plast preparations obtained by the method of Honda *et al.* (*9*) contain intact nuclei, and it seems probable that an isolated nucleus was taken up by the *N. tabacum* protoplast together with the chloroplasts. However, further analyses of individual plants derived from the uptake of chloroplasts into protoplasts will be required for assurance that the phenomenon can be reproduced. In any event, the results from both experiments confirm that in vitro technology can be utilized to alter the genetic makeup of higher plants.

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Sex Pheromone of the Oak Leaf Roller: A Complex Chemical Messenger System Identified by Mass Fragmentography

Abstract. *The sex pheromone of the oak leaf roller,* Archips semiferanus *Walker, is composed of a complex mixture of chemical signals. The attractant component of the pheromone contains a series of tetradecenyl acetates having double bonds in positions 2 to 12. Mass fragmentography of the ozonolysis products of the attractant component was used to locate the double bonds in the various isomers.*

Recent reports on insect phermones have revealed that few insect communication systems involve single chemical messengers. Moreover, sexual signals in some species consist of exact ratios of positional isomers (*1*) as well as geometrical isomers (*2*). The purity of some optical isomers, identified as pheromones, has also been viewed as important in certain insects, as shown by Riley *et al.* (*3*).

The sexual message in some species is composed of different chemicals which elicit separate behavioral responses from the insect. This segmentation of insect behavior in response to different chemical stimuli has been reported in the sex pheromones of Coleoptera (*4*) and in a host-searching kairomone in the Hymenoptera (*5*). In Lepidoptera, a sexual excitant and a sexual attractant have been distinctly isolated from the pheromone extracts of the oak leaf roller moth, *Archips semiferanus* Walker (Lepidoptera: Tortricidae) (*6*). We report here the identification of compounds found in the attractant portion of the oak leaf roller pheromone.

The oak leaf roller moth, a forest defoliator, has recently infested large portions of timberlands in the northeastern United States. In Pennsylvania, tree mortality due to this pest has climbed as high as 90 percent in more than 1 million acres of forest; approximately $70 million has been lost in oak stumpage values alone in this area (*7*). In the process of searching for a control method for the oak leaf roller, a sex pheromone was discovered in the adult female (*8*). Laboratory and field biological assays (*9*) revealed that two chromatographically isolable fractions of the pheromone extracts were involved in the female's sexual message, that is, an excitant and an attractant (*6*). (*Z*)-10-Tetradecenyl acetate was isolated, synthesized, and shown to serve as the major attractant signal (*10*); however, compounds having similar chromatographic properties were unidentified. These compounds represented generally a much smaller portion of the active attractant fraction, and consequently presented a formidable analytical task to identify them.

The analysis of these additional components was conducted as follows. Fifty virgin female oak leaf rollers were anesthetized with carbon dioxide; the last two abdominal segments were excised and ground in a tissue grinder with methylene chloride. The resulting suspension was filtered, and the solvent was evaporated under a stream of nitrogen. The extract was subjected to thin-layer chromatography on silica gel (Brinkmann Silplate F-22) and was subsequently purified by gas chromatography (GC) on nonpolar (SE30) and polar (DEGS) columns, as previously described (*6*). The active portion (*11*) that was known to attract males upwind in our laboratory flight chamber and to trap males in field tests was collected, and half of the product (~ 50 ng) was subjected to computerized gas chromatography–mass spectrometry (GC-MS) (*12*). A full mass spectrum was recorded every second. The result was an unresolved band of peaks which had identical qualitative mass spectra. Key ions included m/e (mass to charge) 254 (M^+, the parent peak), 194 (M^+–HOOC–CH_3), 166, and 61 (H_2OOC–CH_3^+). The spectra were indicative of a mixture of 14-carbon monounsaturated acetates. (No interfering compounds of higher or lower molecular weight were detected by computer search.)

To locate the double bond positions in the acetates, the remaining GC product collected was subjected to microozonolysis (*13*) in highly purified carbon disulfide and analyzed by GC-MS. The ozonolysis products, which were aliphatic aldehydes, were below the limit of detection by mass spectroscopy (< 1 ng). To detect the aldehyde products, the GC-MS was programmed to scan only for a few appropriate ions which were intense in standard aldehyde spectra (*14*). This novel technique, mass fragmentography (also called multiple ion detection), has been utilized in many areas of chemistry (*15*), but this is the first time, to our knowledge, that it has been applied to an insect pheromone study. Since mass fragmentography requires that the GC-MS scan for a few ions rather than over an entire mass range, the sensitivity increases dramat-

Table 1. Aldehydes identified by mass fragmentography in the ozonolysis products of the oak leaf roller attractant pheromone. Glass U-tube columns 1.5 m long were used; columns and temperature programs were: (series 1) Porapak Q, 150° to 250°C at 20°C/min; (series 2) 10 percent DEGS, 70° to 150°C at 20°C/min; (series 3) 10 percent DEGS, isothermal, 165°C. The ions with underlined *m/e* values were the most useful.

Aldehyde	Diagnostic fragments (*m/e*)
Series 1	
CH_3CHO	29
CH_3CH_2CHO	29, 58
$CH_3(CH_2)_2CHO$	29, 72
$CH_3(CH_2)_3CHO$	29, 86
Series 2	
$CH_3(CH_2)_4CHO$	57
$CH_3(CH_2)_5CHO$	57, 70
$CH_3(CH_2)_6CHO$	57, 84
$CH_3(CH_2)_7CHO$	57, 70, 86
$CH_3(CH_2)_8CHO$	57, 70, 96, 112
Series 3	
$CH_3(CH_2)_9CHO$	110
$CH_3(CH_2)_{10}CHO$	110, 140

ically for the identification of candidate compounds; for example, amounts between 25 and 500 pg are normally detected. The ions used to scan for the various aldehyde products are shown in Table 1. Because of the different volatilities of the aldehydes, and to optimize the sensitivity of GC-MS to the ozonolysis products, three separate series were analyzed under different chromatographic conditions. In this manner, analyses of the ozonolysis products of the oak leaf roller attractant fraction showed that aldehydes C_2 to C_{12} were present. (The average amount of aldehyde analyzed varied from approximately 50 to 120 pg.) These results were verified by repeating the procedure six times; each replicate contained two blanks. In no case were any aldehydes found in the blanks (*16*). A sample mass fragmentogram is shown in Fig. 1; the retention times (in spectrum numbers) of the various ion peaks correlate exactly with those for standard aldehydes C_6 to C_9.

The 14-carbon unsaturated acetates associated with the identified aldehydes are listed in Table 2. All the *Z* and *E* monounsaturated acetates in Table 2 have been synthesized and found to have GC retention times within the range for the active attractant. Of the compounds that were available for field testing (*17*) (see Table 2), all attracted male oak leaf rollers; (*Z*)-10-tetradecenyl acetate remained the most active.

The results reported here indicate that the sexual behavior of the oak leaf roller may be mediated by one of the most complex chemical messenger systems as yet elucidated. The complex mixture of requisite intraspecies sexual signals in the roller suggests that this pest may be very primitive compared to other lepidopterans previously studied. If so, closely related species may have evolved from the oak leaf roller by utilizing only a few components of the roller complex. On the other hand, secondary components may have been present, but in amounts too small to be detected, in pheromone studies of related species. Electroantennogram studies (*18*) have shown that the red-banded leaf roller and related tortricids respond to all tested double bond isomers of the 14-carbon monounsaturated acetates, albeit the response is small in some cases.

Table 2. Identified components in the attractant fraction of the oak leaf roller pheromone complex.

$CH_3(CH_2)_{10}CH=CHCH_2OOCCH_3$
$CH_3(CH_2)_9CH=CH(CH_2)_2OOCCH_3$*
$CH_3(CH_2)_8CH=CH(CH_2)_3OOCCH_3$*
$CH_3(CH_2)_7CH=CH(CH_2)_4OOCCH_3$
$CH_3(CH_2)_6CH=CH(CH_2)_5OOCCH_3$
$CH_3(CH_2)_5CH=CH(CH_2)_6OOCCH_3$
$CH_3(CH_2)_4CH=CH(CH_2)_7OOCCH_3$
$CH_3(CH_2)_3CH=CH(CH_2)_8OOCCH_3$*
$CH_3(CH_2)_2CH=CH(CH_2)_9OOCCH_3$*
$CH_3CH_2CH=CH(CH_2)_{10}OOCCH_3$*
$CH_3CH=CH(CH_2)_{11}OOCCH_3$

* The *Z* isomers were found to be attractive to male oak leaf rollers in field tests (*17*). Other isomers were unavailable for the field tests.

Eventual control of the oak leaf roller by use of sex attractants may be best achieved after analyzing the exact ratios of all of the geometrical and positional isomers present in the female. With the analytical methods available, it is unlikely that this can be accomplished easily, and testing large numbers of combinations of these isomers in the field appears impractical. However, a single compound or simple mixture of compounds may be sufficient to effect control by using the communication disruption technique (*19*). In any case, the chemical ecologist is challenged by the increasing knowledge of the complexity of pheromone communication systems, and it is clear that new and sensitive techniques such as mass fragmentography are needed in order to probe further into this expanding area of science.

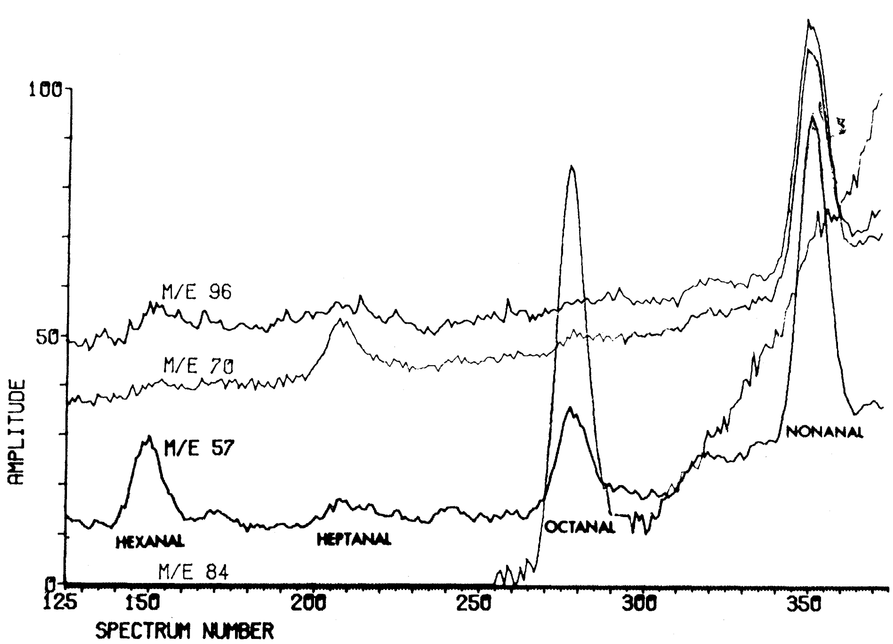

Fig. 1. Sample mass fragmentogram of the ozonolysis products of the oak leaf roller from series 2 (see Table 1).

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Low Frequency Electric Field Induced Changes in the Shape and Motility of Amoebas

Abstract. *Perpendicular and parallel elongation of the giant amoeba,* Chaos chaos (Chaos carolinensis), *have been observed in alternating electric fields over a wide frequency range (from about 1 hertz to about 10 megahertz). The characteristics change as a function of frequency. Simple dielectric forces may be important in the production of these effects.*

Amoeboid cells crawl parallel to a d-c electric field toward the cathode at only a few volts per centimeter (*1*). Mast reported that *Amoeba proteus* extended pseudopodia perpendicular to a 60-hz a-c electric field (*2*). Shape and motility changes in a-c electric fields were reported as early as the 1860's (*3, 4*), but with only limited data on frequency dependence. Recently, shape and orientation changes were reported in many small cells and inanimate particles subjected to radio-frequency electric fields at several hundred volts per centimeter and were attributed to dielectric field induced forces (*5*), including the elongation of erythrocytes (*6*).

We now describe shape, orientation, and motility changes in the giant amoeba, *Chaos chaos (Chaos carolinensis)*, over a wide range of frequencies (1 hz to 10 Mhz) and point out that the simple physical mechanism of dielectric forces may be important for low frequency as well as radio-frequency phenomena.

Chaos, because of its size (0.5 mm or more in length) was used, making handling and observation easier, and its dilute culture fluid—K_2HPO_4 (0.16 m*M*), KH_2PO_4 (0.11 m*M*), $MgSO_4$ (0.05 m*M*), and $Ca(NO_3)_2$ (0.50 m*M*) in glass-distilled water, conductivity 0.20 mmho/cm—reduced electrode polarization and heating. The *Chaos* (*7*) were cultured by standard methods (*8*) and starved for 1 day to eliminate food vacuoles. They were placed in the chamber at least 1 mm from each platinized platinum wire (0.1 mm diameter) electrode between parallel glass surfaces 0.2 mm apart, which had been freshly cleaned with nitric acid to enhance adhesion. Sudden increases, of 30 percent or more, in field intensity were avoided as they often carried past the desired event. Particles of three highly purified long-chain hydrocarbons—octadecane (m.p., 28° to 30°C), eicosane (m.p., 35° to 36.5°C), and docosane (m.p., 43° to 45°C)—indicated the temperature by melting point. The fluid never exceeded 30°C in most of the experiments; it rose above 30°C only where the field exceeded ~ 125 volt/cm.

The three photographs of a single *Chaos* at successive stages of perpendicular elongation (Fig. 1, a to c) were taken within 10 minutes at about 15 volt/cm (root-mean-square) and 316 hz. The character of the elongation changes (which are not rotational) vary with frequency in the following ways: (i) Below ~ 1 hz changes occur, but there is no orientation; (ii) between ~ 1 and ~ 100 hz, pseudopodia extend perpendicular to the field, but complete alignment never occurs; (iii) between ~ 100 hz and ~ 1 khz, parallel pseudopodia withdraw, and perpendicular pseudopodia extend to the extreme of Fig. 1c; (iv) between ~ 1 and ~ 100 khz, *Chaos* loses its pseudopodia and becomes approximately elliptical perpendicular to the field; and (v) above ~ 100 khz, *Chaos* remains elliptical (Fig. 1, d and e), but deforms with its long axis parallel to the field. The fluid circulates violently, and cellular material streams toward the electrodes. The cell is always destroyed.

Field strengths less than ~ 10 volt/cm produce these effects at frequencies below ~ 1 khz. Above ~ 1 khz the required field strength steadily increases. At ~ 100 khz it may exceed 200 volt/cm.

The character of the effects is also a function of field strength. The first observable effect (at a few volts per centimeter) in the middle range of frequencies (~ 10 hz to ~ 1 khz) is an inward drift of the cytoplasm parallel to the field, which the amoeba can overcome up to ~ 10 volt/cm. The ratio of sol to gel does not appear to change. Above ~ 10 volt/cm the perpendicular form shown in Fig. 1, b and c, occurs. Often one pseudopodium forms, rather than the two oppositely directed pseudopodia shown.

Perpendicular elongation up to ~ 15 volt/cm can be repeated many times without apparent damage to the amoeba. The *Chaos* shown in Fig. 1, a to c, experienced elongation twice during the preceding half hour, but was indistinguishable from a normal amoeba within 5 to 10 minutes after the field was removed. Above ~ 15 volt/cm in the middle range of frequencies (~ 10 hz to ~ 1 khz) the ratio of sol to gel increases, the contractile vacuoles swell, sudden internal motions of the cytoplasm occur, the clear hyaline