Note

Inhibition of electrophysiological response to the pheromone of the fall armyworm, *Spodoptera frugiperda*

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In the search for new control methods for the fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae), we present the inhibition of the electroantennographic (EAG) response to the pheromone by 3-octylthio-1,1,1-trifluoropropan-2-one (OTFP). As a member of the trifluoromethyl ketones, OTFP is a potent inhibitor of serine esterases, particularly those of insect antennae. Exposure of antennal receptors to OTFP vapors resulted in a decreased amplitude and 2/3 repolarization time (2/3 RT) of the EAG response to the pheromone. The effect was reversible, with the amplitude and repolarization time being recovered upon suppression of the treatment. The non-fluorinated analogue 3-octylthiopropion-2-one (OTP) did not affect either the amplitude or the 2/3 RT of the EAG response to the pheromone, confirming the key role played by fluorine atoms in this type of chemical. These results may be the basis for new behavioral studies with the aim of considering OTFP in future management strategies of the FAW. © Pesticide Science Society of Japan

Keywords: *Spodoptera frugiperda*, sex pheromone, inhibition, OTFP, EAG.

Introduction

The fall armyworm (FAW), *Spodoptera frugiperda* (Smith), is a major pest of corn, rice, and forage grass in North America, Central America and parts of South America with the greatest damage occurring in the southern and eastern tropical states.¹⁾ This species is frequently treated with insecticides in spite of their harmful effects on human health and the environment and the insect having developed resistance to several insecticides in Mex-

ico.²⁾

The FAW sex pheromone has been studied by a number of authors.^{3–5)} Particularly useful has been the pheromone composition reported by Tumlinson *et al.* (1986) as a mixture of (Z)-9-tetradecen-1-yl acetate (Z9-14:Ac), (Z)-7-dodecen-1-yl acetate, (Z)-9-dodecen-1-yl acetate, and (Z)-11-hexadecen-1-yl acetate in 81:0.5:0.5:18 ratio. This formulation has been very effective in controlling populations of *S. frugiperda* in the USA and the Caribbean basin.³⁾ However, commercial sex pheromone lures from the US and Great Britain have given erratic results in experiments carried out in Mexico and Central America.^{6,7)} For this reason, new methods of control should be explored.

Male moths detect female pheromone components by olfactory receptor cells localized in long sensilla trichodea of the male antennae. After adsorption onto the cuticular surface of the antennae,⁸⁾ pheromones diffuse to the inner cuticular face of the sensory hairs through microscopic pores present in the cuticle of the hair shaft. To diffuse the pheromone into the sensillum lymph, the pheromone is bound to pheromone-binding proteins (PBPs)⁹⁾ and transported through the aqueous lymph to the dendritic membrane. Once bound to the receptor, a series of events not fully understood triggers the action potential of the sensory neurons in the brain.¹⁰⁾ A male-specific G protein-coupled olfactory receptor gene, *Bombyx mori* olfactory receptor 1 (BmOR-1), appears to encode a bombykol receptor.¹¹⁾ After pheromone stimulation, the sensory neuron should return to its original resting potential and this occurs by enzymatic degradation of the pheromone molecules, thereby preventing sensory adaptation.¹²⁾ Two types of antennal-specific odorant-degrading enzymes have been characterized, the esterase from *Antheraea polyphemus*,^{9,13)} and aldehyde oxidases from *Manduca sexta*,¹⁴⁾ *A. polyphemus* and *B. mori*.¹⁵⁾

Trifluoromethyl ketones (TFMKs) are potent inhibitors of a number of serine esterases and proteases, such as acetylcholinesterase, chymotrypsin, human liver carboxylesterase,¹⁶⁾ *etc.* In insects, these chemicals reversibly inhibit the pheromone-degrading esterases present in male olfactory tissues.^{13,17)} The activity of these compounds results from the unique features induced by fluorine, which closely mimics the steric volume of hydrogen at the enzyme receptor sites. In addition, the strong electronegativity of the halogen induces fluorinated ketones to form stable hydrates in aqueous solutions, forming a tetrahedral hemiacetal-type adduct with the active site of the enzyme.^{18,19)}

Inhibition of the enzymatic degradation of odorant molecules may lead to the disruption of pheromone reception, and therefore it may be considered in new future strategies for pest control.^{10,20)} 3-Octylthio-1,1,1-trifluoropropan-2-one (OTFP) is a potent esterase inhibitor which has been reported to alter pheromone detection in *Spodoptera littoralis* and to disrupt male flight to a pheromone source after being exposed to vapors of the chemical.²¹⁾ This compound also induced lower amplitude responses to the pheromone of *A. polyphemus* on two receptor cells

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tuned to (*E,Z*)-6,11-hexadecadienyl acetate, substrate for the sensillar esterase, and (*E,Z*)-6,11-hexadecadienal, not a substrate for the esterase.²²⁾ In this paper, we present our results of the inhibitory effect of OTFP on electrophysiological responses to pheromone esters in another important pest, *S. frugiperda*. The results are compared with those obtained with the non-fluorinated analogue 3-octylthiopropion-2-one (OTP).

Materials and Methods

1. Insects

Larvae of *S. frugiperda* were collected from maize (*Zea mays* L.) at "El Manzano" (Tapachula, Chiapas, Mexico), and brought to the laboratory for rearing on an artificial diet.²³⁾ Pupae were sexed, placed in groups of 20–25 in Petri dishes inside glass boxes (30×30×30 cm), and maintained in a climatic chamber on a 16L:8D photoperiod regime at 25±2°C and 60–70% RH until emergence. Adult insects were collected daily and provided with 10% sucrose solution.

2. Chemicals

OTFP and OTP were prepared by alkylation of 1-octanethiol with 3-bromo-1,1,1-trifluoropropan-2-one and chloroacetone, respectively. Linalool and (*Z*)-9-tetradecenyl acetate (Z9-14:Ac) were purchased from Fluka and Sigma/Aldrich (Toluca, State of Mexico), and their purity determined by gas chromatography was >97%.

3. Electroantennography

Antennal responses of males of *S. frugiperda* to the major sex pheromone component Z9-14:Ac were determined on an electroantennogram (EAG), as previously described.²⁴⁾ The reference electrode was inserted into the neck of 4-day-old males, which had been restrained on a Styrofoam block, and the recording electrode was connected to the tip of the antenna, from which the last subsegments had been previously excised. Both electrodes were filled with saline solution, as reported.²⁴⁾ The signals generated by the antenna were passed through a high-impedance amplifier (NL 1200; Syntech, Hilversum, The Netherlands) and displayed on a monitor equipped with signal processing software (NL 1200, version 2.6; Syntech). A stimulus flow controller (CS-05; Syntech) was used to generate stimuli at 1-min intervals. A flow of humidified pure air (0.7 l/min) was constantly directed onto the antenna through the main branch of a 10-mm diameter glass tube. Test solutions (10 µg/µl) of OTFP, OTP, linalool and Z9-14:Ac were prepared in HPLC-grade hexane. Pheromone stimulation was performed by a puff of air (1 sec, 0.5 l/min) through a Pasteur pipette connected to a lateral branch and containing filter paper (0.5×3.0 cm, Whatman, No. 1) loaded with 10 µg of the chemical. Stimuli were given at 2-min intervals. OTFP and OTP were independently applied by turning on a flux of air (0.1 l/min) passing through another Pasteur pipette containing a filter paper with 50 µg of the compound and connected to a second lateral branch of the glass tube. Three EAG responses to the pheromone were measured at 2-min intervals in pure air, and the average value was considered the response before treatment.

Then, the OTFP or OTP flux was turned on for 2 min and a series of 3 depolarizations was recorded (response during treatment). The airflow containing OTFP or OTP was turned off, and after 5 min of antennal recovery, 3 more stimulations with the pheromone were performed (response after treatment). The absolute EAG responses were normalized and expressed as percentage response relative to that to the standard stimuli (10 µg linalool). Six males were used for OTFP experiments and 5 males for OTP tests and, for each insect, three EAG depolarizations of each treatment (before, during and after) were recorded. We used only one antenna of each male. EAG recordings were stored on a PC microcomputer and analyzed to determine their maximum amplitude and the repolarization time at 2/3 of the baseline (2/3 RT). Both variables are shown in Fig. 1 and 2/3 RT was calculated considering the slope between the last point of the stationary phase time and complete recovery of the antenna equal to 3/3. The differences in EAG responses and 2/3 RT before, during and after treatment were analyzed by the matched-pairs *t*-test using the MINITAB statistical package.²⁵⁾

Results and Discussion

1. Preparation of OTFP and OTP

Both products were prepared as stated above in 64% and 68% yield, respectively. The compounds were fully characterized by IR, ¹H NMR, ¹³C NMR and mass spectrometry.

2. EAG results

From the EAG responses of each insect, we measured the amplitude and 2/3RT, a parameter that delineates the time recovery of the antenna (Fig. 1). Responses to the major pheromone component Z9-14:Ac showed a steep decline in the peak amplitude, followed by a fast return to a plateau which was stable during the stimulus time and slowly returned to the baseline (Fig. 1). As shown in Fig. 2, exposure of the antenna to OTFP-loaded air re-

Fig. 1. Typical shape of an EAG recording of a male antenna of *S. frugiperda* in response to 10 µg (*Z*)-9-tetradecenyl acetate with the measured parameters: EAG amplitude and time to reach 2/3 of repolarization time (2/3 RT). Vertical scale bar=0.5 mV, horizontal scale bar=1 sec.

Fig. 2. Traces of EAG recordings of male antennae of *S. frugiperda* to 10 µg (Z)-9-tetradecenyl acetate before, during and after exposure of the antenna to OTFP-loaded air. Vertical scale bar=0.5 mV, horizontal scale bar=1 sec.

sulted in a significant reduction of the signal amplitude and an increase of 2/3RT. Thus, the amplitude during exposure was 136.2 ± 12.2 vs. 175.7 ± 13.9 before treatment ($t=3.8$; $df=17$; $P<0.01$) and 168 ± 12.8 after treatment ($t=4.59$; $df=17$; $p<0.001$) (Table 1). Similarly, the effect of OTFP on the 2/3 RT was significantly increased from 787.4 ± 41 ms in pure air to

Fig. 3. Relationship between the EAG amplitude and 2/3 repolarization time in *S. frugiperda* measured in clean air (°) and OTFP (▲) loaded air in response to (Z)-9-tetradecenyl acetate.

1204.4 ± 39.6 msec in its presence ($t=9.5$; $df=17$; $p<0.001$) to return to 816.9 ± 56.6 msec after treatment ($t=7.3$; $df=17$; $p<0.001$). The effects of the EAG amplitude and 2/3 RT were fully reversible with both parameters returning to their approximate original values after treatment (Table 1).

The mean EAG amplitude to Z9-14: Ac during exposure of the antenna to vapors of the non-fluorinated compound OTP was not different from that obtained before or after treatment (statistical parameters during/before treatment: $t=0.09$; $df=14$; $p=0.93$; during/after treatment: $t=1.12$; $df=14$; $p=0.13$). Similarly, the 2/3 RT of the EAG responses to the major component of the pheromone during treatment was not significantly different to the values obtained during/before or during/after exposure to the chemical (statistical parameters during/before and during/after treatment: $t=1.26$; $df=14$; $p=0.22$ and $t=0.20$; $df=14$; $p=0.84$, respectively) (Table 1). The values of 2/3 RT were relatively independent of the EAG amplitude and for this reason it was not necessary to correct the time-parameter values as a function of the amplitude (Fig. 3).

The results obtained in this work showed that OTFP significantly affects the electrophysiological responses of *S. frugiperda* males to the major pheromone component both in amplitude and 2/3 RT of the signal. These effects are reversible, the correspon-

Table 1. Mean values of amplitude and 2/3 repolarization time of the EAG responses of male antennae of *S. frugiperda*, before, during and after exposure of the antennal receptors to vapors of OTFP and OTP.^{a),b)}

Stimulus	Parameter	Before	During OTFP	After
Z9-14: Ac	Amplitude (%)	175.7 ± 13.9	$136.2 \pm 12.2^*$	168 ± 12.8
	2/3 RT (msec)	787.4 ± 41.0	$1204.4 \pm 39.6^*$	816.9 ± 56.6
Z9-14: Ac	Before		During OTP	After
	Amplitude (%)	149.1 ± 15.1	156.8 ± 15.2	165.4 ± 11.6
	2/3 RT (msec)	474.7 ± 42.4	562.7 ± 79.1	578.7 ± 80.1

^{a)} Values are relative to those of the standard (linalool). ^{b)} Mean values $\pm$ SEM of 18 replicates from 6 insects in OTFP treatment, and 15 replicates from 5 insects in OTP treatment. Asterisk (*) indicates significant differences ($p<0.05$) in values during OTFP exposure relative to those before or after treatment.

ding initial values being almost completely recovered 5 min after treatment. In contrast, OTP did not alter either EAG parameter, showing once more that the presence of fluorine atoms in this type of compound is necessary for activity. Our results agree with previous reports in which OTFP and other TFMKs elicited a significant reduction of EAG pheromone responses in *S. littoralis*, *Mamestra brassicae*, and *Heliothis zea*.²¹⁾ Moreover, OTFP reversibly lowered the firing responses to the pheromone of *S. littoralis* by the pheromone receptor cell, and this effect resulted in complete inhibition in the presence of high doses of the chemical.²¹⁾ Similarly, OTFP and other isomers of different chain length reduced receptor potential amplitudes to two pheromone components ((*E,Z*)-6,11-hexadecadienyl acetate and (*E,Z*)-6,11-hexadecadienal) in two different receptor cell types of *A. polyphemus* and *Antheraea pernyi*.²²⁾ In this case, when TFMKs were applied after the pheromone at high concentrations, they rapidly inhibited both receptor cells. These results show the antagonistic action of OTFP at the pheromone receptors or PBPs. In this regard, it should be mentioned that (*Z*)-11-hexadecenyl trifluoromethyl ketone (Z11-16:TFMK) almost completely displaced Z11-16:Ac, the major component of the pheromone of *M. brassicae*, bound to a recombinant PBP1 when incubated before or after the pheromone component.²⁶⁾ Additionally, some TFMKs may be bound to PBPs present in the sensory hairs and transported to the sensillum lymph, in competition with pheromone molecules for interaction with pheromone catabolic esterases, as shown in the processionary moth *Thaumetopoea pityocampa*.²⁷⁾ In this context, OTFP displayed remarkable antiesterase activity on antennal extracts of *S. littoralis* ($IC_{50}=5.9\text{ }\mu\text{M}$)^{17,19)} and *Sesamia nonagrioides* ($IC_{50}=16.3\text{ }\mu\text{M}$)²⁸⁾ but not OTP which displayed poor antiesterase activity ($IC_{50}=73\text{ }\mu\text{M}$, unpublished data). The effect of OTFP and other TFMKs on the EAG repolarization time suggests that a decrease of pheromone deactivation because of esterase inhibition. However, since these chemicals also affect responses to pheromones with an alcohol or aldehyde function²¹⁾ which are not degraded by the esterase, other mechanisms of action of these compounds should also be considered.

In conclusion, we have shown that OTFP clearly affects the electrophysiological responses of *S. frugiperda* males to the pheromone; therefore, considering the “*in vivo*” disruption effects displayed in behavioral assays of other insects, such as *S. littoralis* and *Sesamia nonagrioides*,^{21,29)} the chemical may be a good candidate to test in the field in future strategies to control FAW.

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