NAVEL ORANGEWORM HOST ATTRACTANTS

Project No. 86-A7 - Navel Orangeworm, Mite and Insect Research (Part 3)

Progress Report
July 1 - December 31, 1986

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Project No. 86-A7 - Navel Orangeworm, Mite and Insect Research
Navel Orangeworm, Carob Moth Pheromones, and Orangeworm Host Attractants

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Personnel/Cooperators: Parts 1&2 - Dr. Jia-Wei Du, Dr. Rod Youngman
Part 3 - Dr. R. A. Van Steenwyk

Objectives: Part 1. Navel Orangeworm Pheromone - Isolate and identify the secondary pheromone components essential for optimal male navel orangeworm attraction. Part 2. Carob Moth Pheromone - Isolate and identify the sex pheromone of the carob moth. Part 3. Navel Orangeworm Attractants - (a) Isolate and identify additional chemical constituents of almonds utilized by NOW females for host finding and oviposition. (b) Develop and test in the field multiple-point source "attracticide" and oviposition disruption formulations for control of NOW.

Interpretive Summary: Part 1. Using a new high-resolution gas chromatography/electroantennogram (GC/EAG) technique, new information on secondary navel orangeworm (NOW) pheromone components has been obtained. Dr. Jia-Wei Du, head of the pheromone chemistry laboratory at Shanghai Institute of Entomology, China, has made significant progress with this technique while working at U.C. Riverside. Part 2. The GC/EAG technique also has been used to successfully isolate active components of the carob moth pheromone. Chemical structures have been partially elucidated by this technique and by retention times on two different capillary GC columns. Analysis by high-resolution GC/mass spectrometry (GC/MS) is underway to prove definitively the structure of the major and minor components. Attempts are now being made to obtain the appropriate synthetic compounds for behavioral testing in the laboratory wind-tunnel. Part 3. New, previously undetected short-chain fatty acids have been isolated in the volatiles from almond oil using a novel solventless technique developed at OARDC/OSU, Wooster, Ohio, and identified via GC/MS at this same facility. Novel controlled-release carriers have been formulated that will allow the broadcast application of behaviorally active almond constituents in the orchard. These formulations: 1) are meant to disrupt host-finding without insecticides, 2) can be used with conventional spray equipment, and 3) appear not to cause leaf-burn. Using a different approach, the "toxic mummy" for control of spring-emerging females, several formulations of acidulated almond oil soapstock plus insecticide were tested this year for their ability to elicit upwind flight and cause mortality in female NOW. Wind-tunnel bioassays demonstrated these formulations to effectively attract gravid NOW females. Preliminary testing in a large field experiment during hull-split (not expected to be the optimal period for using this technique) resulted in no significant reduction in egg deposition on traps. Use of (non-optimal) racemic pydrin in the formulation, due to a lack of sufficient enantiomerically pure pydrin, may have contributed to the lack of a significant effect on oviposition. More tests are planned for this spring's flight of emerging females.

During the past six months, I have collaborated with Dr. Tom Baker on the attainment of the objectives of Parts 1, 2 & 3; however, during this time, most of my attention has focused on the investigation of host odors that are attractive to the NOW. This report will be restricted to this area. Last year's work elucidated an important role for fatty acids in the long-range host-finding of female NOW, and four long-chain fatty acids were identified from purified extracts of crude almond oil and from the frass of NOW larvae feeding on almonds, both shown to be attractive to ovipositing NOW females. Presentation of these long-chain fatty acids in a wind-tunnel bioassay evoked upwind orientation by females, but short-range behaviors were not elicited. On the other hand, almond soapstock, a waste material of oil processing that contains the free fatty acids removed from crude almond oil (CAO), does elicit full activity when treated to regain the volatile form of the fatty acids. One possibility suggested by these results was the presence of additional active constituents. During this funding period, I have sought to: 1) identify any possible "missing components" responsible for short-range NOW female response and 2) develop a formulation using almond odors for disruption of host finding.

Experimental Procedures:

Most of the techniques used this year are the same as in the previous year. In starting up a new laboratory at the Ohio Agricultural Research and Development Center, I was provided with funding for new instrumentation, such as a high performance liquid chromatograph, gas chromatograph, and mass spectrometer. These have been essential for my work with almond odors. One of the main differences in my approach to identifying the active constituents of almond oil this year has been to focus on the analysis of the volatiles emitted, rather than a direct analysis of the oil itself. To accomplish this, I have designed a device that allows for the collection and GC/MS analysis of plant- and insect-derived volatiles without the use of solvents. This device has at its core a specially made wide-bore capillary tube with a thick stationary phase chemically bonded to its interior, and a six-port valve, which directs carrier gas flows. Volatiles from the material to be analyzed are flushed into the capillary trap where they are absorbed by the stationary phase. After sufficient collection time, the capillary trap, which is connected to the GC/MS via the six-port valve, is ballistically heated so as to desorbed the trapped volatiles and allow their automatic injection onto the GC column without the use of solvents. The volatiles are then analyzed by the GC/MS system.

Results and Discussion:

There are a number of advantages in analyzing volatiles as opposed to solvent extracts when identifying constituents that are responsible for long-range responses. First, this method allows for a much cleaner analysis of active materials by elimination of non-volatile components that can obscure GC and MS analyses. Secondly, since long-range response is dependent on the volatiles emitted and their relative composition in the vapor phase, analysis of this phase is more informative than solvent extracts. The importance of this point is easily demonstrated in the present study by comparison of the GC analysis of solvent extracts of CAO (Figure 1) with that of volatiles from this same material (Figure 2). In the extract, the four long-chain fatty acids, palmitic, linoleic, oleic, and stearic, represent >95% of the constituents that elute from the capillary GC column; however, when the

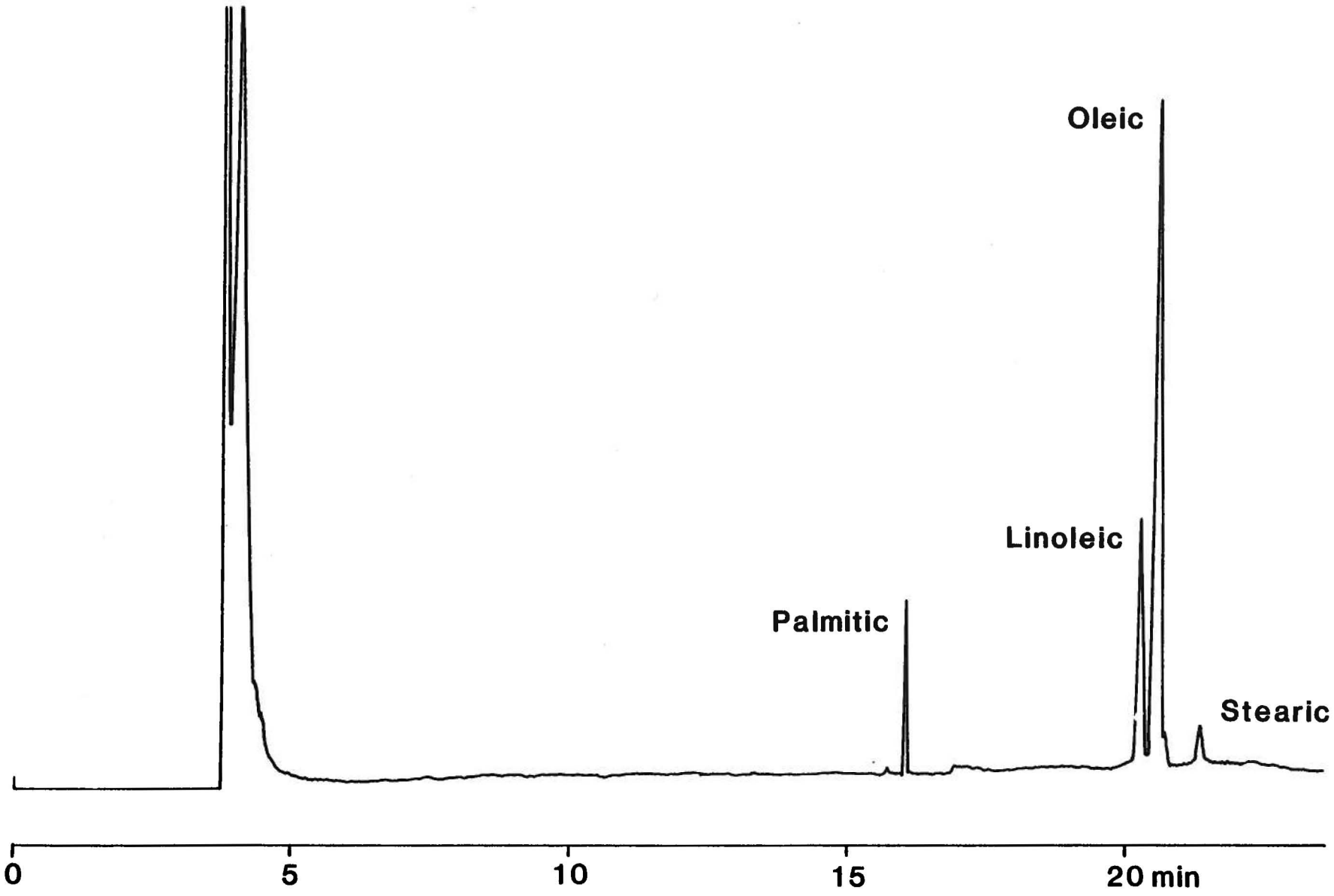

Figure 1. Gas chromatogram of crude almond oil after methylation of fatty acids.

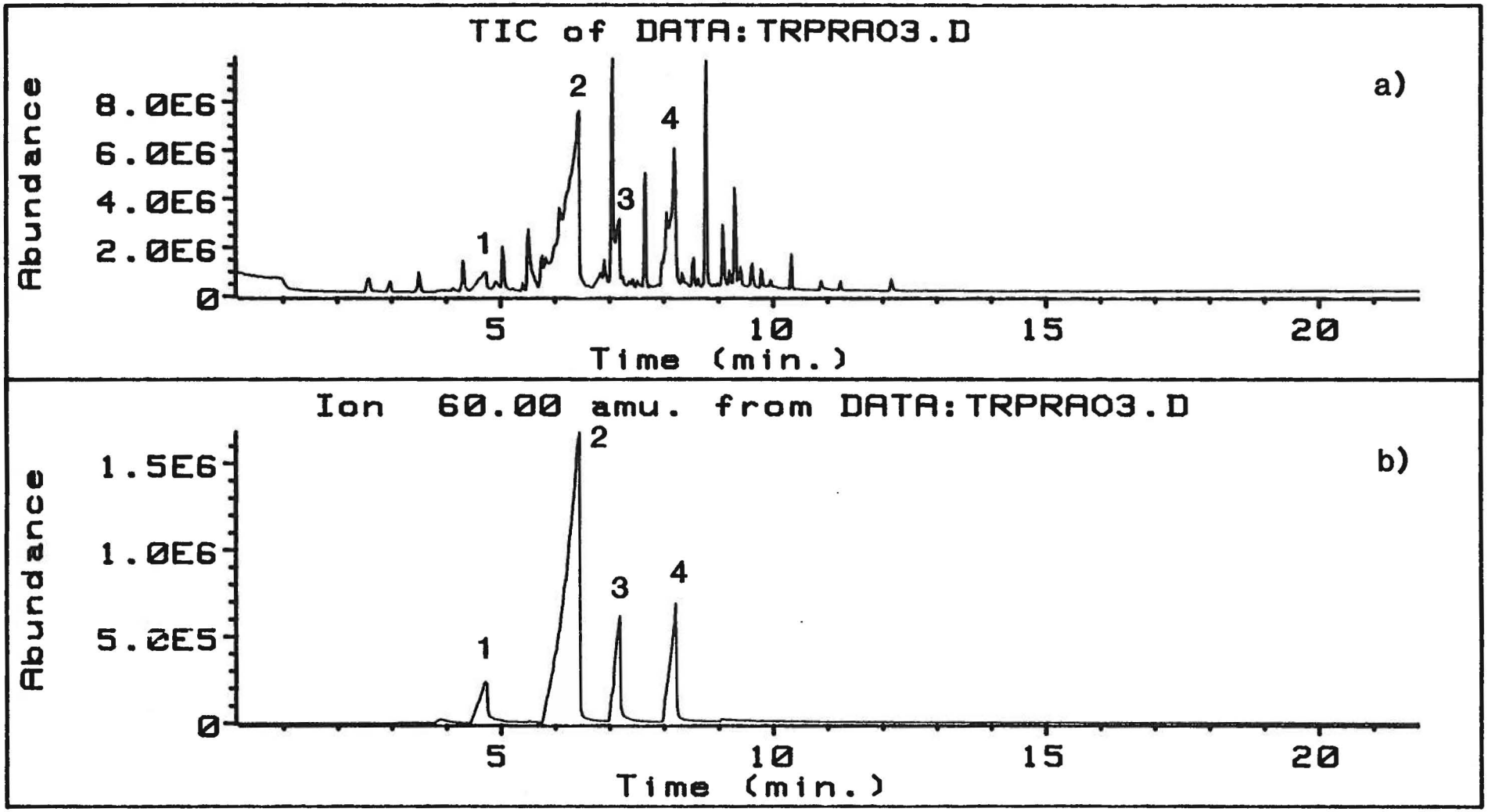

Figure 2. Gas chromatogram of a 30min volatile collection from crude almond oil; a) entire volatile profile, b) mass ion 60 only, to signify saturated fatty acids present in a). 1= Butanoic acid, 2= Hexanoic acid, 3= Heptanoic acid, 4= Octanoic acid.

volatiles that are emitted from this material are analyzed (Figure 2a), many other shorter chain components are apparent. The volatile blend is dominated by four short-chain fatty acids (Figure 2b), which MS analysis has identified as butanoic, hexanoic, heptanoic, and octanoic acids. An analogous situation exists when comparing the two analytical techniques to examine NOW larval frass, as these same short-chain fatty acids are the major constituents of the volatile profile of frass (Figure 3).

The obvious next step is the bioassay of these short-chain fatty acids to measure behavioral activity. Unfortunately, our attempts to do this have been thwarted by a severely reduced response in laboratory females to any of the almond by-products that previously were shown to elicit long-range host-finding. Thus, the percentage of mated females flying uptunnel to 100mg CAO this summer was approximately 10-15%, as compared to 50-60% response in last year's bioassays. This level of response was not sufficient to efficiently compare behavioral response to various treatments, especially since a number of compounds were involved. To try to increase this overall level of response, I engaged in a series of selection and breeding experiments. Females first were tested for their response to CAO. Those that flew 3m upwind to land on the source were captured and then allowed to oviposit on diet in individual rearing jars. The offspring from these females were reared to adulthood and mated with offspring from other females that exhibited the host-finding response. The mated females were then bioassayed and those that showed the entire sequence of odor-source location were again captured and allowed to oviposit on diet. As a result of only two generations of selection, response to CAO is returning to the normal level of response. An interesting and unexpected finding of this selection regime was that the new strain of NOW appears to have a higher level of resistance to *Bacillus thuringiensis*, a pathogen that is the primary limiting agent for rearing high levels of NOW in the laboratory. Thus, the reduced response of females from the laboratory colony may have been due to sublethal effects of *B. thuringiensis* infection. The new selected strain of the NOW is increasing in numbers such that they will soon provide a continuous source of females for bioassay. Thus, measuring the response to the short-chain fatty acids, individually and in various combinations, is forthcoming.

The other area of attention this year has been that of developing a formulation for host attractants that may be sprayed on an orchard for the purpose of multiple-point source "attracticide," or for host-finding disruption. Materials from a number of companies were solicited to act as potential carriers for this formulation. The list of candidates has been narrowed to three, which were chosen because they: 1) provide a system of controlled release where longevity of the active constituents may be greatly enhanced, 2) can be applied using conventional insecticide spray equipment, 3) have already been tested in an agricultural application, and 4) do not appear to cause phytotoxicity, although more extensive testing in almonds is still needed. In addition to the carrier itself, the work on formulation development has also involved working with various additives, such as surfactants, which are necessary for producing an emulsion of the acidulated soapstock, and cross-linkage agents, which modify volatile release rates. At this point, stable formulations have been developed using the acidulated soapstock with all three candidate carriers.

Advances continue to be made in our understanding of the chemistry of host-finding by the NOW, and in our development of the active constituents for use in pest management by the grower. In the laboratory, future work will

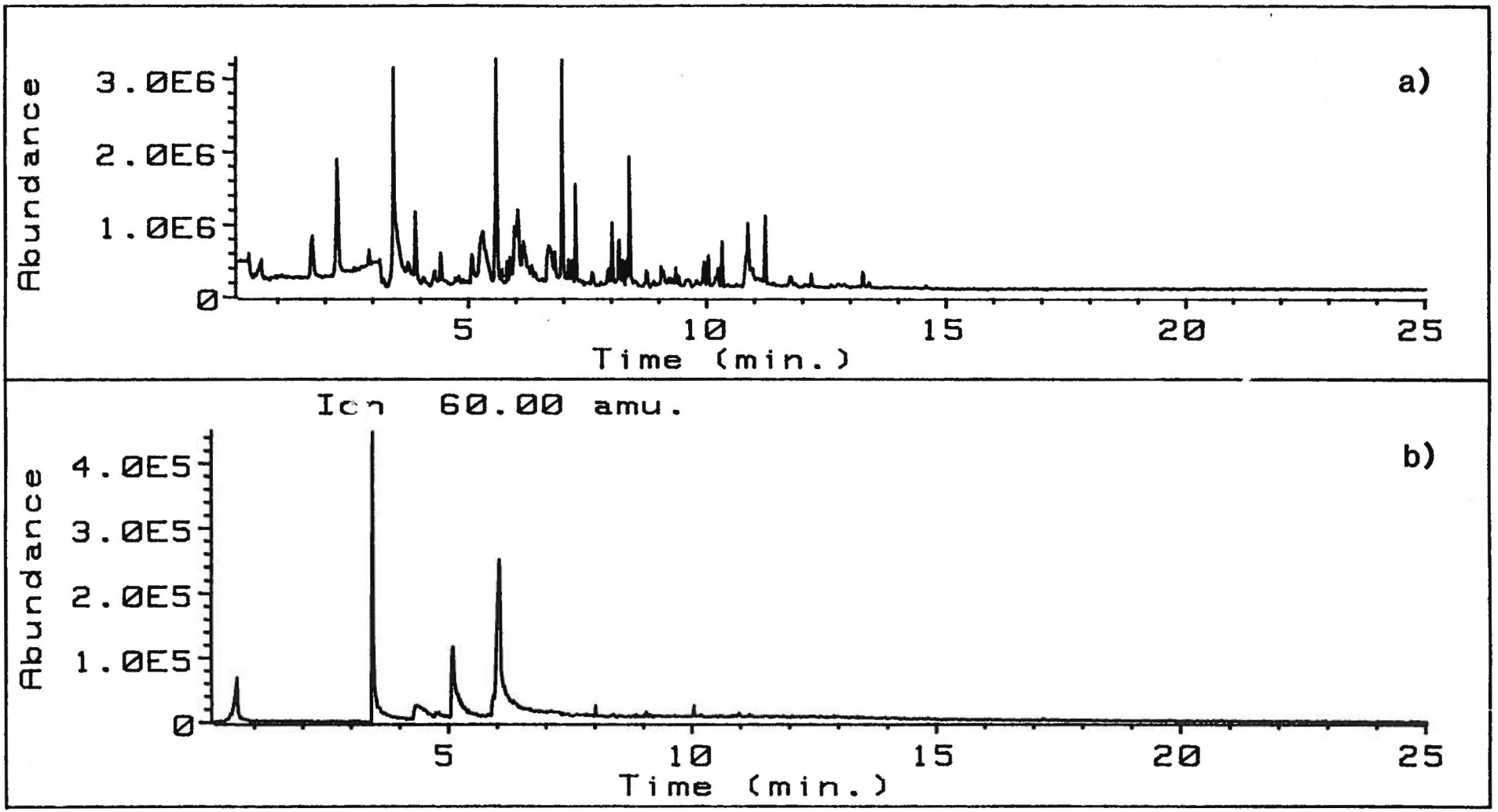

Figure 3. Gas chromatogram of 30min volatile collection from frass of NOW larvae after feeding on almonds; a) entire volatile profile, b) mass ion 60 only, to signify saturated fatty acids present in a).

focus on the wind-tunnel bioassay of female response to the newly identified CAO components. Now that the average response has returned to its previously high level, we will be able to test indivdual components as well as blends of the components in various ratios and release rates. In the field, we will be testing a number of characteristics of the disruption formulations, such as stability after long-term field exposure, compatibility with spray equipment, optimal spray rates, and more extensive testing of plant responses to the applied formulations. Initially, the active material used will be almond soapstock, since I have already demonstrated this material to be a potent attractant; however, I will also be testing other sources of soapstock, e.g., corn, soybean, or safflower, for NOW female attraction. If active, these may act as more reliable and cheaper sources of the attractant. Furthermore, as the chemical identity of the active constituents is elucidated, the economics of synthetic forms will also be investigated.

Publications:

Phelan, P. L. and T. C. Baker. (*in press*). An Attracticide for Control of *Amyelois transitella* (Lepidoptera: Pyralidae) in Almonds. J. Econ. Entomol.

DEPARTMENT OF ANIMAL ECOLOGY ECOLOGY BUILDING UNIVERSITY OF LUND

January 30, 1987

Bob Curtis
Almond Board of California
P.O. Box 15920
Sacramento, CA 95852

Dear Bob,

Enclosed please find a report on our progress on the carob moth and navel orangeworm sex pheromone identification. I apologize for the extreme tardiness of this document. I knew Larry had sent in a report of his progress on the female attractant portion of the grant along with our interpretive summary used at the annual research conference.

We have developed some important new information from Larry's mass spectrum of the major EAG-active component, indicating that our earlier guess at its structure will have to be changed slightly (if the new information holds up). Fortuitously, on my first day here in the lab at the University of Lund, some information on a new pheromone structure from a bark beetle was mentioned, and it led to a discussion on how similar that compound looks in MS to the carob moth's. Now we will do some further examination of the carob moth pheromone using extract from Riverside and their nice system here at Lund. Also Larry is continuing to work on it at OSU, and Dr. Du took 1000 female-equivalents back with him to Shanghai to continue working on it there. So now it really has turned into a circum-global effort!

I hope you are doing well, and that 1987 will be a good year for you and your family.

As always,

Tom

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Progress Report

1986 Research on Carob Moth Sex Pheromone
Isolation and Identification

Work Accomplished in 1986

We continued our successful rearing of carob moth virgin males and females, and also continued to extract pheromone from the pheromone glands of females. We performed intensive gas chromatographic (GC) analysis of this extraction on two different high performance capillary columns. These glands turned out to be particularly messy and difficult to work with. Having unprecedented quantities of extra lipids, we were able to isolate several peaks having electronantennogram (EAG) activity (Figs 1, 2). We developed a new high-resolution combination GC/EAG system, especially for this research on carob moth, and it pinpointed the EAG-active areas on two different columns. We also tested our complete library of 280 different pheromone compounds for activity on carob moth male antennae, and the results are consistent with the GC/EAG results for several suspected compounds. Data from a small set of compounds are presented in Figures 3 and 4. We obtained from colleagues in Sweden some candidate compounds not in our library, and these were also tested for EAG activity, which turned out to be quite high. We obtained preliminary mass spectra on one of the active compounds, in collaboration with Dr. P. L. Phelan of the OARDC/Ohio State University. Flight tunnel tests were initiated, and demonstrated first that we can successfully fly male carob moths to female extract with ca. 60-80% of the males flying 3 m upwind and touching the source. Second, we found the optimum time of male upwind flight to be similar to female calling time, ca. 2 hours before lights-on in the laboratory.

Work Planned for 1987, Winter and Spring

We plan to continue GC fractionation, in order to collect enough purified samples of the EAG-active peaks to get more complete mass spectra from Dr. Phelan at O.S.U. We will also be collecting more material, in order to get mass spectra of the minor peaks having EAG activity. We will begin flight tunnel testing of extract on which micro-chemical reactions have been performed, and will begin testing EAG-active fractions collected from the GC for behavior activity in the wind tunnel. We will continue to rear adult moths and extract pheromone from the glands.

NAVEL ORANGEWORM

Research Performed in 1986

Using high resolution capillary GC chromatography, the ratios of Z,Z-11,13-16:Ald to its corresponding acetate and alcohol were measured. We had not previously definitively isolated the acetate and alcohol in extracts, and now we were able to measure their relative abundance and variation from individual female glands. Data from 20 individual females showed the alcohol to be present at 8.9% of the aldehyde and the acetate to be present at 6.7%. The amounts of alcohol and acetate detected by our improved system were often in the range of 0.1-0.2 ng. Airborne collections from individual females showed that both the alcohol and the acetate are emitted by females along with the aldehyde (Fig. 5). GC/EAG analysis of gland extract showed that the alcohol elicited an EAG response but the acetate response was very low (Fig. 6). The aldehyde evoked very high EAG responses, as expected.

Work Planned for 1987, Winter and Spring

We will test the combination of aldehyde, acetate, and alcohol on male response in the wind tunnel to see if there is any improvement to the blend compared to the aldehyde alone. We realize that the hydrocarbon component(s) as yet unidentified, is the most important missing factor for elevating source location by males, but if we can get some improvement with these compounds now known to be emitted, then this will be a help in elucidating the complete, final blend, including the hydrocarbon. We will continue to extract pheromone from glands, and will send material to P. L. Phelan for mass spectral analysis.

Toxic Running Project

This Spring, working with Bio-Control, Ltd., we are participating in the field testing of the efficacy of their formulation of reconstituted fatty acids plus insecticide for control of emerging females and reduction of subsequent damage. The test will be conducted in China.

Figure 1.

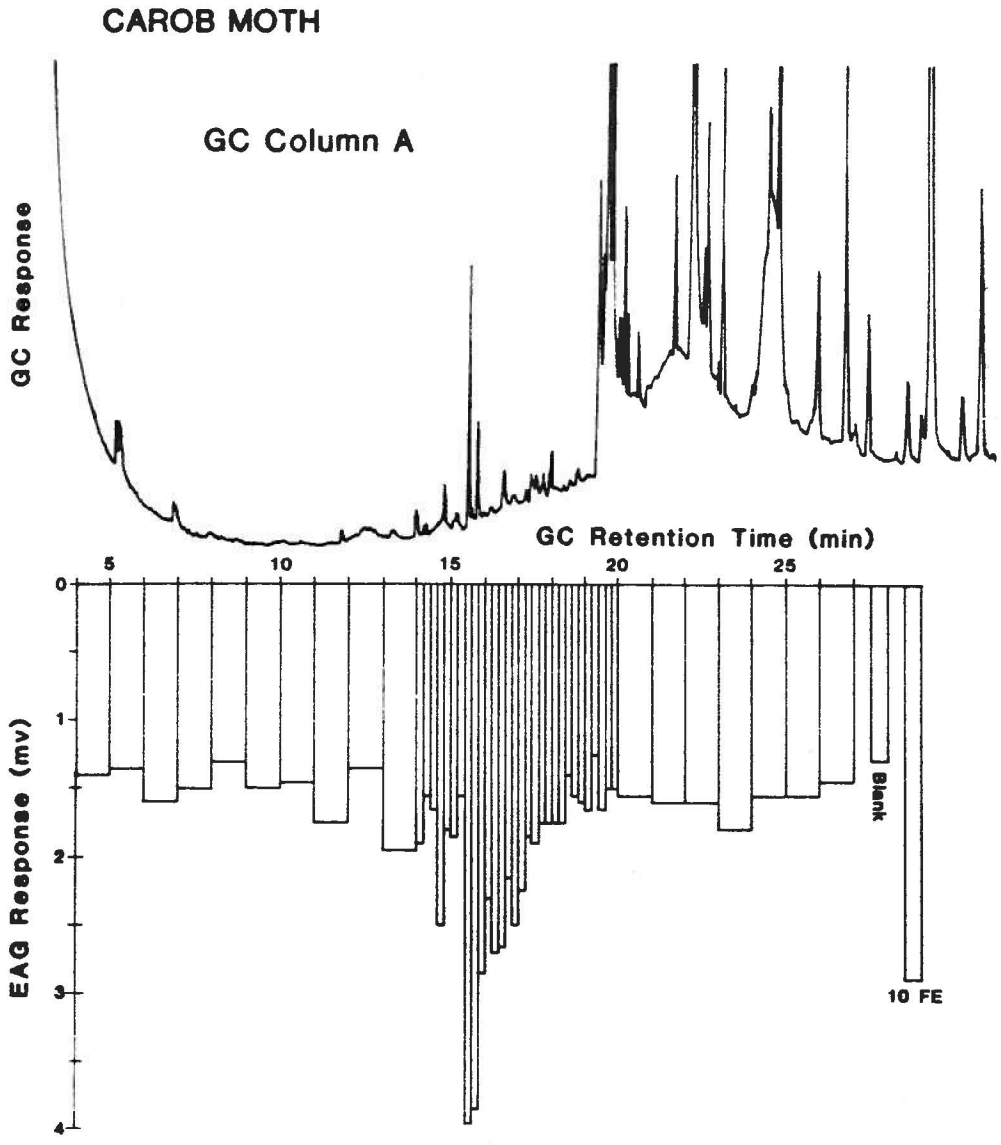

CAROB MOTH
GC Column A
GC Response
GC Retention Time (min)
5
10
15
20
25
0
1
2
3
4
EAG Response (mv)
Blank
10 FE

Figure 2.

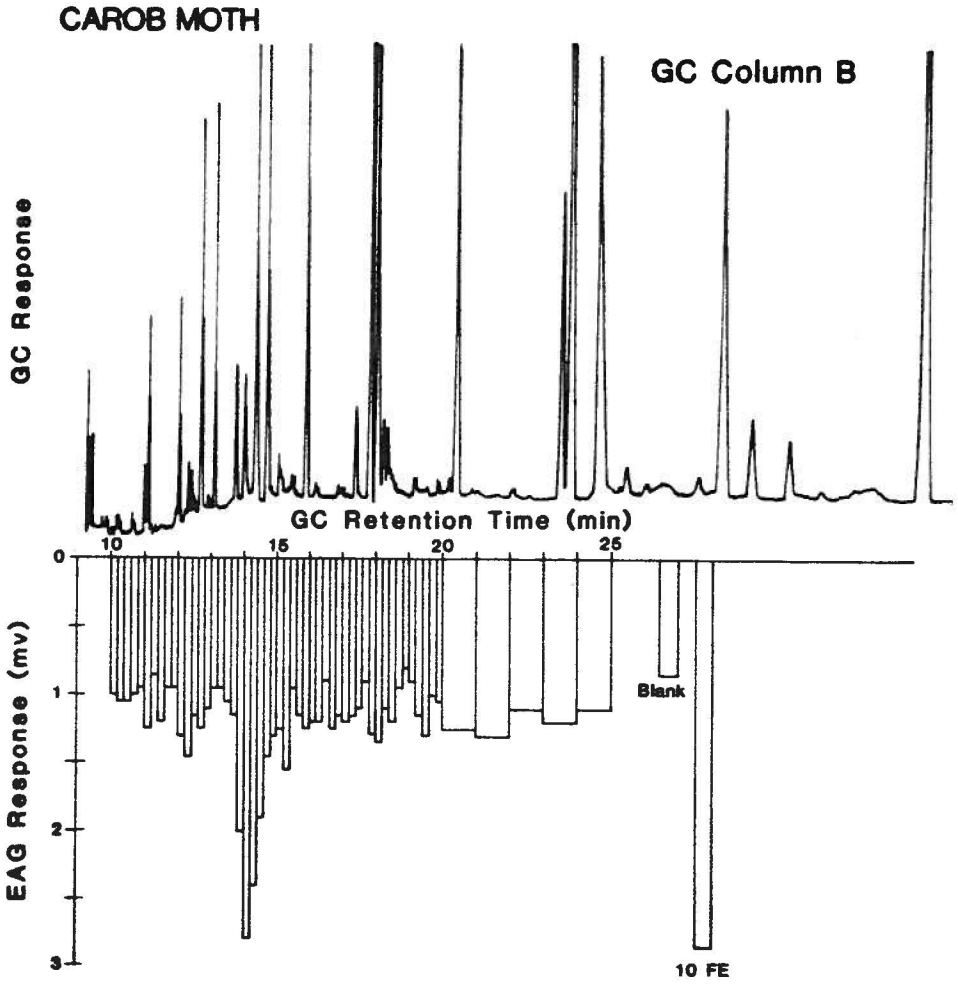
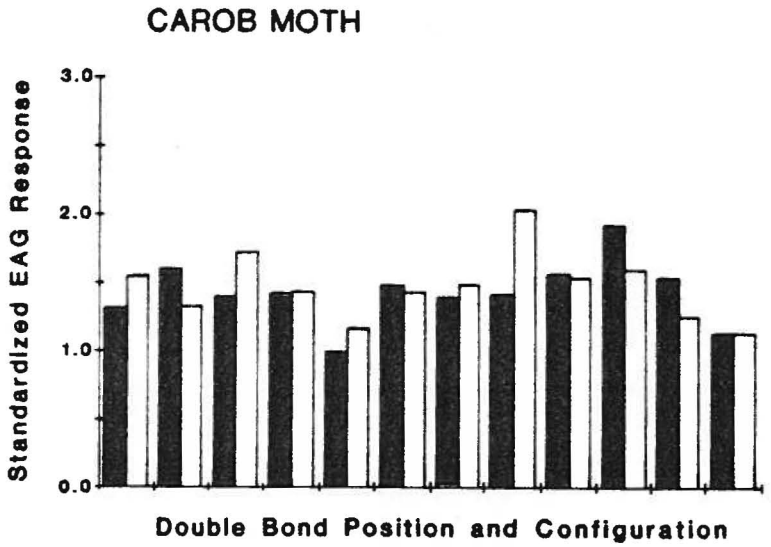

CAROB MOTH
GC Column B
GC Response
GC Retention Time (min)
10
15
20
25
0
1
2
3
EAG Response (mv)
Blank
10 FE

Figure 3.


CAROB MOTH
Standardized EAG Response
3.0
2.0
1.0
0.0
Double Bond Position and Configuration

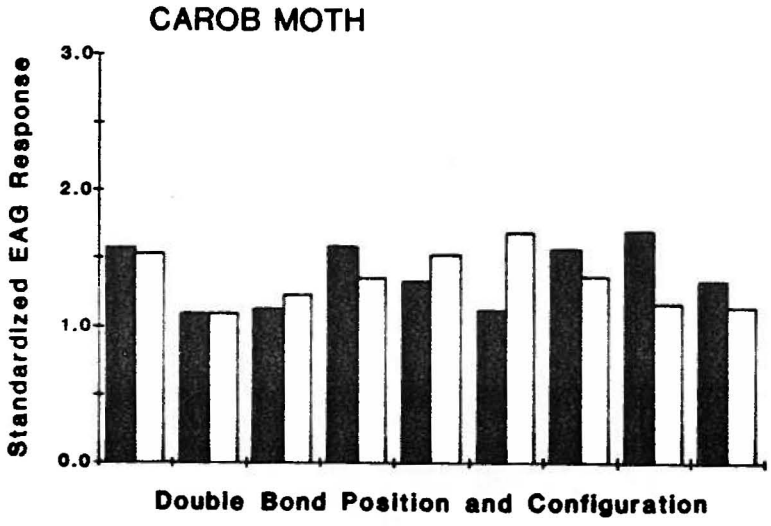
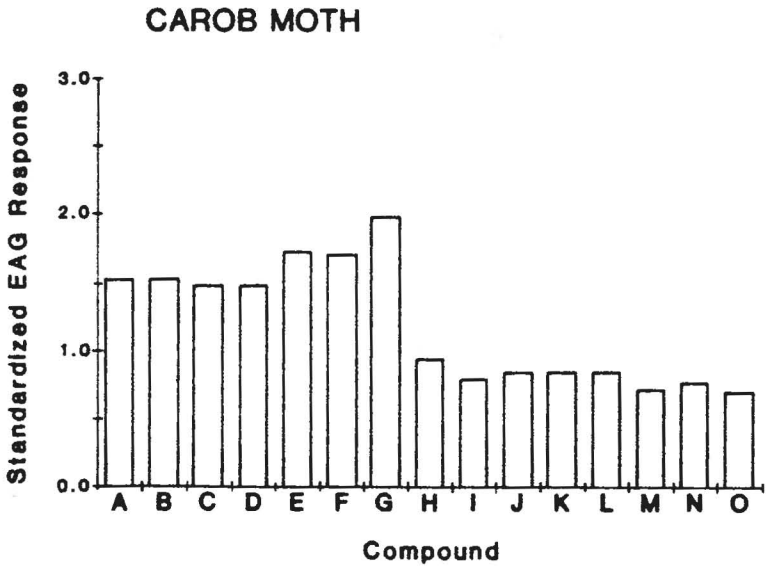

CAROB MOTH
Standardized EAG Response
3.0
2.0
1.0
0.0
Double Bond Position and Configuration

Figure 4.


CAROB MOTH
Standardized EAG Response
3.0
2.0
1.0
0.0
A B C D E F G H I J K L M N O
Compound

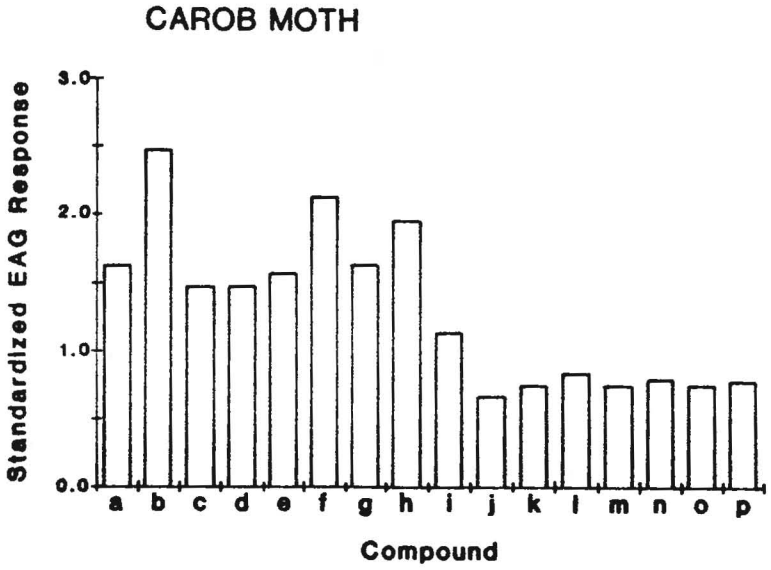

CAROB MOTH
Standardized EAG Response
3.0
2.0
1.0
0.0
a b c d e f g h i j k l m n o p
Compound

Figure 5

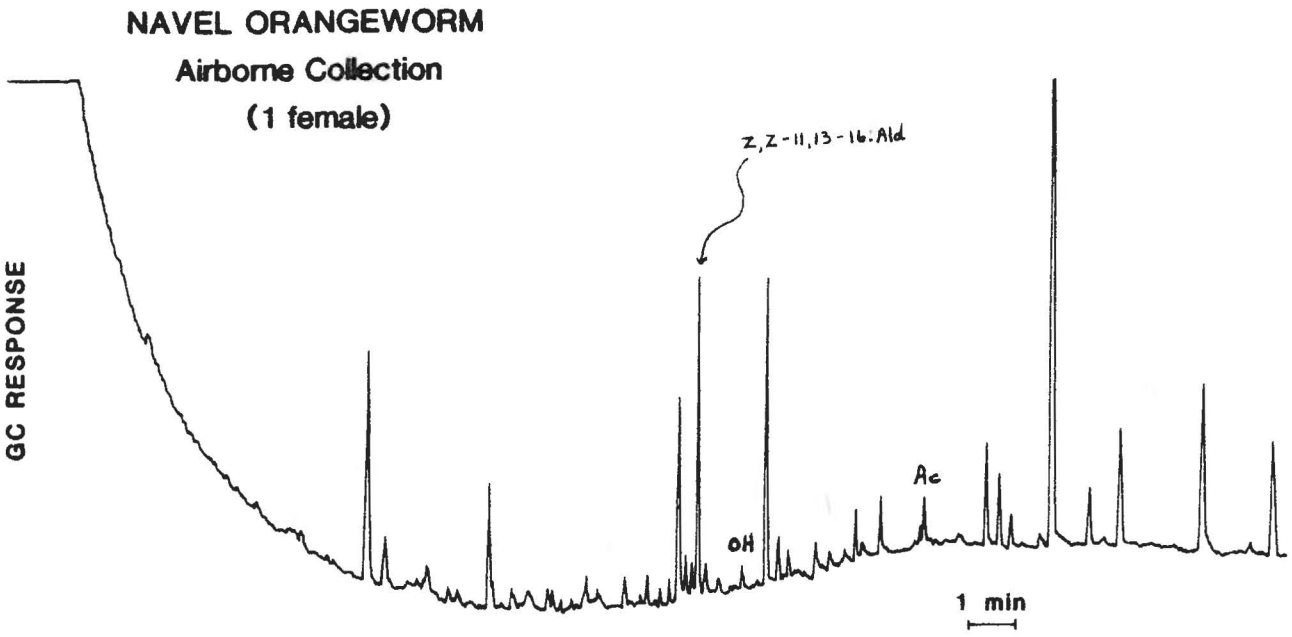


Figure 6.