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).
25. A. K. Minks, W. L. Roelofs, F. J. Ritter, J. C. Persoons, *Science* **180**, 1073 (1973).
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.
* Present address: Department of Entomology, Michigan State University, East Lansing 48823.

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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 ($\leq$ 1 percent) or other compounds are present. However, one is at borderline sensitivity or below when attempting to quantitate or identify such small amounts ($\sim$ 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.

L. B. HENDRY

Department of Chemistry,
Pennsylvania State University,
University Park 16802

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6. Also, application of the EAG technique in searching for insect pheromones normally involves the testing of a limited set of standard compounds (usually straight-chain aliphatics of varying functionality).
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11. We estimate that the amount of various ozonolysis products to be detected at the 1 percent level with so few insects would be in the picogram range, which is below the capabilities of our present instrumentation. Also, using XF-1150 columns, mixtures of positional TDA isomers were inseparable.
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18. Large numbers of males of the fruit tree leaf roller (*Archips argyrospilus*), the black-shielded leaf roller (*Archips griseus*), and the oblique-banded leaf roller (*Choristoneura rosaceana*) were caught in various traps in summer field studies (1971 to 1974) with 11-TDA's.
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21. A detailed study of the release rates of the various quantities of pheromones from several substrates under field conditions may aid in evaluating these data.
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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