diencephalic junction (fig. 1, a). This region may correspond to the tuberculum posterius, which is incorporated into the bulk of the midbrain in higher vertebrates[6]. Therefore, it is of great interest, from a phylogenetic point of view, that a number of catecholaminergic neurons were found in the telencephalon (basal nuclei or paleostriatum) but a few catecholaminergic ones were noticed in the mesencephalodiencephalic junction.

1 Reprint requests should be addressed to I.N., Department of Anatomy, School of Medicine, Fujita-Gakuen University, Toyoake, Aichi 470-11 (Japan).
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Fatal interspecific mating of two *Heliothis* species induced by synthetic sex pheromone[1]

E.A. Stadelbacher, M.W. Barry, A.K. Raina and J.R. Plimmer

Southern Field Crop Insect Management Laboratory, U.S.D.A., Stoneville (Mississippi 38776, USA), Pheromone Programs, Hercon Division, Health-Chem Corp., 1107 Broadway, New York (New York 10010, USA), and Organic Chemical Synthesis Laboratory, U.S.D.A., Beltsville (Maryland 20705, USA), April 27, 1983

Summary. Field tests showed that 3 components of the *Heliothis virescens* sex pheromone when deployed individually, significantly disrupt pheromone communication of males of this species and of *H. zea*. Field and wind tunnel tests indicated that males of *H. zea* are attracted to and mate with the females of *H. virescens* when the atmosphere is permeated with *(Z)*-9-tetradecenal or (*Z*)-11-hexadecen-1-ol. This mating is fatal to both individuals because of morphological incompatbility of their genitalia. Habituation of *H. zea* males to these compounds is the most likely reason for this attraction between 2 species that show reproductive isolation in nature.

The bollworm *(Heliothis zea)* and the tobacco budworm *(H. virescens)* are major pests of a large number of crops in the United States. The average annual loss due to crop damage plus cost of control for these 2 pests in the U.S. was estimated in 1976 to exceed 1 billion dollars[2]. Of comparable inportance are the environmental hazards associated with broad spectrum insecticides used to control these pests. Recent developments in the identification and synthesis of female sex pheromones of many pest insects have created great interest in their use in pest management as trap lures and as mating disruptants[3,4]. The 4 components of the *H. zea* female sex pheromone [(Z)-11-hexadecenal (Z-11-HDAL), *(Z)*-9-hexadecenal (Z-9-HDAL), *(Z)*-7-hexadecenal (Z-7-HDAL) and hexadecanal (HDAL)] are also

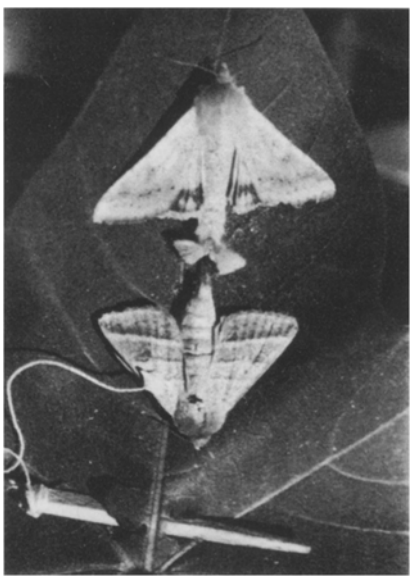
Table 1. Field evaluation of individual components of *Heliothis* spp. pheromones as mating disruptants and /or confusants for *H. zea* and *H. virescens* males by air permeation in cotton fields over a 9-day post-treatment period[a,b]

Treatment	$\bar{X}$ Number feral ♂♂ captured/trap/night in traps baited with:				Mating between tethered *Heliothis* spp. ♀♀ and feral *Heliothis* spp. ♂♂			
	H. zea ♀♀		*H. virescens* ♀♀		% *H. zea* ♀♀ mated to		% *H. virescens* ♀♀ mated to	
	H. zea ♂♂	*H. virescens* ♂♂	*H. zea* ♂♂	*H. virescens* ♂♂	*H. zea* ♂♂	*H. virescens* ♂♂	*H. zea* ♂♂	*H. virescens* ♂♂
Z-11-HDAL	0.03^{c}	0.00^{b}	0.00^{e}	0.58^{d}	1.56^{d}	0.00^{a}	0.00^{e}	7.19^{c}
Z-9-TDAL	$8.53^{b,**}$	0.64^{a}	$18.17^{a,*,**}$	3.03^{c}	38.75^{b}	0.00^{a}	18.75^{a}	15.10^{b}
TDAL	$9.64^{b,*,**}$	0.03^{b}	1.08^{d}	$8.83^{a,*,**}$	28.44^{c}	0.00^{a}	6.25^{c}	29.69^{a}
Z-11-HDOL	$10.72^{b,**}$	0.03^{b}	6.17^{b}	$5.53^{b,*}$	27.19^{c}	0.00^{a}	8.33^{b}	26.67^{a}
Untreated check	$13.44^{a,*,**}$	0.03^{b}	1.56^{c}	$10.61^{a,*,**}$	48.75^{a}	0.00^{a}	1.56^{d}	35.52^{a}

[a] Hypothesis on trap and mating table data were tested using ranks of counts or percentages rather than actual counts or percentages to address potential non-normality of data. [b] Ranks of means followed by different letters in the same column are significantly different at the 5% level of probability by Duncan's multiple range test. * Indicates significant difference between mean ranks between the numbers in columns 1 vs 3 or between the numbers in columns 2 vs 4 for each treatment. Pairwise t-test ($p < 0.05$). ** Indicates significant difference between mean ranks between the numbers in columns 1 vs 2 or between the numbers in columns 3 vs 4 for each treatment. Pairwise t-test ($p < 0.05$).

present in the pheromone of *H. virescens*, which has 3 additional components [(*Z*)-9-tetradecenal (Z-9-TDAL), tetradecanal (TDAL) and (*Z*)-11-hexadecen-1-ol (Z-11-HDOL)][5]. In nature the 2 species do not exhibit any cross attraction. However, we found that when the atmosphere was permeated with Z-9-TDAL or Z-11-HDOL, *H. zea* males were captured in traps baited with *H. virescens* females and also mated with tethered *H. virescens* females, which resulted in the death of both individuals. We report here the results of extensive field and laboratory tests that elucidate disruption of pheromone communication and the basis of fatal interspecific mating of *H. zea* males with *H. virescens* females induced by synthetic pheromones. The results contribute to a better fundamental understanding of recently reported field observation[6].

Three of the components of *H. virescens* pheromone (Z-9-TDAL, TDAL and Z-11-HDOL) and Z-11-HDAL were formulated in 6×6 mm multi-layered polymeric dispensers, and dispersed by a Hercon® Schweizer flake applicator mounted on a high-clearance spray rig at 25 g ai/ha to cotton plots in Mississippi. Each test plot comprised 30, 61-m-long rows of cotton (0.202 ha) separated by similar sized buffer plots. A randomized complete block design with 4 replicates was used. To determine long-range pheromone communication disruption, 2 cone traps, one baited with 3 virgin females of *H. virescens* and the other with 3 of *H. zea*, were placed in each plot. The females were replaced on Monday, Wednesday, and Friday during the 9-day post-treatment test period. Trapped males were removed and identified daily, Monday through Friday. Trap-catch data are expressed as mean numbers of males of each species captured per trap night for a total of 36 trap nights for each species and treatment. Mating disruption and/or confusion was studied with tethered females (the fore-wing of the female moth was glued to one end of a fine string, the other was attached to a fully opened leaf in the main stem terminal of a cotton plant). On 5 occasions during the 9-day post-treatment test period, 3 females of each species were randomly tethered to plants in each of rows 12 and 22 of each plot at 16.00 h and collected at 08.00 h the following day. Records of missing and dead females as well as incidence of intra- and interspecific matings were maintained.



Male *Heliothis zea* (top) and female *H. virescens* (tethered to a leaf) in fatal copulation in a cotton field permeated with (*Z*)-9-tetradecenal.

Permeation of the atmosphere in the test plots with Z-11-HDAL caused almost total disruption of pheromone communication and significantly reduced the trap catch of both species throughout the test period (table 1). This confirms earlier reports of trap catch reduction with Z-11-HDAL[7,8]. This compound also significantly reduced the mating of tethered females of both species.

Permeation of the atmosphere in the test plots with Z-9-TDAL significantly reduced the catch of male *H. zea* and *H. virescens* in traps baited with females of their respective species (table 1). However, while this compound disrupted intraspecific pheromone communication in both species it also induced a significant level of interspecific attraction of male *H. zea* to the pheromone plume of female *H. virescens*. In this treatment, as indicated by t-test analysis, the traps baited with female *H. virescens* captured significantly larger numbers of male *H. zea* than *H. virescens*. Furthermore, the t-test analysis shows the numbers of male *H. zea* captured in the traps baited with female *H. virescens* were significantly larger than the numbers of male *H. zea* captured in the traps baited with female *H. zea*. These trap catch data (which may be indicative of long-range pheromone communication) show that in atmosphere permeated with Z-9-TDAL *H. zea* males prefer or are more strongly attracted to the pheromone plume of female *H. virescens* than to the pheromone plume of their own species. The trap catch data also show for the 1st time that significant (even though low) numbers of male *H. virescens* were attracted to and captured in the traps baited with female *H. zea*. The incidence of mating of tethered female *H. zea* and *H. virescens* with males of their respective species was reduced significantly in the Z-9-TDAL treated plots. As with trap catch, male *H. zea* were attracted by the pheromone plume of the female *H. virescens*. However, in this case they readily copulated with the tethered females. Because the genitalia of these 2 species are morphologically incompatible, such copulations result in the death of both individuals (fig.).

Permeation of the atmosphere with TDAL disrupted intraspecific pheromone communication of *H. zea*, but not *H. virescens*. The disruption resulted in significant reductions in trap catch and mating of *H. zea*. The treatment also caused a low but significant level of interspecific matings between male *H. zea* and female *H. virescens*. Air permeation with Z-11-HDOL caused disruption in intraspecific pheromone communication of both *H. zea* and *H. virescens* that resulted in significant reductions in the catch of male *H. zea* and *H. virescens* in traps baited with conspecific females. Statistical analysis of the data indicated that under these conditions the pheromone plumes of the female

Table 2. Percentage response of *H. zea* male moths to different pheromone mixture in a wind tunnel

Pheromonal stimulus	*H. zea* male response		
	Upwind flight in pheromone plume	Exhibited hair-pencil extension	Attempted to mate with the pheromone source*
H. zea (synthetic)	88.6 a	100.0 a	99.4 a
H. zea + Z-9-TDAL	36.8 b	99.6 a	31.1 c
H. virescens (synthetic)	12.1 c	49.1 b	0.0 d
H. virescens + Z-9-TDAL	80.0 a	93.4 a	60.4 b
H. virescens + Z-11-HDOL	11.8 c	38.7 b	2.8 d

* The interference population for hair-pencil extension and mating response was restricted to those males that flew upwind in the plume. Means in a column not followed by the same letter are significantly different at $p < 0.05$ (t-test).

H. virescens or *H. zea* are equally attractive to male *H. zea*. The Z-11-HDOL treatment caused a significant reduction in intraspecific mating of *H. zea* but not of *H. virescens*. Permeation of the atmosphere with Z-11-HDOL also caused interspecific attraction of male *H. zea* to the pheromone plume of female *H. virescens* and resulted in significant numbers of fatal matings between these individuals. However, the incidence of interspecific mating, was significantly lower than in the Z-9-TDAL treatment.

The 2 compounds (Z-9-TDAL and Z-11-HDOL) were subsequently tested in a 3-m long horse-show shaped wind tunnel made from clear poly-carbonate plastic with a floor of clear Plexiglass®. Air from 2 air conditioner units was blown by a variable speed fan through a Varicel® air filter and cheese cloth screens into the tunnel at a velocity of 50 cm/sec. Light from red colored light bulbs provided an illumination of 2.5 lux. Temperature in the tunnel was maintained at 25 ± 2 °C. Plume width as visualized by smoke from TiCl/4 was about 1 cm at the point of origin and 15 cm at the down-wind end of the tunnel. 20 µl of the synthetic mixtures of the pheromones of *H. zea* (11.5 ng of Z-11-HDAL, 0.45 ng Z-9-HDAL, 0.26 ng of Z-7-HDAL and 1.1 ng of HDAL/µl) or *H. virescens* (same amounts of the compounds as in *H. zea* pheromone plus 0.69 ng Z-9-TDAL, 0.23 ng TDAL and 0.9 ng Z-11-HDOL/µl) were applied to filter paper strips and suspended at 22 cm from the up-wind end of the tunnel. For air permeation 10 µg of the pheromone component was applied to a cotton dental plug and placed behind the blower fan. 2–3-day-old *H. zea* males were released singly from cylindrical cages at the down-wind end and their responses were monitored for 2 min. Five observations were made for each treatment and the test was replicated 14 times.

Results of these tests are given in table 2. Consistent with the field results, permeation of air with Z-9-TDAL caused a significant increase in the number of *H. zea* males that followed the *H. virescens* synthetic pheromone plume. The compound also significantly increased the number of males exhibiting hair-pencil extension (a pre-copulatory behavior) and attempts to mate with the pheromone source. However, permeation with Z-11-HDOL did not cause significant increase in the above responses.

Z-9-TDAL, and possibly Z-11-HDOL, are critical components in the *H. virescens* pheromone that are responsible for pheromone specificity and reproductive isolation. These compounds must permit *H. zea* males to discriminate between *H. zea* and *H. virescens* females in nature. We postulate that habituation to Z-9-TDAL or Z-11-HDOL affects this ability of *H. zea* males to discriminate. Furthermore, when the atmosphere was permeated with Z-9-TDAL, *H. zea* males even appeared to prefer *H. virescens* females to those of their own species, as reflected by higher trap catches.

The occurrence of interspecific matings has only been observed with tethered females and in laboratory cages. The importance of pheromone-induced fatal matings between these 2 species can only be determined after wide area field trials.

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A simple method of obtaining multiple blood samples from the portal vein and the hepatic vein in the rat in vivo

E. Scholtens and G.J. Mulder

Department of Pharmacology, State University of Groningen, Bloemsingel 1, NL-9713 BZ Groningen (The Netherlands), November 26, 1982

Summary. A very simple and rapid technique for inserting a catheter in the portal vein and the hepatic vein in the anesthesized rat in vivo is described. The pointed, saline-containing PE tubing is frozen in liquid nitrogen, whereupon it is used as a 'needle' to insert the catheter into the blood vessel. Multiple blood samples can be obtained from the portal and the hepatic vein at the same time, so that in situ extraction of drugs by the liver can be measured in vivo, since hepatic blood flow is uninterrupted.

Recently a simple and rapid technique for obtaining serial blood samples from the portal vein in the rat has been described[1]. A catheter is inserted into the portal vein of the rat under anesthesia, after first inserting a 23-gauge needle in the vessel; the needle is slightly lifted ventrally to act as a guide for the polyethylene (PE) tubing used for catheterization. When the needle is removed, the catheter is fixed into place by a drop of cyano-acrylate ester cement.

Although this method is an improvement over previous methods, the portal vein still has to be teased free from the mesentery and there is some blood loss. Furthermore, the method cannot be used to catheterize the hepatic vein. The latter would be a very useful technique, because then the extraction of drugs by the liver in vivo could be followed. We present a simplified method for catheterizing both the portal vein and the hepatic vein while leaving the hepatic