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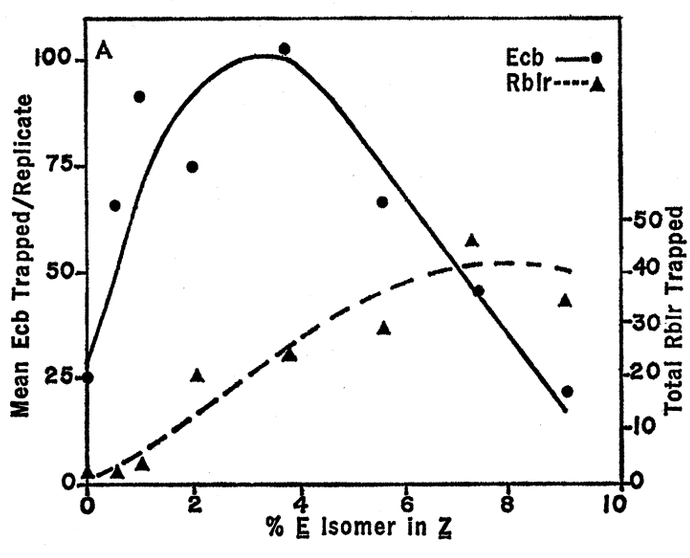
Insect Sex Pheromones: Minor Amount of Opposite Geometrical Isomer Critical to Attraction

Abstract. *(Z)-11-Tetradecenyl acetate is the reported sex pheromone of European corn borer and redbanded leafroller moths. However, geometrically pure preparations of the compound are weakly attractive to these species. Presence of the* E *geometrical isomer in the* Z *is necessary for maximum sex attraction and these moths are "tuned" to respond optimally to specific proportions of* Z *to* E. *This discovery is important to considerations of moth pheromonal specificity, evolution, and in application of knowledge of the pheromones to insect-pest suppression.*

(*Z*)-11-Tetradecenyl acetate has been reported as the sex pheromone of two economically important insect pests—the European corn borer, the Iowa strain *Ostrinia nubilalis* (Hübner) (*1*), and the redbanded leafroller, *Argyrotaenia velutinana* (*2*). However, in studies at Ankeny, Iowa, the European corn borer was only weakly attracted and the redbanded leafroller was not attracted to geometrically pure (*Z*)-11-tetradecenyl acetate (*3, 4*). In contrast, when small amounts of (*E*)-11-tetradecenyl acetate were added to (*Z*)-11-tetradecenyl acetate, the attraction for both species was dramatically enhanced although neither species showed any behavioral response to the *E* isomer alone. Moreover, a defined concentration of the *E* isomer in the *Z* isomer is required for maximum attraction of European corn borer and redbanded leafroller males, and each species responds optimally to different proportions of the geometrical isomers. The finding that the maximum attraction in these species depends on the isomeric proportion provides a useful concept of pheromonal specificity and speciation in Lepidoptera and suggests a potentially practical method of disrupting the chemical communication between the sexes of these two species.

The requirement of the *E* isomer for optimum attraction became obvious in a preliminary field test (*5*). In the test, a total of 66 European corn borer males were caught in traps baited with the pure *Z* isomer, and 171 were caught in the traps baited with an isomeric mixture. Results with the redbanded leafroller were even more striking. None were attracted to traps baited with the pure *Z* isomer, and 127 were attracted to the isomer combination. In a definitive test of the effect of varying proportions of *Z* to *E*, samples of pure (*Z*)-11-tetradecenyl acetate (100 μg) mixed with pure (*E*)-11-tetradecenyl acetate (0 to 10 μg) (*6*) on rubber septa were exposed in the field traps. The results are shown in Fig. 1A. The presence of as little as 0.5 percent of the *E* isomer caused the attraction of European corn borer males to rise sharply, and maximum numbers were caught with the mixture containing 100 μg of *Z* plus 4 μg of *E*. Further increase in the *E* isomer produced decreased attraction, which is in accord with the known inhibitory effect of high concentrations of (*E*)-11-tetradecenyl acetate (*7*). In contrast, the redbanded leafroller showed a continuous rise in captures as the concentration of *E* was in-



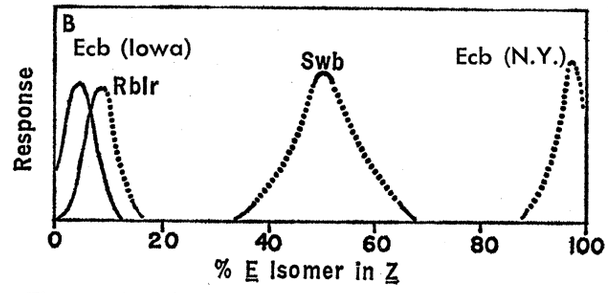


Fig. 1. (A) The response of male European corn borers (*Ecb*) and redbanded leafrollers (*Rbir*) to increasing amounts of (*E*)-11-tetradecenyl acetate added to the *Z* isomer. The experiment was conducted 10 to 31 August 1972 in a randomized block design with three subsamples of each concentration of *E* isomer, and four replications. The standard error for Ecb means plotted is 10.4. Statistical analyses showed quadratic effects were highly significant for both Ecb and Rblr. (B) Sex attraction response maxima of four moth species to different proportions of the *Z* and *E* isomers of 11-tetradecenyl acetate. Dotted lines represent concentrations where no data was obtained; *Swb*, smartweed borer.

creased from 0.5 to 7 percent. Concentrations of *E* isomer that were inhibitory to this species (*8*) lie beyond the 8 percent *E* isomer level.

The data therefore show that the olfactory systems of the European corn borer and the redbanded leafroller are so organized that they permit the detection of small variations in the geometric composition of the attractant and that each species responds optimally to different geometrical proportions of 11-tetradecenyl acetate. This discriminatory capability and specificity of response is not unique. For example, the smartweed borer, *Ostrinia obumbratalis*, is attracted to a 1 : 1 mixture of the *Z* and *E* isomers of 11-tetradecenyl acetate (*9*), but is not attracted to 2 : 1 or 1 : 2 mixtures. Optimum response to a narrow range of ratios of geometrical isomers may therefore be a reasonably general phenomenon among moth species (*10*).

The occurrence and significance of geometrical mixtures in pheromonal secretions, particularly the minor amounts of one isomer in another, may have gone undetected because of the nature of the bioassays and the chemical methods used routinely in investigations of sex pheromones. Moreover, synthesis of the olefinic chemical, particularly of the *Z* isomer, by reduction of the acetylene usually produces 95 to 98 percent (*11*) of the target geometrical isomer instead of the pure isomer. Thus, the finding of high activity for the product could cause an investigator to miss the requirement of the second isomer.

In a survey of the literature on olefinic sex pheromones of Lepidoptera, we found few instances in which the exact geometric composition of the synthetic pheromone was reported. In view of our results, these studies might profitably be reevaluated. We suggest that future studies of pheromones should routinely include an investigation of the response as a function of the geometrical isomer composition of the pheromone.

The capability of moths to distinguish mixtures of geometrical isomers may be of importance in evolutionary considerations. For example, species of moths in certain genera (*12*) and the European corn borer in Iowa and in New York (*13*) are known to respond to opposite geometric isomers of the same compound. Hypothetically, the evolution of such species and strains from a common ancestor could have involved gradual and divergent drift in the specificity of response from one geometric proportion to another. Indeed, the dependence of optimum responses on specific mixtures of geometrical isomers can be illustrated diagrammatically, as it has been in Fig. 1B for the European corn borer (Iowa), the redbanded leafroller, the smartweed borer, and the European corn borer (New York). The solid lines represent existing data, and the dotted lines are speculative. As the diagram suggests, various ratios of the *E* and *Z* isomers will attract predominantly the European corn borer (Iowa) or redbanded leafroller males. The smartweed borer requires a substantially different ratio of the same geometrical isomers, one that does not overlap the ratio for the redbanded leafroller. It is amusing to speculate that optimum attraction of the New York strain of the European corn borer may well be dependent on small concentrations of the *Z* isomer in the *E* isomer.

Table 1. Effect on attraction of distribution of (*E*)-11-tetradecenyl acetate in the field. The experiment was conducted 21 to 27 August 1972. Abbreviations: ♀, four unmated European corn borer females; *Z*, synthetic lure (100 μg of *Z* plus 4 μg of *E*); *E*, (*E*)-11-tetradecenyl acetate.

Replicate	Plot	Male catch			
		European corn borer		Redbanded leafroller	
		♀	*Z*	♀	*Z*
1	*E*-treated	2	34	0	4
	Control	16	217	9	44
2	*E*-treated	7	50	0	3
	Control	9	375	0	19
3	*E*-treated	4	6	1	0
	Control	13	212	0	15
4	*E*-treated	1	11	0	6
	Control	11	178	0	15
Total	*E*-treated	115		14	
	Control	1031		102	

Even though (*Z*)-11-tetradecenyl acetate is the major component of the sex pheromone of the European corn borer in Iowa, the insect is obviously exquisitely sensitive to small amounts of the *E* isomer. Even a few micrograms of *E* isomer placed in insect traps baited with unmated European corn borer females inhibits the attractiveness of these females for the males (*7*). This finding taken in combination with the knowledge that the proportion of the *Z* and *E* isomers perceived by the male is critical to maximum attraction suggested that distribution of the *E* isomer in cornfields might sufficiently alter the geometric proportion of *Z* to *E* perceived by males to reduce their mate-finding efficiency. Therefore (*E*)-11-tetradecenyl acetate (*6*) was formulated on cork granules and distributed in the field (*14*). The results (Table 1) showed that an overall reduction of 89 percent in attraction (compared with control) was achieved by the release of the *E* isomer. The data also demonstrated the greater effectiveness of the synthetic lure compared with the females. The ratio of total male European corn borer attracted by borer females was 14 to 49 (the ratio of the number of males caught in treated area to the number of males caught in control areas) or 71.5 percent reduction in female attractiveness in the areas treated with *E* isomer. Similar data for redbanded leafroller moths in the same experiment showed an 86 percent reduction in attraction due to the inhibiting effect of excessive amounts of the *E* isomer. Thus, owing to the dependence of sex attraction in these species upon geometrical proportions, their chemical communication system is susceptible to disruption. The use of this disruptive technique to reduce mating by insect pests may be an easier method of control to implement than has been thought.

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3. Synthetic (*Z*)-11-tetradecenyl acetate was freed from traces of the *E* isomer by column chromatography on silica gel impregnated with 15 percent silver nitrate by using a mixture of hexane and benzene (1 : 1, by volume) as solvent. The *Z* isomer was then purified further by preparative gas-liquid chromatography (GLC). The product obtained from preparative GLC was verified as pure *Z* isomer by capillary GLC analysis (*4*).
4. Capillary gas chromatography on a support-coated open tubular (SCOT) column; EGSS-X, 15.24 m by 0.05 cm, 150°C, helium carrier gas at 4 ml/min gave near baseline separation of *Z* and *E* isomers of 11-tetradecenyl acetate. Retention times were 8.3 and 8.7 minutes for *E* and *Z* isomers, respectively. By using this chromatographic system, about 0.1 percent of one isomer in the other is detectable.
5. Twenty insect traps (1-gallon cylindrical cartons coated on the inside with Tack-Trap) placed 9.1 m apart and 1.5 m from the ground at the edge of a cornfield were baited

alternately with 100 μg of *Z* isomer and with 92.5 μg of *Z* plus 7.5 μg of *E* isomer on rubber septa. The test was conducted over 12 consecutive nights.
6. The *E* isomer was prepared by sodium metal reduction of the tetrahydropyranyl ether of 11-tetradecynol in anhydrous liquid ammonia followed by acetylation with a mixture of acetic acid and acetic anhydride (10 : 1.5, by volume). Slow spinning band distillation of the product then gave, according to capillary GLC analysis (*4*), pure compound.
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14. Formulation was 12/20-mesh cork plus (*E*)-11-tetradecenyl acetate (7 percent by weight) plus paraffin (8 percent). A chloroform solution of paraffin and *E* isomer was added to the cork granules, and the solvent was evaporated. A "Cyclone" Seed Sower was used to distribute 40 g of granules (2.8 g of *E* isomer) on a row (97.5 m) of corn growing along the perimeter of each of four fields. The exact granule distribution pattern is not known. Ten insect traps baited alternately with a mixture of 100 μg of *Z* isomer plus 4 μg of (*E*)-11-tetradecenyl acetate on a rubber septum and with four unmated European corn borer females were positioned 9 m apart in each of the rows treated with *E* isomer, and ten similar traps were positioned in untreated areas 27.4 m distant, but within the same row treated with *E* isomer.
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Brain Calcium: Role in Temperature Regulation

Abstract. *Perfusion of the preoptic-anterior hypothalamus with excess calcium ion in ground squirrels produces a drop in core temperature. The magnitude of the drop is directly dependent on ambient temperature. Respiration, heart rate, and oxygen consumption are also reduced during perfusion of calcium ion. It is concluded that the depression of body temperature during calcium ion perfusion is due to generalized depression of the neurons of the preoptic-anterior hypothalamus.*

Recently it has been reported that alterations of the electrolyte balance of the cerebrospinal fluid can cause marked changes in deep body temperature. Perfusion of the cerebral ventricles of cats, primates, and rabbits with excess Ca^{2+} causes a drop in rectal temperature, whereas excess Na^{+} results in a rise in rectal temperature (*1*). Direct perfusion of these ions into the posterior hypothalamus causes similar changes in body temperature of primates and cats (*2*).

Myers and Veale (*3*) proposed an ionic mechanism for the internal temperature set point in which the ratio of Ca^{2+} to Na^{+} in the posterior hypothalamus determines the core temperature. We now report that perfusion of excess Ca^{2+} in the preoptic-anterior hypothalamus (POAH) of ground squirrels also causes a marked drop in rectal temperature. The level of the decrease of the body temperature is directly related to the ambient temperature (T_a) and is probably due to generalized depression of autonomic function.

In each of six ground squirrels (*Citellus beecheyi*) a guide tube was implanted stereotaxically and fixed to the skull with dental cement. The tip of the guide tube was located just dorsal to the POAH. The ionic balance of the POAH was selectively altered with a modified push-pull cannula lowered through the guide tube (*4*). A reciprocal infusion pump (Harvard Apparatus) was used to drive and collect the perfusion solutions. The precise location of the area perfused was determined by histological section (Fig. 1).

The animals were restrained in a sealed chamber, and oxygen consumption was monitored continuously with a Beckman G-2 paramagnetic oxygen analyzer. Rectal temperature and air temperature in the chamber were recorded continuously with thermistors. In several of the experiments, heat rate, respiratory rate, and pinna temperature were also recorded. The data were collected on a Vidar digital data acquisition system and stored on magnetic tape for computer analysis and plotting.

Each animal was allowed to equilibrate in the chamber for a minimum of 1 hour prior to perfusion. The perfusion flow was maintained at a constant rate of 20 μl/min; it consisted of normal Ringer solution (NaCl, 154 m*M*; KCl, 5.4 m*M*; and $CaCl_2$, 2.3 m*M*) or Ringer solution that contained either 6.9 or 20.7 m*M* excess concentration of $CaCl_2$. In these solutions the osmolarity was maintained constant by adjusting the concentration of NaCl appropriately.

Table 1. Effect of Ca^{2+} on core temperature and metabolism. Abbreviation: *N*, the number of animals.

N	Concentration of Ca^{2+}	$\Delta\bar{T}_r$* (°C)	T_a† (°C)	O_2 uptake (cm^3 g^{-1} hr^{-1})
2	Normal	− 0.17	25	1.10
1	Normal	− 0.10	12	1.90
6	6.9 m*M* excess	− 1.58	25	0.68
6	6.9 m*M* excess	− 4.51	12	0.81
2	20.7 m*M* excess	− 2.25	25	0.25

* Mean change in rectal temperature. † Ambient temperature.

The effects of 1-hour perfusions are listed in Table 1. Perfusion of normal Ringer solution in the POAH had no effect on rectal temperature or metabolism at a T_a of either 12° or 25°C. When Ringer solution containing an excess of Ca^{2+} (6.9 m*M*) was perfused in the same region, the initial response was a marked drop in the consumption of oxygen followed by a fall in rectal temperature. The decrease in rectal temperature after 1 hour of perfusion was dependent upon the T_a. Animals perfused at a T_a of 12°C showed significantly ($P < .01$) greater drops in rectal temperature than those perfused at a T_a of 25°C. There was no significant difference in oxygen consumption at these two ambient temperatures.

When 20.7 m*M* excess Ca^{2+} was perfused at an air temperature of 25°C, the metabolic rates of two squirrels dropped to less than 0.25 cm^3 of O_2 per gram per hour and rectal temperature dropped only 1.2° and 3.3°C, respectively. At the end of the perfusion period respiration was extremely weak and irregular. The animals were immediately removed from the chamber, artificially respirated, and kept warm with a heat lamp for approximately 2 hours but could not be revived.

Figure 2 shows the effects of changing air temperature during perfusion of the POAH with 6.9 m*M* excess Ca^{2+}. At time zero, the T_a begins to fall and the oxygen consumption increases so as to maintain a stable rectal temperature. At the beginning of perfusion (*On*) the initial response is