The gongylidia constitute the larval diet and so the rate at which they are produced might be expected to have significant importance to the establishment success of foundress queens, growth of worker populations and the production of sexuals. Larger colony worker population would allow an increased foraging range and thus facilitate a qualitative and quantitative expansion of the trophophoric field. Thus the acquisition of a more productive food crop would confer a selective advantage in permitting the exploitation of richer and more abundant substrate resources and would therefore constitute an important factor in attine niche separation. In the absence of sporulation, the only source of more productive fungal strains would be through somatic mutation. We suggest that ancestral selection of high yielding somatic mutants from their cloned fungal symbionts was a major factor in the speciation of the higher attines allowing niche separation through selection for increased queen fecundity and colony population.

In support of this hypothesis is our observation of a declining colony of *T. urichi* whose fortunes were dramatically reversed after providing it with fungal material taken from *Atta cephalotes*. Within a period of 12 months not only did the worker population rise above that of two other colonies of the same species but large numbers of sexuals were produced. Subsequent plate culture of isolates verified that it had indeed adopted the more productive fungal strain.

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Acyl fluorides as reactive mimics of aldehyde pheromones: hyperactivation and aphrodisiac in *Heliothis virescens*[1]

G. D. Prestwich, J. F. Carvalho, Y.-S. Ding and D. E. Hendricks*

*Dept of Chemistry, State University of New York, Stony Brook (New York 11794-3400, USA), and *Subtropical Crop Insects Research, US Dept of Agriculture, Agricultural Research Service, Brownsville (Texas 78520, USA), 7 October 1985*

Summary. Substitution of fluorine for the aldehydic hydrogen provides behaviorally active, chemically reactive pheromone mimics. In male moths of the tobacco budworm *Heliothis virescens*, Z9–14:Acf and Z11–16:Acf cause hyperactivity and irreversible extension of the genitalia in over 80% of treated moths. In addition, a combination of the two components leads to 10–50% of the pairs involving one treated partner becoming locked in copula.
Key words. Acid fluoride; aldehyde; mating disruption; pheromone analogs; *Heliothis;* Lepidoptera; fluorination.

The use of pheromones and pheromone mimics for the suppression of insect populations by mating disruption is becoming practical and environmentally acceptable[2–4]. Substantial quantities of pheromones are required for this management strategy, since saturation of the atmosphere over large areas (> 3 ha) for several days and nights must be achieved to effectively reduce mating. An alternative approach, where insect pheromone receptors would be stoichiometrically and irreversibly modified, would in principle require much less chemical and shorter exposure times. We now report the synthesis of two acyl fluorides which mimic the structures of the two major aldehyde components of the pheromones of *Heliothis* species. With *H. virescens*, these acyl fluorides elicit aberrant sexually-oriented behavioral responses in male moths which suggest the involvement of a novel form of sensory disruption based on receptor modification.

The tobacco budworm, *Heliothis virescens*, uses a 15:1 blend of (Z)-11-hexadecanal (Z11–16:Ald) and (Z)-9-tetradecenal (Z9–14:Ald) as the behaviorally important components[5] of a blend of seven aldehydes identified from female glands[6,7]. The importance of the minor aldehyde and alcohol components in eliciting complete mate-finding and mating behavior by *H. virescens* males has been demonstrated in recent wind-tunnel experiments[8]. Formates[9] and diolefins[10] have been employed as stable aldehyde analogs for mating disruption of *H. virescens* and *H. zea*, and give significant reductions in field populations of these important pests. We envisioned the use of (Z)-11-hexadecenoyl fluoride (Z11–16:Acf) and (Z)-9-tetradecenoyl fluoride (Z9–14:Acf) as reactive analogs of the *H. virescens* pheromone aldehydes. Precedent for this analogy is found in the use of retinoyl fluoride as a mimic of retinal to give specific inactivation of bovine opsin[11]. The inactivation is due to the irreversible formation of a covalent amide adduct with the primary amino group of opsin, instead of the normal reversible Schiff base formation which occurs to give rhodopsin. We postulated that aldehydes might interact with primary amino groups of a putative receptor protein in a 'fast-and-loose' Schiff base formation[12]. Protein conformational changes could then mediate the transduction of the olfactory stimulus into a nerve impulse[13] and resulting behaviors. With this simple model, an acyl fluoride

Number of *H. virescens* moth pairs locked in copula (L) or dead (D) after 5 h exposure to 2 mg of acyl fluoride(s) on a paper wick. Seven moth pairs were evaluated in each of four possible crossed mating combinations (T = treated, N = not treated). Mating success as total spermatophores from 7 ♀ is also shown

		T♂ × T♀	T♂ × N♀	N♂ × T♀	N♂ × N♀
Z9-14:Acf	Day 1	1 L	0 L	0 L	0 L
	Day 4	3♂, 1♀ D	1♂, 1♀ D	1♂, 1♀ D	0 L, 0 D
	Total spermatophores	15	17	17	15
Z11-16:Acf	Day 1	0 L	0 L	0 L	0 L
	Day 4	1 L, 0 D	1 L, 0 D	2 L, 0 D	0 L, 0 D
	Total spermatophores	18	13	17	15
Z9-14:Acf	Day 1	3 L	4 L	1 L	0 L
	Day 4	1♀, 3♂	1♀, 2♂ D	2♀, 1♂ D	0 L, 0 D
+ Z11-16:Acf (1:1)	Total spermatophores	9	13	7	14

with a pheromone-like side chain could bind to the receptor and then form an amide linkage to the protein, thereby locking the sensory transduction mechanism in an activated state (fig.).

Materials and methods. The two acyl fluorides Z9–14:Acf and Z11–16:Acf were prepared by fluorination of the corresponding carboxylic acids with the hexafluoropropene-diethylamine reagent as described in the following representative synthetic procedure. Distilled (140 °C/0.30 torr) Z9–14:Ald (1.18 g, 5.6 mmol) and 1.05 g (6.2 mmol) of $AgNO_3$ were dissolved in 15 ml of 2:1 ethanol-water and treated (dropwise) with 1.06 g (26.4 mmol) of NaOH in 10 ml H_2O at 20 °C. After 1 h, Z9–14:Acid (0.98 g, 77%) was isolated by filtration, evaporation, acidification, extraction with ether, and chromatography on silica gel with hexane-ethyl acetate-acetone 8:2:1. A solution of 500 mg (2.2 mmol) of Z9–14:Acid in ether was added to 1.07 g (4.9 mmol) of 1,1,2,3,3,3-hexafluoropropyldiethylamine[14], the ether was removed under N_2, and the mixture was heated at 60 °C for 2 h. The reaction was cooled, diluted with ether, washed (H_2O, satd. NaCl), dried ($MgSO_4$), concentrated in vacuo, and distilled to give 434 mg (85%) of homogeneous acyl fluoride: IR 1840 cm^{-1} (RCOF); ^{1}H-NMR ($CDCl_3$) δ 2.05 (br d, H–8, 11), 2.52 (t, J = 6 Hz, H–2), 5.33 (t, J = 6 Hz, H–9, 10); HRMS, calcd. for $C_{14}H_{25}OF$, 228.1890; found, 228.1878. Analogous procedures led from Z11–16:Ald to Z11–16:Acf: HRMS, calcd. for $C_{16}H_{29}OF$, 256.2203; found, 256.2218.

The acyl fluoride Z11–16:Acf showed a half-life of 2.5 h on a cotton wick and 2–20 days in anhydrous organic solvents (1 mg/ml). In both cases, the corresponding acid was the only detectable decomposition product. The purity of the acyl fluoride and the extent of hydrolysis to the parent acid were readily assessed by capillary chromatography on a Durabond DB-5 30 m × 0.25 mm fused silica column at 180 °C (T_i)+5 °C/min to 220 °C (T_f).

Moths of both sexes were exposed to the individual acyl fluorides and to a 1:1 combination of the two as follows. Untreated moths were held separately and used in crossed mating experiments. All moths were held in a 14 h L:10 h D photoperiod, not reversed. Chemical exposures were made at 2–3 h after lights on. Acyl fluoride (2 mg/0.2 ml hexane) was applied to filter paper and after 10 min placed in each of 6 cartons containing 5 *H. virescens* adults (15 males and 15 females total). Their immediate responses were noted, and observation was continued for 5 h. After 5 h treated (T) and untreated (N) moths were paired in larger uncontaminated containers for 4 days (7 pairs per combination). Moths were provided with 10% sucrose solution during this period. On day 5, females were dissected and mating success quantified by counting spermatophores in the bursa copulatrix.

Results and discussion. Normally, *H. virescens* males are unresponsive to pheromonal stimuli during daylight. However, for all three tests, 65–85% of all males exhibited an immediate, whole-body response for the first 10 min. This response was sexually oriented. They became alert and hyperactive, walking briskly with wings flat on their backs in a pre-flight attitude, and fluttered the wings. Males extended genitalia and/or claspers half-way. Higher doses of Z9–14:Acf (10 mg) led to irreversible clasper extension in preliminary experiments. Lower doses produced activation without the dramatic, irreversible extension of genitalia. The saturated acyl fluoride 16:Acf did not produce these responses, even at the higher dose. Females did not respond to even high doses of acyl fluorides, remaining quiescent with wings canopied over the abdomen.

The four possible crossed-mating combinations were assessed for aberrant sexual behavior. When either males or females were exposed to the acyl fluorides, particularly the Z9–14:Acf and Z11–16:Acf combination, significant numbers of moths ended up locked (and dying) in copula (table). The unexpected locking of untreated males to treated females indicates that acyl fluorides can adsorb to the female cuticle, and that sufficient residual acyl fluoride remains after 5 h to elicit a behavioral response from males. This effect had been previously observed in the field for interspecific coupling of *H. zea* males to *H. virescens* females in the presence of Z9–14:Ald.[15,16].

From these preliminary experiments, we conclude that the acyl fluorides Z9–14:Acf and Z11–16:Acf are indeed behaviorally active analogs of the *H. virescens* aldehyde pheromones. They are hyperagonists, producing aberrant aphrodisiac-like sexual responses, consistent with the simple receptor model we have proposed. The use of these reactive pheromone analogs for affinity labeling receptor proteins, for altering flight behavior in wind tunnels, and for modifying electrophysiological responses of antennae to pheromone, will be reported subsequently[17]. The use of these reactive pheromone mimics as mating disruptants[18] would require a careful evaluation of their field stability and environmental acceptability.

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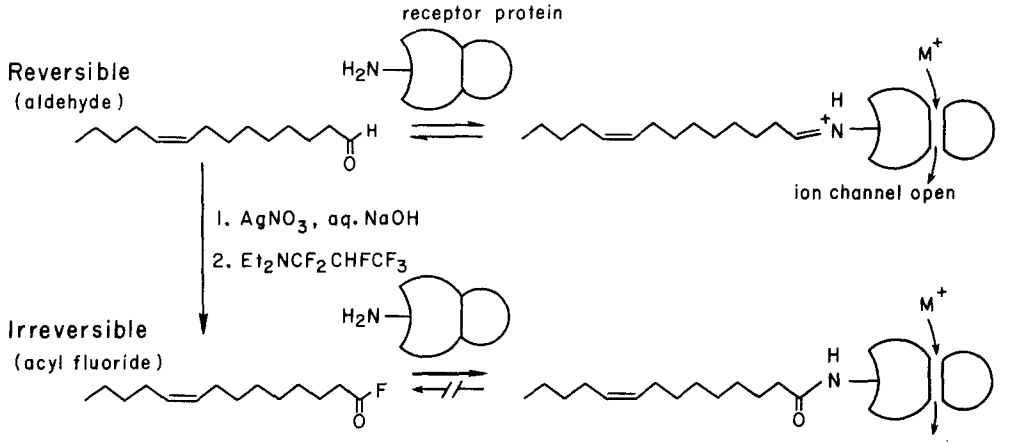

Proposed model for aldehyde action and irreversible mimicry by acyl fluoride, producing hyperagonist effect by locking ion channel in 'open' or by preventing removal of analog from the receptor protein. The synthetic conversion of Z9–14:Ald to Z9–14:Acf is also indicated.

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Correction

L. Hellgren, V. Mohr and J. Vincent: Proteases of Antarctic krill – a new system for effective enzymatic debridement of necrotic ulcerations, *Experientia 42 (1986) 403–404*. Table 2: streptokinase-streptodornase, 10 mg/ml, time of exposure 2 h is − (−), and not (−). Table 4: Trypsin, 10 mg/ml, time of exposure 24 h is − −, and not − − −.

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