Project Number 76-G3

Project Title: Sex Pheromone Testing in Prevention of the Navel Orangeworm

J. A. Coffelt, K. W. Vick and W. T. McClellan
Insect Attractants, Behavior and Basic Biology
Research Laboratory, ARS, USDA, Gainesville, Fl.

SUMMARY

The female sex pheromone of the navel orangeworm has been isolated and identified. Two forms (isomers) of the compound have been synthesized. Following confirmation of biological activity of the synthetic material work will begin on the development of a suitable formulation of the pheromone for orchard use.

PHEROMONE RELEASE bioassay, GC, observations

Females call and release pheromone only during latter 1/3 of scotophase; pheromone not detectable(GC) on surface of pheromone gland during photophase and early scotophase.

B. PHYSIOLOGICAL VARIABLES — METHOD OF ANALYSIS

a. male age — bioassay

maximum male response from 2-4 day-old males; ca. 10 or more fold decrease in response among males older than 4 days; one-day-old males responded less well than did 2-4 day-old individuals but the difference appeared to be slightly less than 10 fold(i.e., on the borderline of the sensitivity of the bioassay).

b. female age — Gas Chromatography

no significant differences in quantity of pheromone from 1-4 day-old females; females older than 4 days generally did not call and were not analyzed re. pheromone content.

c. mating

MALE — bioassay

no significant differences in pheromone response of males mated 24 h before bioassay and unmated males that could not be explained by age differences(see a above).

FEMALE — Gas Chromat.

Erratic results; some mated females seemed to produce and release as much pheromone as did unmated individuals, but other females appeared to contain only about ½ as much pheromone after mating as did comparably aged virgin females.

III. ISOLATION OF THE PHEROMONE

A. Influence of female or extract source on quantity of pheromone obtainable from NOW females

Table 1 summarizes the results of attempts to quantitate obtainable pheromone from field and lab. females as well as pheromone from different sources of lab females.

SOURCE OF FEMALES	SOURCE OF EXTRACT	ng PHEROMONE/ FEMALE	METHOD OF ANALYSIS
laboratory	filter paper	less than 0.5	bioassay
laboratory	abdominal tip	ca. 1.0	GC & bioassay
laboratory	gland dips	0.8-1.7	GC & bioassay
field	gland dips	2-4	GC & bioassay

B. Early work had shown the the quantity of pheromone obtainable by the gland dip technique was at least as high as that obtained by more rigorous methods. Dip extracts had the additional advantage of being "clean" in the pheromone area on both polar and non-polar gas chromatographic columns. Despite the limitation of obtaining pheromone for only about 3 hours/ day, dip preparations were used as a pheromone collection method starting point for the following isolation procedure:

FIGURE 2.

CRUDE PHEROMONE EXTRACT

↓ *

LIQUID CHROMATOGRAPHY (Figure 3)
(silicic acid)

↓ *

LIQUID CHROMATOGRAPHY
($AgNO_3$ on Silica Gel)

↓ *

LIQUID CHROMATOGRAPHY
(Poragel 60A)

↓ *

GAS CHROMATOGRAPHY
(polar columns)

↓ *

GAS CHROMATOGRAPHY
(non-polar columns)

↓ *

PURIFIED (99%) PHEROMONE

Mass Spectrum | UV Spectrum | Chemical tests

Chemical tests:
- hydrolysis
- acetylation
- reduction
- hydrogenation
- ozonolysis

} ALL FOLLOWED BY BIOASSAY, GC, AND MASS SPECTRA OF SIGNIFICANT PRODUCTS

I. BIOASSAY--Pheromone extracts were prepared from calling NOW females using the method of Sower et al. (1973). Serial dilutions of the crude extract were bioassayed using the method and apparatus of Sower et al.(1973b), in which both male activation and orientation (attraction) were used as response criteria. In addition, the extracts were bioassayed using isolated males in a still air olfactometer (5 dram vial). Activation(and wing buzzing) were considered evidence of a positive response in this test.

The results of this series of bioassays are summarized in Fig. 1. The quantitative nature of the bioassay is evident, and the sensttivity of the assay is similar (in terms of female equivalents (FE)) to that which has been reported for a number of moth species. The orientation response in the wind tunnel and the activation response in still air were subsequently used as response criteria while monitoring isolation of the pheromone. Details of the bioassay procedure will be supplied upon request.

II. BIOLOGICAL STUDIES--Following is a brief summary of experiments that were conducted to determine the influence of environmental and physiological variables upon male response to and female production of the pheromone. As above, detailed methods will be supplied upon request.

A. Environmental variables — method of analysis

a. light — bioassay

Maximum male responsiveness at approximately full moonlight; there was a 10-50 fold decrease in male responsiveness with a 10 fold increase in light intensity.

b. temperature — bioassay

Maximum male responsiveness at 68-76^{o}F; male response decreased at temperatures above 78^{o}F. Lower temperatures (less than 68^{o}F. were not tested).

c. time of day — bioassay

MALE RESPONSE

maximum male response during latter half of 10 h scotophase; greater than 1000 fold decrease in male responsiveness during photophase

MATING — OBSERVATION

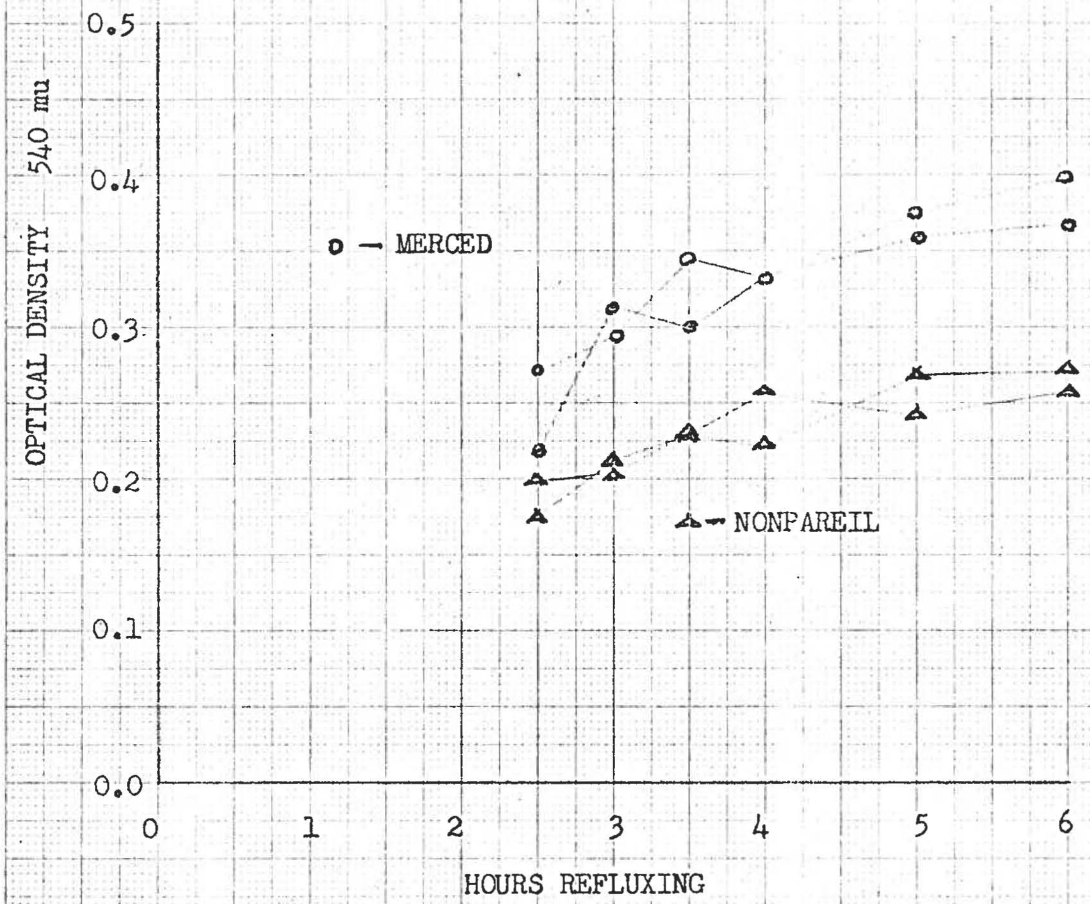
confined to latter 1/3 of 10 h scotophase

The female navel orangeworm was found to produce a single compound that was extractable from the adult and which elicited sexual stimulation in the male. The limited quantities of pheromone that were available for isolation purposes materially slowed the identification of the compound. Isomers of the synthetic pheromone will be available for laboratory testing in January 1977. Extensive laboratory testing of the pheromone will begin by February 1977. Hopefully we will be able to come up with a suitable formulation of the material that will allow for field tests of the material during the 1977 crop year.

References cited

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: 1220-1222.

Sower, L. L., K. W. Vick and J. S. Long. 1973. Isolation and preliminary biological studies of the female-produced sex pheromone of _Sitotroga cerealla_ (Lepidoptera:Gelechiidae). _Ann. Entomol. Soc. Amer._ 66: 184-187.



OPTICAL DENSITY 540 mu
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
HOURS REFLUXING
o — MERCED
△ — NONPAREIL

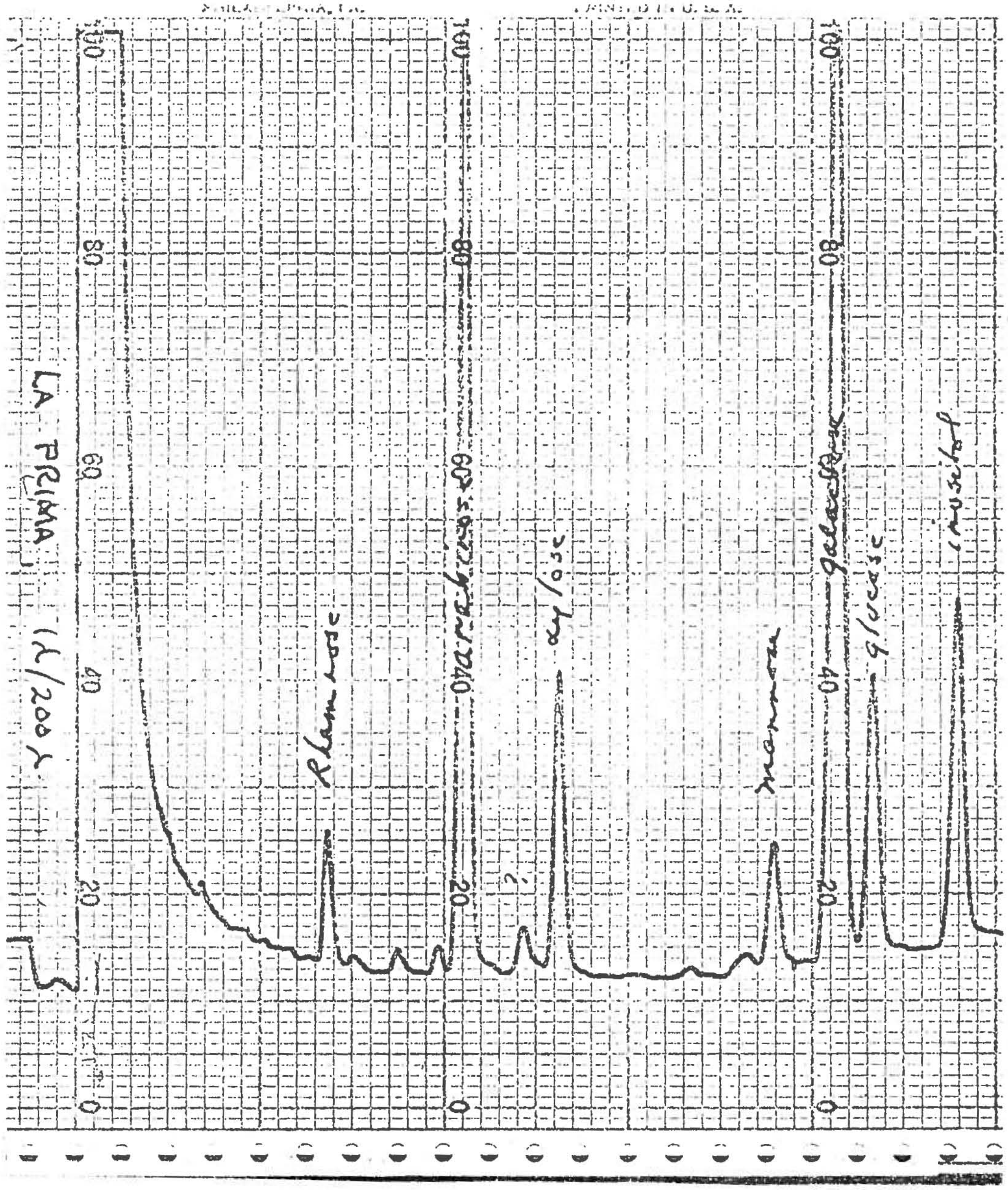


Fig. 3. Gas chromatographic tracing of sugar derivatives obtained from La Prima almond gum after hydrolysis with trifluoroacetic acid and subsequent reduction and acetylation with acetic anhydride.