

81-B2

Project Number 81-B2
Project Title: Navel Orangeworm - Sex Pheromone Blend
Isolation and Identification

NAVEL ORANGEWORM
SEX PHEROMONE BLEND ISOLATION AND IDENTIFICATION

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Progress Report
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OBJECTIVES

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

SUMMARY

This year we have overcome the isomerization problems that prevented full behavioral response from NOW males upon recombination of all gas or liquid chromatographic fractions. By adding a second fraction to the fraction containing Z,Z-11,13-hexadecadienal, the major NOW sex pheromone component, we were able to elicit levels of upwind flight to the source in our wind tunnel and copulatory attempts that were as good as the crude extract. Additionally, these levels were as good as all the fractions recombined. We found that isomerization was related to injector temperature in the gas chromatograph. Our behavioral results confirm our previous findings, that there is a second active fraction that improves upwind flight of males when added to the already-identified component, but in addition these new data demonstrate for the first time that attractancy comparable to females is achieved when the second component is added.

EXPERIMENTAL PROCEDURE

The isolation and identification process can be broken down 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.

The procedures used this year were the same as those described in last year's report, except this year some new capillary gas chromatographic columns were used for step 2. One of these, a 60 meter SE-30 bonded phase-fused silica column (DB-1) was particularly useful for separating the isomers of 11,13-hexadecadienal.

RESULTS AND DISCUSSION

We found that the percentage of E,Z-, Z,E-, and E,E-isomers in our samples increased as the injector temperature increased, thereby giving inconsistent readings of the purity of the Z,Z-11,13-hexadecadienal from females and from our synthetic samples. A sufficiently high injection port temperature is necessary to volatilize the compound for good chromatographic resolution, and at a fairly normal value of 170°C, substantial percentages of minor isomers appeared in our synthetic samples (Fig. 1A). At slightly lower temperatures (130°C) lower percentages of the other isomers appeared (Fig. 1B), and at the lowest temperature used (110°C), the smallest percentages of these minor isomers occurred (Fig. 1C).

Another factor influencing our analyses was the isomerization occurring during our fractionation of samples on the GC. Substantial

Figure 1A.

SE-30 CAPILLARY

INJECTOR TEMP. 170 DEG.

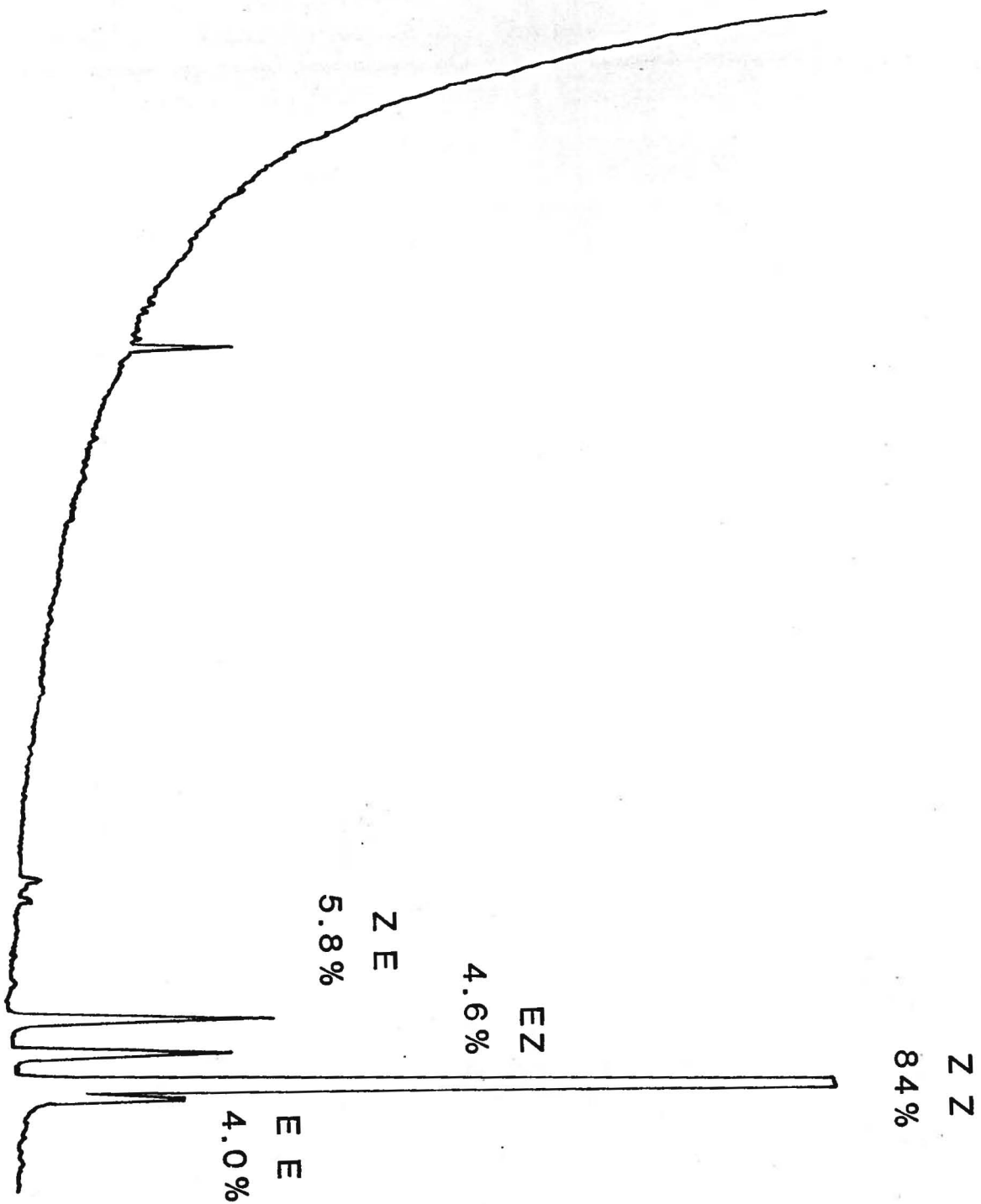


Figure 1B.

SE-30 CAPILLARY
INJECTOR TEMP. 130 DEG.

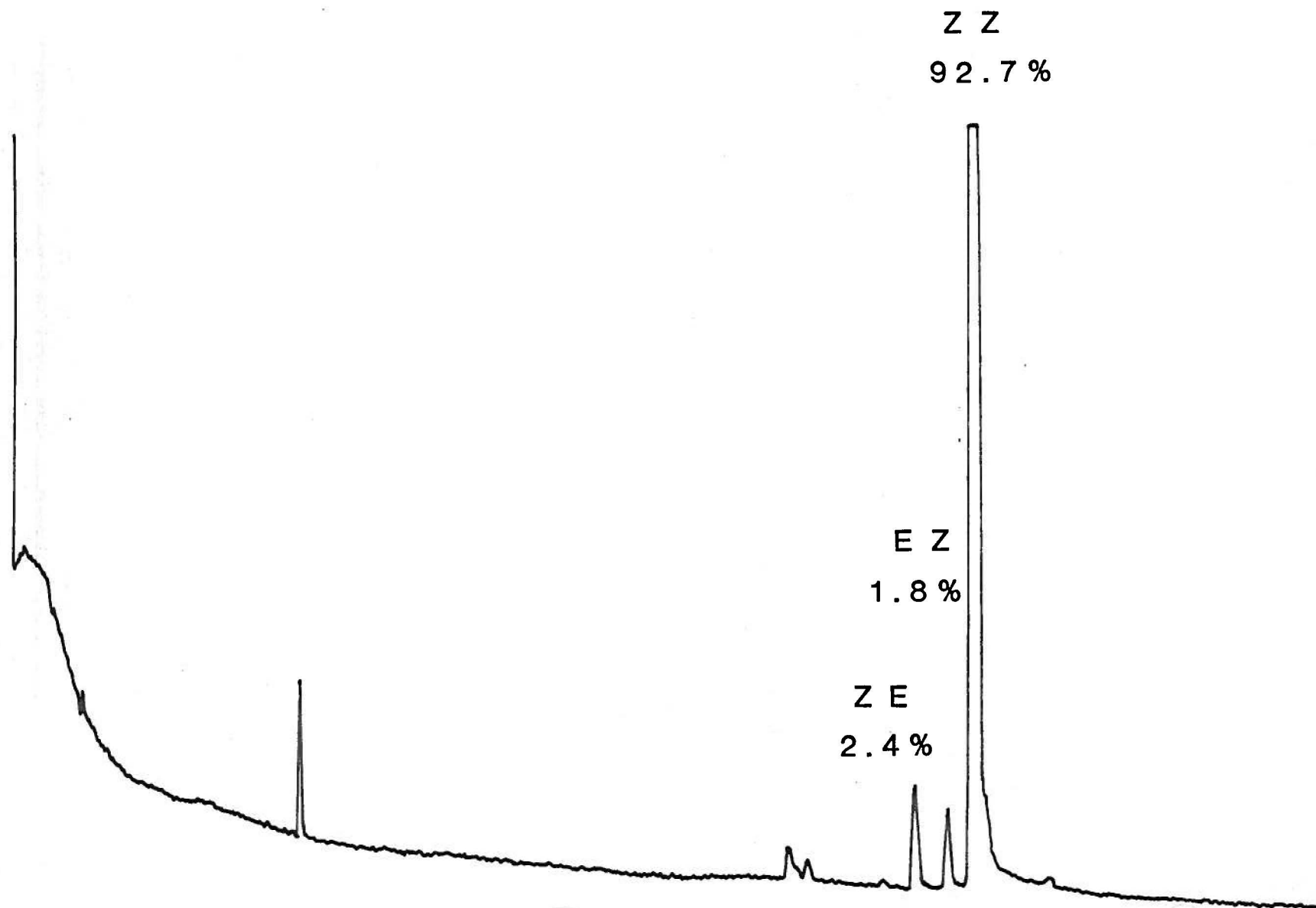


Figure 1C.

SE-30 CAPILLARY

INJECTOR TEMP. 100 DEG.

Z Z
97.6%

E Z
0.6%

Z E
0.7%



amounts of the minor isomers were appearing in the 11,13-hexadecadienal fraction, for instance, after collection from the SF-96 packed column (Fig. 2A) compared to the original sample (Fig. 2B). These changes in purity were affecting the behavioral responses of males in our wind tunnel flight tests, and were likely responsible for the lack of complete upwind flight to the source by males in our previous fractionations (Table 1). We were able to demonstrate repeatedly that a second fraction added to the Z,Z-11,13-hexadecadienal fraction would improve upwind flight responses of males compared to the Z,Z-11,13-hexadecadienal fraction alone (Table 1), but the percentage of males arriving at the source had always been much lower than the crude extract. Even all the fractions recombined had a reduced ability to elicit complete upwind flight to the source (Table 1). We now know that slight alteration of the fractions' chemical makeup during GC collection contributed to this drop in behavioral activity.

We now have overcome this problem with new fractionation procedures that permit the total behavioral activity of the fractions to be retained (Table 2). This has been a major advance, because we now know for the first time that when the second component is identified, a lure comparable to calling females will result. Addition of the second fraction to the Z,Z-11,13-hexadecadienal-containing fraction resulted in percentages of male source location as high as those to crude female extract, which is always the most attractive source we test (Table 2). In addition, intense copulatory responses and wing fanning on the filter paper occurred with this combination of the two fractions, again at levels comparable to the crude extract.

Our ability to retain complete behavioral response after fractionation has allowed us to begin performing chemical tests on the second area

Figure 2A.

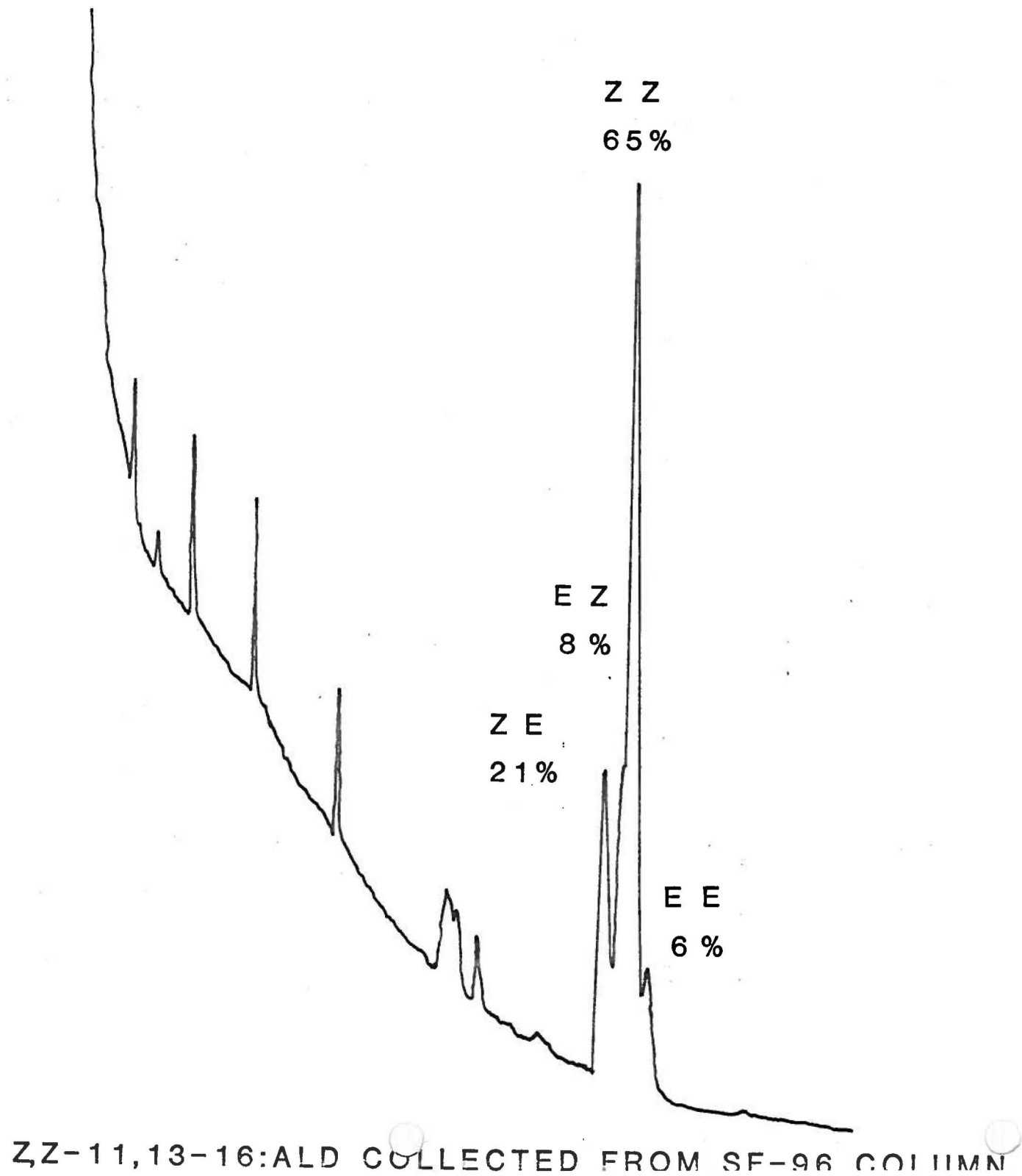
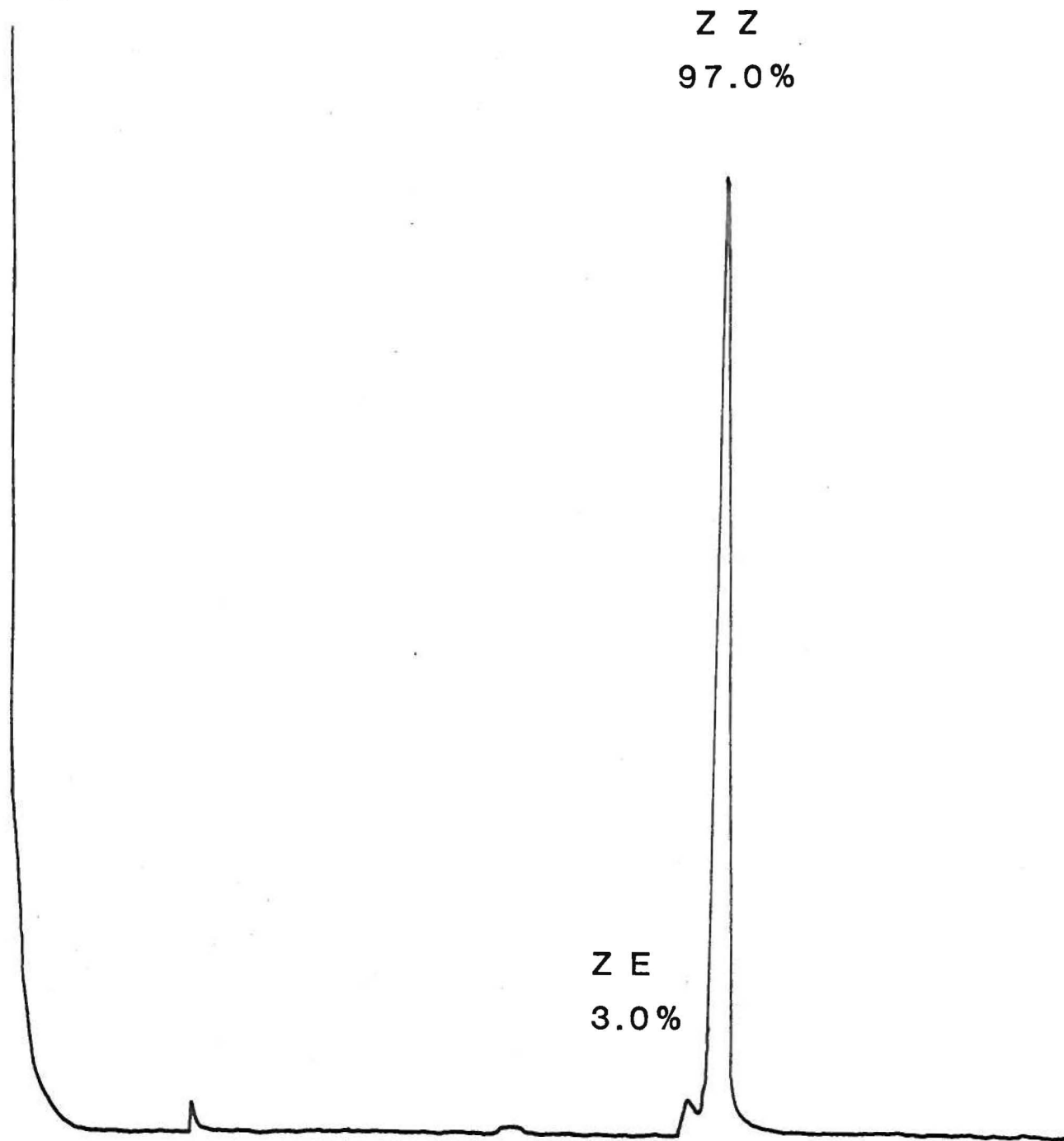


Figure 2B.



○ ZZ-11,13-16:ALD INJECTED I ○ MEDIATELY ONTO CARBOWAX ○ 0-M

Table 1. Lack of complete flight to source in wind tunnel using SF-96 gas chromatographic fractions collected by former methods. Second area of activity (Fraction A) is still apparent due to increased stationary and upwind flights even though no males reached the source.

Treatment	% Males Sitting	% Males Taking Flight	% Stationary Flight	% Flying Upwind	% Reaching Source
Fraction B (Z,Z-11,13-16:Ald)	14%	61%	18%	0%	0%
Fractions A + B	7%	71%	38%	14%	0%
All 5 Fractions	14%	75%	27%	11%	0%

Table 2. Demonstration of complete flight to source in wind tunnel comparable to female gland extract when new fractionation procedures were used and second area of activity was combined with Z,Z-11,13-16:Ald fraction.

Treatment*	% Males Wing Fanning	% Males Taking Flight	% Males Flying Upwind	% Reaching Source
Fraction B (Z,Z-11,13-16:Ald)	76%	78%	37%	1%
Fractions A + B	68%	74%	55%	44%
Female Gland Extract	83%	90%	56%	56%

* N=71, 167, and 36 for Fraction B, Fractions A + B, and extract, respectively.

of activity to determine its chemical structure. These tests include microozonolysis, hydrolysis, acetylation, hydrogenation, plus GC-mass spectral analysis. They will continue to be conducted until the structure of the second pheromone component has been determined and verified with behavioral tests with synthetic material in the laboratory and with field trapping tests. Dr. Wendell Roelofs of the New York State Agricultural Experiment Station at Geneva has agreed to assist, if needed, in the identification effort. His unparalleled expertise in this area, plus his extensive "library" of pheromone compounds, will be invaluable. The field testing of this material will be started in 1982 in cooperation with Dr. Martin Barnes of UC Riverside and Dr. Charles Curtis of the USDA in Fresno.