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**BIOMONITORING  
FOR STORED-PRODUCT INSECTS  
OF MILITARY IMPORTANCE AND  
A REVIEW OF THE LITERATURE  
AND MILITARY APPLICATION**

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<p>An in depth review of current technology and application is provided for the practical use of insect pheromone traps in the military warehouse environment. The recognition, identification, synthesis, and bioassay of insect pheromones is reviewed with respect to eighteen kinds of stored-product insects. A listing is also presented for insect pheromones that can now be used for detection and possible control of stored products insects that might infest military subsistence. Military significance is discussed with regard to surveillance and control of stored-product insects in depot warehouses, commissary storage areas, and troop issue points.</p>										

PREFACE

The work reported herein was done by the Food Packaging and Processing Group, Food Engineering Laboratory, US Army Natick Research and Development Laboratories, Natick, Massachusetts under Project Number 1L162724AH99BE.

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# BIOMONITORING FOR STORED-PRODUCT INSECTS OF MILITARY IMPORTANCE AND A REVIEW OF THE LITERATURE AND MILITARY APPLICATION

## INTRODUCTION

Monitoring of stored-product insects in military warehouses by pheromone traps may provide useful information for the detection and control of insects infesting military subsistence. Food attractants, light traps, and physical traps have been used for this purpose for years in commercial warehouses. Food attractants, however, have the disadvantage in that they usually do not attract over a long distance, and food traps may compete with the natural food and may provide important foci for new infestations. Light traps are dependent on electricity, are effective only at certain wavelengths, and are restricted by space; and certain physical traps also have limited luring capability, for example, perforated, cylindrical grain probes and pitfall traps.

Efforts are now being made to use pheromone traps as a means for producing a highly effective monitoring system for stored-product insects of military importance. The current array of pheromones available for monitoring stored-product insects in military warehouses is summarized in the Appendix, Tables A-1 and A-2.

## TECHNOLOGY APPLICATION

The problem of utilizing pheromone technology in military pest management programs is multi-faceted. There is a definite need for in-depth studies of the biology and behaviour of the various stored-product insects found in the military warehouse environment. There is also a need to determine the most effective rate of release, that is, the proper releasing substrate, and to ascertain the trap sensitivity in military-storage facilities. Satisfying these research needs will lead to innovative pheromone monitoring systems that can be coordinated with military pest management control programs. This is not a simple task, for example, identification, synthesis, and bioassay of candidate compounds is a long, laborious, and expensive process. Although military food storage facilities are often regarded as uniform with stable environments, they are all different with considerable variability. An effective biomonitoring program utilizing pheromones for application in the military warehouse environment is an extremely complex system.

In pheromone research it is necessary to recognize that a chemical may be responsible for an attraction between individual insects, to isolate and identify the specific chemical that is responsible for that attraction, to determine the sensitivity of the pheromone by exhaustive bioassays, and finally to field test the material to determine its efficacy under military warehouse conditions.

## EXPERIMENTAL TECHNIQUE

A basic, chemical protocol may include the following procedure:

1. The insects may be subjected to an extraction process, and after solvent removal, the soluble residue will be subjected to short-path, high-vacuum distillation.

2. The distillate may be extracted with alkali, and the active fraction chromatographed on silica gel using a series of progressively more polar liquids.

3. The active fraction may continually be fractionated by gas chromatography until the active components are isolated in a high state of purity.

4. Synthesis sequences may be designed to confirm structures.

Concurrent with the above procedure, bioassays may be conducted to:

1. Perform a sequence of progressively refined, isolation steps, each monitored by bioassay, until individual compounds are obtained in a high state of purity. Several biologically active compounds may be present, elaborated by the one or more insects. The compounds may be independently and additively active or they may be individually active but show synergistic effects in combination or they may be individually inactive but show activity in combination.

2. Identify the individual components. Usually these are available in milligram or microgram quantities; therefore, identification depends heavily on spectrometric techniques: mass, nuclear magnetic resonance, and optical spectrometry. These techniques give mutually complementary information on chemical structure (Silverstein and Bassler, 1967).<sup>1</sup> Several supplementary chemical techniques are especially effective at the microgram level (Beroza and Acree, 1964; Beroza and Bierl, 1966; Brownlee and Silverstein, 1968).<sup>2-4</sup>

3. Confirm the postulated structures by comparison with rationally synthesized compounds.

Once the synthetic material is available, confirmation must be made to assure that the chemosensory responses are similar to those caused by the natural occurring pheromone.

<sup>1</sup>R. M. Silverstein and G. C. Bassler, 1967. "Spectrometric Identification of Organic Compounds." John Wiley and Sons, Inc., NY, 2nd ed.

<sup>2</sup>M. Beroza and F. Acree, Jr., 1964. A New Technique for Determining Chemical Structure by Gas Chromatography. J. Assoc. Office Agric. Chem. 47:1.

<sup>3</sup>M. Beroza and B. A. Bierl, 1966. Apparatus for Ozonolysis of Microgram to Milligram Amounts of Compound. Anal. Chem. 38:1976.

<sup>4</sup>R. G. Brownlee and R. M. Silverstein, 1968. A micro-preparative gas chromatography and a modified carbon skeleton determinator. Anal. Chem. 40:2077.



## CURRENT RESEARCH EFFORTS

Pheromones are being extracted, identified, and developed by several research organizations. The following is a brief synopsis of what is known about several insect species of military importance.

### COLEOPTERA

#### Dermestid Beetles

An extensive pheromone-monitoring program for dermestid beetles, for example, *Trogoderma* spp., has been underway for several years in the United States. A monitoring system is routinely used by the Plant Protection and Quarantine Program, USDA, APHIS at major port facilities. The pheromone used often attracts *T. variabile*, and it has been used in California to survey the Khapra beetle, *Trogoderma granarium* Everts. Levinson and Levinson (1979)<sup>5</sup> evaluated the pheromone for *T. granarium* in Morocco. *Trogoderma* pheromone traps were also evaluated in military warehouses in California and Tennessee (results of this study will be published in a separate technical report).<sup>5</sup>

Barak and Burkholder (1979)<sup>6</sup> evaluated the alcohol component of the *Trogoderma* pheromone with megatonic acid, the sex pheromone of *Attagenus megatoma* (Fabricius), the black carpet beetle. Traps baited with the pheromone, either singly or in combination, were placed in three warehouses and grain elevator locations in Milwaukee, Wisconsin. Traps baited with pheromones caught significantly more target insects than control traps. A hidden population of *T. variabile* was also detected in one warehouse. The pheromone system also allowed for a precise charting of the seasonal emergence of *A. megatoma* over a two-year period.

Studies of *Trogoderma* trapping in military warehouses are now concerned with proper trap location, density, pheromone concentration, release rate, and trap design. Wheat germ oil and several of its components have been identified in the USDA-Madison Laboratory and are now used to monitor the larval stages of *Trogoderma*. The physical design of the trap has been improved and the most effective enantiomeric composition of the pheromone has

<sup>5</sup>H. Q. Levinson and A. R. Levinson, 1979. Trapping of storage insects by sex and food attractants as a tool of integrated control, In: "Chemical Ecology: Odour Communication in Animals," F. J. Ritter, ed., Elsevier/North Holland Biomedical Press, Amsterdam.

<sup>6</sup>A. V. Barak and W. E. Burkholder, 1976. Trapping Studies with Dermestid Sex Pheromones. Environ. Entomol. 5:111.

been determined (Silverstein *et al.*, 1980),<sup>7</sup> as have the theoretical maximum communication distances for the pheromone (Shapas and Burkholder, 1978).<sup>8</sup>

Cross-attraction seems to be rather general among the several species of *Trogoderma* tested. Species indicating cross attractance to one or both of the two synthetic pheromones of *T. inclusum* are as follows: *T. simplex*, *T. glabrum*, *T. variabile*, *T. sternale*, *T. grassmani*. Of immediate practical use is the finding that *T. granarium* (the Khapra beetle) also responds to a *T. inclusum* extract and to the synthesized compounds.

#### Lesser Grain Borers

The male lesser grain borer, *Rhyzopertha dominica* (Fabricius), produces an aggregating pheromone that attracts both sexes (Khorramshari and Burkholder, 1981).<sup>9</sup> This pheromone produces a "sweetish" odor in grain infested by the borer and in laboratory cultures, and the odor is similar to odors associated with urea compounds. Monitoring studies for this species were conducted in a variety of warehouses in Texas with corrugated floor and aerial mounted traps. The traps collected adult beetles for five weeks.

Since lesser grain borer infestations more often develop deep within storage bins, monitoring becomes somewhat specialized. Although the head space of a bin may be monitored, a more effective method would be the use of grain probes similar to those developed by Loschiavo and Atkinson (1967).<sup>10</sup> Since the adults and larvae spent much of their time deep within the bin, probes baited with the aggregating pheromone could be more closely placed in the infestation site and provide a more precise measurement of adult activity.

#### Other Beetles

Pheromones currently being studied include those of *Tribolium* spp., *Sitophilus* spp., *Oryzaephilus* spp., *Stegobium paniceum* (L.), *Lasioderma serricorne* (Fabricius), *Acanthoscelides obtectus* (Say), the bean weevil, and *Gallosobruchus* spp., seed weevils. Bin probes and bin or floor surface-traps containing pheromones or attractants appear to be a promising monitoring tool for many of these insects.

<sup>7</sup>R. M. Silverstein, R. F. Cassidy, W. E. Burkholder, J. J. Shapas, H. A. Levinson, H. . Levinson, and K. Mori, 1980. Perception by *Trogoderma* species of chirality and methyl branching at a site far removed from a functional group in a pheromone component, *J. Chem. Ecol.*, 6(5): 911-917.

<sup>8</sup>T. J. Shapas and W. E. Burkholder, 1978. Patterns of sex pheromone release from adult females, and effects of air velocity and pheromone release rates on theoretical communication distances, *J. Chem. Ecol.*, 4: 395.

<sup>9</sup>A. Khorramshari and W. E. Burkholder, 1981. Behavior of the lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrichidae) Male-produced aggregation pheromone attracts both sexes. *J. Chem. Ecol.*, 7(1): 33-38.

<sup>10</sup>S. R. Loschiavo and J. M. Atkinson, 1967. Activity of insect growth regulators, hydropene and methoprene, on wheat and corn against several stored grain insects *J. Econ. Entomol.*, 68:688.

The pheromones of *S. pariceum* (drugstore beetles) and *L. serricornis* (cigarette beetle), both important species found in military warehouses, could be used effectively in conjunction with light traps since the adult stages of both species are attracted to light.

## LEPIDOPTERA

Reichmuth *et al.* (1976),<sup>11</sup> investigated the use of pheromone traps for moth control under storage conditions. Experiments were carried out in small rooms, large warehouses, and a chocolate factory in West Berlin during 1975. (Z)-(E)-9, 12-tetradecadien-1-ol acetate vaporized from plastic capsules in sticky traps was successful for monitoring infestations of *Plodia interpunctella* (Hubner) and *Ephestia elutella* (Walker).

Reichmuth *et al.* (1978)<sup>12</sup> were also able to monitor the seasonal fluctuations of *E. elutella* populations in grain stores. Also, their studies in Greece during 1977-1978 pinpointed the seasonal emergence cycles of *Anagasta kuehniella* (Zeller), *E. cautella* (Walker), and *P. interpunctella*. Vic *et al.* (1979)<sup>13</sup> reported on evaluation of eight trap designs utilizing the combined pheromones of *P. interpunctella* and *Sitotroga cerealella* (Oliver).

## MILITARY SIGNIFICANCE/CONCLUSIONS

Biomonitoring for stored-product insects (moths and beetles) that may infest military subsistence items will become an important part of the military pest management program.

As new pheromones, traps, etc., become available, it will be feasible to implement a comprehensive surveillance program in depot warehouses, commissary storage areas, and troop issue points.

When an infestation is detected, increased monitoring could be phased into a control mode that would involve mass-trapping. Such an application would be theoretically feasible, especially if coupled with other control measures including increased commodity inspection, improved sanitation, and spot treatment with pesticides.

In addition, the Military Medical Entomologist or the Facilities Engineer Entomologist could chart the seasonal occurrence of stored product insects associated with storage facilities at various locations. This information could play a major role in an integrated pest management program where precise timing of control measures is so important.

<sup>11</sup>C. von Reichmuth, R. Wohlgemuth, A. R. Levinson, and H. Z. Levinson, 1976. Untersuchungen über den Einsatz von pheromon-bekoderten Klebefallen zur Bekämpfung von Motten in Vorratsschutz, *Z. Angew. Entomol.*, 82: 95.

<sup>12</sup>C. von Reichmuth, H. V. Schmidt, A. R. Levinson, and H. Z. Levinson, 1978. Die Fangigkeit Pheromonbehoderten Klebefallen für Speichermotten (*Ephestia elutella* Hbn.) in unterschiedlich dicht befallenen Getreidelagern, *Z. Angew. Entomol.*, 86: 205.

<sup>13</sup>K. W. Vick, J. Kvenberg, J. A. Coffelt, and C. Steward, 1979. Investigation of sex pheromone traps for simultaneous detection of Indian meal moths and Augoumois grain moths. *J. Econ. Entomol.*, 72: 245.

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**APPENDIX**

Table A-1. Major Pheromone Components of Some Stored-Product Coleoptera (Anobiidae, Bostrichidae, Bruchidae, Dermestidae, Tenebrionidae) in Military Subsistence

Species and Sex which Produce Peromones	Pheromones
<i>Stegobium paniceum</i>	2, 3 Dihydro-2, 3, 5-trimethyl-6 (1-methyl-2-oxobutyl)-4H-pyran-4-one (Kuwahara <i>et al.</i> , 1975, 1978)
<i>Lasioderma serricorne</i>	4, 6 Dimethyl-7-hydroxy-nonan-3-one (Coffelt and Burkholder, 1972; Chuman, 1979 a, b)
<i>Rhyzopertha dominica</i>	1-Methylbutyl ( <i>E</i> )-2-methyl-2-pentenoate and 1-methylbutyl ( <i>E</i> )-2, 4-dimethyl-2-pentenoate (Khorramshahi and Burkholder, 1980; Williams <i>et al.</i> , 1981).
<i>Acanthoscelides obtectus</i>	( <i>E</i> )-(Z)-Methyl-2, 4, 5-tetradecatrienoate (Hope <i>et al.</i> , 1967; Horler, 1970).
<i>Attagenus megatoma</i>	( <i>E</i> , Z)-3, 5-Tetradecadienoic acid (Burkholder and Dicke, 1966; Silverstein <i>et al.</i> , 1967).
<i>Attagenus elongatulus</i>	(Z, Z)-3, 5-Tetradecadienoic acid (Barak and Burkholder, 1977 a, b; Fukui <i>et al.</i> , 1977).
<i>Anthrenus flavipes</i>	(Z)-3-Decenoic acid (Burkholder <i>et al.</i> , 1974; Fukui <i>et al.</i> , 1974).
<i>Tribolium castaneum confusum</i>	4, 8-dimethyldecan-1-ol (Suzuki, 1981).
<i>Trogoderma inclusum/variabile</i>	(Z)-14-Methyl-8-hexadecen-1-ol and (Z)-14-methyl-8-hexadecenal (Burkholder and Dicke, 1966; Rodin <i>et al.</i> , 1969; Cross <i>et al.</i> , 1976).
<i>Trogoderma inclusum/variabile</i>	( <i>E</i> )-14-Methyl-8-hexadecen-1-ol and ( <i>E</i> )-14-methyl-8-hexadecenal (Burkholder and Dicke, 1966; Yarger <i>et al.</i> , 1975; Cross <i>et al.</i> , 1976).
<i>Trogoderma glabrum</i>	( <i>E</i> )-14-Methyl-8-hexadecen-1-ol and (Z)-14-methyl-8-hexadecenal (Burkholder and Dicke, 1966; Yarger <i>et al.</i> , 1976).
<i>Trogoderma granarium</i>	92:8 (Z, <i>E</i> )-14-Methyl-8-hexadecenal (Levinson and Barilan, 1967, Cross <i>et al.</i> , 1976).

Table A-2. Major Pheromone Components of Some Stored-Product Lepidoptera (Gelechiidae and Pyralidae) Found in Military Subsistence

Species and Sex which Produce Pheromones	Pheromones
<i>Sitotroga cerealella</i> <sup>♂</sup>	(Z, E) 7,11-Hexadecadien-1-ol acetate (Keys and Mills, 1968; Vick <i>et al.</i> , 1974)
<i>Ephestia elutella</i> <sup>♂</sup>	(Z, E)-9,12-Tetradecadien-1-ol acetate (Brady, 1973; Brady and Nordlund, 1971; Brady and Daley, 1972; Brady <i>et al.</i> , 1971 a, b; Kuwahara and Casida, 1973; Kuwahara <i>et al.</i> , 1971 a, b).
<i>Plodia interpunctella</i> <sup>♂</sup>	
<i>Ephestia cautelia</i> <sup>♂</sup>	
<i>Anagasta kuehniella</i> <sup>♂</sup>	
<i>Cadra figulilella</i> <sup>♂</sup>	
<i>Amyelois transitella</i> <sup>♂</sup>	(Z, Z)-11,13-Hexadecadienal (Coffelt <i>et al.</i> , 1979 a, b).

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