Project No. 80-B1 (New)

Cooperator:
University of California
Department of Entomology
Riverside, California 92521

Project Leader: Dr. Thomas C. Baker Phone (714) 787-5811

Personnel: Dr. Lyle Gaston, Dr. Martin Barnes, Mr. Curtis Engle

Project: Navel Orangeworm Research
Sex Pheromone Blend Isolation & Identification

Objectives: (1) To isolate and identify the secondary pheromone components essential for optimal male sex pheromone response; (2) through extensive field testing, develop the pheromone blend formulation eliciting optimal trap catch of males.

Progress: The single major pheromone component identified thus far does not attract males very well, and although this component looks promising for mating disruption, a more complete pheromone formulation is needed for monitoring traps. Such traps will be useful in establishing "biofix" points for heat accumulation models such as that of Dr. Martin Barnes and Dr. Keith Oddson, and will be important in monitoring population phenology, especially after hull split. Insecticide spray timing decisions will be made easier.

Plans: Using extensive laboratory bioassay facilities, including two sustained-flight tunnels and an electroantennogram setup, other behaviorally and neurophysiologically "active" fractions in the navel orangeworm sex pheromone extract will be identified. The moths will be reared in the laboratory and collection of female-emitted pheromone will be accomplished by a novel glass-absorption technique developed by Dr. Baker. Chemical separation by gas chromatography and other methods will be performed in Dr. Gaston's laboratory. When new active components are identified, a variety of synthetic blend ratios and dosages will be field tested by Dr. Barnes and Mr. Engle to optimize the blend formulation. Dr. James Coffelt of the USDA/SEA group at Gainesville has generously offered samples of all four geometric isomers of Δ-11, 13-hexadecadienal for use as standards.

Almond Industry Participation $9,973

UNIVERSITY OF CALIFORNIA, RIVERSIDE

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO

SANTA BARBARA • SANTA CRUZ

COLLEGE OF NATURAL AND
AGRICULTURAL SCIENCES
CITRUS RESEARCH CENTER AND
AGRICULTURAL EXPERIMENT STATION
DEPARTMENT OF ENTOMOLOGY
DIVISION OF TOXICOLOGY AND PHYSIOLOGY

RIVERSIDE, CALIFORNIA 92521

Jan. 4, 1981

Dear Dale and Bob,

Please excuse the tardiness of my annual report; I had the flu over Christmas and could not get myself back into the office to work on it. I have enclosed two copies as you requested.

I have some further news. At the Atlanta Entomological Society meetings, Dr. Coffelt renewed his offer of samples for GC standards, and just last Friday I received a package containing not only the aldehydes, but all 4 acetate and alcohol isomers as well. Unfortunately some of the vials were dry, but we may be able to salvage those. He really wants to see this pheromone identified!

Thanks for the delicious dinner at the research conference.

Sincerely,

Tom

Thomas C. Baker

NAVEL ORANGEWORM

SEX PHEROMONE BLEND ISOLATION AND IDENTIFICATION

Progress Report
July 1 - Dec. 31, 1980

T.C. Baker and P.L. Phelan

Division of Toxicology and Physiology
Department of Entomology
University of California, Riverside, CA 92521

OBJECTIVES

The objectives of this project are: 1) to isolate and identify the secondary pheromone components essential for optimal navel orangeworm (NOW) male sex pheromone response; 2) through extensive field testing, develop the pheromone blend formulation eliciting optimal trap catch of NOW males.

SUMMARY

We have made substantial progress during the past 6 months toward meeting the above objective. Despite suffering a decimation of our laboratory NOW colony, we have detected another behaviorally and electrophysiologically active fraction from gas chromatographic (GC) separations. This area was found by electroantennograms (EAG's) of GC fractions on male antennae and by a flying male bioassay in our laboratory wind tunnel to these fractions. The next step is to obtain enough of a highly purified sample of this second active compound to be able to chemically identify, synthesize it, and test it in monitoring traps in the field.

EXPERIMENTAL PROCEDURE

We have broken down the isolation and identification process into six steps:

1) Collect volatiles from female glands or by air extraction
2) Separate compounds into different gas chromatographic fractions
3) Determine neurophysiologically "active" fractions by EAG
4) Determine behaviorally "active" fractions by wind tunnel tests
5) Perform chemical tests on active unknowns to determine chemistry

6) Synthesize compounds and perform wind tunnel and field trapping tests to confirm activity

Our progress has occurred throughout steps 1-4 using the following procedures.

1) Collections of volatiles. Using 4-5-day-old female NOW held on a 16:8hr light:dark photoperiod cycle, we obtained pheromone volatiles by snipping the pheromone glands,located on the abdomen's tip,with micro-scissors and extracting them for 10-15 minutes in redistilled hexane. We also obtained pheromone with our newly-developed air-extraction device (Figure 1) in which nitrogen was passed over forcibly extruded female glands and the pheromone collected on a glass wool plug. The plug was then washed with redistilled hexane.

2) Separation of compounds by gas chromatography. Using packed glass SF-96 and Silar 10-C columns on a Hewlett-Packard model 402 gas chromatograph, we separated the compounds in the extract and found numerous peaks accompanying the major component Z,Z - 11,13 - hexadecadienal (Figure 2). We also found other peaks on our carbowax 20M fused silica capillary column using a Varian 3700 GC. The peaks from SF-96 were re-collected and were the subject of neurophysiological and behavioral assays to determine the potential activities of these compounds on male behavior. Recollection was performed by operating the GC with the flame ionization detector off, and inserting glass capillaries sequentially into the detection port every 2 minutes. Dry ice was placed on the

Figure 1. The airborne pheromone collection device for NOW. Charcoal and glass-wool-filtered N_2 gas at 0.5 ml/sec flows through the lumen and passes over pheromone gland forcibly extruded in a glass tube. A volume of glass wool then adsorbs the pheromone.

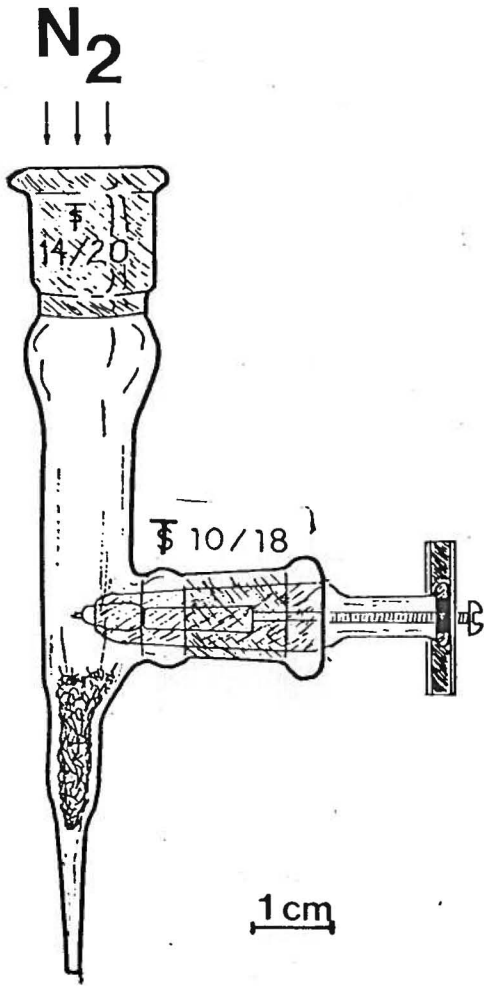


Figure 2. Gas chromatograph of NOW female gland extract on an SF-96 glass column (packed).

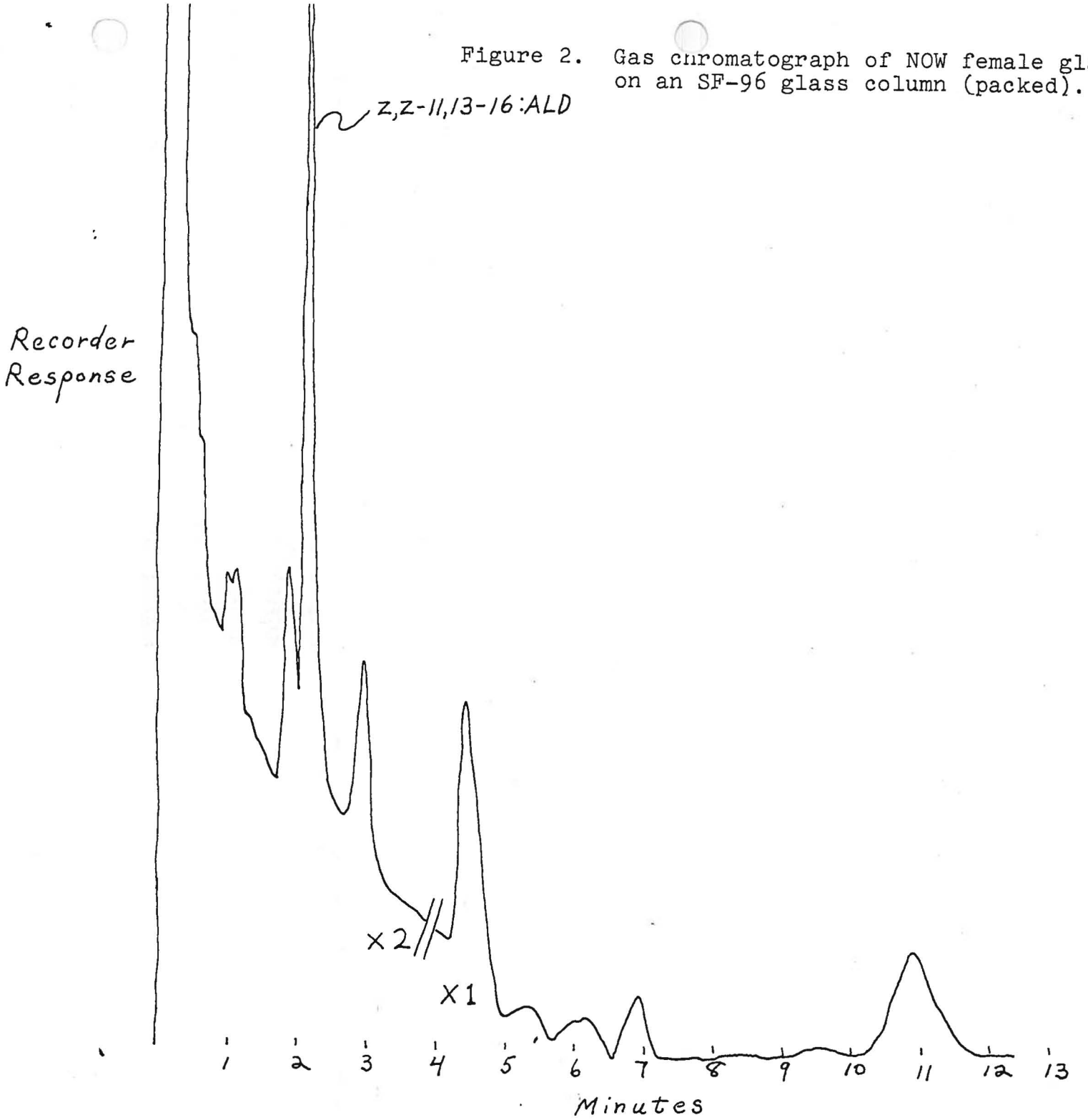
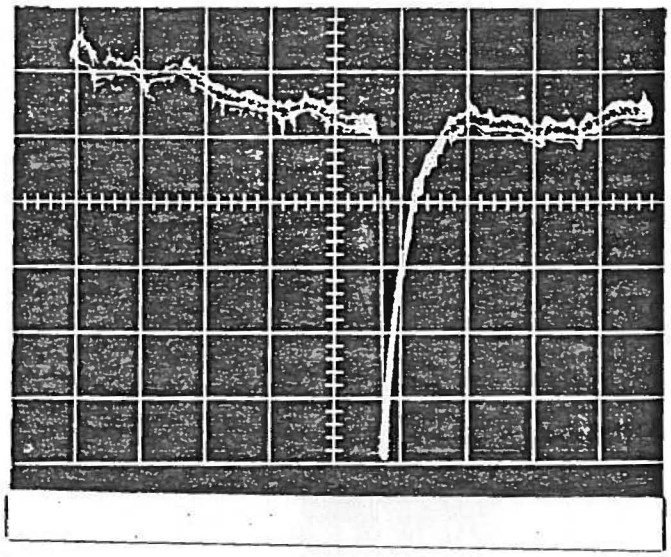


Figure 3. Typical electroantennogram response to the Z,Z-11,13-hexadecadienal fraction from SF-96 column. Each horizontal division is 1 second, each vertical division 1 millivolt.



capillaries to aid adsorption of the compounds onto the internal glass walls of each tube. Tubes were then rinsed with hexane, and the activities of the compounds were tested by behavioral assays. Alternatively, 1 ml of air was puffed through unrinsed tubes for testing of the tubes' contents by electroantennogram (next section).

3) Determination of neurophysiological activity. Electroantennograms (EAG's) to detect active GC fractions were performed as follows. A NOW male's head, complete with antennae, was removed and placed on wax in a dish so that its base contacted a pool of insect Ringer's solution. The terminal antennal segments were excised and contacted by saline solution at the tip of a Pasteur pipet. Silver-silver chloride electrodes contacted the saline in the dish and pipe, and the current changes in the antenna were then fed into,and amplified by, a custom-made amplifier. The responses were displayed on a Tektronix 5113 dual beam storage oscilloscope. Air was puffed through capillary tubes containing GC-collected compounds and into an airstream blowing constantly across the antenna. The sample volatiles thereby impinged on the antennal receptors, creating a depolarization, often amounting to 4 or 5 millivolts for EAG-active samples (Figure 3).

4) Determination of behavioral activity. GC fractions rinsed from the capillary tubes were placed at a concentration of 2 male-equivilents onto a filter paper tab clipped to a cork base. Four-to-five-day-old males held on a 16:8hr light:dark photoperiod regime were acclimated to laboratory wind tunnel conditions and tested

during the last hour of darkness. Tunnel conditions were a wind velocity of approx. 0.5 meters/sec., 24° C, 30-70% R.H., and lessthan 0.3 lux light intensity. After acclimation, males were placed into individual small screen cages and exposed, one at a time, to 2 male-equivilent GC fractions or combinations of fractions on the filter paper. Each paper was placed 3 meters upwind of the males in the tunnel on a 15cm-high platform. Males were observed for the following behaviors: 1) wing fanning in the release cage; 2) taking flight; 3) stationary flight (often a pheromone-mediated behavior preceeding upwind flight); 4) upwind flight; and 5) touching or landing on the filter paper.

RESULTS

We extracted volatile chemicals, including the identified Z,Z-11,13-hexadecadienal from excised female glands as well as from females' airborne emissions. On various GC columns including SF-96 there were consistently 4 or 5 peaks in addition to the major component (Figure 2). The next step in the isolation proces became to collect the GC fractions and test them for EAG activity. Fractions containing no visible peaks were also tested because many times active components may be present in quantities too small to see by GC. The EAG results (Table 1) indicated an EAG-active fraction (fraction A) in addition to the major component (fraction B).

In the course of our GC fractionations we discovered that

Table 1. Electroantennogram responses to gas chromatographic fractions collected from the SF-96 packed column.

Fraction	EAG Amplitude(mv)
A	2.3
B	3.7
C	1.2
D	0.8
E	1.3
F	0.5
G	0.6
H	1.3
I	1.0
J	0.6
K	0.2

isomerization of the Z,Z isomer to the other three isomers (Z,E, E,Z and E,E) was occurring under the conditions of our SF-96 collections. Beginning with about 97% pure Z,Z (Albany International) (as determined on Carbowax 20M capillary column), we injected it onto SF-96 and collected it by dry ice - capillary. The tube was then rinsed and this sample then tested again on the carbowax column. Usually the sample was now only 60-70% pure Z,Z isomer, the rest made up of the other 3 isomers. This isomerization may have affected our wind tunnel assays (see below), and we are currently trying several procedures to overcome this situation.

Several initial wind tunnel behavioral assay conditions were tried, and we found that optimal male upwind flight to the source occurred in the last hour of darkness under less than full moonlight intensity at 24° C. An initial experiment compairing crude female gland extract with the single synthetic component demonstrated the minimal behavioral activity evoked by the Z,Z isomer alone at 1 mg on a rubber septum (Table 2).

An extensive experiment with GC-collected fractions indicated that fraction A contained at least one compound, that, when blended with the Z,Z hexadecadienal fraction (fraction B), resulted in increased behavioral response over B alone (Table 3). However, all responses were relatively lower than to crude extract, and the GC-induced isomerization in these recombined fractions may have contributed to this response reduction.

Table 2. Wind tunnel attraction comparison between female gland extract and single synthetic component. N = 40 for each treatment.

Treatment	% Males Sitting	% Males Taking Flight	% Stationary Flight	% Flying Upwind	% Reaching Source
1 Female-equivalent of Gland Extract	26%	72%	55%	43%	35%
1 milligram Z,Z-11,13-16:ALD	85%	15%	0%	0%	0%

Table 3. Wind tunnel comparison between SF-96 collected gas chromatographic fractions N= 20 for each treatment.

Treatment	% Males Sitting	% Males Taking Flight	% Stationary Flight	% Flying Upwind	% Reaching Source
Fraction B	14%	61%	~~55~~ 18%	~~43~~ 0%	~~35~~ 0%
Fractions A + B	7%	71%	38%	14%	0%
All 5 fractions	14%	75%	27%	11%	0%

DISCUSSION

We are currently trying to refine our fractionation of the NOW extract to more precisely isolate the compound(s) reponsible for the EAG and behavioral activity from fraction A. One complicating factor has been the isomerization of Z,Z-11,13-hexadecadienal to the other three isomers, which probably interfere with the upwind flight responses to fraction A plus the Z,Z isomer. Therefore current work is focusing on the cause(s) of this isomerization and several possible remedies, so that full upwind flight activity will occur in response to the total recombined extract.

We are satisfied that behavioral activity is increased by addition of fraction A (the EAG-active fraction) to B, and the next step will be to collect enough of the purified active compound(s) from A so that chemical tests can be conducted to determine the chemical structure(s). After this has been completed (Step 5, as outlined in Materials and Methods), future work will then center around step 6. After synthesis of the necessary compounds field testing using monitoring traps will be carried out by Drs. Martin Barnes and Curtis Engle of UCR, in conjunction with our research group.

Almond Board Project No. 80-B1: Navel Orangeworm Sex Pheromone Isolation and Identification

Project Leader: T.C. Baker, U.C. Riverside

Objectives

The objective of this research is to develop a navel orangeworm sex pheromone lure that can be used in traps as a population monitoring tool to increase efficiency and timing of insecticide sprays. A sensitive, efficient trap would also be highly compatible with mating disruption using the pheromone as an atmospheric permeant (now under development by Drs. Curtis and Landolt), because it could give the grower an early warning as to when and if the disruptant pheromone should be applied.

Progress in 1980

Collection of Volatiles

During the first 5 months of work on this project we have established a navel orangeworm colony in our laboratory. We now produce about 50-100 virgin adults of each sex per day. We have been accumulating extract from the sex pheromone glands of virgin females and also from airborne emissions using a novel glass-wool collection device. This enabled us to compare the volatile compounds found in the gland to those actually emitted into the air from the gland surface.

Electroantennogram Tests

After separating the volatile compounds obtained above using gas chromatography, we tested the various fractions by electroantennogram and found that besides the single compound already identified there was at least one other active region in the extract. The millivolt response to the major component was 3.7, whereas the second active fraction gave a response of 2.3. Other fractions gave 1.0 mv or less.

Wind Tunnel Flight Experiments

The extract was again fractionated by gas chromatography, and when the major component ((Z,Z)-11,13-16:ALD) and the EAG-active region were combined, they elicited levels of stationary and upwind flight greater than to the major component by itself, and equal to the entire recombined extract. It was apparent though, that the gas chromatographic fractionation may have decomposed some of the compounds, because the amount of upwind flight to the total recombined extract was below that obtained from the crude extract before GC injection. In a separate experiment, the crude extract had caused nearly 40% of the males to fly upwind and touch the source, compared to 0% in response to 1 mg (Z,Z)-11,13-16:ALD on a rubber dispenser. These results demonstrated again that the 1 component by itself causes little or no attraction, and that the other component(s) we are zeroing in on are the key to a good attractant for this species.