Four Species of Noctuid Moths Degrade Sex Pheromone by a Common Antennal Metabolic Pathway[1]

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ABSTRACT Z-9-tetradecenyl acetate (Z-9-14:OAc) is a component in the female sex pheromones of the cabbage looper, *Trichoplusia ni* (Hübner), beet armyworm, *Spodoptera exigua* (Hübner), fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and black cutworm, *Agrotis ipsilon* (Hufnagel). We compared the *in vivo* catabolism of Z-9-14:OAc in time course fashion after the tritiated compound was applied topically to the antennae of males in the four species. Catabolism of tritiated European corn borer, *Ostrinia nubilalis* (Hübner), sex pheromone (Z-11-14:OAc) was monitored concomitantly so direct comparisons could be made between the male borer and the noctuid males. Results showed that catabolism of pheromone in all four noctuid moths proceeded along the same hydrolysis-alcohol oxidation pathway as has been observed in the European corn borer male. Catabolism was mathematically modeled with first-order differential equations as a four-compartment degradative system in which tritiated pheromonal acetate was sequentially converted to tetradecenol, tetradecenoic acid and water. The modeling revealed subtle differences in catabolism from one species to another and that most species exhibited a finite capacity to catabolize the pheromone.

Key Words *Agrotis ipsilon, Ostrinia nubilalis, Spodoptera exigua, S. frugiperda, Trichoplusia ni,,* black cutworm, European corn borer, beet armyworm, fall armyworm, cabbage looper, Z-9-tetradecenyl acetate.

It is generally accepted that male moths use their antennae to detect airborne pheromonal sexual signals emitted by females and that these signals are important in insect reproduction. The pheromones are detected when they interact with receptors on the male antennae. Catabolic enzymes are known to exist on the insect antennae and they are presumably responsible for clearing antennal structures and receptors of resident pheromone by degradation to make the antenna available and highly receptive to fresh incoming pheromonal signals. (Ferkovich 1982, Kasang et al. 1989a, Kasang et al. 1989b, Prestwich et al.

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1989, Klun and Schwarz 1993). Rybczynski et al. (1990) showed that antennal-specific pheromone-degrading oxidases exist in the adults of *Antherea polyphemus* (Cramer) and *Bombyx mori* (L.) and this observation supports the proposition that rapid antennal pheromone metabolism plays an important role in pheromone processing.

Until now, comparative study of i*n vivo* pheromone processing among moths that use a common compound as their part of their pheromone was limited to the European corn borer, *Ostrinia nubilalis* (Hübner) and the redbanded leafroller, *Argyrotaenia velutinana* (Walker). These species use 11-tetradecenyl acetate as sex pheromone. In both species, 11-tetradecenyl acetate i*n vivo* antennal degradation involves a rapid hydrolysis of acetate, oxidation of the alcohol to the corresponding fatty acid, followed by degradation of the acid by β-oxidation (Klun et al. 1992, Klun and Schwartz 1993). The purpose of the present study was to expand the base of knowledge on how pheromone is processed on the antennae of four other economically-important species of moths. The cabbage looper, *Trichoplusia ni* (Hübner), beet armyworm, *Spodoptera exigua* (Hübner), fall armyworm, *Spodoptera frugiperda*, (J. E. Smith), and black cutworm, *Agrotis ipsilon* (Hufnagel), were selected for the study because they each used a common chemical, Z-9-14:OAc, as part of their sex pheromone. We hypothesized that the four species would catabolize radiolabeled Z-9-14:OAc by a pathway similar to that used by the European corn borer and redbanded leafroller. Our study showed that this hypothesis was correct.

Materials and Methods

Selection of species for study. Z-9-14:OAc was known to be a sex pheromone component in at least four noctuids: (1) In 1979, Hill et al. showed that Z-9-14:OAc was part of a binary mixture that comprised the female sex pheromone of the black cutworm. (2) Bjostad et al. (1984) reported that Z-9-14:OAc was a minor component in the cabbage looper female sex pheromone. (3) In the case of the fall armyworm, Tumlinson et al. (1986) determined that the compound was a critical component for attraction of the males in the field. (4) Tumlinson et al. (1990) reported that Z-9-14:OAc along with Z-9-tetradecenol (Z-9-14:OH) and 3 other compounds were emitted by calling virgin beet armyworm females. The exact role of Z-9-14:OAc in the beet armyworm is uncertain. Although the acetate was identified among the volatile compounds emitted by beet armyworm females, field tests showed it inhibited male trap captures. On the other hand Tumlinson et al. (1990) found that a mixture of the alcohol, Z-9-14:OH, and (Z, E)-9,12-tetradecadienyl acetate was the most effective lure for the insect. Because of this ambiguity, we studied catabolism of both tritiated Z-9-14:OAc and Z-9-14:OH in the beet armyworm.

Sources of insects. The cabbage looper and beet armyworm were from cultures maintained at the USDA Insect Attractants, Behavior and Basic Biology Lab., Gainesville, FL. The fall armyworm was received from the USDA Insect Biology and Population Management Research Lab., Tifton, GA, and the black cutworm was obtained from the USDA Corn Insects Research Unit, Ames, IA. All insects were shipped as groups of pupae, and upon receipt, they

were isolated individually in plastic cups and placed in a reverse photoperiod environmental chamber (80% relative humidity; 16 h light-8 h dark, 26°C-20°C) to await adult emergence. After emergence, males were transferred to a screened cage and provided with water. All moths were 2 to 3 days old when they were taken out of scotophase and used in the experiments. European corn borer moths used in the study were reared in our laboratory and were handled similarly.

Tritiated pheromone, high pressure liquid chromatography and radiodetection. Materials and methods used here were identical to those described by Klun et al. (1992). (Z)-[9,10-$^3H_2$]-9-14:OAc (^{3}H-Z-9-14:OAc) and (Z)-[11,12-$^3H_2$]-11-14:OAc (^{3}H-Z-11-14:OAc) were prepared at the National Tritium Labeling Facility, Berkeley, CA by semitritiation of 9- or 11-tetradecynyl acetate by using $^3H_2$ over a stirred cyclohexene (freshly distilled) solution of the acetylenic precursor containing lead-poisoned $Pd/CaCO_3$ Catalyst. ^{3}H nuclear magnetic resonance spectroscopy (Klun et al. 1992) showed that the ^{3}H-Z-9-14:OAc had a specific activity of 39.4 Ci/mmol and ^{3}H-Z-11-14:OAc had 41.6 Ci/mmol. Tritiated alcohol (^{3}H-Z-9-14:OH) and fatty acid (^{3}H-Z-9-14:oic) were prepared from the corresponding acetate using synthetic methods described earlier (Klun et al. 1992). The derivatives were used in metabolic study and/or as chromatographic references. Compounds used in the catabolism studies were repurified at least every 4 to 8 wks. High-pressure liquid chromatographic (HPLC) analyses or purifications were conducted using a Beckman System Gold (Beckman Instruments, Fullerton, CA 92634) chromatographic system equipped with a Beckman model 171 radiodetector. Tritiated water and toluene standards were purchased from NEN, Dupont Company, Boston, MA 02118.

All analyses of the antennal extracts for tritiated pheromone and its catabolites were performed using a Bechman Instruments 25 cm × 4.6 mm (ID) 5 μm Ultrasphere ODS (octadecylsilane coated silica) column, 1 ml/min methanol-water (90:10) pumped as eluant, and using the liquid scintillant cell of the 171 radiodetector with Beckman Ready Flow II scintillant cocktail pumped at 2 ml/min. The NEN tritium standards showed that detection efficiency was 14% for organics and 18% for water.

Pheromone Catabolism in Male Noctuid Moths. In all time-course studies, one antenna of a CO_2-anesthetized moth was treated with a heptane solution containing 160×10^3 disintegrations per min(dpm) tritiated compound (approximately 460 pg). Heptane was the solvent of choice because, when applied topically to a moth antenna, it does not injure the moth nor impair the males' behavioral responsiveness to sex pheromone (Klun et al. 1991). The solution was applied by using a micrometer-driven 50-μl syringe under a dissection microscope. In practice, a 0.25-μl droplet containing 460 pg compound was allowed to form on the tip of the syringe and then the moth's antenna was drawn through the drop to coat it along its length. The quantity of pheromone applied was the smallest quantity of compound that could be used and still remain within radiodetection limits. Following treatment, the antenna was either quickly excised (approximately 30 sec later) into 50 μl chloroform-methanol (2:1, v/v) or the male was transferred to a 30-ml plastic cup and allowed to incubate for 3, 6, or 12 min before the treated antenna was excised and placed into the chloroform-methanol (C-M). Excised antennae were

extracted with the C-M for at least 30 min and then the extract was analyzed by radiodetection-HPLC. The identities of the radiolabeled catabolites were based upon the coincidences of retention times with radiolabeled-reference compounds. For each species of moth, analyses were replicated ten times for each of the four incubation times.

^{3}H-Z-11-14:OAc Catabolism in the European Corn Borer Male. To compare the *in vivo* catabolism in the noctuid males with the European corn borer male, 160×10^3 dpm triated borer sex pheromone was applied to the antennae and they were processed using the same methods as described for the noctuid moths.

Antennal combustion analyses (Evans 1974) were conducted using the European corn borer to validate the efficiency of the C-M extraction procedure. The analysis involved complete combustion of antennae treated with 160×10^3 dpm ^{3}H-Z-11-14:OAc in O_2 after a 5 min incubation; treatment of antennae with the radiolabel followed by a 5 min incubation, C-M extraction and combustion; and combustion after treatment with heptane alone and extraction with C-M. Water formed in the respective combustions was analyzed for tritium by liquid scintillation counting. The combustion analysis was replicated six times.

Mathematical Modeling. Modeling was used to describe the dynamics of *in vivo* catabolism and to provide a statistical basis for comparison of pheromone processing among the five species on an individual antenna basis. The maximum number of radiolabeled substances detected in antennal extracts post topical application of tritiated pheromone was four. These were tritiated acetate, the corresponding alcohol, fatty acid, and water.

$$^3\text{H-Z-9-14:OAc} \xrightarrow{k_1} {}^3\text{H-Z-9-14:OH} \xrightarrow{k_2} {}^3\text{H-Z-9-14:oic} \xrightarrow{k_3} {}^3\text{H}_2\text{O} \xrightarrow{k_4}$$

Therefore, the catabolism was modeled as a four-compartment system (Seber and Wild 1988). The standard mathematical model for this system is a set of linear first-order differential equations with constant coefficients:

$$\frac{d[\text{OAc}](t)}{dt} = -k_1[\text{OAc}](t) \qquad [\text{OAc}](0) = 0$$

$$\frac{d[\text{OH}](t)}{dt} = k_1[\text{OAc}](t) - k_2[\text{OH}](t) \qquad [\text{OH}](0) = 0$$

$$\frac{d[\text{oic}](t)}{dt} = k_2[\text{OH}](t) - k_3[\text{oic}](t) \qquad [\text{oic}](0) = 0$$

$$\frac{d[\text{H}_2\text{O}](t)}{dt} = k_3[\text{oic}](t) - k_4[\text{H}_2\text{O}](t) \qquad [\text{H}_2\text{O}](0) = 0.$$

Where [OAc](t), [OH](t), [oic](t), and [H_2O](t) represent the amounts of radiolabeled compound in each of the four compartments at time t. The solutions to these differential equations yield four curves representing the amounts of the four catabolites over time. The OAc curve decreases asymptotically to zero. The other three curves start at zero, increase to a maximum and then decrease asymptotically to zero. Parameter b represents the amount of tritiated pheromone on the antenna immediately after application. The parameters k_1, k_2, and k_3, are transfer coefficients between the respective pools. The k_4 represented transfer of tritiated water away from the antenna by evaporation and/or diffusion to the insect body. When k_3, the rate constant for transfer from fatty acid to water, approached ∞, the system reduced to three compartments, and k_2 became the rate constant for direct transfer of tritium from alcohol to water.

We used a simple modification of this standard compartment model to account for a time-dependent variation of the transfer coefficients. Our model allowed the transfer coefficients to decrease as time increased. This was done by adding a sixth parameter, s. In our modified system, the transfer coefficients are constant until time s; at this point, the coefficients drop to zero for the remaining incubation times. This represented a time at which catabolism on the system ceased. At the cessation time (s) the amounts of radiolabeled compound in each compartment, [OAc](t), [OH] (t), [oic](t), and [H_2O](t) remained constant over the remainder of the incubation times.

The modified system contained the standard compartment system as a special case. If s was estimated to be greater than the longest incubation time, 12 minutes, then the system was unchanged and did not cease. Consequently, the experimental data were used to determine if the modified system was required.

The same model was used for the catabolism of radiolabeled alcohol in the beet armyworm by letting k_1 equal ∞. In this case, b becomes the initial amount of triated alcohol.

When $t \leq s$, the solutions to the differential equations in the modified compartment system are

$$[\mathrm{OAc}](t) = be^{-k_1 t}$$

$$[\mathrm{OH}](t) = bk_1 \left[\frac{e^{-k_1 t}}{k_2 - k_1} + \frac{e^{-k_2 t}}{k_1 - k_2} \right]$$

$$[\mathrm{oic}](t) = bk_1 k_2 \left[\frac{e^{-k_1 t}}{(k_2 - k_1)(k_3 - k_1)} + \frac{e^{-k_2 t}}{(k_1 - k_2)(k_3 - k_2)} + \frac{e^{-k_3 t}}{(k_1 - k_3)(k_2 - k_3)} \right]$$

$$[\mathrm{H_2O}](t) = bk_1 k_2 k_3 \left[\frac{e^{-k_1 t}}{(k_2 - k_1)(k_3 - k_1)(k_4 - k_1)} + \frac{e^{-k_2 t}}{(k_1 - k_2)(k_3 - k_2)(k_4 - k_2)} \right.$$
$$\left. + \frac{e^{-k_3 t}}{(k_1 - k_3)(k_2 - k_3)(k_4 - k_3)} + \frac{e^{-k_4 t}}{(k_1 - k_4)(k_2 - k_4)(k_3 - k_4)} \right]$$

When $t > s$, the solutions are of the same form, but t is replaced everywhere by s. These equations were fitted to the experimental data for each moth species by the method of iterated, seemingly-unrelated, nonlinear regression using the MODEL procedure of SAS/ETS (SAS 1993). This parameter estimation method fits the four equations simultaneously. Because the four equations share the same parameters, this method can use the data from all the catabolites to estimate the six parameters. The square-root transform-both-sides method was applied to each regression model in order to make the distribution approximately symmetrical and to stabilize the variance of the observed dpm levels (Seber and Wild 1989).

Results and Discussion

Antennal combustion analyses showed that on the average 1.2% of tritium applied to European corn borer antennae remained bound to the antennae after extraction with C-M. This result indicated that the C-M extraction method was highly efficient in removing existing tritiated pheromone and catabolic products from the antennae.

Analyses of the antennal extracts revealed that acetate, alcohol, and water were the only detectable radiolabeled products in all species of moths with exception of the cabbage looper. In looper, the three products plus a large catabolic pool of Z-9-tetradecenoic acid was also observed in individual male antennal extracts. Tetradecenoic acid is a known intermediary metabolite in pheromone *in vivo* catabolism by the redbanded leafroller (Klun and Schwarz 1993) and European corn borer (Klun et al. 1992). Based upon the nature of the radiolabeled products detected in the noctuids, catabolism of pheromone in these moths proceeded along the same hydrolysis-alcohol oxidation pathway as has been observed in the European corn borer and redbanded leafroller.

Results of time-course catabolism studies for the five species of moths are plotted in Fig. 1. The plots show the distribution of tritium in starting material and catabolic products as a function of time radiolabeled pheromone was applied to the antennae. Average predicted values from the mathematical model are overlaid onto the observed median amounts of tritium at four time periods for each insect. Parameter estimates from the compartment modeling are presented in Table 1.

Preliminary analysis of the data revealed substantial lack of fit with the standard compartment model having linear first-order differential equations with constant transfer coefficients. In four cases, the observed amounts of the catabolic products did not appear to decrease asymptotically to zero. For this reason, the compartment model was modified as described earlier to allow the transfer coefficients to decrease with time. The modification is admittedly rough because catabolic processing probably slows gradually rather than ending abruptly at $t = s$. Nonetheless, the simple modification gave a better fit to the observed data and an improved approximation of the catabolic processing (Fig. 1).

Parameter estimates from the compartment modeling are presented in Table 1. Although equal amounts of radioactivity were dispensed from the micrometer driven delivery syringe, the data show that amount of tritium

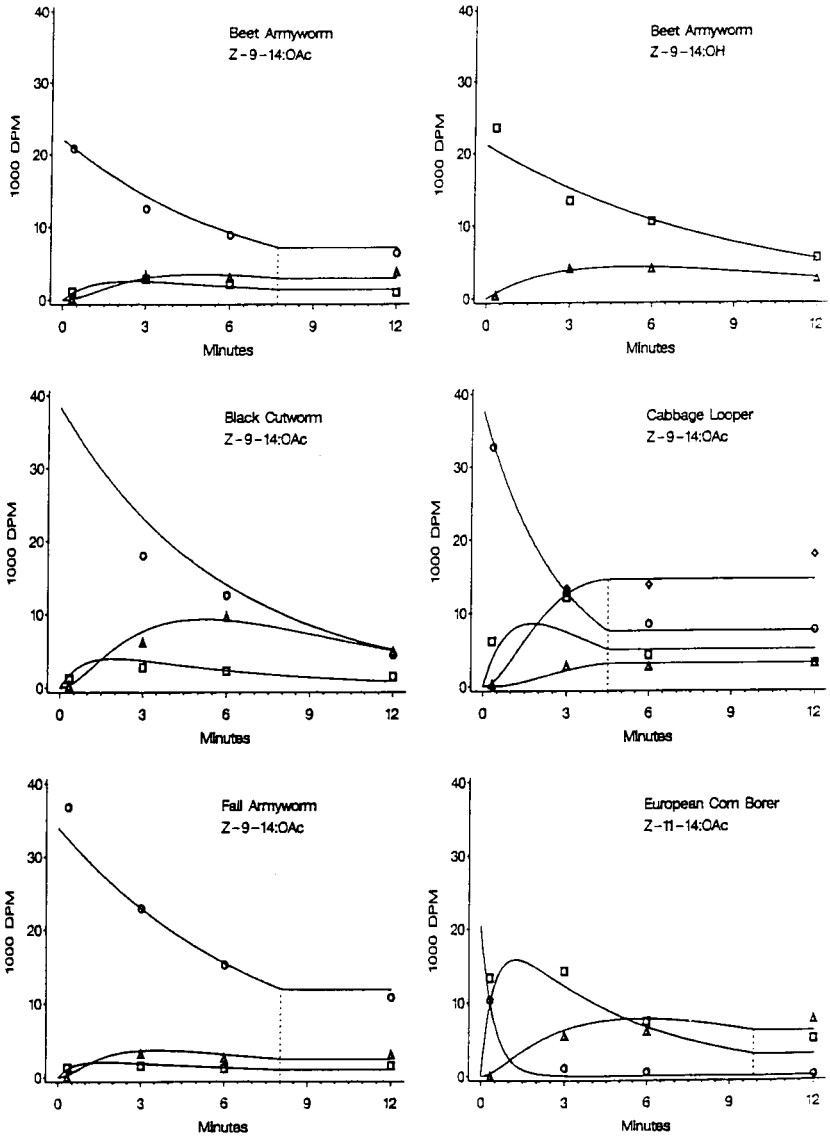

Fig. 1. The time-course distribution of tritium after radiolabeled pheromone was applied to the antennae of five species of moths. The fitted curves induced by the mathematical model are overlaid with the observed amount of tritium in the form of acetate (0), alcohol (□), carboxylic acid (◇), and water (Δ). Each data point represents the median of 10 moths. The dashed vertical line represents the value of s, the estimated cessation time of pheromone processing. The earliest observed OAc dpm value for the black cutworm was off scale (40,040 dpm) and it is not shown in the plot.

Table 1. Parameter estimates (± standard errors) for the compartment modeling of pheromone catabolism in five species of moths.

	Pheromone/Species**					
	Z-9-14:OH	Z-9-14:OAc				Z-11:OAc
Parameter*	BAW	BAW	BCW	CL	FAW	ECB
b	21±1	22±2	38±3	38±2	34±2	20±1.3
k_1	NA	.15±.02	.17±.01	.36±0.2	.13±.01	2.1±.25
k_2	.11±.01	.88±.12	1.2±0.1	.84±.09	1.7±0.3	.20±.023
k_3	∞	∞	∞	.27±.05	∞	∞
k_4	.31±.04	.52±.09	.33±.05	1.1±0.4	.82±.11	.19±.036
s	>12	7.7±1.4	>12	4.5±0.3	8.0±0.9	9.9±1.0

* Units for the parameter estimates are dpm × 10^3 for b, min^{-1} for the k_1, and min for s.
** BAW = beet armyworm, BCW = black cutworm, CL = cabbage looper, FAW = fall armyworm, ECB = European corn borer.

detected immediately after application to the antennae (Parameter b) of beet armyworm and European corn borer was significantly less than that observed in the other three species. The differential amount of tritium adsorbed on the borer and beet armyworm antennae is probably related to the fact that their antennae are smaller than the other species' antennae. Noteworthily, the amount of tritium on the beet armyworm antennae was the same whether the acetate or alcohol was applied. This is good indication that antennal size influenced the amount of radioactivity adsorbed onto the antenna.

Data show that on an individual antennal basis the rates of acetate hydrolysis (k_1) in the noctuids were significantly slower than that seen in the European corn borer (Table 1). Among the noctuids, the black cutworm and fall armyworm catabolized alcohol more effectively than the other two species.

The beet armyworm showed a greater capacity to process alcohol than capacity to process acetate. No cessation of alcohol catabolism was observed within 12 min incubation, while catabolism of the acetate stopped after 7.7 min. Thus, the beet armyworm was more efficient at processing the alcohol pheromonal component that is known to elicit a behavioral output but it was less efficient at degrading the acetate which is known to suppress behavioral output.

Depending on the species, moths evidenced a finite capacity to process the pheromone applied to their antennae. The cabbage looper, beet armyworm (acetate substrate), fall armyworm, and European corn borer had catabolic cessation times (s) of 4.5, 7.7, 8.0, and 9.9 min, respectively. In the black cutworm, cessation of catabolism was not observed within 12 min. We surmise that the differing pheromone processing capacities among the species is reflective of differing non-renewable energy reserves or the amount of catabolic enzymes available to degrade pheromone. We also wonder if species that have low pheromone processing capacity might be more susceptible to mating disruption by using pheromone (Cardé and Minks 1995) than species with higher pheromone-processing capacities.

From our present work, it can be concluded that the four noctuids, European corn borer and redbanded leafroller all catabolize pheromone by a common pathway. This finding is important because if ways can be found to block pheromone catabolism, and thereby cause disruption of sexual communications (and reproduction) in one species, such a blocking agent would likely be effective against all six pests because they use the common catabolic pathway. This realization provides impetus for research to discover compounds that might interfere with the pheromone-degradation pathway used by these important pests.

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