol.* **15**, 863-873.
- GOUINGUENÉ, S. P.; TURLINGS, T. (2002). The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiol.* **129**, 1296-1307.

- KLUN, J. A.; SCHWARZ, M.; UEBEL, E. C. (1991). European corn borer: Pheromonal catabolism and behavioural response to sex pheromone. *J. Chem. Ecol.* **17**, 317-334.
- LEAL, W. S. (1996). Chemical communication in scarab beetles: Reciprocal behavioral agonist-antagonist activities of chiral pheromones. *Proc. Natl. Acad. Sci. USA.* **93**, 12112-12115.
- LILJEFORS, T.; THELIN, B.; VAN DER PERS, J. N. C. (1984). Structure-activity relationships between stimulus molecule and response of a pheromone receptor cell in turnip moth, *Agrotis segetum*. Modification of the acetate group. *J. Chem. Ecol.* **10**, 1661-1675.
- LINDERMAN, R. J.; LEAZER, J.; ROE, R. M.; VENKATESH, K.; SELINSKY, B. S.; LONDON, R. E. (1988). ¹⁹F 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. *Pestic. Biochem. Physiol.* **31**, 187-194.
- LINN, Jr. C. E.; MUSTO, C. J.; ROELOFS, W. L. (2007). More rare males in *Ostrinia*: response of Asian corn borer to the sex pheromone of the European corn borer. *J. Chem. Ecol.* **33**, 199-212.
- LLUSIÀ, J.; PEÑUELAS, J. (1998). Changes in terpene emission and content in potted Mediterranean woody plants under increasing drought. *Can. J. Bot.* **76**, 1366-1373.
- LLUSIÀ, J.; PEÑUELAS, J. (1999). *Pinus halepensis* and *Quercus ilex* terpene emission as affected by temperature and humidity. *Biol. Plant.* **42**, 317-320.
- LÖFSTEDT, C. (1993). Moth pheromone genetics and evolution. *Philos. Trans. R. Soc. Lond. B* **340**, 167-177.
- LOPEZ, J. D.; SHAVER, T. N.; GOODENOUGH, J. L. (1990). Multispecies trapping of *Helicoverpa (Heliothis) zea*, *Spodoptera frugiperda*, *Pseudaletia unipuncta*, and *Agrotis ipsilon* (Lepidoptera: Noctuidae). J. Chem. Ecol. **12**, 3479-3491.
- MASON, C. E.; RICEN, M. E.; CALVIN, D. D.; VAN DUYN, J. W.; HUTCHINSON, W. D.; WITKOWSKI, J. F.; HIGGINS, R. A.; ONSTAD, D. W.; DIVELY, G. P. (1996) *European Corn Borer. Ecology and Management*. North Central Regional Extension Publication **327**, 57. Iowa State University: Ames, Iowa. USA.
- MUSTAPARTA, H. (1997). Olfactory coding mechanisms for pheromone and interspecific signal information in related species of moths. *Advances in Insect Chemical Ecology*. (ed. by Cardé, R. T. i Miller, J. G) 141-164. Chapman & Hall, New York. USA.
- PARÉ, P. W.; TUMLINSON, J. H. (1997a). De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant physiol.* **114**, 1161-1167.
- PARÉ, P. W.; TUMLINSON, J. H. (1997b). Induced synthesis of plant volatiles. *Nature* **385**, 30-31.
- PARRILLA, A.; GUERRERO, A. (1994). Trifluoromethyl ketones as inhibitors of the processionary moth sex pheromone. *Chem. Senses* **19**, 1-10.
- POPHOF, B.; GEBAUER, T.; ZIEGELBERGER, A. (2000). Decyl-thio-trifluoropropanone, a competitive inhibitor of moth pheromone receptors. *J. Comp. Physiol. A* **186**, 315-323.

- POTTING, R. P. J.; LÖSEL, P. M.; SCHERKENBECK, J. (1999). Spatial discrimination of pheromones and behavioural antagonists by the tortricid moths *Cydia pomonella* and *Adoxophyes orana*. *J. Comp. Physiol. A* **185**, 419-425.
- QUERO, C.; ROSELL, G.; JIMÉNEZ, O.; RODRÍGUEZ, S.; BOSCH, M. P.; GUERRERO, A. (2002). New fluorinated derivatives as esterase inhibitors. Synthesis, hydration and crossed specificity studies. *Biorg. & Medi. Chem.* **11**, 1047-1055.
- RENOU, M.; GUERRERO, A. (2000). Insect parapheromones in olfaction research and semiochemical-based pest control strategies. *Ann. Rev. Entomol.* **48**, 605-630.
- RENOU, M.; BERTHIER, A.; DESBARATS, L.; VAN DER PERS, J.; GUERRERO, A. (1999). Actographic analysis of the effect of an esterase inhibitor on male moth responses to sex pheromone. *Chem. Senses* **24**, 423-428.
- RENOU, M.; LUCAS, P.; MALO, E. A.; QUERO, C.; 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. (1994). Inhibition of pheromone action in *Sesamia nonagrioides* by haloacetate analogues. *Pest. Sci.* **41**, 97-103.
- RIBA, M.; SANS, A.; BAU, P.; GROLLEAU, G.; RENOU, M.; GUERRERO, A. (2001). Pheromone response inhibitors of the corn stalk borer *Sesamia nonagrioides*. Biological evaluation and toxicology. *J. Chem. Ecol.* **27**, 1879-1897.
- RIBA, M.; SANS, A.; SOLÉ, J.; MUÑOZ, L.; BOSCH, M. P.; ROSELL, G.; GUERRERO, A. (2005). Antagonism of pheromone response of *Ostrinia nubilalis* males and implications on behavior in the laboratory and in the field. *J. Agric. Food Chem.* **53**, 1158-1165.
- ROSELL, G.; HERRERO, S.; GUERRERO, A. (1996). New trifluoromethylketones as potent inhibitors of esterases. ¹⁹F NMR spectroscopy of transition state analog complexes and structure-activity relationships. *Biochem. Biophys. Res. Commun.* **226**, 287-292.
- SOLÉ, J.; SANS, A.; RIBA, M.; ROSSELL, G.; ROSA, E.; MUÑOZ, L.; BOSCH, M. P.; GUERRERO, A. (2008). Differential activity of non-fluorinated analogues of the European corn borer pheromone. *Chemoecology* **18**, 99-108.
- STRUBLE, D. L.; BYERS, J. R.; McLEOD, D. G. R.; AYRE, G. L. (1987). Sex pheromone components of an Alberta population of European corn borer, *Ostrinia nubilalis* (Hbn.) (Lepidoptera: Pyralidae). *Can. Entomol.* **119**, 291-299.

Conclusions

- Efecte d'algunes TFMCs sobre la inhibició de la percepció de la feromona en *O. nubilalis*.
 - La Z11-14:TFMC presenta un marcat efecte disruptiu del procés de percepció de la feromona en mascles d'*O. nubilalis*.
 - La Z10-13:TFMC també té efectes inhibidors del procés de la percepció de la feromona en mascles d'*O. nubilalis*, tot i que menor que la Z11-14:TFMC.
 - La trifluorometilcetona saturada (14:TFMC), la tetradeceniltrifluoropiruvamida (14:TFPAm) i la (Z)-11-2-tiotetradeceniltrifluorometilcetona (Z11-2S-14:TFMC) són compostos inactius com a inhibidors del procés de percepció de la feromona.
 - Tan sols les TFMCs que presenten una alta similitud estructural amb el component majoritari de la feromona sexual d'*O. nubilalis* poden actuar com a bons inhibidors *in vitro* d'esterases presents a les antenes de l'insecte i al mateix temps tenir activitat *in vivo* com a antagonistes del comportament dels mascles enfront a la feromona.
 - El mecanisme d'acció de les TFMCs és degut a la seva activitat com a inhibidors d'esterases combinada amb la interacció competitiva amb les molècules de feromona per als receptors olfactius antenals o per les *pheromone binding proteins*.
 - L'efecte disruptiu del procés de la percepció de la feromona per part de les TFMCs és dependent de la dosi.
- Utilització de la Z11-16:TFMC per al control dels barrinadors del blat de moro en parcel·les comercials de panís.
 - S'ha comprovat l'eficàcia d'utilitzar la Z11-16:TFMC a una dosi de 80 g/ha per tal de reduir els danys ocasionats per la plaga de *S. nonagrioides* en el cultiu de blat de moro.
 - Aquest mateix producte (Z11-16:TFMC) presenta també una elevada eficàcia per al control dels danys produïts per l'espècie simpàtrica *O. nubilalis*.
 - La cinètica d'alliberament de la Z11-16:TFMC en condicions de camp utilitzant els difusors "inhibitor SN DCF" de SEDQ, ha estat adequada per tal de mantenir una concentració d'inhibidor suficient que ha cobert completament la duració del vol de la segona generació de *S. nonagrioides* i de *O. nubilalis*.

- Altres tipus d'antagonistes feromonals (naturals i sintètics).
 - La Z11-14:MC inhibeix el procés de percepció de la feromona en mascles d'*O. nubilalis*.
 - La inhibició produïda per la Z11-14:MC no és deguda a una interacció amb les esterases de les antenes dels mascles d'*O. nubilalis*. Degut a la seva similitud estructural, aquest compost competeix amb la feromona per als receptors olfactius neuronals
 - Tant la Z11-14:TFMC, la Z11-14:MC i el Z11-16:Ald redueixen el percentatge de resposta a la feromona en túnel de vent, però mitjançant l'alteració de paràmetres de vol diferents.
 - L'alteració de diferents paràmetres de vol per part dels diferents inhibidors és deguda als diferents mecanismes d'acció per part d'aquests.
 - La Z11-14:MC i el Z11-16:Ald tenen una afectació sobre els paràmetres de vol des de l'inici d'aquest, mentre que la Z11-14:TFMC no produceix l'efecte inhibidor fins que l'insecte es troba proper a la font de feromona.
- Compostos orgànics volàtils provinents de plantes hoste d'*O. nubilalis*.
 - S'han identificat diferents compostos orgànics volàtils de plantes senceres de blat de moro.
 - La majoria de compostos han presentat activitat en electroantenografia tant en femelles com en mascles d'*O. nubilalis* i alguns d'aquests també han presentat activitat en bioassais de doble elecció en olfactòmetre.
 - Des d'un punt de vista estructural, no hi ha una relació clara entre estructura i activitat.

