Historic, Archive Document

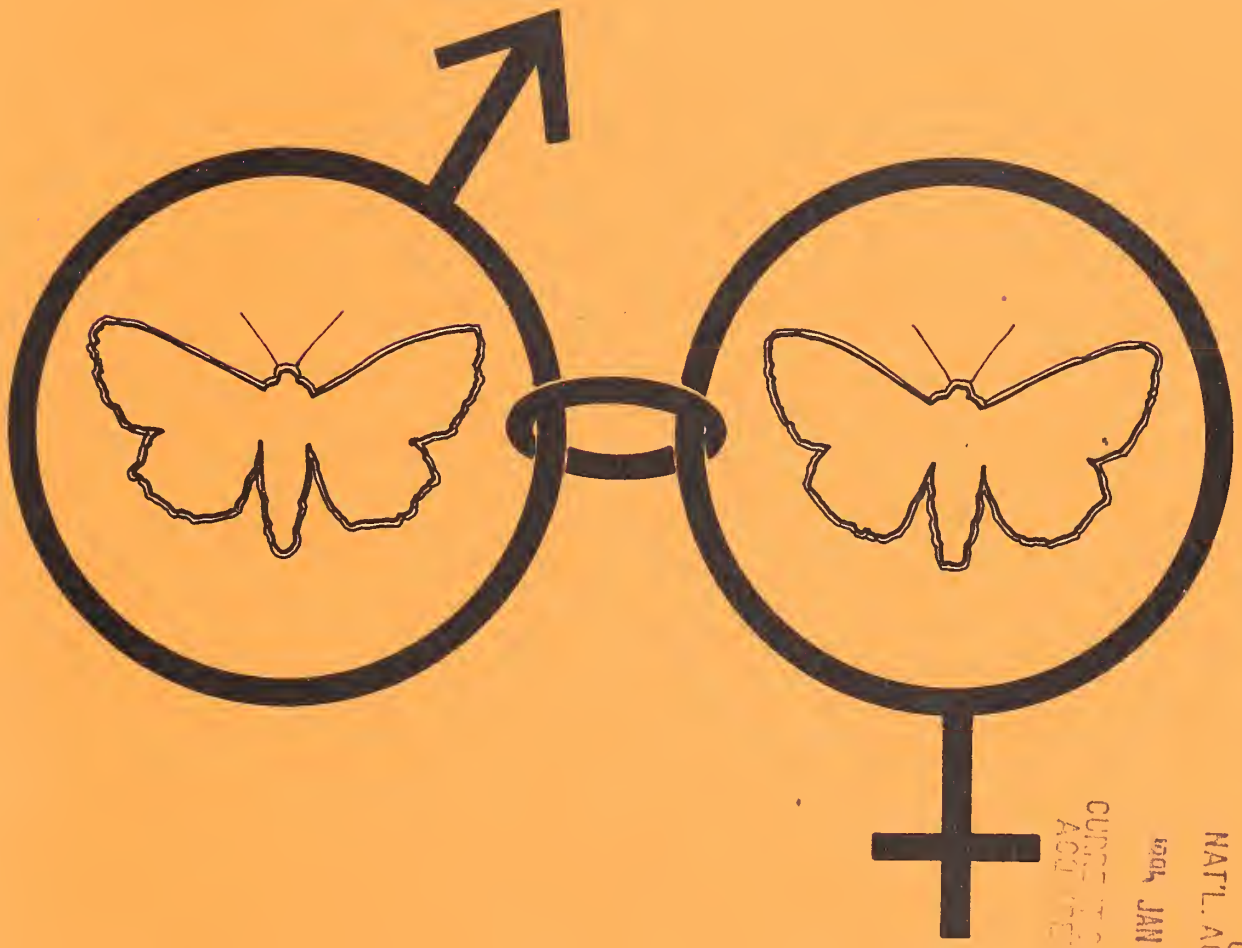
Do not assume content reflects current scientific knowledge, policies, or practices.

aQL469
.U5

INSECT ATTRACTANTS, BEHAVIOR, AND BASIC BIOLOGY RESEARCH LABORATORY

Gainesville, Florida

ANNUAL REPORT–1986



USDA
NAT'L. AGRIC. LIBRARY
1986 JAN 15 P 8:05
CURRENT SERIAL RECORDS
AGRICULTURAL RESEARCH SERVICE

AGRICULTURAL RESEARCH SERVICE
U.S. Department of Agriculture
South Atlantic Area

FOR OFFICIAL USE ONLY

FOR OFFICIAL USE ONLY

ANNUAL REPORT

1986

Insect Attractants, Behavior, and Basic Biology

Research Laboratory

Agricultural Research Service

United States Department of Agriculture

P.O. Box 14565, Gainesville, Florida 32604

This report includes results of research in progress. Such findings when adequately confirmed will be released through established channels. Therefore, this report is not intended for publication and should not be referred to in literature citations.

February 24, 1987

The mission of the Insect Attractants, Behavior and Basic Biology Research Laboratory is to conduct research on the biological processes that promote and maintain insect populations so that these mechanisms may be manipulated for pest management. Our research is based on the premise that biorational pest control requires basic research on unique vulnerabilities in the insect life cycle. The research effort is conducted in five Research Units that focus on the biochemical, genetic, physiological, chemical and physical factors that regulate the behavior, reproduction, development and ecology of pest and beneficial insects.

This year's annual report is based on material submitted by our scientific staff, as well as visiting and cooperating scientists. Interested scientists are welcome to correspond directly with our staff (listed on next page) if they have questions about a particular report. A list of the laboratory's publications for 1986 are found in the Appendix. Reprints may be requested by number.

Herbert Oberlander

HERBERT OBERLANDER
Laboratory Director

Insect Attractants, Behavior and Basic Biology Research Laboratory
P.O. Box 14565
Gainesville, Florida 32604
Dr. Herbert Oberlander, Director

Scientific Staff

Behavior

Dr. C. O. Calkins, Research Leader
Dr. K. A. Draz, Visiting Scientist
Dr. P. J. Landolt, Research Entomologist
Dr. N. C. Leppla, Research Entomologist
Dr. W. J. Lewis[1], Research Entomologist
Dr. J. Sivinski, Research Entomologist
Dr. D. Whitman, Research Affiliate

Behavioral Ecology and Reproduction

Dr. E. R. Mitchell, Research Leader
Dr. T. R. Ashley, Research Entomologist
Dr. J. A. Coffelt, Research Entomologist
Dr. J. R. McLaughlin, Research Entomologist
Dr. E. Roberts, Visiting Scientist
Dr. P. D. Shirk, Research Physiologist
Mr. F. C. Tingle, Research Entomologist
Dr. K. W. Vick, Research Entomologist

Biophysics and Neurophysiology

Dr. J. C. Webb, Research Leader
Dr. H. R. Agee, Research Entomologist
Dr. J. J. Gaffney, Agricultural Engineer
Dr. R. W. Mankin, Research Entomologist
Dr. M. S. Mayer, Research Entomologist
Dr. I. Moore, Visiting Scientist
Dr. E. Orona, Research Associate
Mr. D. C. Slaughter, General Engineer

Bioregulation and Molecular Genetics

Dr. H. Oberlander, Research Leader
Dr. D. W. Bean, Research Associate
Dr. S. M. Ferkovich, Research Entomologist
Dr. P. D. Greany, Research Entomologist
Dr. R. F. Leclerc, Research Associate
Dr. A. M. Handler, Research Geneticist
Dr. S. G. Miller, Research Geneticist
Dr. D. A. O'Brochta, Research Associate
Dr. P. Porcheron, Visiting Scientist
Dr. D. L. Silhacek, Research Chemist

Chemistry

Dr. J. H. Tumlinson, Research Leader
Dr. R. E. Doolittle, Research Chemist
Mr. R. R. Heath, Research Chemist
Dr. S. B. Krasnoff, Research Affiliate
Dr. P. E. A. Teal, Research Scientist (University of Florida)

[1]Tifton, GA

Commercial Phone Numbers for Scientists - (904) 374-

Dr. Herndon Agee	5737
Dr. Thomas Ashley	5761
Dr. Daniel Bean	5759
Dr. Carrol Calkins	5753
Dr. James Coffelt	5719
Dr. Robert Doolittle	5723
Dr. Steven Ferkovich	5767
Mr. Jerome Gaffney	5726
Dr. Patrick Greany	5763
Dr. Alfred Handler	5793
Mr. Robert Heath	5735
Dr. Douglas Whitman	912-382-6904
Dr. Robert Leclerc	5705
Dr. Peter Landolt	5756
Dr. Norman Leppla	5747
Dr. Wallace J. Lewis	912-382-6904
Dr. Richard Mankin	5774
Dr. Marion Mayer	5752
Dr. John McLaughlin	5795
Dr. Steven Miller	5728
Dr. Everett Mitchell	5710
Dr. Herbert Oberlander	5700
Dr. David O'Brochta	5705
Dr. Edward Orona	5738
Dr. Paul Shirk	5720
Dr. Donald Silhacek	5758
Mr. David Slaughter	5751
Dr. Robert Tilden	5779
Mr. Fred Tingle	5769
Dr. James Tumlinson	5730
Dr. Kenneth Vick	5772
Dr. J. C. Webb	5740

To call FTS - 947-7 + list three digets

Contents

Behavior

Chemosystematics of Anastrepha spp. (Diptera:Tephritidae)

D. A. Carlson[1], C. O. Calkins, E. Morales M.[2] and D. H. Habeck[3]

Objectives: To determine if different species of Anastrepha have unique molecular patterns of cuticular hydrocarbons that could be used for species identification, and to determine if these patterns are the same for larval, pupal, and adult stages.

Methods: Gas Chromatography (GC) techniques are used for analysis of cuticular hydrocarbons. The samples are immersed in n-hexane for extraction of cuticular lipids. The washings are then passed through a silica column, and the fractions collected and analyzed on a gas chromatograph with DB-1 columns. Data analysis (pattern recognition, discriminant analysis, stepwise discriminant analysis, etc) of each sample is conducted to establish whther there was a unique pattern.

Results: Important questions have been answered about the changes in cuticular hydrocarbons with regard to age and diet of one Anastrepha species. Analyses of larval hydrocarbons other than A. suspensa have been done on a few wild samples and on two shipments of A. obliqua larvae (the only useful samples from OIRSA). Although the few larvae analyzed show differences in the hydrocarbon pattern, more samples of good quality are needed to obtain good results. A study was done on six different groups of A. suspensa adults according to sex and diet: no diet, regular diet, and sugar diet. Also, selected peak ratios show the similarities among the cuticular hydrocarbons of all groups until 72 hours after emergence, when the production of hydrocarbons changes. The hydrocarbon components with Kovats Index of 3065 and 3250 are produced in larger quantities than the other components in the cuticle and increase as a function of time. Samples of A. ludens and A. obliqua which appeared aged show the presence of a major component with KI of 3042, which is a minor component in A. suspensa, A. striata, and A. serpentina. These are preliminary results. More specimens with accurate records of age and diet are needed to confirm this observation of a unique species-specific compound.

Plans: This study will continue one more year.

[1]Insects Affecting Man and Animal Research Laboratory, Gainesville, Florida
[2]Organismo International, Regional de Sanidad Agropecuaria (OIRSA), San Jose, Costa Rica.
[3]Department of Entomology, University of Florida, Gainesville, Florida

Special Distribution of Sexually Signalling and Mate-Searching Caribbean Fruit Fly, Anastrepha suspensa

J. M. Sivinski

Objectives: To describe details of the lek mating system, i.e., to discover if male signalling location is influenced by male-male aggression and female choice.

Methods: The distribution of virgin males and females was examined in a field cage that contained a tree marked into 15-cm sections. In the field, the positions of males and females were noted relative to the end of branch tips. Aggregations of males and females were captured, and the size of the fly and its position in the group were recorded. In order to determine the distribution of flies in the field, their locations were marked and the degree of clumping was calculated.

Results: Male and female flies had a clumped distribution in the foliage of their guava host plants. Males were no closer to other males than they were to females or than females were to other females. Flies were found often in roughly the same locations over time. However, contemporaries were closer to previous males than subsequent females were to their predecessors. Males were more likely to be found together or with females than were females with other females. Some trees had more flies than others, but there was no regional (northwestern, etc.) preference within trees. Females were no more likely to be found in the vicinity of clumped (lekking) males than near isolated males. About a third of the females taken from inside "leks" had sperm in their spermathecae. In pairs of males within 15 cm of each other, the larger fly tends to be in a position farther up the branch. This may be due to larger males successfully defending preferred territories, i.e., those more attractive to females. Caribbean fruit fly distributions in the field were not entirely consistent with expectations of a simple lek model based on female choice, i.e., the existence of all-female groups, no indication of aggregated male attractiveness, and mated females in leks. It seems possible that males attempting to intercept females accumulate in favorable microhabitats and that leks result from such clumping.

Plans: The qualities; i.e., light, humidity, wind, predator-density, etc.; of spots where females aggregate, need to be determined. Female preference for grouped males, it it occurs, may be best demonstrated in the laboratory using massed vs. single broadcasts of either acoustic or pheromonal displays.

Changes in Female Caribbean Fruit Fly Reproduction Behavior Brought About Through Oviposition

J. M. Sivinski

Objectives: To determine the rate of remating by female Caribbean fruit flies as influenced by the presence of oviposition sites. A number of female characteristics potentially important to detection and control could be changed by ovipositional opportunities. These include mating frequency, life span, and response to pheromones.

Methods: One hundred individual females were caged with food and water and provided access to a male either on a daily or weekly basis. Roughly half had a wax oviposition dome included in order to encourage egg-laying. Sexual behaviors and life spans were noted.

Results: Females with oviposition sites were more likely to remate. Sixty-seven percent of the egg-laying flies exposed to wax domes remated a week following the initial copulation, compared to 10% for those who did not have access to domes. About a third of the egg-laying females mated again on each subsequent week, while those without domes maintained a 10% remating rate. Multiple copulations were typical of flies with oviposition sites. Egg-laying females also mated for longer periods of time. However, the mortality rate was greater for ovipositioning females. Females examined on a daily basis showed a great deal of variation in remating rates.

Plans: The response to pheromones by female flies who have oviposited freely will be compared to that of non-egg-laying females. The implication of multiple mating for sterile-release statistics will be investigated.

Changes in Levels of Naringin and Limonin in Grapefruit Peel Associated with Fruit Maturity and Caribbean Fruit Fly Susceptibility

P. E. Shaw[1], C. O. Calkins, and J. C. Webb

Objectives: To determine if levels of naringin and limonin in the peel decrease with maturity of grapefruit and correlate with increased susceptibility of fruit to attack by the Caribbean fruit fly.

Methods: Fruit were picked weekly from a citrus grove near Merritt Island, Florida, from October 3, 1985, to April 30, 1986. Thirty grapefruit were used each week for the chemical analysis. Naringin values were determined in each extract by the Davis test. Limonin values were determined using the enzyme immunoassay method. Fifty fruit were used each week to determine susceptibility to Caribbean fruit fly attack; 30 fruit were multi-infested (exposed to several mature females for 4 hours), while 20 were infested with a single oviposition. All fruit were held at 27° C and 80% RH. On the fourth day following oviposition, the fruit were examined acoustically for presence of feeding larvae and once thereafter every 24 hours, until the 11th day. After 11 days, any fruit that did not exhibit feeding activity were discarded.

Results: Naringin and limonin values in grapefruit albedo generally decreased with maturity, following the trends observed for these bitter components in grapefruit juice (Tagum et al., 1972; Table 1). Periodically, large decreases in naringin content occurred, as indicated by values underlined in the Table. All but the last sample of April 14, 1986, had unusually large limonin contents. The importance of the periodic differences in content of these two bitter components is unknown. The limonin content in albedo was within the range reported earlier. Levels of naringin in albedo have not been reported previously. Limonin and several other liminoids are known insect-feedig deterrents. No insect antifeedant activity has been associated with the other major bitter component, naringin. The aglycon of naringin, naringenin, does affect insect growth, however. The success of establishment of larvae in grapefruit under two infestation methods is shown in Table 1. Although the mass-infestation rate was high and varied throughout the season, the single infestation rate increased as the fruit continued to ripen and the levels of limonin and naringin decreased.

Plans: Fruit will be collected during 1986-1987. Chemical analyses will be conducted and infestation rates determined to further elucidate the correlations. Naringin and limonin will be tested in artificial diets to determine their effects on larval feeding.

[1]Citrus and Subtropical Products Research Lab, USDA, ARS, Winter Haven, FL

Table 1. Successful infestation rates of Caribbean fruit flies and levels of naringin and limonin in grapefruit throughout the ripening season.

Week Number	% Single-Infested	% Mass-Infested	Naringin ppm	Limonin ppm
1[a]	5.56	45	29,680	91
3	15.00	100	29,440	160
4	20.00	80	31,360	142
5	36.67	85	31,360	114
6	33.33	75	29,920	182
7	40.00	100	30,080	117
10	33.33	89	29,600	125
11	26.67	100	27,920	90
12	13.33	100	24,480	195
13	16.67	100	24,320	69
14	23.33	95	29,120	48
15	40.00	75	28,640	46
16	40.00	100	26,400	35
17	46.67	100	27,840	14
18	60.00	95	22,880	48
19	56.67	95	24,800	18
20	51.72	100	25,280	29
25	40.00	85	22,560	22
26	20.00	85	23,360	16
27[b]	32.14	100	22,480	21

[a] October 31, 1986
[b] April 30, 1986

Pheromone-Mediated Alarm and Attack Behavior in the Southern Yellowjacket, Vespula squamosa

P. J. Landolt and R. R. Heath

Objectives: To determine if alarm behavior observed in V. squamosa is chemically communicated and, if so, to identify the glandular source for chemical isolation and identification.

Methods: Methylene chloride extracts of V. squamosa workers and body parts were applied on filter paper to black 1-gallon paper cans coated with Tacktrap® about 2 m from a colony of V. squamosa. Numbers of attacking wasps caught in the Tacktrap were counted after 2 min. Whole-body extracts were compared to solvent controls, extracts of abdomens were compared to extracts of heads and thoraces, and extracts of the venom sac and gland were compared to extracts of the remainder of the sting apparatus.

Results: Wasps attacked the treated cans in response to wasp whole-body extracts, gaster extracts, and venom sac extracts. Significant responses were not obtained with methylene chloride extracts of the head and thorax, or of the remainder of the sting apparatus (Table 1). Behavior observed in pheromone-elicited attacks included recruitment from the nest, flight initiation, upwind-oriented flight to the source, zigzagging flight and hovering close to the source, and attack behavior.

Plans: The alarm pheromone has been identified by Heath and Landolt. Further tests will be made on the eastern yellowjacket, Vespula maculifrons, to determine if chemically mediated alarm and attack behavior occurs in this species also.

Table 1. Mean number of Vespula squamosa workers trapped within 2 min of application of extracts to filter paper. All extracts were at five wasp-equivalent dosages.

Extract[a]	n	Mean ± SE
Solvent	5	0.4 ± 0.2 a
Whole Wasp	5	13.2 ± 5.6 b
Solvent	10	0.0 ± 0.0 a
Head and Thorax	5	0.2 ± 0.2 a
Gaster	5	43.0 ± 33.8 b
Solvent	14	0.0 ± 0.0 a
Venom Gland and Reservoir	7	9.1 ± 2.6 b
Sting Apparatus	7	1.9 ± 1.7

[a]Means within comparisons followed by the same letter are not significantly different at P>0.05 (Student's t-test).

Effects of Time of Day, Age, Mating, and Release Rate on Female Papaya Fruit Response to Synthetic Sex Pheromone

P. J. Landolt and R. R. Heath

Objectives: To determine how the response of female papaya fruit flies to 2,6-methylvinyl pyrazine (2,6-MVP) changes with time of day, age, and after mating; and to determine the optimum release rate for the response to sex-pheromone.

Methods: Pheromone (2,6-methylvinyl pyrazine) was formulated in glass capillary tubules of different diameters to obtain different release rates. A wind-tunnel bioassay was used to evaluate the sex pheromone response with flies scored for plume-tracking, close-range hovering, and source contact. Mature unmated papaya fruit flies were tested for their response to various release rates of 2,6-MVP from glass capillaries. The optimum release rate was then used in subsequent tests. Mature unmated females were tested every 2 hr from lights-on to lights-off. Females of known ages were tested daily from day 1 to day 10, when they were mated; and from day 11 to day 14, using the optimum release rate and optimum time of day. Since mated females responded to sex pheromone, mature mated females were then tested twice an hour from lights-on to lights-off to compare with the diel response pattern of mature unmated females.

Results: Release rates of 400 to 1,200 nanograms of 2,6-MVP per hour yielded optimum response percentages for plume-tracking, close-range hovering, and source contact. Females were responsive in the flight tunnel to sex pheromone beginning at 5 days of age and peaking at 7-8 days of age. Mated females were also attracted by 2,6-MVP. Optimum response to pheromone was obtained from 5 to 7 hr into a 12-hr photophase for unmated females, and 5 to 11 hr into the photophase for mated females.

Plans: Work on the development of a trapping system for papaya fruit flies is continuing, using a combination of sex pheromone and attractive visual stimuli. Responses of mated female papaya fruit flies to sex pheromone are being investigated.

Develcpment of a Pheromone-Based Trapping System for the Papaya Fruit Fly

P. J. Landolt, R. R. Heath, H. R. Agee,
J. H. Tumlinson, and C. O. Calkins

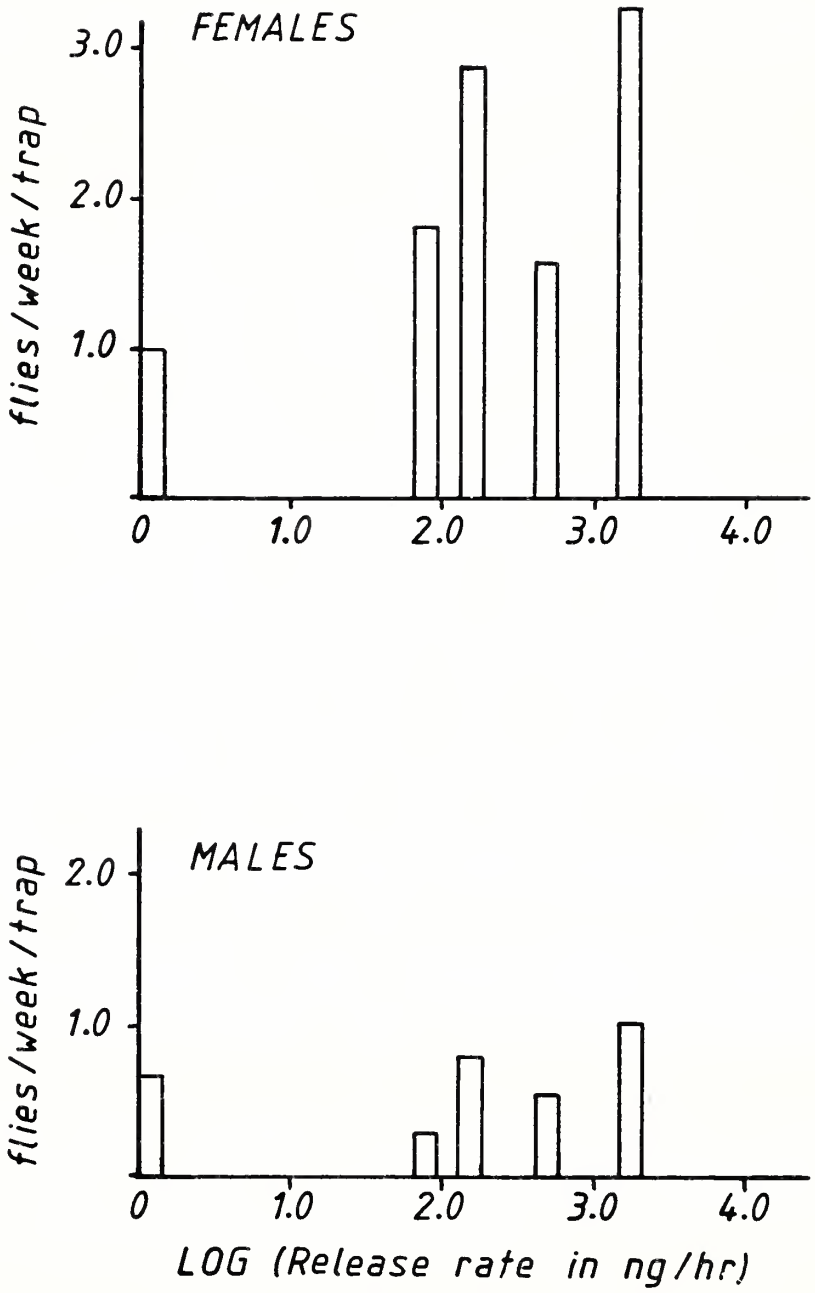
Objectives: To demonstrate the attractiveness of the papaya fruit fly pheromone, 2,6-methylvinyl pyrazine to papaya fruit flies in the field and to develop a method to monitor papaya fruit fly activity in commercial papaya groves.

Methods: A trap developed at the USDA Insect Attractants Laboratory specifically for the papaya fruit fly was tested in four commercial papaya groves in Dade County, Florida. Pheromone was tested in these traps in a formulation providing five release rates of 2,6-methylvinyl pyrazine (0, 90, 200, 400, or 1,200 ng/hr). A randomized complete block design was used with four blocks, each in a different grove. Traps were checked three times per week and replaced weekly for 3 weeks. Data were summarized as weekly trap catch totals.

Results: Female papaya fruit flies were caught on traps with all release rates, including no pheromone. A bimodal response was observed to increasing release rates, with significantly higher trap catches at 200 and 1,200 ng/hr of 2,6-methylvinyl pyrazine (Fig. 1). Males were also caught in traps at all release rates tested. However, none were significantly greater than in the unbaited control.

Plans: Experiments are underway to investigate the bimodal nature of the dose-response observed. Further work will be conducted to determine the potential for control through trapping.

Figure 1


FEMALES
3.0
2.0
1.0
flies/week/trap
0
1.0
2.0
3.0
4.0
MALES
2.0
1.0
flies/week/trap
0
1.0
2.0
3.0
4.0
LOG (Release rate in ng/hr)

Temporal Factors in Reproductive Isolation between *Harrisina americana* and *Acoloithus falsarius*

P. J. Landolt and R. R. Heath

Objectives: To determine if the grapeleaf skeletonizer *Harrisina americana*, and *Acoloithus falsarius*, are sexually isolated by seasonal patterns of adult activity, or diel rhythms in their sex-pheromone response. Both species are attracted to R-(-)-2-butyl (Z)-7-tetradecenoate and are broadly sympatric.

Methods: Male sex-pheromone activity was monitored in Gainesville, Florida, with Pherocon IC traps baited with 500 micrograms of R-(-)-2-butyl (Z)-7-tetradecenoate on rubber septa. Traps were maintained from January to December 1986. To compare seasonal activity, trap catches for both species were recorded as weekly totals. To compare diel activity patterns, traps were checked and numbers caught were recorded hourly from sunrise to sunset on each of 8 days in May 1986.

Results: Three generations or flights were evident from the trap-catch data for the grapeleaf skeletonizer (GLS) during April, June/July, and August/September (Fig. 1). Two generations were evident from *A. falsarius* in April and September (Fig. 2). There were broad overlaps in their seasonal distributions. The two species were separated, however, in their response to sex pheromone by time of day. The GLS males were trapped principally in the early morning hours, while *A. falsarius* males were trapped in mid-afternoon (Figure 3).

Plans: This study is completed. A second, rarer species of *Acoloithus* found in northern Florida, will be studied if a population is located.

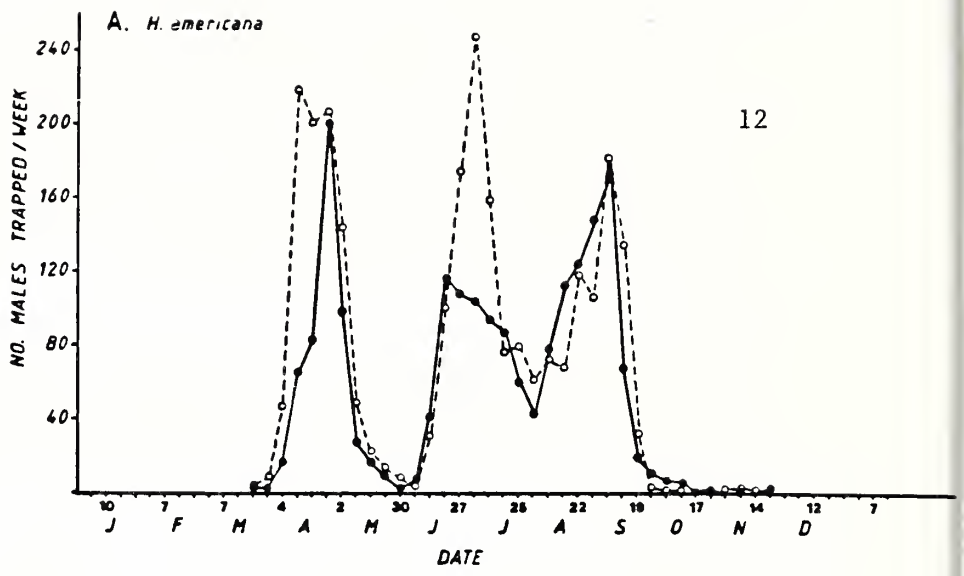


Figure 1

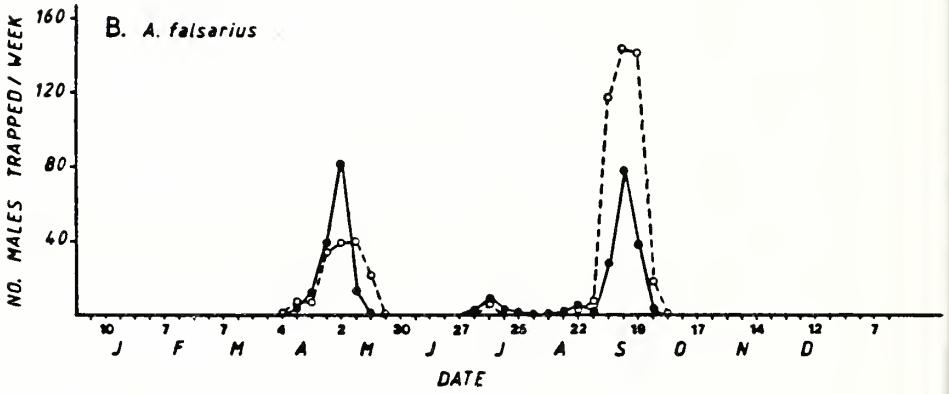


Figure 2

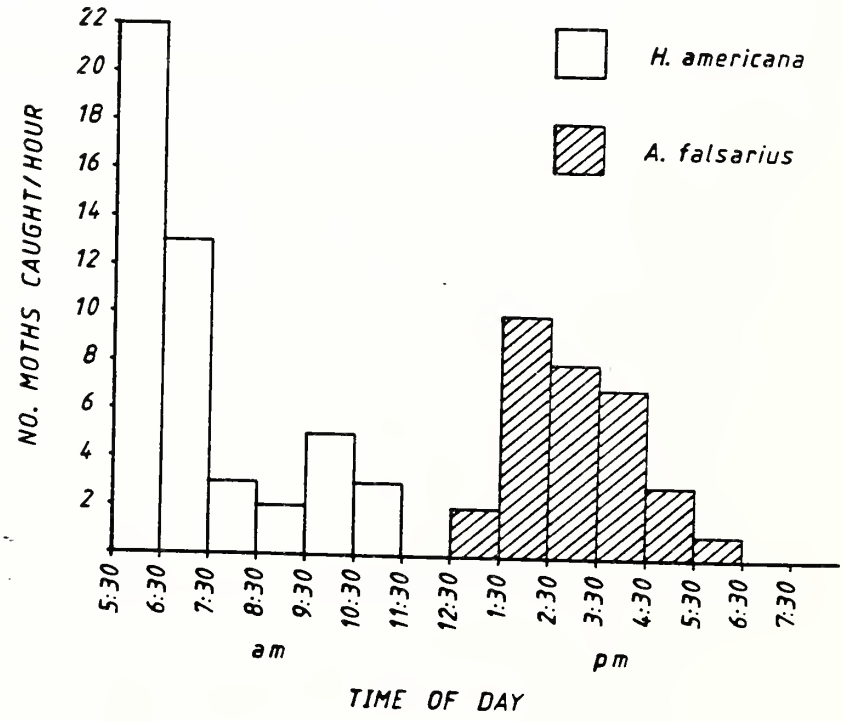


Figure 3

Enantiomers of 2-Butyl (Z)-7-Tetradecenoate as Sex Pheromones/Attractants of Zygaenid moths

P. J. Landolt and R. R. Heath

Objectives: To determine if compounds, identified as sex pheromones/attractants of the western grapeleaf skeletonizer and grapeleaf skeletonizer, are sex attractants for other species within the family, and if any enantiomeric specificity follows taxonomic or zoogeographic lines.

Methods: The R-(-) and S-(+) enantiomers of 2-butyl (Z)-7-tetradecenoate (each greater than 99% purity) were formulated in rubber septa at 500 micrograms/septum. Plastic bucket traps were baited with loaded septa and set up at field sites as trap pairs (one with each enantiomer). Trapping was conducted in Gainesville, Homestead, and Key Largo, Florida; Retalhuleu, Guatemala; Waco, Texas; Fresno, California; Cochise Co., Arizona; St. Croix, Virgin Islands; Cayman Islands; and Whitman Co., Washington.

Results: A total of ten species of Zygaenidae have been collected to date in traps baited with R-(-)- or S-(+)-2-butyl (Z)-7-tetradecenoate. In California, Arizona, and Guatemala, the six species collected were attracted to the S-(+) enantiomer; and in Waco, Texas, one species was attracted to the S(+) and one to the R(-). The two species in Florida were caught in traps baited with the R-(-) enantiomer. Taxonomic determinations have not yet been made on seven of the ten species collected. No zygaenids were collected on St. Croix, the Cayman Islands, or in Whitman County, Washington.

Plans: Efforts will continue to add species to the list by trapping in other areas when possible and to obtain species determinations on material collected to date.

Trapping the Western Grapeleaf Skeletonizer, Harrisina brillians Barnes & McDunnough, with S-(+)-2-Butyl (Z)-7-Tetradecenoate

C. E. Curtis[1], P. J. Landolt, and R. R. Heath

Objectives: To determine the optimum dosage of sex pheromone for trapping male western grapeleaf skeletonizer (WGLS) moths and the effects of contamination of the active S-(+) enantiomer with the R-(-) enantiomer of 2-butyl (Z)-7-tetradecenoate on male WGLS attraction.

Methods: Pherocon IC traps baited with pheromone formulated in rubber septa were set up in a grape vineyard near Fresno, California, in two trapping experiments. The first test was a comparison of dosages of each enantiomer of 2-butyl (Z)-7-tetradecenoate. The dosages were 0, 20, 100, 500, 1,000, and 5,000 micrograms per septum. The second test was a comparison of attractiveness of the optimum dosage of the S-(+) enantiomer with increasing contamination with the R-(-) enantiomer. With the S-(+) enantiomer dosage held at 500 micrograms/septum, 0, 15, 50, 150, and 500 micrograms of the R-(-) enantiomer added per septum were tested. In both experiments a randomized complete block design was used. Traps were checked every half hour or hour during the flight period. Traps baited with two virgin female WGLS were included in some blocks of both experiments. The three blocks of Test A were run for 6 days (18 replicates), as were the three blocks of Test B.

Results: Traps baited with 500 micrograms of S-(+)-2-butyl (Z)-7-tetradecenoate caught more males than traps baited with any other dosage. At all dosages, catches of male WGLS in R-(-) enantiomer-baited traps were very small relative to those in S-(+) enantiomer-baited traps. Addition of the R-(-) enantiomer to the 500-microgram dosage of the S-(+) enantiomer had no effect at 3 or 10% contamination, but significantly reduced trap catches at 30 or 50%. Female-baited traps caught 2.3 times more male WGLS than the best pheromone-baited traps (500 micrograms of S-(+)-2-butyl (Z)-7-tetradecenoate/septum).

Plans: These results suggest the existence of additional components in the sex pheromone of female WGLS that might account for the greater attractiveness of females versus S-(+)-2-butyl (Z)-7-tetradecenoate. Female-produced pheromone should be further analyzed for additional components.

[1]Ecology & Biology/Protection & Quarantine, ARS, USDA, Fresno, California

A Pheromone-Based Trapping System for the Grass Looper, *Mocis latipes*

P. J. Landolt and R. R. Heath

Objectives: To determine the field attractiveness of female pheromone components and to develop a method of monitoring *Mocis latipes* populations.

Methods: Three pheromone blends were tested in rubber septa placed in wire-mesh cone traps or plastic bucket traps. Blend A was a 95:5 mix of (Z,Z,Z)-3,6,9-heneicosatriene and (Z,Z,Z)-3,6,9-eicosatriene. Blend B was a 70:25:5 mix of (Z,Z,Z)-3,6,9-heneicosatriene, (Z,Z)-6,9-heneicosadiene, and (Z,Z,Z)-3,6,9-eicosatriene. Blend C was a 70:30 mix of (Z,Z,Z)-3,6,9-heneicosatriene and (Z,Z)-6,9-heneicosadiene. All three compounds have been reported to be *M. latipes* pheromone components. Septa were loaded with 200 micrograms of a mix. Treatments were randomized initially within trap blocks and traps were rotated one position daily for 6 days.

Results: The greatest number of male *M. latipes* was caught in traps baited with a 200-microgram dosage of Blend C (188 moths/trap/day). Traps baited with Blend B caught 139 male *M. latipes* per trap per day, and those baited with Blend A caught 48.5 male *M. latipes* per trap per day.

Plans: More extensive tests are planned to evaluate other dosages and pheromone blends for attractiveness to *M. latipes* and other *Mocis* sp. males.

Biological Correlates of Learning in the Insect Brain

J. M. Sivinski

Objectives: To use the insect brain structure to predict the relative ability of an insect to learn; particularly, the memorization of qualities of the opposite sex in order to compare potential mates. Protein synthesis during learning occurs in paired brain structures, the corpora pedunculata. If these structures differ predictably in insects with known differences in their ability to learn, then brain differences in species whose learning abilities are unknown may be used to predict behavior. In fruit flies such information might be used to infer the importance of territoriality, lekking, and mate-choice in a particular species.

Methods: Three closely related species of Lepidoptera with large differences in their learning ability were chosen to demonstrate the feasibility of such a study. Multiple brains of each sex were sectioned and stained, and the corpora pedunculata size was compared. Four other species with differing feeding strategies were similarly examined to see if brain structure could be predicted from a species' ecology.

Results: Among three closely related Lepidoptera, the species with the greatest ability to learn had the largest corpora pedunculata. In the other four species, the most generalized feeders had the largest mushroom bodies. This may result from generalists having a more unpredictable environment as to which potential foods are most abundant. It might be profitable to learn the most efficiently exploitable host in a particular locale.

Plans: Diptera with various mating systems will be examined. Particular notice will be made of any sexual dimorphism that might reveal extensive female mate choice or male territoriality.

Sexual "Hot Spots" in the Environments of Flies

J. M. Sivinski

Objectives: To determine if aggregating and territorial Diptera prefer certain locations over time for their social interactions, and if so, what the attractive qualities of these "hot spots" might be. Such locations occur in Caribbean fruit flies, and comparison with the behaviors of other flies might further understanding of tephritid distribution.

Methods: Frequent collections were made of swarming, aggregating, and independently signaling flies, a chironomid, and a dolichopodid that use the same leaves for their respective aggregating, swarming, and territorial behaviors.

Results: The most complete observations to date are on two species of phorid flies, a chironomid, and a dolichopodid that use the same leaves for their respective aggregating, swarming, and territorial behaviors. The phorids are particularly interesting in that they display, in an exaggerated form, the female clumping found in Caribbean fruit fly. This type of extreme sex-role reversal is very rare and may present a previously undescribed mating system.

Plans: To continue collections and observations with emphasis on determining the distribution of tephritid flies.

Benomyl and Folpet as Antimicrobial Agents in Noctuid Larval Diet

F. Adams, M. R. Henderson, A. L. McAshan, and N. C. Leppla

Objective: To test folpet (N-(trichloromethylthio)phthalimide) and benomyl (Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate) for control of fungi in pinto bean/wheat-germ diet used to rear larvae of the velvetbean caterpillar, Anticarsia gemmatalis Hubner, and other noctuid moths.

Methods: Velvetbean caterpillar eggs obtained from a colony maintained in our laboratory for more than 5 years were added to diet-filled containers (300/lid). The diet was prepared for routine use in 18.9-liter batches from which 1.9-liter aliquots were transferred to a blender, combined with folpet or benomyl (1 g/3.8 liters), mixed for 2 min, and poured as equal volumes into two containers. After the diet cooled, lids with eggs were placed on the containers held for ca. 17 to 18 days in a development room maintained at 27° C and 50% RH with a 14-hour photophase. Standard diet served as a control and tests were replicated 3 times (6 containers per treatment).

Results: Both folpet and benomyl eliminated mold growth on the larval diet without affecting insect survival or size (Table 1). However, since the larvae required 1 or 2 extra days to pupate and benomyl reportedly reduces fertility, only folpet was used for routine rearing. During the 4-month period from September through December, folpet was used in 50 of the 100 containers established to rear VBC. A mean of 38% of the standard containers and 18% of those containing folpet had mold growth. Most of this contamination occurred after the containers were established, while they were being sampled for egg-hatch. Therefore, the addition of folpet and a change in larval rearing procedures virtually eliminated the mold problem.

Plans: Folpet (1 g/3.8 liters) will be added during preparation of larval diet for rearing noctuid moths when the incidence of mold reaches epidemic levels.

Table 1. Survival and size of the velvetbean caterpillar and incidence of mold in larval rearing containers using larval diets containing folpet and benomyl

Anti-microbial	% Egg Hatch	% Larvae Pupated	No. Pupae	Wt. Pupae	% Emerg.	% Cont. + Mold[a]
Folpet	91.4±0.8	70.8±26.0	153.5±66.0	273.2±11.6	85.0±0.1	0
Benomyl	91.2±0.8	69.8±18.0	145.8±47.5	277.9±12.0	86.0±0.6	0
Control	90.9±0.3	84.1± 6.3	210.1±65.1	280.1±12.6	79.0±0.8	12.0±3.2

[a] Statistically significant differences occurred only in the percentage of containers with mold.

Courtship of the Velvetbean Caterpillar Moth

N. C. Leppla, R. H. Guy, R. R. Heath and B. Dueben

Objective: To analyze the courtship of velvetbean caterpillar moths, Anticarsia gemmatalis Hubner, relative to the communicative functions of pheromones produced by males and females.

Methods: Ethograms were developed by video-recording the courtship sequences of 60 pairs of moths, describing their behavior into an audio recorder, and then transcribing the coded behaviors. Behavior patterns were organized into a matrix of successful, direct cycling, and unsuccessful courtship. The number of times each pattern occurred and the conditional probabilities of transitions between patterns were determined. These data were used to calculate the frequency with which males proceeded from one behavior pattern to the next in the courtship sequence.

Results: Courtship was composed of seven distinct behavior patterns: approach, display, orientation, thrust, envelopment, mounting, and copulation (Table 1). Most males passed from approach to display only once, 23/29 and 11/18 single transitions for successful and unsuccessful males, respectively. The most common transition, display to antennation, occurred an average of 4.5 times (130/29) for successful males and 13.8 times (249/18) for unsuccessful males. All 29 successful males made the transition from display to antennation more than once and 55% (10/18) of the unsuccessful ones made it more than five times. There was considerable cycling through the remainder of the sequence and successful males cycled much less frequently than those that were unsuccessful. The total number of transitions per respective transitional male was 2.5 (397/150) and 8.6 (712/83). The relative frequency with which successful and unsuccessful males returned from thrust to display was also indicative of the directness of their courtship (68/26 = 2.6 and 166/13 = 12.8). Thus, unsuccessful males remained so, regardless of their remarkable persistence.

Plans: This project has been completed.

Table 1. Number of successful cycling and unsuccessful (in parenthesis) male velvetbean caterpillar moths that proceeded from one behavior pattern to the next in the courtship sequence.

	Number of Transitional Males[a]					
Number of Transitions[b]	Ap Di	Di An	An Di	An T	T Di	T E
1	23 (11)	0 (4)	3 (2)	2 (4)	12 (1)	27 (6)
2	3 (4)	8 (0)	1 (0)	10 (0)	4 (1)	1 (2)
3	3 (2)	7 (2)	2 (1)	6 (3)	5 (1)	0 (0)
4	0 (1)	6 (2)	2 (0)	6 (1)	2 (2)	0 (1)
5+	0 (0)	8 (10)	0 (3)	5 (10)	3 (8)	1 (1)
n	29 (18)	29 (18)	8 (6)	29 (18)	26 (13)	29 (10)
t	38 (31)	130 (249)	19 (39)	108 (207)	68 (166)	34 (20)

[a] Males were considered transitional if they proceeded from one pattern to another: Ap/Di, approach to Display; Di/An, display to antennation; An/Di, antennation to display; An/T, antennation to thrust; T/Di, thrust to display; and T/E, thrust to envelopment.

[b] Total number of transitional males (n) and number of times (t) they passed between indicated patterns. Not included are those that they recycled, 9/29 successful and 13/18 unsuccessful males.

Quality Control in Insect Mass Production: A Review and Model

N. C. Leppla and T. R. Ashley

Objective: To review the current development of insect colonization, strain maintenance, and quality control principles; develop a model based on the fall armyworm, Spodoptera frugiperda (J. E. Smith); and propose an organizational structure for interfacing the functions of methods development, production, product utilization and specialized quality control.

Methods: Each weekday 4 containers of fall armyworm larvae were established using a single cohort of eggs from our laboratory colony (350-400 eggs/unit). A standardized rearing system was employed. The developing insects were tracked for 21 days through completion of the pupal stage. Records were kept on the percentage of egg hatch, container location, number of usable containers, number of acceptable pupae per container, total number of acceptable pupae, pupal weight, percentage of diet surface covered with mold when the pupae were removed from the containers, percentage of adult eclosion, percentage of males that flew to a pheromone source within 2 min., and percentage of mated females out of 20 combined male/female pairs.

Results: Production objectives were achieved during most months; 85 ± 5% egg hatch, nearly all rearing containers usable, 300 ± 50 acceptable pupae per container, 1200 ± 200 total acceptable pupae per cohort, and a male pupal weight of 240 ± 10 mg (Table 1). Adult eclosion was stable at 99 ± 1%, 95 ± 5% of the males mated, and 80 ± 10% of the males flew. During the 5-month period, stable egg hatch provided a consistent number of developing larvae in the containers, averaging 319.0 ± 8.6 acceptable pupae. However, total production for the sixth cohort fell below the projected level because 13% of the containers had to be discarded due to mold growth over more than 65% of the diet surface. Male pupal weight and adult parameters were well within an acceptable range.

Plans: This project has been completed.

Table 1. Production control data ($\bar{x}$ ± standard deviation) for mass rearing in fall armyworm

Cohort[1]	% Egg Hatch	Number[2] Usable Containers	Number Acceptable Pupae per Container	Total no. Acceptable Pupae	Male Pupae Weight mg
1	85.5 ± 6.1	3.2 ± 0.9	358.0 ± 75.0	1147.8 ± 383.4	225.6 ± 13.8
2	83.9 ± 4.2	3.5 ± 0.6	322.5 ± 87.1	1133.3 ± 355.2	217.4 ± 12.1
3	81.4 ± 5.2	3.8 ± 0.7	316.9 ± 75.1	1181.1 ± 344.3	220.1 ± 8.6
4	85.4 ± 11.4	3.5 ± 0.7	322.0 ± 68.4	1122.2 ± 309.8	224.5 ± 9.7
5	85.6 ± 6.2	3.6 ± 0.5	310.9 ± 56.6	1135.0 ± 278.6	238.1 ± 10.9
6	82.5 ± 6.2	3.3 ± 0.9	288.1 ± 37.1	959.4 ± 274.9	248.8 ± 13.9

1 Data typically recorded by shift or day and analyzed for weekly or monthly review.

2 Number of containers established is standardized at 4. Nonusable had more than 65% of the diet surface covered with mold, were held in an inadequate environment, or contained less than 100 pupae.

Responses of the Braconid Parasitoids Microplitis croceipes and M. demolitorto Stereoisomers of the Kairomone 13-Methylhentriacontane

W. J. Lewis, P. E. Sonnet[1], D. A. Nordlund[2]

Objectives: To compare the level of responses elicited by the R- and S-stereoisomers and combinations of these two stereoisomers for each of the subject parasitoid species.

Methods: The responses of females of M. croceipes and M. demolitor to R- or S-stereoisomers of high configurational purity as well as a 1:1 mixture of these enantiomers were tested at three dosage levels; 5, 50, and 500 ng. The kairomone was applied onto a filter paper placed in a petri dish, and the antennation responses of individual parasitoids were scored as they walked past the material. The tests were replicated with three separate groups of parasitoids of each species. The t-test and quadratic regression techniques were used to compare responses to different treatments and dosage levels.

Results: There was a response by both species to the three dosages. Further, there were no differences in responses to the two stereoisomers or the combination by either species at any dosage level. The effects of the two stereoisomers appeared to be fully interchangeable and additive.

Plans: It is not known at this time whether the host and its frass possess both isomers. Further studies to determine this information would assist in assessing the biological significance of these results.

[1]Chemical Modifications Research, ARS, USDA, Wynmoore, Pennsylvania

[2]Cotton Insects Research Unit, ARS, USDA, Cotton Station, Texas

Influence of Host Diet on Host-Oriented Flight-Chamber Responses of *Microplitis demolitor*

F. Herard[1], M. A. Keller[2], W. J. Lewis, and J. H. Tumlinson

Objectives: To evaluate and compare flight responsiveness to host-associated semiochemicals by *M. demolitor* females reared from hosts that were fed plants and hosts that were fed artificial diet.

Methods: The flight responses to host-associated semiochemicals by *M. demolitor* females reared from hosts fed plants were compared to responses of females reared from hosts that were fed artificial diet. Similar comparisons were made between females that were dissected from their cocoons prior to completion of pupation so as to prevent contact with the cocoon versus females that eclosed naturally from their cocoons. Similar comparisons were made with females that were dissected from their cocoons prior to completion of pupation to prevent contact with the cocoon.

Results: Parasitoids reared from plant-fed hosts were significantly more responsive to the semiochemicals than those reared from hosts fed artificial diet. When the parasitoids from the plant-fed hosts were dissected from their cocoons prior to completion of pupation, the responsiveness was significantly reduced. However, the responsiveness was restored when they were provided preflight contact with the empty cocoons from which they were dissected. The cocoons of parasites reared from hosts that were fed artificial diet apparently lack chemicals encountered by the females at times of emergence that are vital to their proper subsequent responsiveness. These chemicals perhaps involve host-associated odors that are incorporated into the cocoons at the time the parasites emerge from their hosts and spin their cocoons.

Plans: Studies are planned to understand better the functional mechanisms involved

[1]European Parasite Laboratory, ARS, USDA, Behoust, France

[2]Agricultural Research Institute, University of Adelaide, South Australia

Influence of Host-Plant Characteristics and Experience on Host-Searching Behavior of the Parasitoid *Microplitis croceipes*

Y. C. Drost[1], W. J. Lewis, J. H. Tumlinson

Objectives: To determine the influence of various factors on the host-searching flight behavior of the parasitoid *M. croceipes*.

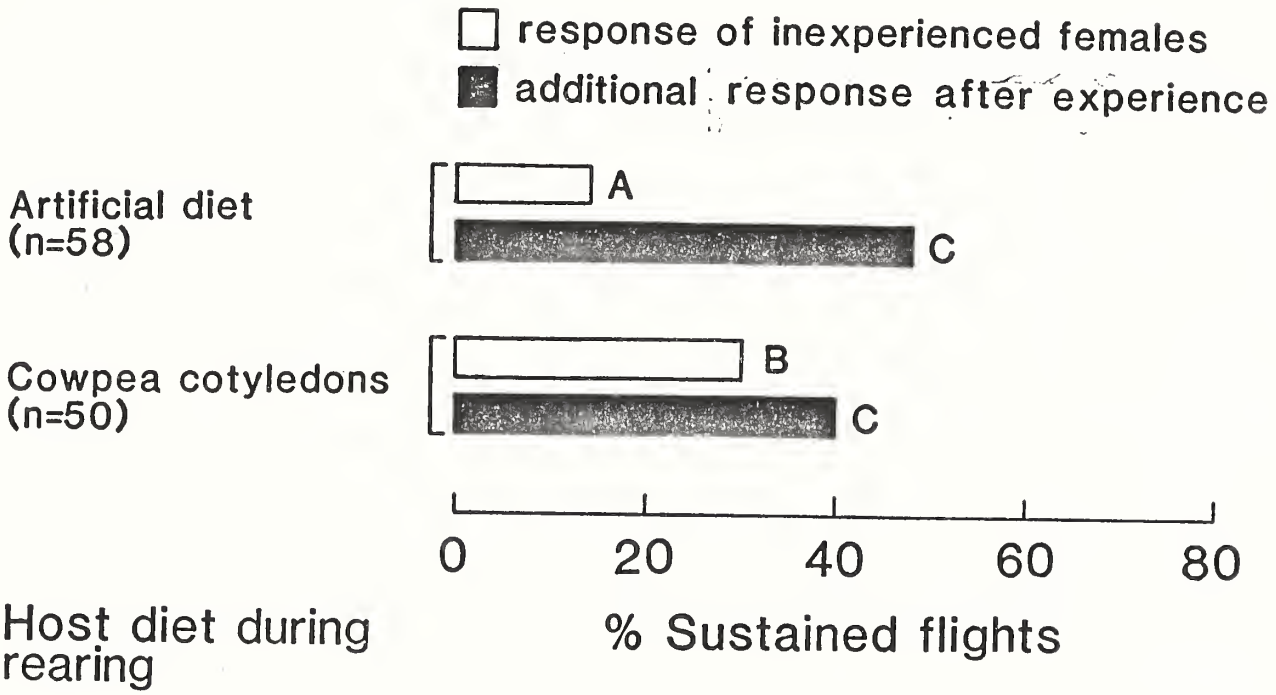
Methods: *M. croceipes* females were reared from hosts fed on different diets and/or were provided different preflight exposures to plant/host complexes. These females were then released into a wind tunnel and their flight responses monitored using previously reported techniques. The factors tested were: host insect diet, species, growth phase, and different parts of host plants; the parasitoid's prior experience with odors from the plant/host complex.

Results: Parasitoids reared from *Heliothis* that were fed on plants were more responsive to odors from *H. zea* feeding on cowpeas than from those reared on hosts fed artificial diet. The responsiveness of *M. croceipes* to volatiles emanating from the plant-feeding *H. zea* varied with the plant species, plant-growth phase, and plant part. The 4- to 5-day-old inexperienced females were more responsive to the host odors than 0- to 1-day-old inexperienced females. Responses to odors of *H. zea* that were feeding on hyacinth beans were significantly higher than to odors from *H. zea* that were feeding on cowpeas. Also, exposure to odors from hosts feeding on hyacinth bean increased the subsequent responsiveness to odors from hosts feeding on cowpea cotyledons (Figure 1).

Plans: This study demonstrates some of the different factors in the environment and the influence of experience on the host-searching behavior of *M. croceipes*. Studies of the mechanisms governing these influences and their interactions are planned.

[1]University of Massachusetts, Dept. of Entomology, Fernald Hall, Amherst, Massachusetts

Figure 1 - Responses of Microplitis croceipes ot odors of Heliothis zea feeding on cowpeas when the parasitoids were reared from hosts fed different diets and subsequent responses after experience. The experience consisted of an oviposition in an H. zea larva and contact with the frass and other materials associated with the host. Similarly patterned bars with different letters indicate significant difference (G test, $P<0.05$).



Behavioral Ecology and Reproduction

Bioassay System for Evaluating Oviposition Allelochemics

E. R. Mitchell and R. R. Heath

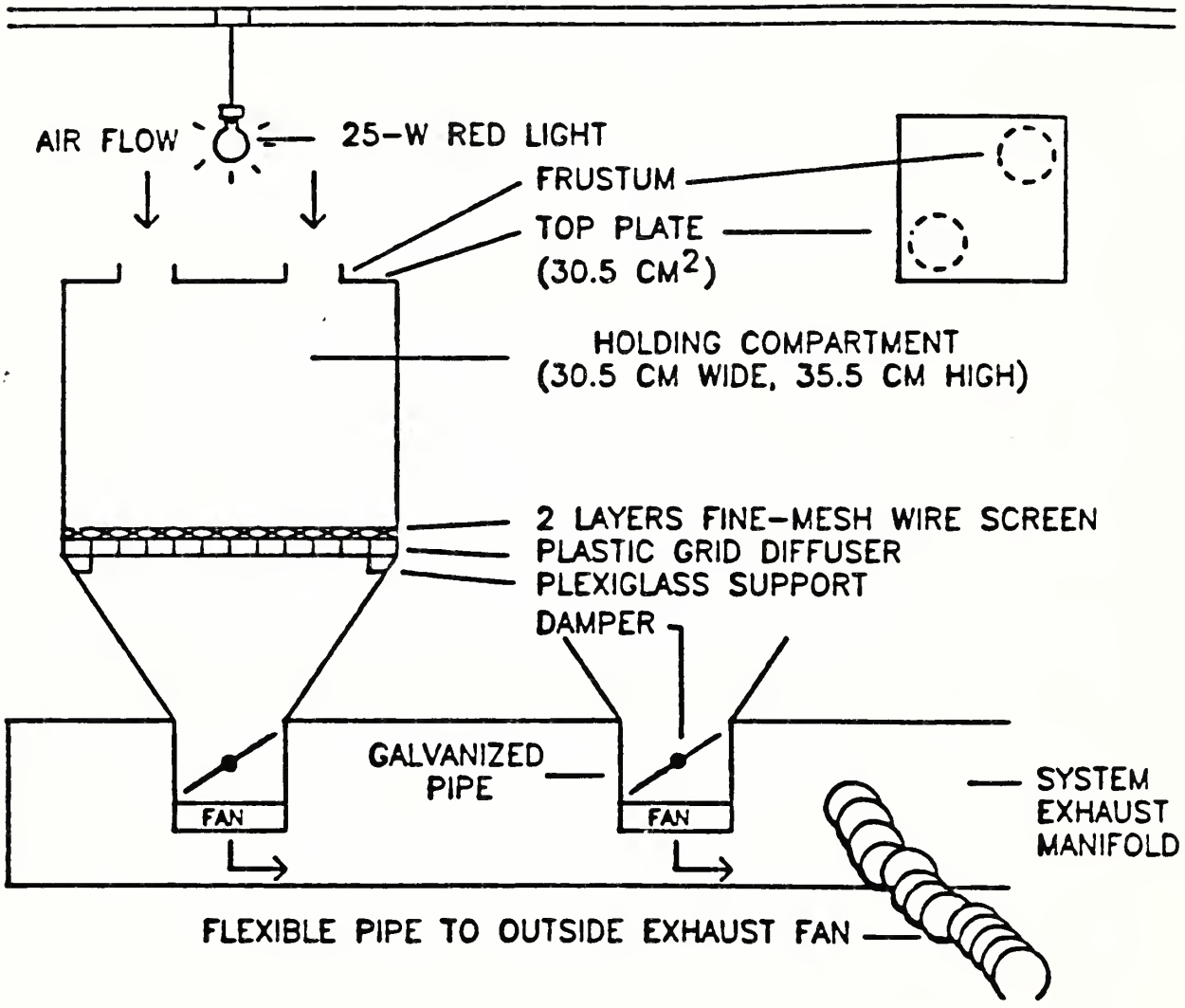
Objectives: Develop a bioassay system that can be used to study the behavioral effects of plant allelochemics on insect oviposition in a controlled environment without need for a constant supply of live plant material as oviposition substrates.

Methods: An olfactometer (Fig. 1) was developed and tested for efficiency against Heliothis subflexa (HS) using methanol washes of whole ground cherry leaves (WLW of GC). Eight chambers were fitted side-by-side on a wooden frame equipped with casters. Two banks of eight cages each were located along opposite walls of a room 3 m long, 2.6 m wide, and 2.1 m high. The room was equipped with timers to turn the fluorescent and incandescent lights off and on (14L:10D). A heat pump controlled room temperature at ca. 26.8° C., and a humidifier maintained the RH at ca. 50. Each chamber contained 10 lab-reared HS females that were confined with eight males for 2 days prior testing. Treatment and control cloths were replaced daily, and the number of eggs on each was recorded and converted to percentages of the total number of eggs deposited in each chamber.

Results: The olfactometer was as effective in demonstrating the presence of an oviposition stimulant for HS from WLW of GC leaves as were choice tests conducted using non-host plants (cotton, tobacco) in cages in the field and greenhouse. In dose-response tests, the threshold of positive vs. no response to the stimulant was within one log-dose unit, compared to controls. This response was consistent in both types of bioassays. The olfactometer described here permits year-round study of the behavioral effects of plant allelochemics on oviposition in a controlled environment.

Plans: The system will be used to complete identification of the HS oviposition stimulant from GC, and to study the effects of allelochemics on the ovipositional behavior of other moth species.

Figure 1. Schematic of plexiglass olfactometer (not to scale) for bioassaying oviposition allelochemics.



Analysis of Pheromonal Communications in Heliothis subflexa and an Associated Hybrid

J. Cibrian-Tovar[1], E. R. Mitchell, and R. R. Heath

Objectives: To elucidate the reproductive behavior of these species and the chemicals associated with specific behavioral events.

Methods: Bioassays with H. subflexa were performed in a plexiglass wind tunnel (1.5 X 0.5 X 0.5 m). Virgin females, free or in cages, were placed in the upwind end of the tunnel. Males were released at the downwind side. The behaviors of these males were monitored during a 3-min period and recorded on videotape for subsequent analysis. The exposed ovipositor segment was removed from calling females during the mating period and immersed in solvent for 45 sec. The behaviors used to categorize the responses of males to crude extracts were those defined in precopulatory and mating studies. Hybrids from mating H. subflexa males with their sibling species, H. subflexa females, were obtained, and F_1 male responses to crude pheromone extract from H. subflexa were evaluated.

Results: H. subflexa male behavioral responses to live females and crude pheromone extract were similar to those described for other lepidopterous species in wind-tunnel bioassays. Hybrid males oriented to and landed on bait dispensers treated with crude pheromone extract.

Plans: Continue studies to gain a better understanding of the behavioral and chemical interactions of H. subflexa and H. virescens and its hybrid for development of practical monitoring systems in the U.S. and Mexico.

---

1 Ph.D. Student, Department of Entomology and Nematology, University of Florida, Gainesville

Behavioral Response of *Heliothis subflexa* to Host Plant *Physalis* Sp. Extracts

F. C. Tingle, R. R. Heath, and E. R. Mitchell

Objectives: To assess behavioral response of *Heliothis subflexa* to extracts of its host plant, ground cherry (Physalis sp.) in laboratory wind-tunnel.

Methods: Individual *H. subflexa* moths from a laboratory colony were released into a plexiglass wind-tunnel and observed for specific time periods while regulated doses of plant extract were released into the tunnel. Behavioral responses such as flight initiation and direction, and contact with extract dispenser, were recorded for the duration of each test with either male or female moths of known age and mating status.

Results: Mated female *H. subflexa* moths 1 to 7 days old demonstrated similar positive responses (59% to 71%) to ground cherry extract. Bioassays with males and virgin females have not been completed.

Plans: When these bioassays are completed, specific behavioral responses will be evaluated, and additional tests will be planned to study the relationships that exist between an insect's ability to perceive the chemistry of a plant and integrate the sensory input into definable behavior.

French-American-Canadian Fall Armyworm Survey Project

E. R. Mitchell and Cooperators

Objective: To determine the seasonal occurrence and potential for fall armyworm adults to migrate annually from areas that harbor endemic populations in the Caribbean Basin and from southern Florida northward, each spring and summer, into uninfested areas of the U.S. and Canada.

Methods: Three or more International Pheromones moth traps baited with a 2-component (2-mg total blend) sex pheromone for the fall armyworm were positioned near pastures and corn fields in French Guiana; Guadeloupe; St. Croix, V.I.; Puerto Rico; Homestead and Gainesville, Florida; Tifton, Georgia; Eastern Shores, Maryland; and Quebec, Canada. The traps were collected and moths were recorded on Tuesday and Friday of each week. The survey was begun in April 1984 and continued over a 2-year period.

Results: Weekly moth trap captures were recorded on computer at Gainesville, Florida. An attempt is being made to correlate moth captures with significant weather patterns that might have been responsible for disseminating the fall armyworm between the Caribbean islands and the U.S. mainland, and northward to Canada. Dr. John Westbrook, meteorologist, USDA, Insect Biology and Population Management Research Laboratory, cooperated in this aspect of the research.

Plans: The survey was terminated in April 1986.

Cooperative Survey of the True Armyworm, *Pseudaletia unipuncta*

J. N. McNeil[1] and E. R. Mitchell

Objectives: To determine the seasonal occurrence of the true armyworm in Alachua County, Florida, and its northward movement to Canada.

Methods: Four Multi-Pher BioControl traps baited with sex pheromone in rubber-septa dispensers supplied by Dr. McNeil were positioned about 1 m above the ground at four different locations near corn fields or pastures in western Alachua County. Trap baits were changed every 4 weeks. The traps were checked each Tuesday and Friday. All true armyworm moths captured were collected and stored in the freezer for shipment to Dr. McNeil, who calculated their wing load and lipid content.

Results: The survey was initiated on June 10, 1986, and terminated on December 23, 1986. The total number of true armyworm meals captured in four traps, June through December, were: June--1, July--45, August--26, September--56, October--10, November--1, and December--2.

Plans: The monitoring programs will continue through 1988.

---

1 Department of Biology, Laval University, Quebec City, Canada

Prediction of *Heliothis virescens* Populations on Tobacco Using Accumulated Degree-Days

F. C. Tingle and E. R. Mitchell

Objectives: To use degree-day (°D) accumulations to estimate population peaks of *Heliothis virescens* adult males, male and female larvae, and larval damage in tobacco fields in Alachua County, Florida.

Methods: Degree-day values were calculated from climatological data for Gainesville, Florida. We collected the insect data from 12 tobacco fields, four fields per year, from 1978-1980. Adult male *H. virescens* populations were monitored using pheromone traps at a density of one trap/ha in all fields except two (C-1 and C-2, Table 1) in 1978, where 22 traps/ha were maintained during the growing season. Trapped moths usually were counted and removed twice weekly. Damage of plant terminals and larval counts also were made twice weekly on 250 to 360 plants per field. In addition to the trap and field data collected from 1978 until 1980, one or more survey traps for *H. virescens* were maintained in this tobacco growing area from April 1, 1979, until January 30, 1986.

Results: Trap catches indicated five adult *H. virescens* peaks beginning in April, with the last peak occurring in early October each year. We estimated from the 1978-1980 trap data a mean of 431 ± 22 °D from January 1 until the first adult population peak. Field-collected data indicated an average of 508 ± 22 and 516 ± 17 °D, respectively, from January 1 until the first larval and plant damage peaks. Estimates of °D between population peaks of adults and larvae and damage peaks are presented in Table 1. Dates of planting, insecticide applications, topping of plants, and harvest were considered for each field, in addition to insect and damage assessments. One trap/ha in the tobacco fields appeared sufficient for indicating the adult population peaks. We calculated from the 1980-1981 and 1983-1985 trap data a mean of 212 ± 7 °D from the first trap catch of *H. virescens* in March of each year to the first adult peak, occurring in April, in tobacco. Additionally, for the period from January 1 to the first adult peak, a mean of 432 ± 17 °D was determined. These mean °D values can be used in population model systems and provide reliable indicators which can be used by crop production consultants and others advising on the control of *H. virescens* in tobacco.

Plans: This project has been completed.

Table 1. Calendar dates of population peaks of Heliothis virescens adults and larva and plant damage peaks, and degree-days between peaks in 12 tobacco fields during the growing season. 1978-1980. Alachua County, Florida.

		Adult Male Peaks					Larval Peaks			Damage Peaks		
Year	Field	1st	2nd	°D	3rd	°D	1st	2nd	°D	1st	2nd	°D
1978	A	4-19	6-6	492	7-21	644	5-8	6-27	634	5-8	6-27	634
	B	4-21	6-5	467	7-20	642	4-20	6-15	616	4-27	6-15	572
	C-1	4-19	6-8	536	7-17	558	4-27	6-15	572	5-1	6-15	546
	C-2	4-17	6-6	511	7-21	644	4-25	6-15	593	4-25	6-16	593
1979	A	4-23	6-15	574	7-24	561	5-3	6-21	565	5-3	6-21	565
	B	4-11	6-8	592	7-20	582	4-12	6-7	566	4-17	6-19	675
	C	4-27	6-13	517	7-18	491	5-3	6-15	484	5-3	6-15	484
	D	4-23	6-13	553	7-18	491	4-26	6-18	585	4-26	6-18	585
1980	A	-	5-30	-	7-11	584	4-24	6-10	509	4-24	6-10	509
	B	4-10	5-30	460	7-15	649	5-1	6-11	469	5-1	6-11	469
	D-1	4-10	6-2	492	7-15	617	4-24	6-9	496	4-16	6-9	551
	D-2	-	5-20	-	7-1	538	4-9	6-3	509	4-16	6-3	462
Mean	Date	4-18	6-5		7-17		4-25	6-14		4-26	6-15	
±S.E.		±2	±2		±2		±3	±3		±2	±2	
	°D			519		583			550			554
				±14		±17			±16			±19

Reproductive Behavior and Exploitation of Sex Pheromones of Lepidopterous Pests: Wind-Tunnel Analysis of the Sex Pheromone Communication of *Heliothis zea*

J. R. McLaughlin and R. R. Heath

Objectives: Characterize the parameters of female pheromone production and male response utilizing highly defined measurements in a wind-tunnel system.

Methods: Highly defined amounts of all combinations of the four identified sex pheromone components of the female sex pheromone were injected into the upwind end of a laboratory wind tunnel. Various behavioral parameters of males, introduced into the chemical plume at the downwind end of the tunnel, were measured. Individual chemicals or blends were released into the air stream at rates equivalent to those released by a calling female (as determined by behavioral analysis, see 1985 annual report) or in log-10 increments below this amount.

Results: Males exhibited oriented flight responses to individual pheromone components and to blends. These behaviors were dose-dependent. No synergistic effects of combinations of "minor" chemicals were evident. Complete behaviors through landing and attempted compulations were rarely exhibited by males exposed to blends lacking (Z)-11-hexadecenal. Upwind orientation to the three "minor" chemicals has not been previously demonstrated. This phenomenon may be responsible for reducing the effectiveness of mating disruption treatments.

Plans: The determination of the female release rate using GC-MS analysis will be completed. Tests will be conducted to determine the response of males to females and synthetic pheromonal blends in the presence of background levels of pheromonal components.

Effect of *Spodoptera frugiperda* Synthetic Pheromone on Behavior of *Cotesia* (= Apanteles) *marginventris*

F. C. Tingle, R. R. Heath, and E. R. Mitchell

Objectives: To determine if blends of synthetic pheromonal components affect the behavior of parasitization of *Spodoptera frugiperda* by *Cotesia* (= Apanteles) *marginventris*.

Methods: Bioassays with pheromonal blends of the *S. frugiperda* synthetic pheromone were conducted in the laboratory to observe any effect on mating by *C. marginventris* and the ability of the parasitoids to locate and successfully parasitize *S. frugiperda* larvae. In the mating study, four virgin pairs of laboratory-reared parasitoids 1 to 2 days old were confined in one-liter plastic containers into which the chemical components were constantly introduced at a specific dosage with an air pump. The parasitoids were observed for 30 minutes for mating or attempted contacts and compared to a control. Similarly, a 0.5-liter plastic container was used to confine one mated *C. marginventris* female with 40 *S. frugiperda* larvae to observe parasitization attempts. After 20 minutes, the parasitoid was removed and the larvae were maintained on artificial diet until they pupated or parasitoids emerged.

Results: Bioassays with a two-component *S. frugiperda* pheromonal blend indicated that doses of 200 and 400 micrograms per 0.5-liter test chamber had no effect on the number of eggs deposited or the number of contacts with *S. frugiperda* larvae by *C. marginventris* females. Bioassays with other doses and mating tests have not been completed.

Plans: To continue bioassays with additional blends of *S. frugiperda* pheromonal components until results are conclusive.

Chemical Ecology of Host Plants in Relation to Insect Behavior and Reproduction: Oviposition Stimulants for the Cowpea Weevil

J. R. McLaughlin

Objectives: To characterize the oviposition behavior of the cowpea weevil in response to chemical and physical cues and in relation to host genotypes.

Methods: (1) Aqueous rinses of seeds of various cowpea varieties were applied to glass beads and assayed as described in previous reports. (2) Cowpea weevil females were exposed for 2 hours to arrays of seeds in petri dishes. Each seed in each array carried a different egg load, usually from 2 to 8 eggs, at the beginning of the test. (3) Bioassays in support of research to isolate and identify chemicals from cowpea seeds responsible for oviposition behavior were conducted.

Results: (1) Some numbered varieties from the International Institute of Tropical Agriculture, Ibadan, Nigeria were found to exhibit marked reduction in the oviposition activity induced by aqueous extracts of their seeds. (2) The number of eggs found on seeds after each test did not differ significantly. Cowpea weevil females appear to be able to accurately distinguish among varying pre-existing egg loads on seeds and partition their eggs among seeds with the least eggs until all resources (seeds) have been equally utilized. (3) Various extraction methods and preliminary separation techniques were assessed, and separation of active materials was achieved.

Plans: Further investigation of varietal differences in oviposition activity will be conducted to determine the causes of the differences noted. The mechanism of resource assessment and partitioning of eggs will be investigated. The program to isolate and identify the oviposition-inducing chemicals will continue.

Development of the Sex Pheromone of the Almond Moth, *Cadra cautella*, for Monitoring Infestations in Stored Products

J. A. Coffelt and R. R. Heath

Objective: To determine the influence of pheromone dosage upon male approach to, and landing on, pheromone-baited traps in the stored-product environment.

Methods: Observations of insect behavior were made in four simulated warehouse rooms (6 X 6 X 2 m), each containing 75 kg of farmer's stock peanuts. Populations of *C. cautella* were established ca. 3 months prior to tests by introduction of male and female pupae. Two wooden platforms (0.3 X 0.3 m) 1 m in height were placed on either side of the bin that contained the peanuts. Pherocon 1C trap liners were baited with 0, 0.001, 0.01, 0.1, 1.0, 10.0, 30.0 or 100 micrograms of an 85:15% binary mixture of formulated pheromone, (*Z*,*E*)-9,12-tetradecadien-1-ol acetate and (*Z*)-9-tetradecen-1-ol acetate, respectively. The numbers of male moths that approached to within 0.5 and 0.25 m of the pheromone source and the number of males that landed on the trap liner within 10 min of pheromone deployment were recorded. Observations of approaches to, and landings on unbaited traps on a given day immediately preceded observations of male responses to pheromone-baited traps; only one dosage was used/day/room. Dosages were rotated from room to room on different days with each day's results defined as one replication. There were six replications for each pheromone treatment. Observations using untreated dispensers were replicated 24 times.

Results: There was no significant difference between the number of males that approached to within 0.5 m of a pheromone source at dosages of 1 microgram or higher. (Fig. 1). A similar result was obtained with regard to approaches to within 0.25 m, except that, at the highest dosage (100 micrograms), fewer males approached to within this distance than at 1 to 30 micrograms (Fig 1). The influence of dosage upon landing (capture) is shown in Fig. 2. A dosage of 1 microgram resulted in greater capture-rate than any of the other experimental dosages. These findings have significant impact upon the selection of the most effective pheromone-release rate.

Plans: The results obtained in the these studies will be used to assess the potential of artificially-induced air streams and pheromone blends to further improve the sensitivity of the pheromone as a tool for monitoring and detecting this and related species.

Figure 1

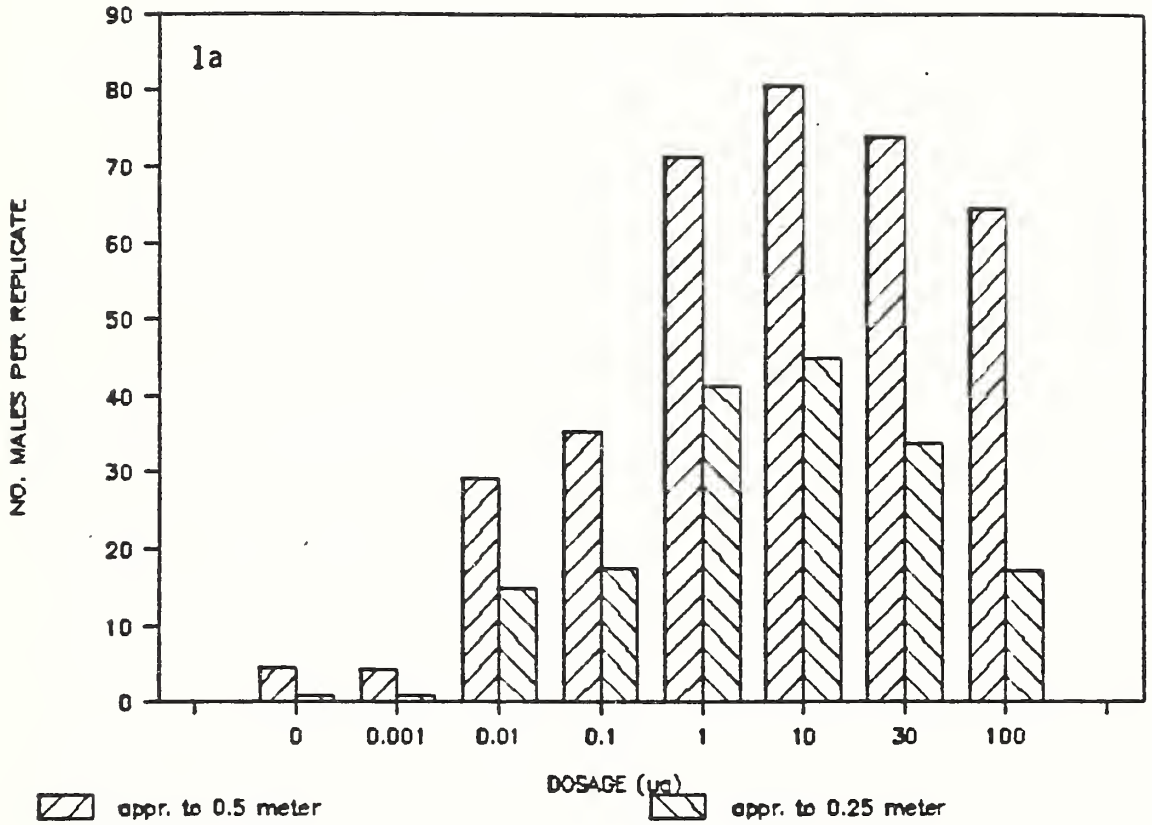
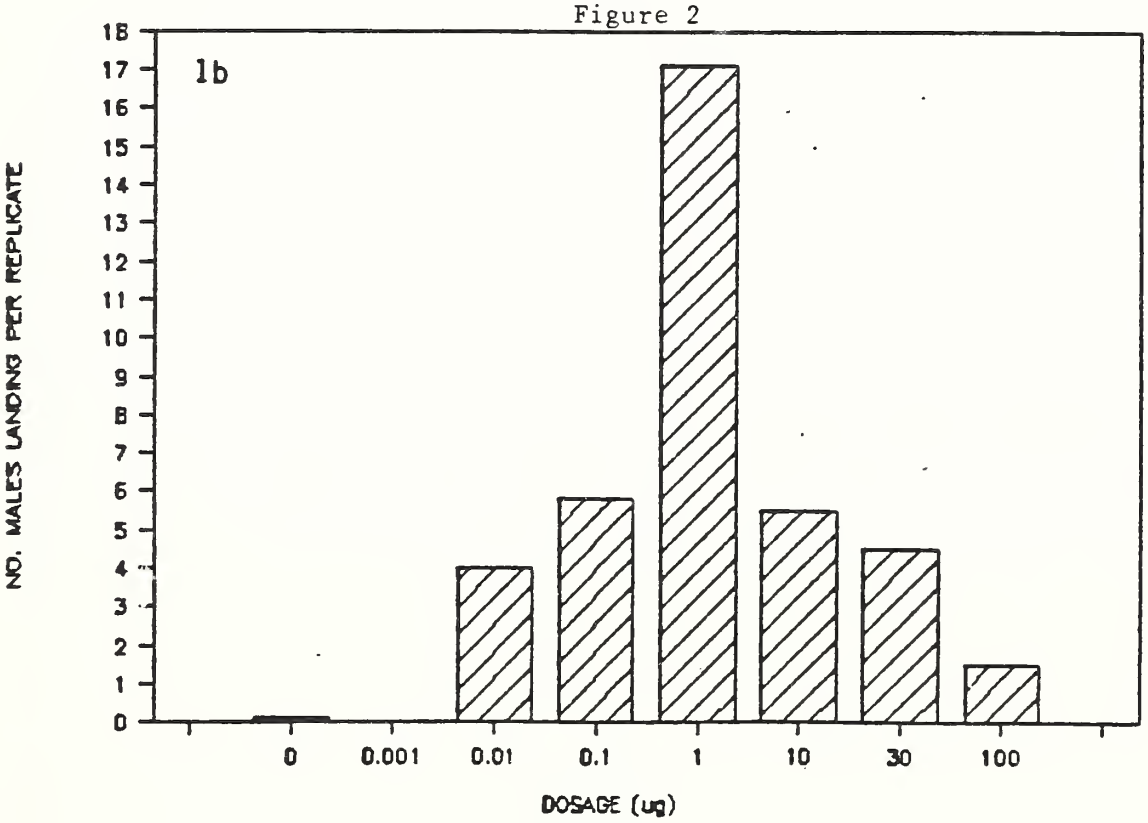


Figure 2



Reproductive Behavior of the Cigarette Beetle, *Lasioderma serricorne*: Male Response to Sex Pheromone Baited Traps

J. A. Coffelt

Objectives: To assess the effectiveness of traps baited with sex pheromone for detecting and monitoring infestations of *L. serricorne*, and to identify optimum dosages for capturing male beetles.

Methods: Synthetic serricornin [(4S,6S,7S)-7-hydroxy-4,6-dimethyl-3-nonanone] (containing less than 0.1% of the inhibitory 4S,6S,7R isomer) was formulated for controlled release and was employed in all tests. Three tests were conducted in 2.8 X 2.8 X 2.8-m rooms as follows: Test 1--2 traps (Serrico) were deployed on the floor of the room near opposite corners; one trap was baited with 10 mg of pheromone, and the other trap was unbaited; 60 virgin males were released into a room at 1200 h. Traps were examined 24 h later and the number of insects captured was recorded. There were 10 replications. Test 2--Sixty virgin males were released into rooms containing an untreated trap and a trap baited with 0.01, 0.1, 1, 10, or 100 mg of pheromone. The number of insects recaptured was recorded 24 h later. There were five replications for each dosage, each replication was done in a different room. Data were converted to percentage recapture. Test 3--Pheromone baits (10-mg) were evaluated after being aged for 0, 3, 10, 20, and 30 days in the field. Mixed sex releases (100 insects of each sex) were made for each aging period with fresh (unaged) baits deployed for comparison at each time period. There were five replications of each variable.

Results: Test 1--A total of 286 males was captured in pheromone-baited traps, while unbaited traps captured 27. Mean recaptures were significantly different at $p=.001$. Test 2--Mean percentage recapture of males in rooms baited with 0, 0.01, 0.1, 1, 10, and 100 mg was 3.8, 7.7, 14.3, 21.1, 29.2, and 18.0, respectively. There was no significant difference between the number of males captured in traps that contained baits of any of the ages tested with mean recaptures of 19, 21.6, 21.8, 20.0, and 23.6 males/trap/day after 0, 3, 10, 20, and 30 days, respectively. Captures of males in unbaited traps and females in baited or unbaited traps were less than 1% of those for males in pheromone traps.

Plans: Studies will be conducted to define the influence of mating, population density, and insect age upon pheromone trap effectiveness. Pheromone-mediated behavior of males will be examined under defined environmental conditions in a laboratory wind tunnel.

Reproductive and Pheromone-Mediated Behavior of the Sweetpotato Weevil, *Cylas formicarius elegantulus*

J. A. Coffelt

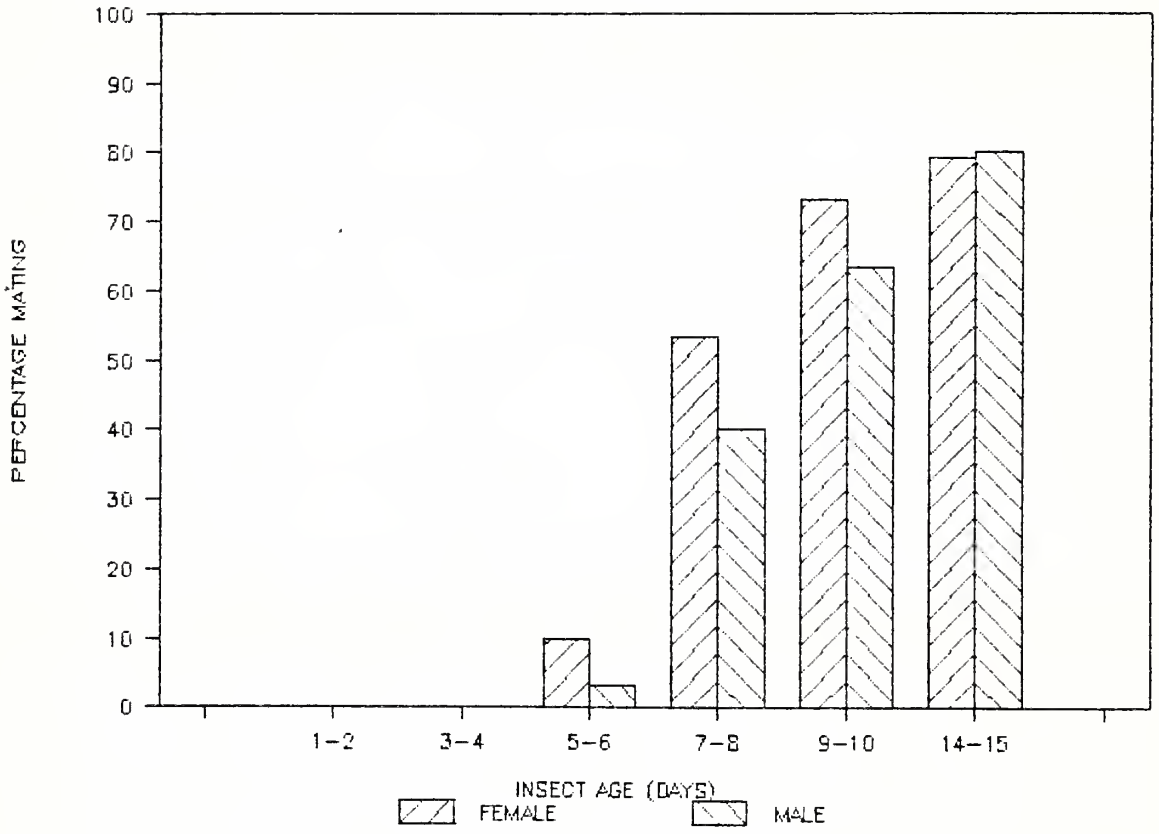
Objectives: To conduct fundamental investigations of the reproductive biology of *Cylas formicarius elegantulus* and to identify behavioral and physiological mechanisms that can be utilized in detection and control.

Methods: Development of sexual receptivity and maturity was examined by conducting a series of single-pair matings of male and female weevils of different ages. Unmated male and female weevils that were 1-2, 3-4, 5-6, 7-8, 9-10 and 14-15 days of age were placed with a sexually mature (over 20 days old) insect of the opposite sex for 24 h. Females then were dissected to determine if insemination had occurred. There were three replications with 10 pairs of each age-group/replication. A second experiment was conducted in which volatile pheromone was collected from females of increasing age (1-2, 3-4, 5-6, 7-8 and 14-15 days old) to identify the age at which pheromone production begins. Samples were diluted in hexane and bioassayed over a range of concentrations from 0.0005 to 0.05 female equivalent days (FED).

Results: The patterns of development of sexual maturity for male and female weevils shown in Fig. 1 indicate that first matings occur at about 5 to 6 days of age for both male and female insects, and that maximum mating rates (over 80%) are reached approximately 1 week later. Bioassays of volatile pheromone from females of increasing age indicated that significant amounts of pheromone can be collected from females as early as 3 to 4 days of age. In the prior test, no mating was observed among 3- to 4-day-old females.

Plans: Additional bioassays of volatile collections from females of different ages will be conducted to confirm the above results. The influence of male age upon responsiveness to pheromone will be determined in both still-air and wind-tunnel bioassays.

Figure 1


PERCENTAGE MATING
100
90
80
70
60
50
40
30
20
10
0
1-2
3-4
5-6
7-8
9-10
14-15
INSECT AGE (DAYS)
FEMALE
MALE

Regulation of Reproduction in the Stored Product Pest, *Plodia interpunctella*

P. D. Shirk

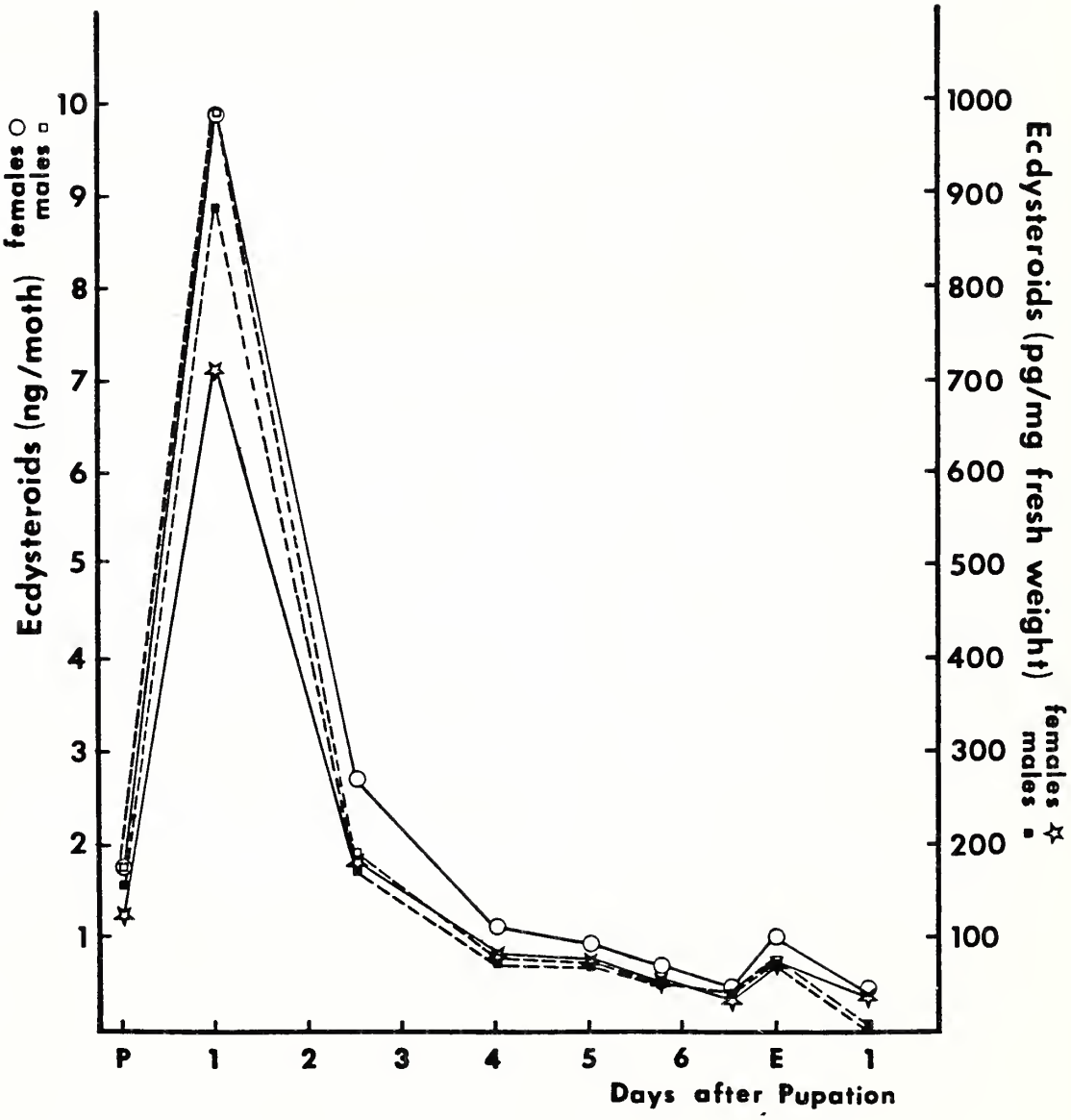
Objective: To determine the endocrine mechanisms that control yolk production in fat body and ovarioles of the Indianmeal moth, *Plodia interpunctella*.

Methods: Early pupae were ligated and either left untreated or injected with varying regimens of 20-hydroxyecdysone. The ligated pupae were either dissected to determine the effects of the hormone on the growth of the oocytes, or injected with ^{35}S-methionine to label the newly synthesized proteins. Additionally, the tissues were dissected from the insects and cultured in vitro. Ecdysteroid titers were measured in both females and males throughout pharate adult development using an ecdysteroid radioimmunoassay.

Results: The eggs of *P. interpunctella* were previously shown to contain four major yolk polypeptides (YPs) of 155,000 daltons (kDa), 69 kDa, 45 kDa, and 33 kDa. When white pupae were ligated, adult development, including the development of the ovaries and vitellogenesis was stopped. If ligation was employed 2 days after pupation, adult and ovarian development continued. Determination of the total ecdysteroids present throughout pharate adult development showed a peak of ecdysteroids at day 1 after pupation (Fig. 1). If ligated white pupae were injected with 20-hydroxyecdysone, adult development continued to completion. However, the development of the oocytes stopped during early vitellogenesis. Continued treatment of the pupae with 20-hydroxyecdysone was not sufficient to support the completion of vitellogenesis. This suggests that additional endocrine components originating in the anterior portion of the insect are necessary for complete egg development to occur.

Plans: Yolk-protein production will be studied further in *P. interpunctella*. The possible roles of neuroendocrine components and ecdysteroids in regulating the expression of the yolk protein genes will be examined.

Figure 1


Ecdysteroids (ng/moth) females ○ males □
10
9
8
7
6
5
4
3
2
1
1000
900
800
700
600
500
400
300
200
100
Ecdysteroids (pg/mg fresh weight) females ☆ males ■
P
1
2
3
4
5
6
E
1
Days after Pupation

Packaging of the Yolk Proteins in the Eggs of the Indianmeal Moth, Plodia interpunctella

P. D. Shirk and K. L. Ogren

Objectives: To determine the structural arrangement of the yolk proteins packaged in the yolk granules of the eggs of the Indianmeal moth, Plodia interpunctella.

Methods: Monospecific antiserum for each of the four yolk polypeptides (YPs) from P. interpunctella were raised in rabbits. Thin section were cut from ovarioles embedded in parafin. The sections were immunocytochemnically stained by reacting them with a monospecific YP antiserum followed by antirabbit antiserum linked with horseradish peroxidase. The sections were viewed with light microscopy. Ultrathin sections of oocytes were cut from ovarioles imbedded in LR White for transmission electron microscopy.

Results: Vitellogenic oocytes were stained uniformly when reacted with the monospecific YP antiserum. Fat body was stained with antiserum to the vitellogenins, whereas follicle cells were not. The follicle cells were stained with antiserum specific for the follicle cell produced protein. The ultrastructure of the maturing oocytes was determined by transmission electron microscopy. The center of the oocytes were filled with yolk granules that were each surrounded by membranes.

Plans: The monospecific antibodies to yolk proteins will be used in conjunction with transmission electron microscopy to establish the ultrastructure of the yolk granules and how each of the yolk polypeptides is packaged in the structure.

Regulation of Reproduction in the Meal Worm, Tenebrio molitor

P. D. Shirk and K. W. Vick

Objective: To determine the physiological and hormonal basis of vitellogenesis in the meal worm, Tenebrio molitor, and to demonstrate the impact of the physiological processes on the reproductive behavior of the adult female.

Methods: To determine those conditions that promoted the greatest egg production, adult females were placed in various environmental conditions, including starvation, dehydration, and non-mating; and the rate of egg production was compared with fed, watered, and mated females. The influence of the anterior endocrine glands on the control of vitellogenesis was examined through the classical techniques of gland extirpation and hormonal replacement. The biosynthetic rate of the vitellogenins under the various conditions of hormonal manipulation was determined by pulse labeling of the newly synthesized proteins with ^{35}S-methionine.

Results: The rate of egg production was greatest when the adult females were fed, watered, and mated. Removal of any of these factors depressed egg production. When the females were maintained as virgins, they matured eggs initially at the same rate as mated females, but after 6 days reduced the rate of egg maturation and began resorbing the mature eggs stored in the oviducts. Egg maturation was dependent upon the presence of the anterior endocrine glands. Decapitation or allatectomy at various times after adult emergence stopped egg maturation. Treatment with a juvenile hormone analogue, methoprene (Zoecon), maintained egg development. However, the rate of vitellogenin synthesis by the fat body was independent of the presence or absence of the anterior endocrine glands.

Plans: The role of mating on egg production will be examined through its effect on the endocrine system. The nature of the control mechanisms regulating the production of vitellogenins by the fat body will be closely examined, as this is the first case where the control of vitellogenin synthesis appears to be autonomous to the fat body.

Biophysics and Neurophysiology

Acoustical Insect Larva Detection System

J. C. Webb, D. C. Slaughter, and C. A. Litzkow

Objectives: To develop an acoustical detection system capable of detecting, continuously monitoring, and storing larval insect acoustical outputs in post-harvest commodities.

Methods: The present detectors and acoustic couplers are being modified to increase their sensitivity, flexibility, and signal-to-noise ratio. The amplifiers and filtering are being modified to reduce size and cost. The computer system is being upgraded so that faster and IBM PC compatible computers can be used.

Results: Two new flexible acoustic couplers have been developed. These permit the same coupler to be used on different sizes of fruit and make the testing of irregular fruit much easier. The detection system has been interfaced to the IBM PC and the HP Vectra. The software that was written for the HP 86B has been modified for the IBM PC and HP Vectra computers. These two computer systems are much faster than the HP 86B and will greatly reduce the data-reduction time.

Plans: Continue to improve the detector and acoustic coupler. This will include the development of a water- or fluid-coupled detector and allow one detector to be used on all sizes and shapes of fruit. The detector system will be modified to reduce the cost of the system. The ultimate goal of the project is to develop the system for accurately detecting insect larvae in post-harvest commodities in quarantine programs.

Feeding Periodicity of Caribbean Fruit Fly Larva in Grapefruit

J. C. Webb, C. O. Calkins, and S. Masuda

Objectives: To determine the feeding periodicity of the Caribbean fruit fly larva in grapefruit.

Methods: Grapefruit was infested by allowing only one female Caribbean fruit fly to oviposit in the fruit. After oviposition, the location was marked on the fruit. The grapefruit was held at 80° F until egg-hatch, and the feeding sounds were heard with the detection system. This usually took 3 to 4 days. The grapefruit was placed on the detector so that the larva was located on one of the sensors. The computer was programmed to monitor the larval feeding activities for 2 to 3 days without pausing. At the end of this period of time, the computer dumped the data on a disk and then continued for another 2- to 3-day period. This procedure was repeated for the life of the larvae. The data were collected in 10-second periods. It then took .15 seconds to store the data in memory. Therefore, the computer was listening to the larvae 98.5% of the time. When the feeding activity stopped, the fruit was cut and examined, and the number of larvae in the fruit was counted.

Results: The larvae in 15 grapefruits were monitored from the day the first sounds were heard until they either pupated or died. Their feeding life-span ranged from 3 days to 31 days, with an average of about 15 days. The number of 10-second collection periods with sound ranged from 10% to 98% on the first day, to nearly 100% on the second and consecutive days. The larvae fed continuously, with only an occasional pause of a few seconds, until the last 1 to 2 days at the end of the larva stage. At this time, the feeding activity decreased until the larvae exited the fruit. Out of the 15 fruit tested, three had two larvae per fruit. The other 12 had only one larva per fruit.

Plans: The feeding periodicity studies will continue for another grapefruit season in the same manner as the one conducted this year. The data then will be published.

Response of Boll Weevils Reared on Natural-Host and Artificial Larval Diets Supplemented with Carotenoids

J. C. Dickens[1] and H. R. Agee

Objectives: To determine whether the addition of carotenoids to the larval diet of the reared boll weevil would restore normal visual sensitivity to the adult weevil.

Methods: Electroretinographic techniques were used to measure the visual sensitivity of compound eyes of boll weevils reared on natural-host diets and artificial diets supplemented with beta carotene or retinyl palmitate.

Results: The compound eyes of boll weevils reared on artificial diets for many generations at the Gast Rearing Laboratory had reduced visual sensitivity when compared to field-collected weevils. Supplementing the larval diet with vitamin A precursors, beta carotene, or retinal palmitate restored the visual sensitivity of the adult weevils to that of weevils reared on the natural host; cotton. The boll weevil eradication program has greatly benefited from this research because weevils with normal vision can be reared for release.

Plans: The project has been completed and results are described in a manuscript that has been accepted for publication.

---

[1]Boll Weevil Research Laboratory, USDA, ARS, Mississippi State

Spectral Sensitivity and Behavioral Responses to Light Quality in the German Cockroach (Orthoptera:Blattellidae)

P. G. Koehler[1], H. R. Agee, N. C. Leppla, and R. S. Patterson[2]

Objective: To determine the spectral sensitivity of the compound eye of the German cockroach, Blattella germanica (Linnaeus), to monochromatic light at wavelengths ranging from 350 to 650 nm and its behavioral responses to selected colors ranging from ultraviolet to red.

Methods: Electroretinogram techniques were used to measure the spectral sensitivity of the compound eyes of ten specimens. The cockroach eye response to monochromatic stimuli from 350 to 650 nm was measured. Behavioral tests were made to determine which bands of light from ultraviolet (350 nm to 380 nm) caused the least disruption of normal behavior and did not cause the evasive reactions elicited by the usual white light inspection flashlight.

Results: The compound eyes of German cockroaches detect monochromatic light at wavelengths from 350 nm to 625 nm with peak sensitivities at the ultraviolet and blue-green regions. Behavioral tests showed that sources emitting ultraviolet or nearly ultraviolet light were responsible for the startle-evasive reaction encountered in roaches illuminated with a white light. Yellow and red light did not cause the startle response. Therefore, a flashlight was equipped with a yellow filter that passed only the yellow-red end of the spectrum to observe German cockroaches without inducing the evasive behaviors.

A manuscript reporting the results of this research was submitted to the Annals of the Entomol. Soc. of Amer. for publication. The commercial community has rapidly utilized the results of this research to enhance the efficiency of detection of cockroaches by pest control operators. They are using a special yellow filter on the inspection flash light that facilitates the observation of the roaches in dark rooms without causing the typical evasive cockroach reaction that occurs when the usual white light is used.

Plans: Additional behavioral tests will be conducted using near monochromatic stimuli to identify the specific wavelengths (5-nm band width) most effective for altering behavior patterns of the cockroach.

---

1 Department of Entomology and Nematology, University of Florida, Gainesville

2 USDA, ARS, Insects Affecting Man and Animals Research Laboratory, Gainesville, Florida

Spectral Sensitivity and Behavior of the Papaya Fruit Fly

H. R. Agee and P. J. Landolt

Objective: To determine the spectral sensitivity of the compound eye of the papaya fruit fly and its color preference for colored spheres.

Methods: Electrophysiological techniques were used to measure the spectral sensitivity of the compound eyes to monochromatic stimuli at wavelengths from 350 nm to 650 nm. A Varian 634S spectrophotometer with reflectance attachment was used to determine the spectral reflectance of papaya fruit at various stages of maturity.

Results: The compound eyes of the papaya fruit fly have two peaks of sensitivity, the first at 475 nm and the second at 500 nm. The 475-nm peak was unique for the papaya fruit fly when compared to the spectral sensitivity of five other species of fruit flies. Ripe papaya fruit reflected wavelengths more efficiently at wavelengths of 550 nm and longer, while the green fruit preferred by ovipositing flies had increased reflectance at 530 nm.

Plans: This project has been completed.

Thoracic Mechanoreceptors in the Wing Bases of *Heliothis zea* (Lepidoptera:Noctuidae) and Their Central Projections

E. Orona and H. R. Agee

Objective: To identify the mechanoreceptors in the wing bases of the bollworm moth, *Heliothis zea*, related to the acoustic response and evasive reaction network.

Methods: The cut end nerves to the mechanoreceptors were allowed to take up and translocate cobalt chloride to selectively stain the axons and cell bodies of the mechanoreceptors. With appropriate intensification, each mechanoreceptor unit was visualized. Methylene blue and other stains were used to stain the axons and cell bodies of the mechanoreceptors, which were studied using stereo and compound microscopes. The wing base mechanoreceptors of thirty moths were studied.

Results: The thoracic mechanoreceptors of the wings and their central projections in the bollworm moth were investigated using cobalt chloride infiltration methods. The different mechanoreceptors, tegula, campaniform sensilla, and chordotonal organ were identified in the wing bases. The forewing and hindwing bases were innervated by two large nerve trunks; IIN1 and IIIN1, respectively. Terminal projections for both wing bases include massive regions within the fused mesothoracic-metathoracic ganglia, as well as direct projections to the subesophageal ganglia. The terminal fields of IIIN1 were exclusively ipsilateral, whereas those of IIN1 also were contralateral. These mechanoreceptors are involved in the evasive flight circuits of the moth.

Plans: This project is completed.

Neuroanatomy of *Heliothis zea*: Acoustic Circuits

H. R. Agee and E. Orona

Objective: To determine the anatomy and neural circuits of the acoustic system of the bollworm moth, *Heliothis zea*.

Methods: The neuroanatomy of the acoustic system is being determined using standard eosin, hematoxylin, and selective cobalt chloride staining of acoustic axons and motor neurons to the wing muscles involved in the evasive behavior network. The neurons for acoustic sense cells (A_1 and A_2) have been stained with cobalt chloride to demonstrate the specific axon and dendritic organization within the central ganglia.

Results: Axons from A_1 and A_2 acoustic receptors enter the lateral margin of the metathoracic ganglion. The A_1 send projections anteriorly through the mesothoracic and prothoracic ganglia toward the brain. Dendritic fibers branch profusely within the metathoracic and mesothoracic ganglia and appear to overlap the motor fibers that leave the same ganglia to drive the wing muscles used in evasive flight behaviors. Most nuclei of the motor nerves to the wing muscles are located in the contralateral side of the mesothoracic-metathoracic ganglia. Usually, one motor-nerve nucleus is located ipsilateral to the muscle group that the nerve unit serves.

Plans: The neuroanatomical studies are continuing, and additional details of the neural circuits in the acoustic system will be developed and coordinated with neurophysiological studies.

Identification of Neural Circuits That Transmit Acoustical Information in the Central Nervous System of the Bollworm Moth

H. R. Agee

Objective: To determine the neural circuits that transmit and process information from the acoustic receptors in the central nervous system of the bollworm moth to modify its behavior.

Methods: Neurophysiological techniques, in combination with a computer-based data-acquisition and analysis system, are being used to track the sensory information flow from the two pairs of acoustic receptors into and through the central nervous system. Intracellular recordings of information flow are recorded through glass micropipette electrodes filled with electrolyte solutions containing special stains to mark the impaled neuron and its dendrites. Cobalt chloride, lucifer, and procion dyes are iontophoretically injected from the recording electrodes into the nerve cell to selectively stain the axon and dendrites.

Results: Cobalt chloride and lucifer staining of the first- and second-order neurons in the acoustic circuit are being identified to develop the neural connections that function in the acoustic reception and evasive behavioral reaction network.

Plans: Additional tracking of the neural connections and networks in the ganglia and brain of the bollworm moth will continue until the complete network is identified.

Heating of Fruit for Quarantine Treatment by the Vapor Heat Method

J. J. Gaffney

Objective: To investigate the various heat and mass transfer relationships involved in heating of loads of fruit by the vapor heat method as a quarantine treatment to kill fruit fly eggs and larvae, and to develop engineering data needed in the design of vapor heat systems.

Methods: A mathematical model was developed to simulate the heat and mass transfer relationships which occur when heated air at high relative humidity is moved through a load of spherically shaped fruit for the purpose of heating of the fruit by condensation of water vapor. Specified initial conditions include fruit temperature, fruit size, size of load, air temperature, air flow rate, and relative humidity. The model accounts for the rapid heat transfer which occurs at the fruit surface during condensation and the rapidly changing conditions of the water vapor content of the air as the air moves through the load. In this process, the rate of condensation, and thus the rate of fruit heating, at any position in the load is a function of the dew-point temperature of the air surrounding the fruit at this position. The dew-point temperature of the air is, in turn, a function of the rate of condensation of water vapor, thus requiring an iterative calculation involving a heat and mass balance between the fruit surface and the air at each incremental layer within the load and at each time step.

Results: The model calculates the time temperature response as a function of position within the fruit and position within the load for specified initial conditions, as well as the rate of condensation as a function of time and position within the load. Results from the model were used to develop data on requirements for the necessary air temperature, relative humidity, air flow rate, and capacity of water vapor injection equipment for use in the vapor heat method. A laboratory scale vapor heat system, including specifications for fruit configuration, air moving equipment, steam generation equipment, and associated control devices and circuitry, was developed for use in vapor heat experiments at other ARS locations.

Plans: The vapor heat model will be revised to incorporate calculations for non-spherical shaped fruit, and to enable the investigation of the heat and mass transfer which occurs during cooling and drying of the fruit following the vapor heat process. Time-temperature lethality data on certain species of fruit fly eggs and larvae will be incorporated into the model to further study the vapor heat process. Engineering assistance will be given to scientists involved in vapor heat experiments at other ARS locations.

Analysis of Heat Transfer During Heating of Fruits in Hot Water for Quarantine Treatment

J. J. Gaffney

Objective: To investigate the heat transfer relationships which occur during heating of fruits in hot water as a method of quarantine treatment to kill fruit fly eggs and larvae.

Methods: A finite-difference mathematical model was developed for analyzing time-temperature relationships in citrus fruits undergoing heating in hot water. The model geometry was that of a composite sphere in order to consider the effects of the differing thermal properties of the flesh and the rind of citrus. Experiments were conducted to measure the convective heat transfer coefficient at the fruit surface for moderate water agitation and for very high agitation provided by bubbling air through the water. A two dimensional heat transfer model was developed to analyze the heat transfer in axisymmetrical shaped fruits, such as papaya. This model was checked for accuracy by comparing model predictions with results from an infinite series analytical solution. Time-temperature lethality data, available in published literature for eggs and first instar larvae of Mediterranean fruit fly, Ceratitis capitata; oriental fruit fly, Dacus dorsalis; and melon fly, Dacus cucurbitae, were used to develop lethality equations which were incorporated into the two dimensional heat transfer model for heating fruits in hot water.

Results: The composite sphere model was used to predict temperatures in citrus fruits being heated in hot water as a function of time, fruit size, position within the fruit, initial fruit temperature, rind thickness, water temperature and amount of water agitation. Results from two dimensional heat transfer model showed significant differences in treatment times required to reach Probit 9 during heating of papayas in hot water for different initial fruit temperatures, water temperatures, fruit sizes, amount of water agitation, and position of insect within the fruit.

Plans: The above-mentioned models will be revised to enable investigation of the heat and mass transfer which occurs during the cooling and drying of the fruit following heating in hot water. Experiments will be conducted to determine time-temperature lethality data for the Caribbean fruit fly, Anastrepha suspensa. Such data will be incorporated into the mathematical heat transfer model for citrus fruits in order to investigate various regimes of heat treatment in hot water, including the study of continued heat treatment which can occur at the center of fruits during the initial portion of the cool down period following heating.

Olfactory Receptor Cell Response to Gland Extracts and Volatile Effluents from Live Virgin Female *Trichoplusia ni*

M. S. Mayer and R. W. Mankin

Objectives: To determine the amount of secondary female sex pheromone gland secretions in the volatile effluent of virgin females.

Methods: A specialized glass chamber designed to be used with a calibrated pheromone delivery system was constructed to confine "calling" virgin females. The volatile effluent from these females was directed over the antenna of a male prepared for single-cell recording. Because the two specialized pheromone receptor cells had been characterized already both quantitatively and qualitatively, we were able to measure the actual emission of two individual pheromone components as they were being released.

Results: The stimulus system, designed so that the actual airborne concentration of pheromonal stimulants could be estimated at continuous intervals throughout the period the female was releasing pheromone, revealed that the HS(a) receptor cell, which is optimally responsive to Z7:12AC, was maximally stimulated throughout most of the period that the female was calling. Conversely, the LS(b) receptor cell, optimally responsive to a secondary pheromone component, was not maximally stimulated. More measurements of the responses by both cells are required to assess whether the secondary component is emitted at regular or irregular intervals. Because this component is not an absolute requirement for either upwind flight or copulatory behavior, its exact role in behavior is not known with certainty, nor is the exact quantity emitted known. With the newly designed equipment, the amount of emission of the secondary pheromone component can be measured and its function can be more firmly established.

Plans: More recordings will be made of the emission of calling females to verify previous measurements of Z7:12AC emission and to quantify the emission of the secondary pheromone component throughout the calling period. Behavioral assays will be performed concurrently to determine the role of the secondary pheromone component.

Quantitative Responses of Two Types of Male *Trichoplusia ni* Pheromone Receptor Cells to Six Female-Produced Sex Pheromone Components

M. S. Mayer and R. W. Mankin

Objectives: To quantify and compare the responses of HS(a) and LS(b) receptor cells to electroantennograms elicited by six female-produced sex pheromone components.

Methods: Electrophysiological recordings of individual receptor-cell responses and electroantennograms (EAG) were made to the six major female sex pheromone components. Prior measurements of pheromone emission from glass tubes reported in 1985 enabled us to measure and compare the responses at the actual airborne concentration at the antenna. Quantitative electroantennogram responses were measured with the usual procedures, which employed the same type of dispenser used for the single-cell recordings.

Results: The EAG responses elicited by a secondary pheromone component were about equal to the responses elicited by Z7:12AC. The other components were about equal to one another and were one to two orders of magnitude less effective stimulants. Responses of individual HS(a) and LS(b) neurons appeared to be in concordance with the EAG. As known, the HS(a) neurons were most sensitive to Z7:12AC, and the LS(b) neurons were about as responsive to the secondary pheromone component. Both neurons, however, were stimulated by all other compounds at concentrations one or two orders of magnitude higher.

Plans: We plan to make more measurements on a larger series of sensilla than presently sampled. Measurements at the naturally-emitted concentrations will be made and the results will be compared with behavioral assays.

Response Characteristics of Pheromone Receptor Neurons on Cabbage Looper Moth Antennae

R. W. Mankin, M. S. Mayer, and A. J. Grant[1]

Objectives: To analyze the responsiveness of olfactory receptor neurons to sex pheromone and relate the responses to insect mate-seeking behavior.

Methods: A microcomputer-based data acquisition and analysis system was developed and interfaced with an electrophysiological work station. Sex pheromone was delivered to the insect antenna using a newly developed calibrated dispenser. The electrical responses of the neurons were recorded and analyzed by user-written software.

Results: A subset of olfactory receptor neurons on male cabbage looper moth antennae have high sensitivity and temporal fidelity of response to component. The stimulus-response relationship for the receptor neurons is described by a power function similar to the behavioral stimulus-response relationship obtained in flight-tunnel bioassays. Analysis of the neuronal response function yields an estimate of the electrophysiological threshold for Z-7:12AC detection and the threshold for discrimination of differences in stimulus intensity, "D"I. As in many other sensory systems, the Weber fraction, "D:I/I (the discrimination threshold divided by the stimulus intensity) decreases as the stimulus intensity increases. Because these neurons are highly sensitive to Z-7:12AC, they appear to be well adapted for detecting pheromone plumes long distances downwind from calling female moths. Furthermore, because they respond quickly to small changes in stimulus intensity at levels expected in female glands emissions, the neurons appear also to be adapted for tracking the rapidly fluctuating concentrations that occur as the pheromone plume breaks up in the turbulent wind stream just downwind of the female.

Plans: This work will be expanded to consider the other sex pheromone components of the cabbage looper. We expect to consider additional insect species as well.

[1]Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

Bioregulation and Molecular Genetics

Bioregulation and Molecular Genetics (Continued)

Ecdysteroid Action on Moth Epithelial Tissues and Cell Lines

H. Oberlander, C. E. Leach, S. Lanka[1], and J. H. Willis[1]

Objectives: To determine the role of ecdysteroids in morphogenesis in a cell line derived from imaginal discs.

Methods: Primary cultures were established from pupal or early pharate adult wings of Hyalophora cecropia. IAL-TND1 cells, derived from wing discs of Trichoplusia ni were maintinaed in continuous culture. The effects of commercially obtained ecdysteroids were assessed through microscopic examination of the cultures.

Results: The spontaneous formation of multicellular, fluid-filled vesicles in primary cultures of pupal wings of Hyalophora cecropia was inhibited by 20-hydroxyecdysone. Physiological levels of ecdysteroids inhibited the ability of larval hemolymph to induce such vesicles in an established cell line (IAL-TND1) derived from imaginal discs of Trichoplusia ni (Hübner). This cell line grew as vesicles for its first year in culture even in the absence of hemolymph, but then converted to growing as multicellular aggregates. Aggregate-conditioned media but not vesicle-conditioned or control media displayed ecdysteroid activity in an imaginal disc bioassay. Larval hemolymph had no influence on the imaginal discs' response to ecdysteroids. Hence, its action in promoting vesicle formation is not to prevent the action of ecdysteroids. We postulate that the change in morphology of TND1 cell cultures from vesicles to aggregates was accompanied by their attaining (or enhancing) the capacity to produce ecdysteroids.

Plans: Analysis of cultures with radioimmunoassays will be employed to determine the ability of established cell lines to produce hormone at various stages.

[1]Department of Entomology, University of ILlinois, Urbana, Illinois 61801

Ecdysteroid-Stimulated Uptake of N-acetyl-D-glucosamine in a Lepidopteran Cell Line Derived from Imaginal Discs

P. Porcheron[1], H. Oberlander and C. E. Leach

Objectives: To determine the mechanisms of hormonal regulation of chitin synthesis in insects.

Methods: IAL-PID2 cells, derived from imaginal discs of Plodia interpunctella were maintained in continuous culture. Chitin precursor uptake was measured by adding radiolabeled N-acetyl glucosamine, and measuring radioactivity in the cells with a liquid scintillation counter. The ability of ecdysteroids to stimulate the uptake of various sugars was compared.

Results: Three cell lines were established in our laboratory from wing imaginal discs of Trichoplusia ni (Hübner), Spodoptera frugiperda (J. E. Smith) and P. interpunctella. We have reported previously some preliminary results that show that an Indianmeal moth cell line, IAL-PID2, responded to exposure to 20-hydroxyecdysone by increased uptake of N-acetyl-glucosamine (GlcNac). In the present work, we investigated more fully the conditions under which 20-hydroxyecdysone stimulates uptake of GlcNac and the possible selectivity of this stimulation with respect to other sugars. The significance of the PID2 cell line as a model system for studying the regulation of uptake of GlcNac is underscored by the present results. The increased uptake of 3-O-methyl-D-glucose, a non-metabolized sugar, after incubation of the cells with 20-hydroxyecdysone raises the possibility that this hormone exerts a fundamental effect on membrane transport per se.

Plans: The PID2 cells are particularly sensitive to stimulation of uptake of GlcNac after treatment with 20-hydroxy-ecdysone, and offer promise of being amenable to a detailed biochemical and cellular analysis of the effects of this hormone on the uptake of chitin precursors in insect cells. These studies will be pursued in collaboration with Dr. Porcheron.

[1]Cytophysiologie des Arthropodes, Universite Pierre et Marie Curie, Paris, France

Kairomonal Stimulation of Oviposition into an Artificial Substrate by the Endoparasitoid *Microplitis croceipes*

R. L. Tilden and S. M. Ferkovich

Objective: To identify factors in the hemolymph of *Heliothis zea* that affect the growth, development and behavior of *Microplitis croceipes*.

Methods: To determine if an oviposition stimulant exists in the larval hemolymph of *H. zea*, solidified agar drops were treated with hemolymph, and then exposed to ovipositing females held in petri dishes. To partially characterize the kairomones, hemolymph was heat-treated, dialyzed, extracted with hexane and enzymatically treated with proteases.

Results: Our preliminary studies into the nature of the oviposition stimulant indicated that the molecule has a molecular weight of less than 12,000 daltons, is somewhat heat sensitive but probably not a protein or peptide since digestion with trypsin and protease had no effect on activity. The kairomone is probably not a lipid since extraction with hexane had no effect on subsequent ovipositional activity.

Plans: Salts, sugars and amino acids off the shelf will be bioassayed as potential ovipositional stimulants. Efforts are underway in cooperation with the Chemistry Research Unit (J. H. Tumlinson and R. R. Heath) to isolate and identify the kairomone by extracting hemolymph with solvents of various polarity and subjecting the samples to analyses by HPLC.

Bioregulation of Synthesis of Macromolecules During Egg Development of the Endoparasitoid, Microplitis croceipes

S. M. Ferkovich and R. L. Tilden

Objective: To study the regulation of protein synthesis in M. crocipes during oögenesis and after oviposition in its host, Heliothis zea.

Methods: Oöcytes were dissected from the ovaries of M. croceipes during various stages of development and from 4th instar host larvae at various times after deposition. The eggs were incubated in tissue culture medium that contained ^{35}S-methionine and/or ^{3}H-leucine for measuring protein synthesis and ^{3}H-uridine for measuring RNA synthesis. Silver stainable proteins as well as newly synthesized proteins were analyzed by one-and two dimensional gel electrophoresis and autoradiography. Actinomycin-D and puromycin were used to inhibit RNA and protein synthesis, respectively.

Results: Protein Synthesis. The patterns of polypeptide synthesis analyzed by one-dimensional SDA PAGE changed quantitively but new polypeptides were not evident in either the oöcytes in various stages of development (located in egg tube, reservoir, and caylx of the ovariole) or during early and late embryogenesis in oviposited eggs. When polypeptide patterns of early and late embryogenesis were compared on two-dimensional SDS gels, synthesis of new polypeptides were evident only after cellularization of the blastoderm (4-6 hr after oviposition). These changes in synthesis after blastoderm formation may be the result of transcription and subsequent translation of zygotic m-RNA.

RNA Synthesis: RNA synthesis continued to diminish throughout oögenesis and into embryogenesis until after blastoderm formation. At this time RNA synthesis returned to the level observed in oöcytes in the egg reservoir. We did not observe a concomittant reduction in protein synthesis with the use of RNA synthesis inhibitors, indicating that translation was dependent on stable maternal RNA rather than zygotic RNA .

Plans: This study has been completed and a manuscript has been accepted for publication. Factors in the host hemolymph and the egg growth factor (Patrick Greany) isolated from Manduca sexta hemolymph will be tested on the rate of protein synthesis as well as the possible synthesis of new proteins in the Microplitis croceipes egg.

Isolation of a Morphogen that Induces Vesicle Formation in a Cell Line Derived from Imaginal Discs of *Trichoplusia ni*

S. M. Ferkovich, H. Oberlander, C. R. Dillard, and C. E. Leach

Objectives: To isolate and characterize the polypeptide morphogen that induces multicellular vesicle formation in an established cell line, IAL-TND1, derived from imaginal discs of *T. ni*.

Methods: Vesicle promoting activity(VPF) was assayed by adding the hemolymph-derived sample to IAL-TND1 cells in multiwell plates that contained test media. The total number of vesicles induced per well was determined after six days of exposure to the hemolymph samples. Test hemolymph was fractionated with various methods of proten purification: gel permeation chromatography, preparative electrophoresis on PAG slab gels, preparative isoelectric focusing in granulated gels, chromato-focusing, and on gel permeation, ion exchange and hydrophobic columns using an HPLC system. Hemolymph was also assayed for VPF activity during each day of the 3rd, 4th, and 5th instar larva.

Results: A polypeptide was purified partially from larval hemolymph that appeared as a 16,600 daltons unit on SDS-PAGE. The polypeptide was highly unstable during separations and storage. It was also isolated from hemolymph using HPLC preparative ion exchange and gel permeation columns' However, the purity attained was not as good as that obtained using chromato- and isoelectric-focusing. When hemocyte-free hemolymph was assayed during larval development, VPF activity was found to be high during each day of the 3rd and 4th instar then significantly declined during the 5th instar.

Plans: A manuscript has been prepared describing purification and properties of the VPF. VPF activity in the hemolymph will be assayed throughout the 4th and 5th instar and during the adult stage. Further work will be undertaken with the HPLC system as a means of purifying enough of the polypeptide to make antibodies. Immuno-chemical techniques will be used to detect and quantify levels of the VPF in the hemolymph and other tissues and to trace and localize the VPF's site of action.

Construction of cDNA and Genomic DNA Libraries for *Heliothis virescens*

S. G. Miller

Objective: To establish genetic repositories for *Heliothis virescens* from which DNA sequences of interest can be readily retrieved.

Methods: A genomic DNA library was constructed from total DNA isolated from moths originating in the Stoneville, MS, *H. virescens* colony. Partial Sau3A digests were cloned into the Bam Hl site of the phage cloning vector EMBL-3, resulting in a library containing 1 x 10^6 independent members (prior to amplification). cDNA libraries were constructed using poly (A)+ RNA isolated from both fat body and testes dissected from late fifth instar larvae. Following ligation of double stranded cDNAs to Eco Rl linkers, each sample was independently cloned into the phage vectors λgt-10 and λgt-11. Yields of independent recombinants varied from one library to the next, and ranged from 5 x 10^4 to 3 x 10^6 independent members.

Results: Most of these libraries have already been screened with either nucleic acid or antibody probes and appear to be representative. As of this writing, these screens have resulted in the isolation of: (1) ß-tubulin cDNA, (from the testes λgt-10 and λgt-11 libraries); (2) a testis-limited homeo box-containing cDNA (from the λgt-10 library); (3) several storage protein cDNA sequences (from the fat body λgt-10 library); and (4) a collection of uncharacterized axonemal protein cDNAs (from the testis λgt-10 library).

Plans: Characterization of these DNA sequences at the structural and functional levels is continuing. It is anticipated that these analyses will culminate in the utilization of full-length copies of genes (retrieved from the genomic library) in appropriate *in vitro* and *in vivo* expression systems.

I would be pleased to make any or all of these libraries available to intereted parties upon request.

Ecydsteroids and Hormonal Regulation of Development in Spodoptera frugiperda

G. Zimowska[1] and A. M. Handler

Objective: To analyze prothoracic gland activity during last instar development in Spodoptera frugiperda, and to determine how this activity and development are influenced by juvenile hormone.

Methods: Animals were raised on an artificial diet under an 18:6 light:dark cycle at 29°C, and developmentally synchronized at the third larval molt. Developmental gates were determined by the timing of changes in body weight, morphology, and behavior. Ecdysteroid content was determined by radioimmunoassay.

Results: Larvae synchronized at the third larval molt and raised under the same conditions divided into two groups based upon the timing of successive molts, growth rate, and the amount of maximum body weight. An analysis of prothoracic gland (PG) activity based upon hemolymph ecdysteroid content was performed during the last larval instar in both groups. In addition, the influence of a juvenile hormone analog, methoprene, on development and ecdysteroid titer was also monitored. In both the rapidly (gate-1) and more slowly (gate-2) developing larvae ecdysteroid titer begins to increase from a low basal level soon after gut purge. Peak levels of hormone (6-8 ng/ml hemolymph) occurred in both groups 42 hr after this time. Sexing of the larvae revealed possible dimorphisms in PG activity. Gate-1 females were delayed by 18-24 hr in their initial ecdysteroid increase, and in both groups females exhibited a delay in decreasing titers from the major peak. The data suggests that a dimorphism exists in the timing of the ecdysteroid peak in developmentally synchronized males and females during metamorphosis, but this must be more clearly determined. As in other lepidopterans, JH treatment influenced the developmental rate of last instar larvae. JH treatment of penultimate and last instar larvae during the first half of the feeding period resulted in delayed development. Treatment near the middle of feeding resulted in accelerated development with molting to supernumerary larvae. Treatment at the end of feeding resulted in larval-pupal intermediates, though no effect upon ecdysteroid titer was observed. The major ecdysteroid peak in animals treated with JH after the feeding period increased at the normal time, but peaked at a relatively low level of 3 ng/ml. Titers prematurely decreased from this level in males, while titers either plateaued at this level or were delayed in decreasing in females. Despite abnormal ecdysteroid titers, possibly due to abnormal PG activity, the pupal molt occurs normally or is slightly accelerated.

Plans: Attention will be focused on clarifying the timing of the metamorphic ecdysteroid peak in males and females. Further analyses on JH titers and metabolism of both JH and ecdysteroids will be pursued.

[1]Department of Invertebrate Physiology, University of Warsaw

An Analysis of Yolk Proteins and Their Synthesis in the Caribbean Fruitfly, Anastrepha suspensa

A. M. Handler and P. D. Shirk

Objective: To identify, isolate, and characterize the major yolk polypeptides in the Caribbean fruitfly as a first step in the isolation and analysis of YP genes.

Methods: Biochemical methods for protein identification and characterization included SDS-polyacrylamide electrophoresis, gel filtration and ion-exchange column chromatography, immunoblot hybridization, and radioactively labelled amino acid incorporation to assay protein synthesis.

Results: An analysis of the regulation of yolk protein (YP) synthesis in the Caribbean fruitfly, Anastrepha suspensa has been initiated. The major polypeptide constitutent of oviposited eggs and vitellogenic ovaries, with a molecular weight of approximately 48 kDa, was identified and isolated by gel-filtration and ion-exchange column chromatography, and SDS-polyacrylamide gel electrophoresis. The major site of synthesis of the polypeptide was the ovary with the first appreciable increase in synthesis occurring at 4 to 5 days after adult eclosion. The polypeptide was also produced by adult fat body and found in the hemolymph, but at markedly lower levels compared to ovaries. A minor denatured hemolymph polypeptide in males co-migrated with the YP, but its identity awaits immunological analysis. Female and male abdomens isolated from 3 to 5 day old adults were tested for YP synthesis in response to 20-hydroxyecdysone and a juvenile hormone analog. The synthetic rate of fat body and ovarian YP did not differ significantly between untreated and hormone treated abdomens. Interestingly though, radioimmunoassay analysis of ecdysteroids indicates a 6- to 7-fold increase in hormone in females near the time of vitellogenesis. The relevance of this sexually-dimorphic ecdysteroid increase in hormone to vitellogenesis and development awaits elucidation.

Plans: A library of cDNA clones made from ovarian mRNA will be screened for a putative YP gene. Screening will be done by hybridization to Drosophila YP genes and by immunological detection in an expression vector system. YP cDNA clones will be used to select genomic clones which will be analyzed with respect to regulatory sequences, those effecting sex-specific expression in particular.

Germline Transformation of the Caribbean Fruit Fly, *Anastrepha suspensa*

D. A. O'Brochta and A. M. Handler

Objective: To determine if *Drosophila* P-element transposons can be used as a vector to achieve germline DNA transformation of *A. suspensa*.

Methods: P-elements are capable of excision as well as transposition. This can result in restoration of gene function previously interrupted by the P-element. The activity of both transposition and excision require certain common biochemical components and therefore excision is another way in which the functionality of the P-element system can be evaluated. Recently Rio et al. (Cell 1986, 44:21) constructed a plasmid which enabled them to detect P-element excision in *Drosophila* cell lines. Prebastoderm M-strain *D. melanogaster* embryos were injected with the excision assay plasmid (pISP) and pUChs $\pi\Delta 23$, a source of transposase. Fifteen hours later the embryos were heat shocked and the plasmids recovered. Plasmids losing the resident P-element are identified after their introduction into the appropriate *E. coli* host.

Results: Eighty-seven of 5×10^4 (freq. = 1.7×10^{-3}) excision assay plasmids recovered from *D. melanogaster* embryos had lost their resident P-element. These excision events only occurred in the presence of transposase.

Plans: The excision assay is a simple method for assessing P-element function in any insect whose embryos can be injected. We plan to conduct this assay in *A. suspensa* as well as other *Drosophila* species.

Quantitative Changes in Storage Protein Content in Hemolymph and Fat Body During Development of the Wax Moth, Galleria mellonella

D. Bean and D. Silhacek

Purpose: To develop a model system for studying the mechanisms of hormonal regulation of storage protein synthesis and uptake in Lepidoptera.

Methods: Wax moths, Galleria mellonella, were reared on artificial diet at 30°C, 70% RH and a 16:8 (L:D) photoperiod. Test insects were selected shortly after molting to the penultimate and final larval instars, pupae and adults and incubated under rearing conditions in small groups for precise staging during the intermolt periods. After appropriate intervals the test animals were chilled, samples of hemolymph and fat body were removed for analyses of storage protein content, and the sex of the animal was noted. Cell-free extracts of the two tissues were analyzed for storage protein content using rocket immunoelectrophoresis. The 82K (82 kilodalton subunit) storage protein was used as an indicator in developing this model system for studying storage protein metabolism.

Results: During the penultimate stadium the 82K storage protein in hemolymph increases from "zero" to 2.5 mg/ml in less than 24 hours. This level dropped to less than 1.0 mg/ml during the molt to the last larval instar. During the first 72 hours of the final stadium 82K storage protein concentration in the hemolymph of female larvae increased to 20 mg/ml, over three times greater than in male larvae. Virtually no 82K protein was found in the fat body during this synthetic period nor during the wandering and spinning stages of development. However, the fat body of both male and female prepupae were found to take up and store nearly all of the circulating 82K protein over a period of 6 hours. During pupal development the fat body loses its store of 82K protein; no corresponding increase of 82K protein in the hemolymph was detected. The fate and function of this protein in adult development is still unclear.

Plans: In vivo alterations in hormonal titers brought on by various environmental stresses and by administration of exogenous hormones will be evaluated in terms of their effects on storage protein metabolism. An in vitro system will be developed for studying hormonal mechanisms that regulate storage protein synthesis and uptake in fat body.

Storage Protein Metabolism During Development of the Waxmoth

C. Malone and D. L. Silhacek

Objective: To determine the subcellular location and movement of storage proteins in fat body cells during synthesis, processing, secretion, uptake, storage and utilization during development of the waxmoth, Galleria mellonella.

Methods: Wax moths were reared on artificial diet at 30°C, 70% RH and a 16:8 (L:D) photoperiod. Test insects were selected shortly after molts to the final larval instar and incubated under rearing conditions in small groups for precise staging during the intermolt periods. After appropriate intervals, the fat body was dissected out of the test insects for embedding and staining.

Results: Colloidal gold as an electron microscopic (EM) immunocytochemical marker was used to identify the role of storage proteins in tissues. The gold, being particulate and electron dense, allows for accurate location of specific binding sites amd the granular structure permits quantitation of the label. The protein A-gold technique, demonstrated in various biological systems has proven reliable for localization of cellular antigenic (82K protein) sites. The protein A-gold complexes with the rabbit antibody bound to the 82K storage protein. This electron dense antibody complex is used to localize 82K protein within cells and subcellular organelles at intervals during waxmoth development. We have developed a protocol for preserving fat body tissue that optimizes morphological preservation while maintaining the antigenicity of the 82K storage protein.

Standard plastic embedding resins (ie., Epon, Araldite, Spurr's) were not used because protein molecules do not retain their antigenicity when exposed to the harsh solvents required to remove the plastic and expose the tissue. As an alternative the more hydrophilic acrylic resins, Lowicryl K4M and LR White were tested. Lowicryl was found to yield less nonspecific binding of the gold probe than LR White and is being used for this study.

The antibody specificity for its antigen (82K Protein) has been demonstrated by Western blotting and immunodiffusion electrophoretic techniques. Working dilutions and incubation times of primary antibody and gold marker are factors that have been optimized. Several dilutions of both primary antibody and labeled marker were tested with various incubation times in order to select the treatment condition maximizing immunocytochemical staining with lowest background.

We have optimized all techniques for using collidal gold as an immunocytochemical marker in following the synthesis, processing, secretion, uptake and storage of 82K storage protein using electronmicroscopy. Our preliminary observations using this technology are encouraging but do not yet allow for definitive conclusions.

Plans: Fat body from precisely aged larvae and pupae will be processed through the colloidal gold method for visualization of 82K storage protein as it is metabolized by the fat body cells.

Isolation and Purification of Serum Proteins from Galleria Mellonella

D. L. Silhacek and C. Murphy

Objective: To develop technology for the rapid isolation of milligram quantitites of plasma proteins for studies on uptake and metabolism.

Methods: Waxmoths were reared on artifical diet at 30°C, 70% RH and a 16:8 (L:D) photoperiod. Experimental insects were selected at the beginning of the wandering stage in the final larval instar. Hemolymph was collected from larvae at 4°C through incisions made in the integument. Lipoproteins were separated by flotation in a density-gradient using the centrifugation procedure described in an earlier report.

Results: The lipoprotein fraction was dialyzed, checked for purity and injected into rabbits for antibody production. A second portion of lipoprotein was separated into two subunits, one heavy (ca. 240K) and one light (ca. 80K) by SDS-PAGE. Antibodies were produced to the 80K subunit. Western blots of the 80K lipoprotein subunit from larvae and the 82K storage protein subunit were probed with the 80K and 82K antibodies; no cross reactivity was evident.

The heavier serum proteins were fractionated by HPLC on anion and cation exchange columns. The three storage proteins, 82K, 76K and 74K were obtained in pure form as evidenced by PAGE and SDS-PAGE. All three proteins were injected into rabbits for antibody production. Western blots of the storage proteins using the different antibodies indicated that 82 K antibody showed no cross reactivity with 76K or 74K storage protein; 76K and 74K antibodies did not react with 82K protein. Although our analyses indicated highly pure preparations of 74K and 76K proteins, considerable cross reactivity was revealed when these two proteins were probed with the 74K and 76K antibodies. Limit digests of 74K and 76K proteins using V8 protease or chymotrypsin indicated little similarity in patterns from PAGE or from Western blots; in fact, marked differences in the susceptability to V8 protease digestion were noted. These observations coupled with the demonstration of lipase activity associated with only 74K protein, indicates that 74K and 76K are different proteins.

Plans: Studies will continue on determining the basis for the 74K and 76K protein-antibody interactions. The receptor responsible for the uptake of 82K storage protein into fat body will be isolated.

Use of Plant Growth Regulators to Manipulate Fruit Susceptibility to Fruit Flies

P. D. Greany, R. McDonald[1], W. Schroeder[1], P. E. Shaw[2], H. Yokomama[3], C. W. McCoy[4], and A. Segarra[5]

Objectives: To reduce the susceptibility of citrus fruits to attack by tephritid fruit flies through use of plant growth regulators known to retard fruit peel senescence (gibberellic acid [GA]) and to affect biosynthesis of peel oils (2-diethylaminoethyl-3,4-dichlorophenylether [DCPTA]).

Methods: (1) Florida tests: Marsh grapefruit trees in a commercial grove near Titusville, FL were sprayed with one of eight solutions with GA alone, DCPTA alone, or a combination of the two, or with water. DCPTA applications were made early in the season, while the fruit were still small. GA applications were made in September, just prior to colorbreak. Fruit samples were collected once each month begining 9/17/86 and were subjected to analyses for peel oil content and peel softness. (2) Peruvian tests: Washington navel oranges in a commercial grove near Lima were sprayed with water, or 20 or 50 ppm GA shortly before colorbreak (April 1986).

Fruit were sampled in April, May, and June to determine infestation by wild Mediterranean fruit flies (Ceratitis capitata). In addition, fruit drop rates were evaluated for treated vs. control trees.

Results: Florida studies: Results to date (through January 1987) indicate that GA applications caused retained peel integrity, based on penetrability, but no effect of DCPTA alone or in combination with GA on oil content was observed to date. Peruvian tests: Fewer flies were produced from GA-treated vs. control fruit, and the magnitude of the effect was directly proportional to the dose employed. However, because the grower sprayed the grove at mid-season, the fly population was vastly reduced and the test will have to be repeated to verify the results. Differential fruit drop rates were markedly different, with only about 50% as many fruit dropping from trees treated with 50 ppm GA and only about 75% as many from 20 ppm trees as compared to the controls. Further tests are needed to establish whether dropped fruit are infested and whether this causes them to drop.

Plans: We plan to initiate tests in Puerto Rico to determine efficacy of using GA to reduce damage by Anastrepha obliqua on mangoes, and on citrus by A. obliqua and A. suspensa. Tests in Peru will be reinitiated. Two manuscripts dealing with the use of GA on citrus and its effect on fruit fly susceptibility are in press.

[1]Horticulture Research Laboratory, USDA ARS, Orlando, FL
[2]Citrus & Subtropical Products Research Lab, USDA ARS, Winter Haven, FL
[3]Fruit & Vegetable Chemistry Laboratory, USDA ARS, Pasadena, CA
[4]University of Florida AREC, Lake Alfred, FL
[5]Agricultural Expt. Station, University of Puerto Rico, Mayaguez

Growth and Development of Microplitis croceipes In Vitro: Requirement for a Host Hemolymph Protein for Initiation of Early Egg Development

P. Greany, S. Ferkovich, and W. Clark

Objectives: To isolate and characterize a protein from host hemolymph that mediates pregermband development by M. croceipes eggs in vitro.

Methods: Partial purification of an egg development stimulating protein (EDSP) was achieved using hemolymph from Manduca sexta, which served as a convenient source of hemolymph. This involved three fractionation steps, including vertical spin density gradient ultracentrifugation, cation exchange chromatography, and gel filtration chromatography. Resultant fractions were bioassayed for activity in stimulating development by newly-laid M. croceipes eggs.

Results: From Bio-Gel P-100 gel permeation chromatography studies, a molecular weight estimate of ca. 60,000-67,000 was determined for EDSP by comparison with the elution volume of known standards. The homogeneity of the protein preparation was confirmed by one dimensional SDS-PAGE. Silver staining revealed a single protein band at Mr = 21,000, suggesting the native protein may exist as an oligomer.

Plans: We will use the protocol developed for Manduca hemolymph to isolate EDSP from the blood of the usual host, Heliothis zea. We then plan to further characterize the protein, define its site of biosynthesis, and investigate its mode of action. With the aid of Drs. Robert Ryan and John Law (Univ. Arizona, Dept. of Biochemistry), we will develop antibodies to EDSP, with the intent of quantifying the titer of EDSP in host hemolymph using host larvae in various instars.

Chemistry

Tobacco Hornworm, Manduca sexta (L.) Pheromones and Associated Behavior

J. H. Tumlinson, M. M. Brennan, E. R. Mitchell, R. E. Doolittle and M. Jackson[1]

Objectives: To isolate and identify the sex pheromone produced by M. sexta females and analyze the behavior of males in response to this pheromone.

Methods: Efforts have been twofold: (1) to obtain more pheromone extract so active components can be isolated and purified for analysis by NMR, and (2) to synthesize the active components. Methods for obtaining gland extract as well as conducting behavioral bioassays in a wind tunnel are the same as described in previous reports. Female tobacco hornworm (THW) pheromone extract has been partially purified by HPLC on a reverse phase HPLC analytical column and analyzed by capillary GC on OV-101 and Carbowax 20M. Synthetic samples have also been partially purified by HPLC and analyzed on Carbowax and OV-101.

Results: Enough information was gathered regarding the possible identity of the one remaining unidentified component of the pheromone blend to undertake the synthesis of several of the stereoisomers of the basic structure of this component. Through these synthetic efforts, including extensive purification by reverse phase HPLC, two isomers were prepared and although they had the correct general structure, did not have the correct stereochemical configuration. During the course of these synthetic and purification efforts, very small quantities of an additional isomer were isolated and partially identified. Spectral data and preliminary bioassay results have narrowed the choices among the remaining steroisomers considerably and have provided strong indications as to the identity of the active isomer. The synthesis of this isomer has been undertaken.

Plans: To synthesize most of the isomers (at least those that are stable enough to isolate and bioassay) of specific active components in order to verify the correct isomers for identification and to test males to the synthetic pheromone blend in the laboratory and field. Several potential pheromone mimics will be synthesized and evaluated for their ability to mimic and substitute for the difficult to obtain component.

[1]USDA, ARS, Oxford, NC

The Effects of Stereostructure on Semiochemical Activity

R. E. Doolittle, T. P. McGovern[1], R. E. Cunningham[2], and J. H. Tumlinson

Objective: Synthesize and evaluate pheromones and other semiochemicals. In particular, develop routes to structures that contain one or more stereocenters so that the effect of exact stereostructure can be determined on insect behavioral response.

Method: The eight stereoisomers of the synthetic insect attractant trimedlure, (a racemic mixture of the t-butyl esters of 2-methyl-4-chloro- and 2-methyl-5-chlorocyclohexane carboxylic acid that had been synthesized earlier; see 1985 Annual Report) which is widely used to monitor infestations of the Mediterranean fruit fly, Ceratitus capitata (Wiedemann), were rigorously purified, analyzed and evaluated in small scale field tests in Hilo, HI. These tests confirm the earlier preliminary results. The 1S,2S,4R enantiomer of isomer C is superior in attractance to male flies over its mirror image and all other isomeric forms. The RR enantiomeric forms of the other three trans isomers were significantly superior to their mirror images. These more extensive tests compared with those of the preliminary report allow us to differentiate between the enantiomers of isomer A and demonstrate that they are not "equally attractive" as was earlier reported.

Plans: The results and data from this project are being incorporated into a manuscript.

[1]USDA/ARS Organic Chemical Synthesis Lab, Beltsville, MD
[2]USDA/ARS Tropical Fruit & Vegetable Research Laboratory, Hilo, HI

Synthesis of Biologically Active Chemicals and Semiochemicals by New Efficient Stereoselective Routes

R. E. Doolittle

Objectives: To develop new efficient synthetic procedures that will allow stereospecific synthesis of complex biologically active molecules in a highly pure state to corroborate assigned chemical structures, confirm biological activity, and provide chemicals for initial field experiments. The preparation of a series of polyunsaturated aldehydes, alcohols and acetates for use as standards and as candidate components of a pheromone blend.

Methods: The technology applied earlier to the synthesis of functionalized conjugated dienes was employed for the sterospecific synthesis of additional compounds of this type.

Results: (E,Z)-11,13-pentadecadienal was synthesized for neurophysiological testing. Methyl(Z)- and (E)-5-tetradecenoate were prepared and characterized and sent for field tests for attraction to the Japanese beetle. The work on methods for the stereoselective synthesis of poly-unsaturated aldehydes was continued, and additional regio and stereoisomers of this type of compound were synthesized. Some of the compounds were utilized as standards and candidate pheromone components in the identification of insect pheromone blends.

Plans: Additional diene, ene-yne, and multiple unsaturated aldehyes, alcohols, and acetates will be synthesized as potential pheromones and pheromone trace components. New technology will be developed and subsequently applied to the stereospecific synthesis of biologically active chemicals in high purity.

Long-Range Attraction of *Microplitis croceipes* to Heliothis Species

F. J. Eller[1], J. H. Tumlinson, and W. J. Lewis

Objectives: To investigate the behavioral role and chemical nature of volatile kairomones which may mediate the long-range attraction of the parasitoid *Microplitis croceipes* (Cresson) to *Heliothis* species.

Methods: A wind tunnel was used to investigate female foraging behavior. Female parasitoids were given an oviposition experience with a *H. zea* larva feeding on cowpeas, these females were then held for 1, 60, or 1440 minutes before being tested in the wind tunnel. To determine the source(s) of the volatile attractant(s), females were tested to the plant-host complex less individual components in addition to being tested to the individual components themselves.

Results: The percent of females making complete flights when held for 1, 60, or 1440 minutes between their oviposition experience and wind tunnel testing were 46, 38, and 31, respectively. The removal of the frass from the plant-host complex was the only treatment which elicited significantly fewer complete flights than the complete plant-host complex elicited. In addition, the frass alone was the only treatment which did not elicit significantly fewer complete flights than that elicited by the complete plant-host complex.

Plans: Volatiles from the plant-host complex will be collected and will be bioassayed for their ability to attract female parasitoids in the wind tunnel. The active compounds will be isolated and identified from these volatile collections.

---

[1]Gruaduate student, Dept. of Entomology & Nematology, Univ. of Florida, Gainesville, FL.

Semiochemical Mediated Long-Range Host Location by Cotesia Marginiventris. A Parasitoid of Several Noctuids

T. C. J. Turlings[1], J. H. Tumlinson, W. J. Lewis, and J. W. A. Scheepmaker

Objectives: To elucidate the behavioral mechanisms and the semiochemicals released by hosts and/or host habitats that mediate the foraging behavior of the larval parasitoid Cotesia marginiventris. Currently, the research focuses on the effect of contacting hosts and/or host products on the parasitoids' responses to semiochemicals. The hypothesis that experience with a particular host species results in a preference for odors associated with that host, is being tested.

Methods: Bioassays that were developed in preliminary studies are now being used to determine the responses of female parasitoids to host associated odors. In a four arm olfactometer females are given the choice to respond to odors of two host/host plant complexes. The complexes consist of host larvae (Fall Armyworm or Cabbage looper) feeding on seedlings (corn or cotton). Prior to the choice tests females are given a 20 second experience with one of the complexes from which the larvae have been removed. The changes in response to the odors offered to the wasps in the olfactometer may indicate whether or not the experience has any effect on the host searching behavior of the wasp.

Results: Earlier results already showed that giving wasps experience with hosts or host products increased their response to semiochemicals dramatically. The choice experiments in the olfactometer demonstrated that experience did not just result in an increase in response, but that the experience also causes a shift in preference in favor of the odor that is associated with the experienced host/host plant complex. The first results indicate that the effect of the host larvae is stronger than that of the plant on which they are feeding. Furthermore, it has been established that this conditioning effect does not require actual ovipositions but that contact with damaged leaves and host products is sufficient.

Plans: Additional olfactometer experiments will be performed. Wasps will be given only part of a complete experience (oviposition of larvae feeding on a seedling), to determine what part of the experience is responsible for the dramatic increase in response and what is actually necessary to cause conditioning.

We will make volatile collections to analyze and identify the chemicals released by hosts in their habitat.

[1] Graduate student, Dept. of Entomology & Nematology, Univ. of Florida, Gainesville, FL.

Sex Pheromone Biosynthesis by Lepidoptera

P. E. A. Teal and J. H. Tumlinson

Objectives: To determine the mechanism of pheromone biosynthesis by female Lepidoptera species.

Methods: The terminal steps in pheromone biosynthesis were studied using in vivo topical application studies in which candidate compounds were applied to the gland surface in either DMSO or H_2O. In vitro studies were also conducted using excised glands and gland homogenates. Incubation periods and concentrations of substrates were varied in order to gain information on the biosynthetic system. Extracts of preparations were made using hexane and analyzed by capillary GC and GS-MS.

Results: Primary alcohols varying in chain length from C_{13} to C_{16}, and in number, position, and geometric configuration of double bonds were applied in dimethyl sulfoxide to the surface of the female sex pheromone glands of Heliothis subflexa (Gn.) and Hydraecia micacea (Esper). Capillary gas chromatographic analysis of extracts of the treated glands indicated that the alcohols were converted to the corresponding aldehydes by H. subflexa females and to the acetates by H. micacea females. Conversions of the alcohols showed no preference for molecular weight, number, position, or geometry of the double bonds in either species. Application of the acetates of the primary alcohols to the gland surface of H. subflexa females resulted in the production of both the corresponding alcohols and aldehydes while neither alcohols nor aldehydes were produced when acetates were applied to the glands of H. micacea. In addition, application of the acetates to the gland surface of Heliothis virescens (F.) resulted in the production of both the corresponding alcohols and aldehydes. However, no evidence was found to indicate that acetates are ever produced by the pheromone gland of females of H. virescens.

In vitro studies on gland homogenates of H. virescens and H. zea in which the gland cells were destroyed by ultra sonnication and osmotic shock indicated that the oxidase responsible for conversion of alcohols to aldehydes is present in the cuticle overlying the cells. The oxidase is tightly bound in the cuticle of H. virescens but can be freed from the cuticle of H. zea by exhaustive homogenation in phosphate buffer.

Plans: The biosynthesis of sex pheromones by hybrid and backcross insects obtained from crosses between H. virescens and H. suflexa will be studied. In addition enzymes responsible for alcohol and aldehyde biosynthesis by females of H. zea, H. subflexa and H. virescens will be isolated and purified.

Male Pheromone of *Heliothis virescens:* Role of Pheromone Components in Chemical Communication

P. E. A. Teal and J. H. Tumlinson

Objectives: To determine the function of the pheromone produced by the hairpencil gland of males of *H. virescens.*

Methods: The effects of extracts obtained from the hairpencil glands of males on the reproductive behavior of males were studied in a wind tunnel. Responses of males to the female sex pheromone were compared with those that occurred when both the female sex pheromone and male gland extracts were presented. These studies were parallelled by studies in which the responses of males to the female pheromone plus the naturally produced pheromone released by other males were compared to those that occurred when the female produced pheromone was released alone. Studies on the behavior of females to the pheromone produced by the hairpencil gland were conducted using pairs of females housed in small screen cages. Air was puffed over either the exposed hairpencil glands held in disposable pipetts or extracts adhering to the inside walls of pipetts and onto the caged females from a distance of ca 1cm.

Results: Studies indicated that males respond equally well to the female pheromone alone or female pheromone plus either naturally released hairpencil pheromone or extracts. Therefore the male produced pheromone does not alter the behavior of conspecific males. Females responded to the hairpencil pheromone of males by becoming quiescent, beginning to call and by attempting to copulate with each other. These behaviors do not occur when air blank samples are used. Therefore the pheromone produced by males of *H. virescens* functions as a species specific signal to females.

Plans: Future studies will address the role of the individual compounds identified from the hairpencil glands of males.

Male Pheromone of Heliothis virescens: Correlation Between Gland Structure, Pheromone Chemistry and Biosynthesis

P. E. A. Teal, J. H. Tumlinson

Objectives: To determine the chemistry of compounds produced by the hairpencil glands of males of H. virescens, identify the terminal steps of biosynthesis of these compounds and correlate these findings with the structure of the hairpencil gland.

Methods: The intact hairpencil glands as well as excised scales associated with the gland were extracted in hexane and/or other solvents. Volatiles released from the glands were collected by passing purified air over exposed glands which carried the compounds either directly into solvent or onto charcoal entrainment filters. Both capillary GC and GC mass spectral analyses were conducted. Studies on biosynthesis were conducted by applying candidate compounds to the glands or scales in DMSO and extracting and analyzing the extracts after various lengths of time. Studies on the structure of the glands were performed by semi thin sectioning of the glands and observation using light microscopy and by scanning electron microscopy.

Results: Analysis of extracts and volatiles indicated that in addition to S16:AC (212.4 ng/male) and S16:OH (22.3ng/male) small amounts of Z11-16:AC, Z7-16:AC, Z9-16:AC and Z11-16:OH were present in the pheromone blend. In addition esterified samples also contained the methyl esters of S14:COOH, S16:COOH and S18:COOH. No Z9-14:AL was found. Studies indicated that pheromone titers increase rapidly after eclosion, then level off at a high level after ca 24 hr. The pheromone is depleted when the hairpencil gland is exposed. After exposure, titers increase to the initial high level within 24 hours. In vivo application of candidate precursors to the surface of the descaled hairpencil gland showed that biosynthesis occurs within the gland cells and proceeds to the alcohol via the acetates. The hairpencil scales serve only to dispense the pheromone and are not involved in biosynthesis. Stuctural studies showed that the hairpencil gland is composed of typical trichogenous units, each unit containing an unmodified epidermal cell, a tormogen cell, a central trichogen cell and associated porous elongate scale. The trichogen cell contains numerous membrane bound lipid vesicles in the apical area which appear to pass through the cell membrane, being stored in a pocket which subtends the scale. The material appears to be forced into the scale and out through the pores when the gland is everted.

Plans: The terminal steps in biosynthesis will be studied further in order to elucidate the complete mechianism of pheromone production. Studies on isolation and purification of the enzymes involved will also be conducted and the results compared with companion studies on females.

Genetics of Pheromone Production by *Heliothis* Hybrid and Backcross Insects

P. E. A. Teal and J. H. Tumlinson

Objectives: To determine the genetic mechanisms regulating the inheritance of pheromone blends by *Heliothis virescens* x *H. subflexa* hybrid and backcross insects.

Methods: Extracts of the pheromone glands of females and hairpencil glands of males were obtained for reciprocal F_1 hybrid insects. Extracts were analyzed by capillary GC and GC- mass spectroscopy. Results were compared with those obtained from studies on the parent species.

Results: Thus far, analysis of the F_1 insects has indicated that the majority of females (95%) from the *H. virescens* (male) x *H. subflexa* (female) hybrid line maintain pheromone titers that are only 10% of those found in the parent species while females of the reciprocal cross maintain titers equivalent to those of the parents. In both groups the ratio of Z11-16:AL to Z9-16:AL is intermediate between those of the parent species and the ratio of S16:AL : Z11-16:AL is distinct in both hybrid groups.

Extracts of hairpencil scales of males of both hybrid lines contain the same ratios and components (a series of fourteen, sixteen and eighteen carbon acids) that are present in extracts of males of *H. subflexa.* None of the acetates or alcohols present in the hairpencil glands of males of *H. virescens* are present in the F_1 hybrid males. This is correlated with differences in the morphology of the hairpencil glands of *H. virescens* and *H. subflexa*. All of the F_1 hybrid males have hairpencil glands similar to *H. sublexa.* Therefore, the genome of *H. subflexa* is dominant over that of *H. virescens* with regard to gland morphology and production of the male pheromone.

Plans: Studies will continue using F_1 hybrids and backcross insects in order to define the genetic basis of pheromone production.

Formulation of Highly Volatile Pheromones

R. R. Heath, and R. H. Murphy

Objectives: (1) To provide a constant release rate of pheromones with vapor pressures in the range equivalent to octane - tetradecane. (2) To provide a predictive model for the release rate of highly volatile pheromones whose vapor pressure is unknown.

Methods: The release rate of compounds from glass capillaries and rubber substrate were obtained. Rate measurements were obtained by collection of volatiles and analysis using gas chromalography. The release rate of the compounds is measured until the half-life is reached or steady-state release is obtained. The release rates are correlated with the relative vapor pressures of the compounds.

Results: Release rate measurements have been obtained for a variety of compounds. The release rate of the compounds formulated using rubber substrates were variable. Release rate of volatile compounds from capillaries were consistent with a predictable change in the release rate per unit of time.

Plans: Additional correlations of the release rate of volatile compounds from capillaries will be obtained. Information available from the research will be used in formulating fruit fly pheromones.

Mediterranean Fruit Fly Pheromone

R. R. Heath, J. H. Tumlinson, D. L. Chambers[1]
J. M. Sivinski, and C. O. Calkins

Objectives: (1) To isolate, identify, and synthesize volatile pheromones produced by Mediterranean fruit flies. (2) To formulate the pheromones for laboratory and field behavioral studies and determine if the pheromone can be used as a lure for monitoringthe population of fruit flies.

Methods: The volatiles emitted by virgin male Mediterranean fruit flies of different ages and at different times in the photophase were collected from flies in Guatemala. The collected volatiles were analyzed by capillary gas chromatography. Nuclear magnetic resonance and mass spectroscopy were used to confirm the structure of the major compounds. The major pheromone components were formulated using rubber septa and capillaries.

Results: The release rate and ratio of the major components produced by the Mediterranean fruit fly have been determined. Spectroscopic data is in agreement with the identification made by Baker et al (J. Chem. Soc., Chem. Commun. 824-825, 1985) that major pheromone components are ethyl (E)-3-octenoate, (E,E)-α-farnesene and geranyl acetate. These chemicals have been formulated using capillaries to provide a release ratio and release rate equivalent to 1,5 and 10 males hours.

Plans: Further analysis of volatiles produced by male Mediterranean fruit flies will be performed. Behavioral analysis of the major pheromone components will be obtained using wild flies in Guatemala.

[1]USDA/APHIS, Guatemala Medfly Station, Guatemala City, Guatemala

Oviposition Stimulant for Heliothis subflexa Obtained from Ground-Cherry

R. R. Heath, E. R. Mitchell, F. C. Tingle, B. Dueben, R. Hines, and W. Copeland

Objectives: To isolate and identify the semiochemicals responsible for stimulating oviposition by Heliothis Subflexa.

Methods: Methanol extracts of ground cherry were purified by gravity flow and high performance liquid chromatography using ether/hexane as the solvent. Individual fractions and fractions in combination obtained from each purification step were bioassayed using two methods. In an oviposition olfactometer (see Mitchell and Heath report) oviposition preference by 3-5 day old mated females was determined for each step of the purification. Similarly, bioassays of 3-5 day old females in a wind tunnel was obtained to the various isolates.

Results: An active fraction was obtained from the gravity flow chromatography purification that was equal in response to that obtained by the crude material. Bioassays in the wind tunnel resulted in an average of 80% source contact with the crude and gravity flow purified material. The mean percent of eggs layed in the oviposition olfactometer was 72% and 76% to the treatment for the crude method extract and active gravity flow fraction respectively.

Plans: Continue with the purification of the active material. After purification is complete, the active compound(s) will be identified and synthesized.

Chemical Analysis of Volatile Pheromones Produced by Anastrepha spp. and the Papaya Fruit Fly

R.R. Heath, J. H. Tumlinson, J. M. Sivinski, P. J Landolt, C. O. Calkins, and D. L. Chambers[1]

Objectives: (1) To isolate, identify, and synthesize volatile pheromones produced by several species of Anastrepha and the papaya fruit fly which are native to Guatemala and may pose a threat to U. S. agriculture; (2) To formulate these pheromones for laboratory and field behavioral studies by cooperating entomologists and to determine the role that these semiochemicals play in fruit fly communication and to develop lures for pest species.

Methods: A volatile collection system will be used to collect volatiles emitted by fruit flies. The volatiles will be collected from males of different ages and at different times during their calling period. Samples will be shipped to Gainesville for analysis by capillary GC, and Mass, IR, and NMR spectroscopy.

Results: The volatile collection system has been installed in Guatemala City at Dr. Chambers' laboratory. The volatiles from several Anestrepha spp. have been collected and analyzed. Preliminary indications are that the pheromone blends of the Anastrepha species analyzed thus far are very similar but that distinct specific differences in composition do occur.

Plans: Additional collections and analysis will have to be made in order to characterize each species pheromone blend.

[1]USDA/APHIS, Guatemala Medfly Station, Guatemala City, Guatemala

PUBLICATIONS LIST

INSECT ATTRACTANTS, BEHAVIOR, AND BASIC BIOLOGY RESEARCH LABORATORY

Reprints may be requested by number.

Address inquiries to:

H. Oberlander, Director
Post Office Box 14565
Gainesville, FL 32604 USA

651. Sivinski, John and J. C. Webb. 1985. The form and function of acoustic courtship of the papaya fruit fly, Toxotrypana curvicauda Gerstäcker (Tephritidae). Fla. Entomol. 68(4): 634-641.

652. Tumlinson, J. H., P. E. A. Teal, E. R. Mitchell, and R. R. Heath. 1985. Sex pheromones of economically important North American Spodoptera species. Proc. Int. Conf. on Integrated Plant Protection, Budapest, Hungary (July 4-9, 1983) 4: 140-146.

653. Guss, P. L., P. E. Sonnet, R. L. Carney, J. H. Tumlinson, and P. J. Wilkin. 1985. Response of northern corn rootworm, Diabrotica barberi Smith and Lawrence, to stereoisomers of 8-methyl-2-decyl propanoate. J. Chem. Ecol. 11(1): 21-26.

654. Curtis, C. E., P. J. Landolt, and J. D. Clark. 1985. Disruption of navel orangeworm (Lepidoptera: Pyralidae) mating in large-scale plots with synthetic sex pheromone. J. Econ. Entomol. 78(6): 1425-1430.

654-1 Ward, C. R., N. E. Urbina, E. Prudott, R. A. Lainez P., L. Crivelli, A. Dominguez, R. Medina, H. Deras and E. R. Mitchell. 1985. Uso de trampas con atrayente sexual para el monitoreo de poblaciones adultas de Spodoptera frugiperda en el norte de Honduras. CEIBA 26(1): 194-199 (Proc. Integrated Pest Management Symposium, Panamerican Agricultural School, Tegucigalpa, Honduras C.A.)

654-2 Ashley, T. R., E. R. Mitchell, and V. Waddill. 1985. Control biologico del Gusano Cogollero, en Florida, E. U. A. CEIBA 26(1): 177-186 (Proc. Integrated Pest Management Symposium, Panamerican Agricultural School, Tegucigalpa, Honduras C.A.)

655. Willis, J. H., H. Oberlander, C. E. Leach, and S. Lanka. 1986. Ecdysteroid action on moth epithelial tissues and cell lines. In Vitro (abstract) 22(3): 12A.

656. Agee, H. R. 1985. Neurobiology of the bollworm moth: Effects of avermectin on neural activity and on sensory systems. J. Agric. Entomol. 2(4): 325-336.

657. Agee, H. R. 1985. Neurobiology of the bollworm moth: Response of neurons in the central nervous system to avermectin. J. Agric. Entomol. 2(4): 337-344.

658. Agee, H. R. 1985. Neurobiology of the bollworm moth: Neural elements controlling behavioral responses to pulsed ultrasound. J. Agric. Entomol. 2(4): 345-350.

659. Ferkovich, Stephen M., and Charles R. Dillard. 1986. A study of uptake of radiolabeled host proteins and protein synthesis during development of eggs of the endoparasitoid, Microplitis croceipes (Cresson) (Braconidae). Insect Biochem. 16(2): 337-345.

660. Greany, Patrick. 1986. In Vitro culture of hymenopterous larval endoparasitoids. J. Insect Physiol. 32(4): 409-419.

661. Sivinski, J., and J. C. Webb. 1986. Changes in a Caribbean fruit fly acoustic signal with social situation (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 79: 146-149.

662. Mitchell, Everett R., and Robert R. Heath. 1986. Pheromone trapping system for the velvetbean caterpillar (Lepidoptera: Noctuidae). J. Econ. Entomol. 79(1): 289-292.

663. Teal, P. E. A., J. H. Tumlinson and R. R. Heath. 1986. Chemical and behavioral analyses of volatile sex pheromone components released by calling Heliothis virescens (F.) females (Lepidoptera: Noctuidae). J. Chem. Ecol. 12(1): 107-126.

664. Teal, P. E. A., and J. H. Tumlinson. 1986. Terminal steps in pheromone biosynthesis by Heliothis virescens and Heliothis zea. J. Chem. Ecol. 12(2): 353-366.

665. Doolittle, R. E., and J. D. Solomon. 1986. Stereoselective synthesis of (Z,E)-3,5-tetradecadien-1-ol acetate: sex attractant for carpenterworm moth, Prionoxystus robiniae (Peck) (Lepidoptera: Cossidae) and effect of isomers and monounsaturated acetates on its attractiveness. J. Chem. Ecol. 12(3): 619-633.

666. Williams, Allen L., Everett R. Mitchell, Robert R. Heath, and Carl S. Barfield. 1986. Oviposition deterrents for fall armyworm (Lepidoptera: Noctuidae) from larval frass, corn leaves, and artificial diet. Environ. Entomol. 15(2): 327-330.

667. Sivinski, John, and Carrol Calkins. 1986. Pheromones and parapheromones in the control of tephritids. Fla. Entomol. 69(1): 157-168.

668. Srinivasan, Asoka, J. A. Coffelt, Phillipa Norman, and Beverly Williams. 1986. Sex pheromone gland of the navel orangeworm, Amyelois transitella: Location, bioassay and in vitro maintenance. Fla. Entomol. 69(1): 169-174.

669. Mitchell, Everett R. 1986. Pheromones: As the glamour and glitter fade -- The real work begins. Fla. Entomol. 69(1): 132-139.

670. Krysan, J. L., P. H. Wilkin, J. H. Tumlinson, P. E. Sonnet, R. L. Carney, and P. L. Guss. 1986. Responses of Diabrotica lemniscata and D. longicornis (Coleoptera: Chrysomelidae) to stereoisomers of 8-methyl-2-decanol propanoate and studies on the pheromone of D. longicornis. Ann. Entomol. Soc. Am. 79: 742-746.

671. Koehler, Philip G., Richard S. Patterson, and J. C. Webb. 1986. Efficacy of ultrasound for German cockroach (Orthoptera: Blattellidae) and Oriental rat fles (Siphonoptera: Pulicidae) control. J. Econ. Entomol. 79: 1027-1031.

672. Miller, Stephen G., Milton D. Huettel, Minh-Tam B. Davis, Eeleen Hickey Weber, and Lee A. Weber. 1986. Male sterility in Heliothis virescens X H. subflexa Bbckcross hybrids: evidence for abnormal processing of mitochondrial transcripts. Mol. and Gen. Genet. 203: 451-461.

673. Agee, Herndon R. 1986. Laboratory-reared boll weevils are visually impaired. J. Econ. Entomol. 79: 900-902.

674. Vick, K. W., P. Koehler, and J. J. Neal. 1986. Incidence of stored-product Phytcitinae moths in food distribution warehouses as determined by sex pheromone baited traps. J. Econ. Entomol. 90: 936-939.

675. McLaughlin, John R. 1986. Manipulation of orientation to pheromones, pp. 163-168. Chapter in Mechanisms in Insect Olfaction. Oxford University Press.

676. Landolt, Peter J., and Robert R. Heath. 1986. A sex attractant synergist for Trichoplusia oxygramma (Lepaidoptera: Noctuidae). Fla. Entomol. 69: 425-426.

677. Tingle, F. C., and E. R. Mitchell. 1986. Behavior of Heliothis virescens (F.) in presence oviposition deterrents from elderberry. J. Chem. Ecol. 12: 1523-1531.

678. Drost, Y. C., W. J. Lewis, P. O. Zanen and M. A. Keller. 1986. Beneficial arthropod behavior mediated by airborne semiochemicals. I. Flight behavior and influence of preflight handling of Microplitis croceipes (Cresson). J. Chem. Ecol. 12(6): 1247-1262.

679. Heath, R. R., J. A. Coffelt, P. E. Sonnet, F. I. Proshold, B. Dueben, and J. H. Tumlinson. 1986. Identification of a sex pheromone produced by female sweetpotato weevil, Cylas formicarius elegantulus (Summers). J. Chem. Ecol. 12(6): 1489-1503.

680. Hildebrand, J. G., A. M. Schneiderman, S. G. Matsumoto, M. M. Brennan and J. H. Tumlinson. 1986. Trans-sexually grafted antennae influence pheromone-directed behavior and development of sexually dimorphic central neurons in the moth Manduca sexta. First Int. Congr. Neuroethol. Tokyo, Sept. 1986 (Abstract).

681. Proshold, F. I., J. L. Gonzalez, C. Asencio, and R. R. Heath. 1986. A trap for monitoring the sweetpotato weevil (Coleoptera: Curculionidae) using pheromone or live females as bait. J. Econ. Entomol. 79(3): 641-647.

682. Tumlinson, J. H., E. R. Mitchell, P. E. A. Teal, R. R. Heath, and L. J. Mengelkoch. 1986. Sex pheromone of fall armyworm, Spodoptera frugiperda (J. E. Smith). Identification of components critical to attraction in the field. J. Chem. Ecol. 12(9): 1909-1926.

683. Landolt, P. J., R. R. Heath, P. E. Sonnet, and K. Matsumoto. 1986. Attraction of Harrisina americana and Acoloithius falsarius Males (Lepidoptera: Zygaenidae) to R(-)-2-butyl-7-Tetradecenoate. Environ. Entomol. 15(4): 969-962.

684. Ashley, Tom R. 1986. Geographical distributions and parasitization levels for parasitoids of the fall armyworm, Spodoptera frugiperda. Fla. Entomol. 69(3): 516-524.

685. Heath, R. R., P. E. A. Teal, J. H. Tumlinson, and L. J. Mengelkoch. 1986. Prediction of release ratios of multicomponent pheromones from rubber septa. J. Chem. Ecol. 12(12): 2133-2143.

686. Mitchell, Everett R. 1986. USDA Technical Bulletin No. 34 -- The legacy of Philip Luginbill. Fla. Entomol. 69(3): 452-455.

687. Miller, Stephen G., and Milton D. Huettel. 1986. Mitochondrial biogenesis during spermatogenesis in Heliothis virescens and H. subflexa and their male-sterile backcross hybrids. Arch. Insect Biochem. & Physiol. 3: 363-380.

688. Schneiderman, Anne M., John G. Hildebrand, Margaret M. Brennan, and James H. Tumlinson. 1986. Trans-sexually grafted antennae alter pheromone-directed behavior in a moth. Nature 323(6091): 801-803.

689. Heath, R. R., and J. H. Tumlinson. 1986. Correlation of retention times on a liquid crystal capillary column with reported vapor pressures and half-lives of compounds used in pheromone formulations. J. Chem. Ecol. 12(11): 2081-2088.

690. Callahan, Philip S. 1986. The possible detection of magnetic monopolar and monopole tachyons. Speculations in Science and Technology 9(1): 51-60.

691. King, E. G., L. F. Bouse, D. L. Bull, R. J. Coleman, W. A. Dickerson, W. J. Lewis, J. D. Lopez, R. K. Morrison, and J. R. Phillips. 1986. Management of Heliothis spp. in cotton by augmentative releases of Trichogramma pretiosum Ril. Z. ang. Entomol. 101: 2-10.

692. Lynn, D. E., and H. Oberlander. 1986. Obtainment of hormonally sensitive cell lines from imaginal discs of Lepidoptera species, pp. C213: 1-12, In vitro invertebrate hormones and genes. In: E. Kurstak [ed.], Techniques In the Life Sciences, C2. Elsevier Scientific Publishers Ireland Ltd.

693. Landolt, P. J., R. R. Heath and N. C. Leppla. 1986. (Z,Z,Z)-3,6,9-Eicosatriene and (Z,Z,Z)-3,6,9-Heneicosatriene as sex pheromone components of Mocis disseverans (Walker) (Lepidoptera: Noctuidae). Environ. Entomol. 15(6): 1272-1274.

694. Nordlund, D. A., W. J. Lewis, S. B. Vinson, and H. R. Gross, Jr. 1986. Behavioral manipulation of entomophagous insects, pp. 105-116. In: E. G. King and J. R. Bradley, Jr. [eds.], Theory and Tactics of Heliothis Management. I. Cultural and Biological Control. Southern Cooperative Series Bulletin 316.

695. Dobson, Ian D., and Peter E. A. Teal. 1986. Field studies of the temporal response patterns of male Diabrotica virgifera virgifera LeConte and D. barberi Smith and Lawrence to 8R-methyl-2R-decyl propanoate. Physiol. Entomol. 11: 405-410.

Appendix 2

Seminars by Visiting Scientists

Dr. John Law, Department of Biochemistry, University of Arizona
"Insect Lipoproteins"

Dr. Patrick Porcheron, Universite Pierre et Marie Curie, Paris, France
"Edysteroid and Integument In Vivo and In Vitro"

Dr. Jo-Ann Fleming, Department of Entomology, Texas A&M University
"Transcriptional & Structural Studies of CfV: The Polydnavirus Endogenous to Campoletis sonorensis Wasps"

Dr. Grazyna Zimowska, Department of Invertebrate Physiology, University of Warsaw
"A Developmental Analysis of Metamorphosis in Spodoptera"

Dr. Imtiaz Ahmad, Chairman, Department of Zoology, University of Karachi
"Agricultural Pests of Pakistan"

Dr. David O'Brochta, Department of Biology, University of California, San Diego
"The Patterns of Growth During the Development and Regeneration of Drosophila Imaginal Discs"

Dr. Miklos Toth, Plant Protection Institute of the Hungarian Academy of Sciences, Budapest, Hungary
"Recent Developments in Pheromone Research on Noctuids, Geometrids, and Tortricids in Hungary"

Dr. Tatsugi Chuman, Central Research Institute, Yokohama, Japan
"Sex Pheromones of the Caribbean Fruit Fly"

Dr. Paul Pener, Hebrew University of Jerusalem
"Endocrinological Control of Mating in Locusts and Grasshoppers"