Control of Insect Behavior by Natural Products

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CONTENTS

PREFACE ix

Pheromone Research with Stored-Product Coleoptera 1
Wendell E. Burkholder

Some General Considerations of Insects' Responses to the Chemicals in Food Plants 21
Vincent G. Dethier

Pheromones of the Honey Bee, *Apis mellifera* L.. 29
Norman E. Gary

The Substances That Control the Feeding Behavior and the Growth of the Silkworm, *Bombyx mori* L.. 55
Yasuji Hamamura

Sensory Responses of Phytophagous Lepidoptera to Chemical and Tactile Stimuli 81
Frank E. Hanson

Aggregation of the German Cockroach, *Blattella germanica* (L.) 93
Shoziro Ishii

Methodology for Isolation, Identification, and Synthesis of Sex Pheromones in the Lepidoptera 111
Martin Jacobson

CONTENTS

Volatile Organic Sulfur Compounds as Insect Attractants with Special Reference to Host Selection 133
Yoshiharu Matsumoto

Pheromones of Social Insects 161
John C. Moser

Insect Antifeedants in Plants 179
Katsura Munakata

1, 3-Diolein, a House Fly Attractant in the Mushroom, *Amanita muscaria* (L.) Fr. 189
Toshio Muto and *Ryozo Sugawara*

Studies on Sex Pheromones of the Stored Grain Moths 209
Minoru Nakajima

Insect Attractants of Vegetable Origin, with Special Reference to the Rice Stem Borer and Fruit-Piercing Moths 225
Tetsuo Saito and *Katsura Munakata*

The Chemistry of Attractants for Chrysopidae from *Actinidia polygama* Miq. 237
Takeo Sakan, Sachihiko Isoe, and *Suong Be Hyeon*

Sex Pheromones of Lepidoptera 249
Harry H. Shorey

Methodology for Isolation and Identification of Insect Pheromones—Examples from Coleoptera 285
Robert M. Silverstein

Pheromones of Bark Beetles 301
David L. Wood

Electrophysiological Investigation of
Insect Olfaction 317
Minoru Yamada

Host Attractants for the Rice Weevil and the
Cheese Mite 331
Izuru Yamamoto and *Ryo Yamamoto*

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PREFACE

The extensive use of pesticides by the more affluent nations of the world has not only created serious problems in pest control but threatens man's health and pollutes his environment. Insecticides in particular are of greatest concern because they can cause death through poisoning, accumulate in man, concentrate in food chains, are often not biodegradable, cause resurgence and resistance in pest populations, and destroy parasites, predators, and pollinators. In the past few years, research aimed at establishing alternative means of pest control has received increased attention. One of the most promising of these is the use of naturally occurring organic compounds that influence insect chemosensory behavior as attractants, repellents, stimulants, deterrents, and arrestants. Many reports have established the presence of such chemosensory behavioral systems, and of particular interest is the number of serious economic pests included in this list.

However, significant advances in pest control utilizing this biochemical approach remain painstakingly slow because of our primitive understanding of insect behavior, problems associated with mass rearing and with isolation and identification of compounds occurring in minute amounts in complex mixtures, synergism and masking, synthesis, and the problems of developing control protocols that utilize synthetic compounds.

The third in a series of three seminars [*Science* **157**, 464 (1967); **160**, 445 (1968)] on new biochemical approaches to pest control, "Control of Insect Behavior by Natural Products," was held January 16-18, 1968 in Honolulu, Hawaii. These seminars were cosponsored by the National Science Foundation and the Japan Society for the Promotion of Science as part of the United States-Japan Cooperative Science Program. The recent efforts of twenty scientists to identify naturally occurring compounds that elicit chemosensory behavior and to describe their modes of action were reported in the context of the meeting theme—collaboration between biologists and chemists. Chemosensory behavior; electrophysiology; isolation, identification, and synthesis of new compounds; and applications to pest suppresion were the key areas discussed. (From: Wood, D. L., Silverstein, R. M., and Nakajima, M., 1969. *Science* **164**, 203. Copyright 1969 by the American Association for

the Advancement of Science.) The papers presented at the third seminar have been revised and up-dated for publication in this volume.

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PHEROMONE RESEARCH WITH STORED-PRODUCT COLEOPTERA

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Table of Contents

		Page
I.	Introduction	3
II.	Insect Rearing	3
III.	Three-Choice "Closed-System" Olfactometer	4
IV.	Multichoice Olfactometer with Air Flow	5
	A. Influence of Age of Female *Attagenus megatoma* on Male Response	6
	B. Influence of Mating of *Attagenus megatoma* on Male Response	7
	C. Pheromones from Female *Trogoderma parabile* and *Trogoderma simplex*	8
V.	Single-Choice Olfactometer	9
	A. Quantitative Assay of *Attagenus megatoma* Pheromone	9

Table of Contents--(Continued)

		Page
	B. Influence of Age of Male *Attagenus megatoma* on Male Response.....	11
	C. Interspecies Attraction with *Attagenus megatoma*, *Trogoderma inclusum*, and *Trogoderma glabrum*.....	12
	D. Response of *Attagenus megatoma* Males After Removal of Distal Portions of the Antennae.....	12
	E. Response of *Attagenus megatoma* Males to the Synthetic Pheromone and Its Isomers.....	13
VI.	Y-Tube Olfactometer.....	14
VII.	Field Trap.....	15
VIII.	Discussion.....	16
IX.	References.....	18

I. Introduction

Sex pheromones appear to play an essential role in the mating behavior of the stored-product Coleoptera. Information on how sex pheromones can be used to influence the behavior of these insects may well permit the development of safer and more effective control measures than are now available.

The earliest evidence of a stored-product beetle sex pheromone was for the yellow mealworm, Tenebrio molitor L. (Valentine, 1931). More recently Tschinkel et al. (1967) have reported that the pheromone is produced by both the female and male and acts as a weak attractant, and that its major role is sexual excitation of the male. A substance produced by the female Trogoderma granarium Everts attracts both females and males and was called a pheromone by Finger (Bar Ilan) et al. (1965). The males have been shown to produce a similar substance that attracts both sexes (Yinon and Shulov, 1967). Levinson and Bar Ilan (1967) described the function and properties of the assembling scent of this insect. A pheromone of the male bruchid, Acanthoscelides obtectus (Say) was reported by Hope et al. (1967). The biological function of this substance is unknown. Sex pheromones that influence the behavior of males are produced by the unmated female adults of the black carpet beetle, Attagenus megatoma (F.) (= A. piceus (Olivier)), Trogoderma inclusum LeC., and T. glabrum (Herbst) (Burkholder and Dicke, 1966). The first successful isolation, identification, and synthesis of a stored-product insect sex pheromone was accomplished with the black carpet beetle (Silverstein et al., 1967).

The present work is an examination of sex attraction in the black carpet beetle with notes on several other stored-product beetles.

II. Insect Rearing

The methods of rearing and handling the black carpet beetle have been described previously by Burkholder and Dicke (1966). The Trogoderma species were reared and handled in a similar manner except that the medium was improved during 1966 by adding to the dog food the following: dry milk, wheat germ and brewer's yeast for a weight ratio of 3:3:3:1. The cigarette beetles, Lasioderma serricorne (F.), were reared on whole wheat flour with approximately

5% brewer's yeast added. The cigarette beetles were sexed as pupae by examination of the genitalia since adult sexual differences are not easily determined.

III. Three-Choice "Closed-System" Olfactometer

The initial discovery of sex pheromones in females of the black carpet beetle, and T. inclusum and T. glabrum, was made in a 3-choice olfactometer that consisted of a modified glass desiccator (Burkholder and Dicke, 1966). The insects, while in the chamber, were in an environment with little air movement, which is similar to that often encountered by stored-product insects in nature. In this olfactometer the insects had the choice of either male or female odor or no odor at all. In the presence of an attractive odor, the male black carpet beetles exhibited the following behavior: (1) a forward and upward extension of the antennae; (2) a "humping" behavior that was brought about by the extension of the first pair of legs until they were nearly straight, and the partial extension of the second pair, which produced an angle of approximately 45° between the body and the substrate; (3) a rapid zig-zag pattern of approach to the attractant with intermittent stops, during which the "humping" behavior occurred; and (4) copulatory attempts with other test males. All of the first 3 responses were necessary before a response to an odor was considered positive. Trogoderma males responded in a similar fashion except for the "humping" behavior.

In tests with L. serricorne this olfactometer was modified by adding Fluon (polytetrafluoroethylene dispersion) to the sides of the arena to keep the insects from crawling up the sides (Radinovsky and Krantz, 1962). Males and females were held separately from the time of adult emergence. They were exposed in groups of 10 to the female and male odors which were obtained by holding the insects for 7 days in 5 dr shell vials with 12.7 mm paper discs on the bottom. The paper discs were then transferred to the chamber for the assay.

The data indicated that the males were attracted only by the female odor (Table I). Little female response to odors of either sex was observed.

Table I

Response of Lasioderma serricorne Males and Females to Odors from Males and Females in a Desiccator Olfactometer

Sex of test insects	Average numbers[a] responding to		
	Female	Male	Control
Male	7.30	0.02	0.02
Female	0.35	0.50	0.52

[a] Averages of 8 replicates, each with 5 observations at 1 min intervals, with 10 insects per replicate.

IV. Multichoice Olfactometer with Air Flow

To determine whether or not males could differentiate between several odors presented at the same time and at greater distances, another olfactometer was developed. The basic design was fan-shaped with the attractant choice available to the test insects at one or more of 5 points equidistant from the place of release (Fig. 1). After preliminary models of wood were built and tested, a unit was constructed of brass and glass. The 2 straight sides were 47 cm long, the curved side was 51 cm long, and the 4 dividers were 12.7 cm long, all being 2.54 cm high and 6.35 mm thick. The distance from the curved side to the apex was 44 cm and the distance to the release point was 39 cm. The plate glass top and bottom were 6.35 mm thick. There was a 1.27 cm hole in the top plate for insertion of the inverted funnel for beetle release. The funnel was a modified Gooch filter tube, 25 mm I.D. and 9 cm long. The funnel was shortened to reduce the depth of its widest part to 20 mm. When the funnel was in the raised position, about a 5 mm opening allowed free movement of the insects into the olfactometer.

A sheet of Whatman filter paper No. 1 covered the

lower plate glass and formed the floor of the chamber. The filter paper and brass unit were sandwiched between the two pieces of plate glass and sealed tightly by means of 10 clamps. The filter paper helped to form a gasket on the bottom, and a 6.35 mm strip of masking tape provided a gasket under the top plate glass. A brass needle valve was attached to the apex of the unit, and a threaded opening was centered in each of the 5 divisions. Brass "Swagelok" fittings with teflon ferrules for 6.35 mm tubing were attached. Each attractant-holding chamber was a 25 ml Erlenmeyer flask fitted with a 19/22 ground glass fitting bearing an inlet tube extending into the flask and an outlet tube for attachment to the olfactometer. The inlet tube was attached by means of nylon "Swagelok" fitting to a Gelman flowmeter.

The unit was attached to a 56 x 56 cm table with four 20 cm legs. This in turn rested on a movable wooden laboratory cart. Suspended 91 cm above the apex of the olfactometer was a 150 w soft-white light bulb. The apparatus was placed in a room separate from other working and insect culturing areas. Room ventilation was provided and attractants from the olfactometer were removed by means of a vacuum system to avoid contamination of the room air. The temperature usually ranged between 25° and 27°C. The relative humidity was regulated at 50 ± 5%.

Air flow through the flasks was regulated at 1/4 l/min. Twenty-five test males were placed in the lowered inverted funnel and allowed to settle for a few minutes before the test. The air flow through the flasks was begun 30 sec before release of the males. Observations were usually made at 1 min intervals for 10 min.

A. Influence of Age of Female Attagenus megatoma on Male Response

A. megatoma male responses to females of various ages were measured in this olfactometer. Ten newly-emerged virgin females were placed on filter paper in Erlenmeyer flasks. The flasks were capped with an aluminum foil covered cork, placed in an incubator, and held for < 1, 2-3, 4-5, and 6-7 days. Females held 24 hr or less from adult emergence were placed in the flasks 3 hr before the test. The males used in this test were 6-7 days old. Two replicates with different flasks and test males were run on separate days.

The data indicate that the females were not attractive until at least 24 hr after emergence (Table II). Male response was highest 6-7 days after female emergence, and was nearly as high 4-5 days after emergence.

Table II

Response of Attagenus megatoma Males to Odors from Females of Various Ages in a Multichoice Olfactometer

Age of female (days)	Numbers responding		
	Replicate		
	I	II	Average
< 1	0.1	0	0.05
2-3	2.6	1.5	2.05**
4-5	6.7	7.8	7.25**
6-7	8.8	8.3	8.55**
Control	0	0.1	0.05

** Values significant at the 1% level.

B. Influence of Mating of Attagenus megatoma on Male Response

As a preliminary to this test a series of petri dishes was set up, each containing 1 male and 1 female of the same age. The following age levels were used: < 1, 1-2, 2-3, 3-4, and 4-5 days following emergence. No copulation was observed in the dishes at the first 3 age levels. Copulation occurred after 4 min at the 3-4 day age level and between 1 and 3 min at the 4-5 day level. Ten pairs were selected from the 4-5 day age level for further testing.