AN ABSTRACT OF THE THESIS OF

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Title: Chemical Ecology of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae): Factors Affecting Biology and Application of Pheromone.

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(Dr. M. T. Aliniazee)

Pheromone biology and behavioral responses to food, pheromone, and traps were investigated in the red flour beetle, *Tribolium castaneum,* (Herbst) (Coleoptera: Tenebrionidae), a serious cosmopolitan pest of stored products. Volatiles were collected from single or multiple beetles by aeration and adsorption on Super-Q, and quantities of the male-produced pheromone 4,8-dimethyldecanal were determined with GC/FID and GC/MS. Virgin males produced about 635 ng of pheromone per day over a 30-day period. Individual male pupae produced 93.4 $\pm$ 24.72 ng/day of pheromone and small but detectable amounts of pheromone were also observed in individual larvae. Males ceased pheromone production in the absence of food, maintained pheromone production after mating, and increased pheromone production under long light conditions. Males in groups of 5, 10 ,or 50 produced no detectable pheromone, but did produce defensive compounds. Males under crowded conditions produced more 2-methyl- and 2-ethyl-1,4-benzoquinones than females treated similarly.

Volatiles from fifty males did not affect pheromone production in a single male, demonstrating that defensive compounds *per se* were not responsible for inhibition of pheromone production.

Both cracked wheat and the processed food "wheat germ nuts" were attractive alone to *T. castaneum* in a laboratory bioassay. Males displayed a higher response to wheat germ nuts than females. Synthetic pheromone tested alone was attractive at low doses in the bioassay (0.001-1.0 μg) but was repellent at higher doses (10 and100 μg). Combinations of pheromone and food elicited responses that were significantly higher than responses to either pheromone or food alone, but this enhancement appeared more additive than synergistic. All three designs of slow-release pheromone lures tested in bioassays exhibited repellency or low activity when first removed from their packages and then elicited high responses after aging. Lures in which pheromone was formulated into laminated polyethylene seemed to give maximum responses for longer periods beginning at an earlier age. Comparison of different traps designs in two-choice experiments in large arenas revealed that traps in which beetles walked up a ramp and fell into a pitfall were superior to traps constructed of corrugated cardboard.

CHEMICAL ECOLOGY OF *TRIBOLIUM CASTANEUM* HERBST (COLEOPTERA: TENEBRIONIDAE): FACTORS AFFECTING BIOLOGY AND APPLICATION OF PHEROMONE.

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DEDICATED TO MY LOVING PARENTS WHO INSPIRED ME FOR THE PURSUIT OF HIGHER EDUCATION AND THOSE HUMANS WHO DIED DUE TO STARVATION AND MALNUTRITION.

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CHAPTER I.

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

The world's population is increasing rapidly, but unfortunately the food supply is not increasing at the same rate. Insects, rodents, birds, and microorganisms consume almost one half of all food produced in the world in spite of the use of pesticides and other technologies to control them. These pests are present in warehouses, elevators, flat storage, mills, food processing plants, bakeries, all kinds of transportation equipment and homes. The level of post harvest losses represents substantial problems that counter modern efforts for higher crop yields (Pimental 1991).

All stored grains and foods decay and lose quality while in storage. Insect pests not only increase deterioration of quality but also reduce quantity. Insects can attack relatively dry stored grains and food at 15-42° C in all parts of the world, and comprise one of the most successful groups of organisms in the storage ecosystem (Sinha 1973). Insect activity fluctuates depending upon environmental factors (Zafar et al. 1987). Several pesticides and fumigants have been used for controlling stored product pests. Some of them have been eliminated because of resistance in the target species or due to health hazards. Recently, the use of several fumigants has been banned in USA (Walter 1991). Presently the choice is between methyl-bromide and phosphine. Fumigants have some limitations too. All are poisonous to humans, some are highly explosive, others have residual effects, and resistance in pest populations is slowly evolving. Similarly, many residual pesticides have also been used against stored product insect pests, but resistance to these is wide spread in stored-product pests. Pesticide resistance has been reported in almost all stored-grain pests

(Champ and Dyte 1976). Although chemical pesticides are relied on extensively to control pests of stored-products, the high cost of the developing new chemicals to replace those withdrawn from use because of pest resistance or regulation is huge (Watson 1979). The need for regulations to prevent environment damage or human health risks have led to increased interest in non-insecticidal methods of pest control. In considering what can be done to prevent storage losses, it is useful to think of a grain storage as an ecosystem (Sinha, 1973) and identify those components of the system that influence deterioration.

To implement any integrated pest management (IPM) program it is important to detect early infestation. Attractant-baited traps are being used to assist in many IPM programs. Various types of insect traps are being used for monitoring insect activity in food storages. Many of these traps include an attractant and a means of capturing and retaining insects. Utilizing a synthetic pheromone is the most important factor in catching moths or beetle pests in traps. A number of international companies are manufacturing pheromone baits and traps. Unfortunately, pheromones that are commercially available for the many pest species have received no more research than the fundamental identification, synthesis, and field bioassay. *Tribolium* pheromone was identified more than ten years ago but its effective use in traps is limited. Many gaps in basic knowledge and improvement of lures have yet to be addressed. Moreover, the use of pheromones, and the fledgling industries that promote and market them, is dwarfed by the magnitude of chemical pesticides in commercial agriculture.

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a cosmopolitan pest of stored products. Both larvae and adults cause devastating damage by feeding externally on both grains and processed food. Adult beetles produce a foul odor from secreted quinones and hydrocarbons and turn flour light pink. Enormous infestation can adversely affect the viscosity and elasticity of flour. *Tribolium* infestation can also cause depletion of the nutritive value of the medium and add contamination in the form of dead animals and exuviae. The economic threshold for pest infestation of stored products is usually zero in developed counties, which consequently offers a challenge to finding effective detection methods.

Tribolium pheromone has been identified as 4,8-dimethyldecanal (Suzuki, 1980). The compound is a male-secreted aggregation pheromone, presumably produced by glands on the prothoracic femora (Faustini et al.1981). Although some studies since 1980 have addressed use of the synthetic pheromone (e.g., Javer et al. 1989, Obeng-Ofori 1991), none have investigated the biological aspects of pheromone production or the behavioral context in which pheromone is used by beetles. Synthetic pheromone is being employed in attempts to monitor *Tribolium*, but currently used traps are believed ineffective (Mullen 1992) and success is very limited. This thesis presents studies conducted to: a) investigate factors that affect pheromone production in *Tribolium castaneum*, b) quantify the amount of volatiles, pheromones and defensive compounds, produced by males and females, c) to examine the relationship between pheromone and other volatiles, d) study the response of red flour beetle to food and pheromone and e) to evaluate the activity of synthetic pheromone in lures and various trap designs.

LITERATURE REVIEW

Tribolium Pheromones:

O'Ceallachain and Ryan (1977) reported that adults of *Tribolium confusum* (J. du Val) secrete two pheromones. The male-produced pheromone attracted both sexes and that produced by the female was attractive to males only, but was not stable. A living source of pheromone from one sex habituated the responding beetles, but made the beetles more sensitive to pheromone from the other sex. These findings were interesting because the male-produced pheromone reached a peak in production after 5 days and the female is reproductively mature after 3 days, yet both sexes respond to the male-produced pheromone immediately after eclosion. A male-secreted aggregation pheromone, 4, 8- dimethyldecanal, was identified from *Tribolium castaneum* and *T. confusum* by Suzuki (1980, 1981). After identifying the pheromone, Suzuki also reported that synthetic pheromone was comparatively less effective than the natural pheromone, and the position of the methyl group around the C-8 carbon was indispensable for the activity (Suzuki , 1981). Both sexes were attracted to ca. 60 ng of crude secretion. Males and females perceive the pheromone on the day of emergence while perception differed between the sexes. Similar studies were conducted by Levinson and Mori (1983), who reported olfactory perception and orientation behavior of female and male flour beetles to single stereoisomers of 4, 8-dimethyldecanal. Their work revealed maximal receptor potentials in electroantennograms and optimal attraction in response to the 4R, 8R(-)-dimethyldecanal enantiomer. They further reported pheromone extracts of prothoracic

femora from unmated male flour beetles elicited higher receptor potentials in the antennae of females than in those of males . Although various authors have reported adult males and females respond to 4,8-dimethyldecanal, the larvae of *T. castaneum* also respond to this synthetic pheromone (Mondal and Port 1984). Larvae of *T. castaneum* were attracted both by contact and vapor of 4,8-dimethyldecanal. A sex pheromone produced by the female *T. castaneum* has also been isolated and identified as Z-2 nonenyl propionate (Rangaswamy and Sasikala 1990). The reported compound induces high rates of matings in unmated males. This compound was isolated from female virgins of *T. castaneum.*

The orientation behavior of *Tribolium castaneum* and *T. confusum* to synthetic pheromone has also been studied (Obeng-Ofori, 1991). Orientation behavior of the two *Tribolium* species responding to different concentrations of synthetic aggregation pheromone in still and moving air was studied in an olfactometer. Analysis of *Tribolium* tracks indicated that the aggregation pheromone stimulated the beetles to walk faster at higher concentrations, to increase the frequency and magnitude of turning, and to decrease track reversal distances and distances between turns. The mean walking speed of both species was lowest at the highest air speed (Obeng-Ofori 1991).

Tribolium Defensive Compounds:

Species in the genus *Tribolium*, and many species of Tenebrionidae in general, possess cuticular reservoirs that secrete and emit defensive chemicals when the insects

are disturbed (Doyen 1985, Sokoloff 1972). These secretions are made up primarily of quinones and hydrocarbons, and in *Tribolium* the common defensive compounds include 2-methylbenzoquinone, 2-ethylbenzoquinone, and 1-pentadecene (Markarian et al. 1978, Howard 1987, Mondal 1985). Quinones and unsaturated hydrocarbons are known to be repellents or deterrents to other insects (e.g. Suzuki et al. 1975), and they can serve an antimicrobial benefit in the food medium (Roth and Eisner (1962). Research on the interaction of defensive compounds and pheromones in *Tribolium* is limited. Faustini and Burkholder (1987) suggested that quinones released by *T. castaneum* under crowded conditions would dissolve (chemically breakdown) the male-produced aggregation pheromone, but no chemical analyses of pheromone production were reported. No literature was found on quantities of defensive compounds or pheromone produced by *Tribolium.*

Food Odor Attraction:

Scientists and stored-product managers have made efforts to use volatile food odors alone or in combinations with naturally produced pheromones to trap insect pests. Miklajczak, et al. 1984 reported that saw toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae), is attracted to certain volatile components of whole and rolled oats as determined by a laboratory pitfall chamber bioassay. They reported that out of more that 100 components detected, 14 were identified and bioassayed. Propanal and formaldehyde were found to be attractive. Similarly the volatile components of wheat germ oil were bioassayed (Nara et al. 1981) and reported

to be responsible for initiating aggregation activity of *Trogoderma glabrum* (Herbst) larvae. A strong aggregation response was observed by the neutral plus basic compound fraction of wheat germ oil, but only a small one by the acidic fraction. The principal compounds were C_{13}-C_{16} saturated and branched hexylbenzenes. Food-grade coconut, corn and sunflower oils have been reported to induce aggregation. Similar kind of studies have been reported by Freedman et al. (1982), who found rolled oats and pentane extracts of rolled oats were attractive to the adults of *O. surinamensis* (L.) when tested in a two-choice olfactometer. Pentane and ether extracts of rolled oats caused aggregation of these beetles when tested in a two choice petri dish bioassay. Barrer (1983) conducted research on six different species of stored grain pests including *T. castaneum.* Trapped odors from stored wheat contributed markedly to the attraction of all six species.

Evidence is now accumulating from various insect species that food or host plant volatiles play a major role in pheromone biology. Some species of bark beetles (Coleoptera: Scolytidae) are well known for their ability to convert host tree monoterpenes into pheromones, and many species require host volatiles as synergists for their aggregation pheromones (Borden 1985). Dickens et al (1990) showed that pheromones from insects in different orders were synergistically enhanced by host plant volatiles. It is apparent now that sex pheromone production in moths occurs when females are in proximity of a host plant , and this is stimulated by plant volatiles (Raina et al. 1992). Walgenbach et. al. (1987) used various food odors with maize weevils, *Sitophilus zeamais* (Motschulsky) in laboratory test arenas, and reported that

weevils strongly preferred the combination of the pheromone, the (4S, 5R) isomer of 5-hydroxy- 4 methyl- 3-heptanone (sitophinone), and cracked wheat. Maize weevils were successfully lured out of a food source only when both cracked wheat and sitophinone were present in the trap. No work to date has addressed the role of food in either pheromone production by *T. castaneum* or in behavioral responses of beetles to pheromone.

Trapping and Monitoring:

Male-produced pheromones have been used quite often for monitoring and trapping of some stored grain beetles. Obeng-Ofori and Coaker (1990) investigated the efficacy of probe traps for *T. castaneum* and other species using various concentrations of synthetic pheromone. Their results indicate that pheromone-baited pitfall and probe traps were over 50% more effective than unbaited traps. A curvilinear relationship between numbers trapped and pheromone concentration indicated that 2.0 mg of pheromone per trap elicited an optimum response for *T. castaneum*. Lindgren et. al. (1985) worked on a method for simultaneous semiochemical-based monitoring of *Cryptolestes ferrugineus* (Stevens) and *T. castaneum*, and concluded that the pheromones of both species can be used together in the same trap in a semiochemical based, pest monitoring systems. Trapping of insects in small bins of stored wheat was conducted during 1984 and 1985 in an unheated building in Winnipeg, Manitoba, Canada (White and Loschiavo 1986). Tubular brass-screen traps were used in the center of the bin to full length. About 57% of released

beetles were trapped in 6 weeks period. *Tribolium* was consistently more abundant in the top 6-10 cm of the wheat while *C. ferrugineus* was more abundant at and near floor level. Traps baited with either wheat germ or adults species caught ca. 16% and unbaited traps caught 6% of the initial *T. castaneum* population during a 5 week period. An efficient multi-species trap of corrugated paper and utilizing plastic pitfall devices containing a vegetable oil based food attractant was developed for the purpose of detecting and monitoring stored product insects (Barak and Burkholder 1985). Cogan and Wakefield (1987) reported that trapping beetles by pitfall and probe traps in bulk grain stores are at least 10 times more effective than conventional sampling methods. They concluded that these trapping methods can be made more sensitive by using both a modification to the trap and by enhancement with a carob food lure. Pitfall traps were found to be more effective when a mesh cover and a carob lure were used. Insect probe traps were more effective when the collection tube was coated with fluon to prevent escape of trapped insects. Although there is a long history of using traps to capture stored-product insects (Barak et al. 1990), there is a great need to specifically address behavioral responses of insects to traps in order to optimize their efficiency for certain pest species.

REFERENCES

Barak, A.V. and W. E. Burkholder. 1985. A versatile and effective trap for detecting and monitoring stored-product Coleoptera. Agri. Ecosys. Environ. 12: 207-218.

Barak, A. V., W. E. Burkholder and D. L. Faustini. 1990. Factors effecting the design of traps for stored-products insects. J. Kansas Entol. Soc. 63: 466-485.

Barrer, P.M. 1983. A field demonstration of odor-based, host-food finding behavior in several species of stored grain insects. J. Stored Prod. Res. 19(3): 105-110.

Borden, J. H. 1985. Aggregation pheromone. pp. 257-285. In: Behavior. (G. A. Kerhut, ed.),

Champ, B. R. and C. E. Dyte. 1976. FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Production and Protection Series No.5. Food and Agriculture Org. of the United Nations. Rome. 297 p.

Cogan, P. M., and M. E. Wakefield. 1987. Further development in traps used to detect low level infestation of beetle pests in bulk stored grain. BCPC MONO No: 37 Stored Product Pest Control. 161-167.

Dickens, J. C., E. B. Jang, D. M. Light and A. R. Alford. 1990. Enhancement of insect pheromone responses by green leaf volatiles. Naturwiss. 77: 29-31.

Doyen, J. T. 1985. Reconstitution of the tribes Ulomini and Triboliini for North and Central America (Coleoptera: Tenebrionidae). Proc. Entomol. Soc. Wash. 87: 512-524.

Faustini, D. C., W. E. Burkholder and R. J. Laub. 1981. Sexually dimorphic setiferous sex patch in the male Red flour beetle, *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae): Site of aggregation Pheromone production J. Chem. Ecolo. 7(2):465-479.

Faustini, D. L. and W. E. Burkholder. 1987. Quinone-aggregation pheromone interaction in the red flour beetle. Animal Behavior 35: 601-603.

Freedman, B., K. L. Milkolajczak, C. R. Smith Jr., W. F. Kwolek and W. E. Burkholder. 1982. Olfactory and aggregation responses of *Oryzaephilus surinamensis* (L.) to extracts from oats. J. Stored Prod. Res. 18:75-82.

Howard, R. W. 1987. Chemosystematic studies of the *Triboliini* (Coleoptera: Tenebrionidae): Phylogenic inferences from the defensive chemicals of eight *Tribolium* spp., *Palorus ratzeburgi* (Wissmann), and *Latheticus orzae* Waterhouse. Ann. Entomol. Soc. Am. 80: 398-405.

Javer, A., J. H. Borden, H. D. Pierce and A. M. Pierce. 1989. Evaluation of Phero-mone- baited traps for monitoring of cucujid and tenebrionidae beetles in stored grain. J. Econ. Entomol. 83:268-272.

Levinson, H. Z. and K. Mori. 1983. Chirality determines Pheromone activity for Flour beetles. Naturewissenschafen, 70:190-192.

Lindgren, B. S., J. H. Borden., A. M. Pierce, and H. D. Pierce Jr. 1985. A potential method for simultaneous semiochemical-based monitoring of *Crytolestes ferrugineus* and *Tribolium castaneum* (Coleoptera: Cucujidae and Tenebrionidae) J. Stored Prod. Res. 21(2): 83-87.

Markarian, H., G. J. Florentine and J. J. Pratt Jr. 1978. Quinone production of some species of *Tribolium.* J. Insect Physiol. 14:785-790.

Mikolajczak, K.L., B. W. Zilkowski, C. R. Smith Jr., and W. E. Burkholder. 1984. Volatile food attractants for *Oryzaephilus surinamensis* (L) from oats. J. Chem. Ecol. 10(2):301-309.

Mondal, K. A. M. S. H. 1985. Response of *T. castaneum* larvae to aggregation pheromone and quinones produced by adult conspecifics. Int. Pest Control, 27: 64-66.

Mondal, K. A. M. S. H. and G. R. Port. 1984. Response of *Tribolium castaneum* larvae to synthetic aggregation Pheromone. Entomol. exp. appl. 36:43-46.

Mullen, M. A. 1992. Development of a pheromone trap for monitoring *Tribolium castaneum.* J. Stored Prod. Res. 28:245-249.

Nara, J. M., R. C. Lindsay. and W. E. Burkholder. 1981. Analysis of volatile compounds in wheat germ oil responsible for an aggregation response in *Trogoderma glabrum* larvae. Agri. Food Chem. 29(1):68-72.

Obeng-Ofori, D. 1991. Analysis of orientation behavior of *Tribolium castaneum* and *T. confusum* to synthetic aggregation Pheromone. Entomol. exp. appl. 60: 125-133.

Obeng-Ofori, D., and T. H. Coaker. 1990. *Tribolium* aggregation Pheromone: Monitoring, range of attraction and orientation behavior of *T. castaneum* (Coleoptera: Tenebrionidae). Bull. Entomol. Res. 80: 443-451.

O'Ceallachain, D. P. and M. F. Ryan. 1977. Production and perception of pheromones by the beetle *Tribolium confusum.* J. Insect Physiol. 23:1303-1309.

Pimental, D. 1991. World resources and food losses to pests. pp. 5-11. In: Ecology and management of food-industry pests (J. R. Gorham ed.) Assoc. official chemist.

Rangaswamy, R. J. and V. B. Sasikala. 1990. Structural investigations of the Pheromones of flour beetles, *Tribolium castaneum*:Part I- sex pheromone of female beetles. Indian J. Chem. 447-450.

Raina, A. K., T. G. Kingan and A. K. Mattoo. 1992. Chemical signals from host plant and sexual behavior in a moth. Science. 255: 592-594

Roth, L. M. and T. Eisner. 1962. Chemical defenses of the arthropods. Ann. Rev. Entomol. 7: 107-136.

Sinha, R. N. 1973. Interrelation of physical, chemical and biological variables in the deterioration of stored grain. In grain storage: Part of a system (Eds R. N. Sinha and W. E. Muir), 15-17. Avi Pub. Co Westport.

Sokoloff, A. 1972. The biology of *Tribolium,* vol. 1 Clarendon Press. Oxford.

Suzuki, T. 1980. 4, 8-Dimethyldecanal: The aggregation pheromone of the flour beetles, *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae). Agri. Biol. Chem., 44(10):2519-2520.

Suzuki, T. 1981. A facile synthesis of 4, 8-dimethyldecanal, aggregation pheromone of flour beetles and its analogues. Agric. Biol. Chem. 45:2641-2643.

Suzuki, T., T. Suzuki, H. M. Huynh and T. Muto. 1975. Hydrocarbon repellents isolated from *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae). Agr. Biol. Chem. 39(11):2207-2211.

Suzuki, T. and R. Sugawara. 1979. Isolation of an aggregation pheromone from the flour beetles, *T. confusum* (Coleoptera: Tenebrionidae). Appl. Entomol. Zool., 14:228-230.

Walgenback, C. A., W. E. Burkholder, M. J. Curtis and Z. A. Khan. 1987. Laboratory Trapping studies with *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Econ. Entomol. 80:763-767.

Walter, V. E. 1991. Fumigation in food industry pests. J. R. Gorham (ed.) 441-457. Association of official Analytical Chemists.

Watson, D. L. 1979. An industry viewpoint on professionalism and ethics in entomology. Bull. Entomol. Soc. Am. 25(3): 235-236.

White, N. D. G., and S. R. Loschiavo. 1986. Effect of insect density trap depth and attractants on the capture of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Cryptolestes furrugineum* (Coleoptera: Cucujidae) in stored wheat. J. Econ. Entomol. 79:1111-1117.

Zafar, A., H. Ali and A. Hussain. 1987. Population patterns of Coleoperous pest insects in flour mills of Faisalabad. Pakistan Entomol. 9: 61-63.

CHAPTER II

FACTORS AFFECTING PHEROMONE PRODUCTION BY MALE *TRIBOLIUM CASTANEUM*

INTRODUCTION

The red flour beetle *Tribolium castaneum* is a renowned pest of stored foodstuffs. *Tribolium* has been reported from almost all over the world. Development of alternate strategies to chemical control based on either using fumigants or grain protectants has been a major goal of stored grain scientists for many years (Aliniazee 1970). Monitoring tools for moth pests of stored products have been used for more than two decades, but attempts to use pheromones for coleopterous pests are quite recent. Unfortunately, the use of pheromones and natural enemies for pest monitoring and control are dwarfed by the size of chemical pesticides in commercial agriculture, stored grain, and processed food industries. Beetles' pheromones can be classified in to two groups , female sex pheromone and aggregation pheromone (Burkholder 1982). Female pheromones are produced by short-lived species and attract males only. Aggregation pheromones are produced by both females and males and attract both sexes. Adult male *Tribolium castaneum* secretes an aggregation pheromone, 4,8-dimethyldecanal (Suzuki, 1980) that is attractive to both sexes. The pheromone is produce from the setiferous glands or patches on the ventral side of the femur (Faustini et al., 1981, 1982). Pheromone extracts of prothoracic femora from unmated male flour beetles elicit higher receptor potentials in the antenna of females than in those of males (Levinson and Mori 1983). The objectives of this research project were to further investigate on the biology of pheromone, quantification and factors that effects its production in the red flour beetle.

MATERIALS AND METHODS

Insect cultures:

An established laboratory colony of *Tribolium castaneum* was used in all experiments. Flour beetles were reared on a mixture of whole wheat flour and brewer's yeast (95:5) in a growth chamber maintained at 27± 1° C, 60% relative humidity and 16:8 (light-dark) photo period. Parent beetles were sifted from cultures one week after inoculation. Pupae were sieved out after three weeks and were sexed using the pygidial characters described by (Ho, 1969). Male red flour beetle pupae were maintained separately on flour until used.

Aeration and collection of volatiles:

For all experiments, insects were placed in 7.5 cm X 2.75 cm cylindrical glass aeration chambers (Fig. 2.1.) that were clamped to a stand and oriented vertically. Chambers were composed of a male and female ground glass joint tapered distally at each end to a 1/4" glass tube. Top and bottom openings to the chamber were loosely packed with glass wool to prevent insect escape while allowing air flow. House vacuum was used to draw air through charcoal and Tenax prefilters into the aeration chamber, and volatiles were trapped upwind on a glass column packed with Super-Q, a solid phase adsorbent for volatile organic compounds (Alltech Assoc., Deerfield, IL). The glass column was a Pasteur pipette for which the tip was removed and the cut end flame polished. Glass wool was used to plug the bottom opening of the column and Super-Q was filled to a level of 2 cm (400-450 mg) and held in place

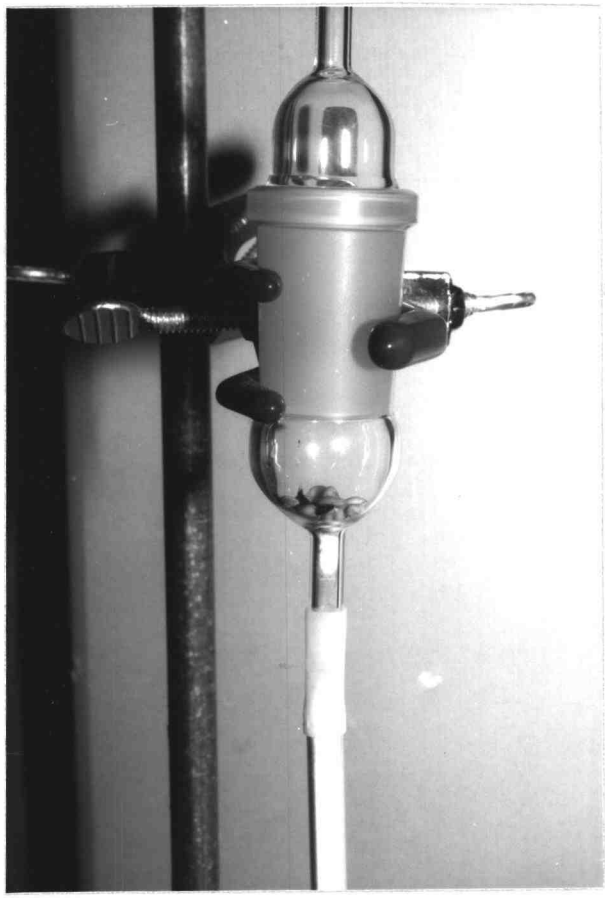


Figure 2.1: The cylindrical glass aeration chamber, 7.5 cm X 2.75 cm, used for collection of volatiles.

with an additional glass wool plug. Air flow rate on each system was maintained at 200 ml/min and monitored with a flow meter located upwind of the Super-Q column. All connections downwind of the Super-Q column were glass-to glass or glass-to-Teflon to avoid contamination and allow for thorough cleaning. Following aeration of insects, the volatiles were extracted from the columns by elution with approximately 700 µl of HPLC-grade hexane and 762 ng of N-dodecane was added immediately as an internal standard. All extracts were held in 1.5 ml glass vials with Teflon-lined septum caps and stored at -20 °C until analyzed. Aeration chambers and associated glassware were washed with acetone, rinsed with distilled water, and baked in an oven at 80° C over night. Following elution of collected volatiles the Super-Q columns were further extracted with methylene chloride followed by pentane, then air-dried before their next use. All aerations were conducted in a room maintained at 27±1° C at 60% RH with a photoperiod of 16:8 (L:D).

Pheromone production by individual beetles

One experiment was conducted to determine the amount of pheromone produced by a single male and to study any quantitative variation in pheromone production over time. Five 2 ± 1 day old adult virgin males were placed individually into glass aeration chambers and each male was provided 0.5 g of cracked wheat as food. Super-Q columns were changed after every two days and continuous aeration was carried out for 30 days (i.e., 15 consecutive two-day collections for each beetle). Analysis of variance (ANOVA) was used to determine if pheromone production

differed over time among the 15 two-day collection periods Two additional experiments were conducted to determine if immature stages of *T. castaneum* were able to produce DMD and at what point pheromone production begins in new adults. In the first experiment ten male pupae were selected randomly from a laboratory colony. The pupae were aerated individually in glass chambers with 0.5 g of cracked wheat and the Super-Q columns were changed daily. Date of adult emergence was noted for each beetle. In the next experiment 11 last instar larvae were randomly selected from a colony and each was set up in individual aeration chambers with 0.5 g of cracked wheat. Each larva was aerated up to the point of pupation, between 30 and 60 hrs. When new pupae were detected a new Super-Q column was applied and an aeration was conducted for 72 hrs, after which a third collection was made up to the point of adult emergence, from 96 to 120 hrs. New adults were aerated for their first 72 hrs of life. Pupae and adults were sexed upon death or at the end of the experiment; larvae that died before pupation could not be sexed.

Effect of food on pheromone production:

Two experiments were conducted to determine if feeding on wheat affects pheromone production and also if exposure to wheat volatiles with no feeding affects pheromone production. A total of ten four-day-old adult males were aerated individually in glass chambers. Five of these males were designated as controls and provided with 0.5 g of wheat for the duration of the study. The remaining five beetles were designated experimental and were subjected to successive time periods with and

without 0.5 g of cracked wheat as food. Super-Q columns were changed after every two days throughout the experiment and the amount of pheromone produced by one beetle was summed for each experimental period. For the first four days of the study, the experimental beetles were provided food, and thus were treated the same as the controls. After these four days of feeding, the wheat kernels were removed from the experimental arenas and the beetles were aerated without food for six days. Food was then reintroduced for two days and again was removed for a final four-day period. Insects in the control group were physically disturbed by shaking the chambers at the time when food was added or removed in the treatment group.

In the food volatiles experiment, eight four-days old adult males were placed individually in aeration chambers without food. A Pasteur pipette, 7.5 cm in length, was filled with 0.5 grams of cracked wheat kernels. Wheat kernels were suspended in the center by glass wool on both ends of pipette. Air was passed through charcoal and Tenax prefilters, through the wheat kernels, and then to the aeration chamber. Sixteen adults of the same age were also aerated individually as controls, eight without any food or food volatiles, designated as negative control, and eight with 0.5 grams of wheat in the arena for feeding, designated as positive control. Beetles were aerated for a total of six days and Super-Q columns were changed at three-day intervals; Pheromone produced by each beetle was summed for the six-day period and differences among treatments were determined with ANOVA.

Effect of mating on pheromone production:

The effect of pairing and mating with a female on pheromone productions was examined by aerating three experimental groups of *T. castaneum*. Four-day-old virgin males were aerated individually, each with 0.5 g food, throughout the course of the study to serve as unmated controls. Two groups of five four-day old males were individually aerated with 0.5 grams of wheat and manipulated with regard to mating status. For one group on day five a virgin female was added to each chamber with a male and maintained as such for the duration of the study. In the other group a virgin female marked on the elytra with white correction fluid was placed with a male for the first four days of the study (day 5 to 8 for beetle age) and then removed. These mated males were then aerated alone for four days, after which a new virgin marked female was added for another four days. This second female was also removed and the male was aerated alone for the last four days. Super-Q columns were changed every two days during the sixteen-day experiment so that there were two collections of volatiles made during each four-day interval. The total amount of pheromone produced in a four-day period was determined and the differences among treatments were analyzed for the four separate experimental periods. The General Linear Models methods of SAS (PROC GLM) was used because of the unbalanced design.

Effect of photoperiod on pheromone production

Pheromone production was measured in three different groups of *T. castaneum* males subjected to different photoperiod during development and aeration. Fifty

mixed sexed beetles from the laboratory colony were liberated in a mixture of whole wheat flour and brewer's yeast (95:5) and were reared in 24 hours continuous light. Another batch of fifty mixed sex beetles was released in wheat flour and brewer's yeast (95:5) and the jar was wrapped completely in aluminum foil for rearing under complete darkness. Adults were removed after one week. From each of these treatmemts, the pupae were sexed and both sexes were kept separate under their respective photoperiod. Pupae were also isolated from the regular laboratory colony and maintained under 16:8 (L:D) photoperiod as a control. Five four-day old adult males from each of the photoperiod treatments were set up individually in glass aeration chambers with 0.5 g of cracked wheat. Chambers with beetles from the all dark treatment were placed in a cardboard box that was made light-tight. Chambers with beetles from the all light treatment were also placed inside a light-tight box, but the box contained a cool incandescent light bulb to provide continuous light. Control beetles were aerated as usual under the 16:8 (L:D) photoperiod of the aeration room. Super-Q columns were changed every two days over the course of the eight-day experiment and the total amount of pheromone produced by each beetle was calculated.

Chemical and analyses

Samples from all experiments were concentrated to 20 µl under a gentle stream of N_2 and were subjected to quantitative analysis by couple gas chromatography-mass spectrometry (GC-MS). Analyses were made using a Schimadzu GC-14A coupled to a

Finigan Model 800 series Ion Trap Detector mass spectrometer. The injector oven was set at 230° C and the heated transfer line to the MS was set at 265° C. The column used was a 30 m x 0.252 mm DB-1 (J & W Scientific), temperature-programmed at 40° C for 30 sec, then 20° C/min to 60° C, held for 1 min, then increased to 280° C at 10°/min, and held at 280° for 20 min. Injection was made with the splitter closed initially, but then opened at 30 sec. Initial studies were conducted with the MS in the full scan mode, recording mass fragments from 35 to 350 amu. An authentic sample of 4(R), 8(R,S)-dimethyldecanal was analyzed for retention time and mass spectrum, which matched the spectrum published by Suzuki (1981). Preliminary studies with volatiles collected from single male *T. castaneum* confirmed the presence of DMD by matching spectrum and retention time with those of the authentic standard. In order to maximize detection ability for DMD in the experiments described above, the MS was operated in the multiple ion detection mode (MID) in which only the characteristic fragment ions m/z=41 and m/z-57 were detected. These ions are common to both the internal standard, dodecane, and DMD. The quantity of DMD in each sample was determined using the Finnigan ITDS software by comparison of the peak area of the internal standard (representing 762 ng in the initial solution) and that of DMD from the MID chromatogram. A calibration curve for these quantifications was developed by analyzing five solutions each containing 762 ng of dodecane and varied known amounts of synthetic DMD.

RESULTS AND DISCUSSION

Pheromone production by one male:

Pheromone was successfully collected and detected from one male in a two-day period for four of the males in this study; a fifth male died early in the experiment. The mean amounts of pheromone produced per day by each of the four males in this study were 605.6 ng, 612.3 ng, 638.3 ng, and 685.3 ng, which indicates little overall variation among males. However, throughout the 30-day aeration of any given male there was great variation, which can be seen in a plot of the mean pheromone production per two-day interval (Fig. 2.2). A fluctuating cycle of two days increase and two days decrease trend of pheromone production was observed over a thirty days period. When the mean amounts produced were compared among two-day intervals, there was no significant difference ($P > 0.05$; ANOVA). The production of DMD remained high up to the last day of the study (1.35 ug). This study is the first to record quantities of DMD produced by individual virgin male *T. castaneum*. The results on longevity of production show that age does not affect pheromone production, at least during the first month of adulthood. This finding of sustained pheromone production over several weeks is congruent with results by Faustini et al. (1981) who found that globules secreted from the setiferous patches on prothoracic femora of male *T. castaneum* over 100 days were attractive to females and presumably contained pheromone.

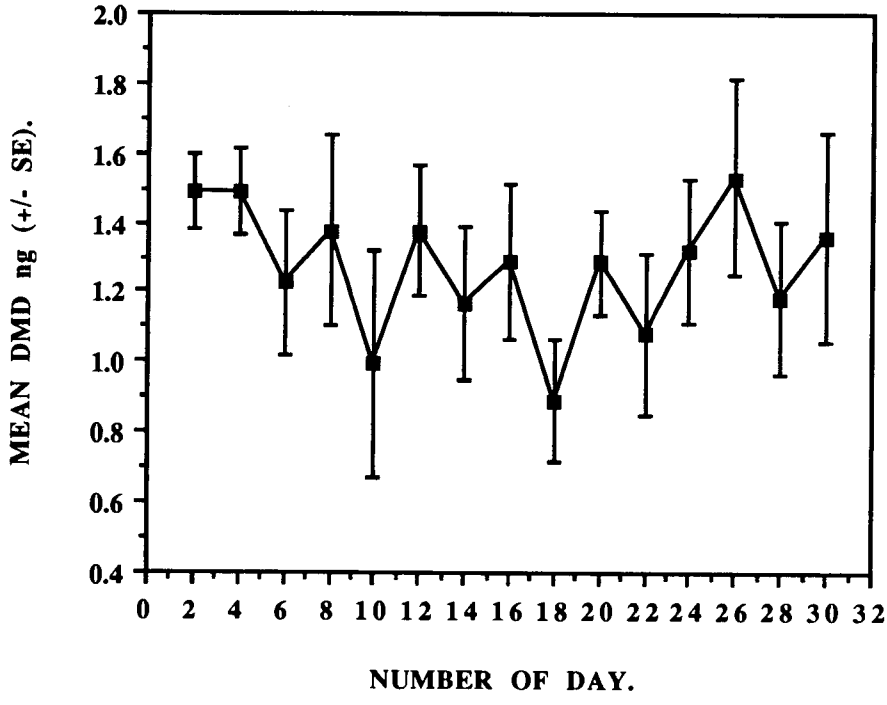


Figure 2.2: Amount of pheromone collected from individual two-days old *T. castaneum*. Graph shows mean DMD (ug) (n=5) and standard error produced per male every 48 hours up to 30 days of age.

Pheromone production by immatures:

The study of pupae was originally designed to observe the time when young beetles initiate pheromone production after eclosion, unfortunately, all beetles died after eclosion to adult. Nevertheless, the results show that male *T. castaneum* produced pheromone during the pupal stage, and that the amounts produced during the early pupal stage (93.4 $\pm$ 24.72 ng/day), five days before adult emergence, were significantly greater than amounts produced one day before emergence (23.80 $\pm$ 8.83 ng/day). The amount further decreased (9.63 $\pm$ 6.27) at the time of adult emergence; in fact no DMD was observed in five individual beetles out of seven newly emerged adults (Fig. 2.3). Sample sizes for each of the days prior to adult emergence varied because of mortality and differential emergence times.

The finding of DMD production in *T. castaneum* pupae was not expected and was very surprising. The identity of DMD in these pupal samples is highly reliable because two independent lines of evidence, GC retention time where a peak was detected, and mass spectra of characteristic ions at that retention time, support the determination. To further confirm the identity, full scan mass spectra were obtained for DMD from several pupal samples. The reduction in DMD production with pupal age may be a true experimental effect of age, or it may be a function of insect stress during the experiment. If age played a factor, then it is intuitive that younger pupae, fresh from the larval stage, have higher energy reserves than older pupae and thus may be able to produce more pheromone. Conversely, pupae are much less hardy than

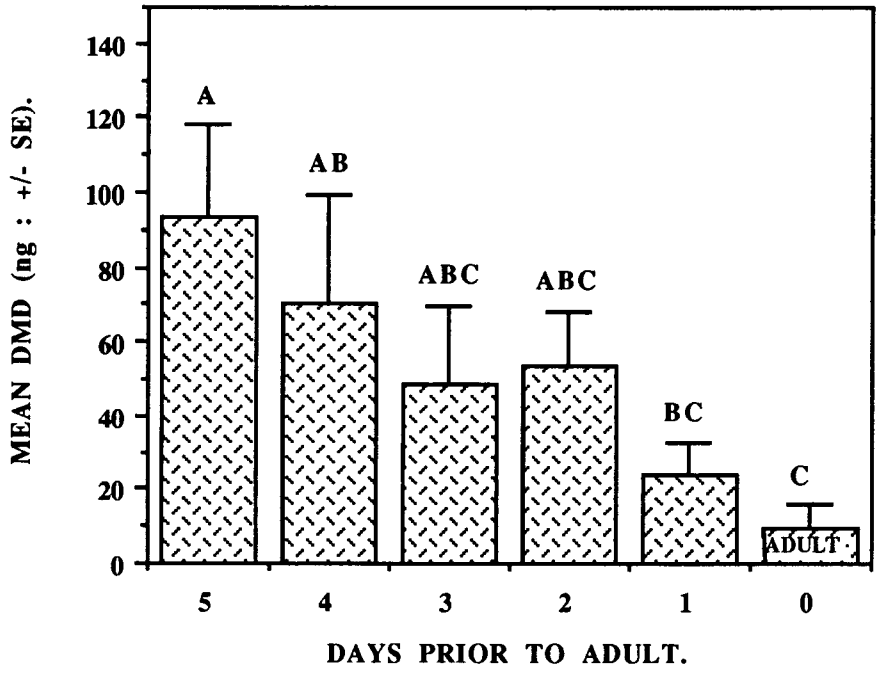


Figure 2.3: Pheromone produced by individual male *T. castaneum* pupae beginning five days prior to adult emergence. Histograms show mean DMD (ng) per day and standard error. Mean amount followed by different letters are significantly different (ANOVA, $P<0.05$, means comparison by Fisher's LSD).

adults, and the handling and aeration of pupae may have unduly stressed them. Unfortunately, mortality of adults precluded determination of the onset of pheromone production in adults. DMD was detected and quantified in individual larvae of *T. castaneum*, and some of these larvae were ultimately determined to be females (Table 2.1). Data are very limited in this experiment due to beetle mortality, but 10 out of 11 larvae were producing detectable levels of DMD, and at least two confirmed females were producing this compound during the larval, pupal, and adult stages. Suzuki (1980) originally identified 4,8-dimethyldecanal from male *T. castaneum* based on the earlier study by Suzuki and Sugawara (1979) who determined there was a pheromone produced by males. Female *T. castaneum* apparently have not been analyzed chemically for the presence of DMD. Suzuki (1985) later reported an attractant from female *T. castaneum*, but provided no proof that females do not produce DMD. Subsequent analyses of more female *T. castaneum* (Chapter II) found no DMD produced by individual adult females older than two days. Therefore, it is likely that females may produce DMD early in their development, but that production is suppressed later in the adult stage. More research is certainly needed in this area. Production of pheromone by larvae and pupae poses a problem in understanding the synthesis and function of the pheromone. Faustini et al. (1981) contend that DMD is secreted by setiferous patches on the prothoracic femora of adult males, although no chemical confirmation of this was provided. It is possible that male pupae have vestiges of such patches, but it is very unlikely that larvae possess homologous structures. DMD is produced at relatively low levels by larvae and pupae (Table 2.1)

Table 2.1:

PHEROMONE PRODUCTION BY INDIVIDL *T. CASTANEUM* LARVAE, PUPAE AND ADULTS.

NO	SEX	LARVAE (Time hrs.)	YOUNG PUAE (72 hrs.)	OLD PUPAE (Time hrs.)	ADULT (72 hrs.)
1	M	00.00 (36)	51.24	14.43 (96)	78.27
2	M	16.36 (36)	63.86	10.09 (96)	811.3
3	M	29.59 (36)	25.51	00.00(96)	32.21
4	M	69.62 (42)	59.30	122.47 (96)	39.19
5	M	148.57 (60)	00.00	106.92 (120)	NA
6	M	16.67 (36)	29.34	100.76 (96)	10.79
7	F	60.33 (66)	43.94	NA	-
8	F	08.60 (60)	34.85	NA	-
9	F	33.98 (60)	46.24	55.77 (96)	13.32
10	F	27.82 (36)	50.67	00.00 (120)	26.26
11	NA	75.40 (60)	NA	-	-

NA= Data "NOT AVAILABLE" due to death of the insect; M = Male; F = Female.

compared to adult males (Fig. 2.3), so this low level may not be biologically relevant with regard to behavioral response of other beetles. However, if male-produced pheromones function primarily to benefit males by attracting females for mating, then early production during the pupal stage may attract females to the vicinity of a male who will eventually be ready for mating.

Effect of food on pheromone production:

The results of the feeding experiment show that there was no significant difference between control and treatment groups when both were provided food, but significant differences occurred when food was removed from the treatment group (Fig. 2.4). Pheromone production drastically decreased to 310.38 $\pm$ 53.67 ng over six days when food was removed, compared to 3123.82 $\pm$ 410.36 for males that had wheat during that period. An increase, equal to control, in DMD production was observed after food was reintroduced to treatment beetles. When wheat was again removed from the treatment chamber, pheromone production was 93.72 $\pm$ 35.91 ng in treatment as compared to 1730.00 $\pm$ 203.97 in control. This reduction was highly significant ($P<0.001$, t-test). Apparently actual contact and feeding on grain is required for production of pheromone. Male *T. castaneum* exposed to volatiles of cracked wheat produced low levels of pheromone that were not different from those produced by beetles with no food, but were significantly lower than amounts produced by beetles actually feeding on cracked wheat (Fig. 2.5). These experiments with food provide

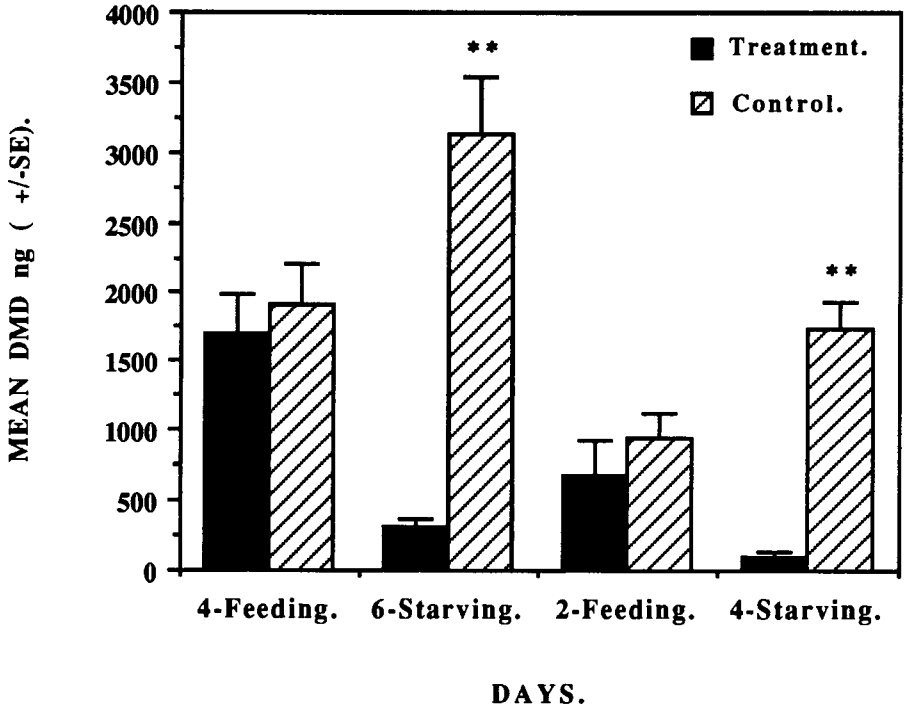


Figure 2.4: Effect of feeding on pheromone production. Histograms show mean DMD (ng) per male and standard error for a given time period and treatment regime. Control is always with food, treatment is either with or without food. Significant difference between treatment and control are indicated as ** ($P<0.01$, t-test).

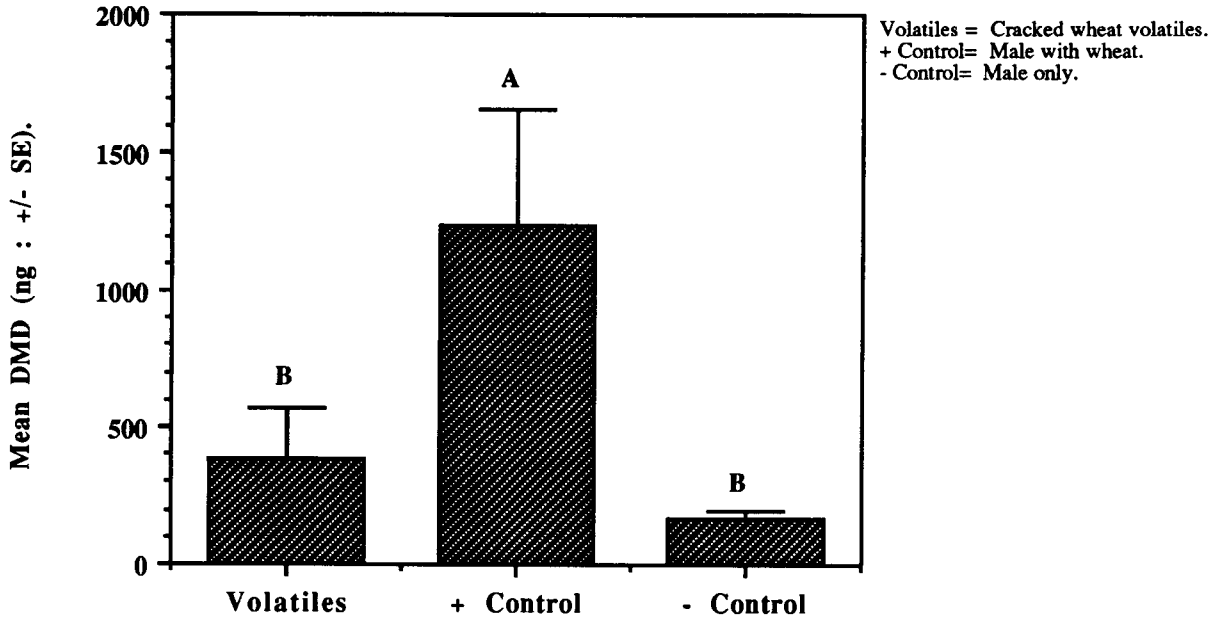


Figure 2.5: Effect of wheat volatiles and feeding on the production of DMD by *T. castaneum*. Histograms show mean DMD (ng) produced per male in six days and standard error (N=8). Mean amount followed by different letters are significantly different (ANOVA, $P<0.05$, means comparison by Fisher's LSD).

strong evidence that the aggregation pheromone production is dependent on feeding. Starvation has a profound effect on the reduction of pheromone release in male red flour beetles, but pheromone production is easily "rescued" by providing food. Feeding has been linked to pheromone production in many other species of beetles, and may be due to one or both of two possibilities (Vanderwel and Oehschlager 1987). Food may provide a direct precursor to the pheromone that is immediately converted upon ingestion or contact. Such is the case in many scolytid beetles that convert terpenes from pine trees into terpene alcohols that serve as pheromones (e.g., Pierce et al. 1987). In some cases with scolytids, simply the exposure to host plant vapors, with no feeding, can induce pheromone production. This is apparently not the case for *T. castaneum* (Fig. 2.5). An alternative is that feeding and gut distention induces a neuro-endocrine reaction that triggers pheromone production as a result of hormones (Renwick and Hughes 1977). The outcome of these experiments supports the idea of sanitation in warehouses. Even if beetles contaminate an area briefly, absence of an easily available food source would prevent attraction of additional beetles. Cleaning of residuals of grains should prevent *Tribolium* from aggregation and consequently reduce the chances of population growth.

Effects of mating on pheromone production:

Mating had no effect on pheromone production in male *T. castaneum* (Table 2.2). Virgin males produced pheromone at the same level as males paired with females for the duration of the study, and unpaired males that had previously mated

Table 2.2:

EFFECT OF MATING ON PHEROMONE PRODUCTION IN *TRIBOLIUM CATANEUM*.

Status	Mean DMD ng (+/- SE) Produced in 4-Days. Age of Beetles (Days).			
	5-8	**9-12**	**13-16**	**17-20**
M only	1659.87 ± 447.47	2137.05 ± 562.82	2407.75 ± 689.62	2632.43 ± 281.00
M & F *	1465.32 ± 506.82	2542.34 ± 426.56	2383.83 ± 345.36	2792.57 ± 183.23
M/M & F **	251.91 ± 78.92	1743.79 ± 481.23	2259.06 ± 428.65	2783.35 ± 64.97
	P > 0.05	**P > 0.05**	**P > 0.05**	**P > 0.05**

M = Male; F = Female.
* Male and female for the entire experiment.
** Male & female from 5-8 days; Male only 9-12 days (female removed); Male and female (new virgin) 13-16; Male only (female removed) 17-20 days.

also produced high levels of pheromone. Sustained pheromone production during and following mating is not typical of insects that have been studied, but may be more prevalent for male-produced pheromones. In moths and fruit flies (Tephritidae) female sex pheromone production decreases after mating, but may resume after a refractory period of no mating (Raina and Menn 1987). Such a refractory period in pheromone production is also true for females of various dermestid beetles (Burkholder and Ma. 1985). Male-produced pheromones generally decline in bark beetles and weevils after mating, but can resume again after subsequent dispersal and location of a new host (Borden 1985).

Effect of photoperiod on pheromone production:

Male *T. castaneum* reared and aerated under 16:8 (L:D) and under total darkness produced statistically similar levels of pheromone, but insects maintained under 24 h light produced nearly four times as much pheromone per day (Fig. 2.6). This result suggests that more light stimulates pheromone release, even when an unnatural photoperiod is applied. Use of 24 h lighting in food storage areas has been suggested for control of stored-product moths because of the disruption this causes to the oviposition cycle (Cox and Bell 1991). Such manipulations of photoperiod would not be advisable for control of *T. castaneum* because the resulting greater production of pheromone may result in high recruitment of new beetles to a site.

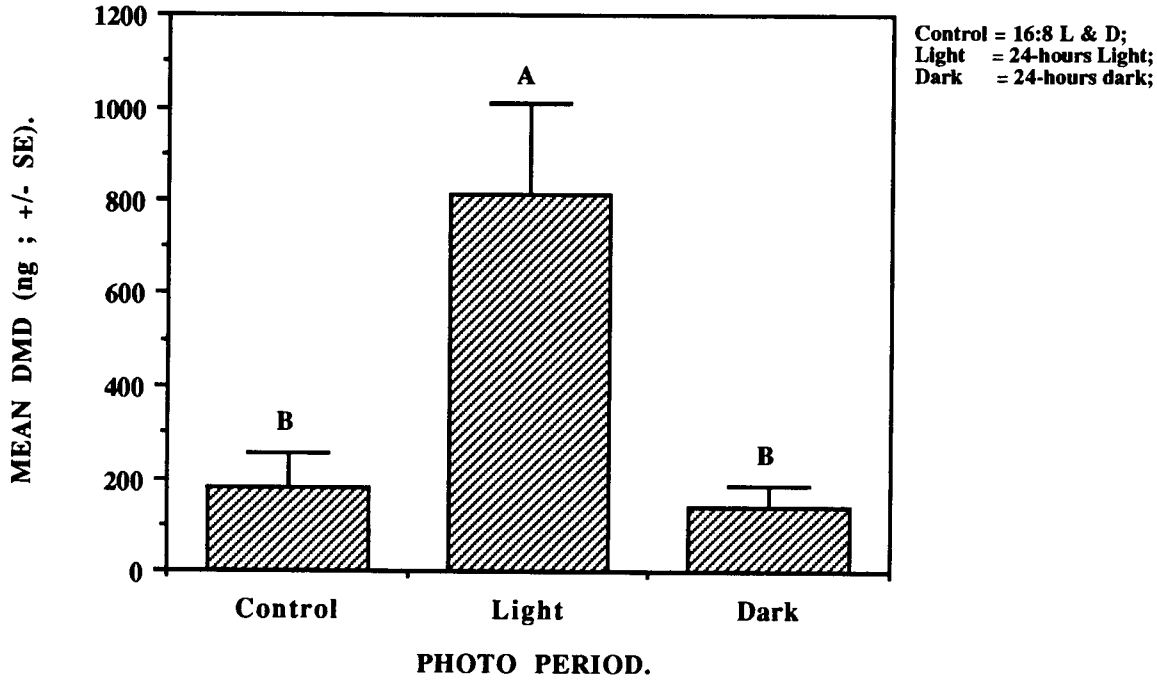


Figure 2.6: Effect of photo period on per day pheromone production in *T. castaneum*. Histograms show mean DMD (ng) per male and standard error for one day. Mean amount followed by different letters are significantly different (ANOVA, $P<0.05$, means comparison by Fisher's LSD).

REFERENCES:

Aliniazee, M. T. 1970. Egg hatch of *Tribolium confusum* and *Tribolium castaneum* (Coleoptera : Tenebrionidae) in different carbon dioxide and nitrogen atmospheres. Ann. Entomol. Soc. Am. 1010-1012.

Borden, J. H. 1985. Aggregation pheromone. pp. 257-285. In: Behavior. (G. A. Kerhut, ed.),

Burkholder, W. E. 1982. Reproductive biology and communication among grain storage and warehouse beetles. J. Ga. Entomol. Soc. 17(2): 1-10.

Burkholder, W. E. and M. Ma. 1985. Pheromone for monitoring and control of stored product insects. Ann. Rev. Entomol. 30: 257-272.

Cox, P. D. and C. H. Bell. 1991. Biology and ecology of moth pests of stored foods. pp 181-193. In: Ecology and management of food-industry pests (J. R. Gorham ed.) Assoc. official chemist.

Faustini, D. L., W. E. Burkholder and R. J. Laub. 1981. Sexually dimorphic setiferous sex patch in the male Red flour beetle, *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae): Site of aggregation Pheromone production J. Chem. Ecol. 7(2):465-479.

Faustini, D. L., J. R. Rowe and W. E. Burkholder. 1982. A male-produced aggregation pheromone in *Tribolium brevicornis* (Le Conte) (Coleoptera: Tenebrionidae) and inter specific response of several *Tribolium* Species. J. Stord prod. Res. 18:153-158.

Ho, F. 1969. Identification of pupae of six species of *Tribolium castaneum* Ann. Entomol. Soc. Am. 62: 1232-1237.

Levinson, H. Z. and K. Mori. 1983. Chirality determines Pheromone activity for Flour beetles. Naturewissenschafen, 70:190-192.

Pierce, H. D., Conn, J. E. Jr., Oehlschlager, A. C. and Borden, J. H. 1987. Monoterpene metabolism in female mountain pine beetles, *Dendroctonus ponderosae* Hopkins, attacking ponderosa pine. J. Chem. Ecol. 13(6): 1455-1480.

Renwick, J. A. A. and P. R. Hughe. 1977. Neural and hormonal control of pheromone biosynthesis in the bark beetle, *Ips paraconfusus.* Physiol. Entomol. 2: 117-123.

Raina, A. K. and J. J. Menn. 1987. Endocrine regulation of pheromone production in Lepidoptera. pp 181-193. In: Pheromone biochemistry. G. D. Prestwich and G. J. Blomquist (eds.). Academic Press.

Suzuki, T. 1980. 4, 8-Dimethyldecanal: The aggregation pheromone of the flour beetles, *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae). Agri. Biol. Chem., 44(10):2519-2520.

Suzuki, T. 1981. A facile synthesis of 4, 8-dimethyldecanal, aggregation pheromone of flour beetles and its analogues. Agric. Biol. Chem. 45:2641-2643.

Suzuki, T. 1985. Presence of another aggregation substance(s) in the frass of the red flour beetles, *Tribolium castaneum* (Coleoptera: Tenebrionidae). Appl. Entomol. Zool. 20(1): 90-91.

Suzuki, T. and R. Sugawara. 1979. Isolation of an aggregation pheromone from the flour beetles, *T. confusum* (Coleoptera: Tenebrionidae). Appl. Entomol. Zool., 14:228-230.

Vanderwel, D. A. and A. C. Oehschlager. 1987. Biosynthesis of pheromones and endocrine regulation of pheromone production in Coleoptera. pp. 175-215. In: Pheromone biochemistry. G. D. Prestwich and G. J. Blomquist (eds.). Academic Press.

CHAPTER III.

EFFECTS OF BEETLE DENSITY ON PRODUCTION OF PHEROMONE AND DEFENSIVE VOLATILES IN *TRIBOLIUM CASTANEUM*

INTRODUCTION

Many arthropods produce and emit defensive chemicals in response to disturbance or attack (Blum 1981). The defensive mechanism of *T. castaneum* is based on defensive compounds released from a reservoir. Quinones and hydrocarbons are secreted by the glands located on the thorax and abdomen (Roth 1943, Markarian et al. 1978). A number of studies have been conducted to identify the defensive compounds in *T. castaneum*. *Tribolium* species produce 2-methyl and 2-ethyl-1,4 benzoquinones (Markarian et. al 1978), 1-pentadecene (von Endt and Wheeler 1971) and various other aromatic compounds and hydrocarbons (Suzuki et. al. 1975, Howard 1987) as defensive compounds.

Faustini and Burkholder (1987) suspected that aggregation pheromone produce by male flour beetle is inhibited by the production of quinone when over crowed, and cause dispersal/repellency to the responding beetles. Quinones inhibit lipid synthesis and induce lipid desaturation (Webb 1966). While preparing to study pheromone production by male *T. castaneum* (Chapter II), a group of males was aerated to search for the pheromone in the volatile effluent. No pheromone was detected, but large numbers of compounds suspected to be defensive quinones and hydrocarbons were found, causing the Super-Q column to appear yellow. Preliminary aerations from females also revealed large amounts of defensive compounds. The present study was designed to study the effects of crowding on production of pheromone and defensive compounds with the following objectives, a) Observe behavioral response of each sex to volatiles produced by different densities, b) Quantify the amount of the pheromone,

4,8-dimethyldecanal, produced by males under different densities, c) Quantify the amount of defensive compounds produced by both sexes under different densities, d) Examine effect of defensive volatiles on pheromone production.

METHODS AND MATERIALS

Response of males and females to volatiles produced by males at various densities:

Adult, four days old, *T. castaneum* males were released in shell vials that had a 7 mm dia. absorbent filter paper disc (Simon and Schuell, Inc., Keene, NH) in the bottom to absorb volatiles (original method of Burkholder and Dicke 1966). The males were released in batches of one, two, five and ten in each vial. The beetles were also provided one cracked wheat kernel and the vials were sealed with cork. Insects were placed in a growth chamber for ten days at 27° C + 1 and 60% R.H. After ten days the filter paper discs were removed from the vials and immediately used as treatment in bioassay.

Bioassay:

These experiments were conducted in two choice pitfall bioassay arena comprised of a glass petri dish (100 x 15 mm) arena (Fig. 3.1). The beetles oriented to one of two holes in the floor of the arena, underneath which were placed treated filter paper or a control (blank) filter paper in 15 x 60 mm petri dish. Pitfall holes were located directly opposite to from each other, 1 cm from the side wall. Once the insects were released, the petri dish cover was placed on the arena. Folded filter paper discs were also placed on the holes of arena, to prevent unintentional slipping of beetles. Bioassay was conducted for 45- minutes in complete darkness at 27° C and 60± 10% R.H . Each experiment was replicated 8 times. A batch of 10 adult males or females was released in the arena under an inverted vial. The beetles were

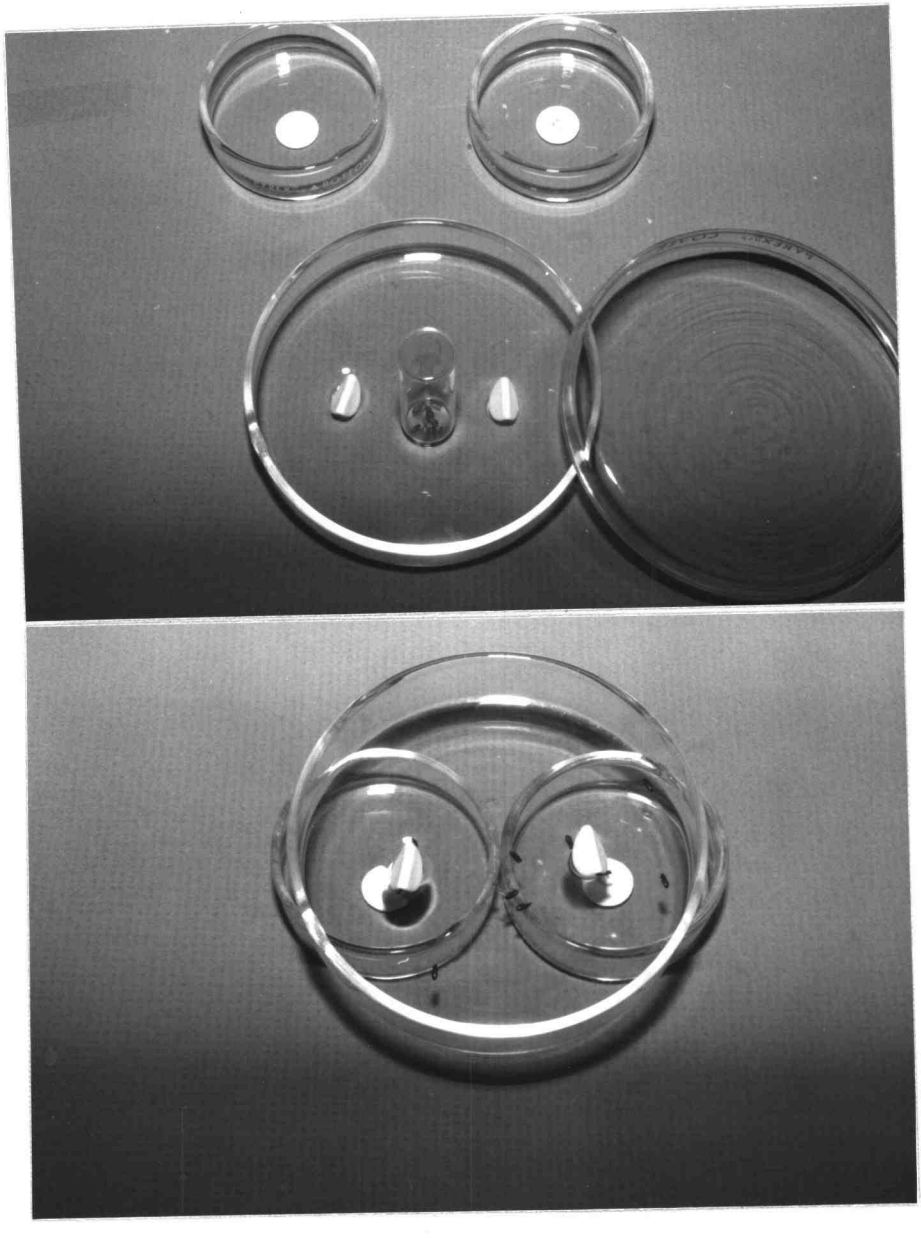


Figure 3.1: Two choice, glass petri dish (100 x 15 mm), pitfall bioassay arena.
Top: Arena prior to beetle release; treatment and control dishes are prepared and test units are being acclimated under a shell vial.
Bottom: Treatment and control collection dishes are in place under pitfall holes and beetles have been released.

confined to the inverted glass vial for 5-7 minutes so they would become calm following handling. Bioassay time began when the glass vial was removed. The number of beetles trapped in treatment and control were counted after 45 min. The percentage responding to the treatment and the control were calculated and compared using the Student's t-test to determine if experimental treatment (volatiles on paper disc) had a significant effect on beetle response. Second, a response index (RI) was calculated to summarize the relative response to treatment and control and to provide a single value that could be used to compare experimental treatments from other two-choice experiments. The response index was calculated as RI = (T - C /Tot) X 100, for which T is the number responding to the treatment, C is the number responding to the control, and Tot is the total number of insects released. Positive RIs indicate attraction to the treatment and negative RIs show repellency; values could theoretically range from -100 for complete repellency to + 100 for complete attraction. RIs were then compared among experiments using ANOVA followed by means comparison using Fisher's protected least significant difference test (PLSD).

Effect of crowding on the production of male and female volatiles:

Four-day-old *T. castaneum* were released at densities of one, five, ten and fifty males or females in glass aeration chambers and volatiles were collected as described in Chapter II. Super-Q columns were changed after every two days, eluted with hexane, and 762 ng of dodecane was added as an internal standard. The samples were analyzed by GC using a Hewlett Packard 5890 instrument equipped with a flame

ionization detector (FID). The column used was a 30 m x 0.252 mm i.d. DB-1 (J and W Scientific, Folsom, CA) with He flow of 1 cm/sec. The injector was set at 255° C, the detector at 280° C, and the column oven programmed at 60° C for 30 sec, then increased to 270° C at 10° C/min, and held at 270° C for 20 min. The injection port was operated in the split mode throughout. FID output went to a Hewlet Packard 3392A integrator recorder and quantities of detected compounds were calculated by comparison of the area under the dodecane peak with areas under sample peaks. Identification of male-produced pheromone and certain known defensive compounds (e.g. Howard 1987) were made by comparison of retention times with authentic standards and by additional analysis with GC/MS (system described in Chapter II). Identities of other compounds were inferred by inspection of mass spectra and estimates of carbon chain length based on retention time.

Effect of volatiles from fifty males on DMD production by one male:

This experiment was designed to determine if the volatiles from beetles at high density have an effect on pheromone production in individual males. Five, 4-6 day-old adult *T. castaneum* males were individually aerated in the glass chambers (as described in Chapter II), but the path of air flow was modified. After passing through charcoal and Tenax pre-filters, the air was drawn through a chamber containing fifty males of the same age with cracked wheat, and then through the chamber containing a single male. Therefore, all the volatiles from fifty males and one male were trapped in a supper Q column attached to vacuum. Five additional males were also aerated

individually as controls, and the air was directly drawn from pre-filters to the chamber. All the column were eluted with hexane (700 ul) and internal standard of dodecane (762 ng) was added. The experiment was conducted for six days. Collected volatiles were analyzed on GC/MS using the quantification method described in Chapter II; amounts of DMD produced by single males in treatment and control were compared using the t-test.

RESULTS AND DISCUSSION

Both male and female *T. castaneum* were significantly attracted to volatiles collected from either one or two males (Fig. 3.2). Females responded significantly higher than males to volatiles from one male (t-test, $P<0.05$), but response to other male densities was similar between the sexes. Conversely, volatiles collected from five males elicited no significant response in either sex, and volatiles collected from 10 males elicited significant repellency in both males and females. These data suggest several explanations for a change in quality or quantity of male volatiles when sampled under different densities. The amount of male-produced pheromone may have decreased under crowded conditions, one or more other compounds acting as repellents were produced under crowded conditions, or such new compounds were produced at higher amounts form a group of males. Defensive compounds, particular quinones and unsaturated hydrocarbons, produced by *Tribolium* species have been implicated as repellents (Suzuki et al. 1975, Mondal 1985). Faustini and Burkholder (1987) showed that quinones would effective dissolve secretions from males presumably containing pheromone, but no behavioral or chemical data were provided to indicate that pheromone integrity had changed. These authors suggested that by dissolution with quinones the pheromone was somehow inactivated. When volatiles were analyzed from male *T. castaneum* at different densities, the pheromone 4,8-dimethyldecanal was detected only in single male aerations and none was found in densities of 5, 10, or 50 beetles (Table 3.1, Fig. 3.3 a and b). Several compounds other than DMD were detected, and the most abundant were identified as 2-methyl-1,4-benzoquinone

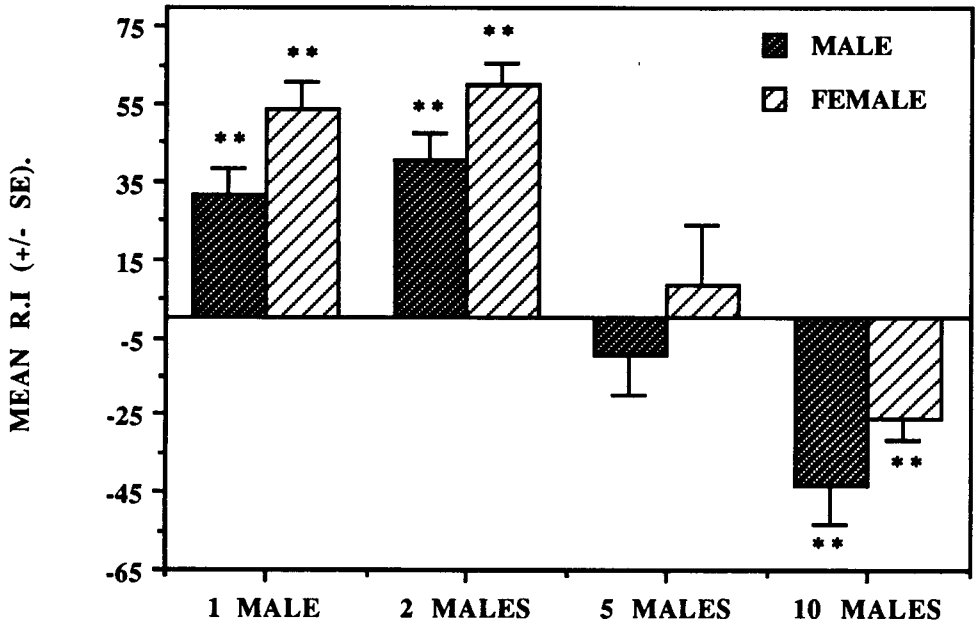


Figure 3.2: Response of males and females in petri dish bioassay to volatiles from males at various densities that was absorbed on filter-paper. Histogram show mean response index (RI, see text) and standard error. Significant difference between treatment and control are indicated as ** ($P<0.01$), ($P<0.05$, ANOVA and LSD).

Table 3.1:

AMOUNT OF QUINONES\PHEROMONE PRODUCED BY ONE MALE IN EIGHT DAYS AT VARIOUS POPULATION DENSITIES.

R.T.	Compd.	Population Densities 1	5	10	50	P-Value
6.52	MQ	499.93 ± 41.82	400.43 ± 91.27	392.69 ± 105.92	327.29 ± 34.26	NS
8.04	EQ	220.54 ± 179.93	500.51 ± 140.24	491.09 ± 151.89	401.22 ± 52.44	NS
11.96	DMD	6036.82 ± 2277.03	ND	ND	ND	-
14.37	Pent.	1678.09 ± 1473.96	2301.0 ± 830.26	1968.54 ± 399.02	1998.03 ± 214.54	NS
15.70	Hexd.	ND	23.96 ± 4.11	11.29 ± 1.61	8.91 ± 1.19	NS
16.69	H.C	29.74 ± 18.85	144.99 ± 66.95	66.22 ± 18.18	78.16 ± 18.18	NS
16.95	H.C	32.11 ± 32.11	48.08 ± 26.89	60.80 ± 13.97	57.18 ± 9.67	NS

NT=Not Detected; NS=Non Significant; R.T=Rention Time; MQ = Methyl-benzoquinone; EQ = Ethyl-benzoquinone; Pent.=Pentadecene;Hexd.=Hexadecene; H.C = Hydrocarbons.

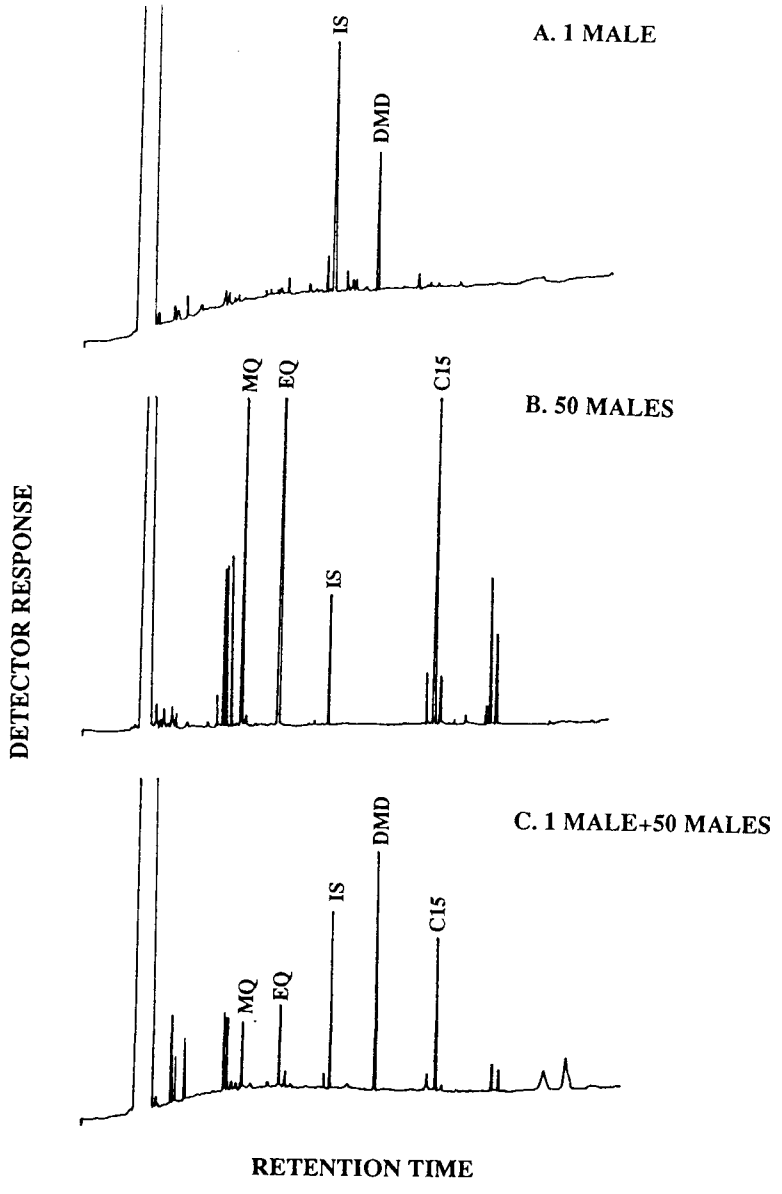


Figure 3.3: Representative chromatograms (GC/FID, see text for conditions) of volatiles from *T. castaneum* males in different density experiments. A: single male, B:A group of 50 males, C: Volatiles from a single male that was exposed to volatiles from 50 males.
Peak designations: MW=2-methyl-1,4-benzoquinone, EQ=2-ethyl-1,4-benzoquinone, IS=internal standard of dodecane, DMD=4,8-dimethyldecanal, C15=1-pentadecene.

(referred to as methyl quinone), 2-ethyl-1,4-benzoquinone (ethyl quinone), and 1-pentadecene. These compounds are well known defensive chemicals in the genus *Tribolium* (Howard 1987), and have been recorded elsewhere as major components of *T. castaneum* volatiles (e.g., Suzuki et al. 1975, Faustini and Burkholder 1987). Quantities were also computed for 1-hexadecene and two unidentified hydrocarbons. When quantities of volatiles were normalized to production per male, and these amounts compared among density treatments, there were no difference in production of defensive compounds among densities (Table 3.1).

Analysis of volatiles from females at different densities detected no DMD at any density, which concurs with all previous research indicating this is a male-produced pheromone. Females produced the same defensive compounds as males, but in several cases males produced more of these than females (Table 3.2). Males produced significantly higher levels of methyl quinone than females at all densities, and more ethyl quinone than females at the 50-beetle density (t-test, $P<0.05$). Levels of pentadecene at all densities and ethyl quinone at densities of 1, 5, and 10 beetles were produced at substantially higher mean values by males over females, but these means had high associated variances and thus were not significantly different.

When volatiles were collected from single males that had been exposed to volatiles of 50 males during aeration, DMD was detected and there was no quantitative difference in this amount compared to that produced by single males that were not exposed to volatiles of others (Fig. 3.4). Inspection of the chromatogram from volatiles collected in

Table 3.2:

AMOUNT OF QUINONES\PHEROMONE PRODUCED BY ONE FEMALE IN EIGHT DAYS AT VARIOUS POPULATION DENSITIES.

R.T.	Compd.	Population Densities 1	5	10	50	P-Value
6.52	MQ	12.80 ± 07.87	43.21 ± 22.42	94.23 ± 35.41	106.19 ± 17.44	**
8.04	EQ	54.75 ± 44.43	75.72 ± 29.74	180.20 ± 77.29	180.47 ± 27.05	NS
11.96	DMD	ND	ND	ND	ND	-
14.37	Pent.	131.34 ± 112.17	472.01 ± 151.57	879.94 ± 348.29	1243.34 ± 280.0	**
15.70	Hexd.	ND	ND	ND	5.81	-
16.69	H.C	ND	17.43 ± 17.34	42.60 ± 32.19	45.47 ± 9.58	NS
16.95	H.C	ND	11.60 ± 03.72	27.57 ± 15.20	31.69 ± 05.18	*

NT=Not Detected; NS=Non Significant; R.T=Rention Time; MQ = Methyl-benzoquinone; EQ = Ethyl-benzoquinone; Pent.=Pentadecene;Hexd.=Hexadecene; H.C = Hydrocarbons.

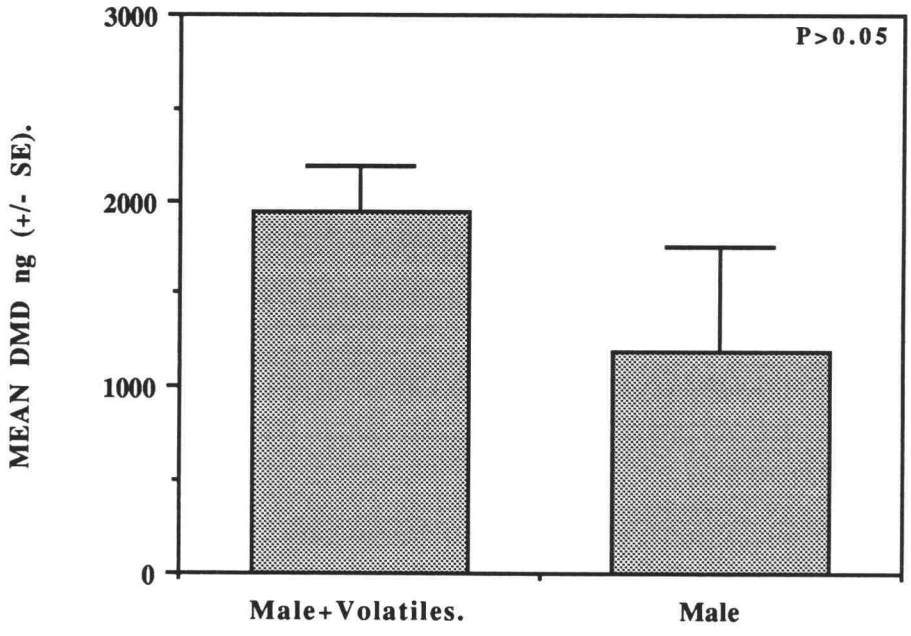


Figure 3.4: Effect of volatiles produced by fifty *T. castaneum* males on DMD production in one beetle. Histogram show mean DMD collected per day and standard error (N=5). (P>0.05, ANOVA and LSD).

this experiment showed that all defensive compounds were being produced, and that the pheromone, presumably contributed by the single beetle, was a prominent peak (Fig. 3.3 c). This experiment clearly demonstrates that the defensive volatiles produced by groups of *T. castaneum* are not responsible for a decrease in pheromone production by male beetles. The hypothesis that quinones disrupt lipid metabolism, and thus pheromone production (von Endt and Wheeler 1971) is not supported. Pheromone produced by a single male may be dissolved by quinones it secretes on its body (e.g. Faustini and Burkholder 1987), but this dissolution should not affect eventual volatilization of the pheromone unless chemical reaction occurs. Rather, a more direct physical phenomenon associated with groups apparently affects pheromone production. Since beetles deprived of eating fail to produce pheromone (Chapter II), it is possible that disturbance caused by groups of beetles would limit feeding by individuals and thus reduce pheromone production. Alternatively, beetles in crowded conditions may perceive the proximity of other males by vision, sound, or physical contact, and cease pheromone production.

The studies above show that production of pheromone by male *T. castaneum* is greatly influenced by crowded conditions, volatiles produced by males under crowed conditions are repellents to beetles, and that males produce more defensive secretions than females. These results conflict somewhat with earlier work aimed at isolating and identifying the aggregation pheromone of *T. castaneum*. By the original work on the isolation and identification of DMD from *T. castaneum*, Suzuki and Sugawara (1979)

aerated 2000 males with 5 g of food in a 500 ml flask and demonstrated attraction of beetles to these volatiles compared to those of females or food only. These same authors claimed that the single pheromone compound (later identified as DMD by Suzuki 1980) was collected in volatiles from 6000 beetles in a 2-l vessel with 30 g of food and purified by column chromatography. Results presented above suggest that it would be very difficult to collect substantial amounts of pheromone from large groups of males, and that volatiles collected from groups would be repellent. However, the present study differs from earlier work by using fewer beetles in much smaller aeration chambers . The actual density of beetles per unit of surface area or volume available may affect the amount of physical contact among beetles, and thus the pheromone production.

REFERENCES

Blum, M. S. 1981. Chemical defences of arthropods. Academic press. New York.

Faustini, D. L. and W. E. Burkholder. 1987. Quinone-aggregation pheromone interaction in the red flour beetle. Animal Behavior 35: 601-603.

Howard, R. W. 1987. Chemosystematic studies of the *Triboliini* (Coleoptera: Tenebrionidae): Phylogenic inferences from the defensive chemicals of eight *Tribolium* spp., *Palorus ratzeburgi* (Wissmann), and *Latheticus orzae* Waterhouse. Ann. Entomolo. Soc. Am. 80: 398-405.

Markarian, H., G. J. Florentine and J. J. Pratt Jr. 1978. Quinone production of some species of *Tribolium.* J. Insect Physiol. 14:785-790.

Mondal, K. A. M. S. H. 1985. Response of *T. castaneum* larvae to aggregation pheromone and quinones produced by adult conspecifics. Int. Pest Control, 27: 64-66.

Roth, L. M. 1943. Studies on the gaseous secretion of *Tribolium castaneum* Duval II. The odoriferous glands of *Tribolium confusum* Ann. Rev. Ent. 7:10-136.

Suzuki, T. 1980. 4, 8-Dimethyldecanal: The aggregation pheromone of the flour beetles, *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae). Agri. Biol. Chem., 44(10):2519-2520.

Suzuki, T., T. Suzuki, H. M. Huynh, and T. Muto. 1975. Hydrocarbon repellents isolated from *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae). Agr. Biol. Chem. 39(11):2207-2211.

von Endt, D. W., and J. W. Wheeler. 1971. 1-pentadecene production in *Tribolium confusum.* Science 172; 60-61.

Webb, J. L. 1966. Enzyme and metabolic inhibitors. Vol. II. New York: Academic Press.

CHAPTER IV

RESPONSES OF *TRIBOLIUM CASTANEUM* TO FOOD AND PHEROMONE

INTRODUCTION

The use of food baits and lures for stored product insects is an old practice (Burkholder 1990). In the last, decade few studies have been conducted to increase trapping strategies to a level where mass trapping could be achieved. Laboratory studies revealed that *Sitophilus oryzae*, an internal feeding pest of stored grain, was attracted to the fresh grain volatiles valeraldehyde, maltol, and vanillin, whereas *Tribolium castaneum,* an external feeding pest of damaged grain, was attracted to rice, soybean, oat, wheat germ and corn oils (Phillips et al. 1993). Similarly, bait bags containing food materials have also been used in Great Britain and other countries (Pinniger et al. 1984). Hodges et al. (1985) used bait bags as an aid to pest management in Indonesian milled rice stores and found that samples represented the same level of infestation that was observed by spear sampling. The disadvantages of the bags are that a rapid insect reproduction may occur within the bags and that can spread if they are lost or unattended. One option to avoid this disadvantage is to use grain extracts or combination of pheromone and food volatiles in traps. An additional problem in using such traps is that once insects are in the grain, it is very difficult to attract them out of the food.

Use of pheromones in combination with food odors holds potential for maximizing trap catch of storage insects. Walgenbach et al (1987) performed some interesting studies to investigate how best to lure *Sitophilus zeamais* out of whole wheat and into a corrugated cardboard trap by using the pheromone 4S, 5R-sitophinone with cracked wheat. In another study, Sinclair and Howitt (1984) reported that in field conditions,

trap catch was significantly higher when traps had both pheromone and grain extract as compared to the traps that had just flour. Phillips et al (1993) showed that an extruded grain-based food product, wheat germ nuts, was attractive to *T. castaneum*. Wheat germ nuts contain soy protein, soy oil, milk protein, wheat germ, and artificial flavors. The objectives of the present study were, a) to investigate behavioral response of *Tribolium castaneum* to odor from wheat and from wheat germ nuts, b) to observe male and female response to food, and c) to determine if food volatiles synergistically enhance response to pheromone.

MATERIALS AND METHODS

Insects:

A laboratory colony of *Tribolium castaneum*, established in 1990 from beetles collected on a farm in Dane County, Wisconsin, was used in all experiments. Beetles were reared on a mixture of whole wheat flour and brewer's yeast (95:5) in a growth chamber maintained at 27± 1° C, 60% relative humidity and 16:8 (light-dark) photo period. Parent beetles were sifted from cultures one week after inoculation, and new adult progeny were removed for bioassay 5-7 days after emergence.

Bioassays:

Two types of bioassays were used in these studies. Experiments with wheat germ nuts employed a two-choice pitfall bioassay (Phillips et al. 1993) in which beetles oriented to one of two holes in the floor of an arena, below which were placed stimulus or control materials in a glass collection dish. Bioassay devices were open steel cans, 25 cm diam. x 20 cm high; 3-cm-diam. pitfall holes were located directly opposite from each other, 4 cm from the side wall (Fig. 4.1) Glass collection dishes were the bottoms of 15 x 60-mm petri dishes. Top edges of petri dishes were coated with Teflon to prevent escape of the trapped beetles. A single layer of wheat grains was placed inside the test arena. The wheat layer provided a substrate for footing and to approximate conditions in stored grain. Wheat kernels were prevented from falling into the pitfall holes by a 12-mesh screen soldered over the holes. The screen allowed easy passage of the insects into the collection dishes.

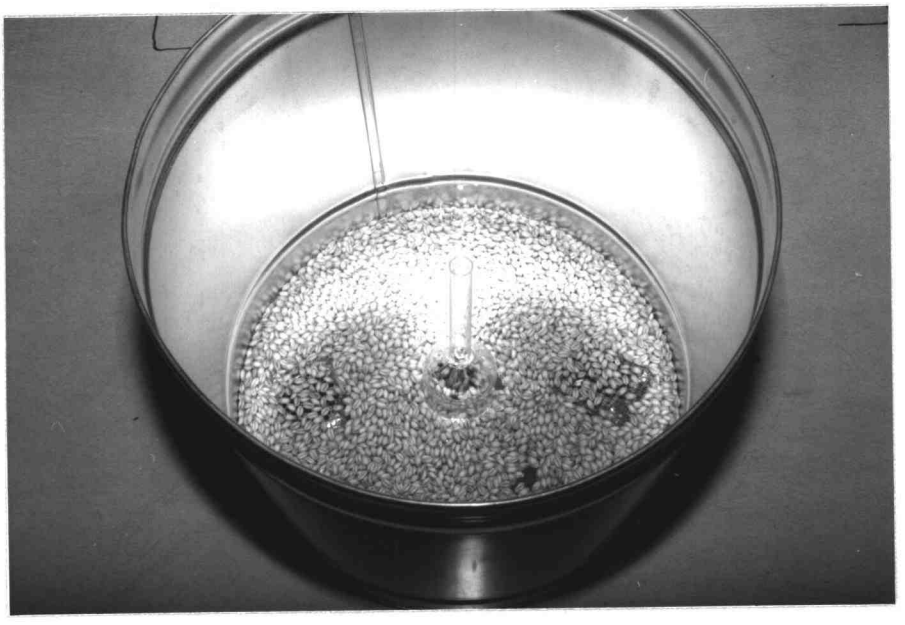


Figure 4.1: Two choice can bioassay arena, 25 cm diam. x 20 cm high x 3 cm diam. Glass funnel harbor test beetles at the central release point; treatment and control dishes are located under screened holes in floor of arena.

Bioassays were conducted for 2 hr in complete darkness at 27° C and 60 ± 10% relative humidity and each experiment was replicated ten times. A batch of twenty adult mixed-sex beetles was released in the arena in each replicate, and the numbers found in the treatment dish, control dish, and remaining in the arena were counted after the experiment. After falling into a glass collection dish, beetles were unable to return to the arena. Before release, the beetles were confined under an inverted glass funnel in the center of the arena for 20 min so they would become calm following handling; the bioassay began when the funnel was removed. Experiments involving wheat kernels utilized another two choice pitfall bioassay, the glass petri dish arena described in detail in Chapter III. Ten adult beetles were released in the glass arena and allowed 45 min to respond.

Studies with Food and Pheromone:

Mixed sex *T. castaneum* were assayed against dosages of 0.5, 1, 2, 2.5, 3, 5 and 7 grams of wheat germ nuts in can arena bioassays. Responses of males and females were separately assessed in can arenas at doses of 0.5, 3 and 5 grams of wheat germ nuts. Response to wheat kernels was studied in petri dish arenas. Berries of soft pastry wheat, 13% moisture, were lightly crushed with a mortar and pestle. Doses of 0.5, 1, 2, and 3 g of wheat were tested against blank controls. Doses of synthetic pheromone 4 (R),8 (R,S)-dimethyldecanal (DMD), were tested for response of *T. castaneum* in both the steel can and petri dish arenas. The synthetic DMD, a gift of Hercon Environmental Co., was determined to be 95+% pure by GC/FID (methods in

Chapter III) and was diluted in hexane at various concentrations before bioassay. Hexane solutions of DMD were applied to a 7 mm dia. absorbent filter paper disc (Simon and Schuell, Inc., Keene, NH) and used similarly in both bioassay systems. Discs loaded with hexane only were used as controls. Dose/response studies of DMD in the can arena assessed doses ranging for 0.001 to 100 μg, and in the petri dish arena the doses ranged from 0.003 to 0.05 μg.

A final series of bioassays investigated the responses of *T. castaneum* to combinations of pheromone with either cracked wheat or wheat germ nuts. Studies with wheat germ nuts utilized the steel can arenas, and studies with wheat utilized the glass dish arenas. Combination experiments used the doses of food and pheromone that elicited response just at or below significant levels when tested separately. Controls in these combination studies were either food only, pheromone only, or a blank dish.

RESULTS AND DISCUSSION

A significant response by mixed sex *T. castaneum* to wheat germ nuts in the can bioassay was observed only at a dose of 3 g of food (Fig. 4.2). Response to controls increased along with response to the wheat germ nuts as dosage increased. High and low doses of wheat germ nuts elicited responses that were not significantly different from those to controls. However, when responses were analyzed separately for males and females, male *T. castaneum* responded positively at higher levels than females when wheat germ nuts were tested at 3 g and 5 g doses (Fig. 4.3). Thus, overall low response by mixed sex beetles may have been due to a combination of a positive significant response by males and lower responses by females. Such differential response between the sexes may reflect a tendency for males to be more sensitive than females in detecting and locating food sources. Since males produce pheromone that attracts females for mating and reproduction, and males only produce pheromone when feeding (Chapter II), then it follows that males might be highly adapted to locating food by responding to odors. Alternatively, a higher response by males in this could simply mean that males are more active than females in the bioassay. Hagstrum and Smittle (1980) reported that male *T. castaneum* had consistently higher tunneling rates than females when studied in flour. In contrast to studies with wheat germ nuts, mixed sex *T. castaneum* gave a clear positive response to cracked wheat grains at 2 and 3 g doses when assayed in the glass petri dish arenas (Fig. 4.4). Although this result is not directly comparable to that with wheat germ nuts because different bioassay designs were used, there was a distinct separation in response of beetles to

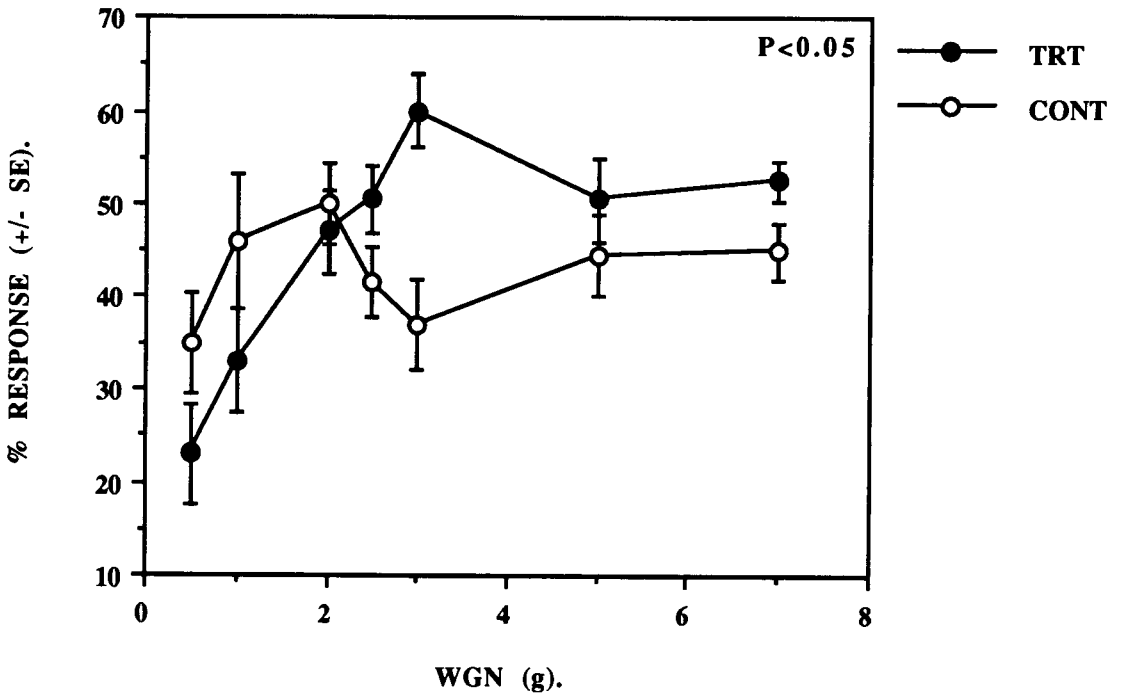


Figure 4.2: Response of *T. castaneum* to various dosages of WGN in steel can arena. Graph shows mean response to treatment and control and standard errors. Significant difference between treatment and control are indicated as ** (P<0.01, t-test).

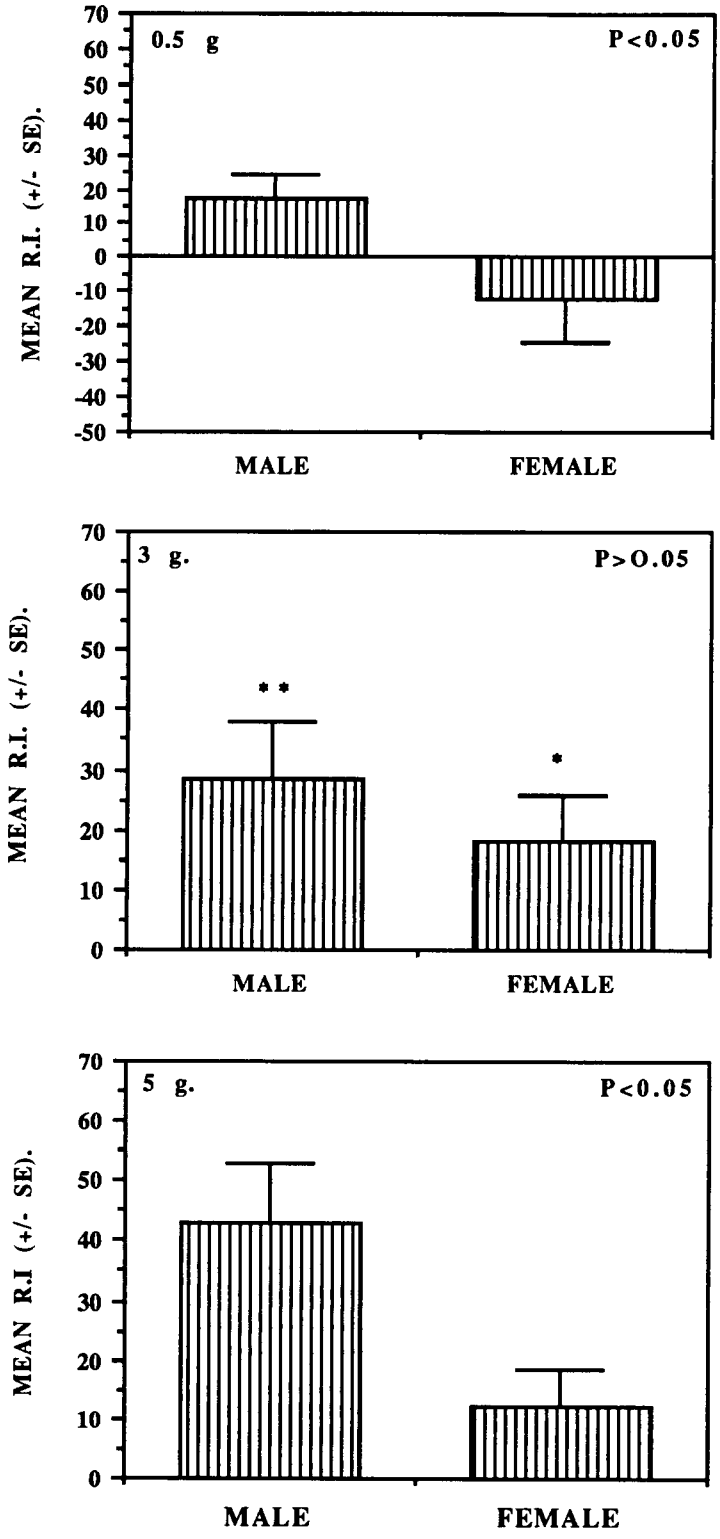


Figure 4.3: Response of male and female *T. castaneum* to various dosages of WGN in steel can arena. Histograms show mean R.I. to and standard errors. RIs were compared between males and females using t-test. Significant difference between treatment and control within a sex are indicated as ** ($P<0.01$) and * ($P<0.05$).

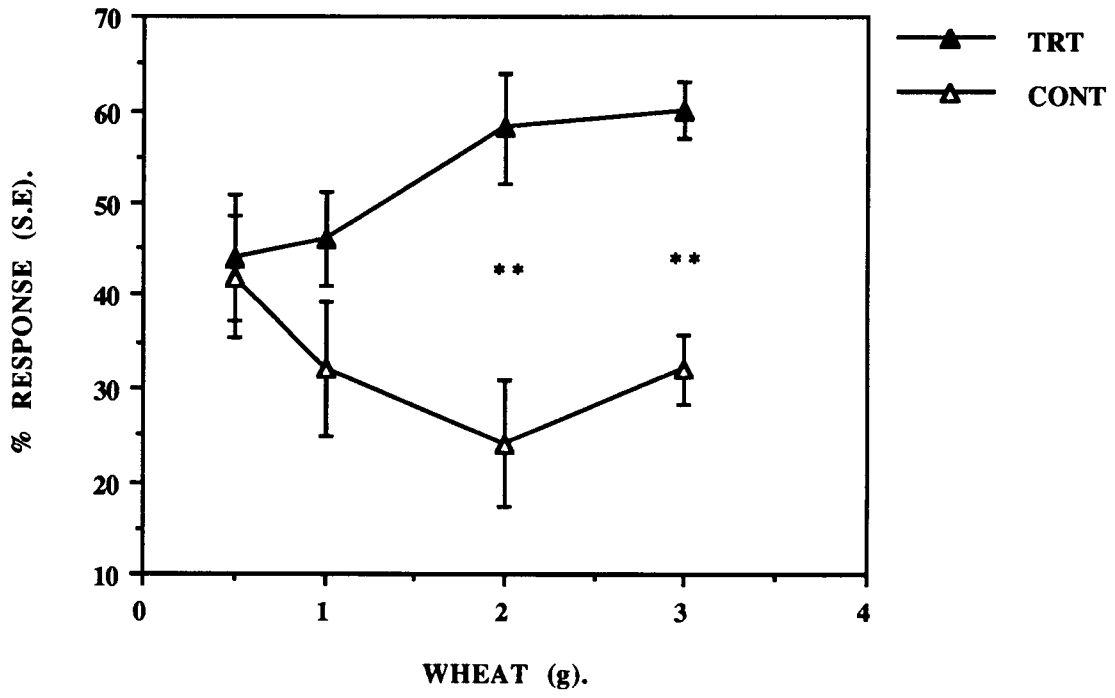


Figure 4.4: Response of *T. castaneum* to various dosages of wheat in petri dish arena. Graph show mean response to treatment and control and standard errors. Significant difference between treatment and control are indicated as ** ($P<0.01$, t-test).

wheat compared to a blank control. The highest response of *T. castaneum* to synthetic DMD in can arenas was elicited by 0.1 μg on filter paper (Fig. 4.5). Response to the lowest dose tested, 0.001 μg DMD, was not significantly different from response to the control. Significant attraction was recorded at 0.01 μg, 0.1μg. 1.0 μg, and 10.0 μg of DMD. However, a significant reduction in response occurred at the highest doses of 10.0 and 100.0 μg DMD. Thus, *T. castaneum* responds to a source of pheromone when released at some optimum level, but levels above this optimum result in decrease of response. Significant repellency was not found with the doses tested, but response was lowered to the point at which there was no significant difference in response to 100.0 μg and an untreated control. High levels of male-produced pheromones are known to elicit reduced behavioral response in other species of beetles (Borden 1985). The high pheromone level presumably signals a crowded resource, thus causing newly arriving beetles to avoid the source of pheromone. Only low doses of DMD were tested in the petri dish arena, and all were significantly attractive compared to the blank control (Fig. 4.6). These data indicate the high sensitivity of the petri dish assay for examining orientation of *T. castaneum* to pheromone. DMD at 0.003 μg was significantly attractive in petri dish arenas, but a similar dose of 0.001 μg was not attractive in the steel can arenas. Combinations of DMD and wheat germ nuts were consistently more attractive to *T. castaneum* than any of the controls tested in two-choice can arenas (Table 4.1). Of particular interest is the finding that a combination of DMD with wheat germ nuts was more attractive than DMD alone (Expts. 2 and 4, Table 4. 1), indicating that the presence of food significantly enhances the activity of

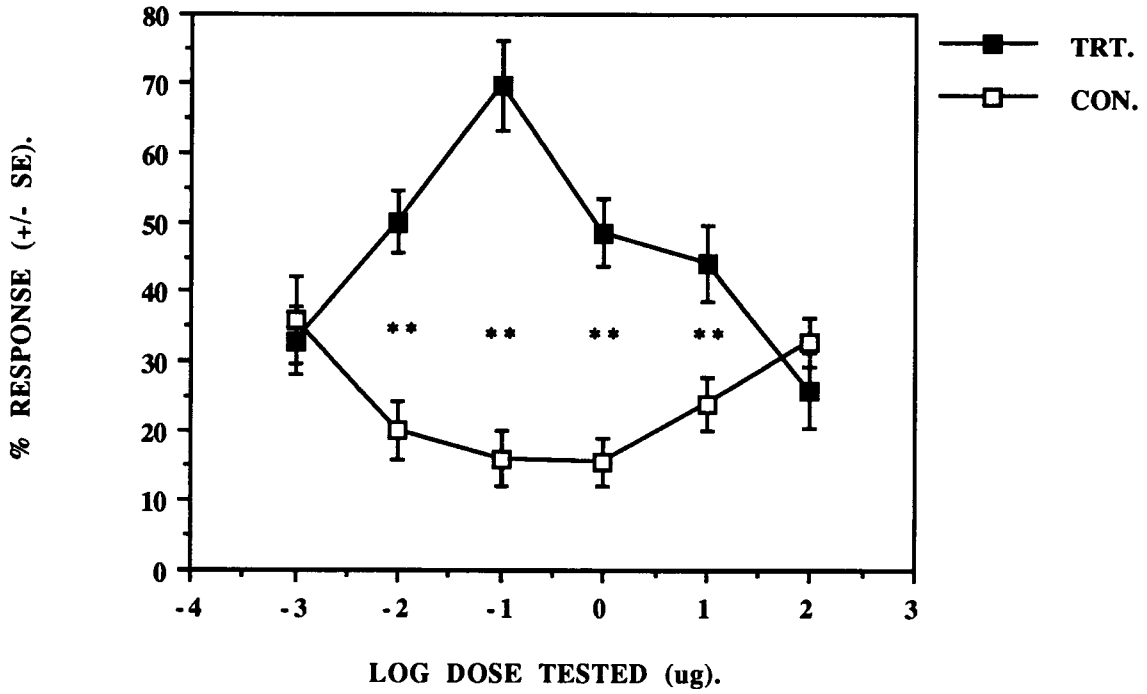


Figure 4.5: Response of *T. castaneum* to various dosages of DMD in steel can bioassay arena.. Graph shows mean response to treatment and control and standard errors. Significant difference between treatment and control are indicated as ** ($P<0.01$, t-test).

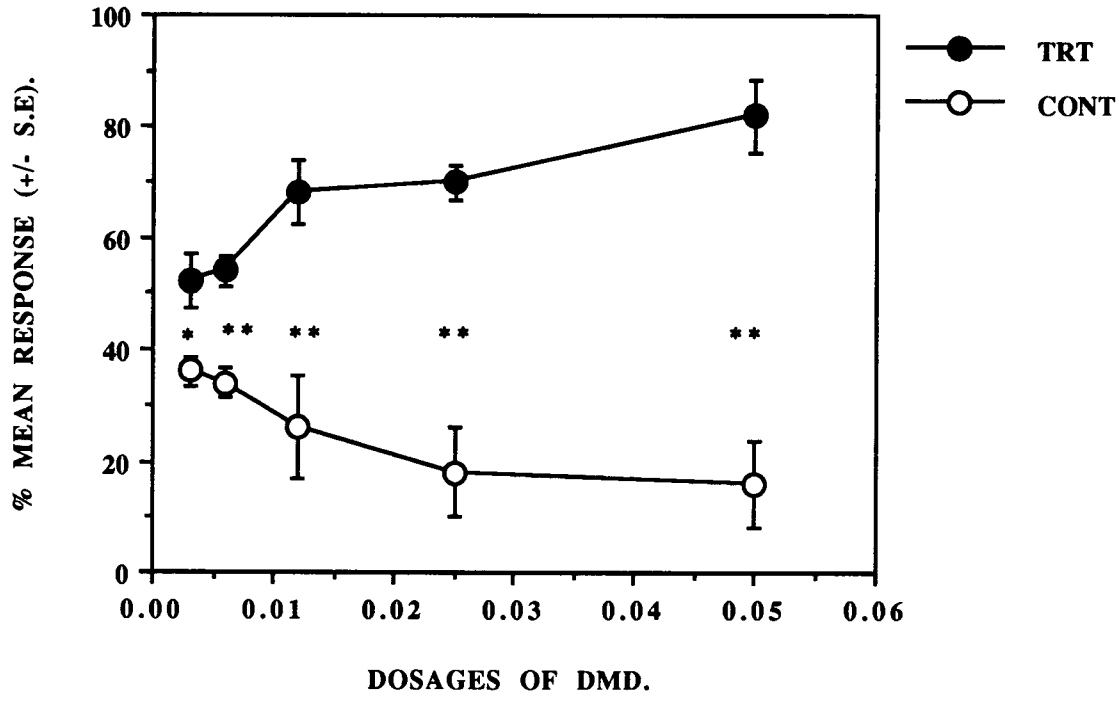


Figure 4.6: Response of *T. castaneum* to various dosages of DMD in petri dish bioassay arena.. Graph show mean response to treatment and control and standard errors. Significant difference between treatment and control are indicated as ** ($P<0.01$, t-test) and * ($P<0.05$, t-test).

Table 4.1:

RESPONSE OF *TRIBOLIUM CASTANEUM* TO VARIOUS COMBINATIONS OF PHEROMONE AND WHEAT GERM NUTS IN STEEL CAN ARENA.

Exp. #	Treatment vs Control	% Response	SE	P-value
1	0.1 ug DMD + 2.5 g WGN	70.00	2.98	**
	vs 2.5 g WGN.	28.00	3.26	
2	0.1 ug DMD + 2.5 g WGN	59.00	2.86	**
	vs 0.1 ug DMD.	37.00	3.34	
3	0.5 ug DMD + 3g WGN	71.00	4.39	**
	vs BLANK.	28.00	4.35	
4	0.5 ug DMD + 3g WGN	57.78	2.51	**
	vs 0.5 ug DMD	41.11	2.6	
5	0.5 ug DMD + 3g WGN	72.00	3.00	**
	vs 3g WGN.	26.50	2.98	
6	0.1 ug DMD + 0.5 g WGN	72.22	4.72	**
	vs BLANK	10.00	2.50	

** $P < 0.05$, student's t-test;
WGN = Wheat germ nuts;
DMD = 4,8-dimethyldecanal.

pheromone. This same phenomenon was observed in at least one study with combinations of wheat and DMD in glass petri dish assays (Table 4.2). When 0.003 μg of DMD combined with 0.5 g wheat was tested against 0.003 μg of DMD only, the combination was significantly preferred (Expt. 6, Table 4. 2), however when 0.006 μg of DMD was used in a combination study (Expt. 3) there was no significant response over DMD only. Combinations of pheromone plus food elicited significant attraction by beetles when tested against blank controls or food only in both series of studies, but this would be expected since the presence of pheromone elicits such a strong response.

Studies with combinations of food and pheromone indicate that response can be increased when using the combination, but this does not demonstrate a synergistic effect. Synergism of multiple semiochemicals is demonstrated when the response to a combination is greater than the sum of the responses to the individual components (Borden 1985). Synergism of insect pheromones is typified in many cases in which one or more separate pheromone are inactive singly, regardless of dose, but that when combined a full behavioral response occurs. Similarly, synergism between host plant (i.e., food) volatiles and insect-produced pheromones has been shown with several species (Silverstein 1970). Research here on *T. castaneum* shows that pheromone alone is behaviorally active and food odor alone is also active. Combination of both pheromone and food odors elicited a significant response over pheromone only in some studies, but these some studies, but these responses were additive at best.

Table 4.2:

RESPONSE OF *TRIBOLIUM CASTANEUM* TO VARIOUS COMBINATIONS OF PHEROMONE AND WHEAT IN GLASS PETRI DISH ARENA.

Exp. #	Treatment vs Control	% Response	SE	P-value
1	0.006 ug DMD + 0.5g Wheat	64.00	8.71	**
	vs BLANK.	28.00	5.83	
2	0.006 ug DMD + 0.5g Wheat	66.00	9.79	**
	vs 0.5 g Wheat.	30.00	8.36	
3	0.006 ug DMD + 0.5 g Wheat	54.00	10.19	NS
	vs 0.006 ug DMD.	42.00	11.99	
4	0.003 ug DMD + 0.5 g Wheat	68.00	5.83	**
	vs BLANK.	20.00	4.47	
5	0.003 ug DMD + 0.5 g Wheat	64.00	3.39	**
	vs 0.5 g Wheat.	25.00	2.69	
6	0.003 ug DMD + 0.5 g Wheat	56.00	2.21	**
	vs 0.003 ug DMD.	39.00	1.79	

** $P < 0.05$; NS = $P > 0.05$, student's t-test.

DMD = 4,8-dimethyldecanal.

Nevertheless, food is very important in the pheromone biology of *T. castaneum*, as feeding is necessary for pheromone production. In the natural context, therefore, pheromone is produced only in the presence of food odors, and responding beetles perceive both.

REFERENCES

Borden, J. H. 1985. Aggregation pheromone. pp. 257-285. In: Behavior. (G. A. Kerhut, ed.),

Burkholder, W. E. 1990. Practical use of Pheromones and other attractants for stored product insects. pp 497-516. In: Behavior-modifying chemicals for insect management :Application of Pheromones and other Attractants .(R. L. Ridgway,R.M. Silverstein and M. N. Inscoe, Eds.) Marcel Dekker, Inc. N.Y.

Hagstrum, D. W., and B. J. Smittle. 1980. Age-and sex-specific tunneling rates of adult *Tribolium castaneum.* Ann. Entomol. Soc. Am. 73: 11-13.

Hodges, R. J., H. Halid, D. P. Rees, J. Meik and J. Sarjono. 1985. Insect traps tested as aid to pest management in milled rice stores. J. Stored Prod. Res. 21: 215-229.

Phillips, T. W., X. -L. Jiang, W. E. Burkholder, J. K. Phillips and H. Q. Tran. 1993. Behavioral responses to food volatiles by two species of stored-product Coleoptera, *Sitophilus oryzae (*Curculionidae) and *Tribolium castaneum* (Tenebrionidae). J. Chem. Ecol. 19: 723-734.

Pimental, D. 1991. World resources and food losses to pests. pp. 5-11. In: Ecology and management of food-industry pests (J. R. Gorham ed.) Assoc. official chemist.

Silverstein, R. M. 1977. Complexity, diversity, and specificity of behavior-modifying chemicals: Examples mainly from Coleoptera and Hymenoptera. pp 231-251. In: Chemical control of insect behavior, theory and application. (Shorey, H. H. and J. J. McKelvey, Jr. eds.) John Wiley & Sons.

Sinclair, -E. R. and C. Howitt. 1984. Tests of pheromones for monitoring infestation of stored products insects. Proced. 4th Australian Applied Entomol. Res. Adelaide. 24-28 September.

Walgenback, C. A., W. E. Burkholder, M. J. Curtis and Z. A. Khan. 1987. Laboratory Trapping studies with *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Econ. Entomol. 80:763-767.

CHAPTER V:

EFFECTS OF DIFFERENT CONTROLLED RELEASE PHEROMONE DISPENSERS AND TRAPS ON RESPONSES OF *TRIBOLIUM CASTANEUM*

INTRODUCTION

Pheromones are effective monitoring tools for a number of stored product pests (Burkholder 1984). For monitoring and detection, synthetic pheromone must be formulated into a controlled release device and used in a trap that is effective, durable, and serviceable. Optimum designs for both the trap and the controlled release device are primary objectives for pest management (Barak et al. 1990). Several designs for controlled release pheromone dispensers have been utilized for different insect species, each one giving characteristic release for specific pheromones (McDonough 1991). Traps of various designs have been developed for stored-product insects, but their efficacy for specific insect species has not been fully investigated.

Despite the early identification of dimethyldecanal as the aggregation pheromone for *Tribolium castaneum* (Suzuki 1980), limited work has been reported on use of the pheromone in trapping systems. Barak and Burkholder (1984) described a trap made of corrugated cardboard with an oil-filled pitfall cup that was effective for several beetle species. They showed that *T. castaneum* was caught in this trap, and eventually this design was patented and commercialized (Barak and Burkholder 1984). Controlled release pheromone lures provided with the cardboard traps are simply rubber septa impregnated with synthetic pheromone. Recently, Mullen (1992) reported a new trap design for *T. castaneum* and compared it with the cardboard trap and two other designs. The objectives of this study were, a) to evaluate the activity and longevity of various controlled release devices for dimethyldecanal that employed three different release designs, and b) to test the effectiveness of several trap designs.

METHODS AND MATERIALS

Insect:

A laboratory colony of *Tribolium castaneum*, established in 1990 from beetles collected on a farm in Dane County, Wisconsin, was used in all experiments. Beetles were reared on a mixture of whole wheat flour and brewer's yeast (95:5) in a growth chamber maintained at 27± 1° C, 60% relative humidity and 16:8 (light-dark) photo period. Parent beetles were sifted from cultures one week after inoculation, and new adult progeny were removed for bioassay 5-7 days after emergence.

Lure Formulations and Bioassay:

Commercially produced lures described below were all formulated with the 4(R), 8(R,S) isomeric blend of DMD at >90% purity. Three general lure designs (Fig. 5.1) studied differed in their formulation and mechanism of release. These designs will be referred to as "septum", "membrane", and "laminate" lures throughout this chapter. Septum lures were provided by Trécé Inc., Salinas, CA, and were composed simply of a red rubber septum (sleeve stopper type), 9.1 x 18.8 mm, onto which a solution of DMD had been applied (loading rate not provided). The pheromone is thus soaked into the rubber and is released slowly over time as a function of the rubber matrix. Membrane lures were from Consep Membranes Inc., Bend, OR, and contained DMD in a reservoir between an impermeable backing material and a plastic membrane through which the pheromone evaporated slowly as a function of membrane characteristics. Membrane lures were flattened rectangular devices that had a circular

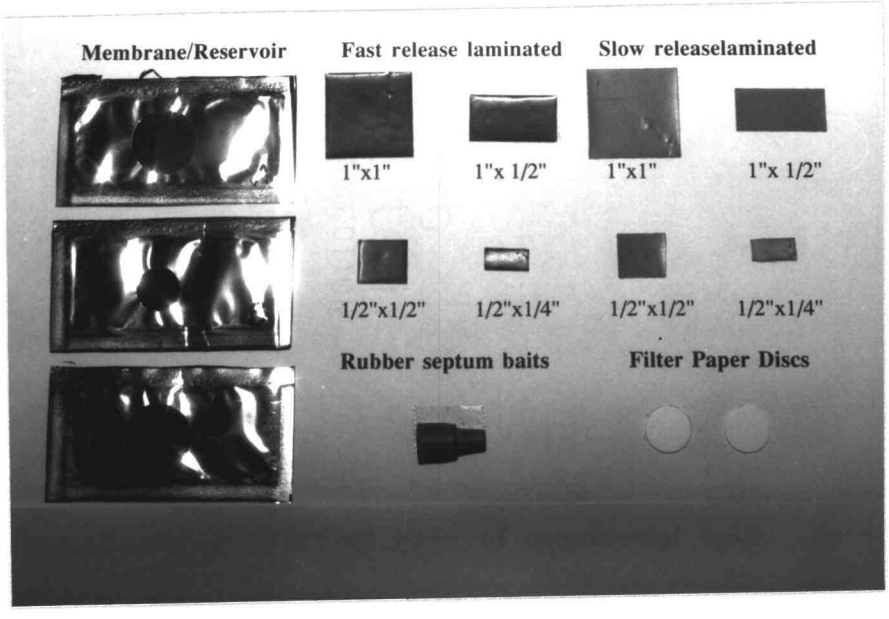


Figure 5.1: Three different slow release pheromone devices, Membrane (A, B, C), Laminated (Various sizes of Slow and Fast) and Rubber Septum, tested in pitfall bioassay; see text for detail.

releasing surface. Three different membrane designs, all loaded with 8.0 mg of DMD, were tested: membrane A had a 0.75 in. dia. circular release area, membrane B had a 0.5 in dia. release area and had an unspecified stabilizer added to the pheromone, and membrane C had a 0.75 in. release area also with a stabilizer added. Laminate lures, provided by Hercon Environmental Co., Emigsburg, PA, were a "sandwich" design in which 1.0 mg of DMD was formulated into a PVC reservoir and placed between an impermeable Mylar bottom and a permeable PVC top piece. A pressure sensitive adhesive was applied to the Mylar backing to aid in application to trap surfaces. The three layers were laminated together under heat and pressure according to the manufacturers specifications. Pheromone release from the laminate lure is primarily through the top permeable layer, and its rate is a function of film thickness, film composition, and total lure dimension (i.e. surface area of release surface). Two different types of laminate lures were tested, each used in four different sizes (sizes were specified in English units by the manufacturer). "Slow" laminate had the permeable upper layer composed of 8.0 mil polymeric PVC, and "fast" laminate had an upper layer of 2-mil regular PVC. Polymeric PVC is stiffer and is less permeable than regular PVC (personal communication, Priscilla McLean, Hercon Environmental). The four sizes of laminate lures tested were 1" X 1", 1" X 1/2", 1/2" X 1/2" and 1/2" X 1/4". Replicate samples of the 12 lure designs described above were subjected to bioassay for activity at different ages (one to two-week intervals) following their removal from the packages. Maximum ages of lures tested were six weeks for septum lures, 12 weeks for membrane lures, and 14 weeks for laminate

lures. Individually identified lures were aged in a fume hood at room temperature between bioassays. A two-choice pitfall bioassay with a steel can arena, described in detail in Chapter IV, was use to evaluate all lures in this study. For all bioassays the test lure was placed in the treatment dish and the control dish was left empty. Twenty adult *T. castaneum* were released in each can arena and given two hours to respond; ten replicates of each lure and age class were deployed.

Quantification of DMD Released from Laminate Lures:

Two slow release laminate lure designs, 1/2" X 1/4" and 1" X 1/2", were studied using volatiles collection and GC analysis to determine their release of pheromone over time. Aeration chambers, collection procedures, and quantitative GC analyses were the same as those reported for live beetles in Chapters Two and Three. Five lures of each size were aerated for one hour immediately after removal from their packages. After Super-Q aeration the lures were aged in a fume hood. The 1/2" X 1/4" lures were analyzed again after one and three weeks, and the 1" X 1/2" lures were analyzed after three weeks.

Trap Designs:

Five different trap designs were obtained from commercial suppliers, and two modifications of one of the designs was made, yielding the following seven designs. 1. Storgard traps (Fig. 5.2), from Trécé, Inc. (Salinas, CA), were made of corrugated fiber board and are 9 cm on a side when folded. Three out of four folds are punched

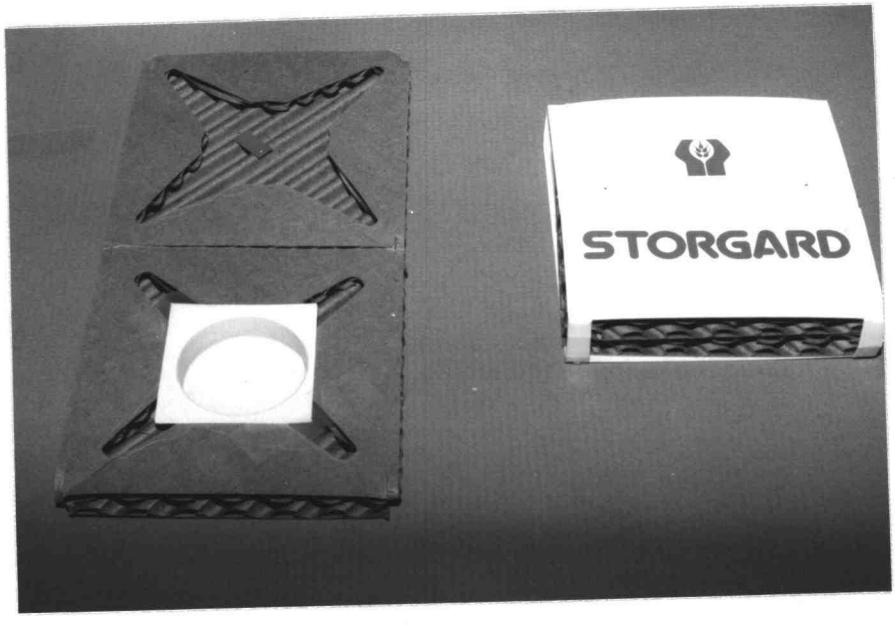


Figure: 5.2: The "Storgard" trap (Tre'ce', Inc. Salinas, CA) of corrugated fiber board, 9 x 9 1 cm, corrugation orientation diagonal.

out to fit in a small cup. Corrugations are oriented diagonally across each section and most flutes lead to the cup that contains oil (Barak and Burkholder 1984). 2. The fundamental Storgard design was modified by using a bigger cup of 4 cm square and 1 cm high that consequently reduced the overall surface area of cardboard (Fig. 5.3). Additionally, the orientation of the corrugations was changed from diagonal to perpendicular in relation to the trap sides, and the direction of corrugations was alternated for adjacent layers. 3. A second modification of the above traps, a circular cardboard trap, was constructed that consisted of four circular, 4.5 cm diam., plates of corrugated paper. All four plates had 4 cm square punchouts. The two bottom circular plates fit the 4 cm square x 1 cm high cup and were mounted on a circular cardboard of the same size. The other two were used as a cover and punchouts openings led into the cup. The two top and two bottom corrugated plates were glued together to make two main top and bottom pieces (Fig. 5.4). The circular corrugated plates were placed on each other so that direction of the corrugations was alternated. 4. The Fuji Trap (Japan Tobacco Co.) was a rectangular ramp-and-pitfall design, approximately 4 x 10 x 1 cm, constructed primarily from hard paper. Responding insects enter ramps on each end of the trap, climb the ramp to the edge of a 4 x 4 x 1 plastic cup. The opening for the cup is covered with cloth netting, onto which beetles crawl, but from which they eventually lose footing and fall onto an oil-soaked pad. The whole trap is covered with a plastic rectangular sleeve that provides support to hang a lure (Fig. 5.5). 5. The Trécé "Flit-trak" (pronounced "flight-track") trap was also a ramp-and-pitfall design and consisted of a wide-mouth plastic cup and an

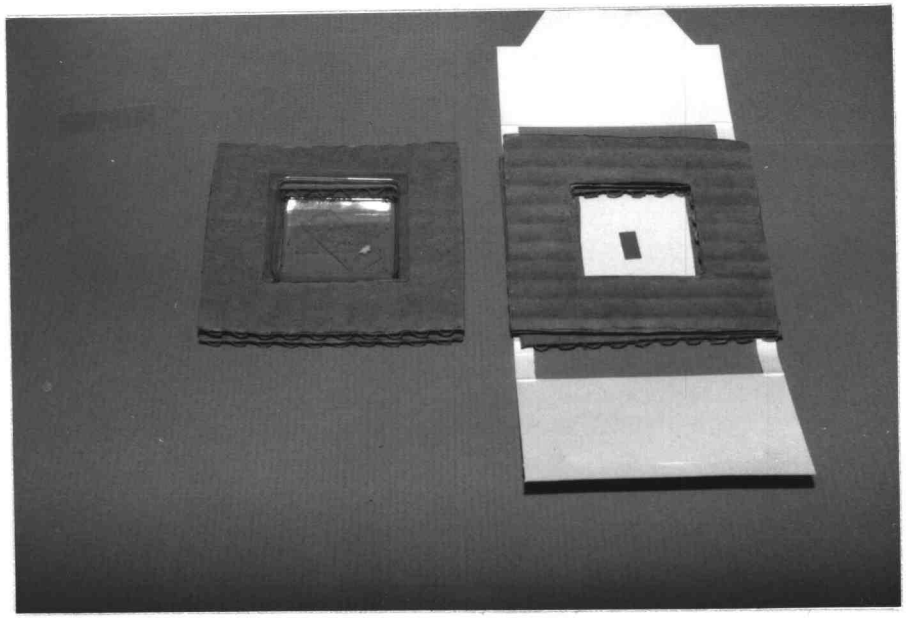


Figure 5.3: The "Modified Storgard" with 4 x 4 x 1 cm cup and perpendicular cardboard orientations.

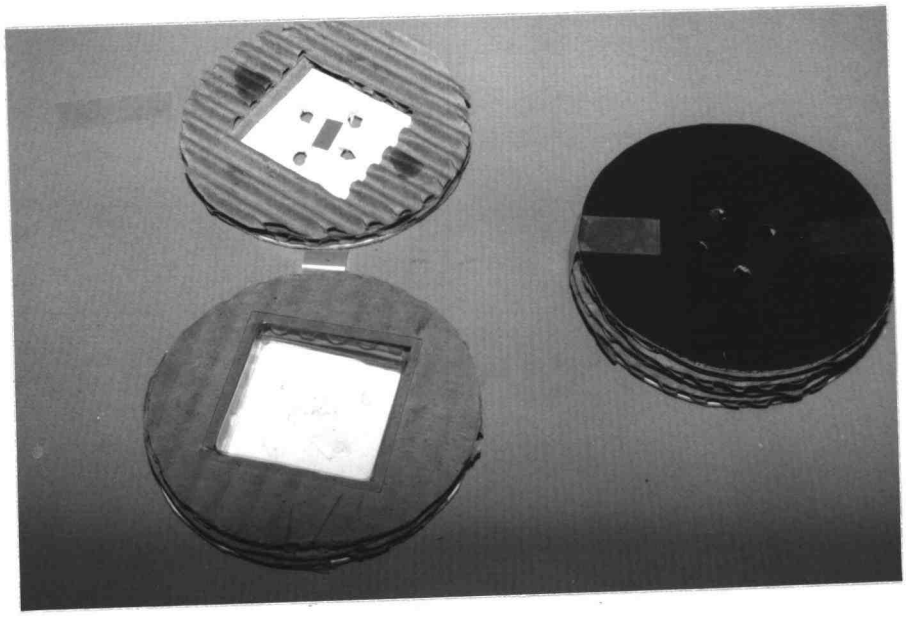


Figure 5.4: A circular cardboard trap "ROUNDED STORGARD". The trap consisted of four circular cardboard plates, 4.5 cm diam., with 4 cm square punchouts.

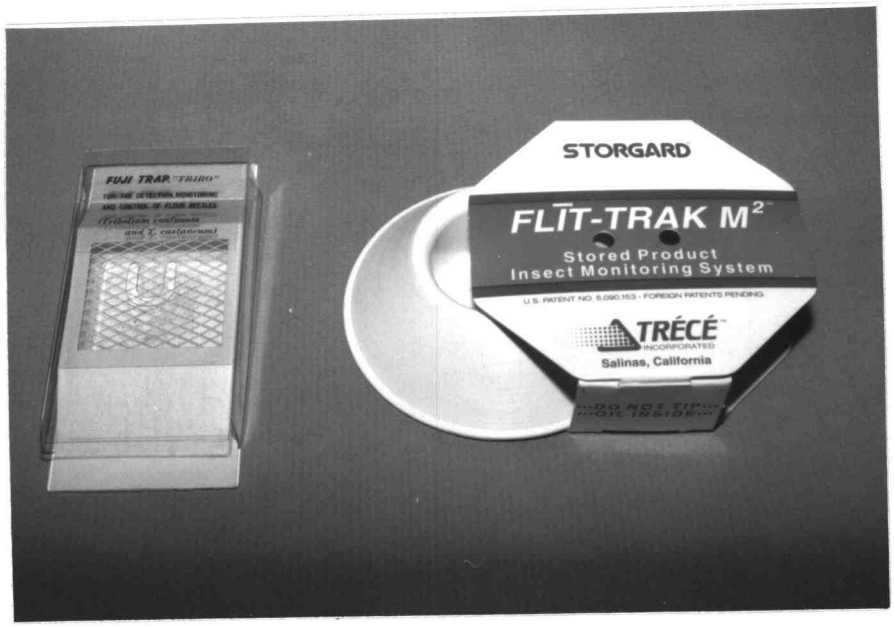


Figure 5.5: Hard paper ramp-and-pitfall design, 4 x 10 x 1 cm, "FUJI TRAP" (JapanTobacco Co.) and wide-mouth plastic cup ramp-and-pitfall "FLIT-TRACK TRAP"; ramp/cup has been removed from sleeve for viewing.

octagonal paper lid and base. The cup is of an inverted cone shape that is 10 cm dia. at the bottom from a which a ramp rises at a 50° angle to a 5 cm dia. top. The ramp had a rough surface to facilitate insect crawling whereas the top edge was smooth so that insects would fall into the cup. The lure was glued to the upper portion of the lid (Fig. 5.5). 6. The Tribolium Trap (Agrisence BCS, U.K.) was simply a single rectangle of corrugated cardboard, 4 x 10 cm, covered with clear plastic. Corrugations were removed from a 3x3 cm area in the middle of the rectangle, leaving the bottom surface of paper that was coated with sticky trapping material; a pheromone lure (laminate type of unknown formulation) was position in the center of the sticky surface. Responding insects were intended to enter the trap at the open ends of the trap and follow the corrugations to the sticky surface (Fig. 5.6). Trapped insects could be seen through the clear plastic cover. 7. The Window Trap (Agrisence BCS) is a ramp-and-pitfall design, 6 X 7.5 X 1 cm with a ramp at 40°, similar to the Fuji trap, but with a sticky surface at the bottom of the pitfall. A permanently affixed slow release laminated lure was on the upper inside surface. The upper cover was made of see clear plastic so that trapped insects could be examined easily (Fig. 5.6). The Window and Tribolium traps were compared only with each other and not with others described above. Because a laminate lure of unknown formulation was provided with both Window and Tribolium traps, experiments were conducted with these when the traps were new and after one and three weeks of aging.

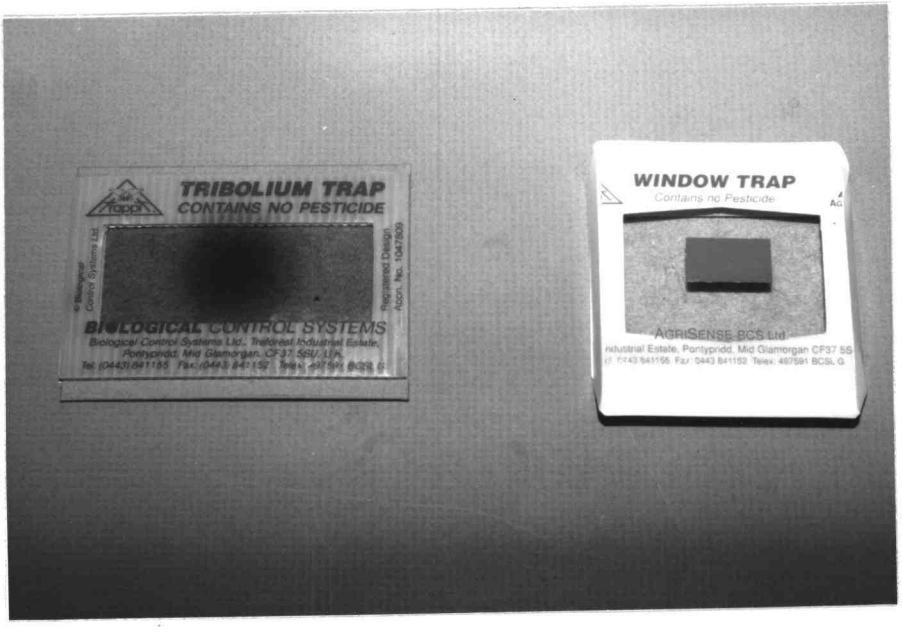


Figure 5.6: Single rectangle corrugated cardboard, 4 x 10cm, "TRIBOLIUM TRAP" and ramp-and-pitfall design, 7.5 x 6 x 1 cm, "WINDOW TRAP".

Trap Bioassays:

All trap experiments were conducted in a 92 X 92 X 9 cm steel tray with one layer of whole wheat grains in the arena (Fig. 5.7). A layer of grains provided a good substrate for moving as well as natural environment to the responding beetles. The sides of the trays were coated with liquid Teflon to prevent insect escape. All trap experiments utilized slow release laminate lures, 1/2" X 1/4", that had been aged 9-12 days in a fume hood. All pitfall traps contained 1/2 ml oil in their reservoirs. The oil was provided by Trécé Inc. and was a mixture of grain and mineral oils. Trécé oil presumably enhanced the attraction of beetles and served to kill the trapped beetles by suffocation. Only two types of traps were tested at a time, and each was placed randomly in the arena a minimum of 15 cm from a side and 30 cm from another trap. Eight replicates of each pair-wise comparison were performed at both high beetle densities (100 beetles released) and low beetle densities (50 beetles released). One-week-old, mixed sex beetles were counted and kept without food for 30-minutes before the experiments. An inverted glass funnel of 2 cm dia. was placed in the center of the tray arena and the test beetles were released into it. Insects were kept confined under the inverted glass funnel for 15-minutes and than were released into the arena by lifting the glass funnel. After releasing the beetles in the arena; the trays were also covered with a screen cover to avoid any insect escape by flight. Bioassay were conducted for 20 hours in complete darkness at 27° C and 60±10% relative humidity, after which the number of beetles caught in each trap was determined. Numbers

Figure 5.7: Steel tray arena, 92 X 92 X 9 cm, with one layer of whole wheat grains used for trap bioassays.

responding to traps were subjected to student's t-test for paired comparisons and denoted by stars in all figures. The data presented as in mean numbers and ± standard error (SE).

Data Analysis:

Raw data from pitfall bioassays were handled in two ways for analysis. First, the percentage responding to the treatment and the control were calculated and compared using the Student's t-test to determine if experimental treatment (e.g., dose of synthetic pheromone or lure design) had a significant effect on beetle response. Second, a response index (RI) was calculated to summarize the relative response to treatment and control and to provide a single value that could be used to compare among experimental treatments from other two-choice experiments. The response index was calculated as RI = (T - C /Tot) X 100, for which T is the number responding to the treatment, C is the number responding to the control, and Tot is the total number of insects released. Positive RIs indicate attraction to the treatment and negative RIs show repellency; values could theoretically range from -100 for complete repellency to + 100 for complete attraction. RIs were compared among ages of lures of the same design by ANOVA followed by Fisher's protected least significant difference test (LSD) for comparison of means. Different lure designs of the same age were also compared using ANOVA and LSD. For trapping experiments the percentage of beetles captured (from a total release of 100 or 50 beetles) in each of the two trap designs tested were compared using Students t-test.

RESULTS AND DISCUSSION

All commercial lure designs elicited significant attraction by *T. castaneum* under certain conditions, but response varied greatly with design and age of the lure (Figs. 5.8-5.11). All of the lures exhibited low activity when first removed from their packages (0 week), and typically elicited maximum response at one or more weeks of age.

Septum Lures. Rubber septum lures (Fig. 5.8) showed highest activity at one week of age (RI=51 $\pm$ 7.48), and yielded similar responses at three and four weeks of age. Significantly lower responses to rubber septum lures were recorded at 6 weeks of age, and these were similar to the initial low response to new lures.

Membrane Lures. Responses to the three membrane lure designs were characteristically low when lures were first taken from the packages, and then showed increased activity with age. Membrane lure A (Fig. 5.9 A) significantly repelled beetles when it was new and elicited no significant responses on weeks one, three and four. Membrane lure B (Fig. 5.9 B) elicited no significant response at zero and one week, while lure C (Fig. 5.9 C) was attractive when newly removed from the package. Maximum attraction occurred on weeks 5 through 12 for lure A, weeks 4 through 8 for lure B, and weeks 5 and 6 for lure C. Attraction declined significantly in the final weeks of the experiment for lures B and C. Upon comparing the three different membrane lures of the same age, there were no significant difference among lures (ANOVA, $P>0.05$) in ages 3 to 12 weeks, although the probability value for

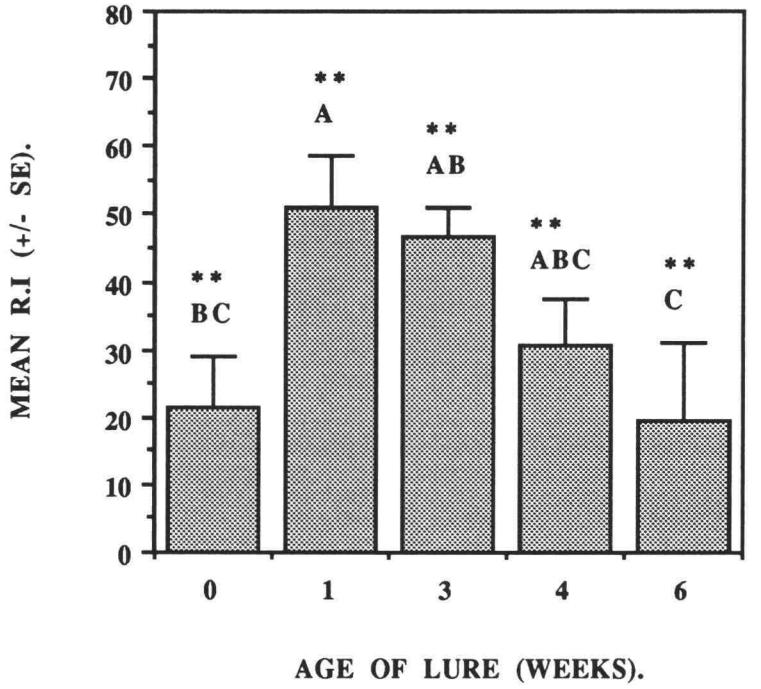


Figure 5.8: Response of *T. castaneum* to rubber septum lures in a two-choice pitfall bioassay. Histogram shows mean response index (RI, see text) and standard error (N=10). Mean RI followed by different letters are significantly different ($P<0.05$, ANOVA and LSD). Significant difference between treatment and control within each age class are indicated as ** ($P<0.01$).

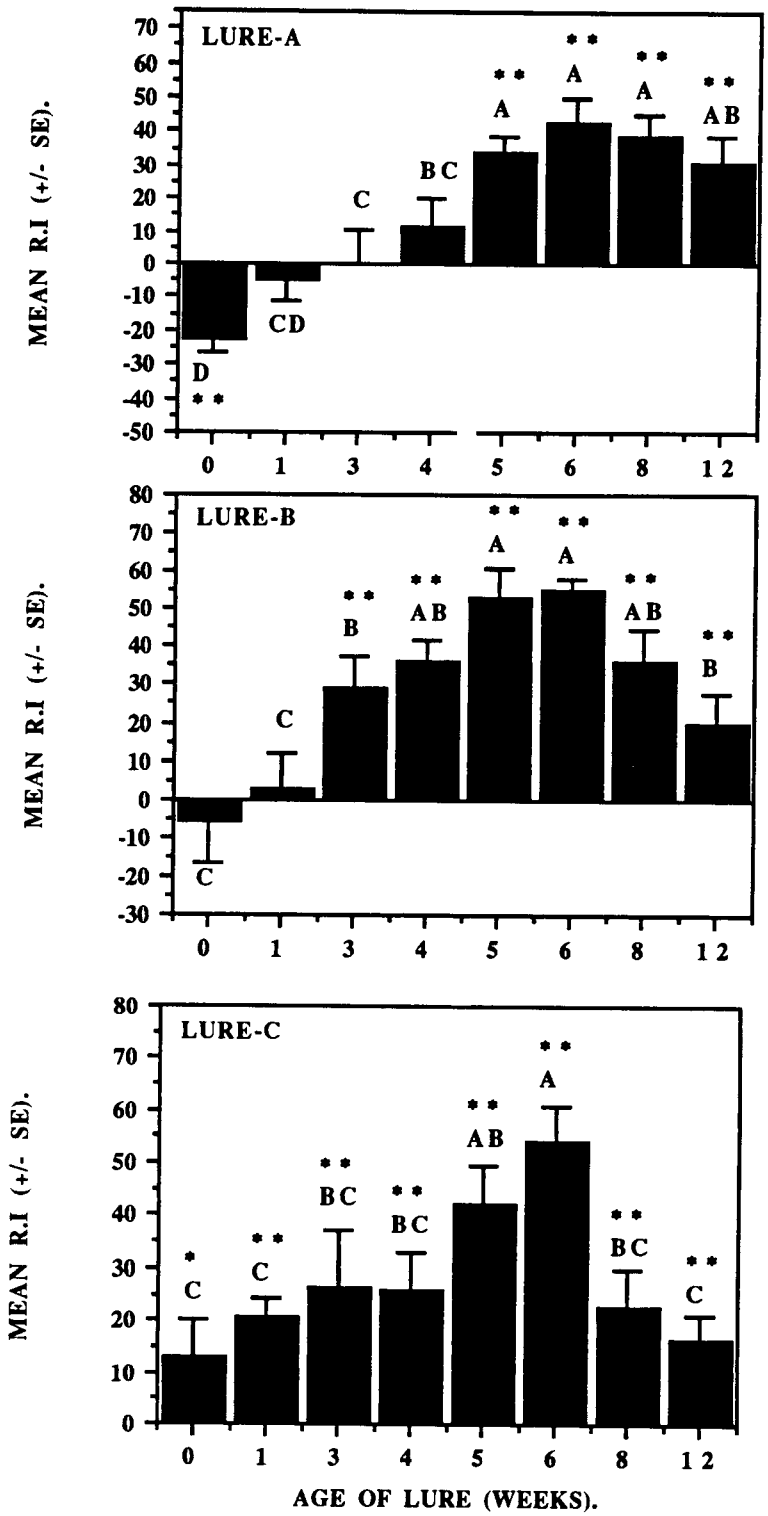


Figure 5.9: Response of *T. castaneum* to membrane lures, A, B, and C, in a series of two-choice pitfall bioassay. Histograms show mean response index (RI, see text) and standard error (N=10). Mean RI followed by different letters are significantly different ($P<0.05$, ANOVA and LSD). Significant difference between treatment and control within each age class are indicated as ** ($P<0.01$) and * ($P<0.05$).

difference at three weeks was P=0.1 and at four weeks it was P=0.07. Brand new septum lures (0 weeks) yielded significantly different responses from each other in all paired comparisons, and at one week the response to lure C was significantly higher than to lures A and B, which were statistically similar in activity. Response characteristics to membrane lures over time may reflect differences in pheromone formulation and release dynamics of the membrane. Lures A and B contained the same pheromone formulation (i.e., no "stabilizer" added), but differed in the surface area of the release membrane. Lure A had the 0.75 in. membrane, presumably was releasing more pheromone than lure B, which had the 0.5 in. membrane, and consequently was repellent early on. Interestingly, Lure C had a large release area (0.75 in.), but possessed a stabilizer in the formulation that may have resulted in slower or more steady pheromone release, and thus better beetle response characteristics.

Laminate Lures. Responses of *T. castaneum* to laminate lures revealed similar patterns over time as those found for membrane and septum lures (Figs. 5.8 & 5.9). Maximum responses to slow laminate lures occurred at one week of age or later (Fig. 5.10). Slow 1" x 1/2" lures and slow 1/2" x 1/4" lures elicited significantly higher responses at three weeks compared to other three-week old slow lures (ANOVA, $P<0.05$). Comparisons among sizes of slow lures at other ages revealed no differences among lures of the same age. Fast laminate lures also revealed increase in attractive response after one week of age followed by an eventual decrease in response at older ages (Fig. 5.11). The four sizes of fast laminate lures did not differ from each

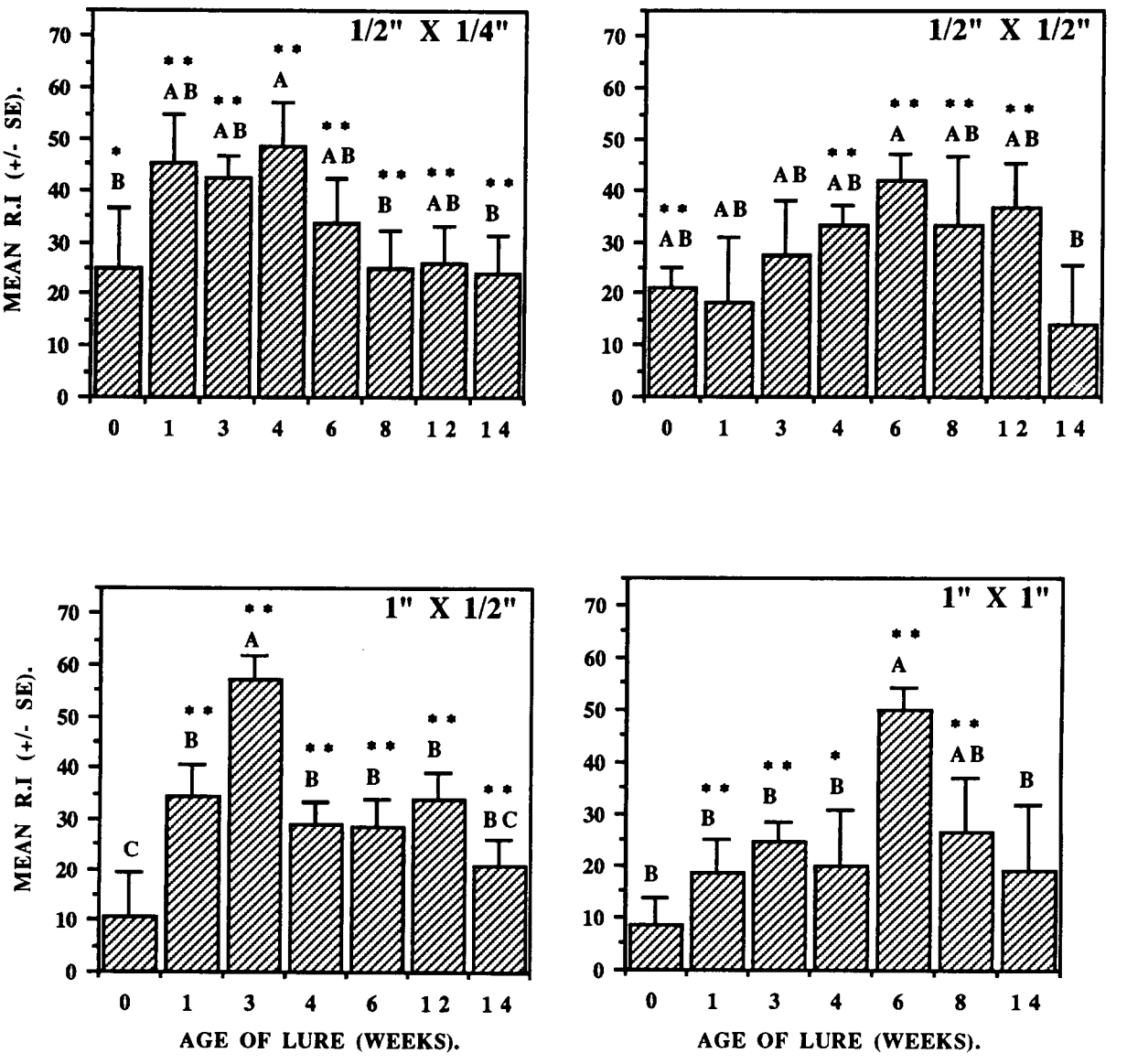


Figure 5.10: Response of *T. castaneum* to different slow release laminated lures in a two-choice pitfall bioassay. Histograms show mean response index (RI, see text) and standard error (N=10). Mean RI followed by different letters are significantly different ($P<0.05$, ANOVA and LSD). Significant difference between treatment and control within each age class within each age class are indicated as ** ($P<0.01$) and * ($P<0.05$).

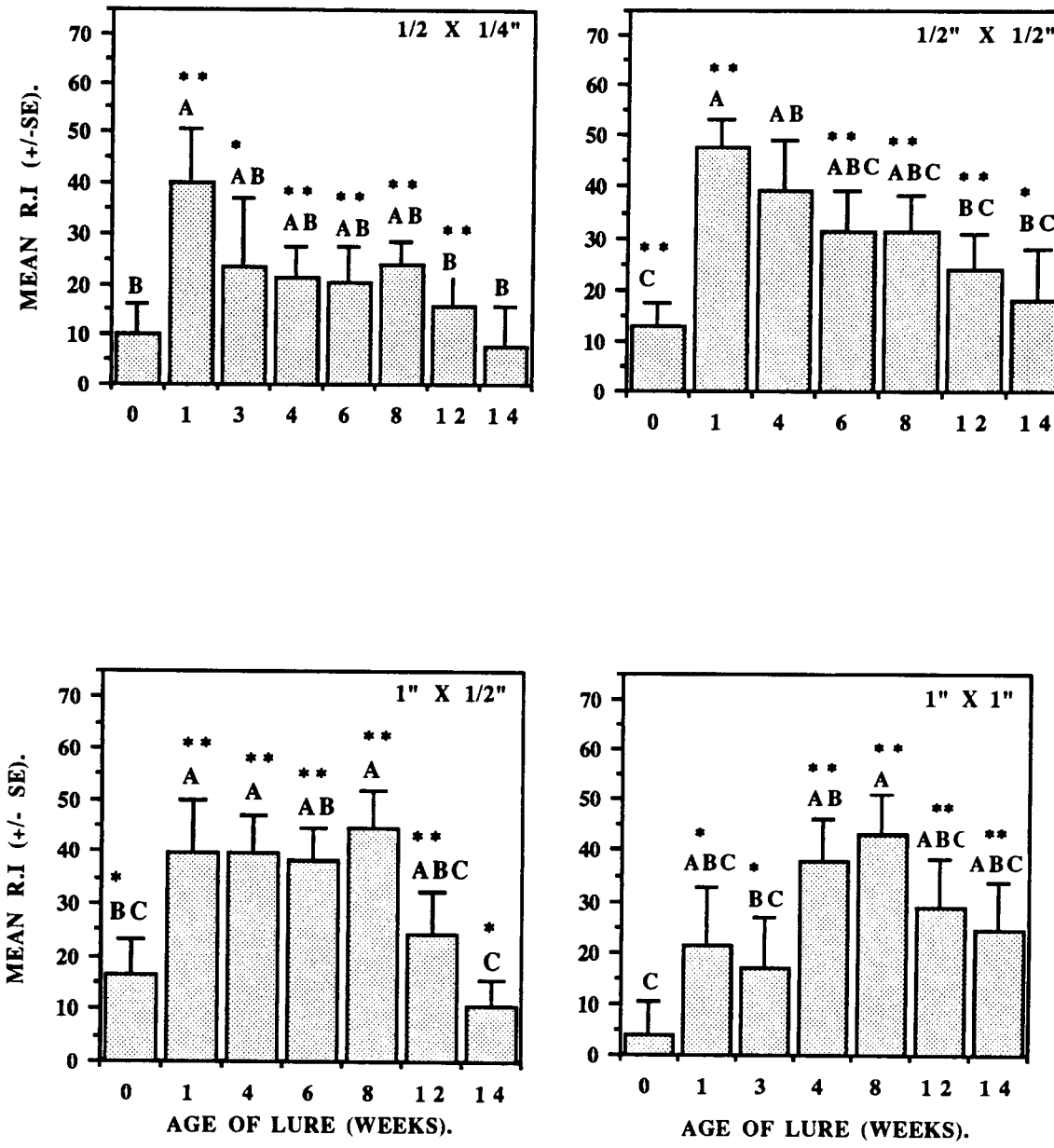
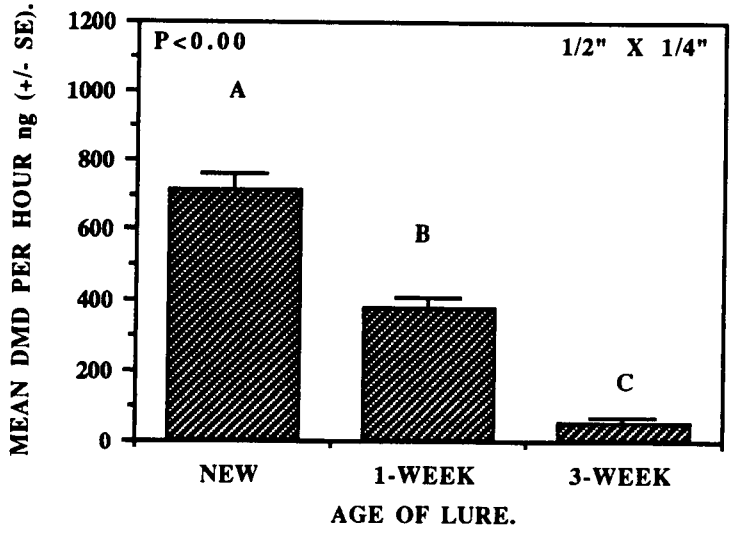


Figure 5.11: Response of *T. castaneum* to different fast release laminated lures in a two-choice pitfall bioassay. Histograms show mean response index (RI, see text)and standard error (N=10). Mean RI followed by different letters are significantly different ($P<0.05$, ANOVA and LSD). Significant difference between treatment and control within each age class are indicated as ** ($P<0.01$) and * ($P<0.05$).

other when lures within each age class were compared (ANOVA, P>0.05). Amounts of DMD release over time were determined for slow laminate lures of 1/2" x 1/4" and 1" x 1/2" (Fig. 5.12). It is clear from these results that the new lures (0 week) that elicited low behavioral response were releasing much higher levels of pheromone than the older lures that were attractive to beetles.

Responses of *T. castaneum* to controlled release formulations of DMD are similar to what was observed for response to different doses of the pheromone (Fig. 4.5). For the dose/response study attraction was typically low at low doses, increased to some optimum level, then decreased sharply at very high doses. The lure studies presented above reflect the same pattern as that for dose/response, but in reverse. Brand new lures (0 week) were presumably releasing at very high levels, and thus are repellent or neutral. As release rate decreases the level of pheromone approaches an optimum level and attraction is highest. As release rate slows even more with age, attractive response diminishes. The ideal controlled release device should emit pheromone at a constant rate over a specified period of time (Roelofs 1979). Our limited chemical analyses of volatiles from two slow release laminate lure designs indicates release rate was not stable over the three-week test period. However, if behavioral response of *T. castaneum* were used to evaluate release rate dynamics and lure efficacy, then one can see that several of the lures tested produced stable response characteristics for several weeks after an initial period of aging. Both constancy of response (similar RIs over several weeks) and level of response (maximum RI) must be considered in evaluating lures for *T. castaneum*. Maximal response was achieved



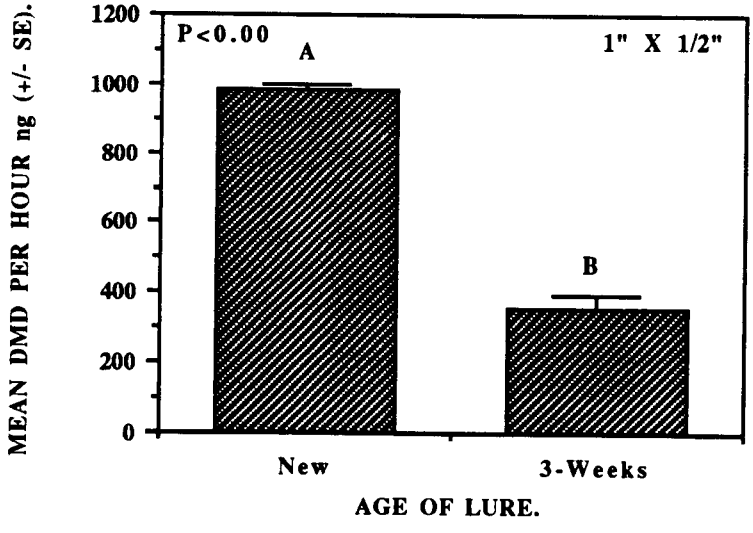


Figure 5.12: Amounts of pheromone released by two sizes of laminated lures at different ages. Volatiles from lures were collected on super-Q and analyzed with quantitative GC (see text for detail).

for periods of three or more weeks with slow laminate 1/2" x 1/4" and fast 1" x 1/2" lures. The slow 1/2" x 1/4" laminate lure was selected for further use in studies on trap design.

A series of two-choice experiments (eight replicates each) were conducted with the five different pitfall-type traps in large tray arenas (Table 5. 1). Initial experiments compared original Storgard with modified Storgard (bigger square cup, corrugations perpendicular to sides) and determined that the modified design captured significantly more beetles under low density conditions (50 beetles released), but that response to the two traps was not statistically different under high density conditions (100 beetles released; Table 5. 1, Expt.1 and 2). Subsequent experiments (Expts. 3 and 4, Table 5. 2) determined that there was no difference in response of beetles to the square Modified Storgard and the Rounded Storgard at either high or low densities of beetles. Thus, Rounded Storgard traps were used in the remaining studies as a comparison with ramp-pitfall designs. The Flit-Trak traps caught significantly more beetles than the Rounded Storgard at both high and low beetle densities (Table 5. 1, Expts. 5 and 6). Similarly, the Fuji trap captured more beetles than the Rounded Storgard at both beetle densities (Table 5. 1, Expts. 7 and 8). Thus, both ramp-pitfall design traps performed better than the corrugated cardboard trap in large arena assays. When Fuji and Flit-Trak traps were compared in the same experiments, there was no significant difference in performance between them at a high density of beetles, but the Fuji trap captured significantly more beetles at low density (Table 5. 1, Expts. 9 and 10). Thus, of the

Table 5.1:

RESPONSE OF *TRIBOLIUM CASTANEUM* TO VARIOUS TRAPS AT LOW AND HIGH DENSITIES.

Exp. #	TRAPS TESTED	DEN.	% MEAN CAUDHT (+/- SE)	P-VALUE
1	Storgard vs Modified Storgard	HD	17.90 + 3.29 25.60 + 3.43	NS
2	Storgard vs Modified Storgard	LD	11.50 + 2.38 26.00 + 2.85	**
3	Modified Storgard vs Rounded Storgard	HD	14.12 + 2.30 17.00 + 1.93	NS
4	Modified Storgard vs Rounded Storgard	LD	20.50 + 3.46 24.50 + 3.89	NS
5	Flight-Trak vs Rounded Storgard	HD	19.25 + 5.00 07.13 + 1.41	**
6	Flit-Trak vs Rounded Storgard	LD	23.00 + 2.93 11.50 + 1.68	*
7	Fuji vs Rounded Storgard	HD	23.00 + 3.23 03.63 + 0.43	**
8	Fuji vs Rounded Storgard	LD	26.00 + 4.44 15.50 + 2.35	**
9	Fuji vs Flit-Trak	HD	26.44 + 5.18 31.89 + 6.99	**
10	Fiji vs Flit-Trak	LD	25.50 + 3.44 12.50 + 2.60	**

HD = High Density (100 insects released); LD = Low Density (50 insects released);
** P-value from student's t-test.

two ramp style traps tested, it appears that the Fuji trap, which employs a straight cardboard ramp at each end, is more effective at trapping *T. castaneum* than the Flit-Trak trap, which employs a circular plastic ramp. The Tribolium trap and the Window trap, which both employ a sticky trapping surface and incorporate a laminate lure of unknown characteristics, were compared in a separate series of large tray arena biaossays. The Tribolium trap caught significantly more beetles than the Window trap under high density conditions when both were new out of their packages (Table 5.2). Since the numbers of beetles captured in this initial experiment were low compared to those with other traps (Table 5.1), it was suspected that the fixed lures were not releasing pheromone at an optimal rate since they were not aged. When Tribolium and Window traps were aged for one and three weeks, however, there were no significant differences between them and overall beetle capture remained very low. The low performance of the Window and Tribolium traps could be due to the lure they used, which was permanently fixed to the traps and presumably was not the same as the lure used for studies of pitfall traps (Table 5.1). These traps also did not include any grain-based oils, which may have accounted for apparently better performance in the pitfall traps.

Very few scientific studies have been conducted with traps for *Tribolium*, and until now none were known that examined efficacy of controlled release pheromone devices. It is clear from these studies that formulation and release rate of pheromone is very important for monitoring response of *T. castaneum*. All commercial pheromone lures tested were repellent when first removed from their packages, but

Table 5.2:

RESPONSE OF *TRIBOLIUM CASTANEUM* TO WINDOW AND TRIBOLIUM TRAPS AT HIGH DENSITY.

EXP. #	TRAPS TESTED	AGE (Wks.)	% MEAN CAUGHT (+/- SE)	P-VALUE
1	Window vs Tribolium	New	03.50 + 0.76 10.83 + 1.96	*
2	Window vs Tribolium	One	4.33 + 0.211 5.83 + 1.17	NS
3	Window vs Tribolium	Three	2.67 + 0.84 8.00 + 2.03	NS

elicited positive responses by beetles after some period of aging. This "burst" effect of slow release pheromone devices, in which a large amount of pheromone is released early in the life of the device as the transfer matrix becomes saturated with pheromone and release equilibrates, is commonly noted (McDonough 1991). These studies also indicate that trap design can be very important in capture of *T. castaneum*. The success of the ramp-pitfall designs may relate directly to a natural tendency of beetles to be negatively geotropic (Mullen 1992). Additionally, ramp traps may allow for more unimpeded release of pheromone from the lure compared to cardboard traps, in which there is not a direct path for escape of the pheromone from the lure to the outside of the trap. These studies provide a framework for future studies that should address efficacy of lures and traps in field situations.

REFERENCES

Barak, A. V., W. E. Burkholder, and D. L. Faustini. 1990. Factors effecting the design of traps for stored-products insects. J. Kansas Entomol. Soc. 63: 466-485.

Barak, A. V. and W. E. Burkholder. 1984. A versatile and effective trap for detecting and monitoring stored-product Coleoptera. Agric. Ecosys. Environ. 12: 207-208.

Burkholder, W. E. 1984. Stored-product insect behavior and pheromone studies: Keys to successful monitoring and trapping. Proceedings of the Third International Working Conference on Stored-product Entomology, Manhattan, Kansas, pp. 20-33.

McDonough, D. L. 1991. Controlled release of insect sex pheromone from a natural rubber substrate. pp. 106-125. In: Naturally occurring pest bioregulators. P.A. Hedin (ed.). ACS Symposium Series 449.

Mullen, M. A. 1992. Development of a pheromone trap for monitoring *Tribolium castaneum.* J. Stored Prod. Res. 28:245-249.

Roelofs, W. L. 1979. Establishing efficacy of sex attractants and disruptants for insect control.. ESA.

Suzuki, T. 1980. 4, 8-Dimethyldecanal: The aggregation pheromone of the flour beetles, *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae). Agri. Biol. Chem., 44(10):2519-2520.

CHAPTER VI:

SUMMARY

SUMMARY

The male produced aggregation pheromone of *Tribolium castaneum*, 4, 8-dimethyldecanal (DMD), was successfully detected and quantified in single males by collection of volatiles on adsorbants and analysis by gas chromatography. The mean amount of pheromone produced per day was 635.37 $\pm$ 18 ng per male. Pheromone production fluctuated with a general pattern of two days up and two days down for the thirty days of observations. This longevity study showed that age had little effect on pheromone production, at least during the first month of adulthood. This study is the first to record quantities of pheromone produced by individual virgin male *Tribolium castaneum.* Small quantities of pheromone were also observed from male pupae. The quantity of pheromone produced five days before adult emergence (93.4 $\pm$ 24.72 ng) was significantly greater than that produced one day before emergence (23.8 $\pm$ 8.83 ng). Pheromone was also detected and quantified from larvae, but data were limited in this experiment due to beetle mortality.

Factors affecting pheromone production were studied. Pheromone production in *T. castaneum* was dependent on feeding. A significant reduction of pheromone was observed in beetles starved for a two-day period. Actual contact and feeding on grain is apparently required for production of pheromone. Pheromone production was significantly higher in beetles exposed directly to grains compared to those exposed to grain volatiles only or to those provided no food. Starvation had a profound effect on the reduction of pheromone release in male red flour beetles, but pheromone production was easily restored by providing food. The results of these experiments

strongly support the practice of sanitation in warehouses. Absence of an easily available food source would prevent *Tribolium* from aggregation and consequently reduce the chances of population growth. Mating studies revealed that mating had no effect on pheromone production in male *T. castaneum.* There was no significant difference in pheromone production between virgin males and males paired with females, and also compared with unpaired but previously mated males. Similarly, male *T. castaneum* reared and aerated under 16:8 (L:D) and under total darkness produced statistically similar levels of pheromone. However, insects maintained under 24 h light produced nearly four times as much pheromone per day as those in long-day or short-day photoperiods. This photoperiod study suggests that more light stimulates additional pheromone release, even when an unnatural photoperiod (e.g. 24 h light) is applied.

The effect of insect crowding on pheromone production was investigated with *T. castaneum.* During preliminary studies we observed that common chemical defensive compounds produced by red flour beetles when aerated in groups. Both male and female *Tribolium castaneum* were significantly attracted to volatiles collected from either one or two males. Female response was significantly higher than males when tested against volatiles produced by one male. Volatiles collected from five males were not attractive to either sex whereas volatiles from ten males elicited significant repellency in both sexes. These data suggested either that male pheromone production was reduced under crowded conditions, or that crowded beetles produced repellents, or both phenomena may occur.

Quantities of pheromone and defensive compounds produced at different beetle density levels were determined. When volatiles were analyzed from male *T. castaneum* at different densities, the pheromone 4, 8-dimethyldecanal was detected only in single male aerations and none was found in densities of 5, 10, or 50 beetles. A number of other compounds were detected at high beetle levels including primarily 2-methyl-1,4-benzoquinone, 2-ethy-1,4-lbenzoquinone and 1-pentadecene. Small quantities of 1-hexadecene and various other hydrocarbons were also observed. No significant difference was observed in production of defensive compounds per individual among the different densities. When single males were exposed to volatiles produced by fifty males, pheromone was detected and no significant difference in quantity was observed compared to that produced by single males that were not exposed to volatiles of others. This experiment clearly demonstrated that the defensive compounds are not directly responsible for reduced production of pheromone. Analysis of volatiles from females at different densities detected no dimethyldecanal at any density, which concurs with all previous research indicating that this is a male-produced pheromone. Females produced the same defensive compounds as males, but in several cases males produced significantly more of these than females.

Behavioral response of *Tribolium castaneum* to food and pheromone was studied in laboratory bioassays. Male *T. castaneum* responded positively to wheat germ nuts, a processed food product, at higher dosage levels than females, suggesting that males were more sensitive to food than females. Since males produce aggregation pheromone upon feeding, it is likely that males are highly adapted to locating food by

responding to odors. Mixed sex beetles also gave a clear positive response to cracked wheat grains.

Response of *T. castaneum* was observed to various dosages of synthetic DMD; the highest response was elicited by 0.1 μg on filter paper. Response to the lowest dose tested, 0.001 μg was not significant. However, a significant reduction in response was observed at the highest doses of 10.0 and 100.0 μg DMD. This suggests an optimum dose at which the response is maximum, beyond this dose the response decreases. Combinations of DMD and wheat germ nuts were consistently more attractive to *T. castaneum* than any of the controls, pheromone or food alone, indicating that the presence of food significantly enhances the activity of pheromone. The same phenomenon was observed in another study with combinations of wheat and pheromone.

Studies were conducted to evaluate the activity and longevity of various controlled release devices for dimethyldecanal and to test the effectiveness of several trap designs for capturing *T. castaneum.* All commercial septum, membrane and laminated lures elicited significant attraction by *T. castaneum* under certain conditions, but response varied greatly with design and age of the lure. All of the lures exhibited low activity when first removed from their package and typically elicited maximum response at one or two weeks of age. Response of *T. castaneum* to controlled release formulations of DMD were similar to what was observed for different doses of the pheromone on filter paper. For the dose/response study, attraction was typically low at low doses, increased to some optimum level, then decreased sharply at very high

doses. The lure studies showed a similar pattern as was noticed for dose/response, but in reverse. New lures were presumably releasing at very high levels, and thus were repellent or neutral. As release rate decreases the level of pheromone approaches an optimum level, and attraction was highest. As release rate slowed even more with age, attractive response diminished.

Five different pitfall-type traps were compared in two-choice, large tray bioassay arenas. When original Storgard and modified Storgard traps (larger cup, perpendicular corrugations) were compared under low beetle density conditions, modified Storgard traps captured significantly more beetles, but no significant difference was observed at higher density. Subsequent experiments showed that there was no difference in response of beetles to the square modified Storgard and the rounded Storgard at either high or low densities of beetles. In another comparison Flit-Trak traps (a ramp-pitfall design) caught significantly more beetles than the rounded Storgard at both low and high densities. Similarly, the Fuji trap (a ramp-pitfall) captured more beetles than the rounded Storgard at both densities. When Fuji and Flit-Trak traps were compared, there was no significant difference in response at high density, but the Fuji trap captured significantly more beetles at low density. Thus, both ramp type traps, Fuji and Flit-trak, performed better than paper cardboard traps (e.g Storgard) in laboratory experiments.

BIBLIOGRAPHY

Aliniazee, M. T. 1970. Egg hatch of *Tribolium confusum* and *Tribolium castaneum* (Coleoptera : Tenebrionidae) in different carbon dioxide and nitrogen atmospheres. Ann. Entomol. Soc. Am. 1010-1012.

Barak, A. V. and W. E. Burkholder. 1984. A versatile and effective trap for detecting and monitoring stored-product Coleoptera. Agric. Ecosys. Environ. 12: 207-208.

Barak, A.V., and W. E. Burkholder. 1985. A versatile and effective trap for detecting and monitoring stored-product Coleoptera. Agri. Ecosys. Environ. 12: 207-218.

Barak, A. V., W. E. Burkholder, and D. L. Faustini. 1990. Factors effecting the design of traps for stored-products insects. J. Kansas Entomol. Soc. 63: 466-485.

Barrer, P.M. 1983. A field demonstration of odor-based, host-food finding behavior in several species of stored grain insects. J. Stored Prod. Res. 19(3): 105-110.

Blum, M. S. 1981. Chemical defences of arthropods. Academic press. New York

Borden, J. H. 1985. Aggregation pheromone. pp. 257-285. In: Behavior. (G. A. Kerhut, ed.),

Burkholder, W. E. 1982. Reproductive biology and communication among grain storage and warehouse beetles. J. Ga. Entomol. Soc. 17(2): 1-10.

Burkholder, W. E. 1984. Stored-product insect behavior and pheromone studies: Keys to successful monitoring and trapping. Proceedings of the Third International Working Conference on Stored-product Entomology, Manhattan, Kansas, pp. 20-33.

Burkholder, W. E. 1990. Practical use of Pheromones and other attractants for stored product insects. pp 497-516. In: Behavior-modifying chemicals for insect management :Application of Pheromones and other Attractants .(R. L. Ridgway,R.M. Silverstein and M. N. Inscoe, Eds.) Marcel Dekker, Inc. N.Y.

Burkholder, W. E. and Dicke, R. J. 1966. Evidence of sex pheromone in females of several species of Dremestidae. J. Econ. Entomol. 59:540-

Burkholder, W. E. and M. Ma. 1985. Pheromone for monitoring and control of stored product insects. Ann. Rev. Entomol. 30: 257-272.

Champ, B. R. and C. E. Dyte. 1976. FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Production and Protection Series No.5. Food and Agriculture Org. of the United Nations. Rome. 297 p.

Cogan, P. M., and M. E. Wakefield. 1987. Further development in traps used to detect low level infestation of beetle pests in bulk stored grain. BCPC MONO No: 37 Stored Product Pest Control. 161-167.

Cox, P. D. and C. H. Bell. 1991. Biology and ecology of moth pests of stored foods. pp 181-193. In: Ecology and management of food-industry pests (J. R. Gorham ed.) Assoc. official chemist.

Dickens, J. C., E. B. Jang, D. M. Light and A. R. Alford. 1990. Enhancement of insect pheromone responses by green leaf volatiles. Naturwiss. 77: 29-31.

Doyen, J. T. 1985. Reconstitution of the tribes Ulomini and Triboliini for North and Central America (Coleoptera: Tenebrionidae). Proc. Entomol. Soc. Wash. 87: 512-524.

Evans, D. E. 1987. Stored Products. In: Integrated Pest Management. (A. J. Burn, T. H. Coaker, P. C. Jepson Eds.): 425-461. Academic Press.

Faustini, D. L., W. E. Burkholder and R. J. Laub. 1981. Sexually dimorphic setiferous sex patch in the male Red flour beetle, *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae): Site of aggregation Pheromone production J. Chem. Ecol. 7(2):465-479.

Faustini, D. L., J. R. Rowe and W. E. Burkholder. 1982. A male-produced aggregation pheromone in *Tribolium brevicornis* (Le Conte) (Coleoptera: Tenebrionidae) and inter specific response of several *Tribolium* Species. J. Stord Prod. Res. 18:153-158.

Faustini, D. L. and W. E.Burkholder. 1987. Quinone-aggregation pheromone interaction in the red flour beetle. Animal Behavior 35: 601-603.

Freedman, B., K. L. Milkolajczak, C. R. Smith Jr., W. F. Kwolek and W. E. Burkholder. 1982. Olfactory and aggregation responses of *Oryzaephilus surinamensis* (L.) to extracts from oats. J. Stored Prod. Res. 18:75-82.

Hagstrum, D. W., and B. J. Smittle. 1980. Age-and sex-specific tunneling rates of adult *Tribolium castaneum.* Ann. Entomol. Soc. Am. 73: 11-13.

Ho, F. 1969. Identification of pupae of six species of *Tribolium castaneum* Ann. Entomol. Soc. Am. 62: 1232-1237.

Hodges, R. J., H. Halid, D. P. Rees, J. Meik and J. Sarjono. 1985. Insect traps tested as aid to pest management in milled rice stores. J. Stored Prod. Res. 21: 215-229.

Howard, R. W. 1987. Chemosystematic studies of the *Triboliini* (Coleoptera: Tenebrionidae): Phylogenic inferences from the defensive chemicals of eight *Tribolium* spp., *Palorus ratzeburgi* (Wissmann), and *Latheticus orzae* Waterhouse. Ann. Entomol. Soc. Am. 80: 398-405.

Javer, A., J. H. Borden, H. D. Pierce and A. M. Pierce. 1989. Evaluation of Pheromone-baited traps for monitoring of cucujid and tenebrionidae beetles in stored grain. J. Econ. Entomol. 83:268-272.

Levinson, H. Z. and K. Mori 1983. Chirality determines Pheromone activity for Flour beetles. Naturewissenschafen, 70:190-192.

Lindgren, B. S., J. H. Borden, A. M. Pierce and H. D. Pierce Jr. 1985. A potential method for simultaneous semiochemical-based monitoring of *Crytolestes ferrugineus* and *Tribolium castaneum* (Coleoptera: Cucujidae and Tenebrionidae) J. Stored Prod. Res. 21(2): 83-87.

Markarian, H., G. J. Florentine and J. J. Pratt Jr. 1978. Quinone production of some species of *Tribolium.* J. Insect Physiol. 14:785-790.

McDonough, D. L. 1991. Controlled release of insect sex pheromone from a natural rubber substrate. pp. 106-125. In: Naturally occurring pest bioregulators. P.A. Hedin (ed.). ACS Symposium Series 449.

Mikolajczak, K.L., B. Freedman, B. W. Zilkowski, C. R. Smith Jr. and W. E. Burkholder. 1983. Effect of oat constituents on aggregation behavior of *Oryzaephilus surinamensis*(L.). J. Agri. Food Chem. 31(1):30-33.

Mikolajczak, K.L., B.W. Zilkowski, C. R. Smith Jr., and W. E. Burkholder. 1984. Volatile food attractants for *Oryzaephilus surinamensis* (L) from oats. J. Chem. Ecol. 10(2):301-309.

Mondal, K. A. M. S. H. 1985. Response of *T. castaneum* larvae to aggregation pheromone and quinones produced by adult conspecifics. Int. Pest Control, 27: 64-66.

Mondal, K. A. M. S. H. and G. R. Port. 1984. Response of *Tribolium castaneum* larvae to synthetic aggregation Pheromone. Entomol. exp. appl. 36:43-46.

Mullen, M. A. 1992. Development of a pheromone trap for monitoring *Tribolium castaneum.* J. Stored Prod. Res. 28:245-249.

Nara, J. M., R. C. Lindsay and W. E. Burkholder. 1981. Analysis of volatile compounds in wheat germ oil responsible for an aggregation response in *Trogoderma glabrum* larvae. Agri. Food Chem. 29(1):68-72.

Obeng-Ofori, D. 1991. Analysis of orientation behavior of *Tribolium castaneum* and *T. confusum* to synthetic aggregation Pheromone. Entomol. exp. appl. 60: 125-133.

Obeng-Ofori, D., and T. H. Coaker. 1990. *Tribolium* aggregation Pheromone: Monitoring, range of attraction and orientation behavior of *T. castaneum* (Coleoptera: Tenebrionidae). Bull. Entomol. Res. 80: 443-451.

O'Ceallachain, D. P. and M. F. Ryan. 1977. Production and perception of pheromones by the beetle *Tribolium confusum.* J. Insect Physiol. 23:1303-1309.

Phillips, T. W., X. -L. Jiang, W. E. Burkholder, J. K. Phillips and H. Q. Tran. 1993. Behavioral responses to food volatiles by two species of stored-product Coleoptera, *Sitophilus oryzae (*Curculionidae) and *Tribolium castaneum* (Tenebrionidae). J. Chem. Ecol. 19: 723-734.

Pierce, H. D., J. E. Conn Jr., A. C. Oehlschlager and J. H. Borden. 1987. Monoterpene metabolism in female mountain pine beetles, *Dendroctonus ponderosae* Hopkins, attacking ponderosa pine. J. Chem. Ecol. 13(6): 1455-1480.

Pimental, D. 1991. World resources and food losses to pests. pp. 5-11. In: Ecology and management of food-industry pests (J. R. Gorham ed.) Assoc. official chemist.

Rangaswamy, R. J. and V. B. Sasikala. 1990. Structural investigations of the Pheromones of flour beetles, *Tribolium castaneum*:Part I- sex pheromone of female beetles. Indian J. Chem. 447-450.

Raina, A. K. and J. J. Menn. 1987. Endocrine regulation of pheromone production in Lepidoptera. pp 181-193. In: Pheromone biochemistry. G. D. Prestwich and G. J. Blomquist (eds.). Academic Press.

Raina, A. K., T. G. Kingan and A. K. Mattoo. 1992. Chemical signals from host plant and sexual behavior in a moth. Science. 255: 592-594

Renwick, J. A. A. and P. R. Hughe. 1977. Neural and hormonal control of pheromone biosynthesis in the bark beetle, *Ips paraconfusus.* Physiol. Entomol. 2: 117-123.

Roelofs, W. L. 1979. Establishing efficacy of sex attractants and disruptants for insect control.. ESA.

Roth, L. M. 1943. Studies on the gaseous secretion of *Tribolium castaneum* Duval II. The odoriferous glands of *Tribolium confusum* Ann. Rev. Ent. 7:10-136.

Roth, L. M. and T. Eisner. 1962. Chemical defenses of the arthropods. Ann. Rev. Entomol. 7: 107-136.

Silverstein, R. M. 1977. Complexity, diversity, and specificity of behavior-modifying chemicals: Examples mainly from Coleoptera and Hymenoptera. pp 231-251. In: Chemical control of insect behavior, theory and application. (Shorey, H. H. and J. J. McKelvey, Jr. eds.) John Wiley & Sons.

Sinclair, -E. R. and C. Howitt. 1984. Tests of pheromones for monitoring infestation of stored products insects. Proced. 4th Australian Applied Entomol. Res. Adelaide. 24-28 September.

Sinha, R. N. 1973. Interrelation of physical, chemical and biological variables in the deterioration of stored grain. In grain storage: Part of a system (Eds R. N. Sinha and W. E. Muir), 15-17. Avi Pub. Co Westport.

Sokoloff, A. 1972. The biology of *Tribolium,* vol. 1 Clarendon Press. Oxford.

Suzuki, T. 1980. 4, 8-Dimethyldecanal: The aggregation pheromone of the flour beetles, *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae). Agri. Biol. Chem., 44(10):2519-2520.

Suzuki, T. 1981. A facile synthesis of 4, 8-dimethyldecanal, aggregation pheromone of flour beetles and its analogues. Agric. Biol. Chem. 45:2641-2643.

Suzuki, T. 1985. Presence of another aggregation substance(s) in the frass of the red flour beetles, *Tribolium castaneum* (Coleoptera: Tenebrionidae). Appl. Ent. Zool. 20(1): 90-91.

Suzuki, T., Suzuki, T., Huynh, H. M. and Muto, T. 1975. Hydrocarbon repellents isolated from *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae). Agr. Biol. Chem. 39(11):2207-2211.

Suzuki, T. and R. Sugawara. 1979. Isolation of an aggregation pheromone from the flour beetles, *T. confusum* (Coleoptera: Tenebrionidae). Appl. Entomol. Zool., 14:228-230.

Vanderwel, D. and A. C. Oehschlager. 1987. Biosynthesis of pheromones and endocrine regulation of pheromone production in Coleoptera. pp. 175-215. In: Pheromone biochemistry. G. D. Prestwich and G. J. Blomquist (eds.). Academic Press.

von Endt, D. W., and J. W. Wheeler. 1971. 1-pentadecene production in *Tribolium confusum*. Science 172; 60-61.

Walgenback, C. A., W. E. Burkholder, M. J. Curtis and Z. A. Khan. 1987. Laboratory Trapping studies with *Sitophilus zeamais* (Coleoptera: Curculionidae). J. Econ. Entomol. 80:763-767.

Walter, V. E. 1991. Fumigation in food industry pests. J. R. Gorham (ed.) 441-457. Association of official Analytical Chemists.

Watson, D. L. 1979. An industry viewpoint on professionalism and ethics in entomology. Bull. Entomol. Soc. Am. 25(3): 235-236.

Webb, J. L. 1966. Enzyme and metabolic inhibitors. Vol. II. New York: Academic Press.

White, N. D. G., and Loschiavo, S. R. 1986. Effect of insect density trap depth and attractants on the capture of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Cryptolestes furrugineum* (Coleoptera: Cucujidae) in stored wheat. J. Econ. Entomol. 79:1111-1117.

Zafar, A., H. Ali, and A. Hussain. 1987. Population patterns of Coleoperous pest insects in flour mills of Faisalabad. Pakistan Entomol. 9: 61-63.