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SEX PHEROMONE RESEARCH WITH THE NAVEL ORANGEWORM

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I. OBJECTIVES AND GOALS:

The objective of this project is to isolate and identify the female-produced sex pheromone of the navel orangeworm in order that field researchers and others concerned with population levels may have a more sensitive monitoring tool than is now available. We also hope to provide field researchers with synthetic pheromone for the purpose of evaluating the material as a mating communication disruptant.

II. ABSTRACT:

Extensive laboratory studies of the sex pheromone biology of the navel orangeworm were conducted to determine the influence of physiological (age, mated status, etc.) and environmental (light, temperature, etc.) factors upon male response to, and female production of the pheromone. Concurrently, studies were undertaken to determine the chemical nature of the pheromone. Pheromone was obtained from laboratory-reared females, and the relative quantity of pheromone obtained was estimated by bioassay. The bioassay consisted of exposing sexually responsive males to serially diluted female pheromone. Crude pheromone extracts were purified initially by means of liquid chromatography, and finally by gas chromatography. Quantitative bioassays were conducted to monitor each purification step. The chemical nature of the pheromone was determined by its chromatographic behavior, functional group analysis, and mass spectrum. Chromatographic analysis and bioassay of pheromone obtained from field-collected females revealed that the "wild" females possess the same compound that was identified from the laboratory-reared females, and that biological activity (bioassay using laboratory-reared males) was equal to that obtained, at similar concentrations, with laboratory-reared females. Plans are underway to begin synthesis of the pheromone.

A. Bioassay: Two different methods of bioassay were employed. In the first, the methods and apparatus of Sower et al. (1973a) were used. Briefly, groups of 10-15 males were placed in 1.8-cm ID X 44-cm acrylic plastic tubes, and exposed to serially diluted pheromone. Two response behaviors were observed; activation and orientation. The former consisted of the onset of locomotory activity, and the latter involved upwind movement to within 4 cm of the pheromone source, 15-20 sec after introduction of the sample. The second method measured activation responses of males held individually in 5-dr vials. Ten sec were allowed for the onset of this behavior. The 2 methods yielded similar dosage-response curves.

B. Biological Studies:

1. Influence of male age upon pheromone-responsiveness--Virgin males that were 1, 2, 3, 4, or 5 days old were exposed to 1 X 10^{-3} female equivalents (FE). Individual males were used only once. Five replications (10 males/rep.) were conducted on different days.

2. Influence of time of day upon pheromone-responsiveness--Unmated male moths, aged 2-4 days, were exposed to 1 x 10^{-3} FE at various times during a 14:10 L:D photoperiod. Individual males were used one time only, and the test was replicated 6 times on different days.

3. Influence of time of day upon female calling behavior: Virgin female moths (50/cage) were observed at 1-2 h intervals during the 10 h scotophase and at 2-4 h intervals during the 14 h photophase, and the percentage of females assuming the pheromone-release posture at those times were calculated. Tests were begun with 1-day-old females and continued for 4 days. The test was replicated 4 times using females from different generations.

4. Influence of time of day upon the quantity of pheromone obtainable from individual females--Pheromone was collected from individual females at several different times during a 24-h period and analyzed by gas chromatography using the methods of Sower et al. (1973b). Ca. 25 females were used at each collection, and 3 replications of each time point were made.

5. Pheromone content of field-collected females--Insects used in this experiment were collected in the San Joaquin Valley and sent to Gainesville for analysis. Last instar larvae were segregated by sex, and held for adult emergence.

Crude pheromone extracts were prepared from 2-day-old virgin females, and the pheromone titer was estimated by means of bioassay and gas chromatography.

C. Chemical Studies

1. Collection of crude pheromone--Pheromone was collected either by solvent rinses of glass containers in which virgin females had been held for varying periods of time, or from female abdominal tips. Material from either source was concentrated to 1-2 ml following filtration and drying. Quantitative bioassays were made using aliquots of this material to estimate the relative amounts of pheromone present. Following this, the extracts were subjected to the initial chromatographic column employed, silicic acid, using the methods described by Sower et al. (1973a). Biological activity was quantitated. Active fractions were subjected to liquid chromatography on a 1.1 x 60-cm column packed with 40 g of 25% $AgNO_3$ on silica gel (200-250 mesh, Applied Science Labs.) using the procedure of Vick and Sower (1973). Active portions were further purified by means of preparative gas chromatography on polar and nonpolar columns. Biological activity was monitored at each step by bioassay.

2. Chemical tests--The purified pheromone was subjected to catalytic hydrogenation, ozonolysis and the usual functional group tests. Each reaction was monitored by bioassay and gas chromatography.

3. Mass spectrum--The chemical ionization mass spectrum of the purified pheromone was obtained. Mass spectra of significant reaction products were also recorded.

IV. RESULTS:

A. Bioassay: The results of a typical bioassay series of the navel orangeworm pheromone are summarized in Fig. 1. The illustrated results were obtained using the vial-type olfactometer, and the quantitative nature of the data are apparent. Concurrent bioassays using the tube-type olfactometer gave similar results for both activation and orientation except that only about 1/10 the concentration was required for any given male response level shown in Fig. 1. In addition, standard errors obtained in the tube-type chamber were 2-3 times as large as those indicated in Fig. 1.

B. Biological Studies:

1. Influence of male age upon pheromone-responsiveness--At 1×10^{-3} FE, the response of 2, 3, and 4-day-old males was 73, 66, and 72%, respectively. These values are not significantly different. The response of 1-day-old males, 39%, was significantly lower than those of 2-4 day old individuals. Five-day-old males responded at an 18% level. This figure is indicative of roughly a 100-fold reduction in responsiveness between days 4 and 5. Standard errors for each test mean were less than 10%.

2. Influence of time of day upon pheromone responsiveness--The results of this series of bioassays are summarized in Fig. 2. Additional bioassays, at higher concentrations (1×10^{-1} and 1×10^{0} FE) during the photophase did not yield any male response above background (ca. 5-8%).

3. Influence of time of day upon female calling behavior: Female navel orangeworms did not assume the calling posture at any time during the photophase, and no pheromone release was observed during the first 5 h of the scotophase. Calling was first observed (7%) during the 6th h of the scotophase, rose to 68% during the 7th h, held at ca. 80% from the 8-10th h, and declined to ca. 10% within 15 min after the onset of the photophase. Age (1-4 days) had no significant influence upon the proportions of females assuming the pheromone release posture.

4. Influence of time of day upon the quantity of pheromone obtainable from individual females--The results of this experiment indicated that the amount of pheromone on the surface of the pheromone gland varies considerably during a 24 h cycle, and that the greatest quantities are found during the peak calling period described in 3 above.

5. Pheromone content of field-collected females--Chromatographic analysis of pheromone extracts of field-collected females revealed that the compound identified from laboratory-bred females is present in the field strain. Field females were found to contain slightly more pheromone than do laboratory-reared individuals. The results of comparative bioassays using extracts of field- and laboratory-raised insects supported the chromatographic data.

C. Chemical Studies:

1. Collection of crude pheromone--Bioassayable quantities of pheromone were obtained from either of the 2 methods employed. Extracts from either of the sources (jar rinses or gland extracts) were, initially, not clean enough for GC analysis. The chromatographic behavior of the pheromone on silicic acid gave some indication of the functionality of the molecule. No loss of biological activity was detected following this treatment. Chromatography on the $AgNO_3$ treated column gave additional purification with no detectable loss of biological activity. Preparative gas chromatography on polar (principally Carbowax 20M) and non-polar (principally OV-1) substrates yielded a substance that was greater than 98% pure on several GC columns and was the only fraction to possess any biological activity. By bioassay, the 50% response level was ca. 1×10^{-6} µg.

2. Chemical tests--Chemical tests, hydrogenation, and ozonolysis of the purified pheromone gave confirm-data that supported chromatographic data.

3. The mass spectrum of the pheromone and the spectra of pheromone reaction products supported and extended the above findings.

DISCUSSION

The femaleproduced sex pheromone of the navel orangeworm has been isolated and identified. Biological activity of the purified material is similar, both qualitatively and quantitatively, to that observed with unpurified material. The compound identified from laboratory-reared females was also found in field-collected females.

The reproductive behavior of navel orangeworms in the laboratory closely parallels that reported to occur in the field. Pheromone release by receptive females, maximum male responsiveness and mating occur during the latter part of the scotophase.

Plans are currently being made to begin synthesis of the pheromone shortly after the first of the year.

The pheromone should find practical use in the field as an adjunct to extant and proposed survey practices, and should be evaluated as a potential mating disruptant.

REFERENCES CITED

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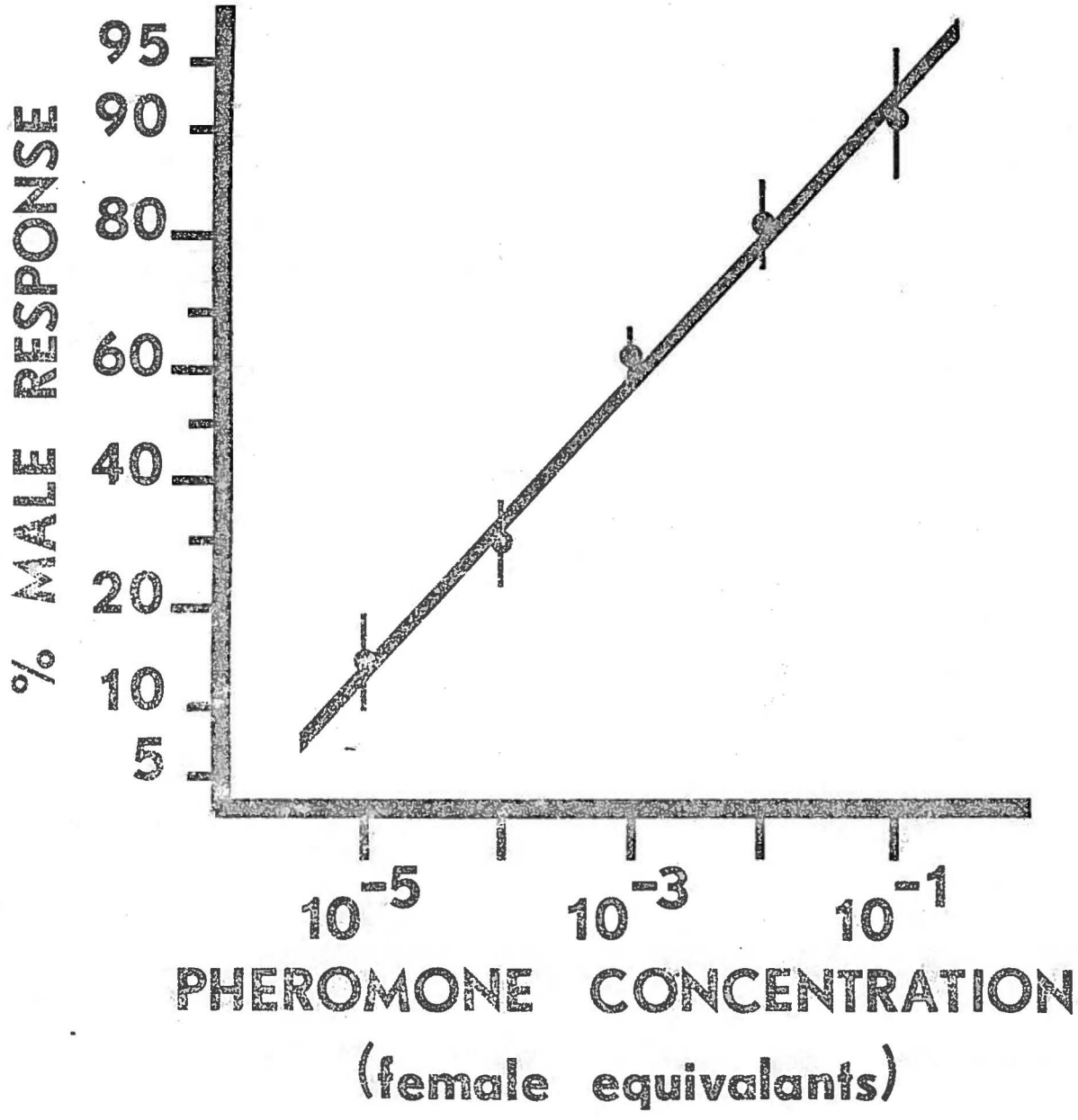
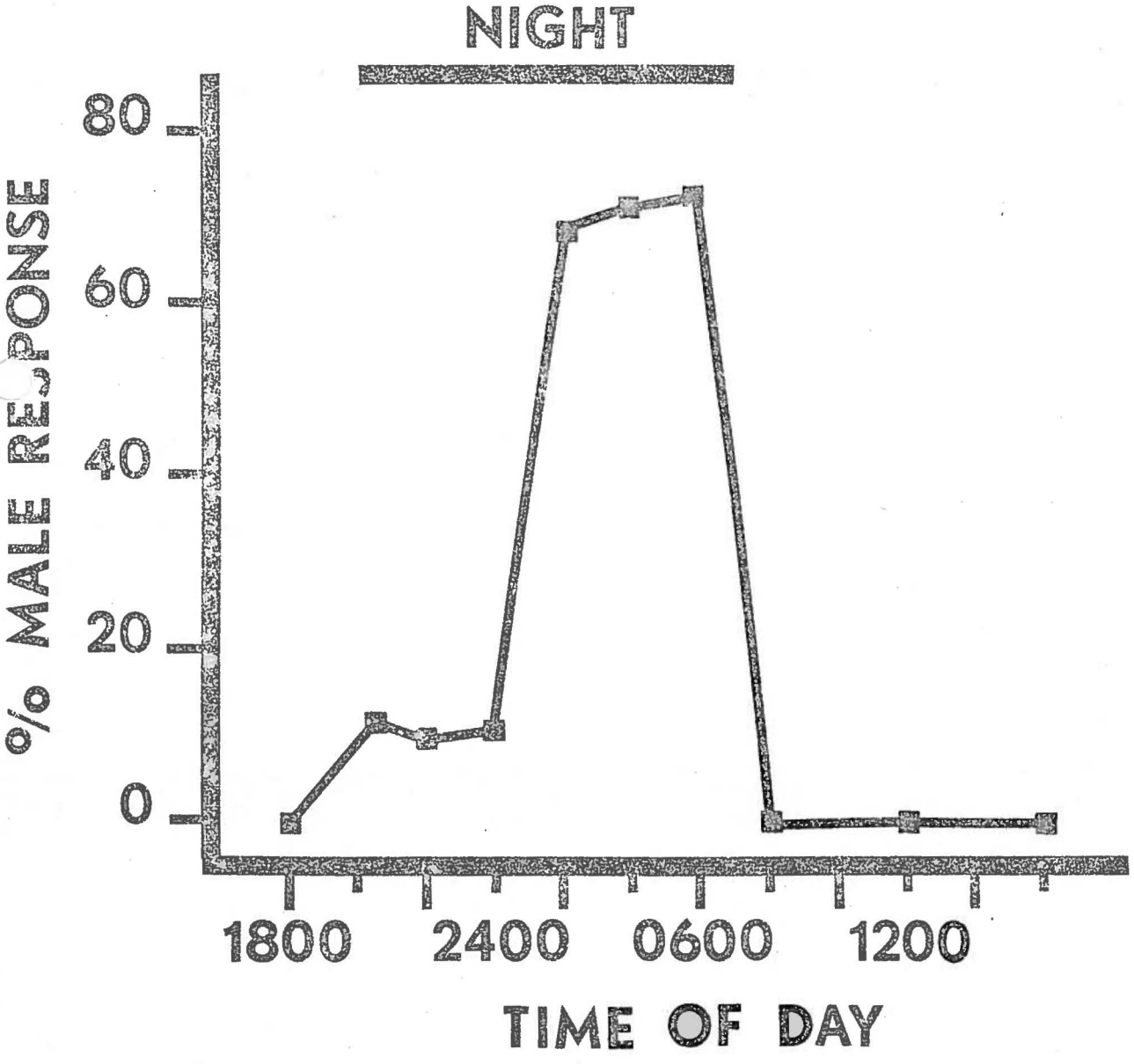

% MALE RESPONSE
95
90
80
60
40
20
10
5
10^{-5}
10^{-3}
10^{-1}
PHEROMONE CONCENTRATION
(female equivalants)

FIGURE 2


NIGHT
% MALE RESPONSE
80
60
40
20
0
1800
2400
0600
1200
TIME OF DAY