Meetings

Pest Control

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, repellants, 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 16–18 January 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 20 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 suppression were the key areas discussed.

After some 30 years of intensive research effort, the chemical basis of food selection by the silkworm larva, *Bombyx mori,* has been elucidated (Y. Hamamura, Konan University, Kobe). The following compounds were isolated from mulberry leaves and identified: attractants—citral, linalyl, and terpinyl acetate, linalol; biting stimulants—β-sitosterol, isoquercitrin, morin; swallowing stimulants—cellulose, potassium phosphate; cofactors—sucrose inositol, silica. When these chemically pure compounds are mixed with agar the larvae will feed and develop as successfully as those fed on the natural host plant.

L. Steiner (U.S. Department of Agriculture, Honolulu) discussed research on the fruit fly attractants that has been conducted by the U.S. Department of Agriculture, Entomology Research Division, at its Hawaii laboratory since 1949. Bait sprays of protein hydrolysate–organic phosphate were developed and used to eradicate four outbreaks of the Mediterranean fruit fly, *Ceratitis capitata,* on the U.S. mainland since 1956. These bait sprays are now widely used around the world to control several pest tephritids. Angelica seed oil, discovered by screening many candidate materials in Hawaii, was used to delimit the Florida infestations in 1956–57. It was later replaced in turn by siglure, medlure, and trimedlure, discovered in a cooperative synthesis and screening program conducted with Division chemists. Cue-lure, the present outstanding attractant for the melon fly (*Dacus cucurbitae*), was developed by the same method. The male lures attract virgin or sperm-deficient females in the absence of males, and hence can elicit behavior like weak sex pheromones. Methyl eugenol combined with 5-percent naled was aerially dispersed at rates of less than 7 pounds of poison per square mile at 2-week intervals to eradicate the oriental fruit fly, *D. dorsalis,* from Rota, Saipan, and Tinian, M.I. The males respond to the poisoned lure before attaining sexual maturity. This outstanding success proves the feasibility of the male annihilation method.

Recent efforts by Japanese scientists to identify pest-insect plant attractants were described. T. Saito and K. Munakata (Nagoya University) reported the identification of *p*-methyl acetophenone (Oryzanone) from the rice plant as one of the attractants for the rice stem borer, *Chilo suppressalis.* The content of this compound was higher in rice plants treated with excessive nitrogen fertilizer and 2,4-D, and lower in plants treated with silicate; the attractivity of the plant paralleled the acetophenone content. However, there was no relationship between the amount of Oryzanone and moth attraction between different rice varieties. The attractant for the fruit-piercing moth (*Oraesia excavata*) has been isolated but the chemistry is not yet known.

Attempts were made to isolate the attractant of the rice weevil *Sitophilus zeamais* from rice grains (I. Yamamoto and R. Yamamoto, Tokyo University of Agriculture). The active principle(s) was contained in the ether extract and showed acidic properties. Results of further fractionation suggest that the active principle is a complex of several compounds. Isolation of the attractant for the cheese mite (*Tyrophagus dimidiatus*) from cheddar cheese was also attempted. The active substance was concentrated in the neutral, noncarbonyl, and alcoholic fraction of a steam distillate. The ordinary nutrient substances such as protein and carbohydrate are, therefore, not involved. As isolation proceeded, a reduction in activity was observed, but the activity was recovered upon recombination of the fractions. Elucidation of the structure of the active principle(s) is in progress.

Nine mustard oils (isothiocyanates) were shown to be effective as attractants for newly hatched larvae of the vegetable weevil, *Listroderes obliquus* (Y. Matsumoto, University of Tokyo). Adults were attracted to, and biting response was elicited by, five of these compounds. Female onion maggot flies, *Hylemya antiqua,* were attracted and an ovipositional response elicited by *n*-propyl disulfide and *n*-propyl mercaptan, which are volatile components of onion bulbs. Field tests confirmed not only the attractiveness of these compounds to onion maggot flies, but also the powerful attraction of methyl disulfide for the female black blowfly, *Phormia regina,* and allyl isothiocyanate for a flea beetle, *Phyllotreta cruciferae.* Twenty-seven sulfur compounds, including sulfides, disulfides, and mercaptans, attracted the newly hatched larvae of the onion maggot.

Houseflies, especially *Musca domestica,* have long been known to be attracted to mushrooms belonging to the Tricholomataceae and Amanitaceae. T. Muto (Tokyo University of Education) and co-workers extracted the attractive components from the fruiting bodies of *Amanita muscaria* and isolated one of them as a colorless, crystalline substance with the molecular formula $C_{39}H_{72}O_5$ and a melting point of 22° to 23°C, which coincides well with 1,3-diolein. Some related compounds were prepared and 1-monoolein was found to be much more attractive than 1,3-diolein. Ethylene glycol monooleate and monolaurate were the most active compounds tested.

Extracts of the plant *Actinidia polygama* has long been of interest because of its peculiar excitatory effect on both vertebrates (Felidae) and invertebrates (Chrysopidae). T. Sakan (Osaka City University) and co-workers isolated several components from the neutral fraction of both leaves and galls that were attractive to lacewing species. These compounds were identified as neo- and isoneomatatabiol, matatabiol, dehydroiridodiol, iridodiol, 5-hydroxymatatabiether, 7-hydroxymatatabiether, and allo-metatabiol. It was remarkable that these cyclopentanoid monoterpene alcohols were found to be attractive only to the male adults of *Chrysopa septempunctata* and *C. japana.* Only 10^{-6} μg of neo- and isoneomatatabiol, and 10^{-3} μg of matatabiol and dehydroiridodiol were needed to evoke a response.

K. Munakata (Nagoya University) reported on his interesting studies of insect antifeeding compounds contained in plant leaves. Extracts of *Cocculus trilolus, Clerodendron tricotomum,* and *Parabenzoin trilobum* produced strong antifeeding activity for the larvae of *Prodenia litura* when applied to sweetpotato leaves. A known alkaloid, isoboldine, was identified from *C. trilobus*; two new terpene crystals, clerodendrin A, $C_{31}H_{42}O_{12}$ and clerodendrin B, $C_{31}H_{44}O_{12}$ were identified from *C. tricotomum*; and a new sesquiterpene, $C_{19}H_{30}O_5$ (tentative structure, shiromodiol diacetate) was identified from *P. trilobum.*

The contributions of the American scientists emphasized insect pheromones. M. Jacobson (U.S. Department of Agriculture, Beltsville) reviewed his pioneering research with the moth pheromones. The first insect sex phenomone was identified and synthesized in 1960 and was isolated from the virgin female gypsy moth, *Porthetria dispar*. The structure of the attractant was shown to be (+)-10-acetoxy-*cis*-7-hexadecen-1-ol, as determined by ultraviolet and infrared spectra, hydrogenation, and oxidation. Also synthesized was an active homolog, (+)-12-acetoxy-*cis*-9-octadecen-1-ol, designated "gyplure," which is used as a field survey tool. Also isolated, identified, and synthesized (by Jacobsen and others) were the sex pheromones produced by female cabbage looper moths (*Trichoplusia ni*), fall armyworm moths (*Spodoptera frugiperda*), and pink bollworm moths (*Pectinophora gossypiella*), shown to be the acetates of *cis*-7-dodecen-1-ol, *cis*-9-tetradecen-1-ol, and 10-propyl-*trans*-5,9-tridecadien-1-ol, respectively.

H. Shorey (University of California, Riverside) discussed the importance of sex pheromone research in providing basic information that may be useful in the design of behavioral control programs. Quantitative bioassays are used to assess male responsiveness to pheromones and other chemicals under various environmental and physiological conditions. They require the establishment of concentration-response curves, that is, the number or proportion of males responding plotted against the logarithmic series of concentrations tested. Essentially all quantitative bioassays to date have been based on the known amount of chemical or extract placed on the substrate for evaporation, with the actual rate of evaporation unknown. Since various substrates and assay techniques are used by different laboratories, results cannot be compared. The ideal quantity is the amount of pheromone per unit volume of air required to induce the specified behavioral response among a specified proportion of males. A consideration of the chemical evolution of lepidopteran sex pheromones suggests that a mechanism is available for the insects to become resistant to such compounds utilized in a control program. More research is necessary to fully elucidate the mechanisms by which male moths orient upwind toward pheromone sources. Control techniques may be based on the use of pheromone-baited traps or on a pheromone-permeated atmosphere to prevent orientation of males to wild females.

The methodology for isolation and identification of insect pheromones with examples from the Coleoptera was presented by R. M. Silverstein (Stanford Research Institute, Menlo Park, California). A mixture of three terpene alcohols constitutes the sex attractant in the frass of male *Ips confusus*; they are compound I—(−)-2-methyl-6-methylene-7-octen-4-ol; compound II—(+)-*cis*-verbenol; and compound III—(+)-2-methyl-6-methylene-2,7-octadien-4-ol. Exo-7-ethyl-5-methyl-6-8-dioxabicyclo [3.2.1]octane is the major component of the sex attractant in the frass of female *Dendroctonus brevicomis*. The synthesized compound is active by itself in laboratory tests, but its activity is enhanced by the terpene hydrocarbon, myrcene, and other components not yet identified. The acid, *trans*-3,*cis*-5-tetradecadienoic, is the sex attractant of female *Attagenus megatoma*.

Laboratory and field bioassy methods utilized in identifying the attractants of *Ips confusus* and *Dendroctonus brevicomis* (see above) were described by D. Wood (University of California, Berkeley). The individual compounds were not active in the laboratory, but the combinations of compound I with either II or III, and the ternary mixture were attractive to both sexes at the submicrogram level. In nature the ternary synthetic mixture was the most potent, and compound I + III was slightly active. Surprisingly, *Ips latidens* was attracted by compound I alone and by I + II. In the laboratory, compound III added to the mixture of I + II eliminated or masked the attraction for *I. latidens*. Two predators of these bark beetles, *Enoclerus lecontei* and *Temnochila virescens chlorodia*, also responded. Attractants produced by sympatric species of *Ips* from different species groups are species-specific while the closely related and allopatric *I. montanus*, *I. lecontei*, and a new species from the same species group are cross-responsive. These species respond to the synthetic compounds at higher concentrations and in different combinations than *I. confusus*.

W. Burkholder (U.S. Department of Agriculture, Madison, Wisconsin) presented his research on the response of the male black carpet beetle, *Attagenus megatoma*, to the synthetic sex attractant (see above). Bioassay conditions for this insect in the laboratory closely approximate those under natural conditions. Preliminary trapping studies with the synthetic attractant have been successful. The sex pheromones of several stored-product species in this genus are apparently not species-specific. The production of the pheromone generally begins 1 day after metamorphsis to the adult and increases with age. Females gradually lose their attractiveness following mating.

Studies of the sex pheromones of moths that infest stored grains were reported by M. Nakajima (Kyoto University). The whole bodies of 94,000 female almond moths, *Cadra cautella*, were extracted with methylene chloride, and after separations of the inactive fractions by precipitation from cold methanol and from cold *n*-hexane, 7.3 grams of a yellow oil were obtained. This was further purified by chromatography on silicic acid. The colorless oil thus obtained (about 240 mg) was active to male moths at a concentration of 0.1 μg/ml. Functional group tests and gas chromatographic behavior of the oil suggested that the pheromone may be an acetate of a C_{14} unsaturated alcohol. The species specificity for sex pheromones of the closely related stored grain moths was also discussed. The male moths of *C. cautella, Plodia interpunctella,* and *Anagasta kuehniella* exhibited sexual excitement to the pheromone extracted from the female moths of not only their own species but also the other species. However, these species failed to respond to the extracted pheromone of *Phralis farinaris.*

Newly emerged nymphs of the german cockroach, *Blattella germanica,* exhibit strong aggregation behavior (S. Ishii, Kyoto University). Nymphs reared individually are delayed in their growth and development. The active principle(s) was found in the feces and in an ether wash of the body surface, especially the posterior portion of the abdomen. Histological studies show that the epithelium of the anterior rectum consists of six rectal pads. The active principle appears to be secreted from the cells lining these pads into the lumen of the rectum and is excreted with feces. Isolation studies of this aggregation pheromone are in progress.

Pheromone systems have reached their highest development in the social insects where they trigger many complex behavior patterns. Best known are the "alarm" substances typified by 4-methyl-3-heptanone, which is coded to release several types of behavior in *Atta texana* (J. Moser, U.S. Forest Service, Alexandria, Louisiana). In the laboratory bioassay, workers are attracted by a concentration of 27 × 10^6 molecules per cubic centimeter, but alarm is released at 270 × 10^6 molecules per cubic centimeter. In the field low concentrations attract and alarm, and high concentrations repel and alarm. The same pheromone compound can be utilized by diverse groups of insects. Citral repels *A. texana* but is a powerful attractant for honeybees. Inquilines whose biologies are closely tied to those of social insects may use the host's pheromones. Roaches, silverfish, millipedes, and beetles following odor-trail substances of their host ants are good examples.

In honeybees, the behavioral interactions that facilitate social organization are coordinated by a complex pheromone system (N. Gary, University of California, Davis). The enclosed nest, characterized by a high population density and precisely regulated environment, enhances the communication potential of pheromones. Certain aspects of intranest behavior were considered, including defense, food acquisition, exchange, and storage, nest construction, and dominance hierarchies and social organization. Alarm pheromones, isopentyl acetate from the sting gland, and 2-heptanone from mandibular glands, elicit aggression. Attractive pheromones (geraniol, citral, and nerolic and geranic acids) are released by foragers near rich food sources. Queen pheromones, primarily 9-oxo-dec-*trans*-2-enoic acid, released inside the hive inhibit ovary development in workers and queen cell construction. Outside the hive, queen pheromones attract drones during mating flights and stabilize swarm clusters.

Three scientists reported on their electrophysiological studies of insect chemoreception. The reaction of an insect to the chemicals in its environment may be immediate or delayed (V. Dethier, Princeton University, New Jersey). Delayed reactions include symptoms of toxicity, growth responses, hormonal changes, reproductive variation, and responses mediated through internal feedback systems operating through the agency of interoceptors. Immediate reactions are overt behavioral responses triggered by extroceptive sense organs. The resulting behavior falls into a number of well-defined categories. Plant chemicals are capable of evoking any or all kinds of these behavior patterns. Most of the reactions are related to feeding. Understanding of the relationship is hampered by an almost total lack of knowledge of the relevant plant chemistry. The operation of the sensory systems involved is becoming increasingly clear as a result of electrophysiological studies. The stimuli that shape feeding are predominantly gustatory and olfactory. The best understood case is that of Cruciferae-feeders. New evidence indicates that marked modification of innate feeding behavior can be brought about by experience.

The chemoreceptors of insects which feed on specific host plants have been studied electrophysiologically by a number of workers (F. Hanson, University of Texas, Austin). As shown by Schoonhoven, some insects have receptors sensitive to phagostimulants which are secondary plant substances. However, no evidence of such was found in *Bombyx mori* by Ishikawa and co-workers; the receptors appear to be stimulated only by nutrients. Recent electrophysiological evidence indicates that the larvae of *Manduca sexta*, the tobacco hornworm, detects volatile secondary plant substances by way of antennal receptors, but may not require specific gustatory phagostimulants other than nutrients.

Antennae of the fruit-piercing moth, *Oraesia excavata*, gave very similar EAG responses to their food attractants and to their repellents (M. Yamada, Nagoya University). Single olfactory receptor cells of this species responded to both materials with an increase in impulse frequency. The single unit activity was extracellularly recorded from the brain of the American cockroach, *Periplaneta americana*. The crude extract of the sex attractant from the female elicited an electrical response from extremely specialized neurons in the brain of the male and female of this species. Many other odorous substances did not excite these neurons.

The papers presented at this seminar will be published early in 1969 by Academic Press, Inc. We recognize the criticism that such symposium volumes are often outdated by the time they are published. Therefore, we have elected to update these articles as of 1 July 1968.

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Calendar of Events

May

2–3. **Laser,** 2nd conf., New York, N.Y. (L. Goldman, Laser Lab., Children's Hospital Research Foundation, Elland Ave. and Bethesda, Cincinnati, Ohio 45229)

2–3. Soc. for **Pediatric Research,** Atlantic City, N.J. (R. E. Greenberg, Dept. of Pediatrics, Stanford Medical Center, Stanford, Calif. 94305)

2–3. American Assoc. of **University Professors,** Minneapolis, Minn. (B. H. Davis, The Association, 1785 Massachusetts Ave, NW, Washington, D.C. 20036)

2–4. Soc. of **Biological Psychiatry,** Miami Beach, Fla. (G. N. Thompson, 2010 Wilshire Blvd. Los Angeles, Calif. 90057)

2–4. Wisconsin Acad. of **Sciences, Arts and Letters,** Whitewater, Wis. (W. Sarles, Dept. of Bacteriology, Univ. of Wisconsin, Madison 53706)

2–5. American **Psychoanalytic** Assoc., Miami Beach, Fla. (H. Fisher, 1 E. 57 St., New York 10022)

3. American Soc. for **Clinical Nutrition,** Atlantic City, N.J. (A. B. Eisenstein, 818 S. Meramec Ave., St. Louis, Mo. 63105)

3. American College of **Psychiatrists,** Bal Harbour, Fla. (M. Sabshin, P.O. Box 6998, Chicago, Ill. 60680)

3–8. American **Ceramic** Soc., 71st., Washington, D.C. (Technical Mtgs. Information Service, 79 Drumlin Rd., Newton Centre, Mass. 02159)

4–5. American Soc. for **Clinical Investigation,** Atlantic City, N.J. (D. H. Nelson, Latter-Day Saints Hospital, Salt Lake City, Utah 84103)

4–6. American Soc. for **Adolescent Psychiatry,** Miami Beach, Fla. (H. D. Staples, 24 Green Valley Rd., Wallingford, Pa. 19086)

4–7. American Inst. of **Chemical Engineers,** 65th natl. mtg., Cleveland Ohio. (The Institute, 345 E. 47 St., New York 10017)

4–7. American **Mining** Congr., Pittsburgh, Pa. (The Congress, 1100 Ring Building, Washington, D.C. 20036)

4–8. American Soc. of **Brewing Chemists,** Baltimore, Md. (Executive Secretary, The Society, 501 N. Walnut St., Madison, Wis.)

4–8. Soc. of **Plastics Engineers,** 27th, Chicago, Ill. (Director, Member Activities, The Society, 65 Prospect St., Stamford, Conn.

4–9. **Electrochemical** Soc., New York, N.Y. (The Society, 30 E. 42 St., New York 10017)

5. **Aerospace Dynamic Balancing,** 2nd symp., San Francisco, Calif. (Technical Mtgs. Information Service, 79 Drumlin Rd., Newton Centre, Mass. 02159)

5–6. **Theory of Computing** Symp., Marina del Ray, Calif. (Technical Mtgs. Information Service, 79 Drumlin Rd., Newton Centre, Mass. 02159)

5–7. Instrumentation Soc. of America, **Aerospace Instrumentation** Symp., Las Vegas, Nev. (Technical Mtgs. Information Service, 79 Drumlin Rd., Newton Centre, Mass. 02159)

5–7. American **Gynecological** Soc., New