Reinvestigation of Oak Leaf Roller Sex Pheromone Components and the Hypothesis That They Vary with Diet

Abstract *The sex pheromone of the oak leaf roller,* Archips semiferanus, *was identified as a specific blend (67 : 33) of* trans-*11- and* cis-*11-tetradecenyl acetates. The pheromone blend of females from a semisynthetic diet and from three oak species did not vary significantly. Males from these diets responded in the laboratory and the field only to treatments approximating the 67 : 33 blend. These findings conflict with the hypothesis that the composition and perception of moth sex pheromones vary with slight changes in diet.*

The sex pheromone systems of closely related tortricid moth species (*1*) have proved to be very interesting, since subtle changes in pheromone component ratios or composition can effect specificity among sympatric species. We have studied the pheromone systems of many tortricid species, including a number in the genus *Archips*, and have found them all, so far, to consist of two to four pheromone components. Reports (*2–4*) on the sex pheromone of the oak leaf roller, *Archips semiferanus*, however, suggested that sexual communication in this tortricid differs decidedly from that in the others by involving at least 21 components. Moreover, the results of the previous *A. semiferanus* studies have led to a proposal of startling new concepts in insect chemical communication and evolutionary biology that demand evaluation. In essence, a recent report (*2*) indicated that slight changes in host plant constituents would be reflected in corresponding changes in the sex pheromone composition. More specifically, it was suggested that oak leaf roller moth females reared on one species of oak would emit a sex pheromone different from those produced by females reared on other species of oak. Differences in the dietary factors were then hypothesized to provide the evolutionary mechanism for diversification of some insect species. This hypothesis and many related ideas were developed from observing that (i) oak leaf roller moth females reared on semisynthetic diet without oak leaves did not produce detectable quantities of pheromone; (ii) the reported attractant pheromone, *cis*-10-tetradecenyl acetate (*5*), was a better attractant than other tetradecenyl acetate isomers for only part of the 2-week adult flight period; (iii) peak attractancy of the various 14-carbon acetate isomers occurred at distinctly different times during the flight period (this led to the interpretation that *A. semiferanus* was evolving subgroups on the different species of oak); (iv) male oak leaf rollers hairpencilled and attempted to copulate with damaged oak leaves; and (v) at least 21 isomeric tetradecenyl acetate compounds were found in oak leaf roller moth pheromone glands, and some of the components of this complex were found in all life stages of both male and female moths, as well as in varying composition in leaves of several oak species.

We have investigated a population of oak leaf rollers in the same geographical area used by the other investigators and our findings directly contradict the new hypothesis and most of the evidence on which it was based. We find that the sex pheromone is a 67 : 33 mixture of *trans*-11-tetradecenyl acetate (t11-14 : Ac) and *cis*-11-tetradecenyl acetate (c11-14 : Ac).

Archips semiferanus larvae (*6*) were reared successfully on a pinto bean–based artificial medium (*7*) without addition of oak leaves or oak extract. The abdominal tips of 2- to 3-day-old virgin females 4 hours into scotophase (16 hours light, 8 hours darkness) were extracted by soaking in methylene chloride. Electroantennogram (EAG) (*8*) assay of 1-minute collections of crude extract effluent from a nonpolar (OV-1) gas-liquid chromatography (GLC) column (*9*) revealed only one area of activity, which coincided with the retention times of standard monounsaturated 14-carbon acetates. Material from the active fractions was collected from a polar (XF-1150) GLC column (*10*), and EAG activity was again found only at the retention times of standard monounsaturated 14-carbon acetates. In the 14-carbon acetate region, XF-1150 GLC tracings of crude female tip extract and the active region collected from OV-1 were identical. Both revealed three very prominent peaks in the same ratios, two of which clearly coincided with the EAG active region (Fig. 1). Upon purification by GLC, components B and C were both highly EAG active but component A was not. The three major components were identified as tetradecyl acetate (14 : Ac), t11-14 : Ac, and c11-14 : Ac, respectively, as follows. Components A, B, and C were shown to be acetates by saponification and reacetylation. Samples of these

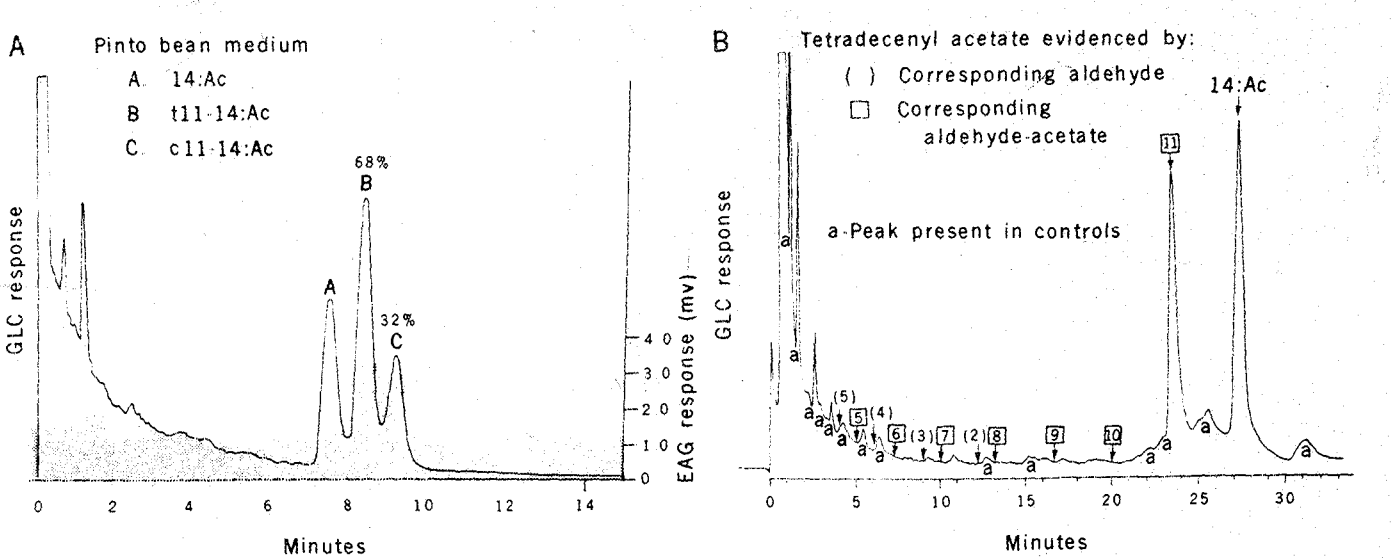

Fig. 1. (A) Tip extract from *A. semiferanus* females reared on pinto bean medium fractionated on an XF-1150 column at 154°C. The GLC tracing of crude extract and the EAG response (shading) to the 14-carbon acetates from crude tip extract collected from OV-1 are shown. (B) GLC tracing (OV-1) of microozonolysis products of the 14-carbon acetates from crude tip extract collected from OV-1.

components collected from GLC were treated with 5 percent KOH in methanol, yielding products having retention times (XF-1150) identical to those of tetradecanol, *trans*-11-, and *cis*-11-tetradecen-1-ol (*11*). Treatment of the hydrolysis products with acetyl chloride gave products possessing the original retention times on XF-1150.

The component mixture was then separated by silver nitrate thin-layer chromatography (TLC) (*12*). Cochromatography of the crude pheromone extract from 50 female tips with 12-carbon acetate standards yielded three TLC bands corresponding to saturated, *trans*, and *cis* compound areas. Each of the three bands yielded a single major peak (*13*) when analyzed by GLC (XF-1150). Precise retention times relative to an internal standard, *cis*-11-pentadecenyl acetate, showed that (i) the component in the top TLC area (saturated compounds) had exactly the same retention value as 14 : Ac (mean ± standard deviation = 0.567 ± 0.002); (ii) the component in the second band (*trans* compounds) had a value matching that of t11-14 : Ac (0.641 ± 0.001 compared to 0.640 ± 0.002 for t11-14 : Ac and 0.628 ± 0.002 for t10-14 : Ac); and (iii) the component in the third TLC band (*cis* compounds) had a value matching that of c11-14 : Ac (0.702 ± 0.004 compared to 0.704 ± 0.002 for c11-14 : Ac and 0.684 ± 0.003 for c10-14 : Ac). The relative retention times for all other *cis* and *trans* 14-carbon acetate isomers were significantly different from those for the isolated compounds.

Microozonolysis of material (2000 to 3000 ng) collected from the 14-carbon acetate region from OV-1 yielded 11-oxoundecyl acetate (*14*). Aside from component A, which was unaltered by ozonolysis (Fig. 1), no evidence was found for the presence of other isomeric 14-carbon acetates (the level of detection was 1 percent of the 11-carbon aldehyde-acetate product). Therefore, the only monounsaturated 14-carbon acetates detected in the pheromone glands were Δ-11-14 : Ac's.

Similar chemical analyses of pheromone gland extracts were performed on *A. semiferanus* that had fed on chestnut oak (*Quercus prinus*), black oak (*Q. velutina*), and white oak (*Q. alba*) in the field (*15*). The XF-1150 GLC profiles of tip extracts from females reared on the three oak species were virtually identical. The GLC profiles of extracts from females reared on oak differed significantly from those of extracts from females reared on artificial medium (Fig. 1) in only one respect. In the former profiles a peak representing about 8 to 12 percent of those in the 14-carbon acetate region appeared before peak A (*16*). The three components in the 14-carbon acetate region of all extracts shared these similarities: (i) identical GLC retention times for equivalent components, (ii) virtually identical ratios (*17*), and (iii) presence in total quantities of 35 to 50 ng per individual.

The components in the 14-carbon acetate region of the pheromone extracts of females from each type of oak were purified by GLC and identified as 14 : Ac, t11-14 : Ac, and c11-14 : Ac by GLC relative retention times and microozonolysis in each case. As in extracts of moths reared on artificial medium, microozonolysis revealed no detectable quantities of other isomeric tetradecenyl acetates when conducted on more than 1000 ng of collected pheromone.

Electroantennogram profiles were recorded for newly emerged *A. semiferanus* males from artificial medium as well as from the various oaks. In the series of 10- to 16-carbon acetates, alcohols, and aldehydes tested, the 14-carbon acetates clearly elicited the greatest antennal responses. No significant differences were evident in the EAG responses of the four male types. Each profile, compiled from ten replicates with at least seven different individuals, was identical to all others. In every case the antennal response was greatest for c11- and t11-14 : Ac (*18*).

Responses of laboratory-reared males to c10-14 : Ac [the reported major attractant of *A. semiferanus* (*5*)], c11-14 : Ac, t11-14 : Ac, and mixtures of c11- and t11-14 : Ac were measured by bioassays with glass tubes suited for observing both excitation and orientation (*19*). At all dosages tested, c10-, c11-, and t11-14 : Ac tested alone elicited no significant response. However, various binary mixtures of c11- and t11-14 : Ac were highly active in bioassays, eliciting wing fanning, orientation to the source of these compounds, and copulatory attempts with other males. In these laboratory behavioral tests, male moths responded to a range of mixtures administered at 10 ng per filter paper dispenser. All those between 80 and 30 percent t11- in c11-14 : Ac elicited significant ($P < .05$) wing fanning (32 to 70 percent) and orientation (9 to 35 percent) responses compared to background; however, highest values were recorded in the range of 50 to 70 percent t11- in c11-14 : Ac. In the same series of tests, ¼ female equivalent of tip extract elicited responses similar to those to 10 ng of the Δ-11 mixtures (64 percent wing fanning and 31 percent orientation response). This is consistent with our findings that female tips contained about 40 ng of c11- and t11-14 : Ac's.

As was true for males from the pinto bean diet, c10-, c11-, and t11-14 : Ac alone elicited no wing fanning or orientation from male *A. semiferanus* from white and chestnut oaks (*20*); but both groups responded similarly (70 to 75 percent wing fanning, 66 to 68 percent orientation) to a 70 percent mixture of t11- in c11-14 : Ac.

Field tests of the pheromone components identified from *A. semiferanus* females and related compounds were conducted at the site of larval collections (*15*). The traps, either Sectar I or Pherocon 1C, were hung throughout the forest at a height of about 1.5 to 2 m (*21*). In test 1, varying dispenser loads of t11- and c11-14 : Ac in the naturally occurring ratio (70 : 30) were tested (Table 1, test 1). One milligram of the mixture on a rubber

Table 1. Field attractance of synthetic compounds and virgin females for male *Archips semiferanus*. Test 1 was conducted on 2 to 6 July with Sectar I traps spaced 15 to 20 m apart; traps within the four replicates were rerandomized daily. Test 2 was conducted on 4 to 6 July with Pherocon 1C traps spaced 25 to 30 m apart; traps within the four replicates were rerandomized daily. Test 3 was conducted on 5 and 6 July with Pherocon IC traps spaced 25 to 30 m apart; traps within the six replicates were rerandomized daily. Means in the same test followed by the same letter are not significantly different at the 5 percent level. Chemicals were loaded on rubber septa.

Treatment	Males per trap (mean)
Test 1. Effect of dispenser dosage	
70 Percent t11- in c11-14 : Ac	
10 μg	4.5 d
100 μg	17.0 c
1 mg	55.0 a
10 mg*	0.0 d
10 μg + 25 percent 14 : Ac	5.5 d
100 μg + 25 percent 14 : Ac	18.0 c
1 mg + 25 percent 14 : Ac	32.0 b
10 mg + 25 percent 14 : Ac*	0.5 d
Unbaited	0.5 d
Test 2. Effect of 14 : Ac	
70 Percent t11- in c11-14 : Ac	
1 mg	91.8 ab
1 mg + 5 percent 14 : Ac	101.1 a
1 mg + 25 percent 14 : Ac	57.0 b
1 mg + 500 percent 14 : Ac	57.0 b
Unbaited	0.0 c
Test 3. Synthetic compounds compared to virgin females	
c11-14 : Ac (1 mg)	0.0 b
t11-14 : Ac (1 mg)	0.0 b
c10-14 : Ac (1 mg)	0.0 b
c4-14 : Ac (1 mg)	0.0 b
70 Percent t11- in c11-14 : Ac (1 mg)	124.2 a
Two (3-day-old) virgin females	81.0 a
Unbaited	0.0 b

*This was dispensed in polyethylene caps (OS-6 Natural Polyethylene Closures, Scientific Products).

septum was very attractive to *A. semiferanus* males and caught significantly more males than did higher or lower dosages (*22*). In the same test, varying dosages of the t11- and c11-14 : Ac mixture were supplemented with 25 percent 14 : Ac, since this compound was found in female extracts in approximately that ratio. At 25 percent, 14 : Ac reduced trap catch by about 40 percent, although the 1-mg dosage remained optimal. Further field tests with higher and lower percentages of 14 : Ac (Table 1, test 2) showed that at very low percentages this compound had little effect on trap catch and that at very high percentages it caused only moderate inhibition.

In test 3 (Table 1) the attractancy of 1 mg of the 70 : 30 mixture proved competitive with a treatment of two virgin females. It is noteworthy that the three pairs of oak leaf roller females from white oak, two pairs from black oak, and one pair from chestnut oak all caught similar numbers of males during the test (*mean* = 81, *mean* = 60, and 120 males per trap, respectively). Various 14-carbon acetate isomers, including c10-14 : Ac, attracted no males. On 9 July 1975, t4-14 : Ac and c5-14 : Ac were added to the experiment. These and the other 14-carbon acetate isomers from test 3 were tested over the remainder of the flight period (*23*). From 5 to 16 July a total of 1456 oak leaf roller males (242 ± 61.6 per trap) were caught in the six replicates of the 70 : 30 mixture of t11- and c11-14 : Ac, while the other treatments caught no males.

In another field test, the ratio of t11- to c11-14 : Ac was varied (Fig. 2). The tetradecenyl acetates were free of other positional isomers (purity > 99.9 percent) and all ratios were established by GLC analysis on 10 percent XF-1150. The range of ratios that caught males was surprisingly narrow. Optimal male catch occurred at 66 percent t11- in c11-14 : Ac and exactly reflected the composition of these components in the female pheromone gland. The narrow range of attractant ratios could be important in reproductive isolation, since other related sympatric tortricids utilize the same two components, but in different ratios (*1*); *Archips argyrospilus* uses a 30 : 70 ratio of t11- to c11-14 : Ac and *Argyrotaenia velutinana* a 7 : 93 ratio. Also, *Archips podana* of Europe utilizes a 50 : 50 ratio (*24*). The first two species utilize dodecyl acetate as an additional component, and some tortricids (*25*) use c9-14 : Ac as a pheromone component. Field tests with c9-14 : Ac and c10-14 : Ac at 2 and 5 percent and dodecyl acetate at 10, 50, and 400 percent of the 1-mg 70 : 30 mixture of t11-14 : Ac and c11-14 : Ac did not increase trap catch. Nevertheless, we do not exclude entirely the possibility that other undetected trace components might influence the attractancy of the 70 : 30 mixture.

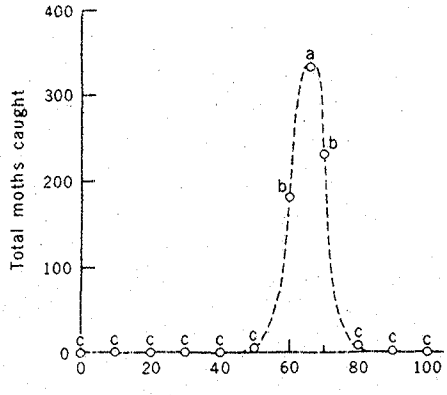

Fig. 2. Trap catches of *A. semiferanus* males with varying ratios of t11- and c11-14 : Ac. This test was conducted from 6 to 16 July 1975 using Sectar traps spaced 15 to 20 m apart. The six replicates were rerandomized each day between 8 and 11 July. Means for the treatments marked by the same letter were not significantly different at the 5 percent level.

In summary, we found that the pheromones produced by female *A. semiferanus* reared on a semisynthetic diet and collected from the foliage of three different oaks did not differ qualitatively or quantitatively. In all cases females produced t11- and c11-14 : Ac in a ratio very close to 67 : 33. Males from the different diets responded identically in EAG tests and laboratory bioassays; in the field, males were trapped only by treatments closely approximating a 67 : 33 mixture of t11- and c11-14 : Ac. Over the entire field season 4242 male *A. semiferanus* were caught by traps containing mixtures of t11- and c11-14 : Ac, while no males were caught by traps containing various 14-carbon acetates alone. These findings contradict the previously reported evidence that *A. semiferanus* pheromone production and perception vary with slight dietary changes (*26*). Although we expect that major changes in the composition of nutrients in an insect's diet could affect pheromone production, we contend that possible slight changes in the 14-carbon acetate composition of oak leaves do not alter pheromone composition and perception in *A. semiferanus* and do not constitute a mechanism for rapid evolution of reproductive isolates.

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6. Egg masses were collected in Moshannon State Forest, Elk County, Pa., December 1974 to February 1975.
7. The diet employed was that of H. H. Shorey and R. L. Hale [*J. Econ Entomol.* **58**, 522 (1965)]; which was supplemented with the vitamin mixture used in the wheat germ diet of G. M. Chippendale and S. D. Beck [*Entomol Exp. Appl* **7**, 241 (1964)] and 0.35 percent Aureomycin, 18 percent soluble (Bio-Serv, Inc.). Egg masses were sterilized by submersion in 10 percent formalin for 30 minutes. One or two larvae were reared on about 8 g of diet dispensed per 30-ml plastic cup. Larval to adult development took 34 to 40 days at 27°C. Rearing success was 60 to 80 percent, with most of the mortality occurring in the first instar.
8. W. Roelofs, A. Comeau, A. Hill, G. Milicevic, *Science* **174**, 297 (1971).
9. We used a glass column 6 feet (1.8 m) long with inside diameter 4 mm packed with 3 percent OV-1 on 100/120 mesh Gas-Chrom Q; N_2 flow was 35 ml/min; the column oven was programmed at 110° to 225°C at 5°/min with an initial hold of 7 minutes. Electroantennogram activity (3 to 4.5 mv) was detected at 23 to 25 minutes. Under the same conditions, tetradecyl and tetradecenyl acetates are eluted at 22 to 25 minutes.
10. A glass column 6 feet long with inside diameter 2 mm packed with 10 percent XF-1150 on 100/120 mesh Chromosorb W was used isothermally at 154°C and an N_2 flow of 35 ml/min.
11. On an XF-1150 column at conditions approximating those given in (*10*), retention relative to that of c11-15 : Ac was, for components A to C, 0.556, 0.631, and 0.697; for saponification products of components A to C, 0.635, 0.723, and 0.798; for reacetylated products of components A to C, 0.555, 0.630, and 0.695; for 14 : Ac, t11-, and c11-14 : Ac standards, 0.555, 0.630, and 0.694; and for 14 : OH, t11-, and c11-14 : OH standards, 0.632, 0.720, and 0.795.
12. Silica gel G plates containing 30 percent silver nitrate were washed by development in 10 percent ether in benzene. Component separation was achieved by development in spectrograde benzene. In our system 14 : Ac, *trans*-, and *cis*-tetradecenyl acetate standards are separated into three discrete bands with R_f values of about 0.64, 0.54, and 0.37, respectively.
13. Several minor peaks were present; however, their retention times did not match those of 14-carbon acetate standards. Similar compounds were present in all thin-layer chromatograms, including controls to which no standards were applied.
14. Microozonolyses were conducted according to the methods of M. Beroza and B. A. Bierl [*Anal. Chem.* **39**, 1131 (1967)]. Ozonolysis products were analyzed on the OV-1 column (*9*) programmed from 100° to 170°C at 3°/min. The Packard series 7400 GLC used was capable of detecting 0.5 ng of product. Aldehyde acetates of a chain length greater than four carbons and aldehydes of a chain length greater than six carbons were detectable under these conditions. 11-Oxoundecyl acetate was eluted at 23.4 to 23.8 minutes.
15. Between 14 and 22 June 1975 about 300 larvae and pupae were collected per oak species from the foliage of felled trees within a natural infestation of *A. semiferanus* on Boone Mountain, 5 km north of Penfield in Elk County, Pa. In this location the forest is predominantly oak. The order of prevalence was chestnut, black, and white, plus a scattering of red and scarlet oaks. This area was heavily defoliated during 1973 and 1974 by *A. semiferanus*, but no defoliation was apparent during 1975. Although collected discriminatively, about 50 percent of the larvae and pupae obtained were parasitized, suggesting that this *A. semiferanus* population was collapsing from parasitism.

16. This compound is not a 14-carbon acetate since it has a retention time significantly shorter than that of any of the 14-carbon acetate standards.
17. The range of ratios of peak B to peak C was 64 : 36 to 70 : 30 (mean = 67.2 : 32.8). The ratios of peak A to peaks B and C varied from 15 to 30 percent within each type of extract. Samples of ten tips each were analyzed in triplicate.
18. Similar EAG results were obtained by Hendry *et al.* (*4*) for a pooled sample of field-collected males.
19. For details of the apparatus see T. Baker [thesis, Cornell University (1975)]. Males were assayed 4 hours into scotophase [16 hours light (1400 lux) and 8 hours darkness (2 lux)] by using a randomized complete block experimental design (12 replicates, 10 males per tube). The key response scored was fanning of the wings for 1 second or longer during the 60-second observation interval. Upwind orientation was based on the number of males crossing a line 10 cm from the pheromone dispenser in the 1-m tubes.
20. Ten replicates were tested, with five males per bioassay tube. Insufficient males from black oak were available for statistically valid bioassays; however, in the three replicates tested these males responded only to the 70 : 30 mixture of t11- and c11-14 : Ac (53 percent wing fanning, 11 percent orientation).
21. Compounds were dispensed in silicone rubber septa (rubber stoppers, sleeve type, 5 by 9 mm, Arthur H. Thomas Company). All experiments were conducted by using a randomized complete block design and, unless otherwise stated, traps were rerandomized within blocks every day. Data were submitted to analyses of variance after being transformed to $(x + 0.5)^{1/2}$. Throughout, means followed by the same letter are not significantly different at the 5 percent level, as determined by Duncan's new multiple range test.
22. Dispenser dosages were varied at decade steps down to 0.01 ng per septum.
23. These treatments were not rerandomized between 11 and 16 July.
24. C. J. Persoons, A. K. Minks, S. Voerman, W. L. Roelofs, F. J. Ritter, *J. Insect Physiol.* **20**, 1181 (1974).
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26. Our findings varied from the previous *A. semiferanus* investigations on several other points. We found that male activity peaks between 11 and 12 p.m. rather than at 3 a.m. At no time either in the laboratory or the field did we observe male oak leaf rollers becoming sexually stimulated in the presence of oak leaves, nor do they possess hairpencils. We found no evidence that a separate "sexual excitant" is involved in the sexual communication of this moth.
27. Supported by the Rockefeller Foundation. We thank S. Loerch and M. Kavanaugh for their assistance in collecting eggs, larvae, and pupae in the field.

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4 September 1975; revised 17 December 1975

Insect Pheromones: Diet Related?

Abstract. *The question of the origin of insect pheromones is discussed in the light of new published information on the communication system of the oak leaf roller. It is concluded that compounds found in diets may be partially responsible for insect sexual behavior and that substructuring of insect populations in ecological and evolutionary time through dietary chemicals remains a hypothesis worthy of further testing.*

Miller *et al.* (*1*) conclude that the oak leaf roller (OLR), and perhaps other tortricids, derive little reproductive capability from food sources and that the hypothesis of Hendry *et al.* (*2*) that "dietary factors may provide an evolutionary mechanism for diversification of certain species" is contradictory to their findings. Considering the apparently bipolar nature of the conclusions in the two studies, I believe that an examination of the methodologies is necessary. Miller *et al.* used a combination of electroantennograms (EAG's) and field testing with standard compounds, analysis of OLR female extracts using chromatographic values in comparison with standards, and functional group analysis (such as ozonolysis) in comparison with standards. These methodologies have led to the point of view that the chemistry and field behavior of the OLR differ from those reported by Hendry *et al.* and that wild and artificially reared females have quantitatively and qualitatively identical pheromone chemistry.

I maintain that the close similarities between chemical signals found in insects and plants are not coincidental and may have important consequence in the coevolution of tortricid insects and their food plants. Furthermore, the utilization by an insect of diet-specific chemicals for olfactory programming remains a viable hypothesis (*2*) [mammalian "imprinting" of chemical messenger systems by exogenous food substances has recently been documented (*3*)]. Ultimately, assessing the validity of these theories will require chemical and biological experiments designed with minimal expectation. In this regard, the theory that dietary chemicals may be exceedingly important in insect reproduction and may be involved in substructuring of insect populations in ecological and evolutionary time remains to be tested.

The experimental results obtained by Miller *et al.* are in overall agreement with our knowledge of the OLR, including the findings that (i) *Z*-11- and *E*-11-tetradecenyl acetate (*Z*-11-TDA and *E*-11-TDA) elicited the greatest male EAG responses; (ii) a mixture of isomeric tetradecenyl acetates (TDA's) was found in female extracts; (iii) the 11-positional TDA isomer is a significant component of the TDA's in pooled extracts of field-collected females; and (iv) tetradecyl acetate is a significant component of female extracts. In our methods of chemical analysis, which were based largely on chromatographic separations and subsequent computerized chemical ionization and electron impact gas chromatography–mass spectrometry (CI-EI-GCMS), we were unable to satisfactorily quantify the amounts of all of the various TDA's. These difficulties were primarily due to the similarities in chromatographic and spectral properties of the isomeric TDA's and the presence of several components of unknown structure in "active" chromatographic fractions. In this regard, Miller *et al.* report an important discovery, that field-reared OLR females contained only two TDA's, *E*-11-TDA and *Z*-11-TDA, in an unusually invariant and discrete ratio, 67 : 33. Miller *et al.* used (i) EAG analysis on OLR male antennae to locate gas chromatographic (GC) "active" peaks in female extracts; (ii) GC of crude field-female extracts on a polar column (XF-1150) for quantitation of exact TDA ratios; and (iii) GC of female extracts on a nonpolar column (OV-1) and subsequent ozonolysis of the active peak to locate double bond positions in various TDA's.

Electroantennograms have been used successfully to locate active chromatographic fractions. With standards, the EAG technique has led to the identification of an impressively large number of insect pheromones (in some cases with just a few antennae) (*4*), and may be an important method for determining "genetically fixed" similarities in the communication systems of closely related insect species. However, chemical substances requisite in the natural pheromone systems of several insects, including tortricids, may give only minimal EAG responses (*5*), while compounds with EAG activity may have little apparent field activity. Hence, some chemical signals that are essential to the natural communication system of an insect may be overlooked by such screening techniques (*6*). For example, does the OLR antennal response shown by Miller *et al.* in their figure 1 at 1 to 2 minutes represent a compound that elicits behavioral activity? Field testing of all chromatographically isolated components of the field OLR extracts and subsequent chemical analyses of active fractions would aid in assessing the complete sexual message in the female (*7*) when compared with results obtained by EAG analyses. Although the EAG technique may be very useful in narrowing down choices of certain types of standard compounds for pragmatic evaluations of field applicability of pheromones, the central nervous system (CNS) of the insect may be intimately involved in the *specificity* of chemoreception [CNS interaction may explain why EAG patterns of individual components of a pheromone system nor-

mally do not quantitatively match the pheromone in the female (*8*)].

The determination by Miller *et al.* (on the basis of GC peak areas) of the invariant 67 : 33 ratio of *E* : *Z*-11-TDA in OLR females (reared on three types of oak) appears very convincing. In our chemical analyses of extracts of field females (collected as pupae on a variety of plants in the summers of 1971 to 1974), another component with similar chromatographic properties was found. The component was identified as tetradecanol (TDOL) (*9*), which has essentially the same GC retention time as *E*-11-TDA (*10*); TDOL was a significant component (as much as 50 percent in some cases) of the pheromone isolate. The presence of such a component in the OLR field female extracts would alter the computed *E* : *Z*-11-TDA ratios considerably. A slight chemical change in the female ratio (to < 50 percent or > 80 percent *E*-11-TDA) would totally inhibit male field attraction [figure 2 in (*1*)]. Quantitation of the *E* : *Z* ratios by Miller *et al.* was predicated on their finding, using ozonolyses on impressively few females, that only the 11-positional isomer exists in field female OLR and that no other TDA's (≤ 1 percent) or other compounds are present. However, one is at borderline sensitivity or below when attempting to quantitate or identify such small amounts (~ 1 percent) by GC or computerized EI-CI-GCMS (*11*).

Although the methodologies used by Miller *et al.* are different from those we used, I find it consistent with our previous analyses that *Z*- and *E*-11-TDA are part of the OLR pheromone system. It is also possible that some OLR female extracts have 67 : 33 ratios of the two TDA isomers. Further studies based on chemical analysis of individual insects whose dietary history has been rigorously kept—that is, field OLR's reared on known specific natural diets (without change of plant) from egg to adult—will aid in the elucidation of variability (temporal or spatial) in OLR pheromones, as has been reported in other Tortricidae (*12*).

One of the most significant reports of pheromone inconstancy is that of Klun (*13*), showing that male European corn borers (Tortricidae) at different geographical locations respond to a variety of radically different TDA mixtures. Moreover, at some locations, the pheromone response of male corn borers appears to be heterogeneous. Cardé *et al.* (*14*) showed that corn borers occurring sympatrically at one site in Pennsylvania have very different pheromone chemistry. The reasons for such geographic and temporal differences in the pheromones within the same insect species have not been experimentally elucidated. To date, we have studied primarily temporal changes of pheromones and related compounds in the OLR and have observed some major differences. An independent study of the chemistry of the field extracts from both groups by spectral as well as chromatographic methods could aid in an overall understanding of the general communication system of the OLR and other tortricids (*15*).

Miller *et al.* found that a narrow range of 1-mg mixtures of TDA's are attractive to field males. This discovery may help to elucidate the natural sexual messenger system in the OLR. In biological testing with field OLR males in flight chambers (*16*) (summers of 1973 and 1974), we were unable to mimic the OLR pheromone system with mixtures of *Z*- and *E*-9-, 10-, and 11-TDA. Male OLR's were not significantly attracted in glass tube bioassays (*17*) to quantities of these agents which approximated the attractive levels in virgin female extracts (10^{-7} to 10^{-8} g) (general excited behavior was not uncommon under a variety of conditions). Moreover, in field tests various TDA mixtures were unsuccessful in attracting males to small traps at concentrations equivalent to those in active female extracts (or in live females) (*18*). The failure of these experiments may have been due to our not using large quantities of pheromone, which we considered unnecessary over the characteristically brief adult flight period of OLR males in the field and which could permanently contaminate bioassay devices in the laboratory. Moreover, since quantities of pheromone greater than 0.01 female equivalent (a female equivalent is the amount of extract from one female) had been found to decrease the attraction of males in the laboratory and field (*16*, *19*), we deduced that the well-known communication disruption or male confusion phenomenon would operate at large doses of pheromone components (*20*). Consequently, we did not test 1-mg quantities of the *Z* : *E* mixture, an amount found to be optimally attractive to field OLR males by Miller *et al.* and an amount approximately 10^5 greater than the quantity in an OLR female (*21*). The use of excessive doses of pheromone components in field trapping experiments requires extreme precautions to prevent contamination from other potentially active components at the level of parts per million or lower (*22*). In a recent study of the European corn borer Klun *et al.* (*23*) found that small amounts of 11-tetradecynyl acetate, a synthetic precursor and common contaminant of *Z*- and *E*-11-TDA, have remarkable biological activity and may be interacting with "the same chemoreception sites on the moth." The analytical difficulties involved in chromatographic separation, detection, and identification of small amounts of precursors or isomeric products in the synthesis of these sex attractants are not trivial, even with present chemical techniques.

If secondary components are essential in the sexual communication system of the OLR, large quantities of TDA's may prove as attractive to males as live virgin females at levels and ratios inconsistent with their presence in the organism. Synergism of sexual attraction in the Tortricidae by apparently innocuous compounds has been established (*4*). In experiments with the OLR, we found that isolated TDA chromatographic fractions of female extracts (*7*) were less attractive than crude female extracts or live virgin females, which suggests that other pheromone components were as yet unidentified.

The conclusion by Miller *et al.* that pheromone production in the OLR does not vary with slight changes in dietary chemicals may be correct. It is based primarily on the finding that OLR females reared on semisynthetic pinto bean diet contain a discrete mixture of TDA's. Using CI-EI-GCMS, we found that 14-carbon acetates are present in a variety of semisynthetic bean diets; pinto beans also contain these compounds (*24*). Definitive statements about the relationship between diet and pheromones in the Tortricidae await thorough chromatographic and spectral analyses at the level of parts per billion for a variety of compounds in the diet, coupled with feeding studies using appropriately labeled materials.

The potential interaction of dietary chemicals and sex pheromones in the OLR may not be limited to female-produced attractants. Male OLR abdominal brushes (*25*) contain benzaldehyde, an "aphrodisiac" produced by males of numerous species of moths (*26*). Only trace amounts of benzaldehyde were found in female OLR's; oak leaves contained relatively large quantities of the compound. The role (if any) of plant-produced benzaldehyde in the OLR communication system remains to be established.

The questions surrounding the origin of insect pheromones remain intriguing but largely unanswered. As previously discussed (*2*), there remain several alternative origins of pheromones: (i) storage or sequestering of chemicals from plants; (ii) degradation or transformation of

plant precursors; (iii) "induction" of pheromone biosynthesis (possibly over multiple generations); (iv) de novo biosynthesis; and (v) production by microorganisms associated with the plant or insect. We believe it is unlikely that all insects will use the same method or methods for procuring pheromone. Schneider *et al.* (*27*) reported the degradation of dietary compounds (alkaloids) in plants to pheromones in danaid butterflies, and Pliske (*28*) discussed the reproductive importance of the attraction of danaids and other Lepidoptera to alkaloid-containing plants. Cardé *et al.* (*29*) found that males of the Oriental fruit moth, an apple-feeding tortricid moth related to the OLR, respond sexually (precopulatory behavior) to a compound which is not a tetradecenyl acetate, namely, dodecanol. We have found that dodecanol is a significant component of apple leaves, a food source of the Oriental fruit moth (*9*). Twelve-carbon compounds of varying functionality have been reported as components of the sexual communication system of other tortricids, including the fruit tree leaf roller (*30*), a member of the same genus as the OLR. The biological activity of the male OLR (*2*) in response to oak leaves could partly involve responses to 12-carbon compounds (*31*) in the plant. [Whether the reported correlation in the Tortricidae of the success of pheromone trapping with the amount of foliage per acre (*32*) is due in part to the behavior mediated by such host plant chemicals remains to be elucidated.]

Whatever mechanisms are involved in the ultimate production of pheromones, investigations such as those by Miller *et al.* are likely to provide insight into the general importance of nutritional chemicals in biological processes.

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22 January 1976

Spatial Frequency–Contingent Color Aftereffects

Abstract. *Two-dimensional Fourier analysis of checkerboards reveals that major components are at a 45° angle to the check edges. After adapting to chromatic checkerboards, subjects who viewed achromatic grating stimuli reported that complementary color aftereffects are aligned with spatial frequency components rather than with the edges in the pattern.*

McCollough attributed orientation-contingent color aftereffects to chromatic adaptation of edge detectors (*1*). Implicit in this interpretation is the assumption that human pattern perception involves a relatively simple feature-extraction mechanism (*2*). Other studies have suggested that spatial properties of visual stimuli are processed by spatial frequency analysis (*3*). There is evidence that the visual system responds to the spatial period rather than to the bar width of grating stimuli (*4*). Our study is an extension of these findings, and emphasizes the importance of frequency analysis in visual perception. We used checkerboard adapting stimuli and square-wave test stimuli (or vice versa) to assess the relative importance of edges and spatial frequency components in the production of contingent color aftereffects. Kelly (*5*) pointed out that checkerboards contain Fourier components oriented at 45° angles to the edges of individual checks. Our results indicate that color aftereffects contingent on pattern involve the orientation of the major two-dimensional Fourier components rather than the orientation of the edges.

The four patterns used in this study were labeled "squares," "diamonds," "verticals," and "obliques" (Fig. 1). The square pattern was a checkerboard with the edges of individual squares oriented vertically and horizontally. The diamond pattern was identical to the square except that the checks were rotated 45° (edges of squares were on the diagonals). Vertical and oblique patterns were square-wave gratings oriented appropriately. The grating spatial frequency was 2.5 cycles per degree of visual angle, and the sides of the checks were 12′ of visual angle (*6*). All stimuli