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Activity of Octylthiotrifluoropropan-2-one, a Potent Esterase Inhibitor, on Growth, Development, and Intraspecific Communication in *Spodoptera littoralis* and *Sesamia nonagrioides*

GADI V. P. REDDY,† CARMEN QUERO, AND ANGEL GUERRERO*

Department of Biological Organic Chemistry, Institute of Chemical and Environmental Research (CSIC), Jordi Girona 18-26, 08034-Barcelona, Spain

A series of experiments were conducted to examine the effect of 3-octylthio-1,1,1-trifluoro-2-propanone (OTFP) on growth, development, and behavior of the cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), and the corn stalk borer, *Sesamia nonagrioides* (Lepidoptera: Noctuidae). The chemical behaved as an oviposition deterrent and, when added to the diet of the second-instar larvae of both insects, reduced diet consumption and growth, pupation, and adult emergence. Treatment of 100−5000 ng of the compound on fourth-instar larvae for 3−24 h, however, did not produce significant differences in the amount of diet ingested. Our results suggest that the effect of OTFP is long-lasting and that the inhibitor is not fully detoxified by the detoxification enzymes of the digestive tract of the insects. In behavioral assays, adult males which had been treated with the chemical at the larval stage were less attracted to the pheromone source than regular untreated males. When *Sp. littoralis* untreated females were used as the attractant source, treated males also displayed significantly fewer contacts with the cage-containing females than untreated or solvent-treated males. In the presence of treated females, only 27% of treated males successfully completed the flight in comparison to animals responding to solvent-treated females (54.5%). By contrast, when *Se. nonagrioides* females, whether they had been subjected or not to the treatment, were used as the attractant source, males were similarly attracted to them regardless of whether they had been treated or not at the larval stage. Analyses of gland extracts of *Sp. littoralis* treated females showed no difference from control insects in the qualitative or quantitative composition of the pheromone blend. The results obtained, in combination with other results previously reported by us (Riba, M.; Sans, A.; Bau, P.; Grolleau, G.; Renou, M.; Guerrero, A. *J. Chem. Ecol.* **2001**, *27*, 1879−1897), provide new and relevant information about the possible utility of these chemicals in future studies directed to the development of new approaches for pest control.

KEYWORDS: ***Spodoptera littoralis*; *Sesamia nonagrioides*; octylthiotrifluoropropan-2-one; OTFP; esterase inhibitor; growth; development; intraspecific communication; maize pests**

INTRODUCTION

It is known that insect development and reproduction are regulated by juvenile hormones (JH) (*1*, *2*). Correlated with a decrease in JH levels in insects is an increase in the rate of JH degradation, and it is believed that degradation by specific JH esterases (JHE) along with changes in the rate of JH biosynthesis is responsible for the regulation of JH titer. In this respect, considerable attention has been paid to the development of selective JHE inhibitors to better understand the biological role of JHE in insect growth and development (*3*, *4*). Extensive research has been conducted on trifluoromethyl ketones (TFMKs) as selective "*in vitro*" and "*in vivo*" inhibitors of JHE in Lepidoptera (*5*−*7*). These studies show that this family of compounds artificially reduce JH metabolism, increase JH concentration, and disrupt normal development and metamorphosis. In this paper, we report for the first time the effect of 3-octylthio-1,1,1-trifluoropropan-2-one (OTFP), a known inhibitor of JHE of *Trichoplusia ni* (Lepidoptera: Noctuidae) (*8*), *Lymantria dispar* (Lepidoptera: Lymantriidae) (*9*), *Ips typographus* (Coleoptera: Scolytidae) (*10*), and *Drosophila melanogaster* (Diptera: Drosophilidae) (*11*) among others, on larval development, pupation, and adult behavior of the Egyptian armyworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), and the corn stalk borer, *Sesamia nonagrioides* (Lef.) (Lepidoptera: Noctuidae). TFMKs are also potent inhibitors of

* Author for correspondence. E-mail: agpqob@iiqab.csic.es.
† Present address: Agricultural Experiment Station, College of Agriculture and Life Sciences, University of Guam, Mangilao, GU 96923, U.S.A.

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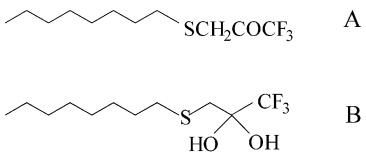
Chart 1. Structure of Octylthiotrifluoropropan-2-one (OTFP, A) and the Corresponding Hydrate (B)



the antennal esterases present in insects' olfactory tissues (*12*−*15*), possibly through formation of a stable hemiacetal of tetrahedral geometry with a serine residue present at the active site of the enzyme (*16*, *17*). In "*in vivo*" experiments, TFMKs have been found to disrupt the orientation flight of *Sp. littoralis* and *Se. nonagrioides* males to pheromone sources (virgin females or synthetic pheromone) (*18*), whereas in the field they displayed inhibition of the pheromone action on conspecific males when mixed with the synthetic pheromone (*13*, *19*).

The Egyptian armyworm is one of the most important polyphagous pests, widely distributed in the Mediterranean region and North and East Africa (*20*). The larvae feed on a wide range of economically important plants, both edible and ornamental. The corn stalk borer is one of the most serious pests of corn, particularly in the Mediterranean basin (below 45°N parallel) and North African countries (*21*). The larvae of the first generation rarely attack maize, but those of the second generation bore the maize stem from the first instars, causing severe damage to the plant. In this paper we also present qualitative and quantitative analyses of the sex pheromone components produced by females which had been treated with OTFP at the larval stage.

MATERIALS AND METHODS

Insects. *Sp. littoralis* and *Se. nonagrioides* were reared in the laboratory at 25 ± 2 °C and 65 ± 10% relative humidity (RH) with a 16:8 h light−dark (L:D) photoperiod on artificial diets slightly modified from those previously reported (*22*). Eggs were deposited by *Sp. littoralis* females on strips of filter paper in a plastic bag, while *Se. nonagrioides* oviposited her eggs on the maize plant (4−5 weeks old) placed inside a plastic cylindrical container (49 cm high × 18 cm i.d.). Groups of *Sp. littoralis* larvae were placed into glass containers (7.5 cm high × 17.5 cm i.d.), while *Se. nonagrioides* larvae were individually located in small plastic boxes (3.0 cm high × 5.5 cm i.d.). In both cases, larvae were provided with small cubes of diet. The lid of the boxes had few small holes to avoid fungal development. When the larvae stopped feeding, sawdust was provided for pupation. Pupae were sexed, and adults were separated daily by age and kept in other plastic containers (18 cm long × 14 cm wide × 9 cm high). Adults were provided with a 10% sucrose solution.

Mating Disruption Assays. OTFP was prepared in our laboratory as previously described (*23*). The compound (98% pure) was a mixture of the ketone and hydrate forms in 64:36 ratio (**Chart 1**). Distillation of the mixture gave the free ketone. To establish the effect of the chemical on mating, 500 ng of OTFP dissolved in 1 mL of nanograde hexane (Merck KGaA, chemical purity ≥99.5%) was sprayed on an intact maize plant of 4−5 weeks old with the aid of a commercial perfume-type atomizer. The solvent was allowed to evaporate for 5 min. Control experiments were run by spraying the same volume of solvent without inhibitor. The plant was then placed into a plastic cylindric container (see above), and four virgin couples (10−35 h old males and females) of *Se. nonagrioides* were released into the cage. Conditions of the room were 25 ± 1 °C, 70−80% RH, and 16:8 L:D photoperiod. The insects were provided with a 10% sucrose solution in cotton wool wads and were allowed to mate and oviposit on the plants for 4 days. The egg-surrounding areas of the plant were carefully cut, and the number of eggs was counted with the aid of a binocular microscope. After 7−8 days, the newly hatched larvae reached the second instar, and then they were counted and the sterilization index was determined. The tests were repeated 10 times.

Feeding Assays. To study the effect of the inhibitor on diet consumption and weight of larvae, several concentrations of OTFP (0.27, 1.37, 2.75, and 13.75 ng/μL) in nanograde hexane were prepared, so that 370 μL of each solution corresponded to a dose of 100, 500, 1000, and 5000 ng of the chemical. These amounts of the inhibitor were sprayed on 10 g of the diet placed on the rearing plastic boxes (see above). We preferred to spray the chemical rather than mixing it with the diet to resemble possible future field experiments. The diet was allowed to dry for 5 min, and then 20 second-instar larvae of *Sp. littoralis* were introduced onto each box. Insects were allowed to feed for 3, 6, 12, and 24 h, while individual *Se. nonagrioides* larvae were fed for 1.5, 3, 6, and 12 h. Twenty *Se. nonagrioides* larvae were also considered in each test. Separate control experiments were run within each experimental series. To establish the effect of the solvent, the same volume of hexane was also sprayed on a similar amount of diet. After feeding for the indicated period of time, the larvae were removed, weighed, and individually placed into boxes containing normal diet (20−25 g). The amount of diet consumed and the weight of larvae were determined every 4−5 days. Growth of larvae refers to the difference in weight of the final instar larvae minus that of the second instar. To ensure reproducibility, the plastic boxes were thoroughly washed with soap and water, rinsed with 10% bleach, and dried before use. Larval mortality, percent of pupation, and adult emergence were also recorded. Similar experiments were performed on fourth-instar larvae of both insects at 3 and 24 h intervals.

Behavioral Assays. To evaluate the possible effect of the chemical on adult behavior, a relatively low dose and a short OTFP treatment were chosen so that no larval mortality or growth disruption was induced. We chose for these assays 500 ng of the inhibitor for 3 h for *Sp. littoralis* and 500 ng for 1.5 h for *Se. nonagrioides*. As described above, groups of 20 second-instar larvae of both insects were fed with treated diet under the specified conditions, followed by normal diet until pupation. Assays were carried out on adults in a 180 cm × 55 cm × 50 cm glass tunnel, as described previously (*24*). Illumination (2−5 lx) was obtained with a dimmed fluorescent red light, and insects were allowed to acclimatize to the tunnel room conditions (22 ± 1 °C, 60 ± 10% RH) for 30−45 min. Just prior to the experiments, hexane solutions of (*Z*,*E*)-9,11-tetradecadienyl acetate, the major component of *Sp. littoralis* pheromone, and the *Se. nonagrioides* pheromone (mixture of (*Z*)-11-hexadecenyl acetate, (*Z*)-11-hexadecenol, and (*Z*)-11-hexadecenal in an 84:15:1 ratio) were applied to the dispensers so that 10 and 1 μg, respectively, were deposited. In the case of *Sp. littoralis*, the major component of the pheromone had elicited in WT an activity on male behavior equivalent to that induced by virgin females (*24*). The dispensers consisted of a brown female-shaped piece of cardboard for *Se. nonagrioides* and a 2 cm × 1 cm piece of filter paper for *Sp. littoralis* (*18*). The solvent was allowed to evaporate, and the dispensers were suspended 18 cm from the top and 40 cm from the upwind end of the tunnel. When virgin females were used as attractants, four individuals of 10−35 h old were placed into 6.5 cm × 4 cm × 3 cm stainless steel cages of 0.2 cm × 0.2 cm mesh. Experiments were conducted in blocks of untreated, hexane-treated, and OTFP-treated males using the synthetic pheromone or control, solvent-treated, or OTFP-treated females. Each time, four insects were released from the upwind end of the tunnel, and the insects were allowed to fly to the source for a total time of 6 min.

Pheromone Gland Extraction and Analysis. To study the effect of OTFP on pheromone production, 1-day-old virgin females, which had been previously treated or not with the chemical at the larval stage, were anesthetized with CO_2. After gently squeezing the abdomen, the glands were removed and rapidly placed into vials containing 100 μL of nanograde hexane and 10 ng of tridecyl acetate as internal standard. After 1 h of extraction at room temperature, the glands were removed and the solutions stored at −80 °C until analysis. GC−MS analyses were performed under electron impact conditions on a Fisons 8000 series gas chromatograph coupled to an MD-800 mass-selective detector. The system was equipped with an HP-FFAP high-performance capillary column (25 m × 0.25 mm i.d.), programmed from 80 °C (1

Table 1. Mean Percent Reduction of Larval Growth (±SE) of *Spodoptera littoralis* and *Sesamia nonagrioides* after Second-Instar Larvae Were Fed with OTFP or Hexane-Treated Artificial Diet for the Specified Time[a]

	treated diet feeding time (h)[b]							
	Sp. littoralis				*Se. nonagrioides*			
dose (ng)	3	6	12	24	1.5	3	6	12
hexane	2.2 ± 0.3a	6.7 ± 0.5a	–1.0 ± 0.4a	6.3 ± 1.6	7.3 ± 3.0a	–4.6 ± 14.9a	5.0 ± 18.3a	2.1 ± 24.8
100	2.7 ± 0.2a	11.6 ± 0.5b	70.0 ± 0.2b	–	8.5 ± 1.3a	21.4 ± 11.8b	42.4 ± 19.5b	–
500	3.1 ± 0.1a	16.4 ± 0.7c	85.3 ± 0.1b	–	10.3 ± 4.2a	24.4 ± 13.9b	61.6 ± 8.6c	–
1000	12.6 ± 0.2b	22.2 ± 0.2d	–	–	11.2 ± 8.1a	29.0 ± 23.0c	–	–
5000	23.2 ± 0.2c	–	–	–	19.8 ± 8.9b	44.2 ± 20.2d	–	–

[a] Figures in a column with different letters are significantly different at $P \leq 0.05$ (LSD test). [b] A negative inhibition value indicates higher growth of the treated larvae than that of control larvae. No value (–) indicates that all treated larvae died.

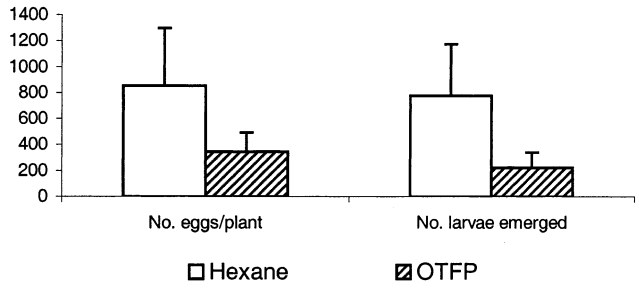


Figure 1. Number of eggs deposited per plant and emerging larvae when four virgin couples were allowed to feed on OTFP-treated maize plants. The tests were repeated 10 times. Bars over the histograms represent standard deviation of the means.

min) to 230 °C at 10 °C/min and then to 300 °C at 12 °C/min. Extracts corresponding to eight glands were concentrated to a 2−3 μL volume and injected under the SCAN mode.

Statistical Analysis. Data concerning the effect of the inhibitor on larval development, pupation, and adult emergence were analyzed for significance using the LSD test ($P < 0.05$). Percentage of responses in the wind tunnel assays was analyzed using the χ^2 test ($P \leq 0.05$) (Statistica, Stat Soft Inc.).

RESULTS

Mating Disruption and Sterility. In *Se. nonagrioides*, the average number of eggs laid per treated plant was 348.1 ± 147.1 (SD), whereas the value in control plants was 854.8 ± 442.7 (SD). This represents a mating disruption of 52.4 ± 18.9%. The average number of larvae that emerged in OTFP-treated plants was 26.2 ± 114.7 (SD), as compared to 780.1 ± 397.7 (SD) in untreated plants, which represents a sterilization index of 68.2 ± 10.3%. The corresponding differences in the two parameters are significant ($P \leq 0.05$) (**Figure 1**).

Growth Reduction. Addition of OTFP to the diet induced larvae of both insects to feed significantly less than control (untreated) larvae or larvae which had been fed with hexane, and therefore a remarkable reduction of growth was noticed (**Table 1**). Thus, in *Sp. littoralis* at 1000 ng dose in a 3 h treatment, the percent reduction of growth was 12.6 ± 0.2, significantly higher than that produced in larvae feeding on hexane-treated diet (2.2 ± 0.3). Treatments for 6−12 h induced higher growth reduction at all doses tested, the effect being dose-dependent. Longer treatments (24 h) were lethal for the insect even at the lowest dose tested (**Table 1**). On the other hand, *Se. nonagrioides* was more sensitive than *Sp. littoralis*, and the larval growth was already impaired after 1.5 h treatment at the highest dose (5000 ng). Here, again, the effect was dose-dependent. Administration of 1000 and 5000 ng of the inhibitor to the diet for 6 h induced larvae to die, whereas no larvae survived longer treatments even at the lowest dose tested (**Table 1**). Hexane elicited no effect on diet consumption in the larvae weight of either insect at any of the doses tested and during the periods of time assayed. The weight of larvae varied from 0.10 to 0.60 mg in *Sp. littoralis* and from 0.18 to 0.42 mg in *Se. nonagrioides*. Treatment of 100−5000 ng of the inhibitor on fourth-instar larvae for 3 and 24 h did not produce significant differences in the amount of diet ingested (data not shown).

Table 2. Percent of Pupation of *Spodoptera littoralis* and *Sesamia nonagrioides* with Respect to the Number of Last-Instar Larvae When the Second-Instars Were Fed with OTFP- or Hexane-Treated Diet for the Specified Time[a]

	treated diet feeding time (h)[b]							
	Sp. littoralis				*Se. nonagrioides*			
dose (ng)	3	6	12	24	1.5	3	6	12
control	88	86a	85a	75	90	95a	85a	90
hexane	88	88a	90a	75	95	90a	90a	95
100	100	86a	25b	–	90	70a	30	–
500	88	63ab	10c	–	90	55b	15c	–
1000	100	38b	–	–	85	40b	–	–
5000	100	–	–	–	75	25c	–	–

[a] Figures in a column of the same insect with different letters are significantly different at $P \leq 0.05$ (LSD test). [b] No value (–) indicates that all treated larvae died.

The feeding inhibition resulted also in a lower percent of pupation, as shown in **Table 2**. No change was observed when the chemical had been applied to *Sp. littoralis* larvae for 3 h and to *Se. nonagrioides* for 1.5 h, but significant pupation reduction was noticed on *Sp. littoralis* at a 1000 ng dose for 6 h (38% of pupae vs 86−88% in control or hexane-treated insects) and at a 100 ng dose for 12 h (25% pupation). Likewise, pupation in *Se. nonagrioides* was significantly lower than that found in control or hexane-treated insects at a 500 ng dose for 3 h (55% vs 90−95% of pupation, respectively) or at a 100 ng dose for 6 h (30% vs 85−90% of pupation, respectively). With regard to adult emergence, only 5−25% of emergence was found in *Se. nonagrioides* when a range of 500−5000 ng dose had been administered for 3 h, and 5−15% emergence was found at a 100−500 ng dose for 6 h (data not shown). In *Sp. littoralis*, all surviving pupae successfully emerged, except those previously treated with 100 and 500 ng of the chemical after 12 h of treatment.

Behavioral Assays. Significantly fewer males treated with OTFP contacted with the source (49.1% of SC, $N = 59$) ($P \leq 0.05$, χ^2 test) in comparison with regular untreated males (68.1% of SC, $N = 47$) (**Figure 2**). The other three parameters studied, TF, HW, and CA, were not significantly different in treated or untreated animals. Here, again, the effect was higher in *Se. nonagrioides*, wherein only 16 treated males out of 44 (36.4%)

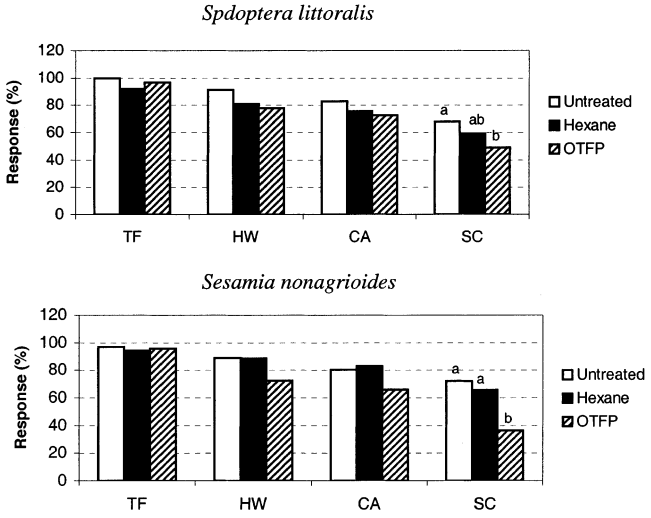


Figure 2. Percentage of response of *Sp. littoralis* and *Se. nonagrioides* males (below), proceeding from larvae treated or not with OTFP, to a synthetic pheromone source in a wind tunnel. TF, taking flight; HW, halfway; CA, close approach; SC, source contact. Bars with different letters are significantly different (χ^2 test, $P \leq 0.05$).

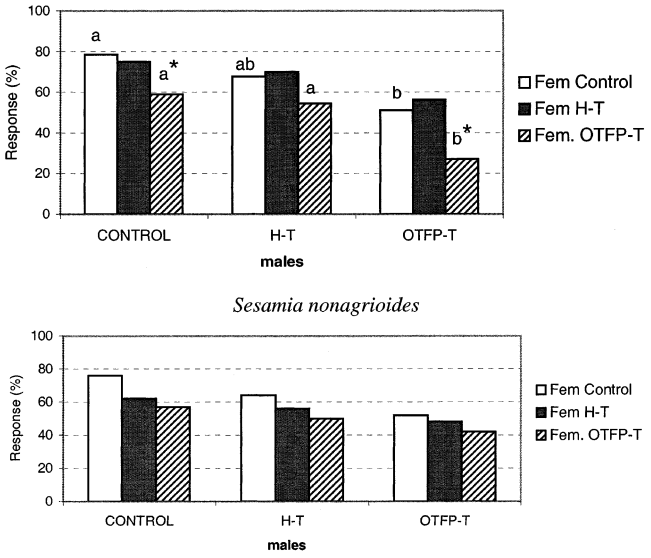


Figure 3. Source contact behavior of *Sp. littoralis* and *Se. nonagrioides* males, proceeding from larvae treated with hexane (H-T), OTFP (OTFP-T), or untreated (control) when attracted to regular females (fem. control), hexane-treated (fem. H-T), and OTFP-treated females (fem. OTFP-T) in a wind tunnel. Letters refer to statistical comparison of behavior of differently treated males when attracted to the same type of females. Asterisks represent significant differences in responses of control and OTFP-treated males when attracted to females treated with the inhibitor (χ^2 test, $P \leq 0.05$). Bars with no letters mean that the values are not significantly different.

successfully contacted the pheromone source in comparison to the untreated (26 insects out of 36, 72.2%) and solvent-treated males (23 out of 35, 65.7%). As in *Sp. littoralis*, the activity of the chemical was only significant at the SC level (**Figure 2**).

On the other hand, when *Se. nonagrioides* females which had been subjected or not to the treatment were used as the attractant source, males were similarly attracted to them regardless of whether they had been previously treated or not. The values corresponding to the SC behavior are presented in **Figure 3**. However, *Sp. littoralis* males proceeding from larvae treated with the inhibitor displayed significantly fewer contacts with

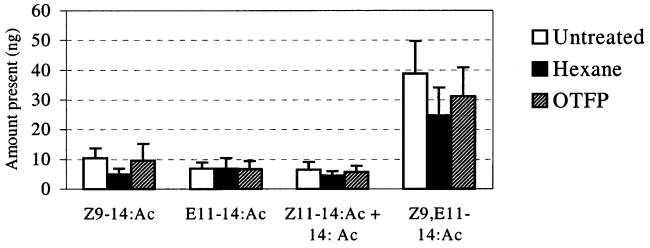


Figure 4. Amount of pheromone components from virgin female glands of *Sp. littoralis* arising from OTFP-treated, hexane-treated, and untreated larvae ($N = 8$ insects per treatment). Bars on columns represent standard deviation of the means.

the cage-containing control females (51.2% of SC, $N = 41$, $P = 0.009$) than untreated (78.6% of SC, $N = 42$) or solvent-treated males (67.7% of SC, $N = 31$). When previously treated females were used as the attractant source, only 27% ($P = 0.002$) of treated males ($N = 37$) successfully completed the flight, in comparison to animals responding to control (59.1% of SC, $N = 44$) or hexane-treated females (54.5% of SC, $N = 33$).

Pheromone Production. Since *Se. nonagrioides* males were behaviorally unaffected by OTFP when they were attracted to treated or untreated females, we did not consider worthwhile evaluation of the pheromone contents in female glands. However, and in view of the effect elicited by the chemical on *Sp. littoralis* (see above), we determined the level of pheromone production in treated females relative to that in control and solvent-treated animals. GC−MS analyses of gland extracts showed an average of 38.7 ± 11.0 ng of Z9,E11-14:Ac, the major component of the pheromone, in untreated females, 24.7 ± 9.3 ng in solvent-treated, and 31.1 ± 9.7 ng in OTFP-treated insects (**Figure 4**). These differences were not significant, nor were the average amounts of the minor compounds present (10.4 ± 3.3, 5.0 ± 1.8, and 9.5 ± 5.5 ng, respectively, of Z9-14:Ac; 6.8 ± 2.1, 6.8 ± 3.5, and 6.7 ± 2.6 ng, respectively, of E11-14:Ac; and 6.4 ± 2.6, 4.5 ± 1.4, and 5.6 ± 2.0 ng, respectively, of Z11-14:Ac and 14:Ac, which were inseparable under several GC conditions). Likewise, the relative ratio of the pheromone components in all types of females was similar (**Figure 4**).

DISCUSSION

OTFP is a highly potent and selective inhibitor of JHE (*25*), 1-naphthyl acetate esterase, acetylcholine esterase, and other esterases associated with insecticide resistance (*26*). However, to our knowledge, no reports have been published on the direct action of the chemical on growth, development, and behavior of adults which had been treated with the compound at the early instars. Our results show that spraying OTFP on the plant significantly disrupts mating in *Se. nonagrioides*, and, moreover, most of the eggs are infertile. In the same vein, administration of the inhibitor to the diet significantly reduced larval growth at sublethal doses in both species. This suggests a secondary antifeedant effect of the chemical, since the results were obtained after the insects had been transferred back to the diet free of the inhibitor. Other important compounds which exert secondary antifeedant activity in *Sp. littoralis* are limonoids present in extracts of the neem tree, *Azadirachta indica* (*27*). These compounds have been traditionally used in several countries to control insect pests by reducing their feeding, survival, and reproduction (*28*), and a variety of commercial formulations of the neem extracts are now available on the market (*29*). Relatively high concentrations of OTFP (≥1000 ng in both insects) in 6−12 h treatments led to starvation. Interestingly,

treatment on fourth-instar larvae of both insects for 3−24 h did not produce significant differences in the amount of diet ingested or in growth. These results agree with others in which third and fifth instars of azinphosmethyl-resistant tufted apple bud moth, *Platynota idaeusalis* (Lepidoptera: Tortricidae), showed low levels of mortality after 24 h of treatment with OTFP, although the chemical synergized the toxic effect of the insecticide (*26*). In our experiments, the compound appears to act on early instars and low doses as a feeding deterrent, and since this effect is dose-dependent it becomes lethal by starvation at high doses. Treatments longer than 3 h produced a decrease of the pupation index and percentage of emergence in both insects. Elden (*30*) also found that some proteinase inhibitors significantly inhibited pupation and adult emergence of alfalfa weevil, *Hypera postica* (Coleoptera: Curculionidae).

The effects of alellochemicals after ingestion are often assessed only on the basis of criteria concerning changes in nutritional indexes. These indexes are only symptomatic and do not establish a cause−effect relationship (*31*). Moreover, discrepancies in levels and types of inhibition between feeding studies using artificial diets and "*in vitro*" midgut analyses have been demonstrated (*32*, *33*). Our investigations show that OTFP acts as an antifeedant, but it is likely that other mechanisms may also be active once the chemical is ingested. In this regard, it should be noted that OTFP has proved to be a good inhibitor of the JHE present in the hemolymph of sixth-instar larvae of *Sp. littoralis* ($IC_{50} = 5.8 \times 10^{-7}$M) (Galcerán and Casas, unpublished), so the chemical may be acting on other esterases or serine proteases of the gut as well. These results are consistent with others in that inhibitors of serine-based enzymes, such as proteinases, reduce larval growth and in some instances cause mortality. This is the case of the nonspecific proteinase inhibitor α_2-macroglobulin that reduced larval growth of the Australian sheep blowfly, *Lucila cuprina* (Diptera: Muscidae), by 62% when incorporated into the artificial diet (*34*). Further studies by Reed and co-workers (*35*) showed that inhibitors of serine proteinases and aminopeptidases caused significant growth inhibition, and in some cases death, of *L. cuprina* larvae after 24 h of treatment, suggesting that these enzymes were one of the major classes involved in protein digestion in the gut and that their inhibition leads to an almost complete blockade of digestion. Other inhibitors of serine-based inhibitors, such as saponins, a group of compounds which protect plants against insect attack, have been shown to reduce larval growth in the flower beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae) (*36*), and in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae) (*37*), among others. Another "*in vitro*" inhibitor of esterases, the hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), present in maize and other cereals, also reduced the relative growth rate of *Se. nonagrioides* larvae, causing larval and pupal mortality when the larvae were fed on maize inbred plants with high DIMBOA content (*38*). The authors suggested that the activity of the compound could also be attributed to the reaction of the hydroxamic acid with nucleophilic residues in the active center of the esterase (*39*), similar to the mechanism of action proposed for TFMKs (*16*, *17*).

To our knowledge, very little work, if any, has been done on the behavioral activity of adult Lepidoptera which have been treated with potential inhibitors at the larval stage. In other orders, such as Coleoptera, Hemiptera, Homoptera, or Diptera, however, some reports have been found. Thus, Murdock et al. (*40*) demonstrated that the fecundity of the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), emerging from artificial seeds treated with E-64, a proteinase inhibitor, was significantly decreased. The chemical was also shown to induce a negative effect on the reproduction potential of the Mexican bean beetle, *Epilacna varivestis* Mulsant (Coleoptera: Coccinellidae) (*41*). Fecundity of two species of adult horn flies, blood-feeding Diptera, was significantly reduced when the flies were fed on artificial diets containing the proteinase inhibitor leupeptin (cysteine) or soybean trypsin (serine) (*42*). Our results show that, when attracted to a pheromone source, treated males of both insects displayed fewer source contacts than control insects, whereas when *Sp. littoralis* treated females were used as attractants source, contact behavior of treated conspecific males also differed significantly from control. One interpretation is that the impaired performance of behavior may be due to a long-lasting effect of OTFP, which may not be fully detoxified by the detoxification enzymes of the gut at the larval stage of the insect. The chemical appears to inhibit, therefore, the arrestant behavior of males. This effect had also been noticed when the chemical was topically applied to the insects' antennae, although in this assay the results were more dramatic since the inhibition of response was remarkable from the first steps of behavior, i.e., wing fanning and taking flight (*18*). The chemical, however, does not affect pheromone production by females and consequently the ability of females to call. In-flight behavior of *Se. nonagrioides* males showed no significant differences when attracted either to a pheromone source or to treated or control females. Perhaps, in this case, the effect of the chemical is not long-lasting and is fully detoxified before the adult develops. Studies directed to address this point are currently underway in our laboratory.

As cited above, JHs regulate many development and reproductive events in insects (*2*, *43*) and the JH titer is mainly modulated by JHE (*3*, *6*, *44*). OTFP has been reported to inhibit JHE of the larval and pupal hemolymph of the gypsy moth *Lymantria dispar* (*9*) and other insects (*8*, *10*, *11*). In *Sp. littoralis* and *Se. nonagrioides*, OTFP is presumably acting on the internal physiology of the insects, and if we assume that this internal action is through the JH pathway, then the results have the significance of expanding our view of the role of the JH pathway in regulating certain reproductive and growth processes. However, whereas this assumption may be applicable in several events, such as diet consumption, larval growth, and adult emergence, in the mating disruption test on the plant, an effect on the chemosensory system of females may be postulated. This chemosensory effect might result in a lower oviposition and, consequently, in fewer eggs hatching.

In summary, we have shown for the first time that the known esterase inhibitor OTFP elicits remarkable effects at nonlethal doses on oviposition, growth, development, and behavior in two important Lepidopteran pests. Although the effects are not entirely parallel in both insects, the results obtained, in addition to other previously reported by us, such as disruption of the chemical communication between sexes and the low acute toxicity to mice (*19*), provide new information which can be useful in studies directed to develop new approaches for pest control, as already proposed for JHE (*45*, *46*).

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