APPLICATION OF PHEROMONES AND BEHAVIOR-MODIFYING TECHNIQUES IN DETECTION AND CONTROL OF STORED PRODUCT INSECTS

WENDELL E. BURKHOLDER
Stored Product and Household Insects Laboratory
Agricultural Research, SEA, USDA
Department of Entomology
University of Wisconsin
Madison, Wisconsin, 53706
U. S. A.

For many years insects that infest stored products have been controlled primarily by chemical pesticides and fumigants. Nevertheless, at present, there is tremendous potential for pest management programs that integrate chemical pesticides, biological chemicals (i.e. pheromones, hormones), other biological control agents (i.e. parasitoids, predators, microbial pathogens), physical methods (including design of proper facilities), culture methods (including sanitation), and legislative controls. There is a need for demonstration projects that incorporate research and education in broad areas of both chemical and nonchemical methods of control. Such projects would coordinate the efforts of all sectors of agribusiness including farmers, shippers, warehousemen, processors, pest control and regulatory personnel, and the consumer. Although we have knowledge of some excellent non-pesticidal control measures, little effort has been made to integrate the most effective procedures in an effective pest management program. My intention here is to discuss ways in which pheromones can be utilized to manage storage insect pests.

The pheromones of a number of important storage pests are known (Table I). I have proposed several strategies for using pheromones to manipulate storage pests (1,2) (Table II). In the first approach, pheromones would be used in traps to monitor and detect the presence, population density, and location of pest species. Then if the level of infestation exceeded a predetermined value, the appropriate corrective measures, which might include a pesticide application, would be taken. This use of pheromones is therefore involved indirectly in the control of pests.

The results obtained during a study of seasonal patterns of emergence of *Attagenus* and *Trogoderma* in warehouses in Milwaukee, Wisconsin show how this strategy works (3). Corrugated paper traps treated with the appropriate pheromones and an insecticide were placed in the warehouses with control traps. Many more males of the target species were recovered from the pheromone traps than from the control traps. In fact, a previously unknown infestation of *Trogoderma variabile* was detected at one site. Pheromones then can be powerful and effective tools for detecting the presence of insect Other trapping studies with the *Trogoderma* pheromones have been made recently in the United States, Mexico, Germany and Australia. For example, trapping has

TABLE I. Major pheromone components of some stored-product insects.

Family-Species and Sex that Produces Pheromones	Chemical Description of Synthesized Pheromones	References
Anobiidae		
Stegobium paniceum (L.) ♀	2,3-dihydro-2,3,5-trimethyl-6-(1-methyl-2-oxobutyl)-4H-pyran-4-one	23,24
Bostrichidae		
Rhizopertha dominica (F.) ♂	1-methylbutyl (E)-2-methyl-2-pentenoate	7,8
	1-methylbutyl (E)-2,4-dimethyl-2-pentenoate	
Bruchidae		
Acanthoscelides obectus (Say) ♂	(E)-(-)-methyl 2,4,5-tetradecatrioneate	6
Dermestidae		
Attagenus megatoma (F.) ♀	(E,Z)-3,5-tetradecadienoic acid	13,25
Attagenus elongatulus (Casey) ♀	(Z,Z)-3,5-tetradecadienonic acid	26,27,28
Anthrenus flavipes LeConte ♀	(Z)-3-decenoic acid	29,30
Trogoderma inclusum LeConte	(Z)-14-methyl-8-hexadecen-1-ol	13,31,32
Trogoderma variabile Ballion ♀	(Z)-14-methyl-8-hexadencenal	
Trogoderma glabrum (Herbst) ♀	(E)-14-methyl-8-hexadecen-1-ol (E)-14-methyl-8-hexadecenal	13,33,32
Trogoderma granarium Everts ♀	92:8 (Z):(E)-14-methyl-8-hexadecenal	34,32

TABLE I. Continued.

Family-Species and Sex that Produces Pheromones	Chemical Description of Synthesized Pheromones	References
Gelechiidae		
Sitotroga cerealella (Olivier) ♀	(Z,E)-7,11-hexadecadien-1-ol acetate	35,36
Phycitidae		
Ephestia elutella (Hübner)	(Z,E)-9,12-tetradecadien-1-ol acetate	37,38,39,40 41,42,43,44
Plodia interpunctella (Hübner)		
Cadra cautella (Walker)		
Anagasta kuehniella (Zeller)		
Cadra figulilella (Gregson)		
♀		

TABLE II. Strategies for manipulation of stored product insects with sex pheromones or other attractants.

I. Surveillance and detection of infestation

II. Control of infestation

1. Communication disruption
2. Mass trapping
3. Lures for insecticide treatments
4. Pheromones as lures in pathogen-dissemination devices

been conducted in California and Idaho to determine the species of *Trogoderma* present in areas where leaf-cutter bees are reared for alfalfa pollination. Also the *Trogoderma* pheromones plus a larval food attractant and a pesticide are being evaluated by USDA-APHIS and the California Dept. of Food and Agriculture for a khapra beetle detection program.

The second basic strategy for using pheromones to manipulate stored product insects involves direct control 1) by disrupting the communication between sexes, 2) by mass trapping, or 3) by luring the insects to non-food materials treated with deleterious agents. As an example of the effectiveness of communication disruption, one application of high levels of pheromone in sealed 208-liter fiber drums was sufficient to significantly reduce mating activity of *Attagenus megatoma* for up to one year (4). Also, Sower et al. (5) reported that in enclosed environments, the mating frequency of the Indian meal moths, *Plodia interpunctella*, was reduced in the presence of the synthetic sex pheromone, (Z,E)-9,12-tetradecadien-1-ol acetate. In fact the effectiveness of any dose of pheromone was markedly increased as population densities were decreased from 10 to 0.2 pairs/m^2 of wall and ceiling surface.

Direct removal of insects from a population (mass trapping) would be an effective control measure if we could remove both sexes. A system of this type may be practical with the pheromone produced by the male bruchid, *Acanthoscelides obtectus* (6) or with the population aggregation pheromone produced by the male lesser grain borer, *Rhizopertha dominica* (7). For example, the lesser grain borer pheromone has been identified and synthesized (8), and recent field tests have demonstrated attractancy (9). The pheromone placed in suspended traps may be effective in attracting flying insects; for crawling insects, it may be effective in traps placed directly on the grain. With an effective pheromone or other attractant, it becomes possible to lure insects to insecticide-treated materials such as corrugated paper, paper chips, or other low-cost and easily disposable materials. The use of miniature traps made from such materials could be placed within the grain mass. By using attractants, less total insecticide would be needed because the insects would focus on the toxicant. The idea of miniature traps is patterned after a successful method of marking grain that was developed several years ago (10) to deter theft: paper chips with a code number corresponding to the owner were mixed with the grain and could be removed easily in the cleaning process before milling and processing. A modified version of the paper chips used in combination with other control agents was discussed by Burkholder (11).

Another means of direct control, as well as detection of infestation, is based on the fact that mechanical disturbance affects the behavior of the granary weevil: weevils appear on the surface of disturbed areas (12). Current studies in my laboratory (9) have demonstrated that other species of grain weevils have a similar response as do *Oryzaephilus* spp. and *Tribolium* spp. I suggest that grain probes could be placed permanently or temporarily in the grain mass to serve either as static traps or, when mechanically vibrated, as active traps. The physical attractiveness of the devices would be enhanced with food attractants or sex or population-aggregation pheromones. Such probes, designed with perforations to allow entry by insects, could be also sampled rapidly for insects by a vacuum device if they were placed at intervals of several meters and sampled weekly or at other regular intervals to determine infestation levels.

An innovative strategy is the use of pheromones as lures for devices that contain entomopathogens. The habitats of storage insects provide ideal conditions for inducing disease epizootics, particularly those caused by desiccation-resistant spore-formers. Even when the habitat is small and the population is localized it can contain highly concentrated populations of insects that have excellent potential for epizootics. With an effective pheromone-baited device, it should be just as easy to expose the attracted insects to a pathogen as to an insecticide. Of course the pathogen would not produce immediate kill. Rather the infected, spore-ladened insect would return to its natural habitat and infect others of its kind. This would seem to be an especially promising method of controlling stored-product insect pests.

The possible use of pheromones with pathogens and other biotic agents for suppression of dermestids was first proposed by Burkholder and Dicke (13) and later elaborated by Burkholder and Boush (14). The concept was put to the experimental test in an effort to suppress populations of the dermestid beetle, *Trogoderma glabrum*. In this trial, the sex pheromone of the beetle (14-methyl-8-hexadecenal) was combined with a protozoan pathogen, *Mattesia trogodermae* (Neogregarinida: Ophryocystidae) (15). Then we evaluated the numerous variables in pathogen transmission under conditions that we felt were highly conducive to population suppression. Because failure at any point in the sequence to bring the insect into contact with the pathogen would doom the strategy, our conditions were optimized: adult males emerged synchronously and prior to females; adult male populations were situated downwind from pheromone-spore-transfer sites; adult males were redistributed after luring and contamination among emerging females with subsequent mating; and dead adults were available as food for next generation offspring.

In this model system, subsequent generations of *T. glabrum* were substantially suppressed by a single introduction of *M. trogodermae* spores into dense populations of adult males via pheromone-baited, spore-transfer sites: high density (32 F_0 adults/m^2) treated populations increased only 4-fold in the 1st posttreatment generation (vs. a 24-fold increase in controls) and fell below pretreatment levels by the 2nd generation (vs. a total 100-fold increase in controls); low density treated populations and also the low density controls (2 F♀ adults/m^2), increased 12-fold during the 1st generation. With the dense population 48-hour exposure of pheromone-spore-transfer sites was sufficient to distribute effective spore doses within a radius of 1.25 m around sites, but spore transfer to the subsequent generations was mainly by larval ingestion of either dead, contaminated adults or larval food that adults had contaminated by contact. Attracted males also attempted copulation with the pheromone source, which increased spore transfer to males. Although the maximum distance the spores were transferred (1.25m) was limited by the size of our experimental arenas, we are confident that in a normal environment the pathogen would be dispersed as far as infected adults could disperse. Such a distance might be substantial since flight is possible by contaminated adults.

Burges and Hurst (16) noted that mortality of storage moths (e.g. *Plodia interpunctella)* in maize storage facilities in Kenya was often sudden and spectacular when the insects were exposed to *Bacillus thuringiensis* though mortality of similar moths exposed in the same way in laboratory jars was only progressive. They therefore suggested that larval cadavers, because they contain so many more *B. thuringiensis* spores than do moth bodies, frass, or eggs, are the most potent source of infective materials and with their attendant crystals, are capable of rapidly killing larvae that feed on them. When they compared the effect of *B. thuringiensis* spores spread on the surface of jars with that of spores applied to one point source on the surface, significantly fewer spores were required to start an epizootic. It is thus possible for initial infestation, and possibly subsequent infections, to begin in newly harvested and untreated grain because of the presence of infected adult moths. Epizootics may also arise because infected larvae have immigrated from adjacent stored grain or residues of food from local farms, transport vehicles, terminal stores or bags contaminated with frass and insect bodies. Moreover, the Burges and Hurst study shows that healthy larvae sometimes feed on larval cadavers even when food other than cadavers is present. Therefore most susceptible larvae could succumb first and provide inoculum to infect the more resistant larvae. Those workers nevertheless suggest that such naturally occurring infections of *B. thuringiensis* rarely curb moth damage to food and that the main effect is to limit moth reproduction in some food residues in stores and mills. Burges (17) believes that predictable control and adequate protection of food can only be obtained by admixing a lethal dose, e.g. 2×10^9 spores/200 g throughout the food to kill most first-instar larvae.

Again since the severe mortality of moth larvae caused by *B. thuringiensis* in Kenya was enhanced by the high concentrations of spores in the larval cadavers, it might be possible to mimic nature by providing a high concentration of spores in an attractive paper chip or other material that would serve as a simulated larval cadaver (SLC). The use of pheromones or other attractants to lure or aggregate larvae or adult stored product insects to sites that contain high concentrations of pathogens would therefore appear to be a promising method of insect population suppression.

Current studies at my laboratory indicate that there are promising larval attractants for dermestid beetles (18). These compounds could be combined with the available adult insect pheromones in an insect suppression system. It should be possible to incorporate baits into small paper chips or other devices that could be used to good advantage in a variety of ways. In grain, for example, a substance containing an attractant, a bait or feeding stimulant, and a pathogen such as *B. thuringiensis* might be formulated to simulate a larval cadaver. A domino effect might follow the introduction of a few SLC's. The initial rapid kill of larvae that would result would produce real cadavers, and their presence would accelerate the suppression of the population even further. A similar procedure was developed for use against the boll weevil, *Anthonomus grandis*, by McLaughlin et al. (19) with the sporozoans *Glugea gasti* and *Mattesia grandis*. Also, Montoya et al. (20) used a feeding stimulant to increase the lethality of a nuclear polyhedrosis virus of *Heliothis* spp.

I suggest a procedure in which the attractant would be selected to attract the young feeding stages that are particularly susceptible to the pathogen. They would then die before inflicting extensive damage on the stored grain. Ideally the SLC would be attractive both physically and chemically, would contain a feeding stimulant to ensure ingestion, and would contain enough pathogen to produce prompt kill. The SLC could be made from any of a variety of materials, corrugated paper, paper straws, or a natural material such as wheat straw, and then coated with the pathogen and attractant. A laminated structure incorporating safe and biodegradable materials would seem to be ideal. Materials such as adjuvants or stickers currently being used in the pesticide industry may be useful in binding pathogen to attractant in the SLC. Distribution of the SLC's in the walls or cracks of empty bins, under conveyors, or in other areas where residual populations might exist may also enhance population suppression.

Another method of effectively distributing a pathogen to stored-product insects is to provide a pheromone- or light-baited device with an open reservoir containing a pathogen such as *Bacillus thuringiensis*. The insects are attracted by the pheromone or light, become dusted with the pathogen, and distribute it within the insect population and habitat. Gard (21) has successfully used light as an attractant to induce night flying insects to disseminate virus material in California cotton fields. The attracted insects were dispersed after they were dusted with virus by using a timer that turned the light on and off in 15-minute cycles.

A critical unknown at this time is whether pheromones and other bioagents can or will be commercially developed. Without industrial development sufficient materials will not be available at reasonable prices. The Stanford Research Institute, under contract from the EPA, has studied the commercial feasibility of innovative pest control agents such as pheromones (22). The potential for commercial development of pheromones for actual control of stored product pests, i.e. mass trapping or mating disruption, was rated as high. In the same study, the likelihood of pest suppression programs involving pheromones of stored product pests was also rated as high. Efficacy, product specificity, market size and incentives, production, storage and field life characteristics, and toxicity were all evaluated.

Investigation of procedures for modifying insect behavior must continue. We need information about detection and control efficacy for all species of the storage-insect-pest complex. Also management procedures should be adaptable to small farms in any part of the world so local materials such as maize cobs, reeds, straw, wood or paper chips would be treated and used in place of manufactured materials. Modern grain handling facilities could easily adapt equipment for removing insects with miniature traps or other treated materials. "Fluidized-bed" or other similar techniques may be an efficient method of removal of both the insect, and the treated materials.

In summary, pheromones can be powerful tools that enhance pest management programs by permitting the early detection and control of storage insects. At the least, such regular monitoring of the storage facility, grain or stored commodity will aid in finding infestations early before extensive damage occurs. However management procedures of the type described might be used to suppress the infestations or to supplement and follow-up on treatment with conventional pesticides. On the basis of preliminary experiments, the prospect for success appears excellent.

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REFERENCES:

(1) Burkholder, W. E., Insect Pheromones and their Applications (Proc. and Forestry, Tokyo (1976) 111.

(2) Burkholder, W. E., "Manipulation of insect pests of stored products", Ch. 20, Chemical Control of Insect Behavior: Theory and Application (H.H. Shorey, J.J. McKelvey, Eds.), Wiley, New York (1977).

(3) Barak, A. V., and W. E. Burkholder, Environ. Entomol. 5 1 (1976) 111.
(4) Burkholder, W. E., J. Econ. Entomol. 66 6 (1973) 1327.
(5) Sower, L. L., W. K. Turner, and J. C. Fish, J. Chem. Ecol. 1 3 (1975) 335.
(6) Horler, D. F., J. Chem. Soc. C (1970) 859.
(7) Khorramshahi, A., and W. E. Burkholder, J. Chem. Ecol. (In press) 1979.
(8) Williams, H. J., R. M. Silverstein, W. E. Burkholder, and A. Khorramshahi, J. Chem. Ecol. (In press) 1979.
(9) Unpublished data, 1978.
(10) Personal communication, Grain Identification Co., Cooperstown, N.D. 1975.
(11) Burkholder, W. E., Prevention and Control of Insects in Stored-Food Products (Proc. Symp. Manhattan, Kansas, 1978) Kansas State Unvi., Manhattan, Kansas (1978).
(12) Browning, H. C., Proc. Zool. Soc. Lond. 116 (1947) 675.
(13) Burkholder, W. E., and R. J. Dicke, J. Econ. Entomol. 66 6 (1973) 1327.
(14) Burkholder, W. E., and G. M. Bousch, Bull. OEPP (Organ. Eur. Mediterr. Prot. Plant) 4 (1974) 455.
(15) Shapas, T.J., W. E. Burkholder, and G. M. Bousch, J. Econ. Entomol. 70 4 (1977) 469.
(16) Burges, H.E., and J. A. Hurst, J. Invertebr. Pathol. 30 (1977) 131.
(17) Burges, H. E., Entomophaga Mem. Hors. Ser., 2 (1964) 323.
(18) Nara, J. M., and W. E. Burkholder, Environ. Entomol. (In preparation) (1978).
(19) McLaughlin, R. E., T. C. Cleveland, F. J. Daum, and M. R. Bell, J. Invertebr. Pathol. 13 (1969) 429.
(20) Montoya, E. L., C. M. Ignoffo, and R. L. McGarr, J. Invertebr. Pathol 8 (1966) 320.
(21) Gard, I. E., Microbial Control of Insect Pests: Future Strategies in Pest Management Systems (Proc. Conf. Gainesville, Florida, 1978) University of Florida (1978).
(22) Ayers, J. H., New, Innovative Pesticides: an Evaluation of Incentives and Disincentives for Commercial Development by Industry (Report, Stanford Research Institute, 1977) Menlo Park, California (1977).
(23) Kuwahara, Y., H. Fukami, S. Ishii, F. Matsumura, and W. E. Burkholder, J. Chem. Ecol. 1 4 (1975) 413.
(24) Kuwahara, Y., H. Fukami, R. Howard, S. Ishii, F. Matsumura, and W. E. Burkholder, Tetrahedron 34 (1978) 1769.
(25) Silverstein, R.M., J. O. Rodin, W. E. Burkholder, and J. E. Gorman, Science 157 (1967) 85.
(26) Barak, A. V., and W. E. Burkholder, J. Stored Prod. Res. 13 (1977) 169.
(27) Barak, A. V., and W. E. Burkholder, J. Stored Prod. Res. 13 (1977) 177.

(28) Fukui, H., F. Matsumura, A. V. Barak, and W. E. Burkholder, J. Chem. Ecol. 3 5 (1977) 541.
(29) Burkholder, W. E., M. Ma, Y. Kuwahara, and F. Matsumura, Can. Entomol. 106 (1974) 835.
(30) Fukui, H., F. Matsumura, M. Ma, and W. E. Burkholder, Tetrahedron Lett. 40 (1974) 3563.
(31) Rodin, J. O., R. M. Silverstein, W. E. Burkholder, and J. E. Gorman, Science, 165 (1969) 904.
(32) Cross, J. H., R. C. Byler, R. F. Cassidy, R. M. Silverstein, R. E. Greenblatt, W. E. Burkholder, A. R. Levinson, and H. Z. Levinson, J. Chem. Ecol. 2 (1976) 457.
(33) Yarger, R. G., R. M. Silverstein, and W. E. Burkholder, J. Chem. Ecol. 1 (1975) 323.
(34) Levinson, H. Z., and A. R. Barilan, Riv. P. 28 (1967) 27.
(35) Keys, R. E., and R. B. Mills, J. Econ. Entomol. 61 (1968) 46.
(36) Vick, K. W., H. C. F. Su, L. L. Sower, P. G. Mahany, and P. C. Drummond, Experientia 30 (1974) 17.
(37) Brady, V. E., Life Sci. 13 (1973) 227.
(38) Brady, V. E., and D. A. Nordlund, 10 (1971) 787.
(39) Brady, V. E., and R. D. Daley, Ann. Entomol. Soc. Am. 65 (1972) 1356.
(40) Brady, V. E., J. H. Tumlinson, III, R. G. Brownlee, and R. M. Silverstein, Science 171 (1971) 802.
(41) Brady, V. E., D. A. Nordlund, and R. C. Daley, J. Ga. Entomol. Soc. 6 (1971) 215.
(42) Kuwahara, Y., and J. E. Casida, Agric. Biol. Chem. 37 (1973) 781.
(43) Kuwahara, Y., C. Kitamura, S. Takahashi, H. Hara, S. Ishii, and H. Fukami, Science 171 (1971) 801.
(44) Kuwahara, Y., H. Hara, S. Ishii, and H. Fukami, Agric. Biol. Chem. 35 (1971) 447.