CHARACTERIZATION OF THE HICKORY SHUCKWORM, *CYDIA CARYANA* (LEPIDOPTERA: TORTRICIDAE: OLETHREUTINAE), SEX PHEROMONE: ELECTROPHYSIOLOGICAL, BEHAVIORAL, CHEMICAL AND FIELD ANALYSIS

By

MICHAEL THOMAS SMITH II

Bachelor of Science
Louisiana State University
Baton Rouge, Louisiana
1976

Master of Science
Louisiana State University
Baton Rouge, Louisiana
1978

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
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ANALYSIS

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Thesis Adviser

PREFACE

The rising costs of insecticides, the shift of once secondary pest species to primary pest status, and the increasing concern over environmental contamination have signalled the necessity for a change in our insecticide use patterns. Insecticide application based upon the calendar date is unacceptable. A sensible alternative would be insecticide application based upon the presence and abundance of the target species. The utilization of sex pheromones could offer the grower the opportunity to monitor the target species for this purpose.

In the present research, the chemical characterization of the hickory shuckworm sex pheromone was undertaken. To this end, since no single analysis or bioassay can substantiate the chemical nature of a sex pheromone, four separate analyses were performed.

To enhance its readability and to expedite its publication, this manuscript has been prepared in a publication format. The introductory chapter (Chapter I) introduces the reader to the pest species, the concept of sex pheromones and outlines the purpose of the four analyses in the characterization process. The subsequent four analyses, although separate entities in themselves, are presented sequentially as they were actually performed. Thus, each of the four analyses are presented in a separate chapter, complete with an introduction, materials and methods, results, discussion, conclusions and bibliography.

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assisted me in this research and during my tenure at Oklahoma State University. Without the dedication and cooperation of so many, this research project could not have become a reality.

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NOMENCLATURE

AOV	analysis of variance
BMC	behavior modifying chemicals
cm	centimeter
CNS	central nervous system
°C	degrees Celsius
EAG	electroantennographic; electroantennogram
F.E.	female equivalent
FID	flame ionization detector
GC	gas chromatography
GLC	gas-liquid chromatography
Hz	hertz
IPM	insect pest management
km	kilometer
m	meter
µg	microgram
ml	milliliter
min.	minute
MS	mass spectrometry
N_2	nitrogen gas
ng	nanogram
P	probability
R.H.	relative humidity

R^2	correlation coefficient squared
SIM	selective ion monitor
t_R	retention time
E-4-12:AC	(E)-4-dodecen-1-ol acetate
E-8-12:AC	(E)-8-dodecen-1-ol acetate
Z-8-12:AC	(Z)-8-dodecen-1-ol acetate
Z-9-12:AC	(Z)-9-dodecen-1-ol acetate
E-10-12:AC	(E)-10-dodecen-1-ol acetate
Z-10-12:AC	(Z)-10-dodecen-1-ol acetate
11-12:AC	11-dodecen-1-ol acetate
12:AC	dodecyl acetate
EE-8,10-12:AC	(E,E)-8,10-dodecadien-1-ol acetate
EZ-8,10-12:AC	(E,Z)-8,10-dodecadien-1-ol acetate
Z-12-14:OH	(Z)-12-tetradecen-1-ol
EE-8,10-12:OH	(E,E)-8,10-dodecadien-1-ol

CHAPTER I

INTRODUCTION

The hickory shuckworm, Cydia caryana (Fitch) (Lepidoptera: Tortricidae: Olethreutinae) is one of the most destructive insect pests of pecan, Carya illinoensis (Wang) K. Koch (Osburn et al., 1963), and has been reported to rank as the most important pest of pecan in six southeastern states (McQueen, 1973). Estimates of annual losses due to damage and control costs range from $1.12-11.3 million in Georgia (Suber and Todd, 1980), while damage estimates in other states are seldom speculated due to damage assessment difficulties.

The hickory shuckworm is native to North America, generally distributed throughout the pecan belt from Georgia and South Carolina in the east, to Texas in the west (Walker, 1928, 1933; Osburn et al., 1963; Osburn et al., 1966). Cydia caryana is multivoltine, with two to five overlapping generations per year. Full grown larvae overwinter in shucks on the ground or on the tree, and pupation within the shucks occurs in late winter and early spring. Moths generally first appear in mid to late February, with peak spring emergence in early to mid April (Van Duyn, 1967; Tedders and Gentry, 1970; Harris, 1973; Harris et al., 1975). Calcote and Hyder (1980) reported adult emergence from overwintering larvae to be bimodal, with peaks in the spring and summer. The spring emergence usually coincides with native hickory fruit development, which preceeds fruit set of pecans by 2 to 3 weeks.

Thus, the spring brood oviposit their eggs on hickory fruit and foliage (Moznette, 1938, 1941), and phylloxera galls on hickory and pecan foliage (Moznette, 1941; Boethel et al., 1974; Calcote and Hyder, 1980). Although many researchers consider this generation to be suicidal, these alternate hosts and late emerging shuckworm both provide adequate shuckworm brood for the increasing populations of each succeeding generation.

During these subsequent generations, female moths oviposit their eggs on young pecan fruit and foliage, and upon hatching, the larvae either bore into the fruit's interior or tunnel within the shuck, depending on the developmental stage of the fruit. Thus, shuckworm damage has been categorized into several types. Prior to shell hardening the shuckworm larvae enter the interior of the fruit to feed and develop, causing fruit abortion (Moznette et al., 1931; Walker, 1933; Moznette, 1941; Todd, 1967; Tedders and Edwards, 1972; Payne and Heaton, 1975). Fifty percent or more of a pecan crop can be lost when there is a light fruit set (Osburn et al., 1963; Phillips et al., 1964; Osburn et al., 1966; Smith et al., 1973). After shell hardening, the larvae are restricted to mining within the shuck. This feeding results in improper kernel deposition due to damage to the vascular bundles located within the shuck, delayed maturation, scarring and discoloration of the shell, and hindrance of normal shuck dehiscence, which interferes with processing (Gill, 1924; Adair, 1930; Moznette et al., 1931; Walker, 1933; Moznette, 1941; Todd, 1967; Tedders and Edwards, 1972; Payne and Heaton, 1975).

In light of the limited success of monitoring and population suppression of C. caryana with blacklight traps (Tedders et al.,

1972; Tedders and Edwards, 1972, 1974; Smith et al., 1973; Calcote and Smith, 1974; Teel et al., 1976; Smith and Tedders, 1978), shuckworm control tactics currently depend heavily upon preventive application of insecticides based on the time when damage occurs (Neel, 1959; Osburn and Tedders, 1969; Boethel and Van Cleave, 1972; Polles and Payne, 1974; Payne and Heaton, 1975; Ellis and Polles, 1976). Additionally, since damage has only been qualitatively determined and is not based upon fruit developmental stage, such control tactics are unacceptable.

An alternate method of monitoring/suppression may be found in the use of behavior modifying chemicals (BMC). Aside from the research by Anderson et al. (1973), which concluded that female C. caryana produce a sex pheromone, such research has received little or no attention.

The term pheromone (Karlson and Butenandt, 1959), taken from the Greek pherein (to carry) and hormon (to excite or stimulate), is applied to semiochemicals used for intraspecific communication between individuals. Pheromones either trigger an immediate and reversible change in the behavior of the recipient (releaser) or induce delayed, lasting responses (primer). Sex pheromones are representative of the former category, releasers.

Sex pheromones, providing the most thoroughly documented cases of long-range communication, are used in signalling sexual receptivity. Usually females release the pheromone which males perceive through specialized sensory receptors on their antennae, although males of some species are the emitters. Following detection, a simple or complex ordered series of behavioral responses ensues, ultimately bringing the sexes together for copulation. Sex pheromones are among the most biologically active substances known, with response thresholds as low

as 2 x 10^2 molecules per milliliter of air reported to induce 50% male silkworm moths (Bombyx mori) to respond within 30 seconds (Boeckh et al., 1965).

Since the first successful sex pheromone identification of B. mori by Butenandt et al. (1959), sex pheromones and parapheromones of hundreds of lepidopterous species have been identified and developed, and some are being utilized in insect pest management (IPM). Utilization of sex pheromonal components in IPM is based upon manipulation of the chemical communication system and mating behavior of the pest species. Therefore, detailed analyses of the chemical communication system in target species is a prerequisite for successful use of the pheromonal compounds. To this end, the chemical language must be properly read and understood (Tamaki, 1979).

The procedures involved in sex pheromone identification generally include the following: confirmation of the presence of a sex pheromone in the target insect species, establishment of a bioassay method to monitor the pheromone titre of various fractions, establishment of conditions for collecting or extracting active materials to maximize the recovery of pheromonal compounds, purification and isolation of each pheromonal component, structural analysis of isolated compounds, synthesis of compounds of suggested structure, comparison of chemical characteristics of the synthetic and the isolated compounds, and then a comparison of the biological activity of the synthetic and the isolated compounds, or comparisons with live insects under natural conditions (Tamaki, 1985). Inclusion of each procedure or their sequence within the research process, is highly dependent upon the resources and objectives of each research program. Therefore, the objectives of the

present research are as follows:

1. Evaluation of male C. caryana relative antennal electrophysiological response specificity, and sensitivity to stimulation by several structural series and dilution series, respectively, of common monounsaturated sex pheromonal components of the tortricids. The resulting electroantennographic (EAG) response spectra should provide structural evidence for modelling the C. caryana sex pheromone.
2. Evaluation of male C. caryana behavioral response to key compounds shown to be perceived by EAG analysis in objective 1. Resulting in-flight behavioral response discrimination should further elucidate a predicted configuration of at least the primary component(s) of the C. caryana sex pheromone.
3. Isolation, fractionation and identification of female C. caryana sex pheromone gland extract by capillary gas chromatography and mass spectrometry. Male C. caryana EAG analysis of whole and fractionated extract will represent the criterion of biological activity.
4. Field evaluation of suspected C. caryana sex pheromonal components, using trap catch as the criterion of biological activity.

Although it is beyond the scope of this text to give a complete review of present knowledge of sex pheromone communication in moths, pertinent literature applicable to techniques employed and relevant in discussions of research results will be cited. For a more complete review of sex pheromones, the reader should refer to Jacobson (1972), Birch (1974), Young and Silverstein (1975), Shorey and McKelvey

(1977), Brand et al. (1979), Ritter (1979), Cardé (1979), Roelofs (1980), Kydonieus and Beroza (1982), and Tamaki (1985) in reference to sex pheromones and their utilization, and to Steinbrecht and Schneider (1980), Bell and Cardé (1984), Hummel and Miller (1984), Baker (1985) and Mayer and Mankin (1985) in reference to behavior and sensory physiology.

Bibliography

Adair, H. S. 1930. The hickory or pecan shuckworm. Miss. State Plant Board 9(4): 1-3.

Anderson, A. L., H. W. VanCleave, and T. L. Payne. 1973. An investigation of a sex pheromone of the hickory shuckworm, Laspeyresia caryana (Fitch). Folia. Entomol. Mex. 25-6.

Baker, T. C. 1985. Chemical control of behavior, pp. 621-672. In G. A. Kerkut and L. I. Gilbert [eds.], Comprehensive Insect Physiology, Biochemistry, and Pharmacology: Behavior, Vol. 9. Pergamon Press, Oxford.

Bell, W. J. and R. T. Cardé [eds.]. 1984. Chemical Ecology of Insects. Sinauer Assoc., Inc., Sunderland, Massachusetts, 524 pp.

Birch, M. C. 1974. Pheromones. American Elsevier, New York. 495 pp.

Boeckh, J., K. E. Kaissling, and D. Schneider. 1965. Insect olfactory receptors. Cold Spring Harbor Symposium Quant. Biol. 30: 263-80.

Boethel, D. J. and H. W. VanCleave. 1972. Dosage-mortality studies on laboratory reared and field collected larvae of the hickory shuckworm. J. Econ. Entomol. 65: 232-5.

Boethel, D. J., J. T. Criswell, and R. D. Eikenbary. 1974. Incidence of hickory shuckworm in galls of Phylloxera spp. on pecan. J. Econ.

Entomol. 67: 692-3.

Brand, J. M., J. C. Young, and R. M. Silverstein. 1979. Insect pheromones: a critical review of recent advances in their chemistry, biology and application. Fortschr. Chem. Org. Naturst. 37: 1-190.

Butenandt, A., R. Beckman, D. Stamm, and E. Hecker. 1959. Über den Sexuallockstoff des Seidenspinner Bombyx mori, Reidarstellung und Konstitution. Z. Naturforsch. B14, 283-4.

Calcote, V. R. and D. E. Hyder. 1980. Late season emergence of shuckworm from overwintering shucks. Proc. S.E. Pecan Growers Assoc. 73: 75-7.

Calcote, V. R. and J. S. Smith, Jr. 1974. Attraction of five pecan insect species to light traps equipped with various colored lamps. J. Econ. Entomol. 67: 461-3.

Cardé, R. T. 1979. Behavioral responses of moths to female-produced pheromones and the utilization of attractant-baited traps for population monitoring, pp. 286-315. In R. Rabb and G. Kennedy [eds.], Movement of Highly Mobile Insects: Concepts and Methodology in Research. North Carolina University, Raleigh.

Ellis, H. C. and S. C. Polles. 1976. Aerial application for control of certain pecan insects. Proc. S.E. Pecan Growers Assoc. 69: 119-21.

Gill, J. B. 1924. Important pecan insects and their control. USDA Farmers Bull. 1364: 7-11.

Harris, E. D., Jr. 1973. Hickory shuckworm. Ga. Ext. Service Leaflet No. 30, Univ. of Ga., Athens. 2 pp.

Harris, E. D., Jr., N. E. McGlobon and H. C. Ellis. 1975. Pecan insects and diseases and their control. Ga. Ext. Bull. 644, Univ.

of Ga., Athens. 20 pp.

Hummel, H. E. and T. A. Miller [eds.]. 1984. Techniques in Pheromone Research. Springer-Verlag, New York. 464 pp.

Jacobson, M. 1972. Insect Sex Pheromones. Academic Press, New York. 382 pp.

Karlson, P. and A. Butenandt. 1959. Pheromones (ectohormones) in insects. Ann. Rev. Entomol. 4: 49-58.

Kydonieus, A. F. and M. Beroza [eds.]. 1982. Insect Suppression with Controlled Release Pheromone Systems, Vol. I and II. CRC Press. 274 pp. (Vol I), 312 pp. (Vol. II).

McQueen, H. F. 1973. Occurrence of pecan insects--a 10-year summary. Proc. S.E. Pecan Growers Assoc. 66: 79-81.

Mayer, M. S. and R. W. Mankin. 1985. Neurobiology of pheromone perception, pp. 95-144. In G. A. Kerkut and L. I. Gilbert [eds.], Comprehensive Insect Physiology, Biochemistry, and Pharmacology: Behavior, Vol. 9. Pergamon Press, Oxford.

Moznette, G. F. 1938. Some important biological factors in the control of the hickory shuckworm on pecan. Proc. S.E. Pecan Growers Assoc. 32: 11-4.

Moznette, G. F. 1941. Progress in the control of the hickory shuckworm of pecan. Proc. S.E. Pecan Growers Assoc. 34: 42-8.

Moznette, C. F., T. L. Bissel and H. A. Adair. 1931. Insects of the pecan and how to combat them. USDA Farmers Bull. 1954. 59 pp.

Neel, W. W. 1959. Shuckworm control tests in Mississippi during 1957-1959, with special reference to foliage sprays and granular insecticides. Proc. S.E. Pecan Growers Assoc. 52: 83-7.

Osburn, M. R. and W. L. Tedders. 1969. Insecticides for control of

the hickory shuckworm on pecan. J. Ga. Entomol. Soc. 4(4): 178-80.

Osburn, M. R., W. C. Pierce, A. M. Phillips, J. R. Cole, and G. L. Barnes. 1963. Controlling insects and diseases of the pecan. USDA Agric. Handb. No. 242, 52 pp.

Osburn, M. R., W. C. Pierce, A. M. Phillips, J. R. Cole and G. E. Kenknight. 1966. Controlling insects and diseases of the pecan. USDA Agric. Handb. No. 240, 55 pp.

Payne, J. A. and E. K. Heaton. 1975. The hickory shuckworm: its biology, effect upon nut quality, and control. Annu. Rep. Northern Nut Growers Assoc. 66: 19-25.

Phillips, A. M., J. R. Large and J. R. Cole. 1964. Insects and diseases of the pecan in Florida. Bull. 619A, Univ. of Fla. Agric. Exp. Sta., Gainesville. 87 pp.

Polles, S. G. and J. A. Payne. 1974. Pecan weevil, hickory shuckworm, and yellow pecan aphids. Proc. S.E. Pecan Growers Assoc. 67: 79-94.

Ritter, F. J. 1979. Chemical Ecology: Odour Communication in Animals. Elsevier, North Holland, Amsterdam. 427 pp.

Roelofs, W. L. 1980. Pheromones and their chemistry, pp. 583-602. In M. Locke and D. S. Smith [eds.], Insect Biology in the Future. Academic Press, New York.

Shorey, H. H. and J. J. McKelvey, Jr. [eds.]. 1977. Chemical Control of Insect Behavior: Theory and Application. J. Wiley and Sons, New York. 414 pp.

Smith, J. S., Jr. and W. L. Tedders. 1978. S-2 blacklight traps for capturing certain pecan insects. Pecan South 5(4): 172-4.

Smith, J. S., Jr., W. L. Tedders and C. R. Gentry. 1973. Blacklight

trapping of the hickory shuckworm moth in Georgia pecan orchards. Trans. Am. Soc. Agric. Eng. 16(1): 127-8.

Steinbrecht, R. A. and D. Schneider. 1980. Pheromone communication in moths sensory physiology and behavior, pp. 635-703. In M. Locke and D. S. Smith [eds.], Insect Biology in the Future. Academic Press, New York.

Suber, E. F. and J. W. Todd [eds.]. 1980. Summary of Economic Losses Due to Insect Damage and Costs of Control in Georgia 1970-1976. Univ. of Ga. Special Publ. No. 7.

Tamaki, Y. 1979. Multi-component sex pheromone of Lepidoptera with special reference to Adoxophyes sp., pp. 169-180. In R. J. Ritter [ed.], Chemical Ecology: Odour Communication in Animals. Elsevier North Holland, Amsterdam.

Tamaki, Y. 1985. Sex pheromones, pp. 145-191. In G. A. Kerkut and L. I. Gilbert [eds.], Comprehensive Insect Physiology, Biochemistry, and Pharmacology: Behavior, Vol. 9. Pergamon Press, Oxford.

Tedders, W. L. and G. W. Edwards. 1972. Effects of blacklight trap design and placement on catch of adult hickory shuckworm. J. Econ. Entomol. 65: 1624-7.

Tedders, W. L. and G. W. Edwards. 1974. A blacklight trap for surveys of hickory shuckworm moths. J. Ga. Entomol. Soc. 9(3): 176-81.

Tedders, W. L. and C. R. Gentry. 1970. Know these pecan enemies--hickory shuckworm and pecan weevil. Progressive Farmer, Aug., 1970, p. 26.

Tedders, W. L., J. G. Hartstack and M. R. Osburn. 1972. Suppression of hickory shuckworm in a pecan orchard with blacklight traps. J. Econ. Entomol. 65: 148-55.

Teel, P. D., H. W. Van Cleave, J. P. Hollingsworth and A. W. Hartsock, Jr. 1976. Spectral sensitivity of the hickory shuckworm to electromagnetic radiation. J. Econ. Entomol. 69: 57-8.

Todd, L. 1967. A spring emergence study of the hickory shuckworm, Laspeyresia caryana. Texas Pecan Growers Assoc. Proc. 46: 80-5.

Van Duyn, J. W. 1967. Emergence of overwintering shuckworm in north Florida. Proc. S.E. Pecan Growers Assoc. 60: 45-6.

Walker, F. W. 1928. The shuckworm, a serious pest of the pecan in Florida. Proc. Ga.-Fla. Pecan Growers Assoc. 211: 26-31.

Walker, F. W. 1933. The pecan shuckworm. Bull. 258, Univ. of Florida Agric. Exp. Sta., Gainesville. 18 pp.

Young, J. C. and R. M. Silverstein. 1975. Biological and chemical methodology in the study of insect communication systems, pp. 75-161. In D. G. Moulton, A. Turk, and J. W. Johnson, Jr. [eds.], Methods in Olfactory Research. Academic Press, New York.

CHAPTER II

ELECTROANTENNOGRAPHIC ANALYSIS OF ANTENNAL RECEPTOR SPECIFICITY AND RESPONSE THRESHOLD

Introduction

Electroantennographic (EAG) techniques have been successfully utilized in the identification of pheromones and host attractants in two primary ways. First, fractions from female gland contents, separated by gas-liquid chromatography (GLC), have either been collected and assayed on male moth antennae (Roelofs, 1979), or the EAG has been linked directly to a gas chromatographic column and recordings made simultaneously from the gas chromatographic and the antennal detectors (Moorhouse et al., 1969; Arn et al., 1975). Secondly, the EAG is used to screen synthetic pheromones and analogues in order to predict the probable chemical constitution of a species' sex pheromone. In this way, potentially attractive candidate compounds can be selected for laboratory and field evaluation (Roelofs and Comeau, 1971; Roelofs et al., 1971). The second approach is more appropriate if there is some preliminary indication of the chemical composition of the sex pheromone or attractants for the species under study (Wall et al., 1976). Preliminary field trapping experiments conducted during 1982 in Oklahoma (M. T. Smith and R. D. Eikenbary, Department of Entomology, Oklahoma

State University), Texas (M. K. Harris, Department of Entomology, Texas A & M University), Louisiana (M. J. Hall, Department of Entomology, Louisiana State University), and Georgia (J. D. Dutcher, Department of Entomology, University of Georgia), in cooperation with W. L. Roelofs (Department of Entomology, Cornell University, Geneva), indicated that EE-8,10-12:AC alone was moderately attractive to Cydia caryana (Fitch) male moths. This attractancy was short-lived and diminished considerably over the duration of the 1982 field season. Therefore, based upon these preliminary data and the taxonomic relationships of pheromone-receptor systems between species of Lepidoptera (Priesner, 1979), and more specifically the Tortricids (Roelofs and Brown, 1982), attractants for C. caryana were thought to be closely related to EE-8,10-12:OH or EE-8,10-12:AC.

We report here results from an EAG analysis which provides evidence for the chemical characterization of the C. caryana sex pheromone. Electroantennograms were recorded from male moth antennae to assess both their relative specificity (responsiveness) and sensitivity (activation threshold) to stimulation by several structural series and dilution series of monounsaturated test chemicals, respectively. Roelofs et al. (1971) used a similar EAG technique in their elucidation of the configuration of a highly active chemical which they proposed as the sex pheromone of Cydia pomonella (L.), the codling moth.

Materials and Methods

Insects

C. caryana has been reared on artificial diet with very limited

success (Schroeder and Osburn, 1969). Thus, test insects used in this study were obtained as full-grown overwintering larvae within pecan shucks from several pecan orchards near Stillwater, Oklahoma. Larvae were kept in a controlled environmental room, simulating Oklahoma environmental conditions, so as to induce normal emergence at a temperature of 22-24°C, 65% R.H. under a 14-hours light/10-hours dark photoperiod. Emergent adults were collected daily, sexed and each sex placed into separate holding cages provided with a solution of 5% sugar-water.

EAG Recording Technique

The EAG is thought to be the expression of generator potentials of many simultaneously stimulated receptor cells with potentially different specificities (Kaissling, 1971; Schneider et al., 1977).

Male hickory shuckworm moths (2- to 4-days old) were anesthetized with carbon dioxide and immobilized on a cork stopper with cellophane tape so that one antenna could be manipulated for electrode attachment without damage. The tip of a glass pipette, containing Ringer solution (Humason, 1974) and a silver-silver chloride electrode was inserted into the hemolymph through the antennal basal membrane, and the tip of the antenna was inserted into the end of another similarly prepared electrode. The EAG records a potential difference between the two electrodes.

Electrodes were connected to the differential amplifier inputs of a Tektronix D-11 storage oscilloscope operated in the infinite input impedance mode. EAG deflections were measured directly from the stored screen image.

Stimulation

Synthetic test compounds were deposited on the inside of a glass tube as a dichloromethane solution. After evaporation of the solvent, the glass tube containing the test compound was connected into a rubber tubing line leading to a glass T tube. Nitrogen gas from a tank was continuously blown through the other side of the T tube and over the antenna. A 2 ml puff of N_2 from a second tank was metered through a solenoid, which then carried the test chemical vapors to the T tube and into the continuous N_2 flow and over the antenna. For a given compound, the chemical evaporation rate, puff concentration and number of chemical molecules that impinged upon dendritic acceptor sites were assumed to be proportional to the concentration of the chemical deposited on the inside of the glass tube. Thus, the stimulating concentration for antennae will be derived from the amount of chemical placed on the glass tube.

Experimental Design and
Evaluation of Results

A strict system of temporal spacing of stimuli was utilized in order to minimize sensory adaptation in the receptors and to avoid absorptive overloading of the antenna. All compounds were tested at least three times. The standard, EE-8,10-12:AC at 100 µg, and a blank N_2 were tested after every three compounds to confirm the stability of the antennal response. Percent responses were calculated relative to responses in millivolts to EE-8,10-12:AC, from the equation (McDonough et al., 1982):

$$\% \text{ response} = \frac{\text{test compound response - air response}}{\text{EE-8,10-12:AC response - air response}} \times 100 \qquad (2.1)$$

The conversion of each response to a percentage of the standard 'normalized' the variability between individual antennal preparations and time-dependent variability in antennal responsiveness (Roelofs and Comeau, 1971; Priesner et al., 1975; Light and Birch, 1979).

Antennal Responsiveness

(Specificity)

To determine whether antennae of male C. caryana perceive various test compounds, relative activities of a series of synthetic chemicals at their saturation levels (100 µg) were EAG assayed. Test compounds included the usual series of geometrical and positional isomers of monounsaturated C12 and C14 acetates and alcohols.

Antennal Sensitivity

(Response Threshold)

To compare the sensitivity of the male moth antennae to those compounds shown to be perceived in the antennal responsiveness bioassay, a range of stimulus intensities for each compound were produced by serial dilutions in dichloromethane. The dilution series varied in three-fold steps from 0.3 ng to 100.0 µg.

Results

Antennal Responsiveness

None of the responses to the monounsaturated C12 and C14 acetates and alcohols approached that elicited by the standard, EE-8,10-12:AC.

In general, responsiveness was greatest among the C12 acetate group (Figure 1), where E-10-, Z-9- and E-8-12:AC elicited significantly greater responses than all other C12 acetates ($P < 0.05$). Although not significantly different from each other and yet significantly less than the three previously mentioned isomers, Z-8- and Z-10-12:AC elicited significantly greater responses than the remaining monoene C12 acetates (except 11- and E-4-12:AC). No significant responses were elicited by any C14 acetate (Figure 2), and all C14 acetate responses were significantly less than those to the E-8-, Z-8-, Z-9-, E-10-, and Z-10- C12 acetates ($P < 0.05$).

Regardless of double bond position or configuration, no significant responses ($P > 0.05$) were elicited by any of the C12 alcohols (Figure 3). However, the E-10- isomer produced the greatest response, indicating the positional importance of unsaturation at C-10. Among the C14 alcohols, significant response ($P < 0.05$) was elicited by only the Z-12-14:OH (Figure 4).

Antennal Sensitivity

The response parameters resulting from serial dilution stimulations by compounds selected from the antennal responsiveness tests, which include the E-8-, Z-9- and E-10- 12C acetates, Z-12-14:OH, and EE-, EZ-, and EE- and EZ-8,10-12:AC C12 acetates, are summarized in Table I. To facilitate comparison of the important trends in the data, the mean EAG responses for each sample concentration were used in the analysis. This minimized the influence of variablility in response at each concentration for each compound, which may arise from: (1) physical factors which alter the relative accessibilities of compounds to the receptor

Figure 1. Male Hickory Shuckworm Moth Mean Percent EAG Responses to Monounsaturated 12-Carbon Acetates

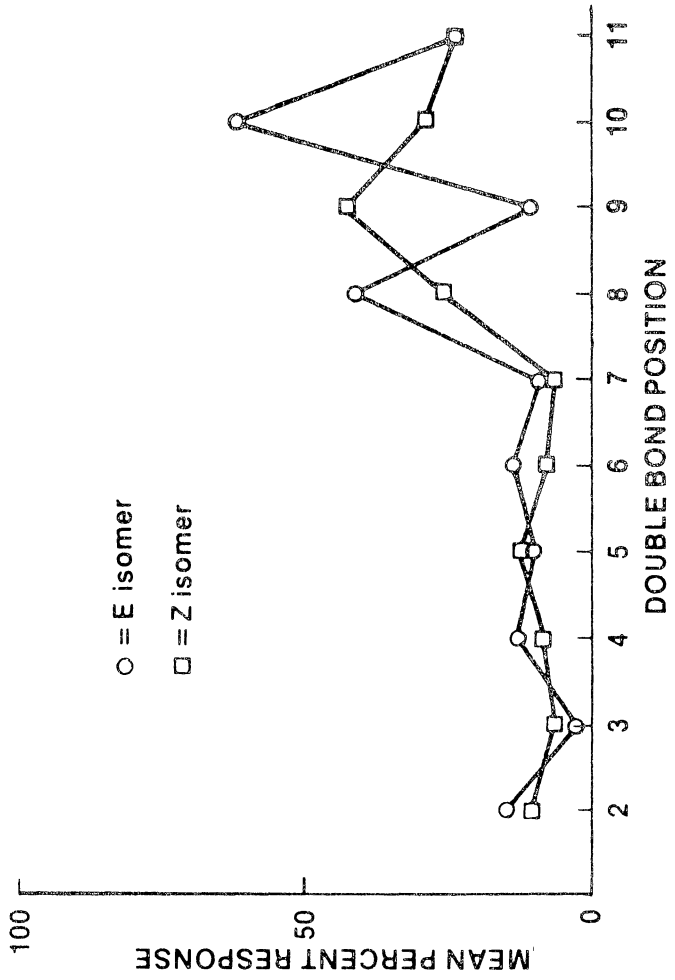

MEAN PERCENT RESPONSE
100
50
0
2
3
4
5
6
7
8
9
10
11
DOUBLE BOND POSITION
○ = E isomer
□ = Z isomer

Figure 2. Male Hickory Shuckworm Moth Mean Percent EAG Responses to Monounsaturated 14-Carbon Acetates

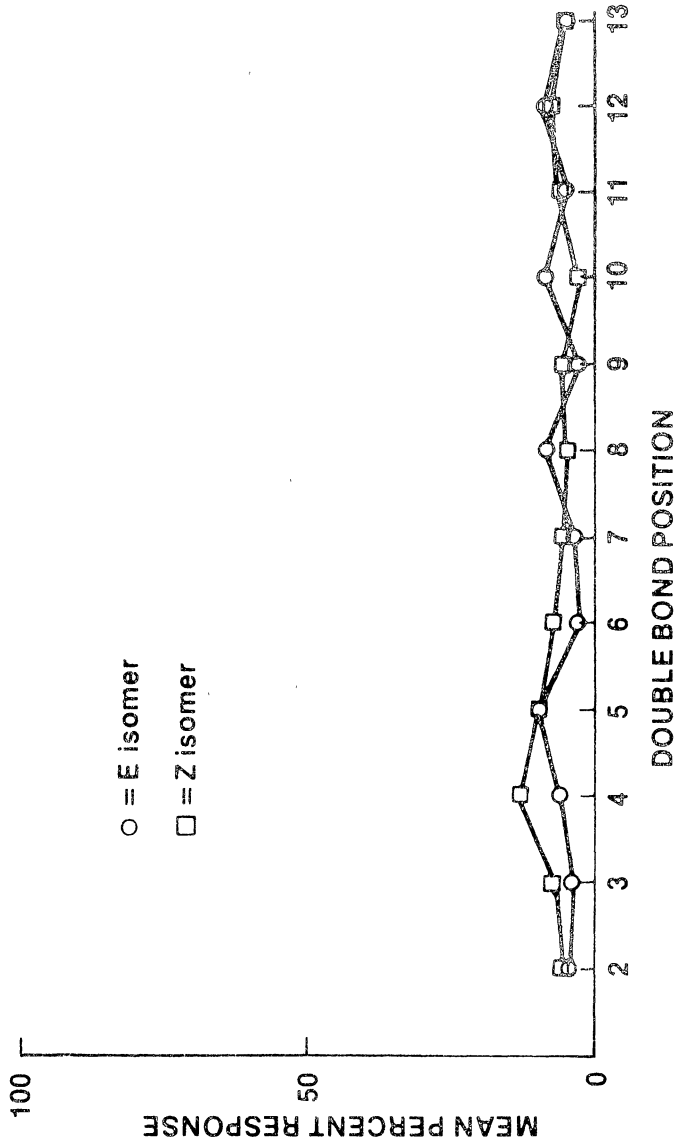

MEAN PERCENT RESPONSE
100
50
0
2
3
4
5
6
7
8
9
10
11
12
13
DOUBLE BOND POSITION
○ = E isomer
□ = Z isomer

Figure 3. Male Hickory Shuckworm Moth Mean Percent EAG Responses to Monounsaturated 12-Carbon Alcohols

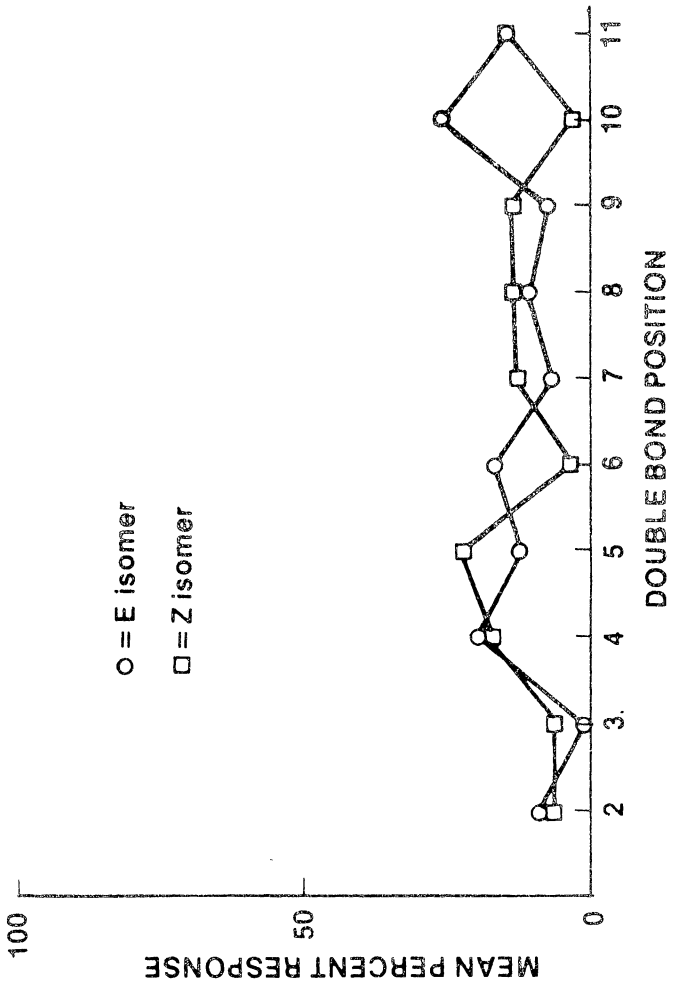

MEAN PERCENT RESPONSE
100
50
0
2
3
4
5
6
7
8
9
10
11
DOUBLE BOND POSITION
O = E isomer
□ = Z isomer

Figure 4. Male Hickory Shuckworm Moth Mean Percent EAG Responses to Monounsaturated 14-Carbon Alcohols

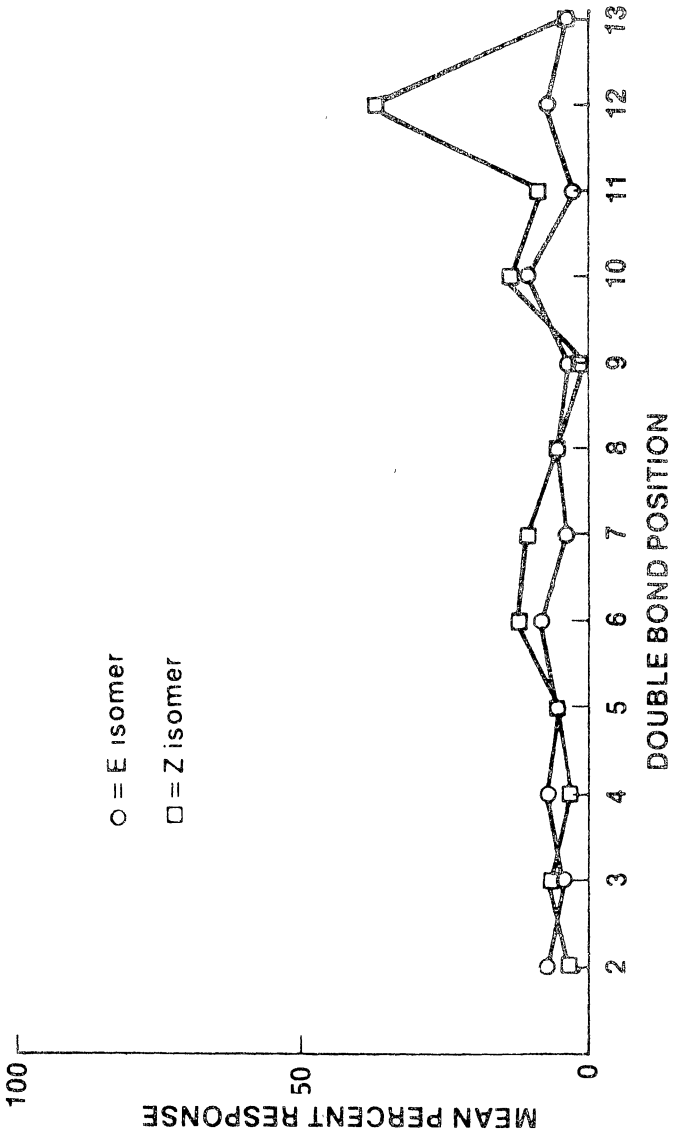

MEAN PERCENT RESPONSE
100
50
0
2
3
4
5
6
7
8
9
10
11
12
13
DOUBLE BOND POSITION
○ = E isomer
□ = Z isomer

TABLE I

CYDIA CARYANA ANTENNAL SENSITIVITY RESPONSE PARAMETERS

Compound	Model		R^2	X-Intercept (log_{10} ng)			Number Molecules at X-Intercept (X 10^{-11})
	Y-Intercept ± S.E. (% Response at 1 ng)	Slope ± S.E. (% Response/ng)		Lower Limit	Calculated	Upper Limit	
EE-8,10-12:AC	31.02 ± 3.93	13.41 ± 1.39 a [1]	.90	-3.94	-2.31 a [2]	-1.01	0.132
EE- & EZ-8,10-12:AC	20.81 ± 7.80	14.90 ± 2.76 a	.74	-4.41	-1.40 a	0.73	1.081
EZ-8,10-12:AC	8.25 ± 3.48	11.39 ± 1.23 a	.90	-2.20	-0.72 a	0.52	5.076
Z-12-14:OH	-2.76 ± 4.52	14.24 ± 1.60 a	.89	-1.24	-0.19 a	1.45	44.401
E-8-12:AC	9.39 ± 5.58	12.11 ± 1.98 ab	.79	-3.18	-0.78 a	1.07	4.474
E-10-12:AC	22.41 ± 4.72	10.73 ± 1.07 ab	.80	-4.14	-2.09 a	0.05	0.216
Z-9-12:AC	13.52 ± 1.58	7.96 ± 0.56 b	.95	-2.69	-1.70 a	-0.83	0.533

[1] Slope values followed by the same letter are not significantly different (P = 0.10, Confidence Interval Method) (Snedecor and Cochran, 1967).

[2] X-Intercept values followed by the same letter are not significantly different (P = 0.10, Confidence Interval Method) (Sokal and Rohlf, 1981).

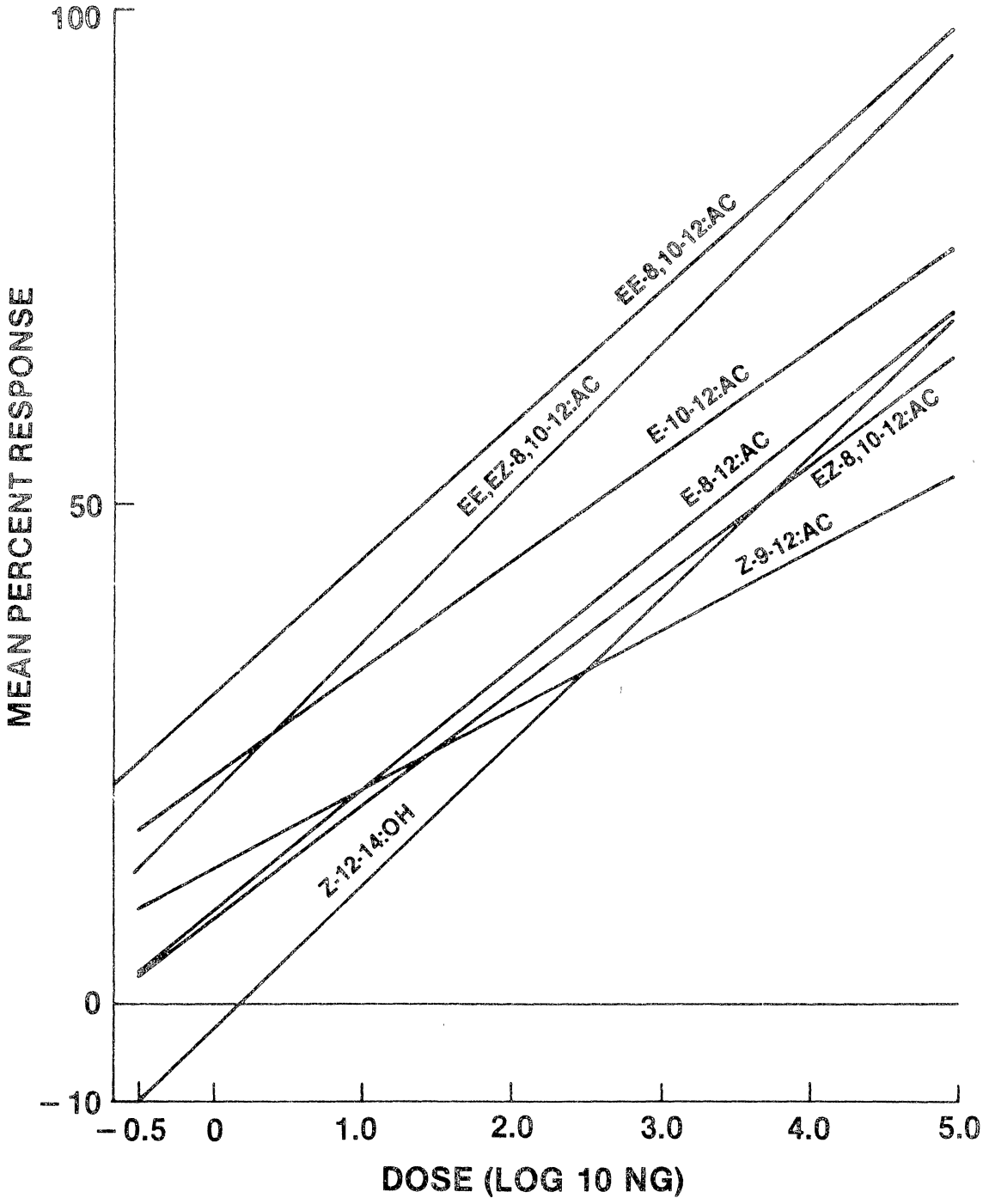
neurons; (2) physiological factors which change the levels of excitability of the receptor neuron system from one odor presentation to the next; and (3) intrinsic receptor neuron properties, such as the kind, number and location of individual receptor sites, which are differentially distributed from one receptor to another (O'Connell, 1975). The slopes of the response functions and their X and Y intercepts were obtained by computing a straight line fit with regression analysis.

Our results show primarily that male C. caryana antennae are most sensitive to the standard, EE-8,10-12:AC, within the range of concentrations tested. The correlation coefficients relating receptor response to the log of stimulus intensity in every case had an absolute value greater than 0.74 and each response stimulus displayed a significant positive linear log dose-response relationship (Figure 5). However, it should be noted that E-8-12:AC and Z-12-14:OH also showed significant quadratic log dose-response functions, with R^2 values of 0.89 and 0.96, respectively, versus 0.79 and 0.89, for their respective linear functions.

The slope of the response function can be taken as a measure of the gain of individual receptors to increasing stimulus intensity. Stimulation with Z-9-12:AC produced a response curve whose slope was significantly different ($P = 0.10$) from those produced with EE-8,10-12:AC, EE- and EZ-8,10-12:AC, EZ-8,10-12:AC and Z-12-14:OH.

The X-intercept may be regarded as an estimate of a given receptor's response threshold. Our results showed no significant differences in response thresholds ($P = 0.10$) between response functions, but provided information on the minimum number of stimulus molecules needed to elicit a detectable EAG response for each response function.

Figure 5. *Cydia caryana* Predicted EAG Response


100
50
0
– 10
MEAN PERCENT RESPONSE
– 0.5
0
1.0
2.0
3.0
4.0
5.0
DOSE (LOG 10 NG)
EE-8,10-12:AC
EE,EZ-8,10-12:AC
E-10-12:AC
E-8-12:AC
EZ-8,10-12:AC
Z-9-12:AC
Z-12-14:OH

Discussion

Antennal Responsiveness

The position of unsaturation and its configuration within a stimulus molecule have a marked effect on antennal response. In both the C12 acetates and alcohols, the E-10- isomer elicited the response of greatest magnitude. This is further illustrated in the Z-12- isomer of the C14 alcohols, where lengthening the carbon chain by two CH_2 units merely displaced the preferred position of unsaturation by two carbon atoms.

Although the position of unsaturation at C-10 produced the largest EAG response of any monoene C12 acetate, double bonds at C-8 (E configuration) and C-9 (Z configuration) also produced significantly larger responses. This response pattern suggests that the E-8- double bond is an important feature of the pheromone structure as well as the E-10-, indicating that the pheromone may contain a conjugated E-8-, E-10- system. Wall et al. (1976) similarly found that in the pea moth, Cydia nigricana, larger EAG responses were elicited by the E-10-, Z-9-, and E-8- isomers of the monounsaturated C12 acetates.

This same EAG profile was also obtained when male codling moth, Cydia pomonella, antennae were subjected to monounsaturated C12 alcohols (Roelofs et al., 1971). Strong responses produced by the C12 alcohols unsaturated at C-8, C-9, and C-10 were subsequently used to predict the EE-8,10-12:OH pheromonal structure. Roelofs et al. (1971) also suggested, but never confirmed, that positional isomers unsaturated in the intermediary position (C-9) can probably interact with the binding sites of the C-8 and C-10 double bonds.

EAG analysis of standard series of synthetic compounds is generally considered to be unreliable in predicting minor pheromone components. However, known minor pheromone components generally elicit responses below those produced by primary components, yet usually still detectable above responses to the majority of test compounds. Thus, although in no way conclusive, the intermediate responses elicited by the Z-8- and Z-10- isomers of the C12 acetates may suggest their importance as minor C. caryana sex pheromone components.

Antennal Sensitivity

The maximally effective stimulus compound is the 'key' compound and the primary feature in classifying receptor response; it is also the point of reference in all statements of structure-activity relationships (Priesner, 1979). Although it is accepted that a receptor may have its threshold for a 'key' substance, it may also respond to other compounds but only at higher concentrations. Therefore, the chemical specificity of pheromone receptors is best described by their dose-response relationships to a series of related test compounds (Kaissling, 1977).

Our results demonstrate that fewer molecules of EE-8,10-12:AC are necessary to elicit an electrophysiological response in the antennae of male C. caryana than all other compounds tested. Thus, we conclude here that EE-8,10-12:AC is the 'key' stimulus compound whose structure most likely resembles a biologically active compound for which C. caryana possess a pheromone neuron acceptor.

Although each stimulus displayed a linear logarithmic stimulus-response curve, the additional quadratic feature of the E-8-12:AC and

the Z-12-14:OH response curves should be viewed in light of the probable protein nature of the dendritic membrane acceptor molecules. Such cooperative allosteric binding within and/or between spatially distinct sites occurs in many proteins, i.e., hemoglobin's binding by oxygen.

Under the limitations of the EAG technique, i.e., the summation of receptor potential in numerous receptor neurons over an undefined antennal area, we can only conjecture that differential gain in responsiveness arises when stimulus compounds are perceived by receptor neurons having different intrinsic receptor membrane properties (number, distribution or kind of receptor sites, i.e., number of ion channels opened by the binding of stimulus molecules with acceptor site protein molecules).

Payne and Finn (1977) utilized regression analysis to evaluate mean percent EAG response of female Galleria mellonella (L.) to standard vs. log concentration curves for two conspecific male pheromones, n-nonanal and n-undecanal. They hypothesized that female G. mellonella antennae posses two heterologous populations of acceptors since regression curves for these two pheromones were of significantly different slopes. Payne and Finn (1977) confirmed their hypothesis by the differential adaption technique. Kaissling (1971), O'Connell (1975) and Light and Birch (1979), have also utilized EAG response vs. concentration curves to imply specificity of receptor neurons.

Since the EE-8,10-12:AC, EZ-8,10-12:AC, and EE- and EZ-8,10-12:AC share a common slope and are structurally quite similar (differing only in geometrical configuration about the C-10 unsaturation), it is highly probable that these compounds share the same acceptors (designated here as acceptor a).

Conversely, however, we hypothesize that a second population of acceptors (designated as acceptor b) exists for Z-9-12:AC. Structural similarities shared by the E-8-12:AC and E-10-12:AC with compounds utilizing either of the two proposed heterologous populations of acceptors, results in their ability to bind with either acceptor a or b.

Although Z-12-14:OH shares a common slope with EE-, EZ- and EE- and EZ-8,10-dodecadien-1-ol acetate when considering the linear feature of their response curves, the additional quadratic feature of its (Z-12-14:OH) response curve and its obvious structural differences precludes conjecture on its acceptor homologousity with acceptor a. Although inconclusive without additional evidence based on the differential adaptation technique, the additional quadratic features of both the Z-12-14:OH and E-8-12:AC response curves suggests that these compounds may utilize acceptors distinct from acceptors a and b.

Again, under the limitations of the EAG technique, we can only conjecture that differential response thresholds for stimuli utilizing homologous acceptors arises when quantitative differences in binding strengths (affinity) exists between the odor molecules and the acceptor site protein.

Payne (1969) recorded EAGs from male antennae of Trichoplusia ni (Huber) to the synthetic pheromone (cis-7-dodecenyl acetate) and parapheromones (analogous compounds). He revealed that the greater a parapheromone differs in structure from cis-7-dodecenyl acetate, in the site of unsaturation or carbon chain length, the more of the parapheromone is needed to elicit an EAG response, thus increasing its response threshold (Payne et al., 1973).

Given the likelihood that all compounds tested share homologous

acceptors (acceptor a), except Z-9-12:AC, our results likewise demonstrate an increasing response threshold (or decreasing affinity) in the order EE-8,10-12:AC < E-10-12:AC < EE- and EZ-8,10-12:AC < E-8-12:AC < EZ-8,10-12:AC < Z-12-14:OH. The response threshold for Z-12-14:OH is 336 times that for the key stimulus compound, EE-8,10-12:AC (significantly different at $P = 0.14$). In addition, taking their structural differences into account, this further precludes any conclusion regarding the homologousity of their dendritic acceptors.

Conclusions

The antennal responsiveness and sensitivity results provide an example in which the EAG bioassay of monounsaturated standards can be used to predict the position and unsaturation of a possible diunsaturated sex pheromonal component, thus elucidating in part the relationship between chemical structure and electrophysiological activity (Roelofs and Comeau, 1971; Roelofs et al., 1971; Wall et al., 1976). The physiological requirements for orientation to a distant odor source are hypothesized to be: (1) a highly specific and sensitive response threshold; (2) a logarithmic stimulus-response curve in which a small increase in stimulatory molecule concentration elicits a significantly increased neural output; and (3) a rapid recovery rate from adaptation (Boeckh et al., 1965; Kaissling, 1971; Seabrook, 1978; Light and Birch, 1979). Although the later condition was not tested here, the two former requirements were fulfilled in male C. caryana. Therefore, it is highly probable that the E-8-, E-10- conjugated double bond system of a dodecadien-1-ol acetate is a critical chemical structural component of a C. caryana sex pheromone.

We propose that at least two heterologous populations of acceptors may exist (acceptors a and b). We also conjecture the existence of an additional acceptor for Z-12-14:OH and E-8-12:AC, distinct from acceptors a and b. Although inconclusive, we suggest that Z-8- and Z-10- dodecen-1-ol acetates, as well as Z-12-14:OH and E-8-12:AC, should be investigated as possible minor pheromone components.

Bibliography

Arn H., E. Stadler, and S. Rauscher. 1975. The electroantennographic detector - a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. Z. Naturforsch. 30: 722-5.

Boeckh, J., K. E. Kaissling, and D. Schneider. 1965. Insect olfactory receptors. Cold Spring Harbor Symposium Quant. Biol. 30: 263-80.

Humason, G. L. 1979. Animal Tissue Techniques. 4th ed. W. H. Freeman and Co., San Francisco. 661 pp.

Kaissling, K. E. 1971. Insect olfaction, pp. 351-431. In L. M. Beidler [ed.], Handbook of Sensory Physiology IV. Springer, Berlin.

Kaissling, K. E. 1977. Structure of odour molecules and multiple activities of receptor cells, pp. 9-16. In J. LeMagnem and P. MacLead [eds.], Olfaction and Taste, Vol. 6. IRL London.

Light, D. M. and M. C. Birch. 1979. Electrophysiological basis for the behavioral response of male and female Trichoplusia ni to synthetic female pheromone. J. Insect Physiol. 25: 161-7.

McDonough, L. M., J. A. Kamm, D. A. George, C. L. Smithhisler, and S. Voerman. 1982. Sex attractant for the western lawn moth, Tehama bonifatella Hulst. Environ. Entomol. 11: 711-14.

Moorhouse, J. E., R. Yeadon, P. S. Beevor, and B. F. Nesbitt. 1969.

Method for use in studies of insect chemical communications. Nature 223: 1174-5.

O'Connell, R. J. 1975. Olfactory receptor responses to sex pheromone components in the redbanded leafroller moth. J. Gen. Physiol. 65: 179-205.

Payne, T. L. 1969. Electrophysiological investigation of sex pheromone reception in noctuid moths, with special reference to the cabbage looper, Trichoplusia ni (Hübner) (Lepidoptera: Noctuidae). Ph.D. dissertation, University of California, Riverside. 115 pp.

Payne, T. L. and W. E. Finn. 1977. Pheromone receptor system in the females of the greater wax moth, Galleria mellonella. J. Insect Physiol. 23: 879-81.

Payne T. L., H. H. Shorey, and L. K. Gaston. 1973. Sex pheromones of Lepidoptera: XXXVIII. Electroantennogram responses in Autographa californica to cis-7-dodecenyl acetate and related compounds. Ann. Entomol. Soc. Am. 66(3): 703-4.

Priesner, E. 1979. Specificity studies on pheromone receptors of noctuid and tortricid Lepidoptera. In F. J. Ritter [ed.], Chemical Ecology: Odour Communication in Animals. Elsevier/N. Holland, Amsterdam.

Priesner, E., M. Jacobson, and H. J. Bestmann. 1975. Structure-response relationships in noctuid sex pheromone reception. An introductory report. Z. Naturforsch. 30c: 283-93.

Roelofs, W. L. 1979. Electroantennograms. Chemtech 9: 222-7.

Roelofs, W. L. and R. L. Brown. 1982. Pheromones and evolutionary relationships of Tortricidae. Annu. Rev. Ecol. Syst. 13: 395-422.

Roelofs, W. L. and A. Comeau. 1971. Sex pheromone reception:

electroantennogram responses of the redbanded leafroller moth. J. Insect Physiol. 17: 1969-82.

Roelofs, W. L., A. Comeau, A. Hill, and G. Milicevic. 1971. Sex attractant of the codling moth: characterization with electro-antennogram technique. Science 174: 297-9.

Schneider, D., W. A. Kafka, M. Beroza, and B. A. Bierd. 1977. Odor receptor responses of male gypsy and nun moths (Lepidoptera: Lymatriidae) to dispalure and its analogues. J. Comp. Physiol. 113: 1-15.

Schroeder, W. J. and M. R. Osburn. 1969. Rearing the hickory shuckworm, Laspeyresia caryana, on artificial diet, with notes on biology. Ann. Entomol. Soc. Am. 62: 1401-3.

Seabrook, W. D. 1978. Neurobiological contributions to understanding insect pheromone systems. Annu. Rev. Entomol. 23: 471-85.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods, 6th ed. The Iowa State University Press, Ames. 593 pp.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry. 2nd ed. W. H. Freeman and Co., San Francisco. 859 pp.

Wall, C., A. R. Greenway, and P. E. Burt. 1976. Electroantenno-graphic and field responses of the pea moth, Cydia nigricana, to sex attractants and related compounds. Physiol. Entomol. 1: 151-7.

CHAPTER III

ANALYSIS OF *CYDIA CARYANA* IN-FLIGHT BEHAVIOR TO PERCEIVED SYNTHETIC COMPOUNDS AND CORRELATION WITH ELECTROANTENNOGRAM (EAG) RESPONSE

Introduction

Sex pheromone communication in many species of Lepidoptera involves a complex sequence of behaviors exhibited by males to a single or multicomponent chemical signal released by conspecific females (Cardé, 1979). Behavior is the end result of integrative processes, receiving input via a large number of modalities. Thus, the ability of a given sex to perceive a pheromone does not necessarily mean it will display a stereotyped behavioral response or respond at all (Birch, 1971). Knowledge of what information is transmitted by nerve impulses, how it is integrated by the central nervous system (CNS), or even the difference between excitatory and inhibitory impulses is still quite limited and often highly speculative.

Because the antennae of Lepidoptera usually have a large number of specialized receptors for perception of a specific pheromone(s), the electroantennogram (EAG) provides a more realistic prediction of behavior than that of other insect orders (Schneider et al., 1967; (Priesner, 1968). Previously it was postulated and subsequently

demonstrated that a lepidopterous sex pheromone evokes greater EAG potentials than other insect orders because of this great number of olfactory receptors specific for the sex pheromone (Grant, 1970; Roelofs and Comeau, 1971; Roelofs et al., 1971a, b). However, since the EAG is a summated antennal receptor potential (Schneider, 1969), which per se unlikely conveys information to the CNS, behavior may not always be predictable from the evoked EAG (Mayer, 1973). Therefore, where the EAG is employed for determination of compounds that an insect may perceive as odors or as a tool in pheromone identification (Roelofs et al., 1971a, b), it is essential that correlative behavioral analyses be conducted to determine a chemical's biological activity.

In the present study, we report results of experiments designed to examine Cydia caryana (Fitch) behavioral responses to synthetic compounds shown to be perceived at low response threshold levels in an EAG analysis (Chapter I). Principally diagnostic in nature, this paper, therefore, reports additional evidence of the probable chemical characterization of at least the primary component(s) of the C. caryana sex pheromone.

Materials and Methods

Insects

C. caryana male moths were obtained as full-grown overwintering larvae within pecan shucks from a pecan orchard in Sparks, Oklahoma. Larvae were maintained in a controlled environmental room, simulating Oklahoma environmental conditions so as to induce normal emergence at

a temperature of 22-24°C, 65% R.H. under a 14-hours light/10-hours dark photoperiod. Unmated emergent male moths were caged and provided with a solution of 5% sugar-water. All males were tested between 2 and 3 days after eclosion.

Wind Tunnel

In the research reported here, a flight tunnel modeled after that designed by Miller and Roelofs (1978), equipped with a patterned moving floor, was used to study flight behavior and orientation of C. caryana. The moving floor was not utilized in this study. The light intensity inside the tunnel was 0.5 lux. Full moonlight is ca. 0.3 lux (Baker and Cardé, 1984). A fan and set of screen baffles downwind were used to draw in air and minimize turbulence in an air flow set at 30 cm sec^{-1}. An exhaust fan at the downwind end of the tunnel also aided in the removal of pheromone-laden air which was vented to the outside. The room housing the wind tunnel was isolated from the colony room and both rooms were maintained at similar environmental conditions.

Plume Characterization

Prior to testing, the plume was visually characterized as to its relative position, shape and flow within the tunnel using titanium tetrachloride, which upon exposure to air forms titanium dioxide smoke (Miller and Roelofs, 1978). With very little turbulence in the tunnel, the resultant plume, filamentous in nature, had discrete boundaries particularly within 1 m of the smoke source. The plume then widened to approximately 30 cm by the time it reached the downwind end of the tunnel.

Stimulation

Lower test compound dosages are best for discriminating among behavioral responses to various treatments. Therefore, a dilution series of concentrations varying in 10-fold steps from 0.1 ng to 100.0 µg, plus a solvent blank were prepared in dichloromethane. Red rubber 1F type septa (The West Co., Phoenixville, PA) dispensers were used so as to ensure a fairly constant emission rate of stimulus compounds over the duration of the assay. Because the stimulus compounds have long half-lives, their actual release rates represent only a small percentage of the applied dosages (Butler and McDonough, 1979, 1981; McDonough and Butler, 1983). Septa impregnated with experimental compounds were positioned in the tunnel 40 cm above the floor and 10 cm from the upwind end of the tunnel on removable wire brackets. Adsorption onto the sides of the tunnel was minimized by positioning the test compound source so that even with lateral and vertical spread of the plume, it did not make contact with the sides of the tunnel.

Testing Procedure

During the initial five hours of the scotophase wire release cages (measuring 15 cm x 10 cm x 4 cm), containing individual C. caryana male moths, were hung at the downwind end of the tunnel, carefully aligned directly in the pheromone plume's center axis. Testing moths individually allowed for greater detailed observations and eliminated the potential influence of group effects upon behavioral response. Following a 5 minute acclimation period to allow moths to settle, the release cage was opened, the chemical source inserted, and behavioral responses

observed for a total of 10 minutes.

Data Collection and Analysis

Given the diagnostic nature of the experiments reported here, and the speed with which data needed to be gathered and analyzed (due largely to the uncertain availability of C. caryana male moths), the behavioral response criterion chosen for odor discrimination was chemically induced upwind flight within the characterized plume. This upwind flight in the characterized odor plume was scored as to its presence or absence for each moth.

Non-responders were moths either remaining stationary, and/or those making a positive phototactic flight to the lights. Tests with non-responders were terminated after 5 minutes.

Positive responders were those moths that became activiated, flew out of the release cage and made upwind anemotactic flight within the characterized plume. It should be stressed that the behavioral response criterion, as defined here, is not simply activation from a quiescent state. Whereas activation merely results from signal detection, upwind flight in the characterized plume involves integrative processes in the central nervous system (CNS) in which recognition would occur in the process of orienting to the odor plume after taking flight (Wiley and Richards, 1982). To enhance discrimination of odor test treatments, positive responders were scored on three additional in-flight behavioral responses:

(1) upwind plume flight, with repeated approaches, retreats, and reapproaches to the odor source; Fair (F), Good (G), Excellent (E).

(2) hovering (stationary flight) at close proximity (2-5 cm) downwind of the odor source during plume flight; None (N), Fair (F), Good (G), Excellent (E).

(3) contact with the odor source; yes (+), no (-).

Each candidate lure was replicated ten times (or 10 moths per test lure) and no moths were reused on any given day. Percentage of responders for each lure was calculated from the 10 moths tested ([number responders/10] X 100). Septa support wire brackets and moth release cages were removed and thoroughly rinsed with acetone prior to reuse.

Results

Since the behavioral criterion, upwind flight within the characterized plume, was the only behavior to provide discernable odor discrimination, the other three behaviors scored will only be discussed briefly in a qualitative description of the generalized behavioral sequence of the positive responders.

Experiment 1: Comparison of Upwind Flight Behavior Among C. caryana Male Moths to Various Dosages of Known Perceived Synthetic Compounds.

Upwind flight in the characterized plume was elicited by four of the ten compounds when tested individually (Table I). Although EE-8,10-12:AC and Z-9-12:AC elicited the behavioral response criterion at all dosages tested, EE-8,10-12:AC elicited a slightly higher percent response than Z-9-12:AC at their peak response levels (70% and 60%,

TABLE I

EXPERIMENT 1. CHEMICALLY INDUCED UPWIND FLIGHT BEHAVIOR OF MALE C. CARYANA ELICITED BY VARIOUS DOSAGES OF KNOWN PERCEIVED SYNTHETIC COMPOUNDS.

Chemical	Dose [1] .1 ng	1 ng	10 ng	100 ng	1 µg	10 µg	100 µg
E-8-12:AC	-	-	-	-	-	-	-
Z-8-12:AC	-	-	-	-	-	+ [2]	+
E-9-12:AC	-	-	-	-	-	- [3]	-
Z-9-12:AC	+	+	+	+	+	+	+
E-10-12:AC	-	-	-	-	-	-	+
Z-10-12:AC	-	-	-	-	-	-	-
EE-8,10-12:AC	+	+	+	+	+	+	+
EZ-8,10-12:AC	-	-	-	-	-	-	-
EE-EZ-8,10-12:AC	-	-	-	-	-	-	-
Z-12-14:OH	-	[4]	[4]	[4]	-	-	-

[1] Dose represents the concentration impregnated into red rubber septa.

[2] The + symbol designates those C. caryana male moths displaying upwind flight within the characterized plume (positive responders).

[3] The - symbol designates those C. caryana male moths failing to display upwind flight within the characterized plume (non-responders).

[4] Behavioral response within the characterized plume was erratic, particularly in close proximity of the odor source, making behavior difficult to interpret. A number of these moths landed upwind of the odor source.

respectively) (Figure 1). Both Z-8-12:AC and E-10-12:AC also elicited the behavioral response criterion, but only at greatly increased dosage levels, 10^5 and 10^6, respectively, times that for EE-8,10-12:AC and Z-9-12:AC. Z-12-14:OH elicited an unusual, seemingly directed response, with some flight within the characterized plume. However, the erratic, excited behavior displayed by C. caryana to this 14C monounsaturated alcohol was difficult to interpret in the present bioassay, particularly in light of the repeated occurrence of landing upwind of this odor source.

Experiment 2: Comparison Among Responses to Various Ratios of Z-9-12:AC : EE-8,10-12:AC Binary Mixtures at a Fixed Dosage.

Based upon the results in Experiment 1, binary mixtures of various ratios of Z-9-12:AC and EE-8,10-12:AC at a fixed total concentration of 10 ug were assayed in Experiment 2. Responses of male C. caryana showed very little difference over the range of ratios tested (Figure 2). It should be noted that attractancy was in fact generally reduced in response to all binary mixtures relative to the response when either compound was tested individually at comparable concentrations. Maximum response of 50% occurred at four of the five ratios above 7:3, Z-9-12:AC : EE-8,10-12:AC, inclusive.

Figure 1. Percent Moths Displaying Upwind Flight Behavior Within the Characterized Plume in Response to Various Dosages of EE-8,10-12:AC, Z-9-12:AC, Z-8-12:AC and E-10-12:AC

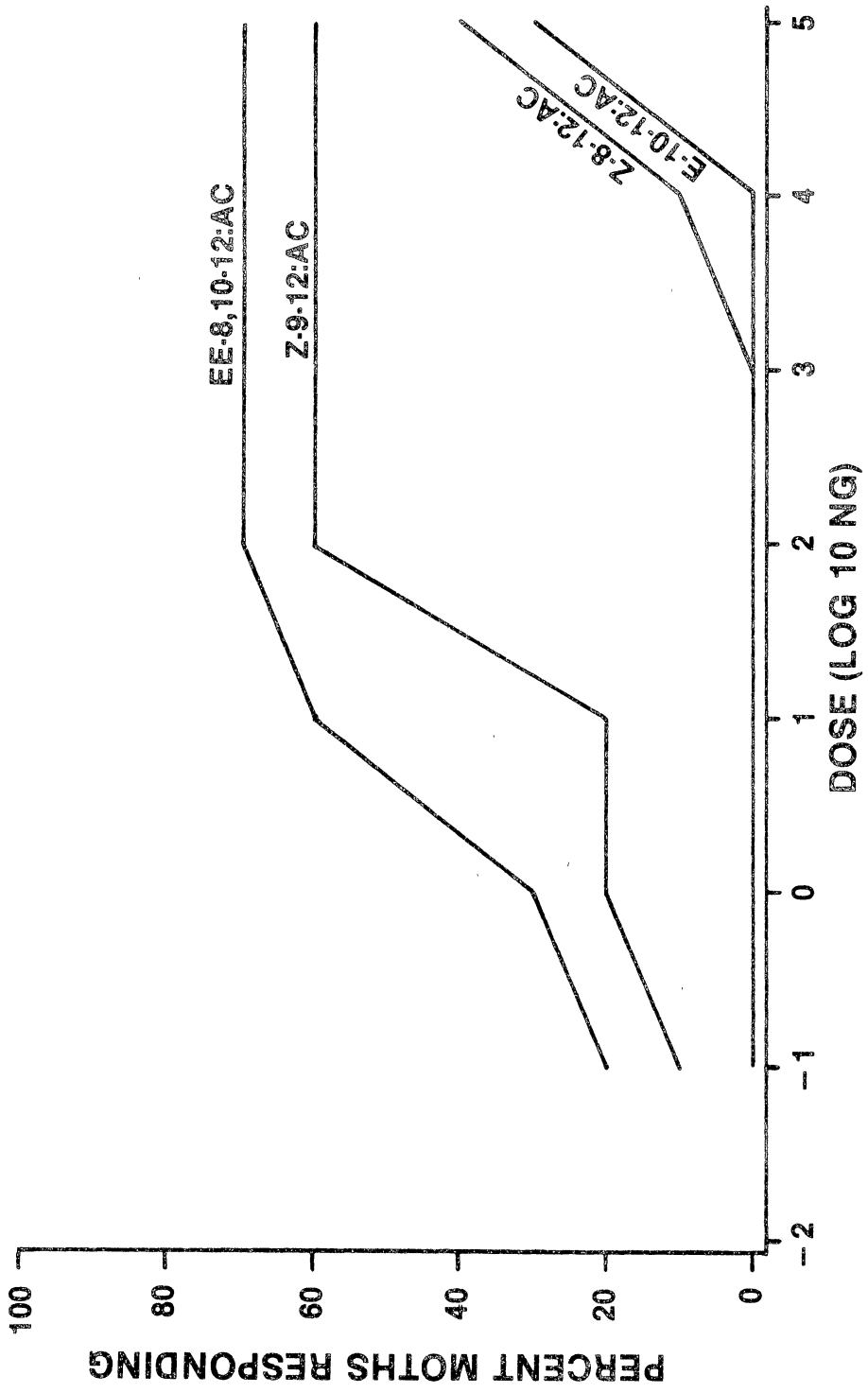
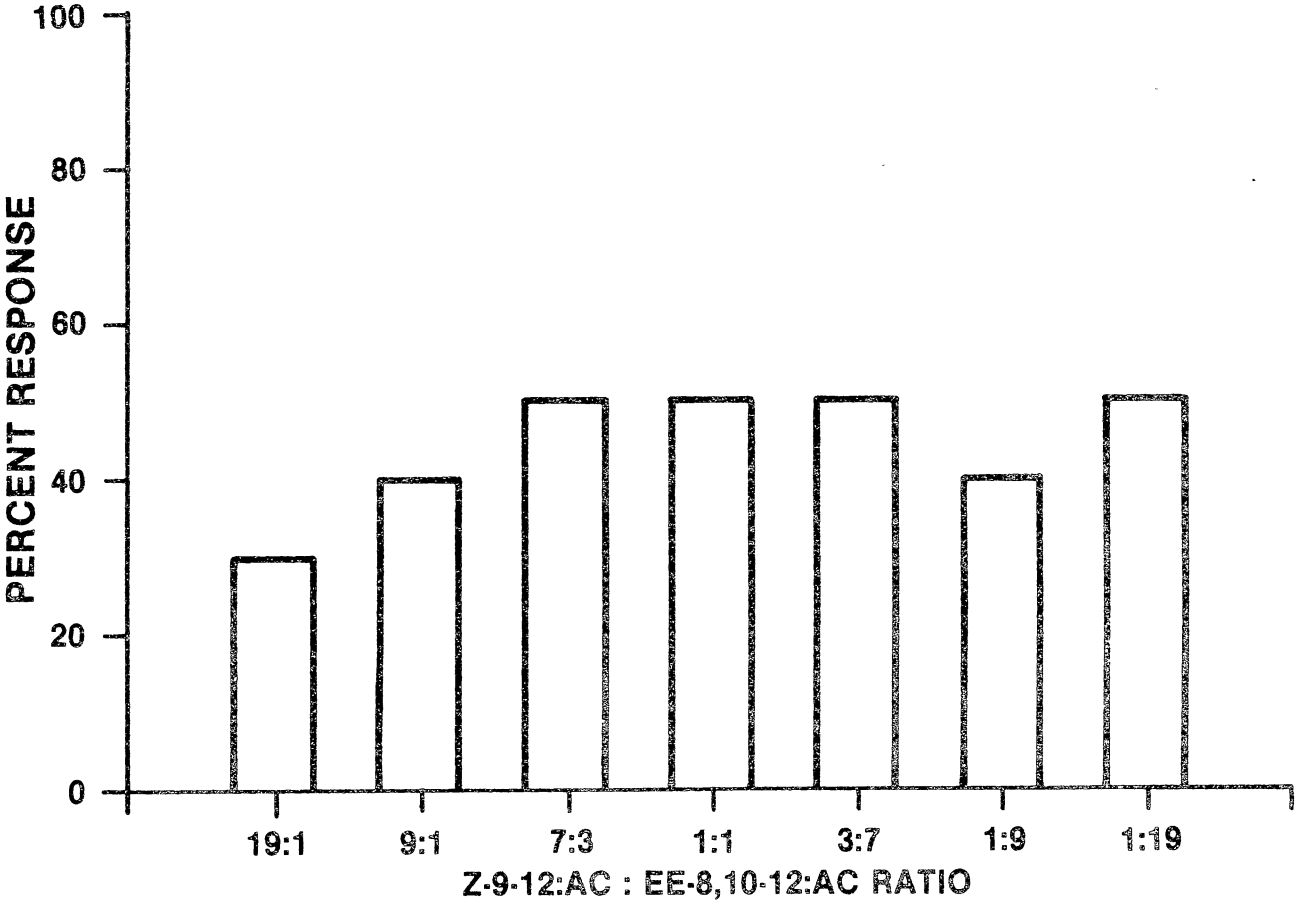

EE-8,10-12:AC
Z-9-12:AC
Z-8-12:AC
E-10-12:AC
PERCENT MOTHS RESPONDING
0
20
40
60
80
100
−2
−1
0
1
2
3
4
5
DOSE (LOG 10 NG)

Figure 2. Percent Moths Displaying Upwind Flight Behavior Within the Characterized Plume in Response to Various Binary Mixture Ratios of Z-9-12:AC and EE-8,10-12:AC (10 µg)


100
80
60
40
20
0
PERCENT RESPONSE
19:1
9:1
7:3
1:1
3:7
1:9
1:19
Z-9-12:AC : EE-8,10-12:AC RATIO

Experiment 3: Comparison Among Responses to Various Dosages of a 7:3 Binary Mixture of Z-9-12:AC : EE-8,10-12:AC.

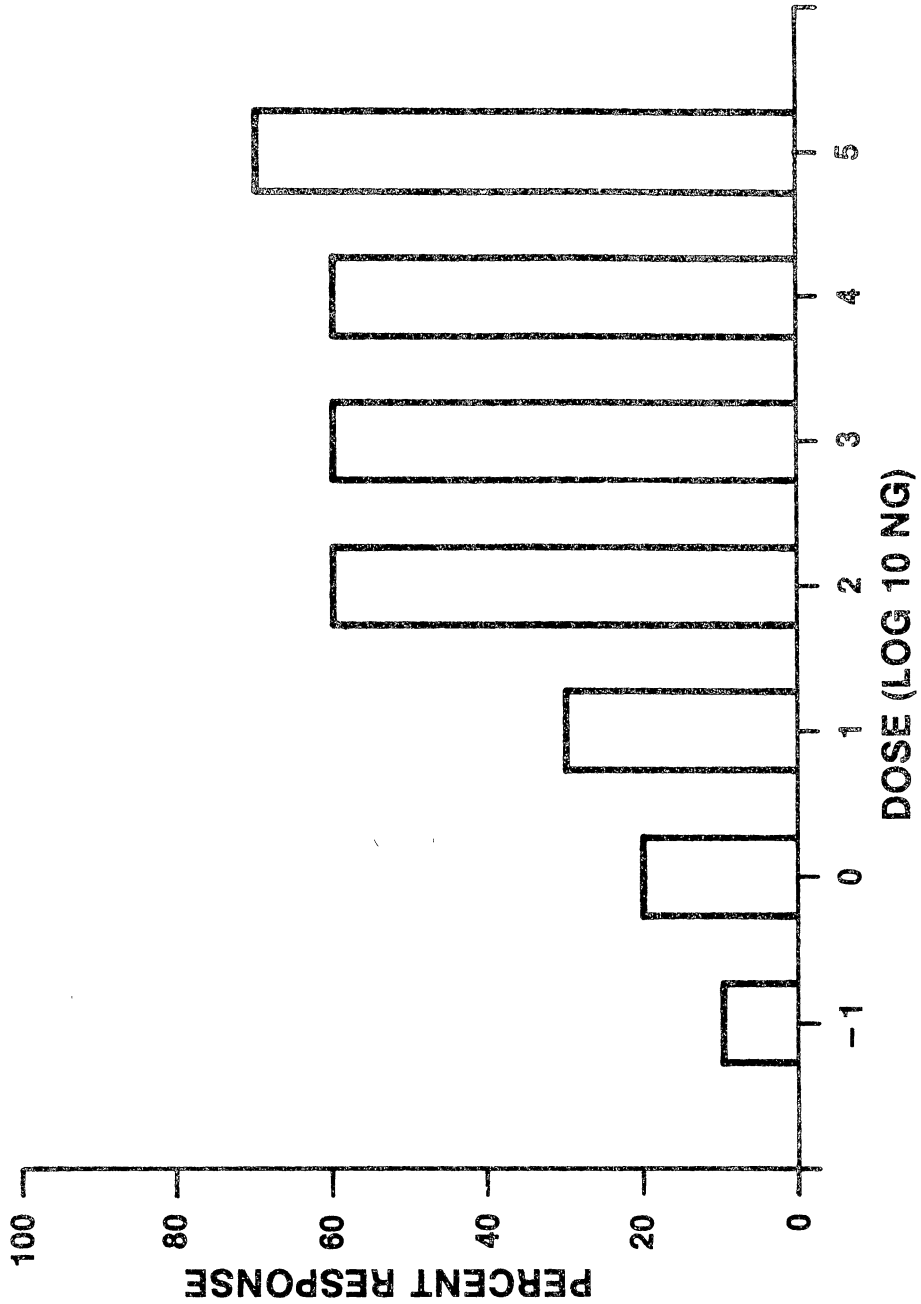
Based upon the results in Experiment 2, a 7:3 binary mixture of Z-9-12:AC : EE-8,10-12:AC, at dosages ranging in ten-fold steps from 0.1 ng to 100 µg was assayed in Experiment 3. Only when the dose reached 100 ng did the percent response increase above that elicited by any ratio assayed in Experiment 2 at the 10 µg level. Unlike the results in Experiment 2, response levels obtained in Experiment 3 were similar to those in Experiment 1 at dosages greater than or equal to the 100 ng level (Figure 3).

Generalized Behavioral Description of Positive Responders.

C. caryana male moths displaying the behavioral response criterion in the three preceeding experiments followed a pre-copulatory behavioral sequence common in many lepidopterous moth species.

C. caryana were generally activated and took flight within the first minute of exposure to the odor source. The moths' flight track along the axis of the odor plume, particularly within one meter of the odor source, had a characteristic zigzag pattern of successive lateral reversals along the upwind trajectory, predominantly in the horizontal plane. Although the zigzag lateral reversals represent an internally generated (pre-programmed) pattern of flight, external information about the spatial structure of the pheromone plume, i.e., narrowness of

Figure 3. Percent Moths Displaying Upwind Flight Behavior Within the Characterized Plume in Response to Various Dosages of a 7 : 3 Binary Mixture of Z-9-12:AC and EE-8,10-12:AC


PERCENT RESPONSE
0
20
40
60
80
100
DOSE (LOG 10 NG)
-1
0
1
2
3
4
5

the plume, can influence the zigzag path (Cardé, 1984).

Flight speed was initially quite rapid, particularly within the first meter, and then slowed considerably (inverse orthokinesis) within one meter of the odor source. The decrease in the rate of forward progress appeared to be coupled, in general, with an increase in the frequency of turning (direct klinokinesis). The result was a narrower zigzag path and a slowing of the speed of progress up the plume as the moth experienced increasing odor intensity closer to the odor source. Thus, increasing stimulus strength apparently decreased the rate of upwind movement, allowing for more clear observations of in-flight hovering. Similar flight behavioral patterns have been reported among others, for the gypsy moth, Lymantria dispar (L.) (Cardé, 1984), and pink bollworm, Pectinophora gossypiella (Saunders) (Farkas et al., 1974).

Reversing anemomenotaxis, following upwind anemotaxis and hovering, often occurred a number of times in succession prior to landing downwind of the odor source. In no instance did C. caryana male moths approach any closer than 2-5 cm of, nor land on the odor source.

Discussion

Tamaki (1985) suggests that data on lepidopterous insects apparently indicate that the structural specificity is not necessarily high for certain pheromonal components. Structural specificity of multicomponent pheromonal systems could be lower than that of single component systems, and the specificity possibly depends on the function or role of each component. Depletion of one double bond from a doubly

unsaturated pheromone molecule (Takahashi et al., 1971; Hummel et al., 1973), or changes in the position of a double bond or in the chain length of a pheromone component (Gaston et al., 1971), generally depress activity 10^1 to 10^5 fold. However, such altered compounds often remain attractive at higher concentrations.

Compounds Eliciting the
Behavioral Response Criterion
at all Concentrations
(EE-8,10-12:AC and Z-9-12:AC).

Roelofs and Cardé (1977) reported that moth sex pheromone systems characterized chemically within the last decade have demonstrated the widespread occurrence of multicomponent systems. They suggested that hierarchical behavioral responses may be evoked by increasing concentrations of the entire blends, or in other multicomponent systems, the first steps of the behavioral sequence may be mediated by one or more primary component(s). Continuing the behavioral sequence past initial phases governed by the primary components would require the presence of both the primary components and additional chemical stimuli, the secondary (minor) components.

In the present study, both EE-8,10-12:AC and Z-9-12:AC readily elicited the behavioral response criterion independently, which is most often considered to be the function of primary sex pheromonal components (Experiment 1). Although the various binary ratios and concentration mixtures (Experiment 2 and 3, respectively) failed to increase response above that obtained when either compound was tested independently (Experiment 1), the individual low response thresholds suggests

that both compounds may be or closely resemble primary sex pheromonal components of C. caryana. Additional supportive evidence for this hypothesis can be drawn from their EAG analysis (Chapter II), given the often correlative nature of EAG and behavioral responses (Mayer and Mankin, 1985), i.e., chemicals eliciting the greatest behavioral response also generate the EAG response with the greatest amplitude. As reported in Chapter II, EE-8,10-12:AC and Z-9-12:AC elicited the highest and third highest EAG responses at 100 µg test levels, respectively, and C. caryana probably possess two types of acceptor populations for these two compounds.

C. caryana behavioral response appears to be, after structural specificity for certain key compounds, more concentration dependent than ratio dependent when the two suspected primary sex pheromonal components are presented in binary mixtures. If in fact EE-8,10-12:AC and Z-9-12:AC are primary components: (1) a critical ratio may not have been assayed in Experiment 2 that might have increased percent response above that elicited in Experiment 1 where both were tested independently, and/or, (2) not unlike other Olethreutinae, i.e., Cydia nigricana (Wall et al., 1976), C. caryana may have built into its receptor-CNS integrative behavioral system a certain degree of plasticity which may represent past or future components of reproductive isolation resulting from various selection pressures. Concentration and dosage do not appear to be governing response independently. Therefore, to adequately test these two components in binary mixtures, extensive dose-ratio behavioral analysis should be conducted either in a flight tunnel or under natural field conditions.

Compounds Eliciting the
Behavioral Response Criterion
Only at Elevated Concentrations
(Z-8-12:AC and E-10-12:AC).

Compounds eliciting the behavioral response criterion only at greatly elevated dosages, which includes Z-8-12:AC and E-10-12:AC in the present study, are either, (1) minor sex pheromonal components, or (2) closely resemble a primary sex pheromonal component (parapheromones). Separate arguments can be made for either case.

As Minor Sex Pheromone Components. Although minor sex pheromone components are seldom attractive alone, cases do exist contrary to this general concept. Sekul and Sparks (1967) identified the sex pheromone of the fall armyworm, Spodoptera frugiperda (Noctuidae), as Z-9-14:AC. This compound alone was attractive, but was not competitive with virgin females in terms of its attractancy. Later, Sekul and Sparks (1976) identified Z-9-12:AC from virgin females, which was proposed as the primary sex pheromone by Jones and Sparks (1979). Jones and Sparks thus considered Z-9-14:AC to be a minor component, although it was attractive alone. Similarly, Roelofs (1979) reported that the California red scale, Aonidiella aurantii (Maskell), had two pheromone components, 3-methyl-6-isopropenyl-9-decen-1-yl acetate and Z-3-methyl-6-isopropenyl-3,9-decadien-1-yl acetate, and each were independently active. Therefore in the present study, Z-8-12:AC and E-10-12:AC may be minor sex pheromonal components.

As Parapheromones. Perception of a pheromone and parapheromone

either takes place through the same or different receptor sites on the dendrites of the receptor. However, the fact that parapheromones elicit the same behavioral response as pheromone, although at higher concentrations, is evidence that they probably stimulate the same receptor (Payne, 1974). Thus, if a compound closely resembles and is able to bind with the acceptor of a major pheromonal component, it may in fact result in the display of some degree of sexual attractancy.

Priesner (1969) reported that all substances that activated the bombykol receptor cells of the male Bombyx mori (which included its own pheromone, other pheromones, bombykol derivatives in higher concentrations and certain nonpheromone substances in much higher concentrations) elicited an upwind flight response similar to the behavioral response criterion reported here for C. caryana. In such a system (considered a labeled line system), the presence of activity in a specialist cell codes the odor quality (Mountcastle, 1968).

Kaissling et al. (1978) reported that a second cell within the same sensillum trichodeum housing the cell responsive to bombykol (EZ-10,12-16:OH) had been shown to respond to bombykal (EZ-10,12-16:AL). The bombykol cell also responded at high concentrations of bombykal and furthermore, bombykal alone was able to elicit some of the component sexual behaviors at concentrations 10^4 times that of bombykol. Thus encoding in B. mori appears consistent with across-fiber patterns (coding by inputs from a number of cells with overlapping spectra), with effects of the aldehyde centrally determined.

Finally, Renou et al. (1981) identified Z-11-16:AL as the major sex pheromone component of the leek moth, Acrolepiopsis assectella Zeller, and studied its responses to this pheromone and five analogues. They

demonstrated a graded discrimination of Z-11-16:AL and the five analogues at three concentrations by monitoring the progressively higher-order behaviors. At the lowest concentrations, four of the five analogues elicited the lowest order behavior, but only the pheromone elicited the complete behavioral hierarchy. Only at concentrations 10^4 times higher did the analogues elicit behaviors above the lowest order.

In the present study, because E-10-12:AC elicits the behavioral response criterion only at dosage levels 10^6 times that for EE-8,10-12:AC, and differs structurally from this probable doubly unsaturated pheromone molecule by only the depletion of one double bond, it (E-10-12:AC) is most likely a parapheromone. Additional supportive evidence for this hypotheses may be drawn from the EAG analysis (Chapter II) in which results showed that E-10-12:AC elicited the highest response of all monoenes tested (second only to the EE-8,10-12:AC diene), and indicated that both compounds (E-10-12:AC and EE-8,10-12:AC) probably stimulate the same acceptors.

Also in the present study, Z-8-12:AC elicits the behavioral response criterion only at elevated dosage levels, 10^5 times that for Z-9-12:AC. Coupled with EAG analysis (Chapter II), Z-8-12:AC may be or structurally resemble a minor sex pheromone component due to its intermediate EAG response level.

Evidence for the status of E-10-12:AC and Z-8-12:AC as parapheromones or as minor pheromone components can only be developed from a thorough chemical analysis of conspecific female moth sex pheromone gland contents coupled with additional behavioral analysis.

Compounds Eliciting No Response Within the Range of Concentrations Tested.

Lack of behavioral response to a compound tested alone is not evidence of non-sensation nor behavioral insignificance. Therefore, the remaining perceived compounds should be evaluated extensively either in flight tunnel tests or under natural field conditions.

Conclusions

Behavioral evidence presented here suggests that EE-8,10-12:AC and/or Z-9-12:AC resemble or are C. caryana sex pheromonal components. Z-8-12:AC and E-10-12:AC are either parapheromones eliciting attractive responses only at elevated concentrations, or are minor pheromone components. The behavioral significance of Z-12-14:OH was difficult to interpret in the present bioassay.

Failure of C. caryana male moths to land on or at the odor source conveys the complex processing and integrative steps in the CNS which are involved in neurological orienting and the mating behavioral sequence. Lack of an essential chemical cue(s) and/or nonchemical cues, such as visual, tactile and auditory stimuli, may be involved in the orientation and mating behavioral sequence. Although this bioassay utilized flight in wind to analyze responses made in-flight, which are highly integrated and probably the most discriminating in pheromone research, performance in a flight tunnel could result in less "specificity" of response compared to field behavior. Thus, field bioassays involving the capture of males are the ultimate test of a

compound's activity to the environmental complexities often simplified in flight tunnel tests (Baker and Linn, 1984).

EAG and Behavioral Correlation

Two correlative methods for obtaining information about an insect's response to its sex pheromone or suspected pheromone components, as well as gaining insight into the functioning of the CNS, include the EAG and the bioassay of a behavioral response criterion, i.e., wing fanning or flight response (Mayer and Mankin, 1985).

The EAG, to reiterate, measures the potential difference induced between the distal and proximal tips of an antenna during odor stimulus, and thus potential difference is proportional to the sum of the generator potentials of all the stimulated sensory cells of the antenna. These potentials are characterized by low frequency (< 1 Hz) changes and occur in a reasonably well-ordered manner in response to various odorants. However, the CNS does not likely react to these potential changes, but rather it reacts to trains of action potentials from individual olfactory cells which synapse in the antennal lobe of the brain. Therefore, the EAG cannot be used to predict the actual input to the brain. In general, however, it has been shown that those insects that exhibit a pronounced behavioral response to an odor also react to that odor with a definite EAG.

The correlation of EAG responses to discriminatory behavior is limited primarily by the fact that input from non-olfactory stimuli are jointly processed and integrated by the CNS prior to induction of the behavioral response. Although behavioral bioassay response does not necessarily reflect solely the reaction to pheromone, it is a valuable

aid in determining if an insect perceives a particular compound and if a compound is likely to be a major component of a species sex pheromone. Failure to respond behaviorally cannot be used, however, as a determinant of the discriminatory range of a receptor cell to groups of odorants, nor should it warrant the disregard of the potential behavioral significance of a particular perceived compound. Although interactions of the responses to quality and quantity complicate the linkage between the behavioral response and peripheral sensation of pheromones, these two bioassays jointly play critical roles in the chemical characterization of a species sex pheromone.

Bibliography

Baker, T. C. and R. T. Cardé. 1984. Techniques for behavioral bioassays, pp. 45-74. In H. E. Hummel and T. A. Miller [eds.], Techniques in Pheromone Research. Springer-Verlag, New York.

Baker, T. C. and C. E. Linn, Jr. 1984. Wind tunnels in pheromone research, pp. 75-110. In H. E. Hummel and T. A. Miller [eds.], Techniques in Pheromone Research. Springer-Verlag, New York.

Birch, M. C. 1971. Intrinsic limitations in the use of electroantennograms to bioassay male pheromones in Lepidoptera. Nature 233: 57-8.

Butler, L. I. and L. M. McDonough. 1979. Insect sex pheromones: evaporation rates of acetates from natural rubber septa. J. Chem. Ecol. 5(5): 825-37.

Butler, L. I. and L. M. McDonough. 1981. Insect sex pheromones: evaporation rates of alcohols and acetates from natural rubber septa. J. Chem. Ecol. 7(3): 627-33.

Cardé, R. T. 1979. Behavioral responses of moths to female produced pheromones and the utilization of attractant-baited traps for population monitoring, pp. 286-315. In R. L. Rabb and G. G. Kennedy [eds.], Movement of Highly Mobile Insects: Concepts and Methodology in Research. Univ. Graphics, North Carolina State University, Raleigh.

Cardé, R. T. 1984. Chemo-orientation in flying insects, pp. 111-124. In W. J. Bell and R. T. Cardé [eds.], Chemical Ecology of Insects. Sinaver Assoc., Inc., Sunderland, Massachusetts.

Farkas, S. R., H. H. Shorey, and L. K. Gaston. 1974. Sex pheromones of Lepidoptera. Influence of pheromone concentration and visual cues on aerial odor trail following by males of Pectinophera gossypiella. Ann. Entomol. Soc. Am. 67: 633-5.

Gaston, L. K., T. L. Payne, S. Takahashi, and H. H. Shorey. 1971. Correlation of chemical structure and sex pheromone activity in Trichoplusia ni (Noctuidae), pp. 167-173. In D. Schneider [ed.], Olfaction and Taste IV. Proc. 4th Intl. Symp., Starnberg, Germany.

Grant, G. G. 1970. Electrophysiolgical and histological studies on the cabbage looper: electroantennogram responses to the female pheromone and male hairpencil scent and anatomy of their glandular sources. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg.

Hummel, H. E., L. K. Gaston, H. H. Shorey, R. S. Kaae, K. J. Byrne, and R. M. Silverstein. 1973. Clarification of the chemical status of the pink bollworm sex pheromone. Science 181: 873-5.

Jones, R. L. and A. N. Sparks. 1979. (Z)-9-Tetradecen-1-ol acetate. A secondary sex pheromone of the fall armyworm, Spodoptera frugiperda

(J. E. Smith). J. Chem. Ecol. 5: 721-5.

Kaissling, K. E., G. Kasang, H. J. Bestmann, W. Stransky, and O. Vostrowsky. 1978. A new pheromone of the silkworm moth Bombyx mori. Sensory pathway and behavioral effect. Naturwissenschaften 65: 382-4.

Mayer, M. S. 1973. Electrophysiological correlates of attraction in Trichoplusia ni. J. Insect Physiol. 19: 1191-8.

Mayer, M. S. and R. W. Mankin. 1985. Neurobiology of pheromone perception, pp. 96-144. In G. A. Kerkut and L. I. Gilbert [eds.], Comprehensive Insect Physiology, Biochemistry and Pharmacology: Behavior, Vol. 9. Pergamon Press, Oxford.

McDonough, L. M. and L. I. Butler. 1983. Insect sex pheromones: determination of half-lives from formulations by collection of emitted vapor. J. Chem. Ecol. 9(11): 1491-501.

Miller, J. R. and W. L. Roelofs. 1978. Sustained-flight tunnel for measuring insect responses to wind-borne sex pheromones. J. Chem. Ecol. 4(2): 187-98.

Mountcastle, V. B. 1968. Medical Physiology. The C. V. Mosby Co., St. Louis, Missouri.

Payne, T. L. 1974. Pheromone perception, pp. 35-61. In M. C. Birch [ed.], Frontiers of Biology: Pheromones, Vol. 32. North-Holland Publ. Co., Amsterdam.

Priesner, E. 1968. Die interspezifischen Wirkungen der Sexuallock stoffe der Saturniidae (Lepidoptera). Z. vergl. Physiol. 61: 263-97.

Priesner, E. 1969. A new approach to insect pheromone specificity, pp. 235-240. In C. Pfaffmann [ed.], Olfaction and Taste III.

Rockefeller Univ. Press, New York.

Renou, M., C. Descoins, E. Priesner, M. Gallois, and M. Lettere. 1981. Etude de la phéromone sexuelle de la teigne du poireau Acrolepiopsis assectella (A study of the sex pheromone of the leek moth, Acrolepiopsis assectella.) Entomol. Exp. Appl. 29(2): 198-208.

Roelofs, W. L. 1979. Production and perception of Lepidopterous pheromone blends, pp. 159-168. In F. J. Ritter [ed.]. Chemical Ecology: Odour Communication in Animals. Elsevier/North-Holland.

Roelofs, W. L. and R. T. Cardé. 1977. Responses of Leipidoptera to synthetic sex pheromone chemicals and their analogues. Ann. Rev. Entomol. 22: 377-405.

Roelofs, W. and A. Comeau. 1971. Sex pheromone perception: electroantennogram responses of the red-banded leaf roller moth. J. Insect Physiol. 17: 1969-82.

Roelofs, W., A. Comeau, A. Hill, and G. Milicevic. 1971a. Sex attractant of the codling moth: characterization with electroantennogram technique. Science 172: 297-9.

Roelofs, W. L., J. P. Tette, E. F. Taschenberg, and A. Comeau. 1971b. Sex pheromone of the grape berry moth: identification by classical and electroantennogram methods, and field tests. J. Insect Physiol. 17: 2235-43.

Schneider, D., B. C. Block, J. Boeckh, and E. Priesner. 1967. Die Reaktion der Männlichen Seidenspinner auf Bombykol und seine Isomeren: Elektroantennogramm und Verhalten. Z. Vergl. Physiol. 54: 192-209.

Sekul, A. A. and A. N. Sparks. 1967. Sex pheromone of the fall armyworm moth: isolation, identifcation, and synthesis. J. Econ.

Entomol. 60: 1270-72.

Sekul, A. A. and A. N. Sparks. 1976. Sex attractant of the fall armyworm moth. USDA Tech. Bull. 1542, 1-6.

Takahashi, S., C. Kitamura, and Y. Kuwahara. 1971. The comparative pheromone activity of acetates of unsaturated alcohols to males of the almond moth. Botyu-Kagaku 33: 24-6.

Tamaki, Y. 1985. Sex pheromones, pp. 145-191. In G. A. Kerkut and L. I. Gilbert [eds.], Comprehensive Insect Physiology, Biochemistry and Pharmacology: Behavior, Vol. 9. Pergamon Press, Oxford.

Wall, C., A. R. Greenwaay, and T. E. Burt. 1976. Electroantennographic and field responses of the tea moth, Cydia nigricana, to sex attractants and related compounds. Physiol. Entomol. 1: 151-7.

Wiley, R. H. and D. G. Richards. 1982. Adaptation for acoustic communication in birds: sound transmission and signal detection, pp. 131-181. In D. E. Kroodsma and H. H. Miller [eds.], Acoustic Communication in Birds, Vol. 1. Academic Press, New York.

CHAPTER IV

CHEMICAL AND ELECTROANTENNOGRAPHIC ANALYSIS OF FEMALE *CYDIA CARYANA* SEX PHEROMONE GLAND EXTRACT

Introduction

Anderson (1972) previously reported that "...female hickory shuckworm produce a compound within its body that elicits a response from the male hickory shuckworm following the definition of a sex pheromone....," and that "...the source of the excitatory odor is located in the last five abdominal segments of the female moth's body...." Given this confirmation of the presence of a sex pheromone in *Cydia caryana* (Fitch), the present study was undertaken to isolate and identify the component(s) of the sex pheromone of *C. caryana*.

One of the major problems in efforts to decode a pheromone for insect control is the identification of all compounds used in any particular pheromone system (Roelofs, 1980). The procedure for sex pheromone identification generally includes: (1) establishment of conditions for collecting or extracting active materials to maximize the recovery of pheromonal compounds, (2) establishment of a bioassay method to monitor the pheromone titre of various fractions, (3) purification and isolation of each pheromone component, (4) structural analysis of isolated compounds, (5) synthesis of compounds of suggested

structure, (6) comparison of chemical characteristics of the synthetic and isolated compounds, and then (7) a comparison of the biological activity of the synthetic and the isolated compounds, or comparisons with live insects under natural conditions (Tamaki, 1985).

The major problem in the identification of sex pheromones has been the small amounts of biologically active material in a large amount of chemically similar inactive material (Gaston, 1984). Although most efforts at pheromone collection prior to 1972 involved laborious solvent extraction of either whole insects or excised parts (Jacobson, 1972), knowledge of sex pheromone gland morphology and physiology has resulted in glandular extraction techniques useful in obtaining relatively pure pheromone material (Jefferson et al., 1968; Sower et al., 1972; Sower et al., 1973). Additionally, the advent of such physiochemical analysis techniques as capillary gas-chromatography, selective ion mass spectrometry, and high performance liquid chromatography, have enabled the achievement of high levels of detection sensitivity and resolution (Golub and Weatherston, 1984). In the present study both gas chromatography (GC) (polar and non-polar, and packed and capillary columns) and selective ion mass spectrometry (MS) were utilized for physical purification and identification of the C. caryana female sex pheromone gland extract.

An electroantennogram (EAG) technique which utilizes the summated potential of pheromone receptors of olfactory sensilla on antennae was developed (Schneider, 1957), used in conjunction with gas chromatography (Moorehouse et al., 1969) and subsequently established as a convenient bioassay method for sex pheromones (Roelofs, 1977). EAG derived information has been of invaluable service in the study of

insect sex pheromone chemistry, either to predict active structures from response to related molecules, or to monitor biological activity during isolation and purification of attractants from natural sources (Arn et al., 1975).

In the present study, the EAG technique was utilized in this later way, functioning much like a GC detector. In this way, each fraction of a gas-liquid chromatographic (GLC) fractionated crude extract can be checked for EAG activity and the active areas correlated with retention times of reference standards to obtain structural information on possible pheromone components. Since many corresponding pheromone analogues are often extracted from pheromone glands and exhibit weak EAG activity, pheromone component activity must be demonstrated in subsequent behavioral analysis. In many cases, however, since pheromone components do not elicit behavioral responses unless mixed together in the proper proportions, behavioral analysis can involve tremendous replication of a multitude of recombined fractions. On the other hand, since antennal receptors respond to individual components, recombinations are not necessary with the EAG technique. Thus, EAG analysis can quickly provide an excellent method for locating the major pheromone component(s) and can give indications of some less abundant minor components (Roelofs, 1978).

Materials and Methods

Insects

C. caryana were obtained as full-grown overwintering larvae within pecan shucks from a pecan orchard in Sparks, Oklahoma. Larvae were

maintained in a controlled environmental room, simulating Oklahoma environmental conditions so as to induce normal emergence at a temperature of 22-24°C, 65% R.H. under a 14-hours light/10-hours dark photoperiod. Unmated emergent adults were collected daily, sexed, and each sex placed into separate holding cages provided with a solution of 5% sugar-water.

Collection of Pheromone

Female moths, 2 to 3 days after eclosion, were collected 45 minutes to one hour after the initiation of the scotophase and placed in a refrigerator for 10 to 30 minutes prior to dissection. The glandular area, which lies dorsally between the 8th and 9th abdominal segments as a modified intersegmental membrane, was everted under pressure, excised and extracted in dichloromethane at room temperature for 15 minutes. The solution was then removed with a syringe and kept at -20°C.

Gas Chromatography

The following analytical columns were used for fractionating C. caryana female sex pheromone gland crude extract:

(1) Column A (non-polar) -- a silanized glass column (1.8 m X 2.3 mm OD) packed with 3% dimethyl silicone (SE-30) on 80/100 mesh Gas Chrom Q and operated at 110°C for 8 minutes and then temperature programmed at 4° per minute to 200°C and held at this temperature.

(2) Column B (polar) -- a silanized glass column (1.8 m X 2.3 mm OD) packed with ultrabond Carbowax 20M on 80/100 mesh Gas Chrom Q and operated at 120°C for 16 minutes and then

temperature programmed at 4° per minute to 200°C and held at this temperature.

The following capillary GC column was used for chemical analysis of biologically active fractions:

(3) Column C (non-polar) — a capillary column (60 m x 0.25 mm ID) of fused silica with crosslinked methyl silicone bonded to the column wall (DB-1) and operated at 80°C for 2 minutes and then temperature programmed at 20° per minute to 170°C and held at this temperature.

Retention times (t_R) relative to known standards and candidate pheromone compounds based on EAG and behavioral studies were used to provide information on the presence or absence of the pheromone candidates.

Purification of Sex Pheromone

Purification of pheromone components from crude extracts of C. caryana sex pheromone glands was effected by collection from a GC (Hewlett-Packard 5710A) equipped with a strip chart recorder, a flame ionization detector (FID) (burning produces charged particles which the collector turns into a current), and a glass-lined column effluent splitter (with an adjustable valve positioned for one part of effluent to the detector and ten parts to the collection trap). Effluent was collected in glass tubing cooled in a mixture of dry ice and acetone. Collection efficiency of model compounds was approximately 80%.

GC analysis of biologically active fractions determined by EAG analysis was effected by utilizing a GC (Hewlett-Packard 5880A) equipped with computerized data collection.

Bioassay

The criterion for biological activity determination of fractionated extract was via electroantennographic (EAG) response in 2- to 4-day old C. caryana male moths. Collected fractions were rinsed from the glass tubing traps with dichloromethane and deposited on the inside of EAG glass tubes. After evaporation of the solvent, the glass tubes containing the various fractions were bioassayed by the EAG procedure as described previously in Chapter II.

Gas Chromatograph - Mass Spectrometry (GC-MS)

A quadrupole mass spectrometer (Hewlett-Packard 5970) with computerized data collection and a GC inlet (Hewlett-Packard 5790) was used. A dimethyl silicone (DB-1) capillary column (60 m x 0.25 mm ID) was held at 80°C for 2 minutes and then temperature programmed at 20° per minute to 190°C and then held at this temperature.

Mass spectra of candidate pheromone compounds based on EAG and behavioral studies were used to provide information on the presence or absence of the pheromone candidates.

Results

EAG Analysis of GC Fractionated Extract from a Non-Polar Column

Fractions of 94 C. caryana female equivalents (F.E.) were collected off of the non-polar column A (SE-30) in the volatility range for C10 to C20 acetates. Based upon a predetermined 80% recovery efficiency

utilizing known amounts of certain standards, each of six fractions containing approximately 75 F.E. were collected. EAG analysis of 70 F.E. in each fraction showed that only fractions 3 and 4 elicited significant responses in male C. caryana (Figure 1). Fraction 3 encompassed the retention time corresponding to 12:AC, and fraction 4 that of both EE-8,10-12:AC and Z-12-14:OH.

Subsequent capillary GC analysis of fraction 3 indicated a peak with a structural resemblance to 12:AC based upon their respective retention times (t_R [peak] = 14.383 minutes; t_R [12:AC] = 14.388 minutes) (Table I). A second peak in fraction 3 was also detected at a retention time indicative of a dodecenyl acetate other than Z-9-12:AC (t_R [dodecenyl acetate] = 14.255 minutes).

Capillary GC analysis of fraction 4 indicated a peak with a structural resemblance to EE-8,10-12:AC based upon their respective retention times (t_R [peak] = 15.938 minutes; t_R [EE-8,10-12:AC] = 15.943 minutes) (Table I). There was no peak in fraction 4 corresponding to the retention time for Z-12-14:OH.

EAG Analysis of GC Fractionated
Extract from a Polar Column

Fractions of 39 C. caryana F.E. were collected off of the polar column B (Ultrabond Carbowax 20-M) in the volatility range for C10 to C20 acetates. Based upon a predetermined 80% recovery efficiency utilizing known amounts of certain standards, each of seven fractions containing approximately 30 F.E. were collected. EAG analysis of each fraction showed that only fractions 2, 4 and 6 each elicited significant responses in male C. caryana (Figure 2). Subsequent capillary GC

Figure 1. Mean Percent EAG Response Elicited by Each of Six Fractions of Cydia caryana (70 F.E.) Sex Pheromone Collected on Column A (SE-30 Packed Column). (S1) 10:AC (t_R = 8.0 minutes); (S2) 12:AC (t_R = 15.0 minutes); (S3) EE-8,10-12:AC (t_R = 16.5 minutes); (S4) Z-12-14:OH (t_R = 17.3 minutes); (S5) 16:AC (t_R = 26.3 minutes).

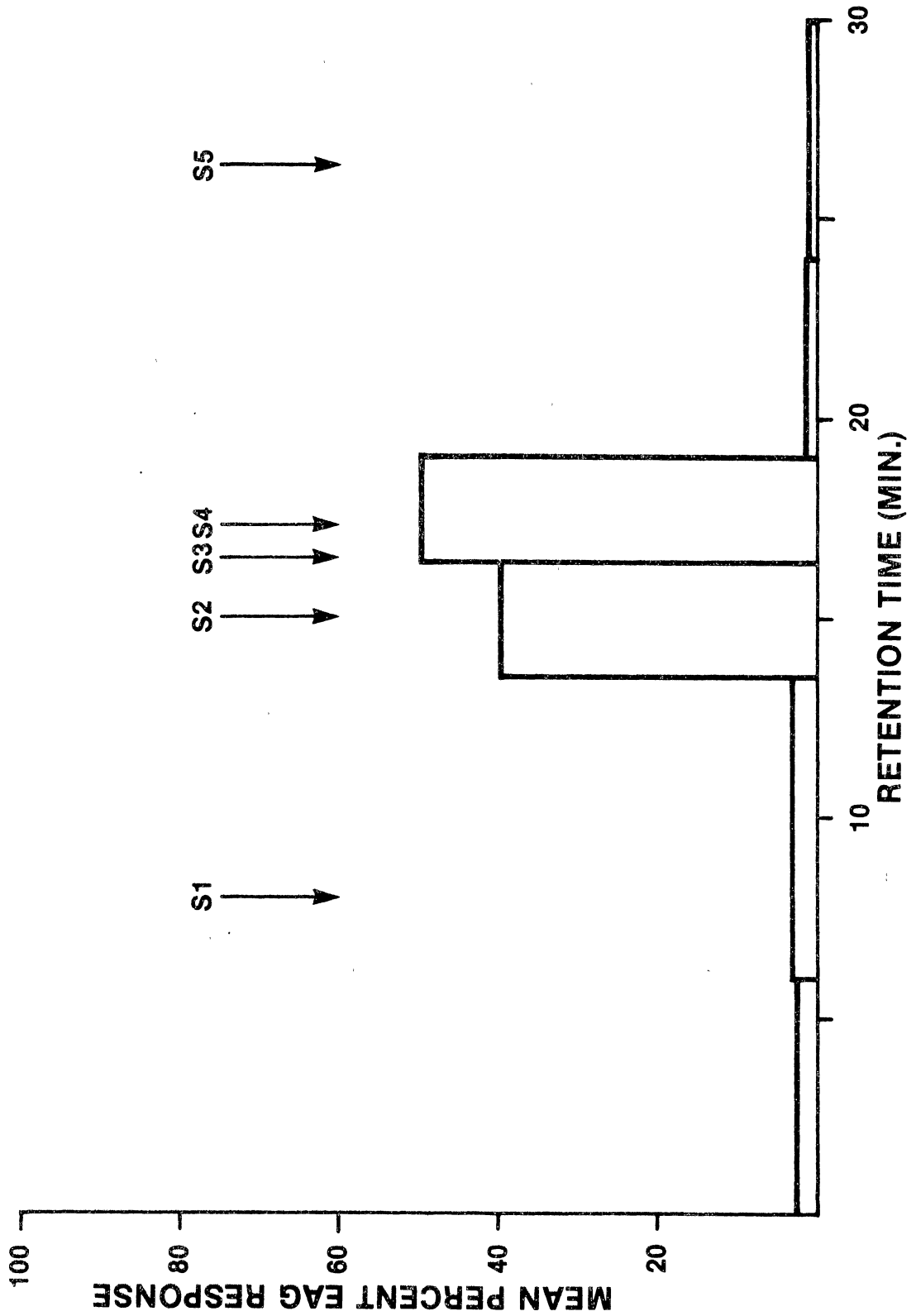

S5
S3S4
S2
S1
100
80
60
40
20
MEAN PERCENT EAG RESPONSE
10
20
30
RETENTION TIME (MIN.)

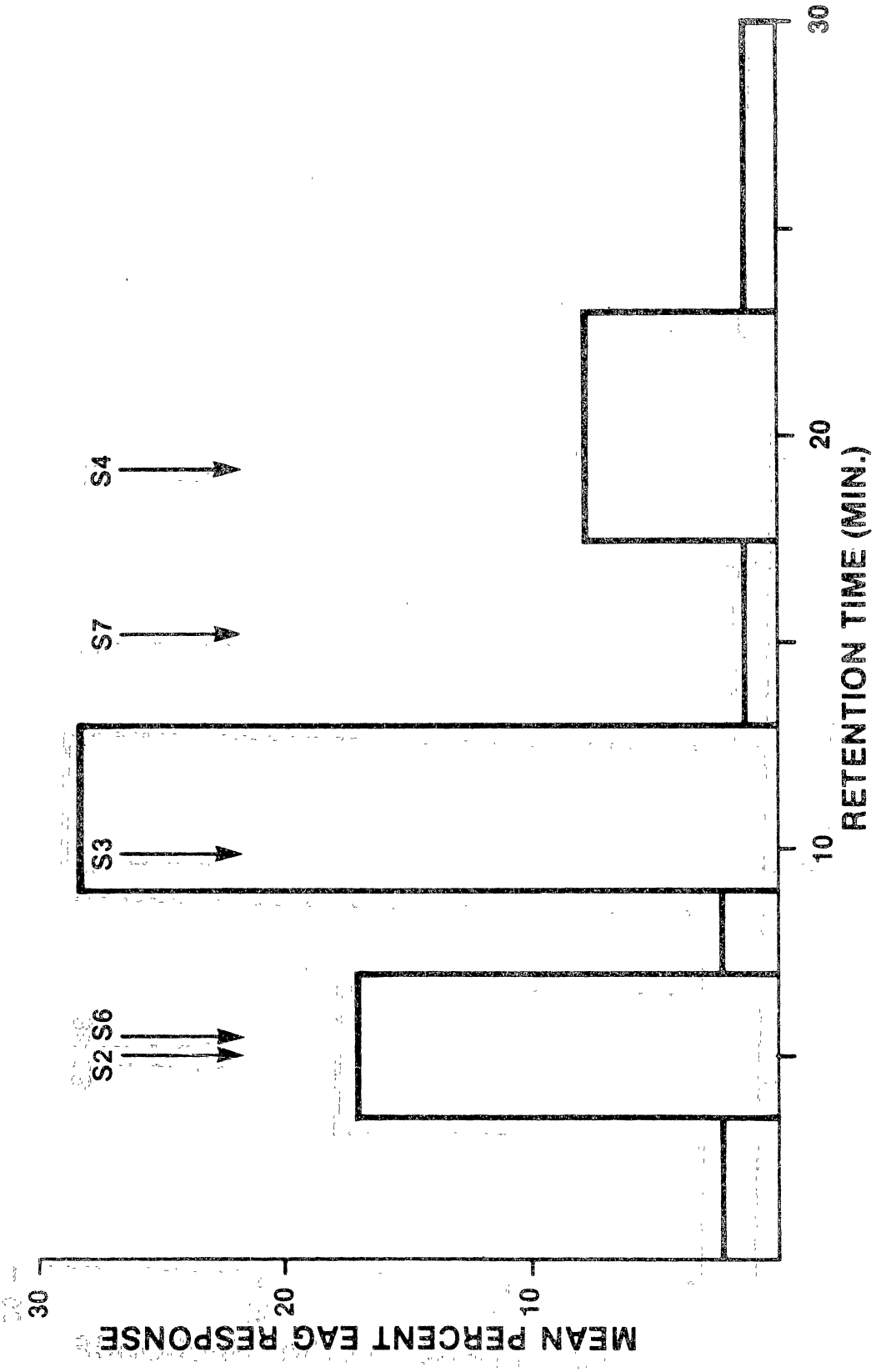
TABLE I

RETENTION TIMES OF PHEROMONE CANDIDATES AND COMPOUNDS IN EAG ACTIVE FRACTIONS

GC (SE-30) Collected Fraction or Standard Compounds	Retention Time on the Capillary GC Column (DB-1) (minutes)
Fraction 3	14.255 1/
	14.383 1/
Z-9-12:AC	14.327
12:AC	14.388
Fraction 4	15.938 1/
EE-8,10-12:AC	15.943
Z-12-14:OH	16.853

1/ Retention times of peaks from collected fractions in the range of pheromone candidates.

Figure 2. Mean Percent EAG Response Elicited by Each of Seven Fractions of Cydia caryana (30 F.E.) Sex Pheromone Extract Collected on Column B (Ultrabond Carbowax 20-M). (S2) 12:AC (t_R = 5.00 minutes); (S6) Z-9-12:AC (t_R = 5.50 minutes); (S3) EE-8,10-12:AC (t_R = 9.90 minutes); (S7) 14:AC (t_R = 15.25 minutes); (S4) Z-12-14:OH (t_R = 19.25 minutes).


MEAN PERCENT EAG RESPONSE
30
20
10
RETENTION TIME (MIN.)
10
20
30
S2 S6
S3
S7
S4

analyses was not possible because of a shortage of material. Therefore, utilizing as a reference the t_R values of selected diagnostic compounds, as well as suspected pheromonal compounds based on EAG and behavioral analysis, we can only speculate on the structural characteristics of the active compounds in these three fractions. Fractions 2, 4 and 6 encompassed retention times corresponding to 12:AC (t_R = 5.00 minutes) and Z-9-12:AC (t_R = 5.50 minutes), and EE-8,10-12:AC (t_R = 9.90 minutes), and Z-12-14:OH (t_R = 19.25 minutes), respectively.

GC-MS Analysis

Based upon the GC-EAG analysis, coupled with the results from EAG model compound screening (Chapter II) and behavioral analysis (Chapter III), the following standard compounds were selected for GC-MS analysis: E-8-12:AC, Z-8-12:AC, E-9-12:AC, Z-9-12:AC, 12:AC, EE-8,10-12:AC and Z-12-14:OH. Analysis of these standard compounds provided retention time values, and fragmentation information (via resultant diagnostic ion molecular weights), respectively, for each compound (Table II).

GC-MS analysis (operated in the selective ion monitor mode, SIM) of 18 F.E. of C. caryana whole extract (Figure 3) shows evidence of: (S1) a dodecenyl acetate at t_R 15.98 minutes, indicative of Z-8-12:AC and/or E-9-12:AC; (S2) dodecyl acetate (12:AC) at t_R 16.16 minutes; and (S3) a dodecadienyl acetate at t_R 17.46, which matches t_R of EE-8,10-12:AC.

Quantitative evaluation of peak areas indicated that the dodecenyl acetate, dodecyl acetate and dodecadienyl acetate are present at 1.0,

TABLE II

GC-MS ANALYSIS (SIM [1] MODE) OF STANDARD COMPOUNDS

Compound	t_R [3] (min.)	Diagnostic Ion Fragments (m/z) [2]				
		M^+ [4]	H_2OAC [5]	M-60 [6]	M-18 [7]	M-46 [8]
E-8-12:AC	15.87	-	61	166	-	-
Z-8-12:AC	15.98	-	61	166	-	-
E-9-12:AC	15.98	-	61	166	-	-
Z-9-12:AC	16.10	-	61	166	-	-
12:AC	16.16	-	61	168	-	-
EE-8,10-12:AC	17.46	224 [9]	61	164	-	-
Z-12-14:OH	18.22	-			194	166

[1] SIM -- Selective ion monitor.

[2] m/z -- Mass per unit charge.

[3] t_R -- Retention time (minutes) based upon peakfinder analysis.

[4] M^+ -- Molecular weight of molecular ion minus one electron.

[5] H_2OAC -- Molecular weight of acetic acid ($C_2H_5O_2$); indicative of an acetate.

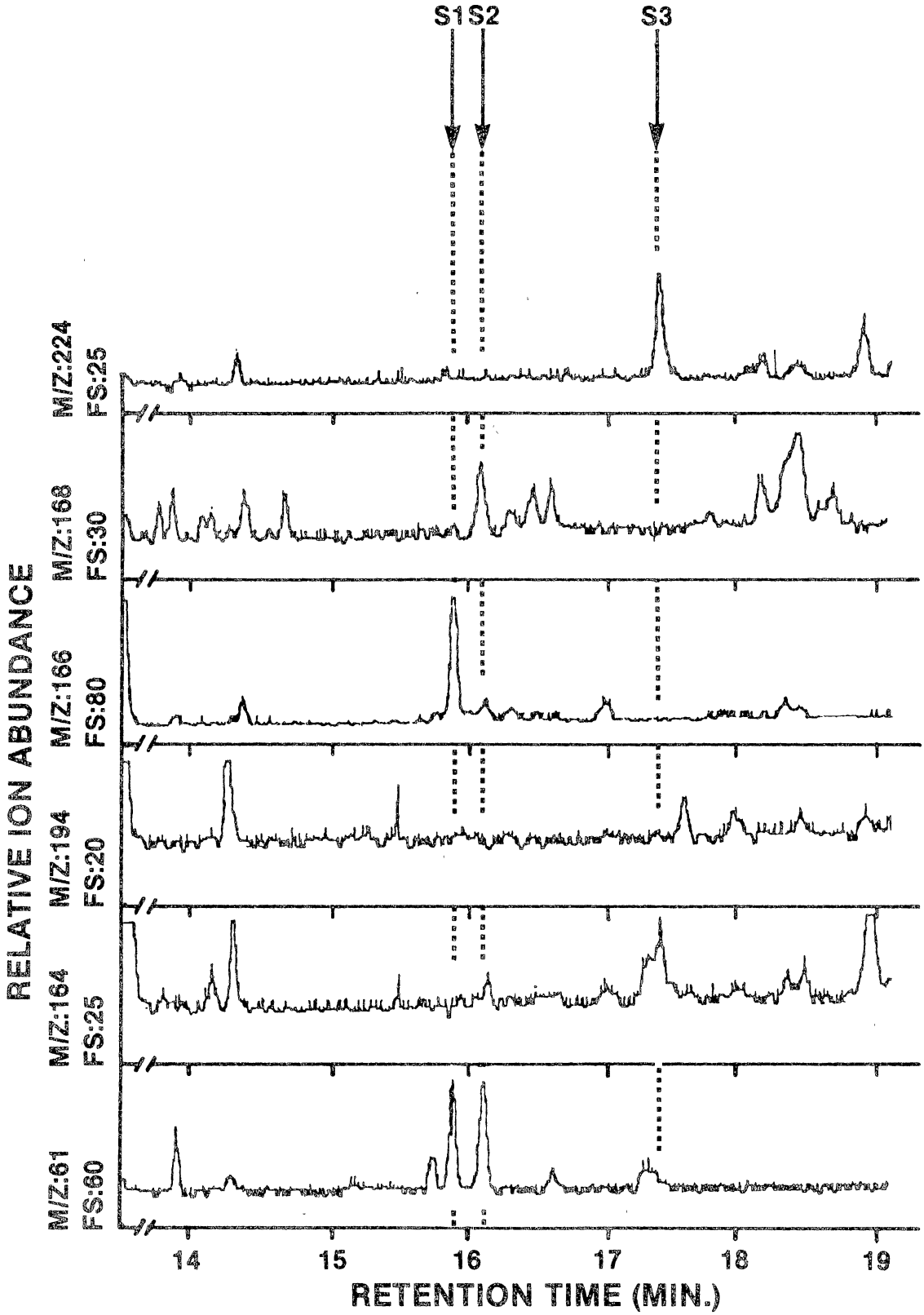
[6] M-60 -- Molecular ion minus the acetate group.

[7] M-18 -- Molecular ion minus water; indicative of an alcohol.

[8] M-46 -- Molecular ion minus water and ethylene; indicative of an alcohol.

[9] Molecular ion diagnostic for conjugated diene systems.

Figure 3. GC-MS SIM Analysis of 18 F.E. of Cydia caryana Sex Pheromone Whole Extract. (S1) a dodecenyl acetate; (S2) dodecyl acetate (12:AC); (S3) a dodecadienyl acetate.


S1
S2
S3
M/Z:224
FS:25
M/Z:168
FS:30
M/Z:166
FS:80
M/Z:194
FS:20
M/Z:164
FS:25
M/Z:61
FS:60
RELATIVE ION ABUNDANCE
14
15
16
17
18
19
RETENTION TIME (MIN.)

0.75 and 0.5 ng per female, respectively (2:1.5:1 ratio).

Due to the lack of additional crude extract, fractionated extract with corresponding retention times greater than 15.07 minutes was recovered from selective active EAG samples. The resultant mass spectrum (Figure 4) provides additional evidence for EE-8,10-12:AC at t_R 17.42.

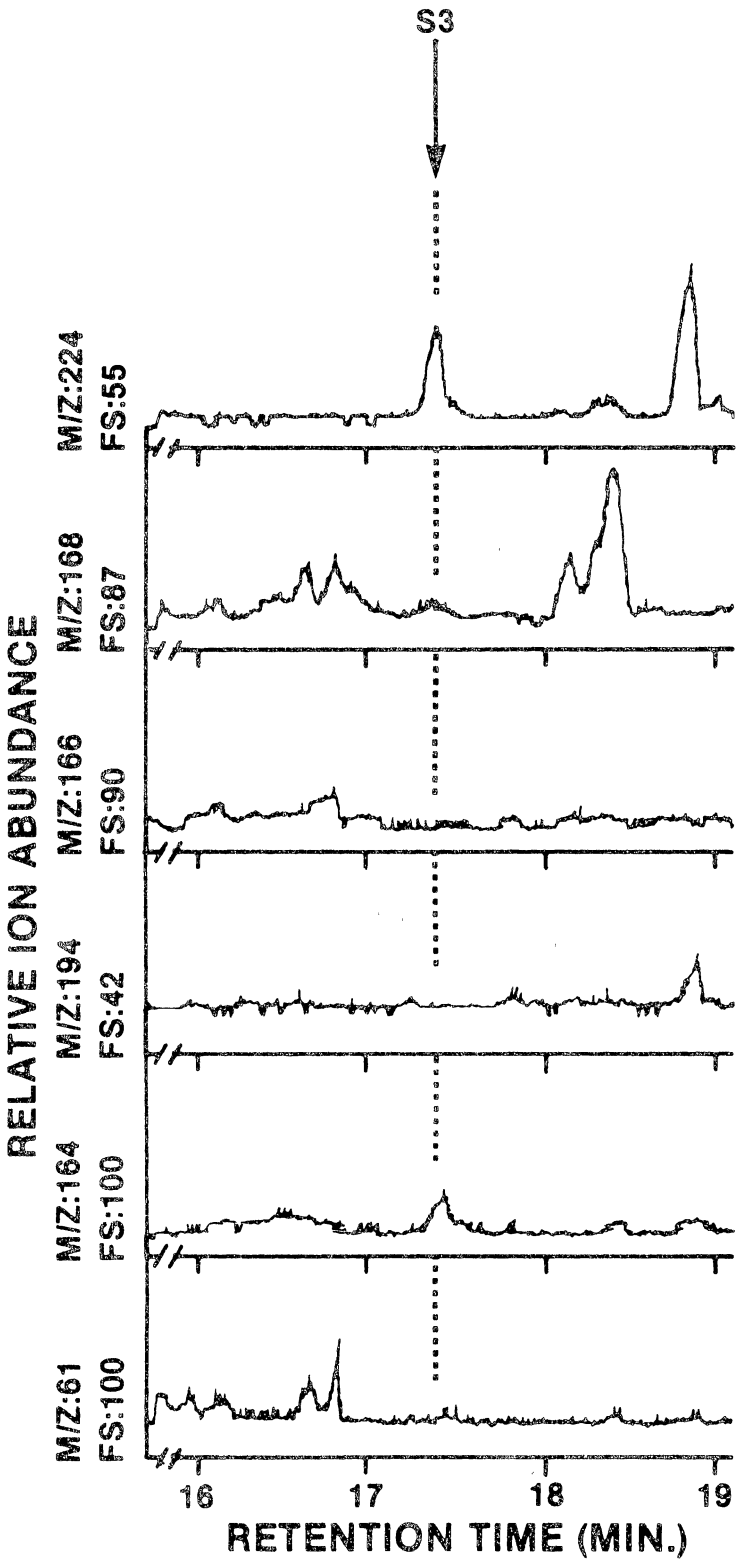
Scanning for Z-9-12:AC and Z-12-14:OH at t_R 16.10 and 18.22, respectively, provided no evidence for their presence in either SIM analysis.

Discussion

Most species of Lepidoptera whose pheromonal systems have been studied extensively utilize aliphatic straight-chain compounds, such as hydrocarbons, epoxy-hydrocarbons, alcohols, acetates, aldehydes and ketones. Tamaki (1985) lists about eighty compounds as female sex pheromonal components from more than 120 lepidopterous species. The carbon chain length ranges from 10 to 21, with those having 12, 14 and 16 carbon numbers comprising about 70% of the total compounds.

Some lepidopterous families seem to produce characteristic sex pheromone compounds. Roelofs and Brown (1982) reported that all female pheromonal components so far identified in the Tortricidae are compounds with 12 or 14 uninterrupted carbon atoms in the main chain of their structure. They related these structural similarities of known sex attractants of the Tortricidae for development of phylogenetic relationships within this family based upon speculative evolutionary schemes for pheromone biosynthetic pathways. The two major subfamilies, the Olethreutinae and the Tortricinae, each possess their own

Figure 4. GC-MS SIM Analysis of Cydia caryana Fractionated Sex Pheromone Extract Recovered from EAG Samples. (S3) a dodecadienyl acetate.


S3
RELATIVE ION ABUNDANCE
M/Z:61
FS:100
M/Z:164
FS:100
M/Z:194
FS:42
M/Z:166
FS:90
M/Z:168
FS:87
M/Z:224
FS:55
16
17
18
19
RETENTION TIME (MIN.)

structural characteristics. While most Tortricinae species utilize 14 carbon alcohols, aldehydes or acetates as major pheromonal components, most Olethreutinae species utilize compounds having 12 uninterrupted carbons in the main chain. The results presented herein, which provide evidence for Z-8-12:AC and/or E-9-12:AC, 12:AC and EE-8-10-12:AC, comply with this 12 carbon chain length scheme.

Sex attractants have been reported for 66 species of Olethreutinae. However, sex pheromone components have been chemically identified for only 21 species, and only two of which are Cydia species (Roelofs and Brown, 1982; Chisholm et al., 1985; Tamaki, 1985). The small number of pheromone identifications for Olethreutinae may be because Olethreutinae characteristically produce very small quantities of sex pheromone, unlike the Tortricinae. Additionally, the two to five components usually involved in these complex pheromone systems further hinders detection, isolation, and identification of secondary components, often present at only 0.1 to 10 percent of the primary component. Therefore, the research reported here represents one of relatively few chemical identifications of a sex pheromone for this genus and subfamily.

Compounds reported as sex pheromone components of Olethreutinae include: E- and Z-8-, E- and Z-9-, EE- and EZ-8,10- and EZ-7,9-12:AC, 12:AC, Z-8- and E-11-14:AC, ZZ-8,10-16:AC, and Z-8- and EE-8,10-12:OH. Components reported for Cydia species include only EE-8,10-12:AC (Cydia nigricana) and EE-8,10-12:OH (Cydia pomonella) (Roelofs and Brown, 1982). Therefore, the compounds identified for C. caryana herein are not unknown sex pheromone components of the Olethreutinae.

It should be noted that sex pheromone gland tissue extraction is not always the best method for obtaining the pheromone since closely

related analogues of pheromone components are frequently present in these extracts and, in some cases, pheromone components are not stored in detectable quantities. The presence of additional related compounds can complicate the study since their behavioral function in the pheromone system, or lack thereof, is difficult to determine (Roelofs, 1980).

The small quantity of pheromone material collected in this study precluded analyses of every peak in each active fraction. Thus, only suspected pheromone candidates based upon the previous EAG and behavioral studies were searched. Furthermore, SIM analysis can be utilized only to determine the presence or absence of suspected compounds in the gland extract, and can not be used to structurally characterize previously unknown materials.

Conclusions

The heretofore GC-EAG and GC-MS SIM analyses of C. caryana sex pheromone gland extract established the presence of a dodecenyl acetate (Z-8-12:AC and/or E-9-12:AC at 1.0 ng per female), dodecyl acetate (12:AC at 0.75 ng per female), and a dodecadienyl acetate (corresponding to t_R of EE-8,10-12:AC at 0.5 ng per female). Lack of adequate extract precluded confirmation of the sites of unsaturation and their geometrical configuration in the later compound.

Presence of biological activity in an additional extract fraction (fraction six eluted from the Ultrbond Carbowax 20-M column), coupled with the minute quantities determined for the identified compounds, strongly implicates the existence of at least a fourth possible component whose t_R is consistent with a tetradecen-1-ol. It is very

likely that additional components may be found when an effluent collection technique is employed.

The correlation between female sex pheromone components and male receptor key compounds seems to be the rule in Lepidoptera. However, it must be emphasized that this correspondence is not a strict one for Tortricidae, as potential pheromone compounds may regularly be found in a female secretion without an apparent equivalent in the male receptor system (Kaissling, 1979). Thus, although the chemical and EAG evidence presented here reports the existence of certain compounds in the female sex pheromone gland extract and their perception by conspecific male moths, respectively, only field evaluation will substantiate the biological function of these compounds as C. caryana sex pheromone components.

Bibliography

Anderson, A. L. 1972. An investigation of a sex pheromone of the hickory shuckworm, Laspeyresia caryana (Fitch). M.S. thesis, Texas A & M University, College Station. 54 pp.

Arn, H., E. Stadler, and S. Rauscher. 1975. The electroantennographic detector - a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. Z. Naturforsch. 30c: 722-5.

Chisholm, M. D., D. W. Reed, E. W. Underhill, P. Palaniswany, and J. W. Wong. 1985. Attraction of Tortricid moths of subfamily Olethreutinae to field traps baited with dodecadienes. J. Chem. Ecol. 11(2): 217-30.

Gaston, L. K. 1984. Technique and equipment for collection of volatile chemicals from individual, natural, or artificial sources, pp.

215-21. In H. E. Hummel and T. A. Miller [eds.], Techniques in Pheromone Research, Springer-Verlag, New York.

Golub, M. A. and I. Weatherston. 1984. Techniques for extracting and collecting sex pheromones from live insects and from artificial sources, pp. 223-85. In H. E. Hummel and T. A. Miller [eds.], Techniques in Pheromone Research, Springer-Verlag, New York.

Jefferson, R. N., H. H. Shorey, and R. E. Rubin. 1968. Sex pheromones of noctuid moths. XVI. The morphology of the female sex pheromone glands of eight species. Ann. Entomol. Soc. Am. 61: 861-5.

Kaissling, K. E. 1979. Recognition of pheromones by moths especially in Saturniids and Bombyx mori, pp. 43-56. In F. J. Ritter [ed.], Chemical Ecology: Odour Communication in Animals. Elsevier/N. Holland, Amsterdam.

Moorehouse, J. E., R. Yeadon, P. S. Beevor and B. F. Nesbitt. 1969. Method for use in studies of insect chemical communication. Nature 223: 1174-5.

Payne, J. A. and E. K. Heaton. 1975. The hickory shuckworm: its biology, effect upon nut quality, and control. Ann. Rep. Northern Nut Growers Assoc. 66: 19-25.

Roelofs, W. L. 1977. The scope and limitations of the electroantennogram technique in identifying pheromone components, pp. 147-65. In N. McFarlane [ed.], Crop Protection Agents. Academic Press, London.

Roelofs, W. L. 1978. Chemical control of insects by pheromones, pp. 419-64. In M. Rockstein [ed.], Biochemistry of Insects, Academic Press, New York.

Roelofs, W. L. 1980. Pheromones and their chemistry, pp. 583-602.

In M. Locke and D. S. Smith [eds.], Insect Biology in the Future, Academic Press, New York.

Roelofs, W. L. and R. L. Brown. 1982. Pheromones and evolutionary relationships of Tortricidae. Ann. Rev. Ecol. Syst. 13: 395-422.

Schneider, D. 1957. Electrophysiologische untersuchungen von chemo- und mechanoreceptoren der antenne des seidenspinners Bombyx mori L. Z. Vergl. Physiol. 40: 8-41.

Sower, L. L., J. A. Coffelt, and K. W. Vick. 1973. Sex pheromone: a simple method of obtaining relatively pure material from females of five species of moths. J. Econ. Entomol. 66: 1220-2.

Sower, L. L., H. H. Shorey, and L. K. Gaston. 1972. Sex pheromones of Lepidoptera. XXVIII. Factors modifying the release rate and extractable quantity of pheromones from females of Trichoplusia ni (Noctuidae). Ann. Entomol. Soc. Am. 65: 954-7.

Tamaki, Y. 1985. Sex pheromones, pp. 145-91. In G. A. Kerkut and L. I. Gilbert [eds.], Comprehensive Insect Physiology, Biochemistry and Pharmacology: Behavior, Vol. 9. Pergamon Press, Oxford.

CHAPTER V

FIELD EVALUATION OF POTENTIAL *CYDIA CARYANA* SEX PHEROMONE COMPONENTS

Introduction

The development of an insect pest management system which incorporates behavior modifying chemicals (BMC) that function as insect attractants usually involves certain steps according to Roelofs (1979): (1) chemical characterization of the chemical(s) and documentation of their potency by laboratory and field tests; (2) determination of the optimum release rate, component ratio and trap design; (3) determination of the optimum trap spacing and density; and (4) correlation of trap catch with population density and/or crop loss.

The research reported here, as part of step 1, was undertaken to provide initial field verification of results culminating from our laboratory bioassays of *Cydia caryana* (Fitch) male moth perception and behavioral response patterns, and conspecific female moth sex pheromone gland extract chemical analysis. These field trapping experiments, used here as a bioassay in the characterization and identification of the *C. caryana* sex pheromone, were intended to determine relative activities of different compounds or compound combinations utilizing the number of male moths captured in traps as the criterion for biological activity.

Materials and Methods

Test Location and Trap Placement

Field tests were conducted near Red Rock, Oklahoma, approximately 51 km north of Oklahoma State University, in a commercial pecan orchard. Pherocon 1C wing traps (Zoecon Corp., Palo Alto, California) were suspended within the lower canopy of pecan trees approximately 6.2 m above ground level. Traps were set out in rows (replicates), with treatments assigned at random within each replicate, and traps were at least 20 m from any other trap and usually separated by at least one pecan tree.

Design

The ideal experimental design should minimize variation in trap catch caused by differences in population density in different areas of the test site and also minimize interactions between different treatments (Cardé and Elkinton, 1984). Replicating treatments, if extensive enough, will tend to obviate the difficulties inherent in sampling clumped populations. However, the tactic of extensive replication itself may be insufficient to overcome distributional uneveness, particularly where 10 to 20 replicates are plainly impractical (Roelofs and Cardé, 1977). Thus, the objective of the experimental design should be to ensure that treatment interactions and population clumpedness affect all treatments as equally as practicable. Cardé and Elkinton (1984), suggests that rerandomization and/or rotation of treatments within replicates (or blocks) as often as the traps are sampled can aid in

reaching this objective. Therefore in this study, traps were checked daily, the number of C. caryana moths recorded and removed, and traps rotated by advancing each trap one position within each replicate. This ensured that each trap occupied every position within each replicate during the duration of the test periods.

Interpretation

In trapping experiments used for verification of pheromone identity, comparison is sometimes made between attractiveness (trap catch) of synthetic compounds versus natural pheromone emitted by caged conspecifics. In this study, the unavailability of C. caryana female moths prevented their inclusion. However, unbaited traps containing blank septa were used to establish that trap catches were not due to the attractiveness of the traps or septa themselves, nor due to random flight of moths into the traps.

Since relatively few treatments will likely be attractive and many more treatments unattractive during initial trapping experiments, it is essential to verify the presence of the target species during the test periods. In this study, blacklight traps (one per replicate) were run periodically before, during and following the test periods, thus enabling the distinction between unattractivenss and the absence of C. caryana.

Septa and Chemicals

Treatment compounds were prepared for use by dissolving them in dichloromethane and impregnating them into red rubber 1F septa (The West Co., Phoenixville, PA). Candidate pheromone chemical compounds

were 98% pure by GC analysis, with less than 1% of their respective geometrical isomers.

1984 Field Trapping Experiment

In the 1984 field trapping experiments, three 14-day tests were conducted: August 14 to 28, September 6 to 20; and October 4 to 18. Utilizing results from the previous EAG, behavioral and chemical analyses, three replicates of eight treatments at three concentrations were tested. One unbaited trap per replicate was also included. Light traps were run periodically for 48- to 72-hour intervals from August 13 to October 19.

1985 Field Trapping Experiment

In the 1985 field trapping experiment, one 28-day test was conducted from May 15 to June 11. Utilizing the results from the 1984 field trapping experiments, three replicates of two treatments at six concentrations were tested. Two unbaited traps per replicate were also included.

The unbaited traps and the Z-8-12:AC baited traps were reloaded on a weekly basis, while the EE-8,10-12:AC baited traps were reloaded three times per week. Reloading traps ensured the integrity of the odor quality being emitted from the septa. This is particularly important for the conjugated diene, which isomerizes very rapidly. Light traps were run for 24-hour intervals prior (day 0), during (days 7, 14, and 21), and following (day 28) the test period.

Results

1984 Field Trapping Experiment

Light trap catches verified the presence of C. caryana moths during the test period, but at very, very low population levels (average 24-hour catch was 4.6 moths, ranging from 0 to 18 moths).

Likewise, resulting trap catches in the treatment traps were also very low, making statistical analysis impractical (Table I). However, particularly in light of the zero trap catch in the unbaited traps, it is readily apparent that both Z-8-12:AC (100 µg dose) and EE-8,10-12:AC (1, 10 and 100 µg doses) are attractive to C. caryana under natural field conditions.

1985 Field Trapping Experiment

Light trap catches verified the presence of C. caryana moths during the test period, but only at low population levels (average 24-hour catch was 7.9 moths, ranging from 12 to 26 moths).

Trap catch data in the treatment traps were transformed to $[(Xi + 1)^{\frac{1}{2}}]^{\frac{1}{2}}$, where Xi is the average number of moths captured in treatment by concentration trap i. Cardé and Elkinton (1984) recommend transformation of trap catch data when analysis of variance (AOV) is contemplated since one of the basic assumptions regarding homogeneity of residual variances is rarely satisfied. Transformers, such as the one used here, tend to stabilize the variance or correct for the nonhomogeneity of residual variances (Snedecor and Cochran, 1980). Therefore, transformed trap catch data was submitted to an analysis of variance and Duncan's multiple range test (Table II).

TABLE I

1984 FIELD TRAPPING EXPERIMENT, RED ROCK, OKLAHOMA [1,2,3]

Chemical	Number Moths Captured [4] Dose (µg) 1	10	100	Total	Percent of Total Moths Captured
Z-8-12:AC	0	2	20	22	31.4
E-9-12:AC	0	0	0	0	0.0
Z-9-12:AC	0	0	1	1	1.4
Z-11-14:OH	0	0	0	0	0.0
EE-8,10-12:AC	13	4	12	29	41.4
EE-8,10-12:AC : Z-8-12:AC	2	0	3	5	7.1
EE-8,10-12:AC : E-9-12:AC	2	6	2	10	14.3
EE-8,10-12:AC : Z-9-12:AC	2	0	1	3	4.3
Blank	0	0	0	0	0.0

[1] Three 14-day tests conducted from August 14-28, September 6-20, and October 4-18.

[2] Pheromone traps were placed at 2.0 to 6.2 m above ground level and spaced 20 m between traps.

[3] Light trap catches during the test period averaged 4.7 moths per trap per night.

[4] Total number male C. caryana moths captured over 42 days in three replicates.

TABLE II

1985 FIELD TRAPPING EXPERIMENT, RED ROCK, OKLAHOMA [1,2,3]

Z-8-12:AC		EE-8,10-12:AC	
Dose (µg)	Mean [4]	Dose (µg)	Mean [4]
3000	1.27 a [5]	30	2.25 a [5]
300	1.24 a	100	2.24 a
10	1.23 a	10	2.03 a
100	1.20 a	300	1.94 a
1000	1.10 a	1000	1.93 a
30	1.06 a	3000	1.20 b
0	1.00 a	0	1.00 b

[1] One 28-day test period from May 15 to June 11.

[2] Pheromone traps were placed at 6.2 m above ground level and spaced 20 m between traps.

[3] Light trap catches during the test period averaged 7.9 moths per trap per night.

[4] Means represent the transformed average number (averaged over three replicates) of male C. caryana moths captured in each treatment by concentration combination.

[5] Treatments means followed by the same letter are not significantly different at $P > 0.05$ (Duncan's [1955] multiple range test).

Analysis of variance for Z-8-12:AC indicated that there were no significant differences ($P > 0.05$) between the mean trap catch for the various dosages, including the unbaited traps. There was, however, a significant difference ($P < 0.05$) between replicates.

Analyses of variance for EE-8,10-12:AC indicated that the mean trap catch for the 3,000 ug dose and the unbaited traps (which were not significantly different at $P > 0.05$) were significantly less ($P < 0.002$) than that for all other dosages. There was once again a significant difference ($P < 0.03$) between replicates.

Discussion

During the 1984 trapping experiments, although trap catches were very low, the attractiveness of both Z-8-12:AC and EE-8,10-12:AC was elucidated. Those chemical combinations showing some attractiveness may be doing so in response to the attractiveness of the EE-8,10-12:AC component portion of the combinations.

During the 1985 trapping experiments, although trap catches in Z-8-12:AC baited traps (at all dosages tested) showed no significant difference from the unbaited traps, the fact remains that Z-8-12:AC baited traps did catch some moths at all dosages, while the unbaited traps failed to catch any moths. Also during 1985, although there were no significant differences in trap catch between EE-8,10-12:AC baited traps at dosages from 30 to 1,000 µg, the general trend indicated an obvious optimum dosage between 30 and 100 µg, probably closer to the 30 µg level. The significant drop in trap catch at the 3,000 µg level of EE-8,10-12:AC, may indicate that late behavioral modes are being diminished. This same effect was found in the oriental fruit moth,

Grapholitha molesta, which exhibited normal attraction and mating responses to a comparatively narrow range of pheromone concentrations. High rates of pheromone emission, although eliciting attraction well downwind, caused arrestment of upwind progress near the source, resulting in virtually no trap catch (Cardé et al., 1975; Baker and Cardé, 1979; Baker and Roelofs, 1981).

Significant difference between replicates, for both Z-8-12:AC and EE-8,10-12:AC, provides evidence for the idea of a non-random (clumpedness) population distribution of C. caryana in the field and also supports the need for trap catch data transformation.

Conclusions

EE-8,10-12:AC is or closely resembles a primary component in the natural sex pheromone of C. caryana. Future field trapping experiments should attempt to screen the other three geometrical isomers of this conjugated diene. Z-8-12:AC alone, although much less attractive than EE-8,10-12:AC, does exhibit some ability to attract and capture C. caryana moths. Obvious future field trapping experiments should incorporate these two compounds at a wide variety of ratios to test for a synergistic effect of Z-8-12:AC on EE-8,10-12:AC trap catch. Due to the low population levels experienced during 1984, a thorough examination of Z-9-12:AC and Z-12-14:OH, and various binary and tertiary mixtures of the conjugated diene, EE-8,10-12:AC, plus these monoenes should be conducted.

Bibliography

Baker, T. C. and R. T. Cardé. 1979. Analysis of pheromone-mediated

behavior in male Grapholitha molesta, the Oriental fruit moth (Lepidoptera: Tortricidae). Environ. Entomol. 8: 956-68.

Baker, T. C. and W. L. Roelofs. 1981. Initiation and termination of Oriental fruit moth male response to pheromone concentrations in the field. Environ. Entomol. 10: 211-8.

Cardé, R. T., T. C. Baker, and W. L. Roelofs. 1975. Ethological function of components of a sex attractant system for Oriental fruit moth males, Grapholitha molesta (Lepidoptera: Tortricidae). J. Chem. Ecol. 1: 475-91.

Cardé, R. T. and J. S. Elkinton. 1984. Field trapping with attractants: methods and interpretation, pp. 111-29. In H. E. Hummel and T. A. Miller [eds.], Techniques in Pheromone Research. Springer-Verlag, New York.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-41.

Roelofs, W. L. 1979. Establishing efficacy of sex attractants and disruptants for insect control. Entomol. Soc. Am. 97 pp.

Roelofs, W. L. and R. T. Cardé. 1977. Responses of Lepidoptera to synthetic sex pheromone chemicals and their analogues. Annu. Rev. Entomol. 22: 377-405.

Snedecor, G. W. and W. G. Cochran. 1980. Statistical Methods, 7th ed., Iowa State University Press, Ames. 593 pp.

APPENDIXES

APPENDIX A

CHAPTER II:

ELECTROANTENNOGRAPHIC ANALYSIS OF ANTENNAL RECEPTOR SPECIFICITY AND RESPONSE THRESHOLD

TABLE I

MEAN PERCENT EAG RESPONSE OF *CYDIA CARYANA* TO STIMULATION BY A STRUCTURAL SERIES OF 12-CARBON ACETATES (100 µg)

Geometric and Positional Isomer	N	Mean Percent EAG Response	Duncan Mean Separation [1]
E10	4	74.16	a
Z9	5	49.25	b
E8	5	47.55	b
Z8	4	29.68	c
Z10	5	28.69	c
11	4	22.88	cd
E4	3	19.40	cde
E2	4	14.69	def
Z2	3	13.68	def
E6	4	13.36	def
Z5	4	12.32	def
Z4	3	10.96	def
Z3	3	10.27	def
E9	4	10.14	def
E5	4	9.48	ef
Z7	3	8.44	ef
E7	4	8.40	ef
Z6	4	7.74	ef
E3	3	2.87	f

[1] Numbers followed by the same letter are not significantly different at $P < 0.05$ (Duncan's multiple range test).

TABLE II

MEAN PERCENT EAG RESPONSE OF *CYDIA CARYANA* TO STIMULATION BY A STRUCTURAL SERIES OF 14-CARBON ACETATES (100 µg)

Geometric and Positional Isomer	N	Mean Percent EAG Response	Duncan Mean Separation [1]
Z4	3	13.090	a
E5	3	9.287	ab
Z5	3	9.287	ab
Z10	3	8.557	ab
E8	3	8.507	ab
E12	3	8.430	ab
Z6	3	7.180	ab
Z12	3	7.117	ab
Z3	3	6.857	ab
Z11	3	5.973	ab
Z2	3	5.877	ab
E4	3	5.843	ab
Z9	3	5.590	ab
Z7	3	5.270	ab
13	3	5.193	ab
E11	3	4.923	ab
E2	3	4.827	ab
Z8	3	4.220	ab
E3	3	4.037	ab
E7	3	3.187	ab
E9	3	2.623	ab
Z10	3	2.453	ab
E6	3	2.363	b

[1] Numbers followed by the same letter are not significantly different at $P < 0.05$ (Duncan's multiple range test).

TABLE III

MEAN PERCENT EAG RESPONSE OF *CYDIA CARYANA* TO STIMULATION BY A STRUCTURAL SERIES OF 12-CARBON ALCOHOLS (100 µg)

Geometric and Positional Isomer	N	Mean Percent EAG Response	Duncan Mean Separation [1]
E10	3	25.830	a
Z5	4	22.047	ab
E4	6	19.347	abc
Z4	6	17.137	abc
E6	3	16.427	abc
11	3	13.933	abc
Z9	5	13.484	abc
Z8	5	12.978	abc
Z7	5	12.658	abc
E5	6	11.942	abc
12	5	11.776	abc
E8	6	10.645	abc
E2	3	8.753	abc
E9	5	7.502	bc
Z2	3	6.547	bc
Z3	3	6.547	bc
E7	6	6.298	bc
Z6	3	2.947	c
Z10	3	2.717	c
E3	3	1.320	c

[1] Numbers followed by the same letter are not significantly different at $P < 0.05$ (Duncan's multiple range test).

TABLE IV

MEAN PERCENT EAG RESPONSE OF *CYDIA CARYANA* TO STIMULATION BY A STRUCTURAL SERIES OF 14-CARBON ALCOHOLS (100 µg)

Geometric and Positional Isomer	N	Mean Percent EAG Response	Duncan Mean Separation [1]
Z12	6	37.138	a
Z10	3	13.253	b
Z6	3	12.160	b
Z7	3	10.740	b
E10	3	10.583	b
14	3	10.157	b
E6	3	8.627	b
Z11	3	8.043	b
E2	3	7.267	b
E12	3	7.190	b
E4	3	7.103	b
Z3	3	6.267	b
E8	3	5.517	b
Z8	3	5.407	b
E5	3	5.293	b
Z5	3	5.050	b
E3	3	4.397	b
E7	3	3.797	b
13	3	3.733	b
E9	3	3.333	b
Z2	3	3.233	b
Z4	3	3.150	b
E11	3	2.547	b
Z9	3	0.000	b

[1] Numbers followed by the same letter are not significantly different at $P < 0.05$ (Duncan's multiple range test).

TABLE V

CALCULATIONS FOR THE CONFIDENCE LIMITS ABOUT THE SLOPE BASED UPON
$CI = SLOPE \pm t_{alpha/2\ (E\ df)}(SE)$

Compound	Slope	Alpha = 0.10	Alpha = 0.05	Alpha = 0.01
EE-8,10-12:AC	13.4061	± 2.5241 10.8820-15.9302	± 3.1036 10.3025-16.5097	± 4.4145 8.9916-17.8206
EZ-8,10-12:AC	11.3882	± 2.2337 9.1545-13.6219	± 2.7465 8.6417-14.1347	+ 3.9064 7.4818-15.2946
EE- and EZ-8,10-12:AC	14.9027	± 5.0068 9.8959-19.9095	± 6.1562 8.7465-21.0589	± 8.7563 6.1464-23.6590
E-8-12:AC	12.1111	± 3.5782 8.5329-15.6893	± 4.3997 7.7114-16.5108	± 6.2579 5.8532-18.3690
E-10-12:AC	10.7297	± 3.0300 7.6997-13.7597	± 3.7256 7.0041-14.4553	± 5.2991 5.4306-16.0288
Z-9-12:AC	7.9649	± 1.0103 6.9546-8.9752	± 1.2423 6.7226-9.2072	± 1.7670 6.1979-9.7319
Z-12-14:OH	14.2435	± 2.8973 11.3462-17.1408	± 3.5625 10.6810-17.8060	± 5.0671 9.1764-19.3106

TABLE VI

MEAN SEPARATION TEST FOR SLOPE BASED UPON CONFIDENCE LIMITS CALCULATED AT THREE LEVELS OF ALPHA

Compound	Slope	Alpha Level 1/		
		0.10	0.05	0.01
EE- and EZ-8,10-12AC	14.9027	a	ab	a
Z-12-14:OH	14.2435	a	a	a
EE-8,10-12:AC	13.4061	a	a	a
E-8-12:AC	12.1111	ab	ab	a
EZ-8,10-12:AC	11.3882	a	ab	a
E-10-12:AC	10.7297	ab	ab	a
Z-9-12:AC	7.9649	b	b	a

1/ Identical letters within each column (alpha level) indicate that respective slopes are not significantly different.

TABLE VII

CALCULATIONS FOR THE NUMBER OF MOLECULES AT THE X-INTERCEPT (RESPONSE THRESHOLD)

	FW [1]	Grams at X-Intercept ($X\ 10^{+12}$)	Moles [2] ($X\ 10^{+13}$)	Number [3] Molecules ($X\ 10^{-11}$)
(E,E)-8,10-dodecadien-1-ol acetate [4]	224	4.9	0.21875	0.13173
(E,Z)-8,10-dodecadien-1-ol acetate [4]	224	188.8	8.4286	5.0757
(E,E)- & (E,Z)-8,10-dodecadien-1-ol acetate	224	40.2	1.7946	1.0807
(E)-8-dodecen-1-ol acetate	226	167.9	7.4292	4.4739
(E)-10-dodecen-1-ol acetate	226	8.1	0.35841	0.21583
(Z)-9-dodecen-1-ol acetate	226	20.0	0.88496	0.53292
(Z)-12-tetradecen-1-ol	212	1,563.1	73.731	44.401

[1] FW = Formula weight.

[2] Number gram moles = grams at X-intercept $\times \frac{1 \text{ mole}}{FW}$ or $\frac{1 \text{ mole}}{FW} = \frac{X}{\text{g at } X}$

[3] Number molecules at response threshold $= \frac{6.02205 \times 10^{23}}{1 \text{ mole}} \times$ number moles

[4] E = Entfernung (across); Z = Zusammen (together)

TABLE VIII

FORMULAS UTILIZED IN CALCULATING THE CONFIDENCE LIMITS ABOUT THE X-INTERCEPT [1]

1. $D = b^2 - t^2_{0.05(n-2df)} \times S^2b$

2. $SS_x = \frac{MS_E}{S^2b}$

3. $\bar{X} = \frac{B_o - \bar{Y}}{-b}$

4. $H = \frac{t_{0.05(n-2df)}}{D} \times \left[S^2_{Y\,x} \left(D \left(1 + \frac{1}{n}\right) + \frac{(Y_i - \bar{Y})^2}{SS_x}\right)\right]^{\frac{1}{2}}$

5. $L = \bar{X} + \frac{b\,(Y_i - \bar{Y})}{D} \pm H$

[1] Sokal, R. R. and F. J. Rohlf. 1981. Biometry, 2nd ed., W. H. Freeman & Co., San Francisco. 859 pp.

TABLE IX

RINGER SOLUTION FORMULA

Sodium Chloride	0.9 g
Potassium Chloride	0.042 g
Calcium Chloride	0.025 g
Distilled Water	100.0 ml

TABLE X

CALCULATIONS FOR CONFIDENCE LIMITS ABOUT THE SLOPE
FOR LINEAR AND QUADRATIC FUNCTIONS [1/]

Compound	Function	Slope	Alpha = 0.10	Alpha = 0.05	Alpha = 0.01
E-8-12:AC	Linear	12.1111	± 3.5782 8.5329-15.6893	± 4.3997 7.7114-16.5108	± 6.2579 5.8532-18.3690
	Quadratic	2.7188	± 0.5436 2.1752-3.2624	± 0.6684 2.0504-3.3872	± 0.9507 1.7681-3.6693
Z-12-14:OH	Linear	14.2435	± 2.8973 11.3462-17.1408	± 3.5625 10.6810-17.8060	± 5.0671 9.1764-19.3106
	Quadratic	3.1344	± 0.3624 2.7720-3.4968	± 0.4456 2.6888-3.5800	± 0.6338 2.5006-3.7682

[1/] When considered linear, difference designations follow those given in Appendix Table VI. When considered quadratic, both slopes, which are not significantly different from one another, are significantly different from all other slopes, and thus designated by 'c' based upon the Confidence Interval Method.

APPENDIX B

CHAPTER III:

ANALYSIS OF *CYDIA CARYANA* IN-FLIGHT BEHAVIOR TO PERCEIVED SYNTHETIC COMPOUNDS AND CORRELATION WITH ELECTROANTENNOGRAM (EAG) RESPONSE

TABLE I

EXPERIMENT 1. COMPARISON AMONG RESPONSES TO VARIOUS DOSAGES OF KNOWN PERCEIVED SYNTHETIC COMPOUNDS

		Dose							
Compound	Behavior	0	0.1 ng	1.0 ng	10.0 ng	100.0 ng	1.0 µg	10.0 µg	100.0 µg
Z-8-12:AC	a [1]	0	0	0	0	0	0	10	40
	b [2]	-	-	-	-	-	-	F	F
	c [3]	-	-	-	-	-	-	F	G
	d [4]	-	-	-	-	-	-	-	-
Z-9-12:AC	a	0	10	20	20	60	60	60	60
	b	-	F	F	F	E	E	E	E
	c	-	F	F	F	E	G	G	G
	d	-	-	-	-	-	-	-	-
E-10-12:AC	a	0	0	0	0	0	0	0	30
	b	-	-	-	-	-	-	-	F
	c	-	-	-	-	-	-	-	F
	d	-	-	-	-	-	-	-	-
Z-12-14:OH	a	0	0	30 [5]	10	10	0	0	0
	b	-	-	F	F	F	-	-	-
	c	-	-	F	N	N	-	-	-
	d	-	-	-	-	-	-	-	-

TABLE I (Continued)

Compound	Behavior	Dose 0	0.1 ng	1.0 ng	10.0 ng	100.0 ng	1.0 µg	10.0 µg	100.0 µg
E,E-8,10-12:AC	a	0	20	30	60	70	70	70	70
	b	-	F	F	E	E	E	E	E
	c	-	G	G	E	E	E	E	E
	d	-	-	-	-	-	-	-	-

1/ Behavior a = percent moths displaying upwind flight within the characterized plume.

2/ Behavior b = upwind plume flight with repeated approaches, retreats, and reapproaches. Fair (F), Good (G), Excellent (E).

3/ Behavior c = hovering in close proximity downwind of the odor source during plume flight. None (N), Fair (F), Good (G), Excellent (E).

4/ Behavior d = contact with the odor source. Yes (+), N (-).

5/ When stimulated with dosages greater than or equal to 1.0 ng, C. caryana male moth behavior deviated by appearing to be erratic and excited, particularly in close proximity of the odor source. Thus, behavior was difficult to score. Additionally, responding moths (1.0, 10.0, and 100.0 ng stimulus intensities) often landed directly upwind of the odor source.

TABLE II

EXPERIMENT 1. TALLY SHEET FOR CYDIA CARYANA BEHAVIORAL RESPONSES TO STIMULATION WITH VARIOUS DOSAGES OF Z-8-12:AC IN A FLIGHT TUNNEL

		Moth									
Dose [1]	Behavior	1	2	3	4	5	6	7	8	9	10
0	a [2]	-	-	-	-	-	-	-	-	-	-
	b [3]										
	c [4]										
	d [5]										
0.1 ng	a	-	-	-	-	-	-	-	-	-	-
	b										
	c										
	d										
1.0 ng	a	-	-	-	-	-	-	-	-	-	-
	b										
	c										
	d										
10.0 ng	a	-	-	-	-	-	-	-	-	-	-
	b										
	c										
	d										
100.0 ng	a	-	-	-	-	-	-	-	-	-	-
	b										
	c										
	d										

TABLE II (Continued)

Dose	Behavior	Moth 1	2	3	4	5	6	7	8	9	10
1.0 µg	a	-	-	-	-	-	-	-	-	-	-
	b										
	c										
	d										
10.0 µg	a	-	-	+	-	-	-	-	-	-	-
	b			F							
	c			F							
	d			-							
100.0 µg	a	+	-	-	-	+	+	+	-	-	-
	b	F				F	F	F			
	c	G				F	G	G			
	d	-				-	-	-			

1/ Dose represents the concentration impregnated into red rubber septa.

2/ Behavior a = upwind flight within the characterized plume. Responders (+), Non-responders (-)

3/ Behavior b = upwind plume flight with repeated approaches, retreats and reapproaches. Fair (F), Good (G), Excellent (E)

4/ Behavior c = hovering in close proximity downwind of the odor source during plume flight. None (N), Fair (F), Good (G), Excellent (E)

5/ Behavior d = contact with the odor source. Yes (+), No (-)

TABLE III

EXPERIMENT 1. TALLY SHEET FOR CYDIA CARYANA BEHAVIORAL RESPONSES TO STIMULATION WITH VARIOUS DOSAGES OF Z-9-12:AC IN A FLIGHT TUNNEL

		Moth									
Dose [1]	Behavior	1	2	3	4	5	6	7	8	9	10
0	a [2]	-	-	-	-	-	-	-	-	-	-
	b [3]										
	c [4]										
	d [5]										
0.1 ng	a	-	-	+	-	-	-	-	-	-	-
	b			F							
	c			F							
	d			-							
1.0 ng	a	-	-	-	+	-	-	+	-	-	-
	b				F			F			
	c				F			F			
	d				-			-			
10.0 ng	a	-	+	-	+	-	-	-	-	-	-
	b		F		F						
	c		F		F						
	d		-		-						
100.0 ng	a	+	-	+	+	+	-	-	-	+	+
	b	E		F	G	E				E	E
	c	E		G	E	E				F	G
	d	-		-	-	-				-	-

TABLE III (Continued)

Dose	Behavior	Moth 1	2	3	4	5	6	7	8	9	10
1.0 µg	a	+	+	-	-	-	+	+	+	-	+
	b	E	G				G	G	E		G
	c	F	E				G	G	G		G
	d	-	-				-	-	-		-
10.0 µg	a	+	-	+	+	+	+	-	-	+	-
	b	E		E	E	E	G			G	
	c	G		G	E	G	G			F	
	d	-		-	-	-	-			-	
100.0 µg	a	-	+	+	+	-	-	+	+	+	-
	b		E	G	E			E	E	E	
	c		G	G	G			G	E	G	
	d		-	-	-			-	-	-	

1/ Dose represents the concentration impregnated into red rubber septa.

2/ Behavior a = upwind flight within the characterized plume. Responders (+), Non-responders (-).

3/ Behavior b = upwind plume flight with repeated approaches, retreats and reapproaches. Fair (F), Good (G), Excellent (E).

4/ Behavior c = hovering in close proximity downwind of the odor source during plume flight. None (N), Fair (F), Good (G), Excellent (E).

5/ Behavior d = contact with the odor source. Yes (+), No (-).

TABLE IV

EXPERIMENT 1. TALLY SHEET FOR CYDIA CARYANA BEHAVIORAL RESPONSES TO STIMULATION WITH VARIOUS DOSAGES OF E-10-12:AC IN A FLIGHT TUNNEL

		Moth									
Dose [1]	Behavior	1	2	3	4	5	6	7	8	9	10
0	a [2]	-	-	-	-	-	-	-	-	-	-
	b [3]										
	c [4]										
	d [5]										
0.1 ng	a	-	-	-	-	-	-	-	-	-	-
	b										
	c										
	d										
1.0 ng	a	-	-	-	-	-	-	-	-	-	-
	b										
	c										
	d										
10.0 ng	a	-	-	-	-	-	-	-	-	-	
	b										
	c										
	d										
100.0 ng	a	-	-	-	-	-	-	-	-	-	-
	b										
	c										
	d										

TABLE IV (Continued)

Dose	Behavior	Moth 1	2	3	4	5	6	7	8	9	10
1.0 µg	a	-	-	-	-	-	-	-	-	-	
	b										
	c										
	d										
10.0 µg	a	-	-	-	-	-	-	-	-	-	-
	b										
	c										
	d										
100.0 µg	a	+	-	-	-	+	+	-	-	-	-
	b	F				F	F				
	c	F				N	F				
	d	-				-	-				

1/ Dose represents the concentration impregnated into red rubber septa.

2/ Behavior a = upwind flight within the characterized plume. Responders (+), Non-responders (-).

3/ Behavior b = upwind plume flight with repeated approaches, retreats and reapproaches. Fair (F), Good (G), Excellent (E).

4/ Behavior c = hovering in close proximity downwind of the odor source during plume flight. None (N), Fair (F), Good (G), Excellent (E).

5/ Behavior d = contact with the odor source. Yes (+), No (-).

TABLE V

EXPERIMENT 1. TALLY SHEET FOR CYDIA CARYANA BEHAVIORAL RESPONSES TO STIMULATION WITH VARIOUS DOSAGES OF Z-12-14:OH IN A FLIGHT TUNNEL

		Moth									
Dose[1]	Behavior	1	2	3	4	5	6	7	8	9	10
0	a[2]	-	-	-	-	-	-	-	-	-	-
	b[3]										
	c[4]										
	d[5]										
0.1 ng	a	-	-	-	-	-	-	-	-	-	-
	b										
	c										
	d										
1.0 ng	a	-	-	+[6]	-	-	+[6]	+[6]	-	-	-
	b			F			F	F			
	c			F			F	F			
	d			-			-	-			
10.0 ng	a	-	-	-	-	-	-	-	-	+[6]	-
	b									F	
	c									N	
	d									-	
100.0 ng	a	-	+[6]	-	-	-	-	-	-	-	-
	b		F								
	c		N								
	d		-								

TABLE V (Continued)

Dose	Behavior	Moth 1	2	3	4	5	6	7	8	9	10
1.0 µg	a	- 6/	- 6/	- 6/	- 6/	-	- 6/	- 6/	- 6/	-	-
	b										
	c										
	d										
10.0 µg	a	-	- 6/	- 6/	- 6/	- 6/	- 6/	-	-	- 6/	-
	b										
	c										
	d										
100.0 µg	a	-	- 6/	- 6/	- 6/	- 6/	- 6/	-	-	-	-
	b										
	c										
	d										

1/ Dose represents concentration impregnated into red rubber septa.

2/ Behavior a = upwind flight within the characterized plume. Responders (+), Non-responders (-).

3/ Behavior b = upwind plume flight with repeated approaches, retreats and reapproaches. Fair (F), Good (G), Excellent (E).

4/ Behavior c = hovering in close proximity downwind of odor source during plume flight. None (N), Fair (F), Good (G), Excellent (E).

5/ Behavior d = contact with the odor source. Yes (+), No (-).

6/ When stimulated with dosages greater than or equal to 1.0 ng, C. caryana male moth behavior deviated by appearing to be erratic and excited, particularly in close proximity of the odor source. Thus, behavior was difficult to score. Additionally, responding moths (1.0, 10.0, 100.0 ng stimulus intensities) often landed directly upwind of the odor source.

TABLE VI

EXPERIMENT 1. TALLY SHEET FOR CYDIA CARYANA BEHAVIORAL RESPONSES TO STIMULATION WITH VARIOUS DOSAGES OF EE-8,10-12:AC IN A FLIGHT TUNNEL

		Moth									
Dose[1]	Behavior	1	2	3	4	5	6	7	8	9	10
0	a[2]	-	-	-	-	-	-	-	-	-	-
	b[3]										
	c[4]										
	d[5]										
0.1 ng	a	-	-	+	-	-	+	-	-	-	-
	b			F			F				
	c			G			G				
	d			-			-				
1.0 ng	a	-	+	-	-	+	-	-	-	+	-
	b		F			F				F	
	c		G			G				G	
	d		-			-				-	
10.0 ng	a	-	+	+	-	+	-	+	-	+	+
	b		G	E		E		E		E	G
	c		F	E		G		E		E	E
	d		-	-		-		-		-	-
100.0 ng	a	+	-	+	-	+	+	-	+	-	+
	b	G		E		E	G		G		E
	c	E		E		E	E		G		E
	d	-		-		-	-		-		-

TABLE VI (Continued)

Dose	Behavior	Moth 1	2	3	4	5	6	7	8	9	10
1.0 µg	a	+	+	+	+	-	-	+	-	+	-
	b	E	E	G	E			E		E	
	c	E	E	G	G			E		E	
	d	-	-	-	-			-		-	
10.0 µg	a	+	+	+	-	+	+	-	+	-	+
	b	E	G	E		E	E		G		E
	c	E	E	E		E	E		E		G
	d	-	-	-		-	-		-		-
100.0 µg	a	+	-	-	-	+	+	+	+	+	+
	b	E				G	G	E	E	G	E
	c	E				G	G	E	E	E	G
	d	-				-	-	-	-	-	-

1/ Dose represents the concentration impregnated into red rubber septa.

2/ Behavior a = upwind flight within the characterized plume. Responders (+), Non-responders (-).

3/ Behavior b = upwind plume flight with repeated approaches, retreats and reapproaches. Fair (F), Good (G), Excellent (E).

4/ Behavior c = hovering in close proximity downwind of the odor source during plume flight. None (N), Fair (F), Good (G), Excellent (E).

5/ Behavior d = contact with the odor source. Yes (+), No (-).

TABLE VII

EXPERIMENT 2. COMPARISON AMONG RESPONSES TO VARIOUS RATIOS OF A BINARY MIXTURE OF Z-9-12:AC AND E,E-8,10-12:AC (TOTAL DOSE OF 10 µg)

Ratio	Dose		Behavior			
	Z-9-12:AC	EE-8,10-12:AC	a [1]	b [2]	c [3]	d [4]
19 : 1	9.5	0.5	30	F	G	-
9 : 1	9.0	1.0	40	G	G	-
7 : 3	7.0	3.0	50	G	E	-
1 : 1	5.0	5.0	50	G	G	-
3 : 7	3.0	7.0	50	G	E	-
1 : 9	1.0	9.0	40	G	E	-
1 : 19	0.5	9.5	50	G	E	-

[1] Behavior a = percent moths displaying upwind anemotactic flight within the characterized plume.

[2] Behavior b = upwind plume flight with repeated approaches, retreats, and reapproaches. Fair (F), Good (G), Excellent (E).

[3] Behavior c = hovering in close proximity downwind of the odor source during plume flight. None (N), Fair (F), Good (G), Excellent (E).

[4] Behavior d = contact with the odor source. Yes (+), N (-).

TABLE VIII

EXPERIMENT 2. TALLY SHEET FOR CYDIA CARYANA BEHAVIORAL RESPONSES TO STIMULATION WITH VARIOUS Z-9-12:AC : EE-8,10-12:AC RATIOS (10.0 µg) IN A FLIGHT TUNNEL

		Moth									
Ratio	Behavior	1	2	3	4	5	6	7	8	9	10
19 : 1	a [1]	-	+	-	-	-	+	+	-	-	-
	b [2]			F				F	F		
	c [3]			F				G	G		
	d [4]			-				-	-		
9 : 1	a	+	-	+	-	-	-	+	+	-	-
	b	G		F				G	G		
	c	G		G				G	G		
	d	-		-				-	-		
7 : 3	a	+	-	+	+	-	-	-	-	+	+
	b	G		G	G					E	G
	c	E		E	E					E	G
	d	-		-	-					-	-
1 : 1	a	-	+	+	-	+	-	-	+	+	-
	b		G	G		F			F	G	
	c		G	F		F			G	G	
	d		-	-		-			-	-	
3 : 7	a	-	+	+	+	+	-	-	+	-	-
	b		G	G	G	F			G		
	c		E	E	E	G			E		
	d	-	-	-	-	-			-		

TABLE VIII (Continued)

Ratio	Behavior	Moth 1	2	3	4	5	6	7	8	9	10
1 : 9	a	+	-	-	-	-	+	-	-	+	+
	b	E					G			G	G
	c	E					G			E	E
	d	-					-			-	-
1 : 19	a	+	-	+	-	-	-	-	+	+	+
	b	G		E					G	G	G
	c	E		E					E	G	G
	d	-		-					-	-	-

1/ Behavior a = upwind flight within the characterized plume. Responders (+), Non-responders (-).

2/ Behavior b = upwind plume flight with repeated approaches, retreats and reapproaches. Fair (F), Good (G), Excellent (E).

3/ Behavior c = hovering in close proximity downwind of the odor source during plume flight. None (N), Fair (F), Good (G), Excellent (E).

4/ Behavior d = contact with the odor source. Yes (+), No (-).

TABLE IX

EXPERIMENT 3. COMPARISON AMONG RESPONSES TO VARIOUS DOSAGES OF A 7 : 3 BINARY MIXTURE OF Z-9-12:AC : EE-8,10-12:AC

Dosage (ng)	Behavior			
	a [1]	b [2]	c [3]	d [4]
0.1	10	F	G	-
1.0	20	F	G	-
10.0	30	G	G	-
100.0	60	G	G	-
1,000.0	60	G	G	-
10,000.0	60	G	G	-
100,000.0	70	G	G	-

[1] Behavior a = upwind flight within the characterized plume. Responders (+), Non-responders (-).

[2] Behavior b = upwind plume flight with repeated approaches, retreats and reapproaches. Fair (F), Good (G), Excellent (E).

[3] Behavior c = hovering in close proximity downwind of the odor source during plume flight. None (N), Fair (F), Good (G), Excellent (E).

[4] Behavior d = contact with the odor source. Yes (+), No (-).

TABLE X

EXPERIMENT 3. TALLY SHEET FOR CYDIA CARYANA BEHAVIORAL RESPONSES TO STIMULATION WITH VARIOUS DOSAGES OF A 7 : 3, Z-9-12:AC : EE-8,10-12:AC BINARY MIXTURE IN A FLIGHT TUNNEL

		Moth									
Dose[1]	Behavior	1	2	3	4	5	6	7	8	9	10
0.1 ng	a[2]	-	-	+	-	-	-	-	-	-	-
	b[3]				F						
	c[4]				G						
	d[5]				-						
1.0 ng	a	-	-	-	-	-	-	-	+	+	-
	b								F	F	
	c								G	G	
	d								-	-	
10.0 ng	a	-	+	-	-	-	+	+	-	-	-
	b		G				F	G			
	c		G				F	G			
	d		-				-	-			
100.0 ng	a	+	+	+	+	-	-	+	+	-	-
	b	G	F	G	E			G	G		
	c	G	N	G	G			G	G		
	d	-	-	-	-			-	-		

TABLE X (Continued)

Dose	Behavior	Moth 1	2	3	4	5	6	7	8	9	10
1.0 µg	a	+	-	-	+	-	-	+	+	+	-
	b	G			F			G	G	G	
	c	G			G			G	G	G	
	d	-			-			-	-	-	
10.0 µg	a	+	+	+	-	-	+	+	+	-	+
	b	G	G	G			F	G	G		G
	c	G	G	F			N	G	G		G
	d	-	-	-			-	-	-		-
100.0 µg	a	-	+	-	+	+	+	+	-	+	+
	b		F		G	G	G	F		G	G
	c		G		G	G	G	G		F	N
	d		-		-	-	-	-		-	-

1/ Dose represents the concentration impregnated into red rubber septa.

2/ Behavior a = upwind flight within the characterized plume. Responders (+), Non-responders (-).

3/ Behavior b = upwind plume flight with repeated approaches, retreats and reapproaches. Fair (F), Good (G), Excellent (E).

4/ Behavior c = hovering in close proximity downwind of the odor source during plume flight. None (N), Fair (F), Good (G), Excellent (E).

5/ Behavior d = contact with the odor source. Yes (+), No (-).

TABLE XI

CALCULATION OF EVAPORATION RATES FROM SEPTA IN THE FLIGHT TUNNEL

Compound	E-8-12:AC	Z-8-12:AC	E-9-12:AC	Z-9-12:AC	E-10-12:AC	Z-10-12:AC	Z-12-14:OH	E,Z- or E,E-8,10-12:AC
$t_{\frac{1}{2}}$ (days) [1]	36.1	36.8	38.4	44.8	38.4	38.4	110.0	46.5
Dose [2]	Evaporation Rates (log_{10} ng/hr) [3]							
0.1 ng	- 4.10	- 4.10	- 4.12	- 4.19	- 4.12	- 4.12	- 4.58	- 4.21
1.0 ng	- 3.10	- 3.10	- 3.12	- 3.19	- 3.12	- 3.12	- 3.58	- 3.21
10.0 ng	- 2.10	- 2.10	- 2.12	- 2.19	- 2.12	- 2.12	- 2.58	- 2.21
100.0 ng	- 1.10	- 1.10	- 1.12	- 1.19	- 1.12	- 1.12	- 1.58	- 1.21
1.0 µg	- 0.10	- 0.10	- 0.12	- 0.19	- 0.12	- 0.12	- 0.58	- 0.21
10.0 µg	0.90	0.90	0.88	0.81	0.88	0.88	0.42	0.79
100.0 µg	1.90	1.90	1.88	1.81	1.88	1.88	1.42	1.79

[1] $t_{\frac{1}{2}}$--Half lives (Butler and McDonough 1979, 1981; McDonough and Butler, 1983).

[2] Concentration impregnated into septa.

[3] Evaporation rate (ng/hr) = $Mt_{\frac{1}{2}}^{-1}$ In2 ; where M = dose impregnated into the septa.

APPENDIX C

CHAPTER IV:

CHEMICAL AND ELECTROANTENNOGRAPHIC ANALYSIS OF FEMALE *CYDIA CARYANA* SEX PHEROMONE GLAND EXTRACT

TABLE I

FRACTIONATION OF CYDIA CARYANA (94 F.E.) SEX PHEROMONE GLAND EXTRACT ON COLUMN A (SE-30) AND SUBSEQUENT EAG ANALYSIS OF COLLECTED FRACTIONS (70 F.E.)

Retention Time Interval		EAG Analysis		Standards	
Fraction	(min.)	Mean %	Response (S.E.)	Compound	t_R (min.)
Solvent blank		0.39	(0.68)		
1	0.0- 6.0	2.52	(1.74)		
2	6.0-13.5	3.35	(1.78)	10:AC	8.0
3	13.5-16.4	39.82	(18.49)	12:AC	15.0
4	16.4-19.0	49.43	(17.80)	EE-8,10-12:AC	16.5
				Z-12-14:OH	17.3
5	19.0-24.0	1.40	(1.53)		
6	24.0-30.0	1.32	(2.28)	16:AC	26.3

TABLE II

FRACTIONATION OF CYDIA CARYANA (39 F.E.) SEX PHEROMONE GLAND EXTRACT ON COLUMN B (ULTRABOND CARBOWAX 20-M) AND SUBSEQUENT EAG ANALYSIS OF COLLECTED FRACTIONS (30 F.E.)

Retention Time Interval		EAG Analysis		Standards	
Fraction	(min.)	Mean % Response	(S.E.)	Compound	t_R (min.)
1	0.0- 3.5	2.26	(2.01)		
2	3.5- 7.0	17.12	(3.01)	12:AC	5.00
				Z-9-12:AC	5.50
3	7.0- 9.0	2.26	(2.01)		
4	9.0-13.0	28.27	(6.29)	EE-8,10-12:AC	9.90
5	13.0-17.5	1.29	(1.12)	14:AC	15.25
6	17.5-23.0	7.75	(5.04)		
				Z-12-14:OH	19.25
7	23.0-30.0	1.28	(2.00)		

APPENDIX D

CHAPTER V:

FIELD EVALUATION OF POTENTIAL *CYDIA CARYANA* SEX PHEROMONE COMPONENTS

TABLE I

1984 FIELD TRAPPING EXPERIMENT. LIGHT TRAP CATCHES OF MALE *CYDIA CARYANA* [1/]

Date	Number Nights	Total Number Moths Captured		
		Rep 1	Rep 2	Rep 3
Aug. 13	2	0	0	0
Aug. 15	2	0	0	0
Aug. 19	2	3	2	1
Aug. 25	2	6	4	2
Aug. 28	3	11	4	29
Sept. 4	3	54	10	3
Sept. 12	3	53	5	3
Sept. 20	3	45	6	6
Oct. 3	3	49	43	0
Oct. 19	3	17	0	0

[1/] Average number moths captured per trap per night was 4.6 moths, ranging from 0 to 18 moths.

TABLE II

1985 FIELD TRAPPING EXPERIMENT. TOTAL (28 DAYS) TRAP CATCHES OF MALE *CYDIA CARYANA* IN PHEROCON 1C WING TRAPS CONTAINING SYNTHETIC CHEMICALS

Dose (µg)	Total Number Moths Captured					
	Z-8-12:AC			E,E-8,10-12:AC		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
0	0	0	0	0	0	0
10	1	2	1	33	6	17
30	0	1	0	82	7	17
100	3	0	1	26	17	31
300	8	0	0	33	6	9
1,000	2	0	0	35	9	5
3,000	6	0	1	0	1	3

TABLE III

1985 FIELD TRAPPING EXPERIMENT. LIGHT TRAP CATCHES OF MALE *CYDIA CARYANA* [1/]

Day	Number Nights	Total Number Moths Captured		
		Rep 1	Rep 2	Rep 3
0	1	13	12	12
7	1	26	19	18
14	1	7	2	4
21	1	3	2	0
28	1	0	0	0

[1/] Average number moths captured per trap per night was 7.9 moths, ranging from 12 to 26 moths.

2.

VITA

Michael Thomas Smith

Candidate for the Degree of

Doctor of Philosophy

Thesis: CHARACTERIZATION OF THE HICKORY SHUCKWORM, CYDIA CARYANA (LEPIDOPTERA: TORTRICIDAE: OLETHREUTINAE) SEX PHEROMONE: ELECTROPHYSIOLOGICAL, BEHAVIORAL, CHEMICAL AND FIELD ANALYSIS

Major Field: Entomology

Biographical:

Personal Data: Born in New Orleans, Louisiana, February 7, 1954, the son of Edward T. and Bobbye C. Smith, Jr. Married to Billie J. Banker on August 7, 1975. Daughter, Meghan C. Smith, born June 14, 1980.

Education: Graduated from East Jefferson High School, Jefferson, Louisiana, in May, 1972; received a Bachelor of Science degree in Forest Management from Louisiana State University in August, 1976; received a Master of Science degree in Entomology from Louisiana State University in December, 1978; completed requirements for the Doctor of Philosophy degree at Oklahoma State University in December, 1985.

Professional Experience: Graduate Research Assistant, Department of Entomology, Louisiana State University, August, 1976, to December 1978; Research Assistant, Department of Entomology, Kansas State University, April, 1979, to January, 1980; Research Associate, Department of Entomology, University of Missouri (UMC), January, 1980, to December, 1981; Graduate Research Assistant, Department of Entomology, Oklahoma State University, April, 1982, to present.

Professional Organizations: Entomological Society of America; Entomological Society of Canada; International Society of Chemical Ecology; Gamma Sigma Delta; Sigma Xi.