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BARD

FINAL REPORT

PROJECT NO. US-1208-86

**Chemical Characterization and Species
Specificity of Sex Pheromones of Female
Looper Moths
(Lepidoptera: Noctuidae: Plusiinae)**

6 P.J. Landolt, R.R. Heath, E. Dunkelblum, M. Kehat

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Appendix VI

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Title

Chemical characterization and species specificity of
sex pheromones of female looper moths (Lepidoptera

Noctuidae Plusiinae)

Investigators' Names
(Principal listed first)

P. J. Landolt

R. R. Heath

Ezra Dunkelblum

Moshe Kehat

Investigators' Institutions

USDA, ARS, P. O. Box 14565

Gainesville, FL 32604

Institute of Plant Protection

Volcani Center

Bet Dagan, 50-250, Israel

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Peter Landolt
Signature
Principal Investigator

Herbert Oberlander 1-4/91
Signature
Institution's Authorizing Official

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- A: "Identification of female sex pheromones of Autographa biloba and Anagrapha falcifera (Lepidoptera: Noctuidae)"
- B: "Identification of a male cabbage looper sex pheromone"
- C: "Temporal partitioning of dual sexual attraction strategies in Trichoplusia ni (Lepidoptera: Noctuidae)".

C. Abstract.

The sex pheromones of females of 9 species of looper moths (Noctuidae: Plusiinae) were chemically characterized. Results for Trichoplusia ni were similar in the US and Israel laboratories, confirming earlier accounts of a 6 component blend comprised of Z7-12:Ac, Z5-12:Ac, 11-12:Ac, Sat12:Ac, Z7-14:Ac, and Z9-14:Ac. The female pheromone of Chrysodeixis chalcites is similar, but missing Z5-12:Ac and possessing Z9-12:Ac. Three other species appear to also use Z7-12:Ac as the major female sex pheromone component, but at much lower release rates than in T. ni and including Z7-12:OH as a minor component. Autographa biloba in the US uses these two chemicals and Z-5-12:Ac as the female pheromone. Anagrapha falcifera, also in the US, and Autographa gamma in Israel, use only very small amounts of Z7-12:Ac and Z7-12:OH. Cornutiplusia circumflexa in Israel uses the Z7-12:OH as the major female sex pheromone component, with smaller amounts of Z7-12:Ac. Rachiplusia ou and Mouralia tinctorides, both in the US, also possess Z7-12:Ac in female abdominal tip extracts, with as yet unidentified minor components (1 in R. ou and 2 in M. tinctorides).

The two species of Argyrogramma studied in the U.S., A. verruca and A. basigera, use Z5-10:Ac as the principal female pheromone. A. basigera female pheromone also possesses lesser amounts of Z5-10:OH and another unidentified minor component.

The specificity of sex pheromone blends of Plusiinae noctuids appears to be maintained by several chemical parameters. Minor components that are inhibitory to interspecific interactions among species studied include Z-5-12:Ac, Z-9-14:Ac, and Z-7-12:OH. Other parameters evident are pheromone release rate, the use of ten carbon acetates and alcohols instead of a 12 carbon system, and shifting from Z-7-12:Ac to Z-7-12:OH as the major component.

D. OBJECTIVES OF ORIGINAL RESEARCH PROPOSAL:

The original objectives of the research proposed were to 1). determine the pheromone chemistry of females of a number of species of Plusiinae in the southeastern U.S. and in Israel, 2). determine pheromone release rates and ratios for the same species, 3). to determine pheromone characteristics eliciting optimum male attraction responses, and 4). to assess the roles of pheromone components in preventing interspecific attraction among sympatric species. During the conducting of this research project, it was discovered that the cabbage looper male releases a sex attractant pheromone, adding a new dimension to mate finding strategies in this group. Because the overall focus of the project was to build an understanding of the sex pheromone chemistry and behavior of looper moths and to develop lures and traps, the study of this novel and important behavior was

included and is considered to fall within the original scope and intent of the objectives.

E. BODY OF REPORT:

INTRODUCTION

The noctuid moth subfamily Plusiinae includes a number of major pests, commonly referred to as loopers, that destroy vegetable, forage, and fiber crops in many areas of the world. These include cabbage looper, Trichoplusia ni, soybean looper, Pseudoplusia includens, clover looper, Rachiplusia ou, celery looper, Anagrapha falcifera, gamma moth, Autographa gamma, tomato looper, Chrysodeixis chalcites, and others. For some species, lures and traps based on the chemistry of the female sex pheromone are used to monitor populations and time control measures. However, effective lures are not available for many species, and complete blends that may be more attractive are not known for other species. Moreover present lures are not species specific because of shared pheromone chemistry among species in this group. Better monitoring methods and new control technology based on environmentally safe methods are needed for all species to reduce crop losses, production costs, and pesticide use.

The cabbage looper was one of the first moths for which a sex pheromone was identified (Berger 1966). The same chemical, Z-7-dodecenyl acetate, has been found to be a sex pheromone or sex attractant for nearly all Plusiinae species studied to date, and has been tested as a monitoring attractant for several species and as a mating disruptant for the cabbage looper. Further research on the cabbage looper pheromone (Bjostad et al 1984) and tomato looper (Dunkelblum et al. 1987) as well as other Noctuidae has revealed a surprising complexity to female moth sex pheromone chemistry. The cabbage looper female sex pheromone is comprised of 6 components, the tomato looper 5, and the soybean looper 5 (Linn et al 1987). Our knowledge of the female pheromone chemistry of these species suggests that Plusiinae generally utilize Z-7-12:Ac as the principal or major component but also possess multiple minor components.

It has been hypothesized that the chemical complexity of these pheromones provides species specificity and the avoidance of courtship and mating mistakes between species. This specificity is due to overall blend characteristics (a chemical gestalt) (Linn et al 1988) and/or to interspecific inhibitors in blends (Landolt and Heath 1987). A complete knowledge of the minor components making up female sex pheromones of the pest plusiine moths in an area, and determination of what provides behavioral reproductive isolation among sympatric and synchronic species, would provide a better

understanding of why these moths possess such complex pheromone blends and how they function to maintain signal specificity. Also, such information should facilitate the development of synthetic species specific blends for use in pest management programs. Currently, it is not uncommon to capture multiple species of Plusiinae in traps baited with commercially available lures, resulting in misleading information on pest numbers and cycles.

Materials and Methods

Moths of all species were reared in the laboratory generally using the methods of Guy et al (1985), on a semisynthetic medium with a pinto bean base. Colonies were established in the laboratory from field collected material in both the U.S. and Israel, in most cases from mated females collected with light traps or traps baited with phenylacetaldehyde. These included T. ni in both laboratories, A. falcifera, Autographa biloba, Argyrogramma basigera, and Rachiplusia ou in the U.S. and C. chalcites, Autographa gamma, and Cornutiplusia circumflexa in Israel. Efforts in the U.S. to rear Agrapha oxygramma and Mouralia tinctoides using these methods were unsuccessful. Small numbers of F-1 generation M. tinctoides were reared on a host plant. In both the U.S. and Israel laboratories, moths were sexed as pupae and held in 25 cm wide plastic screen and aluminum cages with a solution of sucrose and honey on cotton as food. Pupae were moved daily to new cages providing moths of discrete ages for experiments. Cages of males and females were kept in separate environmental chambers on a 14:10 light:dark cycle, at 24 ± 1 C, and $60 \pm 10\%$ RH.

Samples of pheromone from females were obtained as extracts of pheromone glands or as volatile collections made from airflow passed over calling females. Gland extracts were prepared by snipping off the last 2 abdominal segments while gently squeezing the abdomen to forcibly extrude the abdominal tip and expose the intersegmental glands. The cut tips were dropped into 200-300 μ l of solvent (usually hexane) in a microvial, and the tips removed after one hour. Extracts were further concentrated under a stream of nitrogen as needed. Volatile collections were made from airflow over calling females using systems similar to that reported in Landolt and Heath (1987). Extract preparations were also made of male cabbage loopers, parts of males, and of male hairpencils. Volatile collections were made from airflow over males using a design similar to that used with females, but combining airflow over rubber septa loaded with female pheromone, airflow over host plants, and airflow over males. These methods are reported in detail in Landolt and Heath

(1987,1989, 1990).

Extracts were analyzed by capillary gas chromatography, using cool on-column injectors and flame ionization detectors. Structural elucidation of suspect peaks found in GC analyses was accomplished principally via analyses by gas chromatography/mass spectrometry and ozonolysis to determine bond position and geometry of certain compounds. Additionally, infrared and nuclear magnetic resonance spectra were obtained on male cabbage looper compounds, with chiral determinations on linalool made using capillary gas chromatography (Landolt and Heath 1990; Appendix B).

In most cases, synthetic chemicals needed were commercially available, with the exception of d-linalool, which was extracted for these studies from coriander seeds and purified by preparative GC.

Flight tunnel bioassays were used in these studies to verify attractive responses to identified pheromone blends, to investigate interspecific pheromonal interactions, and to elucidate the novel cabbage looper behavior involving male production of a sex attractant. Flight tunnels used are described in Snir et al (1986) and in Landolt and Heath (1987). For some species preliminary tests were conducted of heterosexual intraspecific attraction responses, using only live insects, to determine diel periodicity to behavior.

Moths tested in flight tunnel bioassays for attraction responses were scored for upwind flight, oriented upwind flight in the odor plume, and source contact, within a 2 minute time period following release. Moths were tested individually, usually with 5 tested sequentially per treatment.

A volatile effluent delivery system was built for testing odors in the flight tunnel, comprised of a glass holding chamber for live insects and or plants, through which filtered metered air was pumped and vented into the flight tunnel at one liter per minute. This system, described in Landolt and Heath (1990) was used in some cases to test moth responses to generated odors without the complications of sounds and visual stimuli in the tunnel.

Results

Chemical analyses of gland extracts and volatile collections resulted in initial characterizations or confirmations of female pheromones for Trichoplusia ni, Autographa gamma, Cornutiplusia circumflexa, Chrysodeixis chalcites, Autographa biloba, Anagrapha falcifera, Rachiplusia ou, Argyrogramma basigera, Agrapha oxygramma, and Mouralia tinctorides (Appendix A, unpublished

data). Qualitative results of these analyses, combined with related work conducted prior to this project on Pseudoplusia includens and Argyrogramma verruca, are summarized in Table 1. Of the 12 species covered, 10 possessed Z-7-12:Ac, and 7 of those appeared to possess it as the major component. Cornutiplusia circumflexa and A. falcifera were unusual in possessing the Z-7-12:OH as the major component. The two species of Argyrogramma use Z-5-10:Ac instead of Z-7-12:Ac as the major pheromone component (Heath and Landolt 1987, unpublished data). The number of minor components found in association with Z-7-12:Ac ranged from 0 for R. ou to 5 for T. ni, with 4 minor components for P. includens and C. chalcites.

Assessments were made of release rates of some species and pheromone amounts in glands were quantified for most. As such, pheromone amounts ranged widely among the species from less than one ng to nearly 500 ng per female (Table 2). Only C. chalcites was similar to the cabbage looper in pheromone amounts. Autographa gamma, A. falcifera, R. ou, and C. circumflexa all possessed small amounts in the glands, with likely very low release rates.

Flight tunnel assessments were made for several species of male attraction to synthetic identified female pheromone blends. Such confirmation of complete responses of males to pheromone were obtained for A. falcifera, A. basigera, A. gamma, and C. circumflexa. Flight tunnel determinations of inhibitory effects of interspecific pheromone signals were obtained for increased amounts of Z-7-12:OH, and for both Z-5-12:Ac and Z-9-14:Ac inhibiting male A. gamma sex attraction, Z-7-12:OH inhibition of male response in T. ni and C. chalcites, Z-5-12:Ac inhibition of male C. chalcites attraction, and Z-9-12:Ac inhibition of male T. ni pheromonal responses.

Field trapping tests confirmed lure effectiveness for A. gamma, C. chalcites, and C. circumflexa. Field test confirmation was also obtained for exclusion of males of T. ni, C. chalcites, and A. gamma with Z-7-12:OH added to pheromone blends, and of C. chalcites male exclusion with the addition of Z-5-12:Ac to blends.

Laboratory flight tunnel tests of female cabbage looper attraction to males confirmed the pheromonal sex attraction response to male odor and to extractable material in the terminal hairpencils (Landolt and Heath 1989). Three compounds were identified from active GC fractions of hairpencil extracts, as d-linalool, p-cresol, and m-cresol (Landolt and Heath 1990, Appendix B). Flight tunnel bioassays of synthetic blends formulated on filter paper discs confirmed the attraction of unmated female cabbage loopers to the blend of these three components in a ratio of 1 to 0.2 to 0.03 respectively. For attraction to contact the dispenser in the assay $p=0.37$ for the synthetic blend and $p=0.34$ for attraction to hairpencil extract, $n=35$. In this test, elimination of linalool reduced

attraction ($p=0$), as did elimination of the cresols from the blend ($p=0.09$). D-linalool as part of the blend was more attractive ($p=0.42$) than the blend with l-linalool ($p=0.1$) or racemic linalool ($p=0.16$), but was equivalent in attractiveness to hairpencil extract ($p=.44$, $n=50$) (Appendix B).

Volatile collections were made from airflow over males, using the system described previously, to document the release of attractive chemicals identified from the male hairpencils were being released (Landolt and Heath 1990, Appendix B). Males in the volatile collection system were also exposed to synthetic Z-7-12:Ac formulated in rubber septa to elicit hairpencil exposure, and to cabbage odor as airflow from over a cabbage plant vented into the glass chamber holding males. The pheromone blend previously identified was recovered from volatile collections with males exposed to Z-7-12:Ac and males exposed to both Z-7-12:Ac and cabbage odor. Mean amounts of pheromone collected were 5.4 ± 2.6 ng from males exposed to Z-7-12:Ac, 0 from males exposed to cabbage odor, and 9.8 ± 4.7 ng from males exposed to both.

Additional flight tunnel studies were conducted to determine the effects of female pheromone or host odor on both male pheromone release and female response to male pheromone. A volatile effluent delivery system was put together that vented airflow from over males exposed to host odor or female pheromone into the flight tunnel. Female moths were tested for attraction responses to the airflow from the empty system (control), from airflow over males, from airflow over males exposed to Z-7-12:Ac, and from airflow over males exposed to cabbage odor. Females were more attracted to males exposed to cabbage odor ($p=0.44$) than to the odor of cabbage ($p=0.16$) or to males alone ($p=0.10$). Similarly, females were more attracted to males exposed to Z-7-12:Ac ($p=0.6$) than to Z-7-12:Ac ($p=0$) or to unexposed males ($p=0.07$).

Female cabbage looper attraction to males was also investigated in field cages monitored with a video system (Appendix C). Males were enclosed in small screen cages and placed on a stand within the large field cage. Unmated females were released within the field cage and their activity at the smaller cage containing males was monitored throughout the night. This test was replicated 5 times and then repeated with females within the smaller cages and males released within the large field cage to document the time of night and incidence of male attraction to the females. In this test apparent female attraction to males occurred principally at dusk, but with some activity throughout much of the night. Male attraction to females occurred later, in the 3rd to 8th hours of the 10 hour night.

Preliminary trapping tests were conducted to further document attraction under field conditions and to begin an effort to develop trapping systems for female loopers (Appendix C). One

experiment was conducted using *Heliothis* cone traps baited with cages of 5 live males, and placed in a large screened pool enclosure set up in a field. Four traps were used, two baited and 2 unbaited, with trap positions switched daily for 10 days, and 1083 unmated female cabbage loopers released in the enclosure over the 10 day period. A total of 108 were caught in male baited traps versus 4 in unbaited traps, demonstrating some attraction under outside conditions and also the feasibility of trapping females with such a trap design. Similar attempts to trap cabbage loopers in the field were unsuccessful because of very low populations of moths, as evidenced by a lack of males captured in traps baited with the female pheromone Z-7-12:Ac.

Discussion

The results of these studies include several new findings concerning the use of pheromones as sex attractants by Plusiinae and the means of maintaining both signal specificity and behavioral reproductive isolation among sympatric and synchronic species with this subfamily of noctuid moth.

It was originally anticipated that the principal means of providing signal specificity among the species of concern would involve the role of minor components making up species specific blends, but including Z-7-12:Ac as the principal or major component, as appears to be the case in the cabbage looper. The soybean looper, *P. includens*, the tomato looper *Chrysodeixis chalcites*, *A. biloba*, and *A. oxygramma*, appear to fit this pattern. All 5 species utilize Z-7-12:Ac as the major component of the female sex pheromone. Two compounds in the *T. ni* blend (Z-5-12:Ac and Z-9-14:Ac) inhibit male *P. includens* response. Z-5-12:Ac is also inhibitory to *C. chalcites* males, while the Z-7-12:OH in *C. chalcites*, *A. biloba*, and *A. oxygramma* pheromones are inhibitory to *T. ni* males. Also, *A. biloba* possess Z-5-12:Ac which is inhibitory to *P. includens*. Thus, it may be possible to make species specific lures for all of these species, except for *P. includens* pheromone which will still attract *T. ni*.

Outside of this group of 4 species, the signal specificity appears to be a result of 3 additional parameters: pheromone release rate, the replacing of Z-7-12:Ac as the principal component with Z-7-12:OH, and the use of a system based on Z-5-10:Ac, instead of Z-7-12:Ac. Four species investigated in these studies appear to possess very small amounts of pheromone relative to *T. ni*, providing behavioral isolation by likely inhibition of responses to high release rates, as was demonstrated in the field for *A. gamma* and *T. ni*. This in itself provides some degree of signal specificity and also the

means to design lures for one species and excluding others. Two of these species appear to use Z-7-12:OH as the major component, but also possess Z-7-12Ac; A. falcifera and C. circumflexa. The other 2 species, A. gamma and R. ou, use very small amounts of Z-7-12:Ac, and A. gamma also possess the Z-7-12:OH. The 2 species of Argyrogramma studied both use z-5-10:Ac as the principal component, thus probably avoiding interspecific interactions with other Plusiinae. It is obvious from the information obtained to date on this project that the pheromone complexity within the subfamily is greater than anticipated but provides the means for the development of specific blends.

The discovery of male cabbage looper production of a female attractant pheromone from the hairpencils provides new exciting avenues of research on the development of female lures for control technology, but also adds another dimension to the consideration of how species use pheromone characteristics to sort out heterospecifics from conspecifics. An additional factor is the possibility that host plants may play important roles in cabbage looper sexual behavior (Landolt and Heath 1990). There are as yet many unanswered questions regarding how and why male cabbage loopers attract females, and it will be of interest to find out if similar behavior occurs in other Plusiinae and in other noctuid subfamilies. Also, it is anticipated that the lures and traps developed for females may be more successful in reducing populations than similar technology now available for trapping males.

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Table 1. Pheromone components identified from female gland extracts on volatile collections for species of Plusiinae studied. U. S. and Israel combined.

Species	C10:AC	C10:OH	<———— C12:AC ————>					C12:OH	C13:AC	C14:AC	C:14:AC	Other
	Z5	Z5	SAT	Z5	Z7	Z9	All	Z-7	Z-8	Z-7	Z-9	
Trichoplusia ni			X	X	X		X			X	X	
Pseudoplusia* includens			X		X		X					XX
Chrysodeixis chalcites					X	X	X		X		X	
Agrapha oxygramma					X	X		X			X	
Argyrogramma basigera	X	X										
Argyrogramma* verruca	X											
Autographa gamma					X			X				
Autographa biloba			X		X			X				
Rachiplusia ou					X							
Anagrapha falcifera				X	X			X				
Cornutiplusia circumflexa					X			X				
Mouralia tinctoides					X							X

* Data from published analyses prior to this project.

Table 2. Pheromone amounts in extracts of female glands for species of Plusiinae

Species	in Gland Extract ng/gland	in Volatiles ng/hr.	References
Trichoplusia ni		256 ± 130.8	Landolt & Heath 87
Pseudoplusia includens		96 ± 28.8	Landolt & Heath 87
Chrysodeixis chalcites	460±135		Snir et al 1987
Agrapha oxygramma			
Autographa biloba	8.4±5.6	22.7±12.1	
Anagrapha falcifera	2.1±2.3	<1	
Autographa gamma	2-3		
Rachiplusia ou	<1	<1	
Cornutiplusia circumflexa	1-2		
Mouralia tinctoides			
Argyrogramma verruca	60 ng	-	Heath & Landolt 87
Argyrogramma basigera			

F. DESCRIPTION OF COOPERATION

This project was designed to make maximum use of two similar laboratories but also of the existence of similar but different guilds of Plusiinae in the southeastern US and in Israel. In both countries, this included the cabbage looper moth, *Trichoplusia ni*, which has been studied intensively in the past, as the model for pheromone studies and as the central focus of research in both areas. It was hoped that shared discoveries and progress in either laboratory would have direct implications and results in the other laboratory. Also, duplication of findings in two separate systems would lend more weight to our results.

Cooperation throughout the execution of the project consisted principally of multiple shared findings and discoveries which facilitated more rapid progress, and shared methods and approaches. This cooperation was most important in the investigation of female pheromones containing small amounts of chemicals and including the Z-7-12:OH. The nature of the compound and also the rate of production and release of pheromone in several species in both countries made collection and analysis of natural material difficult. New approaches and methods developed to deal with this included modifications to the volatile collection systems and capillary GC technology. Dr. Dunkelblum visited the Gainesville laboratory in June 1988, during which much of the discussions on the problem and some technology transfer took place. Dr. Kehat also visited the Gainesville laboratory, during which several discussions were made of the approaches and methods used for behavioral analyses, of the ongoing work on the male cabbage looper pheromone and theoretical implications of the results of those studies. As a direct result of those discussions with both Dr. Dunkelblum and Dr. Kehat, agreement was reached on the pursuit of additional cooperative projects on moth pheromones, part which was submitted as a new BARD proposal.

G. EVALUATION OF RESEARCH ACHIEVEMENTS:

The objectives set forth in the original research proposal have largely been met with the conclusion of this project by the characterization of female pheromones of 9 species of looper moths and the determination of multiple factors contributing to the specificity of the natural pheromone blends. It is now possible to provide fairly species specific sex attractant blends for the pest species in the southeastern U.S. and in Israel. The results of our studies have provided a much more comprehensive assessment and understanding of how such a complex of closely related species maintains clear signal channels through quantitative and qualitative differences in pheromone chemistry.

Additionally, the discovery of female T. ni attraction to male pheromone and the possible effects of host plant volatiles on pheromone release and pheromonal responses in both sexes is a significant accomplishment, but which also contributed to a broader understanding and new way of thinking about mate finding strategies in the Plusiinae.

H. LIST OF PUBLICATIONS:

Heath, R. R., P. J. Landolt, B. D. Dueben, R. E. Murphy, and R. E. Schneider. Identification of a male cabbage looper sex pheromone. J. Chem. Ecol. (in prep).

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