1978 Annual Report

California Almond Board Project 78-H2

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University of California
Davis/Parlier

Project: Navel Orangeworm Research

Identification of Food and Oviposition Attractants

Oviposition Biology

I. Objectives: 1) To extract, isolate, and identify the NOW oviposition attractant(s); 2) to simplify the egg trap and monitoring techniques by using a synthetic chemical as the attractant source; 3) to attempt using the oviposition attractant as a control technique for NOW; and 4) to determine the temperature requirements for NOW egg development as an aid in timing chemical sprays for NOW control.

II. Summary: Egg deposition by NOW females was monitored with egg traps throughout the 1978 season in Fresno County. Seasonal laying patterns were similar to those observed in previous years (1974-77). Chemical controls for NOW were applied starting May 13, near the second peak of oviposition activity. Detailed analysis of the 1978 plots indicated that the spray date was ca. one week late; it should have started about May 4-6 at the beginning of the second period of egg laying.

Comparison of three sprayed plots to an untreated check showed damage reductions to nut meats ranging from 64.3-89.5%. It was also observed that the timing of the NOW sprays on May 13 coincided closely to the optimum timing for spring controls of peach twig borer, resulting in significant reductions in PTB moth activity through August.

Laboratory studies confirmed that the lower threshold for NOW egg development is ca. 57°F; the upper threshold is ca. 90-95°F.

Results of oviposition attractant bioassays have been encouraging. Continued fractionation of active extract groups should lead to isolation of the attractive components within a reasonable time.

III. Experimental Procedures: Egg traps used during 1978 were standard Pherocon IV traps made by Zoecon. Bran bait was made in the laboratory according to previously described methods. Pheromone traps for peach twig borer were the Pherocon I-C model, using standard commercial rubber septum pheromone dispensers. All traps were counted and serviced once/week, except during April-June when they were counted 2x/week.

Nut samples from the chemical control plots were taken on Sept. 12-13 by machine knocking nuts to the ground and raking these nuts into a pile at each of 15 sample sites (trees) per treatment. A composite sample of ca. 1000 nuts was removed from each sample site, fumigated for 24 hours and then dried at ca. 120°F for four days. From each of these samples 200 nuts were then hand cracked and evaluated for damage.

Bioassays of oviposition attractants were conducted in a greenhouse screen cage at Parlier. Materials included standard egg traps and a revolving wheel olfactometer for flying moths (see manuscript enclosure).

IV. Results and Discussion: Seasonal monitoring of NOW egg deposition showed a normal pattern of oviposition during 1978 at Caruthers, Fresno County (Fig. 1). First eggs from overwintered NOW were collected on egg traps the last week of March, with the first spring

peak of oviposition April 6-13. A two-week decline was then followed by the expected second spring period oviposition, which peaked May 11-18.

First generation moths began emerging and laying eggs the first week in July, which corresponded (again) closely to the beginning of Nonpariel hullsplit. Heavy oviposition then occurred during August (2nd generation moths) and October (3rd generation).

Chemical control of navel orangeworm was evaluated in large plots applied by the grower at Caruthers. One plot (A) received winter clean-up and a dormant spray of Diazinon and oil. Three other plots (B, C, D) had sanitation and dormant treatments, and also were sprayed for NOW in May with Guthion at 2.5 lbs. a.i./acre. Chemical treatment in May was started on May 13, coinciding to first egg hatch plus 10 days. However, post-application evaluation of this timing indicated that the sprays were ca. one week later than optimum, and should have been applied when the first eggs began to hatch.

Results of the chemical plots (Table 1) showed significant reductions in NOW damage in all three blocks sprayed in May. It was also observed that the May spray timing (May 13) coincided closely to the suggested May spray timing for peach twig borer in Fresno County (May 6). The Guthion treatments for NOW appeared to have a marked effect on PTB moth populations up to September (Fig. 2). After this time, however, PTB populations again increased to levels approaching those in the check (A) plot that had received only the dormant spray. The net effect of these sprays on PTB was that twig borer damage to nut meats was negligible. There was no correlation

between number of mummies per acre in February in sprayed plots and percent damaged meats in September (Table 1).

Laboratory and field studies on NOW eggs confirmed that the lower threshold for development is ca. 57°F, while the upper threshold is near 95°F. Eggs held at a constant 55°F eventually collapse without hatching, while eggs held at 57-60°F have a very low percentage hatch. Constant temperatures above 90-95°F have a retarding effect on NOW egg development. These data will be evaluated with a computer day-degree program this winter to establish the validity of the proposed thresholds, and to determine the accumulated day-degree values between oviposition and hatch under field conditions.

Isolation and identification of NOW oviposition attractants has continued, with the cooperation of Dr. W. G. Jennings and Mr. Fong-Yi Lieu, Department of Food Science, U. C., Davis. Extracts of attractant materials are prepared at Davis and are then bioassayed at Parlier according to procedures previously described (Rice et al. 1978). Recent results of some of these extract bioassays (Table 2) are quite encouraging; it is hoped that rapid identification of these attractive fractions will lead to field evaluation of single and/or multiple component synthetic attractant in the spring of 1979.

V. Publications:

Rice, R. E., M. M. Barnes, and C. E. Curtis. 1978. Integrated pest management in almonds. Calif. Agric. 32(2):18. February.

Rice, R. E., and R. A. Jones. 1978. Mites in almonds and stone fruits. Calif. Agric. 32(4):20-21. April.

Rice, R. E. 1978. Insect and mite pests of almonds, in Almond Orchard Management. Accepted for publication (Jan. 1979). U. C. Press.

Rice, R. E., F. Y. Lieu, W. G. Jennings, and L. L. Sadler. 1978. A laboratory bioassay for oviposition by navel orangeworm moths (Lepidoptera: Pyralidae). Accepted for publication, Canadian Entomologist, Dec. 1978.

Table 1. Control of navel orangeworm, Caruthers, Fresno County, 1978.

Treatment[1/]	No. mummies per acre[2/]	Damaged[3/] nuts	Percent[4/] damage
Check	666	143	4.77 a
Plot C	518	51	1.70 b
Plot D	185	34	1.13 bc
Plot B	1392	15	0.50 c

1/All plots received winter sanitation programs and dormant sprays. Plots B, C, D treated with azinphosmethyl on May 13-28, 1978.

2/Calculated on 87 trees per acre; 50% Nonpareil, 25% NePlus, 25% Milow. Averages from 10 trees per variety in each treatment, Feb. 1978.

3/Totals from fifteen 200-nut samples per plot, Sept. 12-13, 1978.

4/Significant at 5%, Duncan's Multiple Range Test.

Table 2. Bioassays of candidate NOW oviposition attractants, Parlier, Calif., 1978.

Extract fraction	Total eggs collected		
	Test 78-80[1/]	78-82[1/]	78-84[2/]
1	106	114	39
2	109	78	27
3	300	175	93
4	576	173	151
5	-	90	25
6	11	11	-
7	9	-	-
8	11	-	-
Hexane check	18	5	12

1/ 3 replicates.

2/ 4 replicates; all fractions in Test 84 from fraction 4 in Tests 80/82.

FIGURE 1.

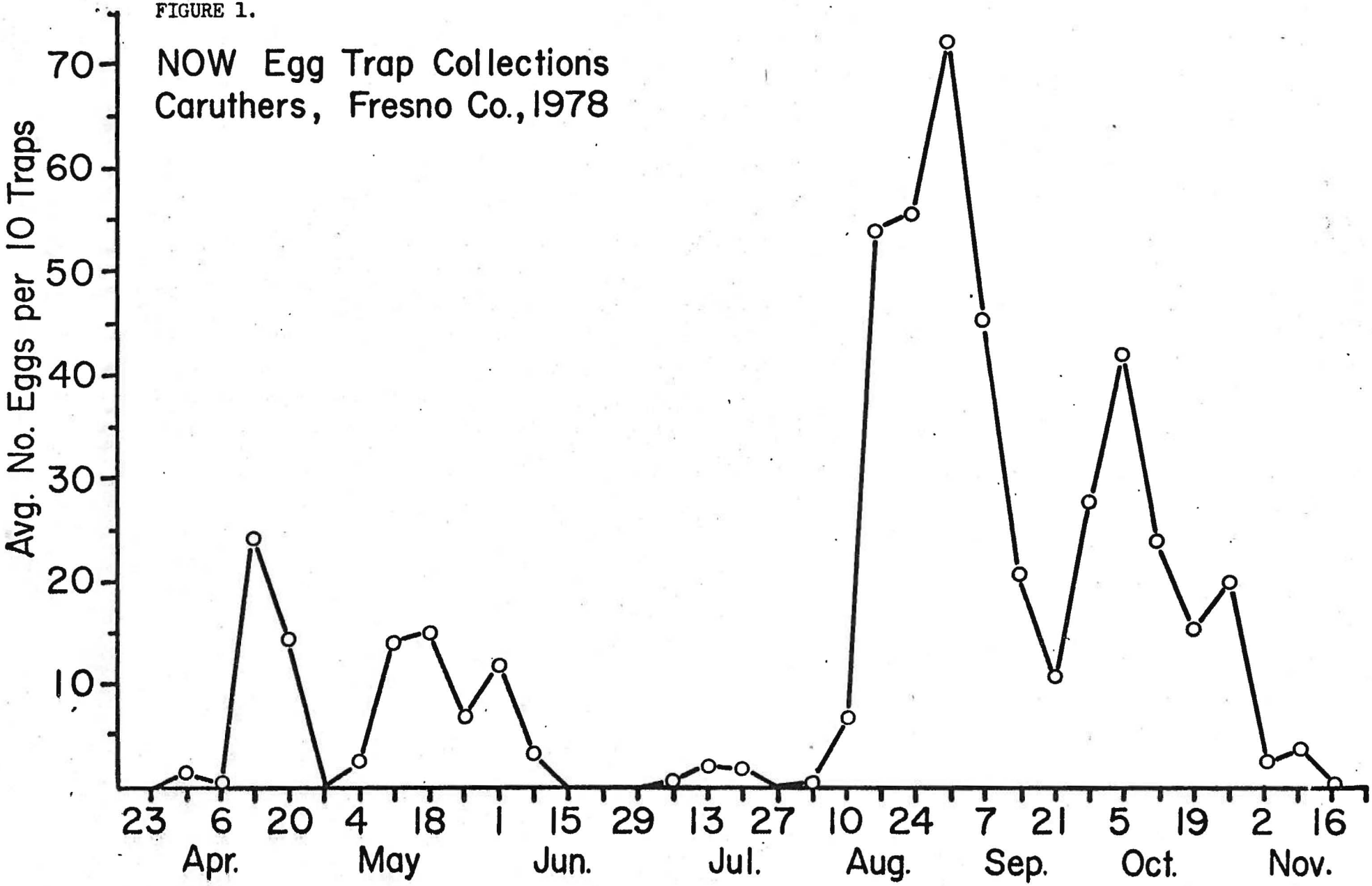
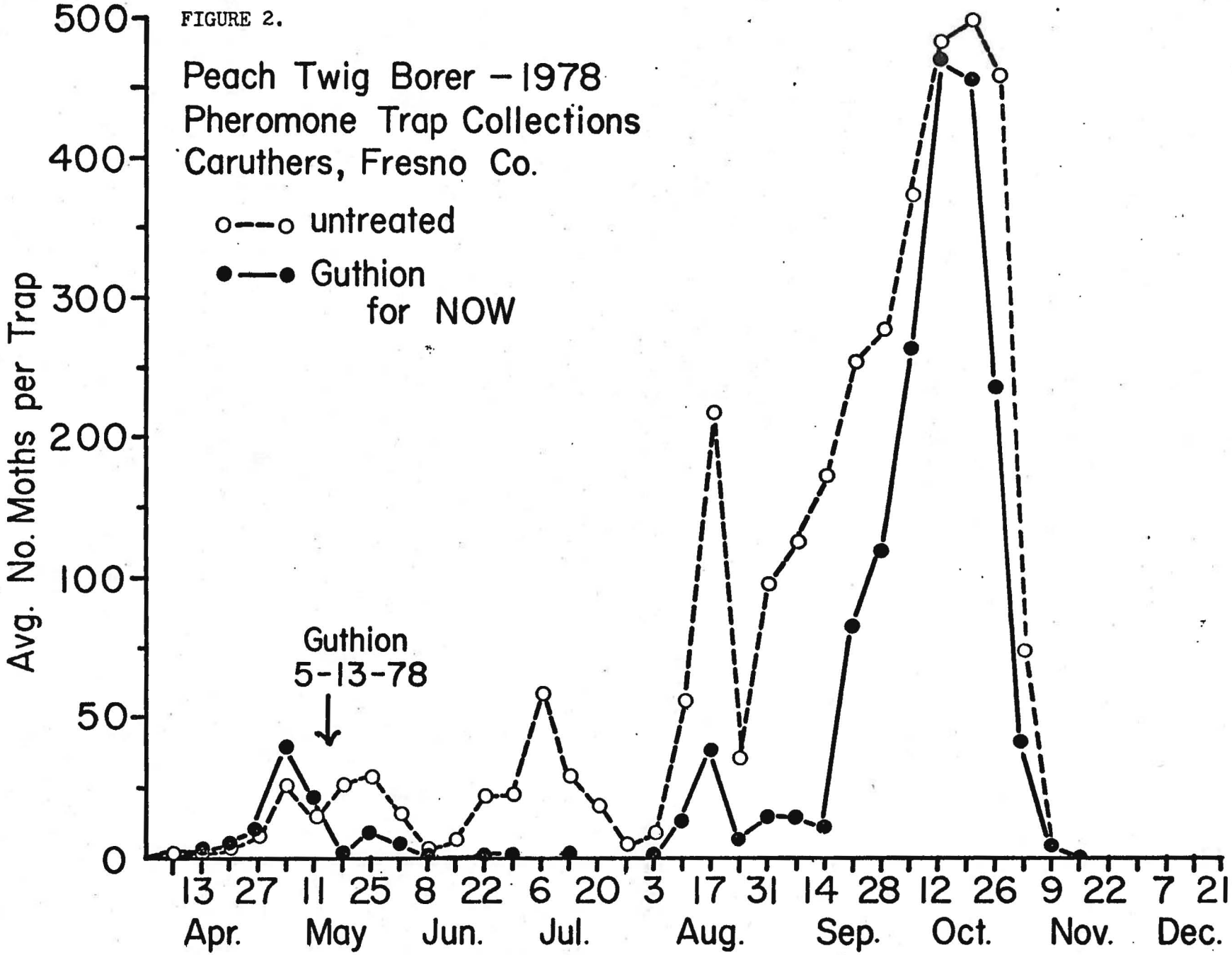

NOW Egg Trap Collections
Caruthers, Fresno Co., 1978
Avg. No. Eggs per 10 Traps
70
60
50
40
30
20
10
23
6
20
4
18
1
15
29
13
27
10
24
7
21
5
19
2
16
Apr.
May
Jun.
Jul.
Aug.
Sep.
Oct.
Nov.

FIGURE 2.


Peach Twig Borer – 1978
Pheromone Trap Collections
Caruthers, Fresno Co.
o—o untreated
●—● Guthion for NOW
Avg. No. Moths per Trap
500
400
300
200
100
50
0
Guthion
5-13-78
13 27 11 25 8 22 6 20 3 17 31 14 28 12 26 9 22 7 21
Apr. May Jun. Jul. Aug. Sep. Oct. Nov. Dec.

For: Canadian Entomologist

Ed. only: Corresp. to:

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A Laboratory Bioassay for Oviposition by Navel Orangeworm Moths (Lepidoptera: Pyralidae)

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Abstract

A rotating wheel olfactometer was adapted for laboratory evaluation of oviposition attractants for the navel orangeworm, Paramyelois transitella (Walker). The bioassays were conducted inside a large screen cage in a glasshouse with controlled temperature and humidity. Flying female moths responded to attractant volatiles emitted from egg traps on the olfactometer and oviposited on the surface of the traps.

The attraction to, and subsequent oviposition on, almond nuts by female navel orangeworm moths, Paramyelois transitella (Walker), has been well documented (Wade 1961; Caltagirone, et al. 1968; Curtis and Barnes 1977). Following the discovery that the navel orangeworm (NOW) also responds to non-host sources of the attractant and oviposits on synthetic substrates (Rice 1976), research was initiated to identify the chemicals responsible for host-finding and/or oviposition. This paper describes a bioassay technique to screen candidate NOW oviposition attractants. Initial laboratory bioassay tests with Y-tube olfactometers and small sleeve cages proved unsatisfactory because females tended to oviposit indiscriminately when influenced by oviposition stimuli in confined conditions.

Materials and Methods

A walk-in screened cage was constructed in a standard glasshouse at the San Joaquin Valley Agricultural Research and Extension Center, Parlier, CA. The screen material was 16-mesh mosquito netting. The cage was 2.1 m high, 3.1 m wide and 5.5 m long.

The bioassay apparatus, which was placed inside the cage, was a modified version of the revolving wheel olfactometer described by Tashiro et al. (1969) for work with scale insect pheromones. The plywood wheel of the olfactometer (Fig. 1) had a diameter of 1.22 m. Twelve stiff wires were placed in holes ca. 0.3 m apart around the perimeter of the wheel and adjusted so their tips were 0.46 m from the edge, thereby giving the olfactometer a diameter of 2.13 m. The tips of the wires were ca. 0.55 m apart around the periphery of the wheel. The wheel was placed on a phonograph turntable and rotated at 5.0 rph by a barbeque rotisserie motor.

Test materials were placed inside standard NOW egg traps (Pherocon IV traps, Zoëcon Industries, Palo Alto, CA.) suspended from the tips of the wires. Solid materials, e.g. nut meats, were placed directly on the bottom of the traps; ground or pulverized materials were placed in shallow steel planchets in the traps. Liquids were pipetted onto filter paper in planchets. Usually 3 or 4 replicates of each material were randomized on the olfactometer, thus allowing 3 or 4 materials to be bioassayed in each trial. A "standard" treatment, consisting of 2 to 15 g/rep of wheat bran and water (mixed 1:1 w/w), was usually included in each bioassay. A population of 35-50 mated female NOW moths of mixed ages was maintained in the cage for most bioassays. While it was recognized that differing numbers of eggs were being laid during each test, efforts to control precisely the maternal age, number of females present, and eggs laid were considered impractical. The glasshouse was maintained at 22-28°C, 30-50% R.H., and natural photoperiod. Tests were normally started just prior to sunset and were allowed to run until about 8:00 a.m. the following morning.

Results and Discussion

Moths responded to attractant odors by laying eggs on the trap surfaces, similar to responses obtained in field studies on NOW oviposition (Rice et al. 1976). Table 1 shows the range of response to various treatments by ovipositing females.

Test 1 indicates that of the four components of standard NOW bait (Rice 1976), wheat bran is the source of oviposition stimulus. However, glycerine and water are necessary to maintain moisture levels for long-term field use of the bait. Test 2 shows that production and release of attractant volatiles and/or oviposition stimuli are not related to ageing (or fermentation) of the bran. Test 3 shows that relatively

small amounts of bran can induce oviposition. Test 4 shows that the response of ovipositing females to bran was not reduced by the presence of two volatile synthetic chemicals. Similar results were observed with other synthetics and organic solvents.

Test 5 compares a natural host source of the oviposition attractant (almonds) to a non-host source (bran). In this test water was also added to the check traps and some oviposition occurred on them, in contrast to previous tests (test 1). However, test 5 was conducted with an excessive number (ca. 100) of female moths in the cage. In this situation females tend to oviposit more heavily than usual on nonattractive substances and checks, perhaps because of stimulation by attractants and subsequent competition for oviposition sites. Tests 6 and 7 show that an oviposition attractant can be extracted from ground almond meats by organic solvents. Various quantities of bran were used in different tests to establish a standard amount that would provide consistent attraction of moths, but also minimize competition with other treatments. Two g of bran and water have been used as a standard in most recent bioassays.

Rotation of the olfactometer was very important. If the wheel stopped during a test, a strong position effect became apparent among the replicates. This made it difficult to analyze the data and detect differences among treatments.

This bioassay technique is suitable for screening potential NOW oviposition attractants and evaluating them under conditions similar to those in the field. The object of this work is to use synthetic attractants in a NOW management system. Prospective uses include monitoring populations with standard commercial egg traps and inducing oviposition and suicidal hatch of eggs on nonfood substrates.

Acknowledgment

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Figure Caption

Fig. 1a. Rotating wheel olfactometer with egg traps in place.

Fig. 1b. Barbeque rotisserie drive assembly for olfactometer, showing pressure sensitive contact wheel.

Table I. Response of ovipositing navel orangeworm moths to various substances. Parlier, CA. 1977-78.

Test No.	Treatment	No. eggs deposited[1/]
1	5 ml H_2O	1.7a
	5 ml glycerine	2.7a
	5 ml honey + H_2O (1:1)	2.7a
	7.5 g bran + H_2O	27.7 b
2	15 g bran + H_2O, aged 0 days	91.0a
	15 g bran + H_2O, aged 2 days	78.3a
	15 g bran + H_2O, aged 5 days	68.0a
	15 g bran + H_2O, aged 8 days	81.3a
3	Blank trap	3.3a
	3.8 g bran + H_2O	19.0 b
	7.5 g bran + H_2O	25.0 b
	15.0 g bran + H_2O	31.3 b
4	Blank trap (check)	.0.0a
	10.0 µl pentane	0.0a
	10.0 µl n-pentanal	0.0a
	7.5 g bran + H_2O	25.7 b
5	2.0 ml H_2O	35.5a
	2.0 g ground almond meats + H_2O	129.8 b
	2.0 g bran + H_2O	84.8 b
6	Blank trap	3.3a
	10.0 µl ether extract of almond meats	49.7 b
	3.8 g bran + H_2O	90.7 b
7	Blank trap	1.8a
	10.0 µl methanol extract of almond meats	14.5 b
	2.0 g bran + H_2O	26.5 b

1/Means of 3 or 4 replicates per treatment. Means followed by different letters are significantly different at P = 0.05 (Duncan's new multiple range test).