

MONITORING THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM*  
(HERBST) (COLEOPTERA: TENEBRIONIDAE) AND  
OTHER STORED-PRODUCT INSECTS WITH  
TRAPS IN FLOUR MILLS

BY

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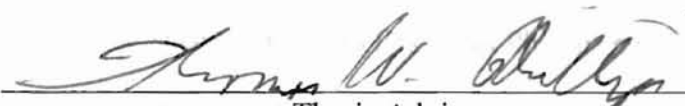
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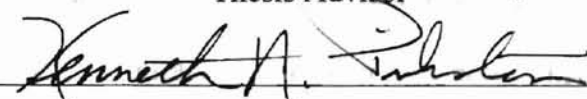
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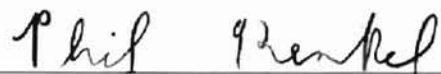
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## PREFACE

The first chapter of this thesis is a literature review that provides a discussion of relevant issues related to the research presented in subsequent chapters. Chapters two through four are formal papers presenting research performed for this degree program and are written in compliance with the publication policies and guidelines for manuscript preparation with the Entomological Society of America.

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PROJECT REPORT IN FLOUR  
MOUSE-TRAP FOR  
TABLE OF CONTENTS

Chapter	Page
I. LITERATURE REVIEW.....	1
Introduction.....	2
Insect Pests in Food Processing Centers and Flour Mills.....	3
Methyl Bromide.....	4
<i>Tribolium castaneum</i> and <i>T. confusum</i> .....	6
Trapping of <i>Tribolium</i> and other Stored-Product Beetles.....	9
<i>Plodia interpunctella</i> .....	14
Pheromone Trapping of <i>P. interpunctella</i> .....	15
Study Objectives.....	16
References Cited.....	17
II. RESPONSE OF <i>TRIBOLUM CASTANEUM</i> AND OTHER STORED-PRODUCT BETTERES TO TRAPS AND SEMIOCHEMICALS.....	26
Abstract.....	27
Introduction.....	28
Materials and Methods.....	32
Experimental Insects.....	32
Traps Evaluated and Pheromone Lures.....	32
Semiochemicals.....	33
Bioassays.....	35
Tray Assay.....	35
Two-choice Pitfall Assay.....	36
Field Experiments.....	39
Results.....	43
Response of <i>T. castaneum</i> to food oils.....	43
Response of <i>T. castaneum</i> to Host and Host-related Materials and Compounds.....	43
Response of <i>T. castaneum</i> and other Stored-Product Beetles to Traps.....	44
Effects of Pheromone and Oil on <i>T. castaneum</i> Response to Pitfall Traps.....	45
In-Flight Response of <i>T. castaneum</i> to Pheromone- Baited Traps.....	46

	Discussion.....	47
	References Cited.....	53
III.	MONITORING STORED-PRODUCT BEETLES IN FLOUR MILLS WITH PARTICULAR ATTENTION TO <i>TRIBOLIUM CASTANEUM</i> .....	92
	Abstract.....	93
	Introduction.....	94
	Materials and Methods.....	96
	Trapping Sites and Study Design.....	97
	Mill 1.....	97
	Mill 2.....	99
	Data Analysis.....	100
	Results.....	102
	Discussion.....	105
	References Cited.....	112
IV.	CAPTURE OF <i>PLODIA INTERPUCTELLA</i> WITH PHEROMONE-BAITED TRAPS IN AND AROUND FLOUR MILLS.....	136
	Abstract.....	137
	Introduction.....	138
	Materials and Methods.....	140
	Traps.....	140
	Mill Trapping Studies.....	140
	Outdoor Moth Dispersion Study.....	142
	Data Analysis.....	143
	Results.....	145
	Discussion.....	147
	References Cited.....	150

## LIST OF TABLES

Table	Page
Chapter II	
2.1. Summary of food oils tested.....	57
2.2. Response of <i>T. castaneum</i> to various food oils.....	58
2.3. Response of <i>T. castaneum</i> to oils that had previously tested attractive.....	59
2.4. Response of <i>T. castaneum</i> to various concentrations of a hexane wheat extract.....	60
2.5. Response of <i>T. castaneum</i> to wheat extract + DMD.....	61
2.6. Response of <i>T. castaneum</i> to four doses of volatile compounds associated with stored-grain microorganisms.....	62
2.7. Response of <i>T. castaneum</i> to the green leaf volatile compounds.....	64
2.8. Response of <i>T. castaneum</i> to various compounds + DMD.....	66

## LIST OF FIGURES

Figure	Page
<b>Chapter II</b>	
2.1. Standard design and modified pitfall traps.....	68
2.2. Mean response index (RI) of <i>T. castaneum</i> to each of the individual pitfall trapping oil components as well as the oil mix in the pitfall bioassay.....	70
2.3. Mean response index (RI) $\pm$ SE of <i>T. castaneum</i> to pitfall trapping oil samples with and without the antioxidant BHT added, in the pitfall bioassay.....	72
2.4. Response of <i>T. castaneum</i> to three unbaited trap designs in the metal tray bioassay.....	74
2.5. Response of <i>T. castaneum</i> to Trapper and Window sticky traps, with and without DMD lures in the metal tray bioassay.....	76
2.6. Captures of three species of stored-product beetles by pitfall and Detector sticky trap designs in a flour mill.....	78
2.7. Difference in weight (g), from dust accumulation, of uncovered and covered pitfall traps after one week in a flour mill.....	80
2.8. Mean % $\pm$ SE response of <i>T. castaneum</i> to standard and modified pitfall trap designs in the metal tray bioassay.....	82
2.9. Capture of <i>T. castaneum</i> by standard and modified pitfall traps.....	84
2.10. Response of <i>T. castaneum</i> to the pitfall trap components in the metal tray bioassay.....	86
2.11. Capture of <i>T. castaneum</i> in modified pitfall traps with or without DMD lures within three locations of a flour mill.....	88
2.12. Capture of <i>T. castaneum</i> in multiple funnel traps with and without DMD pheromone lures outside a flour mill.....	90

### Chapter III

3.1. Layout of mills 1 & 2.....	114
3.2. Total capture of stored-product beetle pests throughout mill 1 during 1997.....	116
3.3. Total capture of stored-product beetle pests throughout mill 1 during 1998.....	118
3.4. Total capture of stored-product beetle pests throughout mill 2 during 1998.....	120
3.5. Capture of the three most commonly trapped beetle pests by location within mill 1 during 1997.....	122
3.6. Capture of the three most commonly trapped beetle pests by location within mill 1 during 1998.....	124
3.7. Capture of the three most commonly trapped beetle pests by location within mill 2 during 1998.....	126
3.8. Trap capture throughout mill 1 and counts of <i>T. castaneum</i> from six load-out system tailings during 1997.....	128
3.9. Trap capture throughout mill 1 and counts of <i>T. castaneum</i> from six load-out system tailings during 1998.....	130
3.10. Correlation of <i>T. castaneum</i> trapped in mill 1 to those sampled from bulk load-out tailovers during 1998.....	132
3.11. Capture of <i>T. stercorea</i> , <i>C. ferrugineus</i> , and <i>T. castaneum</i> throughout mill 2 during 1998.....	134

### Chapter IV

4.1. Placement of traps around mill 2 for the moth dispersion study.....	153
4.2. Capture of <i>P. interpunctella</i> by location within mill 1 during 1997.....	155
4.3. Capture of <i>P. interpunctella</i> by location within mill 1 during 1998.....	157
4.4. Biweekly capture of <i>P. interpunctella</i> throughout mill 1 during 1997.....	159
4.5. Weekly capture of <i>P. interpunctella</i> throughout mill 1 during 1998.....	161

4.6. Comparison of biweekly capture of <i>P. interpunctella</i> in traps outside, and on ground floors within mill 1 during 1997.....	163
4.7. Weekly capture of <i>P. interpunctella</i> during 1998.....	165
4.8. Regression of <i>P. interpunctella</i> captures at various distances from a flour mill....	167

Introduction

1-10

considerable area, in post-harvest

1-11

of the area, in their posts, etc.

**CHAPTER I**

1-12

1-13

1-14

**LITERATURE REVIEW**

1-15

1-16

1-17

1-18

1-19

1-20

1-21

## Introduction

Stored-product insects pose a considerable threat to post-harvest food commodities. Although estimations of dollar loss due to these pests are difficult to determine; however, Pimentel (1991) placed the amount at \$5 billion per year to post-harvest food commodities in the United States (Pimentel 1991). Furthermore, worldwide food losses to stored-product insects are estimated to be 5-10% (Pedersen 1978, Burkholder 1990). It is therefore a constant challenge to those in the food industry to control the damage done by these insects.

Beetles in the genus *Tribolium*, in particular the red flour beetle, *Tribolium castaneum* (Herbst), and the confused flour beetle, *Tribolium confusum* Jacquelin du Val, are major pests of many stored-food products and are commonly found in cereal processing facilities such as flour mills. Present pheromone based trapping systems for monitoring *Tribolium* have been perceived as ineffective (Phillips 1994, Trematerra et al. 1996), and therefore efforts to improve on these systems are of considerable interest.

The Indianmeal moth, *Plodia interpunctella* (Hubner) is a worldwide pest on many stored food commodities (Sinha & Watters 1985). Traps baited with the moth's sex pheromone are commonly used and successful at monitoring this pest's activity (Vick et al. 1990). Although several trapping studies have targeted this moth, relatively little has been done to explore the outdoor dynamics of this species relative to stored-product facilities.

Methyl bromide (MB) is commonly used as a structural fumigant in flour mills and other food processing facilities (Chakrabarti 1996). This compound will be banned for use in the U. S. on 1 January 2005, under the Clean Air Act. Researchers and



industry personnel are scrambling to discover and develop alternative control methods. Trapping to monitor insect activity within the context of an integrated pest management (IPM) program will likely increase in practice and necessity as a result of the MB ban.

This project sought to explore ways of enhancing *Tribolium* trap effectiveness by assessing the attraction of a variety of food materials and compounds for attraction to *T. castaneum*. Trap designs and modifications were also assessed for attraction to *T. castaneum* and other stored-product beetles. In addition, trapping studies were conducted during 1997 and 1998 at two flour mills, targeting *T. castaneum* and *P. interpunctella*. These studies included outdoor trapping for *P. interpunctella* with respect to the mills.

#### **Insect Pests in Food Processing Centers and Flour Mills**

Flour mills and other cereal processing facilities host several species of stored-product insects. These types of facilities provide an ideal habitat in particular to secondary storage insects (e.g., *T. castaneum* and *P. interpunctella*) that cannot infest sound grains. Good (1937) surveyed common insect pests in flour mills by sampling the elevator boots in 17 mills throughout Missouri, Kansas, and Oklahoma. *Tribolium* were the most common pests, present in 78% of all samples, and 97% of all infested samples. *P. interpunctella* is common within flour mills also (Cotton et al. 1945). These moths are not commonly found in milling machinery or flour stocks; however, they offer a considerable threat to packaged flour as the gravid females seek a suitable oviposition site (Phillips & Strand 1994). Other common pests of flour mills include species in the genera *Oryzaephilus*, *Trogoderma*, *Cryptolestes*, *Sitophilus* (Agricultural Research Station 1913, Cotton et al. 1945) and the less economically important pests in the genera

*Ahasverus* (Woodroffe 1962) and *Typhaea* (Jacob 1988), which seem to prefer storage molds, but may also feed on grain and grain products directly.

The flour mill environment may be viewed as two separate compartments, the product milling stream, and the physical area outside the product line including machinery, walls, floors, etc. Mill elevator boots or bulk stored flour bins are areas where *Tribolium* are commonly found and likely represent a final stop for these beetles because these areas provide abundant food supply and are relatively dormant environments. Although directly sampling the flour stock to assess infestation is important and a good way to make decisions on control measures, knowledge of pest activity outside the milling stream can lead to control measures that would protect the product from infestation. A system of trapping throughout the mill can provide this knowledge and identify "hot spots" of insect activity that allow for more precise control measures. Chemical or physical spot treatments of problem areas could then be applied, thus avoiding, or delaying, the need of costly mill-wide fumigations (Vick et al. 1990). Pheromone trapping can also provide data on the dynamics of a pest population that would allow for better timing of control measures (Silverstein 1981; Chambers 1990; Vick et al. 1990; Phillips 1994, 1997)

### **Methyl Bromide**

Methyl bromide (MB) is commonly used in food processing facilities as a structural fumigant for control of pests. Benefits of using MB over alternative methods include reduced exposure time, rapid dispersion, little to no residues and lower production down-time costs (Chakrabarti 1996). However, this fumigant is scheduled to be banned by 1 January 2005 as it is suspected as an ozone-depleting compound. This

deadline, stipulated by the U. S. Clean Air Act, is in accordance with the Montreal Protocol, a treaty among 167 countries that calls for a 100% phase-out of MB by 2005 in developed nations, including the U. S.

Alternative control treatments to MB include the use of phosphine gas and controlled atmosphere methods including CO<sub>2</sub> and heat treatments. However, phosphine is difficult to use for structural fumigation because it is highly corrosive to copper, and may damage any equipment such as electrical devices that use copper contacts. Phosphine also requires a much longer exposure time than methyl bromide for effective kill, and many species have developed resistance to it (Annis & Waterford 1996). Heat treatments are another alternative (Bell 1996), but are difficult to use effectively because of the challenge of maintaining sufficient temperatures for an adequate amount of time to achieve satisfactory kill, particularly in many older buildings that are difficult to seal due to abundant cracks and other losses of structural integrity. Furthermore, certain equipment cannot withstand the extreme temperatures and must be removed, increasing the cost of treatment in time and labor. Sealing the structure is also a problem with CO<sub>2</sub> treatments, and exposure time is much greater than with MB (Bell 1996). One of the most promising alternatives presently being studied involves a combination treatment using heat, CO<sub>2</sub>, and low levels of phosphine (Mueller 1994).

An IPM program can be considered an alternative to MB fumigation and will likely increase in implementation in food processing facilities with the ban of MB (Phillips 1997). An IPM program calls for close monitoring of insect activity to include insect trapping (Flint & van den Bosch 1981). Therefore, an objective of this research was to explore implementing trapping programs within mills. Trapping within a facility

immediately before and after chemical treatment has been used to estimate fumigation impact on the pest populations (Levinson & Buchelos 1979, Sifner & Zdarek 1982), and was another goal of the present study.

### ***Tribolium castaneum* and *T. confusum***

*Tribolium* (Coleoptera: Tenebrionidae) are dark reddish brown beetles, approximately 4 mm in length with a flattened oval body (Sokoloff 1974). Development from egg to adult may take as few as 30 days. Eggs are laid singly throughout its food source, are kidney shaped, nearly transparent and small (0.6 mm in length by 0.35 mm in width) (Brindley 1930). Furthermore, the eggs are sticky and readily adhere to fine particles such as flour, making them very difficult to recognize (Good 1936). Good (1936) found that incubation lasts from 3-5 days at 30° C. Larvae are mostly white, elongate and cylindrical ranging in length from approximately 1.2 mm (first instar), to 6.0 mm (sixth instar) (Brindley 1930). Development of larvae ranges from 22 to 100 days depending on food source and environmental conditions (Good 1936). Mature larvae will come to the surface of the food medium to pupate. The period of pupation ranges from six to twelve days (Good 1936). Adult *Tribolium* may begin mating one to two days after emergence. The beetles have been known to live as long as three years. Males may be fertile their entire lifetime and females can lay eggs for over one year, averaging 327 eggs laid per female (Good 1936). Adults are fairly resistant to starvation; Good (1936) found the period of survival without food to range from 18 to 54 days.

There are several practical implications of the biology of *Tribolium*. All active stages of the insect feed on stored food products, which makes them a pest throughout their lifetime. The insects are long lived and are capable of reproducing during most of

their adult life; thus, they offer an incredible potential for exponential population growth. Finally, because these beetles can survive for extended amounts of time without food, they can cause infestations in facilities that have discontinuous food sources.

There is no apparent seasonal activity-regulating behavior among *Tribolium* beetles. In heated facilities, the beetle remains active all year round. However, in unheated structures, cold temperatures retard activity. Immature stages are more susceptible to the cold, and adults may be the only life stage present in these situations. *T. castaneum* tends to prefer warmer climates than *T. confusum* (Good 1936).

*Tribolium* adults are highly mobile and will soon infest all the available food in a warehouse or mill if not controlled. As a species, *T. castaneum* is more apt to dispersal than *T. confusum*. Based on this and other observations, Zeigler (1976, 1977, 1978) concluded that *T. castaneum* is a primary colonist, and *T. confusum* a secondary colonist. Although both species have well-developed wings, only *T. castaneum* has been observed in flight.

Male *T. castaneum* and *T. confusum* produce an aggregation pheromone (4,8-dimethyldecanal) that was first identified and synthesized by Suzuki (1981). In laboratory experiments, the greatest amount of pheromone was produced when the beetles were feeding in relatively low density (Hussain 1993, Hussain et al. 1994).

Beetles in the genus *Tribolium*, like many tenebrionid beetles, produce defensive secretions in the form of quinones from adult odoriferous glands (Alexander & Barton 1943). Quinones are highly oxidizing and are even toxic to the beetle itself (Roth & Howland 1941). The secretion of quinones into flour leads to contamination of the medium and is believed to contribute to beetle emigration (Ogden 1969; Ziegler 1976,

1977, 1978). While the function of these secretions is often defensive in other Tenebrionidae species, this is likely not an important function for *Tribolium* infesting stored food as they have relatively few natural enemies; however, these secretions have been proposed to play an interesting role that contributed to the success of this beetle as a stored-product insect. The secretions are toxic to micro-organisms in food sources and likely play an important ecological function to the beetle by keeping their food material relatively free of micro-organisms that might otherwise make the habitat unsuitable (Van Wyk et al. 1959). Engelhardt et al. (1965) observed that mutant *T. confusum* produced greatly reduced amounts of quinones, and that the flour they were infesting became moldy and caked, therefore, unsuitable to the beetle.

*Tribolium* are known to infest a wide variety of both animal and plant-produced products. Good (1936) listed over 100 food items that *T. castaneum* could be found infesting. In addition to whole grain and cereal products, this list includes: animal matter such as hides and bird skins; preserved insect specimens; pollen and possibly dead insect matter in bee cells; milk powder; wood, particularly ash and pine, likely as scavengers, not feeding on the wood itself; plant products such as yams, garlic, cured tobacco, dried cornstalks, snuff, and orris root; and also the spices of nutmeg and ginger.

Sokoloff (1974) described *Tribolium* as an opportunistic generalist feeder. He concluded that *Tribolium*'s natural habitat was likely as a scavenger under the bark of trees, feeding primarily on eggs and pupae of its own species and others, and on fungi, bacteria and carbohydrates secondarily. This conclusion is in accordance with the occasional observance of *Tribolium* under tree bark today (Andres 1931, Good 1936). Furthermore, many other beetles in the family Tenebrionidae commonly inhabit tree bark

(Borror et al. 1989). *T. castaneum* has additionally been documented to occur in rodent burrows and ant nests (Khare & Agrawal 1964). Despite their origin, *T. castaneum* and various other species of the genus *Tribolium* have long been associated with stored-food. Beetle remains, believed to be that of *T. confusum*, were found in a jar of presumed cereal product of a pharaonic tomb, dated at 2500 B.C (Andres 1931).

From the above information, it may be observed that this beetle is a potential pest of several foodstuffs. Therefore, improving traps for this insect is of great concern to many producers and retailers. Also, since flour is not likely this beetle's original diet, a variety of other food based trap lures may be attractive to them.

*Tribolium* causes damage in a couple of ways, first, they contaminate their food source by their mere presence, and also with frass, exuviae and dead insect body parts (Mondal & Port 1994). In addition, the release of quinones by the beetles imparts a pungent odor to their food source, will discolor flour to a grayish pink and reduce its elasticity and viscous properties (Payne 1925). The effects of quinone-contaminated food to mammals have been investigated and are suspected to be a carcinogenic (El-Mofty et al. 1989, 1992).

### **Trapping of *Tribolium* and Other Stored-Product Beetles**

Trapping with in a food-storage facility offers some unique challenges. Traps must overcome factors such as competing food attractants, alternative shelter and possible competing pheromone production by other insects of the same species. Stejskal (1995) demonstrated that traps for *T. castaneum* were progressively less effective in laboratory experiments when paper shelters were introduced into the arena. Trap efficacy was further diminished when food was added, and least when food and shelter



where added together. In many cases, abundant alternative shelter is present in the vicinity of an insect trap including machinery, shelving, holes and cracks in walls and floors, etc. These areas commonly contain spilt food products also, which sustain insect populations. These sources of shelter and food likely reduce the movement of the beetles and reduce trap capture.

DeCoursey (1931) designed a trap made of corrugated paper baited with flour to successfully trap the confused flour beetle out of corn and flour. Because of the habit of *Tribolium* to seek shelter, corrugated paper has been a popular choice for a trapping material. Although this trap was effective in isolating the beetle from infested materials, it did not serve to kill the beetles; therefore, traps had to be destroyed after use to prevent propagating the insect.

Pinniger (1975) developed a trap known as a bait bag, which consisted of a mesh envelope that served both to hold a food bait, and as a sieve to separate insects from food when inspecting the traps. Pinniger evaluated various food baits within this trap and found that a bait of wheat, groundnuts and carobs was most attractive to several species of stored product insects (Pinniger et al. 1984). These traps also require frequent observation and servicing, as they do not kill insects and might be an unintentional source of further infestation.

A major advance in the development of more effective *Tribolium* traps was the incorporation of synthetic *Tribolium* aggregation pheromone. Barak & Burkholder (1985) designed a pitfall type trap using *Tribolium* pheromone constructed from four layers of corrugated paper in a 9 cm square. This trap also used an oil lure consisting of a mixture of mineral and wheat germ oils combined with a pentane extract of raw rolled



oats. The oil bait was useful in attracting the beetle species *Oryzaephilus surinamensis*, *T. confusum*, and *Trogoderma variabile*, and also served to suffocate the insects that fell into the pitfall cup. This design was patented, and sold commercially for several years.

The trap designed by Barak & Burkholder was eventually replaced with a trap first designed by Mullen (1992). This trap also used an oil and pheromone bait; however, instead of using corrugated paper, this trap took advantage of the negative geotaxis habit of the insects by using an inverted concave ramp that had a 4.0 cm hole drilled in the center. Insects crawling up the ramp would then fall into the center pitfall into the oil bait. Mullen found this trap to be superior to previous designs. This latest design is currently sold as the storgard Flit Trak® M<sup>2</sup> by Trécé, Inc. (Salinas, CA), and is used in much of the lab and field research of this project.

Dethier et al. (1960) clarified definitions of chemicals that elicit insect behavior. He made distinctions among chemicals that act as arrestants, locomotor stimulants, attractants, and repellents. He noted that the same chemical might elicit one, or more, of the above responses depending on factors such as concentration. All references to a chemical or food material that elicits an attractant response will assume his definition, "Attractant - a chemical which causes insects to make oriented movements towards its source." An arrestant can be defined as a chemical that causes insects to aggregate when coming into contact with it. Chemicals acting as arrestants have been confused with attractants because the same end result occurs. However, the method by which aggregation occurs differs in the two.

Many insects use semiochemicals produced from, or associated with, their host in food location (Phillips 1997). Several traps for stored-product pests have incorporated

food-based lures both in combination with pheromone and as the only attractant (Chambers 1990, Pinniger 1990, Phillips 1997). An objective of this research was to screen potential food materials and single volatile compounds associated with many host plants for attractiveness to *T. castaneum*. Better understanding of semiochemicals attractive to *T. castaneum* will allow for more effective traps to be used for monitoring *Tribolium* or possibly even control. A good example of this type of work is the extensive research that has been done to identify attractants associated with food and food-related microorganisms to beetles in the genus *Oryzaephilus* (Pierce et al. 1981, 1990, 1991a; Freedman et al. 1982; Mikolajczak et al. 1984; Stubbs et al. 1985).

Attractive responses by *Tribolium* to semiochemicals associated with the beetles' common food sources have been demonstrated. Willis & Roth (1950) studied the olfactory attraction of *T. castaneum* to flour at various moisture contents and discovered that the beetle was attracted to flour based on olfactory stimuli when starved from 2 to 7 days. Seifelnasr et al. (1982) demonstrated attraction of *T. castaneum* to whole wheat, wheat endosperm and wheat germ extracts, with wheat germ extracts being most attractive. Phillips et al. (1993) observed attractive responses from *T. castaneum* to rice, soybean, oat, wheat germ, and corn oils. Hussain (1993) found that *T. castaneum* was significantly attracted to wheat germ nuts, a processed food product. He also observed that this same food product was able to enhance the attractiveness of DMD to the beetle.

*Tribolium* have been documented to feed on fungi associated with stored grain, and some of the olfactory responses to these fungi have been assessed. Imura (1991) when comparing the feeding habits of *T. castaneum* to *T. freemani* noted that both species developed well on a diet of the fungus *Alternaria alternata*. Van Wyk et al. (1959)

identified *Aspergillus glaucus*, *A. flavus*, *A. candidus*, and an unidentified species of the genus *Penicillium* as fungal species associated with *T. confusum*. These species are also common storage fungi (Abramson et al. 1980, Seitz & Sauer 1992). Van Wyk also reported unidentified bacteria that were isolated from the insect's gut, and found within the food of *T. confusum*. Based on olfactory stimuli, the beetles were attracted in laboratory experiments to both treatments of this bacteria added to flour, and flour plus the fungi, significantly more than to autoclaved flour alone. The response of *Tribolium* to the lightweight compounds produced from the metabolic processes of microorganisms associated with the beetle and its food should be investigated further. These compounds may significantly enhance *Tribolium* attraction to pheromone and/or grain based food lures when used in combination by simulating the olfactory cues of an ideal habitat to the beetles.

Compounds such as 3-methyl-1-butanol, 1-octen-3-ol, 3-octanone, and 1-octanol have been identified as some of the volatiles associated with storage fungi in the genera *Aspergillus*, *Penicillium* and *Alternaria* (Abramson et al. 1980, Seitz & Sauer 1992). It is interesting to note that 1-octen-3-ol, as well as being a fungal volatile, is also a known aggregation pheromone for *Oryzaephilus* spp. and *Ahasverus advena* (Waltl), the foreign grain beetle (Pierce et al. 1989, 1991b). Food baits containing volatiles such as these may be capable also of attracting *Tribolium*, as well as other insect pests. Volatiles associated with grain bacteria have also been identified (Seitz & Sauer 1992), and should be examined for potential food bait enhancement.

### *Plodia interpunctella*

Adult moths are 9 mm in length, with a 20 mm wing span, trunk whitish yellow in color. The most striking characteristic of these insects is the copper color with two dark-brown lateral bands on the distal half of the forewings (Zakladnoi & Ratanova 1973).

Eggs are laid in groups, or singly, in or near a larval food source and incubate for 2-17 days. Larvae are whitish in color, go through five instars, and complete development in 13 to 288 days, depending on temperature. The mature larva spins a cocoon in which to pupate. The pupal stage lasts for an average of 15 days. Development from egg to adult takes an average of 26 days at 30°C and 70% relative humidity. Adults are short-lived (5-13 days), and do not feed. They begin mating as soon as one hour after emergence (Sinha & Watters 1985).

In heated facilities the moth multiplies throughout the year. In unheated buildings, mature larvae enter diapause as a combined result of lower temperature and shorter photoperiod (Tzanakakis 1959). The moth averages five generations a year (Sinha & Watters 1985). Unlike *Tribolium*, the larval stage is the only damaging life stage of this insect.

Adult female moths produce Z-9, trans-E-12-tetradecandienyl acetate (ZETA) as a component of their sex pheromone (Brady & Nordlund 1971; Brady et al. 1971; Kuwahara et al. 1971). This compound is also a pheromone component of other stored-product moths of the subfamily Phycitinae.

Like *T. castaneum*, *P. interpunctella* is a pest on a wide variety of foods. They are known to infest stored grains, cereal products, nuts, dried fruits, chocolate, dried roots, herbs, and dead insects (Hill 1990). The moth larvae damage foodstuffs by

contamination with exuviae, fecal matter, dead insects, and like material. In addition, larvae also contaminate food with webbing, which can clog milling and grain movement machinery (Cox & Bell 1991).

### **Pheromone Trapping of *P. interpunctella***

Traps using synthetic ZETA as a lure are effective and widely used to trap male Phycitinae moths for survey and detection (Chambers 1990, Vick et al. 1990). Pheromone baited traps have proven successful at detecting otherwise unknown infestations of these moths (Vick et al. 1981). Although many studies have focused on capture of phycitine moths inside storage and food processing structures (e.g., Hoppe & Levinson 1979, Vick et al. 1986), only a few studies have attempted to document outdoor activity of *P. interpunctella*.

In an early study of *P. interpunctella*, Ganyard (1971) used traps baited with virgin females as natural pheromone sources and captured < 1 per trap per day at numerous outdoor locations far from grain storages. A limited trapping study by Vick et al. (1981) with synthetic pheromone found *P. interpunctella* and the almond moth, *Cadra cautella* (Hubner), inside a food warehouse, but no moths were trapped outdoors on the loading dock. Cogburn & Vick (1981) trapped similar high numbers of *C. cautella* both inside and immediately outside rice storage bins, but recorded very low numbers of this moth at field sites further away. Vick et al. (1987) placed pheromone traps for four species of storage moth pests in five outdoor locations along a 56 km transect from a peanut warehouse and trapped substantial numbers of *P. interpunctella* only in the yard immediately outside the structure. These early studies suggest that outdoor occurrences of moths such as *P. interpunctella* and *C. cautella* can be attributed to emigration from

nearby storages, and that these species do not breed in wild habitats. Some researchers have concluded that moth infestation inside a food processing facility is mainly attributed to the introduction of infested product (Levinson & Buchelos 1979) rather than by immigration of adults from outdoor locations.

### Study Objectives

The specific objectives of this research can be divided into three broad areas that correspond with the three following chapters of this thesis. The first set of objectives, which correspond to chapter II were: 1) Evaluate grain and food oils and other compounds from plant and microbial origin, as attractants and pheromone synergists for *T. castaneum*; 2) Evaluate traps for *T. castaneum* in laboratory and field, with specific goals of comparing pitfall with sticky traps, effects of pheromone and oil on response to traps, the potential for improving pitfall trap performance with dust protection, and evaluate response of the beetle in flight to a pheromone baited trap. Objectives of chapter III were: 1) determine pest species present and their distribution in space and time, 2) monitor *T. castaneum* activity before and after methyl bromide fumigation to assess efficacy of treatment, and 3) correlate *T. castaneum* trap capture to *T. castaneum* counts from direct sampling of the product. Specific objectives of chapter IV were: 1) to observe variation in moth activity in space and time both inside and outside a flour mill. 2) determine *P. interpunctella* activity before and after methyl bromide fumigations to obtain a relative measure of fumigation impact on the moth population, and 3) evaluate the outdoor dispersion of the moth at various distances from a second mill.

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CHAPTER II

RESPONSE OF *TRIBOLUM CASTANEUM* AND OTHER STORED-PRODUCT  
BEETLES TO TRAPS AND SEMIOCHEMICALS



## Abstract

A series of laboratory and field experiments were performed to assess the responses of *Tribolium castaneum* (Herbst) and other stored-product beetles to various semiochemicals, traps, and trap components. Of the single volatile compounds assayed, 3-methyl-1-butanol showed the most promise as an attractant to *T. castaneum*. An experiment to evaluate the effects of aging on the trapping oils with and without the antioxidant butylated hydroxytoluene (BHT) added revealed that the oil with BHT became repellant to the beetle over time. A commercial *Tribolium* pitfall trap was superior in both laboratory and field experiments over the other floor trap designs assessed at capturing *T. castaneum*. In field experiments, *Typhaea stercorea* (L.) and *Ahasverus advena* (Stephens) preferred a sticky trap to the pitfall trap. The synthetic *Tribolium* aggregation pheromone lure is an important component of the pitfall trap's efficacy to *T. castaneum*. Although the food-based pitfall trap-trapping oil was not found to be attractive to *T. castaneum* when assayed alone, it did have value as an enhancer of the pheromone bait when the two were used together in the trap. A dust cover modification made to go over the pitfall trap was effective in protecting the trap from dust, although the trap was still vulnerable to dust contamination from sanitation techniques that used compressed air to blow down the mill floors. Capture of *T. castaneum* in the modified trap performed as well as the standard trap design in a non-dusty area of a flour mill, and significantly superior over the standard trap in a dusty area. *T. castaneum* responded in flight outside a flour mill preferentially to multiple funnel traps with pheromone lures over traps without pheromone.

## Introduction

Beetles of the genus *Tribolium*, in particular the red flour beetle, *Tribolium castaneum* (Herbst), and the confused flour beetle, *Tribolium confusum* Jacquelin du Val, are major pests of many food commodities. A current method of monitoring *Tribolium* spp. is the use of pheromone and food-oil baited traps (Burkholder & Ma 1985). Despite improvements to *Tribolium* trap efficacy in recent years (Mondal & Port 1994), these beetles are still thought to be rather difficult to monitor via trapping (Phillips 1994, Trematerra et al. 1996). Therefore, efforts to identify attractive materials that could make traps more sensitive, and to evaluate and improve current *Tribolium* traps designs, are of considerable interest.

Male *T. castaneum* produce an aggregation pheromone when actively feeding at relatively low density (Hussain 1993, Hussain et al. 1994b). Suzuki (1981) first identified and synthesized this pheromone as 4,8-dimethyldecanal (DMD) from males of both *T. castaneum* and *T. confusum*. The incorporation of synthetic DMD lures into *Tribolium* traps was a major improvement to trap efficacy.

Commonly used *Tribolium* traps include the Storgard Flit Trak® M<sup>2</sup> (Trécé, Inc. Salinas, CA) (Mondal & Port 1994), which was manufactured after the prototype designed by Mullen (1992). The trap is a ramp-pitfall design which utilizes both DMD pheromone and a food-based oil as attractants (Chambers 1990, Pinniger 1990, Barak et al. 1991). Other common trap designs include floor mounted adhesive traps that are used unbaited or baited with *Tribolium* pheromone lures (Barak et al. 1991, Mondal & Port 1994).

The trapping environment can affect the efficacy and longevity of traps (Barak et al. 1991). Tingle & Mitchell (1975) during moth trapping studies demonstrated that adhesive traps could be detrimentally affected by dust. Large amounts of flour dust is produced as a result of milling processes in flour mills and has been shown to lower efficacy of sticky and pitfall traps (Chapter III). These observations led to the development of a dust-cover modification to the pitfall trap.

An issue of concern when using a food-based oil attractants in traps is the effect of age on oil attractiveness. Natural processes such as auto-oxidation alter the chemical composition of the material causing rancidity. Although this can negatively affect the attractiveness of oil lures, there is evidence that it can actually increase attraction. Barak (1989), while developing a trap for *Trogaderma granarium* Everts tested several oils for their attraction to the beetle and noted how the beetle responded to them over time. He observed that while oat, pumpkin, and sesame oils were more attractive than wheat germ oil while fresh, their attractiveness quickly diminished with age and wheat germ oil actually increased in attractiveness over time. Barak referred to an oxidation theory proposed by O'Donnell et al. (1983) to explain these data, which suggested that the auto-oxidation of unsaturated fatty acids results in the formation of volatile carbonyls and aldehydes that are potentially attractive to insects. Because wheat germ oil has a high composition of unsaturated fatty acids relative to the other oils tested (Tamaki et al. 1971), Barak suggested that the results from his study were consistent with O'Donnell's theory. Butylated hydroxytoluene (BHT) is a common synthetic phenol used as a food additive that reduces the rate of auto-oxidation in oils and fats (Sherwin 1985), and has not been studied as a preservative in oil baits for *Tribolium*.

Semiochemicals associated with food sources have often been demonstrated as co-attractants or synergists with pheromones (Chambers 1990, Phillips et al. 1993, Phillips 1997, Pinniger 1990). Lightweight compounds produced from the metabolic processes of microorganisms associated with *Tribolium* and its food may act as a strong attractant to the beetle either alone, or may significantly enhance the beetles' attraction to pheromone and/or grain based food lures when used in combination. Some work has been done to explore the odors from microorganisms associated with *Tribolium*. Van Wyk et al. (1959) identified *Aspergillus glaucus*, *A. flavus*, *A. candidus*, and an unidentified species of the genus *Penicillium* as fungal species associated with *T. confusum*. He also reported unidentified bacteria that were isolated from the insect's gut, and found within the food of *T. confusum*. Based on olfactory stimuli, the beetles were attracted in laboratory experiments to both treatments of this bacteria added to flour, and flour plus the fungi, significantly more than to autoclaved flour alone. These findings hold potential for the enhancement of attractants in traps for *Tribolium*.

Six-carbon alcohols and aldehydes known collectively as green leaf volatiles are natural products of plant lipid degradation and are ubiquitous among plants (Visser et al. 1979). They have been shown to enhance the attraction of pheromones in other insect species (Dickens et al. 1990), and may be attractive to *Tribolium* as well.

Objectives of this present study were: 1) Evaluate grain and food oils and other compounds from plant and microbial origin, as attractants and pheromone synergists for *T. castaneum*; 2) Evaluate traps for *T. castaneum* in the laboratory and field, with specific goals of comparing pitfall with sticky traps, effects of pheromone and oil on response to

traps, the potential for improving pitfall trap performance with dust protection, and evaluate response of the beetle in flight to a pheromone baited trap.

## Materials and Methods

### Experimental Insects

All laboratory experiments were conducted using established colonies of *T. castaneum*. The insects were reared on whole-wheat flour and brewer's yeast (95:5) in standard quart jars (0.95 l). Colonies were maintained in a growth chamber at 28° C, 70% relative humidity, in complete darkness. Insects used in bioassays were four to six weeks old and were separated without food from their colonies 24 h prior to the experiment to enhance their olfactory response, and to ensure uniform starvation.

### Traps Evaluated and Pheromone Lures

Four trap designs, as well as a modification to one of the traps, were evaluated in field and laboratory experiments. The pitfall trap consisted of a 10 cm plastic circular ramp roughed to allow insects to crawl up with a cup in the center that held the food/oil bait, which acted as an attractant and also as a killing agent by suffocation (Fig. 2.1a). The inner edges of the cup were smooth to facilitate insects falling into the cup. The trap came with a cardboard cover that provided moderate dust and debris protection, as well as a means to hold the rubber septum impregnated with DMD. The traps contained 0.5 ml of oil for all lab experiments and 1 ml in field experiments. The pitfall trap modification consisted of a 10 cm PVC end-cap that replaced the standard cardboard cover (Fig. 2.1b). The cap rested on four plastic beads glued on its lower rim to allow beetles clearance to the ramp-pitfall under the cap. A 2 mm hole was drilled in the top of the cap to receive the pheromone septum lure.

Three sticky traps were assessed in these studies. The Detector trap (AgriSense, Palo Alto, CA) was made of cardboard with a 9.5 X 6.5 cm sticky area. The trap folded in on itself top to bottom to provide protection to the sticky surface. The Trapper Monitor insect trap was provided from Bell laboratories. This trap was also made of cardboard with a 7.5 X 6 cm sticky area. The window sticky trap was provided by Agri Sense (Barak et al. 1991, Mondal & Port 1994). The trap's sticky surface was enclosed under a transparent plastic cover (window) approximately 3mm above the floor of the trap. Insects enter the trap through short ramps positioned on both sides of the trap and step or fall off onto the sticky surface. All sticky traps were tested both without pheromone lures and with the DMD pheromone lures provided with the pitfall trap.

Multiple funnel traps (Lindgren 1983) were constructed of plastic with several funnels approximately 19 cm in diameter, with a 5.5 cm hole at the bottom. Insects flying into the trap strike the underside of a funnel, and fall through the lower funnels into the collecting cup at the bottom of the trap. Strips of vapona were placed in the cup to kill captured insects. Traps used in this study were eight tier (eight funnels).

The pheromone lures provided with the Trécé pitfall traps were used in the laboratory trap assessments experiments and field experiments when traps were pheromone baited. When used in laboratory experiments, the lures were aged for three days in a fume hood prior to use to avoid a repellent release rate immediately after opening (Hussain 1993, Hussain et al. 1994a).

### **Semiochemicals**

A series of materials were assayed with the goal of identifying substances attractive to *T. castaneum*. The response of the beetle to a hexane extract of wheat was



assessed. The extraction procedure involved crushing a total of 982.3 g of sound wheat in four parts for 30 s in a blender. The crushed wheat was then placed in hexane for 42 h, after which the extract was filtered from the crushed wheat through a funnel lined with filter paper. The extract was distilled down to 10 ml making a 98.23 mg/ml wheat equivalent (MGE) extract. The extract was diluted (1/10) in hexane three times making concentrations of 9.82, 0.982, and 0.0982 MGE. The following food-based oils were assessed for attraction to *T. castaneum*: wheat germ, sesame, walnut, flax, corn, castor, grapeseed, safflower, sunflower, hemp, peanut, avocado, vegetable, apricot, olive, canola, almond, and coconut. Oils were purchased from a local specialty food store and were stored in complete darkness at 5° C. Oil manufacturers were contacted, if possible, in order to obtain information regarding processing, manufacturer date and oil shelf life (Table 2.1). The literature was reviewed relative volatile compounds given off by fungi and bacteria associated with wheat and other grains in order to compile a list of compounds to be assessed as potential attractants to *T. castaneum* (Hougen et al. 1971, Abramson et al. 1980, Seitz & Sauer 1992). The following fungal and bacterial volatiles were identified and purchased for evaluation: 1-octen-3-ol, vinyl acetate, phenylacetaldehyde, anisole, 3-methyl-1-butanol, 3-methyl-2-butanone, 3-octanone, 2-methyl phenol, 1-octanol, and styrene. The following green leaf volatiles were tested: hexanal, hexanol, *t*-2-hexanal, *c*-2-hexen-1-ol, *t*-2-hexen-1-ol, *c*-3-hexen-1-ol, and *t*-3-hexen-1-ol. All these chemicals were obtained commercially (Aldrich Chemical Company, Milwaukee, WI) and were  $\geq 90\%$  pure. A neat sample of DMD was provided from Trécé Inc. and was diluted to various concentrations in hexane for use in pheromone synergism experiments in the two-choice pitfall bioassay (see below).



Trécé Inc. provided fresh samples of the trapping oil used in the pitfall traps for the oil aging study. Two samples of the oil were provided, one with BHT added (as sold commercially), and the other without BHT. Samples of each of the individual components of the trapping oil were also provided for an isolated assessment of *T. castaneum* response to each component.

### **Bioassays**

**Tray Assay.** A tray bioassay was used to evaluate the response of *T. castaneum* to different trap designs and components (Hussain et al. 1994a). The bioassay consisted of a 92 X 92 X 9 cm steel tray with one layer of whole-wheat grains in the arena. A layer of grains provided a good substrate for moving as well as a natural environment to the responding beetles. The traps were tested one per tray, placed in the same randomly determined position in the arena a minimum of 15 cm from the tray sides. Experiments were set up in a randomized complete block design with blocks occurring on successive days. All experiments were replicated a minimum of four times. One hundred adult mixed-sex *T. castaneum*, were introduced into the center of the arena under an inverted 6 cm plastic petri dish for 15 min to allow them to calm and adjust to environmental conditions. The bioassay began after the beetles were released and a screen was placed over the tray to prevent escape. Bioassays were conducted for 20 h (2:00 p.m. – 10:00 a.m. of the following day) in total darkness at  $28 \pm 2^\circ \text{C}$  and  $70 \pm 10\% \text{ r.h.}$ , after which the number of beetles captured was recorded.

A series of four experiments were conducted using the metal-tray bioassay. In the first experiment the response of *T. castaneum* was assessed to trap designs without any attractants. The treatments were: 1) Detector sticky trap unbaited, 2) Trapper Monitor

sticky trap unbaited, and 3) pitfall trap unbaited (mineral oil in cup reservoir). The experiment was replicated four times. A second series of bioassays were conducted to compare the response of the beetle to the Trapper and Window sticky traps, both without an attractant and with DMD pheromone lures. The treatments were: 1) Trapper sticky trap without pheromone, 2) Trapper sticky trap with DMD lure, 3) window trap without pheromone, and 4) window trap with DMD lure. The experiment was replicated four times. In the next experiment the response of *T. castaneum* to each of the pitfall trap components was assessed. The assay contained the following treatments: 1) control trap, no pheromone nor oil baits, 2) trap with oil bait and no pheromone lure, 3) trap with pheromone and no oil bait (mineral oil in trap), and 4) trap with pheromone and oil baits. The experiment was replicated eight times. The fourth series of bioassays compared the pitfall traps with the standard cardboard cover to the PVC cap modified trap; both trap treatments used pheromone and food/oil lures. The experiment was replicated eight times.

Results from each of these experiments were analyzed in SAS (SAS Inst. 1996) by an analysis of variance (ANOVA) using PROC MIXED on arcsine square root transformed percent count data. In this, and all following ANOVA procedures, the null hypothesis was no significant difference ( $\alpha = 0.05$ ) in capture among the trap treatments. If ANOVA results were significant, a mean separation test (LSD) was performed.

**Two-choice Pitfall Assay.** Experiments to evaluate *T. castaneum*'s response to the wheat extracts, food-based oils, pitfall trapping oil and volatile compounds employed a two-choice pitfall bioassay similar to that used by Pierce et al. (1981). Beetles oriented to one of two holes in the floor of an arena, below which were placed stimulus or control

materials. Bioassay arenas were 9 cm glass petri dishes modified with two holes in the bottom approximately 6 mm in diameter, on opposing sides of the dish, 8 mm from the sides. The surface of the Petri dishes was roughed up with sandpaper to facilitate the beetle's footing and allow them to right themselves when overturned. Under each hole was placed a 13×45 mm vial with either the test or control substance. The following treatment amounts will apply to all like materials assayed unless noted otherwise. When testing oils, 0.1 ml of the test oil was compared to 0.1 ml of heavy mineral oil as the control. In preliminary experiments, beetles had no significant preference between the choice of heavy mineral oil or an empty vial. The mineral oil was an ideal control substance because the beetles were suffocated when falling into it, just as they were when falling into the various oils to be screened for attraction. Single volatile compounds were tested by applying 10 µl of the compounds in aliquots of hexane solutions to a 13 mm filter paper disk in the bottom of the vials. The control vial contained a filter paper disk with 10 µl of hexane alone. Two tables were constructed from plywood and pine lumber to allow for positioning of vials, and to support the petri dishes above them. Each table could accommodate twenty dishes, allowing four treatments of ten replications to be done at a time. Treatments were assigned in a completely randomized design across the forty available positions. A batch of 10 adult mixed-sex beetles was used in each dish. Prior to releasing beetles, they were held under a 17 mm glass vial for 15 min to allow them to calm and adjust to environmental conditions. The bioassay was run for 1 h in total darkness at  $28 \pm 2^\circ \text{C}$  and  $65 \pm 10\%$  r.h., after which the numbers of beetles found in the treatment dish, control dish, and remaining in the arena were recorded. Results were reported using a response index (RI), calculated by dividing the number of beetles

responding to the treatment vial by the total number of beetles out of ten responding to either the treatment or control vials, multiplied by 100 ( $[T/(T + C)] 100$ ). RI values could theoretically range from 0 for complete repellency, to 100 for complete attraction. Ten replicates were performed for each test material or combination of materials studied, unless otherwise noted. A one-population t-test was performed to evaluate the hypothesis that the mean RI was equal to 50 using SAS PROC UNIVARIATE. Materials or compounds exhibiting RI values significantly greater, or less, than 50 were deemed “attractive” or “repellant”, respectively.

A series of seven experiments utilized the two-choice pitfall bioassay. In the first experiment the response of *T. castaneum* to the wheat extract was assessed. The stock concentration, as well as the three dilutions, were assessed. The second series of assays involved evaluating *T. castaneum*'s response to the wheat extracts in combination with DMD to determine if any enhancement/synergism occurred when they were added together. These assays involved three treatments: 1) The 9.82 MGE wheat extract, 2) 0.000001 µg of DMD, and 3) the extract and DMD together. The third series of bioassays evaluated the response of *T. castaneum* to the eighteen food-based oils. The oils that tested attractive in either the study reported, or in preliminary assessments (corn, sesame, walnut, hemp, apricot kernel, wheat germ, and flax), were then assayed an additional time in two groups. The first assay was performed to observe the beetle's response to corn, sesame and walnut oils, and the second to apricot kernel, flax and hemp oils. A fourth series of experiments assessed the beetle's response to the four individual components of the pitfall trapping oil, as well as the oil mix. Because there were five treatments and 40 available bioassay devices, each oil component and the oil mix were

replicated only eight times. A fifth series of experiments tested the effect of aging on *T. castaneum*'s response to pitfall trap-trapping oil samples with and without BHT. Ten ml of each of the oils were placed in 50 ml beakers, covered with a filter paper disk, and left under a fume hood. Each week, for four weeks, samples from the oils in the beakers (aged) were compared to samples from the stock oils that were stored in darkness at 5° C (fresh). There were four treatments: 1) fresh trapping oil with BHT (fresh control with BHT), 2) fresh trapping oil without BHT (fresh control without BHT), 3) trapping oil aged one-four weeks with BHT, and 4) trapping oil aged one-four weeks without BHT. The sixth series of bioassays evaluated the response of *T. castaneum* to the 10 bacterial and fungal single volatile compounds, and the seven green leaf volatiles. These were evaluated in four doses (0.0001 µg, 0.01 µg, 1 µg, and 100 µg). The last series of experiments sought to identify volatiles that had a synergistic/enhancing effect on the attractiveness of the beetle when combined with DMD. All compounds that did not test repellent when tested alone in the previous series of experiments were used. This bioassay consisted of three treatments: 1) 1 µg of the volatile compound alone, 2) 0.000001 µg of DMD alone, and 3) 1 µg of the volatile compound + 0.000001 µg of DMD. The dose of DMD was determined by doing a dose response experiment in the two-choice bioassay where concentrations of DMD were reduced by 1/10 until a concentration was found that was not significantly attractive to the beetle alone.

### **Field Experiments**

Experiments were done in a flour mill (mill 1 of Chapters III & IV) in order to evaluate various traps, trap modifications, and trap components in field conditions. Comparison of the pitfall trap to the Detector sticky trap capture on the most commonly

trapped stored-product beetles (*T. castaneum*, *T. stercorea*, and *A. advena*) was done from 1 May to 26 November 1997. Traps were placed in paired treatment blocks on five floors of the mill (floors three to eight, Fig. 3.1), as well as an area above the bulk stored flour bins. Trap positions were not changed during the study. A total of 26 blocks were placed throughout the mill, 13 biweekly observations were taken and pheromone lures were replaced every four weeks. The contents of the pitfall traps were placed in a ziplock bag and taken back to the laboratory for identification under a dissecting scope. The sticky floor traps were collected, replaced with a new trap, and taken to the laboratory for identification of their contents. The mean beetle per trap counts were square root transformed and analyzed through an ANOVA (PROC MIXED) for differences among trap treatments.

A study was done in an attempt to both quantify the amount of dust accumulated in various areas of mill 1 during a one-week interval, and to assess the efficacy of the pitfall trap dust cover modification at protecting the trap from dust. The experiment consisted of the following two treatments: 1) ramp/pitfall portion of the pitfall trap with no cover, and 2) the ramp/pitfall portion of the pitfall trap covered with the PVC cap modification. The traps were placed in paired treatments (randomized complete block design). Mineral oil was placed in both trap treatments. Four distinct areas of the mill were chosen to place the traps, 1) the third floor of the mill, which was very dusty and also cleaned daily with a compressed air blow-down, 2) the second floor packing area, which was dusty and was cleaned once a week with compressed air blow-down, 3) the area below the bulk stored product, which was dusty and was not cleaned during this experiment, and 4) the warehouse area that was relatively dust free (see Chapters III &



IV). The traps were weighed in ziplock bags prior to deployment in the mill, and again after collecting. Any insects trapped during the study were removed so as to account only for increased weight due to dust and debris. The difference in the two weights was recorded and analyzed by treatment and mill location using PROC MIXED.

The response of *T. castaneum* to the standard design and modified pitfall traps was evaluated in two areas of the mill. The first experiment was conducted in a low dust area (warehouse 1, see chapters III & IV). This was done to assess the efficacy of the modified pitfall traps in field conditions, without dust interference. In addition to evaluating the PVC end-cap modification, the role of the *Tribolium* aggregation pheromone to *T. castaneum* capture was evaluated by in this experiment by employing the following four treatments: 1) standard trap design (cardboard cover) pheromone and food/oil baited, 2) standard trap design food/oil baited with no pheromone, 3) modified trap design (PVC cap) pheromone and food/oil baited, and 4) modified trap design, food/oil baited with no pheromone. Another experiment was done in a dusty mill area (feed area, see chapters III & IV) consisting of the following two treatments: 1) standard trap design (cardboard cover), pheromone and oil baited, and 2) modified trap design (PVC cap), pheromone and oil baited. In both experiments, treatments were arranged in a randomized complete block design, 3 m between each trap, 6 m between blocks. Three blocks were set up for the two-treatment experiment (feed area), and six for the four-treatment experiment (warehouse 1). Both experiments were conducted for sixteen days from 16 June to 2 July 1998. Traps were checked and serviced twice in eight-day intervals, and re-randomized halfway through the experiment. An ANOVA was performed using PROC MIXED on the square root transformed trap captures.

The response of *T. castaneum* to the eight tier funnel traps was assessed in an experiment setup outside, approximately 100 m north of the mill. Trap treatments were arranged in a randomized complete block design consisting of a trap with no attractant and one with a DMD pheromone lure. Traps were placed  $\approx$  2 m high attached to either a fence outlining the mill property or on utility poles within the mill yard, approximately 10 m apart. Traps for blocks one and two were placed on 16 June 1998. Block three was added on 17 September, and block four was added on 24 September. All traps were monitored until 5 November 1998. Traps were checked and re-randomized weekly, the trap contents were removed, placed in a ziplock bag, and taken to the lab for identification. The *Tribolium* aggregation pheromone lures provided with the pitfall trap pitfall traps were used and replaced every four weeks. Data were sorted by month, then an ANOVA (PROC MIXED) was performed on the square root transformed trap capture counts.



### Response of *T. castaneum* to food oils

When the commercial trapping oil components were assessed individually, components A and D were significantly repellent, components B and C, as well as the mix of all the components, showed no significant difference from the response to control (Fig. 2.2). Results from the oil aging study are presented in Figure 2.3. The oil with no BHT was repellent after aging for one week, and neutral on weeks two through four. The oil with BHT was neutral after aging for one week, and repellent on weeks two through four. Of the eighteen food oils assessed for attraction to *T. castaneum*, walnut, hemp and apricot kernel oils all tested significantly attractive (Table 2.2). The test statistic for corn and sesame oils were nearly significant ( $0.05 < P < 0.1$ ). When the oils that tested significantly or moderately attractive were tested an additional time in two groups (Table 2.3), *T. castaneum* showed no significant response to any of these materials.

### Response of *T. castaneum* to Host and Host-related Materials and Compounds

No significant response was observed to any of the four concentrations of the wheat extract (Table 2.4). When this extract was tested with DMD there was no significant response (Table 2.5). Among the bacterial and fungal volatile compounds assayed, *T. castaneum* responded preferentially to anisole at 100  $\mu\text{g}$ , and to 3-methyl-1-butanol at 0.0001  $\mu\text{g}$  (Table 2.6). Three-methyl-2-butanone and 2-methylphenol tested nearly attractive at one of the concentrations assessed. One-octen-3-ol, phenylacetaldehyde, 2-methylphenol and 1-octanol were either significantly, or nearly

significantly, repellant at at least one of the concentrations assessed. None of the green leaf volatiles tested significantly attractive at any of the amounts assessed (Table 2.7). *Trans*-2-hexenal was nearly significantly attractive at .01 g; *t*-2-hexen-1-ol was nearly significantly attractive at 0.0001 g, and significantly repellant at 1 g and 100 g. *Cis*-3-hexen-1-ol, hexanol, and *t*-3-hexen-1-ol all were either significantly, or nearly significantly, repellant at one of the amounts assessed. In the pheromone synergism assay, 3-methyl-1-butanol was found to enhance attraction of *T. castaneum* to DMD (Table 2.8).

### **Response of *T. castaneum* and other Stored-Product Beetles to Traps**

The results of the experiment to assess the response of *T. castaneum* to the pitfall and two sticky traps without attractants are plotted in Figure 2.4. There was a significant difference in capture by the three traps with significantly more beetles captured by the pitfall trap. Capture by the Detector sticky trap, and the Trapper Monitor sticky trap were not significantly different. The response of *T. castaneum* to the Trapper and the window sticky traps with and without pheromone lures is plotted in Figure 2.5. This experiment constituted a 2x2 factorial arrangement of treatments in a randomized complete block design. The pheromone by trap interaction was significant. When the two traps without pheromone lure were compared there was no significant difference in capture ( $F = 0.14$ ;  $df = 1, 9$ ;  $P = 0.7174$ ). When the catch of the Trapper sticky trap was analyzed with and without the pheromone lure it had similar capture ( $F = 1.26$ ;  $df = 1, 9$ ;  $P = 0.2915$ ). The window sticky trap; however, had significantly higher capture with the DMD lure than it did without pheromone ( $F = 55.81$ ;  $df = 1, 9$ ;  $P = 0.0001$ ). The capture of *T. castaneum*, *T. stercorea*, and *A. advena* by the Detector sticky floor trap and the pitfall trap within a

flour mill are plotted in Figure 2.6. Capture by the pitfall trap was significantly higher for *T. castaneum* than the Detector sticky trap. However, *T. stercorea* and *A. advena* were captured significantly higher in the Detector sticky trap over the pitfall trap.

The difference in weight due to dust accumulation of the traps both with and without the PVC cap cover are plotted in Figure 2.7. No significant difference was observed in the increase of pre and post-study trap weights among treatments within the third floor ( $F = 0.22$ ;  $df = 1, 6$ ;  $P = 0.6584$ ), and second floor ( $F = 2.18$ ;  $df = 1, 6$ ;  $P = 0.1907$ ) as well as among the warehouse treatments ( $F = 0.06$ ;  $df = 1, 6$ ;  $P = 0.8169$ ). Traps placed below the bulk storage area did exhibit a significant difference in weight among treatments. The relative dustiness of each mill location can be observed by noting the uncovered trap weight difference for each of the four mill locations. The dustier areas of the mill (third & second floors and the area below bulk flour bins) experienced an increase in weight due to dust of anywhere from 0.62 to 1.14 g. The average weight change for the uncovered traps in the warehouse area was not different.

When the standard pitfall trap (cardboard cover) was compared to the modified pitfall trap in the metal tray laboratory assay, the two had statistically similar capture of *T. castaneum* (Fig. 2.8). When these two traps were compared in non-dusty (warehouse) and dusty (feed area) areas of mill 1, significantly more beetles were captured by modified traps in the feed area (Fig. 2.9). Capture was not different in the warehouse area, and the additional two treatments in this area, which consisted of the standard and modified traps without pheromone lures, captured no *T. castaneum*.

#### **Effects of Pheromone and Oil on *T. castaneum* Response to Pitfall Traps**

Results from the four treatment pitfall trap lab assessment are plotted in Figure 2.10. This experiment constituted a 2x2 factorial arrangement of treatments. Pheromone by oil interaction was significant. The trap treatment with oil attractant alone did not have different capture of *T. castaneum* from control (mineral oil) ( $F = 0.11$ ;  $df = 1, 21$ ;  $P = 0.7404$ ). The trap with pheromone alone captured significantly more beetles than control ( $F = 11.65$ ;  $df = 1, 21$ ;  $P = 0.0026$ ). The treatment of both oil attractant and pheromone lure had significantly higher capture than control ( $F = 66.10$ ;  $df = 1, 21$ ;  $P = 0.0001$ ) as well as significantly higher capture than pheromone alone ( $F = 22.27$ ;  $df = 1, 21$ ;  $P = 0.0001$ ). When the capture of the *T. castaneum* by modified pitfall traps with oil and pheromone lures was compared to that of the same trap without the pheromone lure, there was no significant difference in capture among the two traps in location 1 (Fig. 2.11). Capture was significantly different within location 2 and location 3.

#### **In-Flight Response of *T. castaneum* to Pheromone-Baited Traps**

Capture was not significantly different among trap treatments during July (Fig. 2.12), but was significantly higher in the pheromone-baited traps during August, September and October.

## Discussion

The response of *T. castaneum* to the pitfall trapping oil was unexpectedly low in both two-choice assays that evaluated it. Furthermore, the observation that over time the oil with BHT became repellant where the oil without BHT remained neutral was unexpected. The effect of age on the oil with BHT may be explained by the conversion of the BHT to quinones as the antioxidant absorbed O<sub>2</sub> as a process of blocking oil auto-oxidation (Kikugawa et al. 1990). Quinones, if present, may have been perceived by the beetle causing them to be repelled.

As far as the author is aware, many of the food oils assessed in this study had not been previously evaluated for attraction to *Tribolium*. Of the oils that elicited either significant, or nearly significant attraction, corn, sesame, and walnut oils come from foods that the beetle is known to feed on (Sokoloff 1974). Sesame oil was attractive in these experiments despite eliciting no significant response in previous assessments (Phillips et. al 1993). Though Phillips et al. found wheat germ oil to be an effective attractant, and this oil was attractive in the preliminary assessments of this study, it did not attract *T. castaneum* in the experiments presented. This lack of repeatability was also observed in the follow-up assessment of the oils that had previously been attractive to *T. castaneum*. More in-depth and controlled assessments should be made of these oils in regards to the response of *T. castaneum* before any definite conclusions are drawn. More of these oils may have been attractive to the beetle if they had been evaluated earlier. From Table 2.1 it can be noted that many of these oils were past their estimated shelf lives. Therefore, their attractiveness may have been diminished (Barak 1989) even though they were stored in darkness at 5° C. The method of processing of these materials

as provided by the manufacturer should be carefully considered when noting these data (Table 2.1). For instance, refined oils are generally produced for the purpose of cooking and the manufacturer in processing has the goal of removing as many impurities from the oils as possible (Mounts 1985). The resulting product consists almost exclusively of triglycerides, and many of the lighter volatile compounds that would otherwise serve as olfactory cues to the beetle have been removed. Therefore, it is likely that the beetle could not recognize these highly refined oils. The results suggest this, of the food oils that elicited an attractive response from *T. castaneum*, all with the exception of corn oil, were pure, unrefined oils.

No significant response from *T. castaneum* was observed from the hexane wheat extract when assessed alone or with DMD. Seifelnasr et al. (1982) assessed the response of *T. castaneum* to ether extracts of whole wheat flour, whole wheat kernels, as well as wheat germ, bran, and endosperm fractions. All the extracts elicited a significantly higher response from control. Perhaps the polar ether extracts isolated compounds more active in inducing an attractive response than did the non-polar hexane extract in this study.

Of the 16 volatile compounds evaluated, 3-methyl-1-butanol seems to offer the most promise as a both an attractant and enhancer of DMD. This compound tested attractive at 0.001  $\mu\text{g}$  when assessed alone, and increased attraction to 0.000001 DMD when the two were evaluated together. Three-methyl-1-butanol is a common odor component of fungi in the genera *Aspergillus* and *Penicillium*, both common post-harvest storage fungi (Seitz & Sauer 1992). Borjesson et al. (1989) reported the volatile compounds of four fungi species grown on wheat. They noted that 3-methyl-1-butanol

was associated predominantly with early growth stages of *Aspergillus flavus* and *Penicillium cyclopium*. Perhaps because this compound seems to be associated with early growth of storage fungi, it may cue the beetle to a relatively uncontaminated and thus preferential habitat, which would explain the attractive responses. Conversely, the response of the beetle to 1-octen-3-ol was moderately to significantly repellent at all concentrations assayed. In addition to being a common fungal volatile, this compound is also an aggregation pheromone of *Oryzaephilus* spp. and the foreign grain beetle, *Ahasverus advena* (Waltl) (Pierce et al. 1989, 1991). Furthermore, these beetles prefer moister habitats and therefore habitats that more suitable for large amounts of fungi than does *T. castaneum* (Sinha & Watters, 1985). Therefore, this compound may cue *T. castaneum* of an unsuitable or less preferred habitat.

The overall low response of the beetle to the volatile compounds along with the repeatability difficulties among the food-oils tested led the author to question the efficacy of the pitfall bioassay device used to assess these materials. It is possible another assay method (e.g., wind tunnel or Y-tube assays) could have better identified compounds and materials that were attractive to the beetle. However, the value of the pitfall assay is that it mimics the conditions by which the beetle would respond to a pitfall trap. Therefore, identifying attractive materials by another assay device more sensitive than the pitfall assay may be of little value since the goal of this research was to find compounds and materials that would improve pitfall trap efficacy.

Much more work could, and should, be done to further evaluate these and other microbial and host chemicals. For instance, it might be discovered that although many of the single compounds that were tested alone elicit no attractive response from the beetle,



that combinations with other microbial and host volatiles are successful at attracting *T. castaneum*, thus leading to an improved *Tribolium* lure.

Another area that should be addressed is that of identifying the most attractive single compounds to the beetle from flour, corn, rice, and other common *Tribolium* foods. Perhaps a bait could be formulated that incorporates the most attractive compounds from the beetles food sources, and attractive microbial volatiles along with pheromone as an optimal lure. This bait could possibly allow for a nonperishable oil such as mineral oil to be used as a trapping medium, thus avoiding food oil-aging issues.

The pitfall trap proved superior to the Detector and Trapper sticky traps in capture of *T. castaneum* in all experiments that compared them. Interestingly, *T. stercorea* and *A. advena* were captured more competently by the Detector sticky trap over the pitfall trap in the flour mill assessment. Perhaps these two beetles are simply more susceptible to the sticky traps, or they are repelled by the *Tribolium* pheromone.

Interesting results were observed from experiments to isolate and evaluate specific pitfall trap components. The four treatment assessment of this trap in the laboratory revealed again that the trapping oil alone did not attract *T. castaneum*; however, when used in combination with the pheromone lure the oil was able to significantly enhance capture over the trap with pheromone alone. The value of the *Tribolium* pheromone was confirmed in this experiment as well as three others. As mentioned before, the treatments lacking pheromone lures in the experiment to compare the modified and standard pitfall traps (warehouse area) captured no *T. castaneum*. Additionally, results from the experiment done in the mill to compare capture of *T. castaneum* in modified pitfall traps with and without pheromone lures revealed a



significantly higher capture by the traps with pheromone in two of three locations. It is likely that the first location (third floor of mill) did not support a high enough beetle population to reveal differences in trap capture. Another possibility for the lack of significant difference is that the traps in this area may have been lowered in efficacy due to dusty conditions and mill floor cleaning techniques (see below & Chapter III). Finally, the funnel traps with pheromone lures also captured more *T. castaneum* than those with no attractants. It is noteworthy that this study is the first the author is aware of which documents this beetle responding to its pheromone in flight.

The PVC end-cap modification seems to improve trap efficacy in dusty mill environments. The modified trap showed no inhibitory effects in capture of *T. castaneum* in dust-free laboratory and field environments. Furthermore, when these two traps were compared in a dusty area the modified trap captured significantly more beetles.

The dust accumulation study produced interesting results regarding various areas of the flour mill, and the efficacy of the dust cover trap modification in protecting the trap. The third floor traps experienced a relatively large amount of weight gain due to dust. Furthermore, the dust cover did not offer protection from the dust as evidenced by the statistically similar amount of weight increase of both covered and uncovered traps. However, the area below the bulk flour bins did have significantly different weight increase. Noteworthy as well is that the uncovered trap experienced the greatest dust weight increase of all areas. Therefore, the cap was apparently efficient in protecting the trap from dust. This difference can be attributed to the frequency and technique of floor cleaning used in these areas. The area below the bulk stored flour, although the area of greatest dust accumulation was undisturbed during the study. Conversely, the third floor

of the mill was cleaned daily during the study with compressed air blow-downs. These blow-downs were apparently able to force dust up under the cap, causing an increase in weight. Thus, traps were efficiently protected from even the largest amounts of dust if this technique of floor cleaning was not used; where it was used, the efficacy of the cap modification was diminished.

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**Table 2.1. Summary of food oils tested.**

Oil	Processing	Age when Assayed	Estimated Shelf Life
Almond	*	*	*
Apricot kernel	EP	12 mo	12 mo
Avocado	CP, R, D	27 mo	24 mo
Canola	EP, R, B, D	13 mo	12 mo
Castor	CP	*	*
Coconut	E, R, B, D	*	*
Corn	EP, R, B, D	15 mo	12 mo
Flax	*	*	*
Grapeseed	EP	11 mo	12 mo
Hemp	CP	21 mo	12 mo
Olive	*	*	*
Peanut	E, R, B, D	15 mo	30 mo
Safflower	E, R, B, D	14 mo	30 mo
Sesame	CP	19 mo	12 mo
Sunflower	EP, R, B, D	15 mo	12 mo
Vegetable	EP, R, B, D	15 mo	12 mo
Walnut	CP	27 mo	12 mo
Wheat germ	E	9 mo	12 mo

EP, Expeller Pressed; CP, Cold Pressed; E, Extracted (Hexane); D, Deodorized; B, Bleached; R, Refined. \*, Unable to locate information.

**Table 2.2. Response of *T. castaneum* to various food oils.**

Oil	Mean RI $\pm$ SE	P-value
Walnut	77.06 $\pm$ 5.10	0.0005 ***
Hemp	65.88 $\pm$ 3.54	0.0015 **
Apricot kernel	61.07 $\pm$ 4.54	0.0374 *
Corn	64.42 $\pm$ 6.62	0.0576
Sesame	70.30 $\pm$ 6.77	0.0682
Almond	45.23 $\pm$ 7.28	NS
Avocado	47.39 $\pm$ 5.96	NS
Canola	45.98 $\pm$ 5.60	NS
Castor	42.60 $\pm$ 5.35	NS
Coconut	55.49 $\pm$ 8.96	NS
Flax	55.48 $\pm$ 6.70	NS
Grapeseed	43.92 $\pm$ 7.57	NS
Olive	41.18 $\pm$ 8.12	NS
Peanut	47.25 $\pm$ 8.09	NS
Safflower	56.13 $\pm$ 8.67	NS
Sunflower	47.94 $\pm$ 5.99	NS
Vegetable	54.92 $\pm$ 7.71	NS
Wheat germ	56.21 $\pm$ 7.03	NS

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; NS not significant ( $P > 0.1$ );  $n = 10$ . RI =  $([T/(T + C)] 100)$ . Null hypothesis RI = 50, alternative hypothesis RI  $\neq$  50.



**Table 2.3. Response of *T. castaneum* to oils that had previously tested attractive.**

Assay 1

Oil	Mean RI $\pm$ SE	P-value
Corn	54.78 $\pm$ 6.68	NS
Sesame	54.60 $\pm$ 7.17	NS
Walnut	51.95 $\pm$ 4.90	NS

Assay 2

Apricot kernel	42.25 $\pm$ 6.02	NS
Flax	44.42 $\pm$ 3.78	NS
Hemp	49.16 $\pm$ 4.89	NS

NS, not significant ( $P > 0.1$ );  $n = 10$ .  $RI = ([T/(T + C)] 100)$ . Null hypothesis  $RI = 50$ , alternative hypothesis  $RI \neq 50$ .

**Table 2.4. Response of *T. castaneum* to various concentrations of a hexane wheat extract.**

Concentration (MGE)	Mean RI $\pm$ SE	P-value
98.2	41.62 $\pm$ 8.08	NS
9.82	46.84 $\pm$ 6.28	NS
0.982	45.31 $\pm$ 7.89	NS
0.0982	52.94 $\pm$ 4.97	NS

NS, not significant ( $P > 0.1$ );  $n = 10$ .  $RI = ([T/(T + C)] 100)$ . Null hypothesis  $RI = 50$ , alternative hypothesis  $RI \neq 50$ .

**Table 2.5. Response of *T. castaneum* to wheat extract + DMD.**

Extract/DMD	Mean RI $\pm$ SE	P-value
extract (9.82 MGE)	46.33 $\pm$ 4.28	NS
DMD	41.36 $\pm$ 7.15	NS
extract + DMD	41.80 $\pm$ 4.52	NS

NS, not significant ( $P > 0.1$ );  $n = 10$ .  $RI = ([T/(T + C)] 100)$ . Null hypothesis  $RI = 50$ , alternative hypothesis  $RI \neq 50$ .

**Table 2.6. Response of *T. castaneum* to four doses of volatile compounds associated with stored-grain microorganisms.**

Compound	Dose ( $\mu\text{g}$ )	Mean RI $\pm$ SE	P-value
anisole	100	64.78 $\pm$ 3.44	0.0020 * *
	1	49.82 $\pm$ 6.73	NS
	0.01	43.96 $\pm$ 6.66	NS
	0.0001	57.69 $\pm$ 6.18	NS
3-methyl-1-butanol	100	53.25 $\pm$ 5.96	NS
	1	43.93 $\pm$ 5.45	NS
	0.01	47.67 $\pm$ 5.77	NS
	0.0001	68.05 $\pm$ 6.52	0.0217 *
3methyl-2-butanone	100	53.58 $\pm$ 5.67	NS
	1	49.25 $\pm$ 7.77	NS
	0.01	46.00 $\pm$ 4.02	NS
	0.0001	59.10 $\pm$ 4.70	0.0851
2-methylphenol	100	35.18 $\pm$ 7.12	0.0669
	1	60.93 $\pm$ 5.83	0.0936
	0.01	49.29 $\pm$ 5.64	NS
	0.0001	55.66 $\pm$ 8.87	NS
1-octen-3-ol	100	37.33 $\pm$ 5.86	0.0589
	1	32.99 $\pm$ 7.61	0.0522
	0.01	30.20 $\pm$ 7.62	0.0288 *
	0.0001	37.02 $\pm$ 5.19	0.0339 *
1-octanol	100	21.41 $\pm$ 5.17	0.0004 * *
	1	54.93 $\pm$ 5.81	NS
	0.01	43.10 $\pm$ 7.77	NS
	0.0001	47.22 $\pm$ 6.62	NS

**Table 2.6. (continued)** *Response to the green leaf volatile compounds.*

Compound	Dose ( $\mu\text{g}$ )	Mean RI $\pm$ SE	P-value
3-octanone	100	48.97 $\pm$ 8.31	NS
	1	56.61 $\pm$ 6.30	NS
	0.01	54.19 $\pm$ 5.37	NS
	0.0001	40.78 $\pm$ 8.89	NS
phenylacetaldehyde	100	35.25 $\pm$ 7.49	0.0804
	1	41.93 $\pm$ 5.47	NS
	0.01	34.71 $\pm$ 4.51	0.0080 **
	0.0001	40.01 $\pm$ 6.47	NS
styrene	100	41.91 $\pm$ 7.33	NS
	1	57.51 $\pm$ 8.04	NS
	0.01	43.25 $\pm$ 7.17	NS
	0.0001	41.93 $\pm$ 6.98	NS
vinyl acetate	100	57.16 $\pm$ 5.70	NS
	1	54.56 $\pm$ 3.00	NS
	0.01	51.92 $\pm$ 7.89	NS
	0.0001	47.00 $\pm$ 8.16	NS

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; NS, not significant ( $P > 0.1$ );  $n = 10$ .  $\text{RI} = ([T/(T + C)] 100)$ .

Null hypothesis  $\text{RI} = 50$ , alternative hypothesis  $\text{RI} \neq 50$ .

**Table 2.7. Response of *T. castaneum* to the green leaf volatile compounds.**

Compound	Dose ( $\mu\text{g}$ )	Mean RI $\pm$ SE	P-value
hexanal	100	52.56 $\pm$ 4.69	NS
	1	50.97 $\pm$ 7.05	NS
	0.01	46.62 $\pm$ 7.14	NS
	0.0001	47.00 $\pm$ 5.57	NS
t-2-hexenal	100	42.92 $\pm$ 7.80	NS
	1	64.85 $\pm$ 6.85	0.0582
	0.01	58.72 $\pm$ 6.68	NS
	0.0001	48.82 $\pm$ 6.21	NS
hexanol	100	36.48 $\pm$ 7.13	0.0906
	1	41.25 $\pm$ 7.04	NS
	0.01	45.80 $\pm$ 6.13	NS
	0.0001	60.70 $\pm$ 7.57	NS
c-2-hexen-1-ol	100	41.61 $\pm$ 8.03	NS
	1	45.49 $\pm$ 7.10	NS
	0.01	58.58 $\pm$ 8.03	NS
	0.0001	45.12 $\pm$ 6.26	NS
t-2-hexen-1-ol	100	36.27 $\pm$ 4.51	0.0140 *
	1	35.42 $\pm$ 5.03	0.0176 *
	0.01	49.14 $\pm$ 7.02	NS
	0.0001	67.17 $\pm$ 8.38	0.0708
c-3-hexen-1-ol	100	33.94 $\pm$ 5.90	0.0235 *
	1	48.10 $\pm$ 6.31	NS
	0.01	45.27 $\pm$ 5.84	NS
	0.0001	43.19 $\pm$ 6.12	NS

**Table 2.7. (continued)**

response to various volatile compounds + DMU.

Compound	Dose ( $\mu\text{g}$ )	Mean RI $\pm$ SE	P-value
<i>t</i> -3-hexen-1-ol	100	26.11 $\pm$ 4.95	0.0009 **
	1	45.67 $\pm$ 6.87	NS
	0.01	62.21 $\pm$ 6.72	NS
	0.0001	61.11 $\pm$ 6.30	NS

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; NS, not significant ( $P > 0.1$ );  $n = 10$ .  $\text{RI} = ([\text{T}/(\text{T} + \text{C})] 100)$ .

Null hypothesis  $\text{RI} = 50$ , alternative hypothesis  $\text{RI} \neq 50$ .

**Table 2.8. Response of *T. castaneum* to various volatile compounds + DMD.**

Compound	Mean RI ± SE	P-value
anisole	55.46 ± 6.60	NS
DMD	43.75 ± 3.97	NS
anisole + DMD	52.30 ± 4.52	NS
3-methyl-1-butanol	47.27 ± 2.90	NS
DMD	49.30 ± 4.42	NS
3-methyl-1-butanol + DMD	66.11 ± 7.25	0.0534
3-methyl-2-butanone	45.38 ± 6.24	NS
DMD	53.79 ± 2.38	NS
3-methyl-2-butanone + DMD	48.83 ± 7.61	NS
2-methylphenol	56.97 ± 8.56	NS
DMD	51.48 ± 6.38	NS
2-methylphenol + DMD	49.42 ± 6.38	NS
3-octanone	60.62 ± 4.12	0.0297 *
DMD	67.83 ± 5.06	0.0065 **
3-octanone + DMD	60.25 ± 7.78	NS
Styrene	54.72 ± 5.02	NS
DMD	57.78 ± 5.39	NS
Styrene + DMD	48.06 ± 3.55	NS
vinyl acetate	47.17 ± 6.72	NS
DMD	63.86 ± 7.18	0.0858
vinyl acetate + DMD	42.21 ± 6.41	NS
hexanal	52.98 ± 5.25	NS
DMD	79.17 ± 3.62	0.0001 **
hexanal + DMD	56.77 ± 6.20	NS
<i>t</i> -2-hexenal	48.89 ± 8.95	NS
DMD	55.19 ± 7.76	NS
<i>t</i> -2-hexenal + DMD	61.74 ± 5.92	0.0785

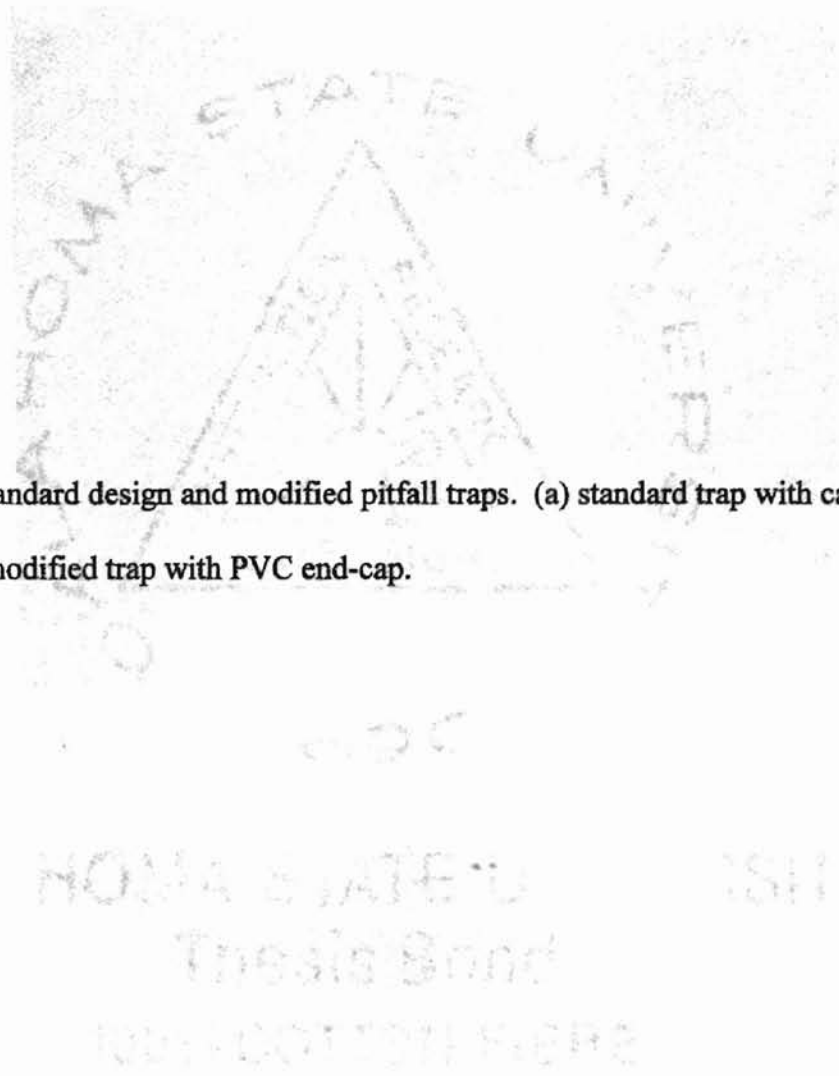


**Table 2.8. (continued)**

Compound	Mean RI $\pm$ SE	<i>P</i> -value
hexanol	43.55 $\pm$ 7.41	NS
DMD	70.14 $\pm$ 5.59	0.0057 **
hexanol + DMD	53.58 $\pm$ 7.53	NS
<i>c</i> -2-hexen-1-ol	35.40 $\pm$ 6.40	0.0484 *
DMD	70.10 $\pm$ 5.80	0.0071 **
<i>c</i> -2-hexen-1-ol + DMD	51.22 $\pm$ 3.45	NS
<i>t</i> -2-hexen-1-ol	42.31 $\pm$ 6.33	NS
DMD	58.67 $\pm$ 7.44	NS
<i>t</i> -2-hexen-1-ol + DMD	59.10 $\pm$ 7.14	NS
<i>c</i> -3-hexen-1-ol	51.31 $\pm$ 7.50	NS
DMD	75.11 $\pm$ 4.68	0.0005 **
<i>c</i> -3-hexen-1-ol + DMD	66.20 $\pm$ 5.70	0.0193 *
<i>t</i> -3-hexen-1-ol	28.45 $\pm$ 6.80	0.0114 *
DMD	78.36 $\pm$ 9.40	0.0166 *
<i>t</i> -3-hexen-1-ol + DMD	52.83 $\pm$ 7.50	NS

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; NS, not significant ( $P > 0.1$ );  $n = 10$ . RI =  $([T/(T + C)] 100)$ .

Null hypothesis RI = 50, alternative hypothesis RI  $\neq$  50.



**Fig. 2.1.** Standard design and modified pitfall traps. (a) standard trap with cardboard cover. (b) modified trap with PVC end-cap.



Fig. 2.2

trapping

variance

and 7.5%

capture

11


total

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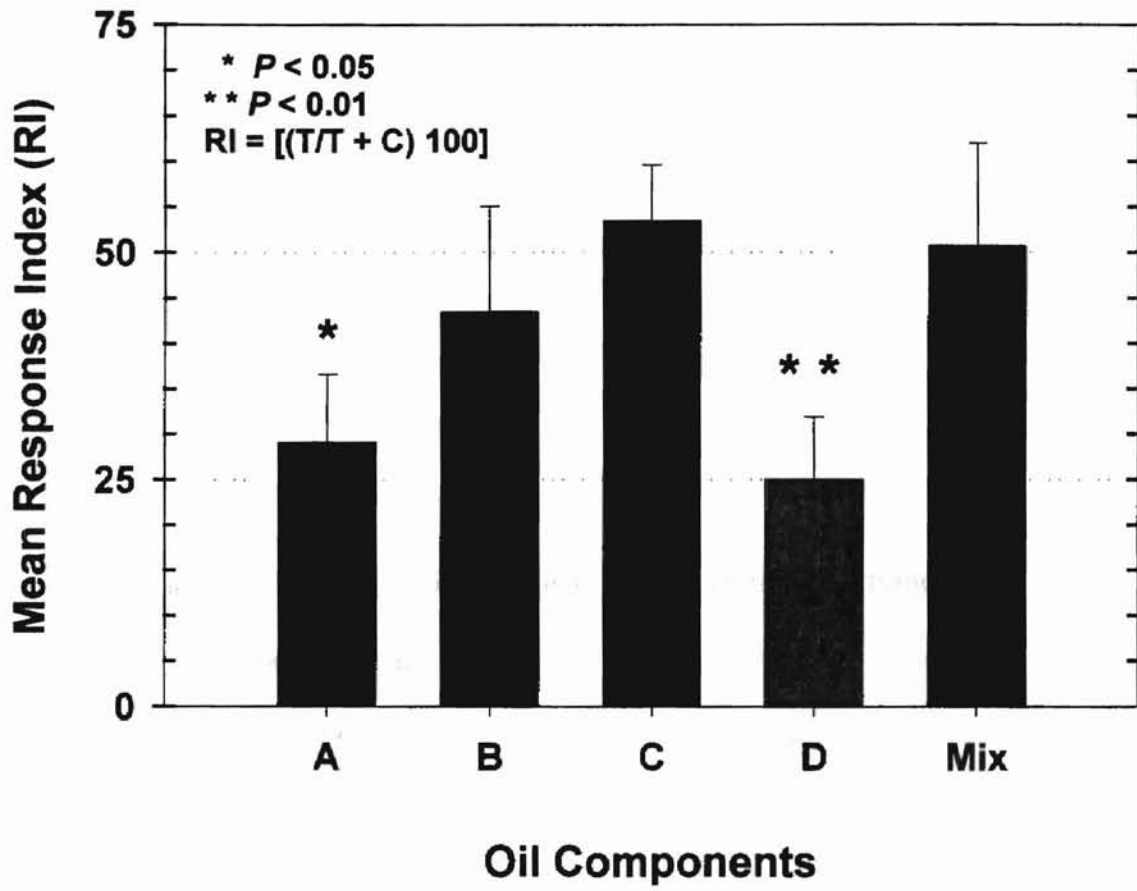
0.026

were not

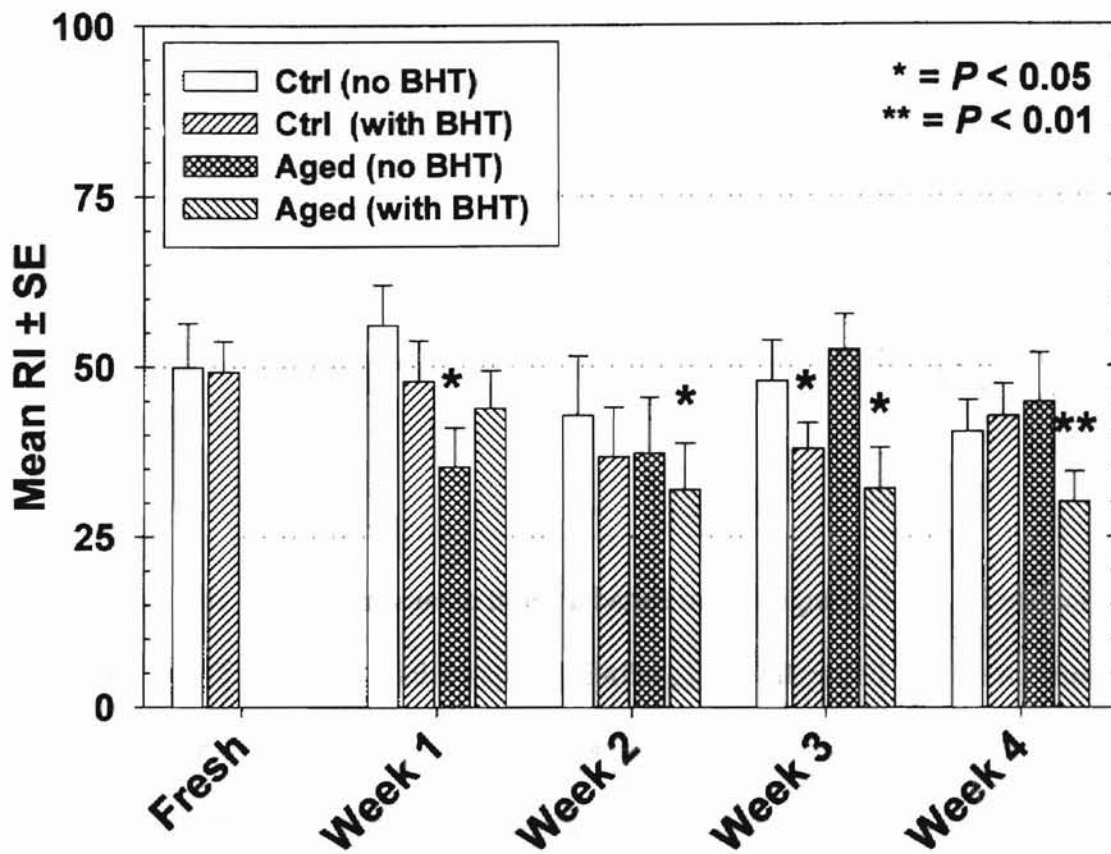
10



**Fig. 2.2.** Mean response index (RI) of *T. castaneum* to each of the individual pitfall trapping oil components as well as the oil mix in the pitfall bioassay. An analysis of variance revealed that components A and D had a significantly repellent RI ( $P = 0.026$  and  $0.0083$ , respectively). RI's of all other components, as well as the oil mix, were not significantly different than 50 (neutral). Each treatment was replicated eight times.  $RI = [T/(T+C)]100$ . Null hypothesis  $RI = 50$ , alternative hypothesis  $RI \neq 50$ .



**Fig. 2.3.** Mean response index (RI)  $\pm$  SE of *T. castaneum* to pitfall trapping oil samples with and without the antioxidant BHT added, in the pitfall bioassay. The control oil without BHT (stored at 5° C) tested neutral throughout the study. The control oil with BHT (stored at 5° C) tested neutral all weeks of the study except for week 3 where it tested significantly repellent (RI = 35.25  $\pm$  5.75,  $P$  = 0.0304). The aged oil without BHT tested significantly repellent on week 1 (RI = 37.89  $\pm$  3.84,  $P$  = 0.0117), and neutral all other weeks. The aged oil with BHT tested neutral on week one and significantly repellent on weeks 2-4, (RI = 31.86  $\pm$  6.84,  $P$  = 0.0264), (RI = 32.05  $\pm$  6.04,  $P$  = 0.0157) and (RI = 30.08  $\pm$  4.46,  $P$  = 0.0016), respectively.

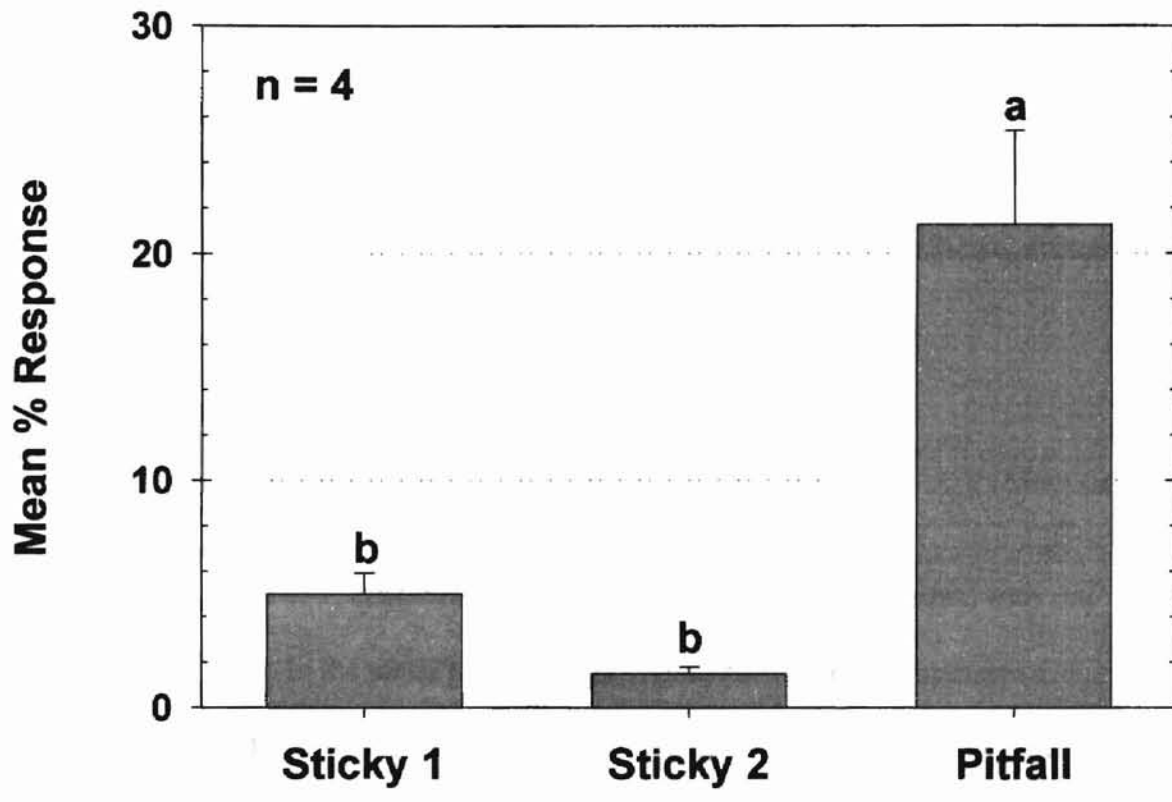


$RI = [T/(T+C)]100$

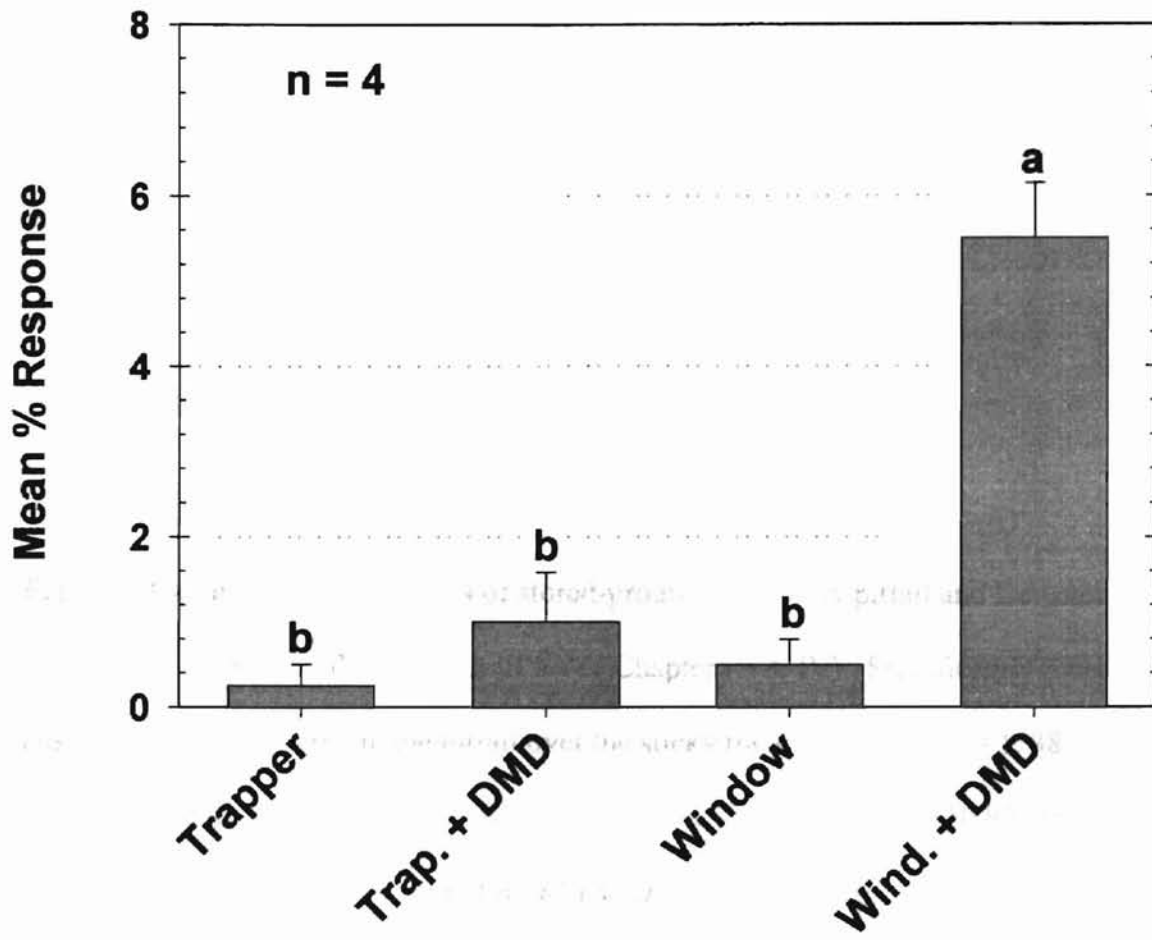
n = 10

Fig. 2.4. Response of *T. castaneum* to three unbaited trap designs (Sticky 1, Detector sticky trap; Sticky 2, Trapper sticky trap; and Pitfall, Flit Trak pitfall trap) in the metal tray bioassay. Analysis of variance revealed a significant difference in trap capture ( $F = 18.84$ ;  $df = 2$ ;  $P = 0.0026$ ). Means with the same letter are not significantly different. Percent captures were arcsine square root transformed for ANOVA.





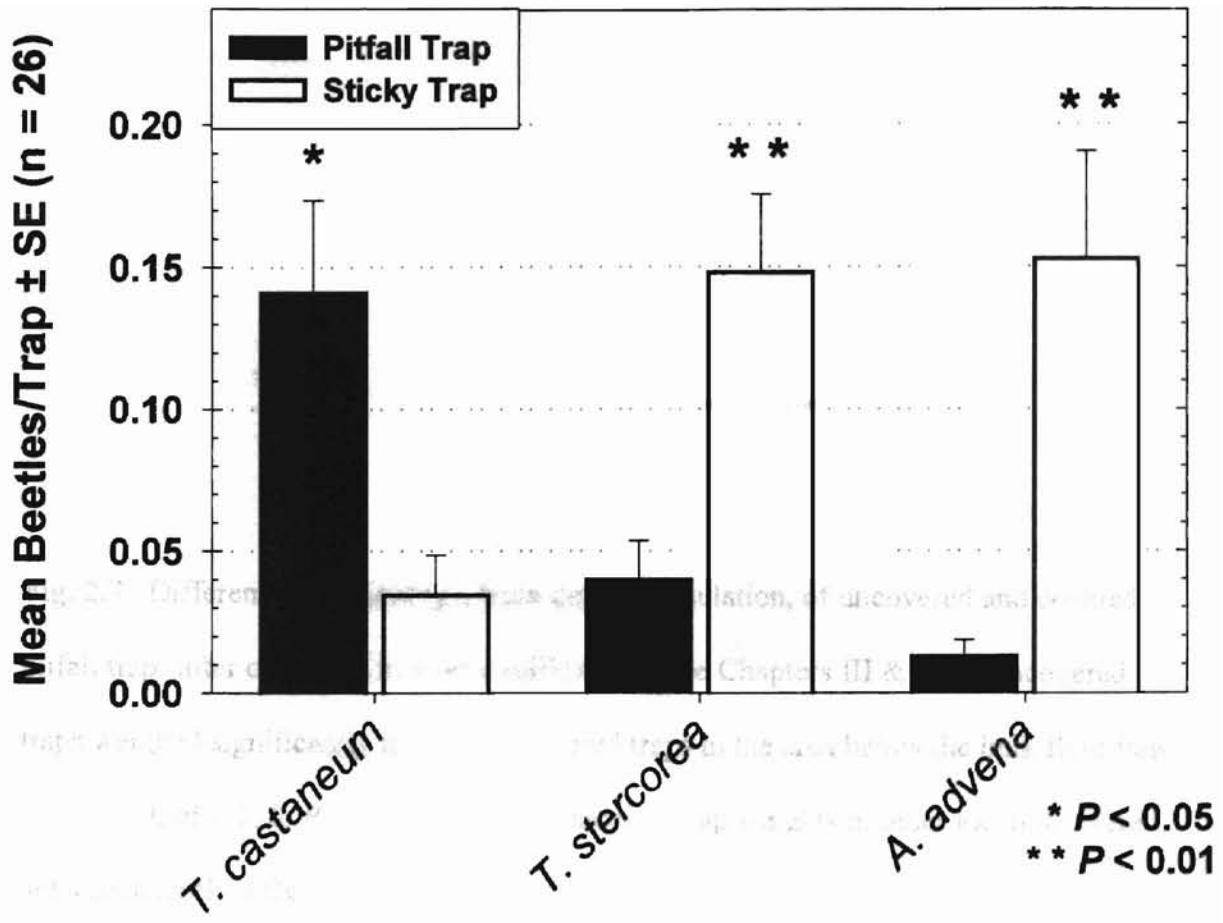
**Fig. 2.5.** Response of *T. castaneum* to Trapper and Window sticky traps, with and without DMD lures in the metal tray bioassay. Analysis of variance revealed a significant pheromone by trap interaction ( $F = 20.16$ ;  $df = 1, 9$ ;  $P = 0.0015$ ). Means with the same letter are not significantly different. Percent captures were arcsine square root transformed for ANOVA.



Species/Trip → SE (n = 20)

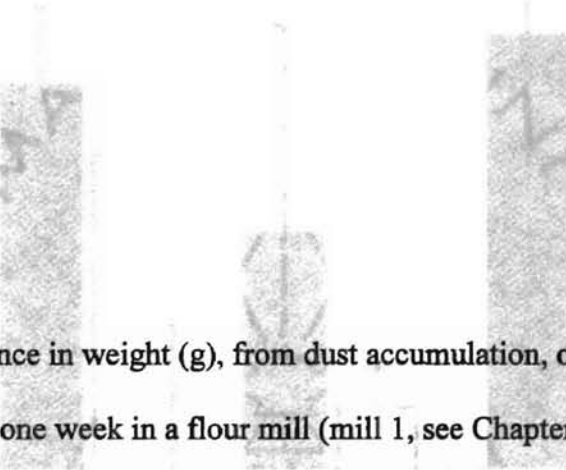
□ Pitfall  
□ Sticky

**Fig. 2.6.** Captures of three species of stored-product beetles by pitfall and Detector sticky trap designs in a flour mill (mill 1, see Chapter III & IV). Significantly more *T. castaneum* were captured in the pitfall over the sticky traps ( $F = 6.59$ ;  $df = 1, 38$ ;  $P = 0.0143$ ). The sticky trap captured significantly more *T. stercorea* ( $F = 10.92$ ;  $df = 1, 38$ ;  $P = 0.0021$ ), and significantly more *A. advena* ( $F = 10.95$ ;  $df = 1, 38$ ;  $P = 0.0021$ ) than the pitfall trap. Results are from 13 biweekly observations from 26 pitfall and 26 sticky traps. Count data were square root transformed for ANOVA.

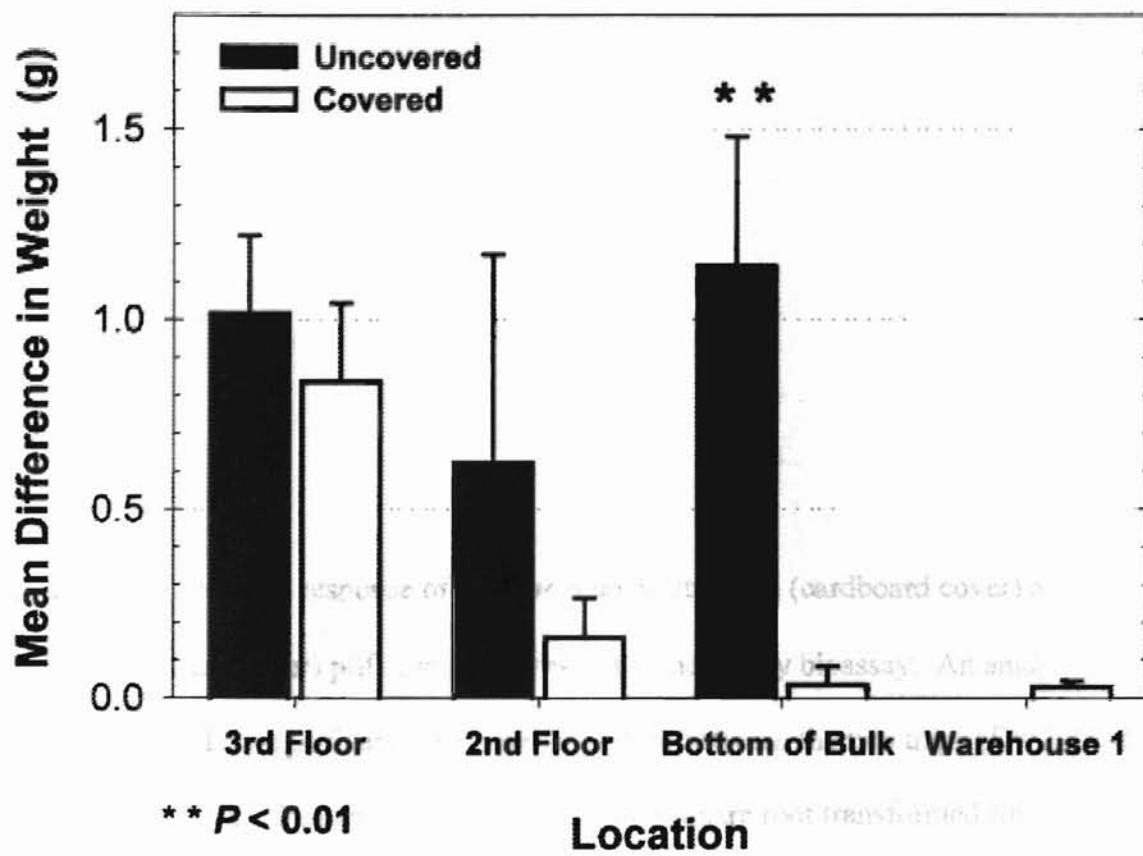


(b) Difference in Weight (g)

Uncovered  
Covered

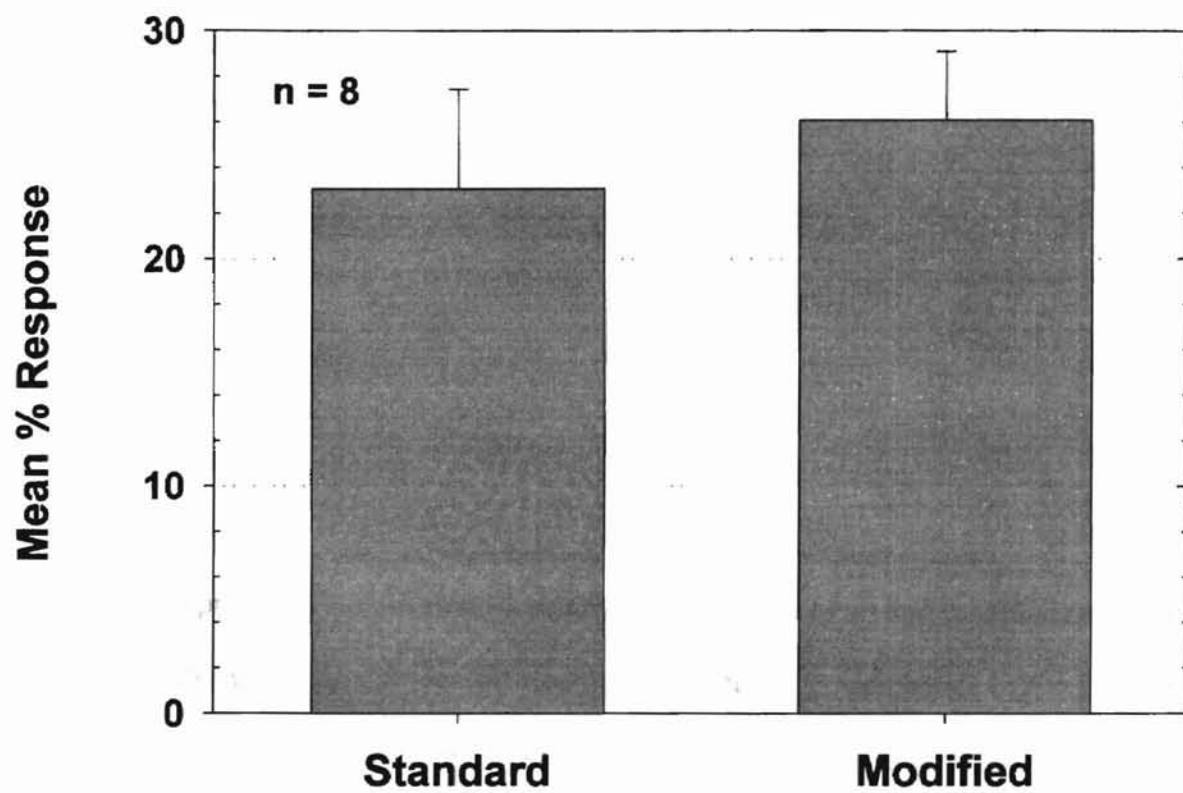


**Fig. 2.7.** Difference in weight (g), from dust accumulation, of uncovered and covered pitfall traps after one week in a flour mill (mill 1, see Chapters III & IV). Uncovered traps weighed significantly more than covered traps in the area below the bulk flour bins ( $F = 18.59$ ;  $df = 1, 6$ ;  $P = 0.0050$ ). Differences of trap weights in other locations were not significantly different.



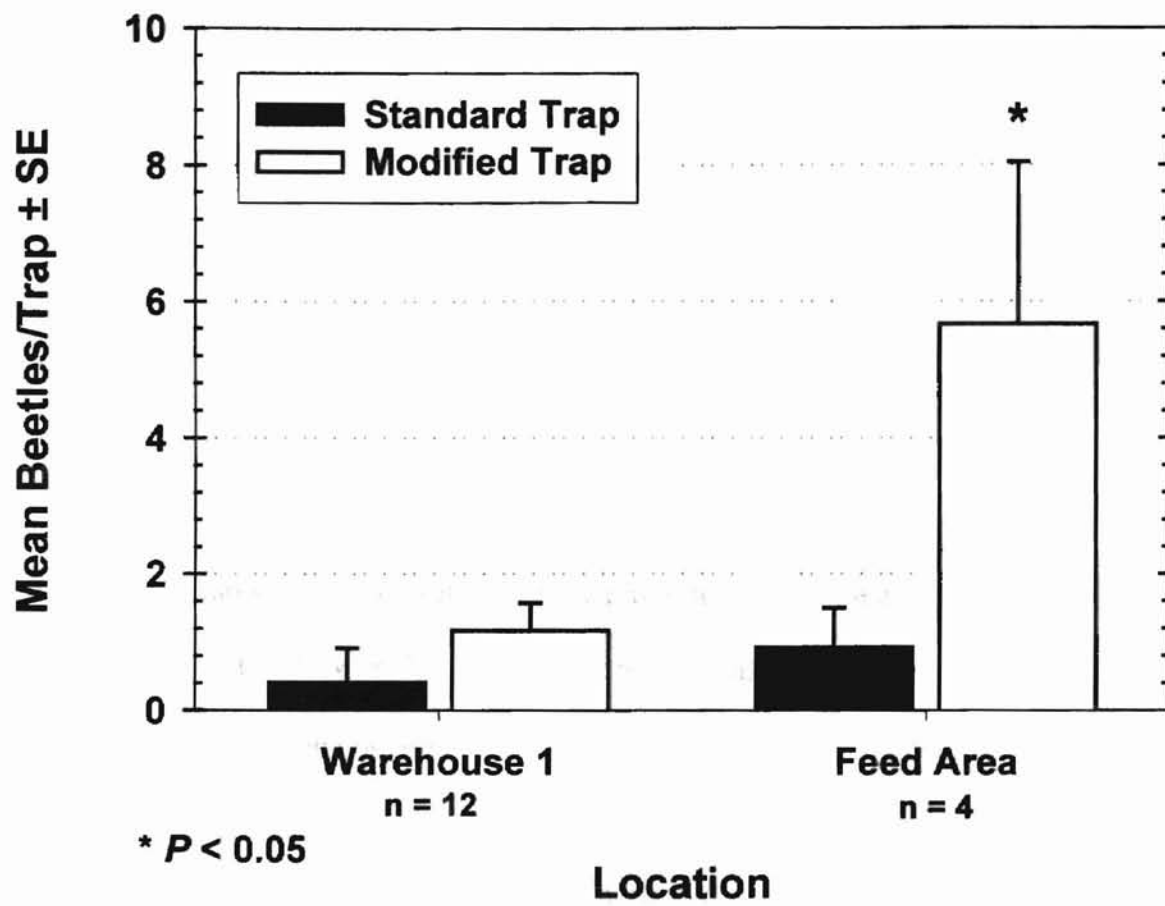
**Fig. 2.8.** Mean %  $\pm$  SE response of *T. castaneum* to standard (cardboard cover) and modified (dust-cap cover) pitfall trap designs in the metal tray bioassay. An analysis of variance revealed no significant difference in capture between the two traps ( $F = 2.08$ ;  $df = 8, 12$ ;  $P = 0.1218$ ). Percent captures were arcsine square root transformed for ANOVA.



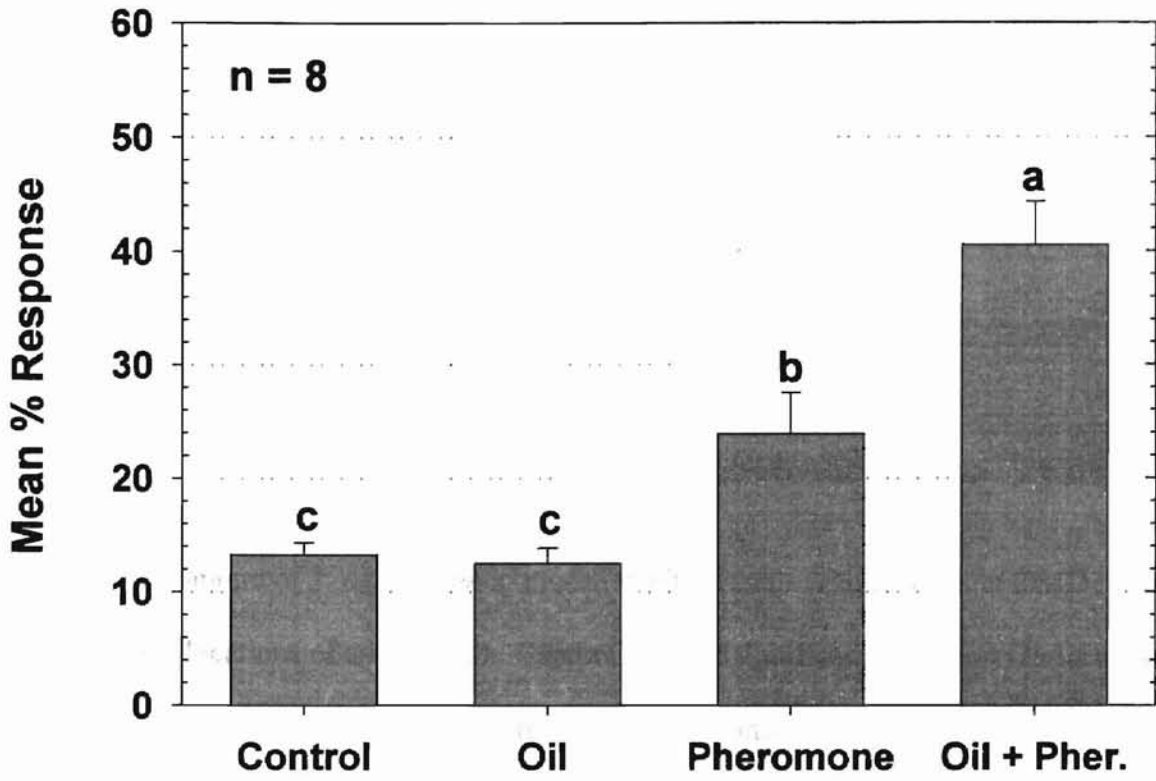


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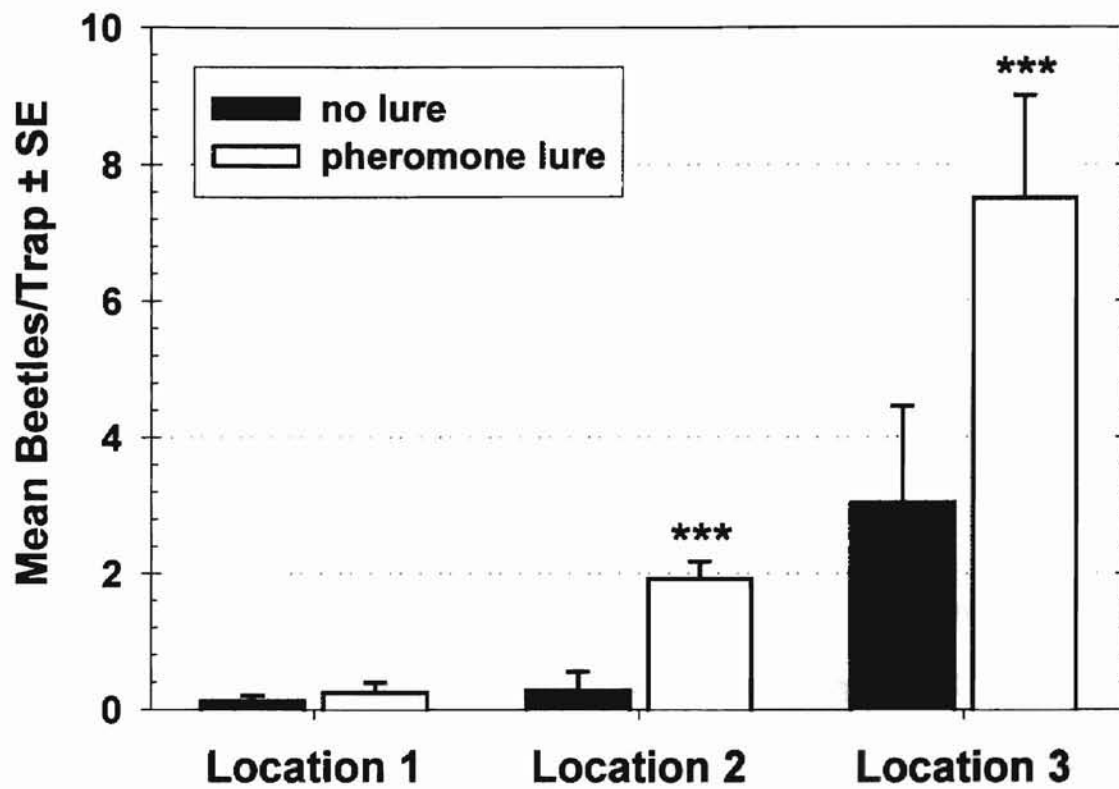
**Fig. 2.9.** Capture of *T. castaneum* by standard (cardboard cover) and modified (cap dust cover) pitfall traps. Capture was not significantly different in warehouse 1 ( $F = 2.14$ ;  $df = 12, 11$ ;  $P = 0.1097$ ). In the feed area, the modified trap captured significantly more beetles ( $F = 4.79$ ;  $df = 4, 3$ ;  $P = 0.1144$ ). Warehouse 1 was a relatively dust-free environment (see dust accumulation study) where the feed area was relatively dusty.  $N = 4$  for the feed area study, and  $n = 12$  for warehouse 1 study. Count data were square root transformed for ANOVA.



**Fig. 2.10.** Response of *T. castaneum* to the pitfall trap components in the metal tray bioassay. Treatments were: 1) control (1 ml mineral oil, no pheromone lure), 2) Oil (1 ml trapping oil, no pheromone lure) 3) Pheromone (1 ml mineral oil, pheromone lure), and 4) Oil + Pher. (1 ml trapping oil, pheromone lure). Analysis of variance revealed a significant interaction among oil and pheromone in the trap ( $F = 12.77$ ;  $df = 1, 21$ ;  $P = 0.0018$ ). Means with the same letter are not significantly different. Percent captures were arcsine square root transformed for ANOVA.



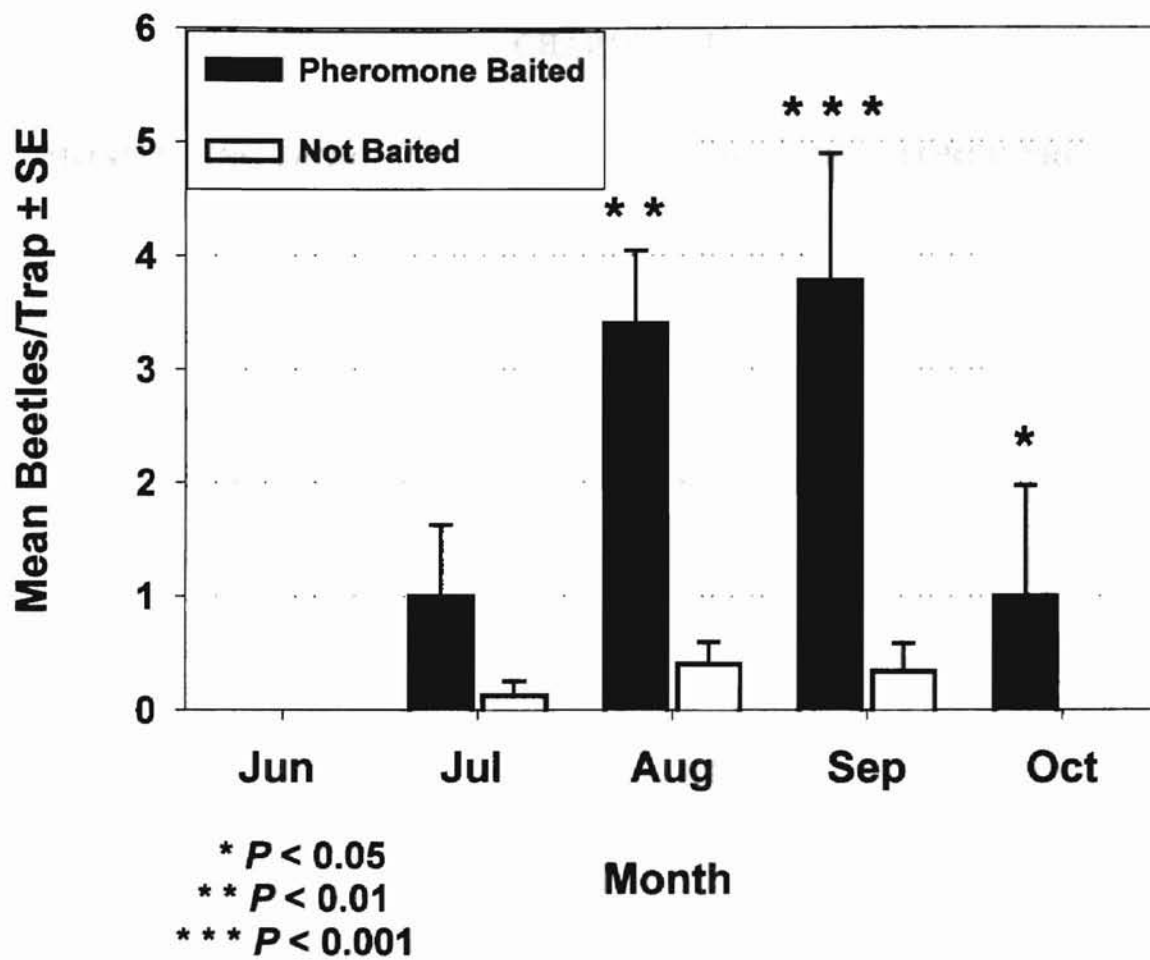
**Fig. 2.11.** Capture of *T. castaneum* in modified pitfall traps with or without DMD lures within three locations of a flour mill. Capture was not significantly different in location 1 ( $F = 0.48$ ;  $df = 1, 5$ ;  $P = 0.5207$ ). Capture was significantly higher in pheromone traps in location 2 ( $F = 0.115.65$ ;  $df = 1, 5$ ;  $P = 0.0001$ ) and location 3 ( $F = 475.36$ ;  $df = 1, 5$ ;  $P = 0.0001$ ). Results are from the following: in location 1, six weekly observations among four, two-treatment blocks; in location 2, six weekly observations among two, two-treatment blocks; and in location 3, four weekly observations from one, two-treatment block. Counts data were square root transformed for ANOVA.



\*\*\*  $P < 0.001$

**Fig. 2.12.** Capture of *T. castaneum* in multiple funnel traps with and without DMD pheromone lures outside a flour mill (mill 1). Capture was not significantly different among trap treatments during July ( $F = 3.06$ ;  $df = 1, 11.5$ ;  $P = 0.1071$ ). Capture was significantly higher in pheromone traps during August ( $F = 18.32$ ;  $df = 1, 11.5$ ;  $P = 0.0012$ ), September ( $F = 28.56$ ;  $df = 1, 11.5$ ;  $P = 0.0002$ ), and October ( $F = 5.82$ ;  $df = 1, 11.5$ ;  $P = 0.0336$ ). Count data were square root transformed for ANOVA.





Abstract

Introduction

Materials and Methods

### CHAPTER III

## MONITORING *TRIBOLIUM CASTANEUM* AND OTHER STORED-PRODUCT BEETLES IN FLOUR MILLS

## Abstract

The red flour beetle, *Tribolium castaneum* (Herbst) and other insect pests common to mills and food processing facilities were targeted during 1997 and 1998 trapping studies conducted in two flour mills. Objectives of the studies were: 1) use insect traps to determine pest species present and their distribution in space and time, 2) monitor *T. castaneum* activity before and after methyl bromide fumigation to assess efficacy of treatment, and 3) correlate *T. castaneum* trap capture to *T. castaneum* counts from direct sampling of the product. *T. castaneum* was the most commonly trapped beetle during both years in mill 1. In mill 2, *Typhaea stercorea* (L.) and *Cryptolestes ferrugineus* (Stephens) were both captured in higher numbers than *T. castaneum*. In mill 1, trap capture was higher overall during 1998 for most of the species compared with capture during 1997, likely due to the dust cover modification made of the pitfall trap used during 1998, as well as more frequent trap servicing during 1998. Trap capture was also evaluated by location within the mills and a significant difference was found in the capture of *T. stercorea* during both years in mill 1. In some cases, *T. castaneum* captures were significantly reduced following fumigations; however, in no cases were beetle population eliminated. These results indicate either that the fumigations were not entirely effective, or that the beetles were entering the mill immediately after treatment. When trap captures of *T. castaneum* were compared with counts of this beetle from samples of siftings from the flour, the test for correlation coefficient of the 1998 data were nearly significant.

## Introduction

Pest management in food processing is critical to the industry because: 1) their facilities support persistent pest populations, 2) processing is the last place where pests can be eliminated before marketing, 3) FDA and EPA regulations limit certain pest management approaches and tools available in processing, and 4) limited time constraints require that most processors run 24 h per day with little time to correct pest problems.

Beetles in the genus *Tribolium*, in particular the confused flour beetle, *T. confusum* Jacquelin du Val, and the red flour beetle, *T. castaneum* (Herbst), are major pests of many stored-food products and are commonly found in cereal processing facilities such as flour mills (Agricultural Experiment Station Bull. 1913, Cotton et al. 1945). Good (1937) surveyed insect pests in 17 flour mills throughout Missouri, Kansas, and Oklahoma. Beetles in the genus *Tribolium* were found in 78% of all samples, and present in 97% of all infested samples.

Methyl bromide (MB) is a compound commonly used as a structural fumigant by many flour mills. This chemical is preferable to other fumigants because it is effective against a wide variety of pests, relatively fast acting and leaves little to no residues (Chakrabarti 1996). However, MB is scheduled to be banned 1 January 2005 under the Clean Air Act. Therefore, users of this compound need to find viable alternatives. Trapping to monitor *Tribolium* and other mill pests plays an important role within an integrated pest management (IPM) program (Flint & van den Bosch 1981), and will likely increase in practice and necessity with the ban of MB. Current stored-product insect trapping methods include the use of pheromone and food baited traps (Burkholder & Ma 1985, Chambers 1990, Phillips 1997).

This study was conducted in 1997 and 1998 in two flour mills with the following objectives: 1) determine pest species present and their distribution in space and time, 2) monitor *T. castaneum* activity before and after methyl bromide fumigation to assess efficacy of treatment, and 3) correlate *T. castaneum* trap captures to *T. castaneum* counts from direct sampling of the product.

## Traps

Two trap designs, as well as a modification to one of the traps, were used in these studies. The Storgard Flit Trak® M<sup>2</sup> (Trécé Inc. Salinas, CA) is a ramp-pitfall trap baited with a grain-based oil and *Tribolium* aggregation pheromone, and were used throughout the 1997 study (Fig. 2.1a). This trap was designed after a prototype by Mullen (1992). The trap is constructed of a 10 cm plastic circular ramp in the shape of an inverted cone. The ramp portion of the trap is roughed to facilitate insect footing. A cup ( $\approx 4$  cm in diameter) in the center of the trap holds the oil bait that is designed to act both as an attractant and a trapping medium. A filter paper disk lines the bottom of the cup to facilitate removing its contents. The trap comes with a cardboard cover that provides moderate dust and debris protection to the oil cup, as well as a means to hold the rubber septum impregnated with synthetic *Tribolium* aggregation pheromone, 4,8-dimethyldecanal (DMD) (Suzuki 1981). Traps were serviced by removing the contents and placing them in a sealed plastic bag, depositing approximately 1 ml of fresh trapping oil in the cup reservoir on a new filter paper disk, and placing a new pheromone lure in the trap, if needed. Pheromone lures were replaced every four wk. The contents of the pitfall traps were taken back to the laboratory for identification under a dissecting scope.

A modification of the pitfall trap was constructed in an attempt to improve trap efficacy in dusty environments and was used throughout the 1998 study (Fig. 2.1b). The modification consisted of a durable 10 cm PVC end-cap that replaced the standard cardboard cover. The cap rested on four plastic beads glued on its lower rim to allow

beetles clearance to the ramp-pitfall under the cap, and to allow release of volatile and attractants. A 2 mm hole was drilled in the top of the cap to receive the pheromone lure.

Sticky traps were also used during the 1997 trapping study to monitor insects at the floor level. The Detector trap (AgriSense, Palo Alto, CA) is made of cardboard with a 9.5 X 6.5 cm sticky area. The trap folds in on itself top to bottom to provide protection to the sticky surface. The traps use no pheromone or food attractants and are designed to capture any insect crawling onto them. These traps are not reusable, so were removed, replaced with a new trap, and the old trap was taken to the laboratory for identification.

### **Trapping Sites and Study Design**

**Mill 1.** Mill 1 was relatively large, producing 750,000 kg of flour per day. Trapping studies occurred during 1997 from 1 May to 26 November, and during 1998 from 16 June to 5 November. Traps were deployed in three main areas of the mill. Floors three through eight, which consisted of the milling areas where wheat was processed into flour (Fig. 3.1). Each milling floor was approximately  $\approx 25 \times 50$  m. Areas above and below the 36 bins containing the bulk stored flour were also monitored for insects; each measured  $\approx 20 \times 20$  m. Finally, insects were sampled in one of the two mill warehouses (warehouse 1 of chapter IV). The warehouse was divided into two separate areas: the main warehouse ( $\approx 65 \times 85$  m), where the packaged product was stored before shipping by rail or truck, and the feed area ( $\approx 10 \times 15$  m) that contained systems for processing animal feeds. Traps were placed in non-random paired treatment blocks containing one pitfall and one sticky trap, 3 m between each trap and each block, along the perimeter of the rooms. Trap placement was not changed throughout the 1997 study. A total of 36 pitfall traps and 26 sticky traps were placed throughout the mill.

Four, two-treatment blocks were placed on each of the mill floors (four pitfall traps and four sticky traps on each); two blocks above bulk storage bins (two pitfall and two sticky traps). Pitfall traps only (no sticky traps) were deployed below the bulk storage area (two traps), in the feed area of the warehouse (four traps), and in the main warehouse (four traps).

During the 1998 study, only the modified pitfall trap was used because of its design to reduce dust interference and the relatively poor performance of *Tribolium* capture by the sticky traps in 1997 (Chapter II). Floors four through eight were not included in the 1998 study because capture was low among these floors during the 1997 study. The first and second mill floors were used, which were not used during the 1997 study, and the third floor was again used. Two traps were placed on the first floor ( $\approx 12 \times 50$  m); this area was adjacent to a loading dock and experienced a lot of traffic from forklifts transporting bagged product. Four traps were placed on the second floor, which was used for flour packaging and was the same dimensions as floors three through eight ( $\approx 25 \times 50$  m). This area was chosen because the load-out tailings (see below) were collected, and the potential existed for capturing insects escaping the tailing collection bags.

Live *Tribolium* adults were monitored directly from product flow out of the bulk stored flour bins. Accumulated tail-over material from the six load-out systems that transported the flour from bulk storage bins to various packaging and bulk shipment systems was inspected regularly by mill staff. Tail-over or tailings refers to the particles of debris too large to pass through selective sieves in sifting devices. As the product is transported from storage to packaging and bulk load-out, it is directed through a final sift



that removes any foreign matter, including insects, and is collected as tail-over. Data from tail-over samples were used by mill personnel to assess insect infestation and plan control measures in the mill. There was interest in what relationship would be observed between *T. castaneum* sampled in tail-overs, and the number of beetles trapped throughout the mill. During the 1998 study, trap capture below the bulk stored flour bins was high enough to allow a correlation of it to the tail-over counts as well. The area below the bulk flour bins was isolated because it was in closest proximity to the bins, and therefore a closer correlation may have been observed between these trapping data than from the data collected from trapping data from throughout the entire mill. Mill 2 sampling data of the bulk product tailings were collected as well, but low beetle counts prevented a meaningful comparison.

Methyl bromide fumigations occurred on 31 May and 30 August 1997, and on 4 July 1998. The fumigant was applied to all areas of the mill used in trapping. The stored product bins, which were not used in trapping, but from which tail-over data were collected, were fumigated with magnesium phosphide on the same date of methyl bromide application. Phosphine from metal phosphides is generally used as opposed to methyl bromide on products such as bulk flour and wheat because it is better able to penetrate into the commodity. Nevertheless, to ensure adequate phosphine penetration, the bins had to contain less than 18,000 kg of flour out of a  $\approx$  60,000 kg capacity. Any bins containing more than 18,000 kg of flour were therefore not treated.

## Mill 2

This mill was smaller than mill 1, producing 225,000 kg of flour per day. Trapping occurred from 24 June to 5 November 1998. The mill consisted of four floors,

as well as a basement that contained the product elevator boots (Fig 3.1). The mill floors were divided into two separate areas: the main milling area ( $\approx 25 \times 50$  m), and the warehouse area. The warehouse area was north of the milling area, corresponding to the basement, and floors one through three of the main mill. A doorway on each floor connected these two areas of the mill. The warehouse area contained the bulk storage bins, and various equipment and supplies. From the basement up to the second floor, the warehouse areas were comparable in size to the milling areas ( $\approx 25 \times 50$  m), and the third floor warehouse was  $\approx 25 \times 25$  m. Two modified pitfall traps were placed in the perimeters of each milling floor (basement to fourth floor) and in each warehouse area (basement up to the 2nd floor); one trap was placed in the third floor warehouse area. Methyl bromide was applied on 1 August 1998 to all areas of the mill sampled by trapping.

### Data Analysis

The total numbers of stored-product beetle pests trapped (larvae and adult) for a given year in each mill were plotted as totals, and as functions of date and location. The total number of the most commonly trapped beetles are plotted for each years' study in each mill. The number of beetles captured were combined for the pitfall and sticky traps for the 1997 study due to the lower competency of the sticky traps for *Tribolium* (Chapter II), and only from the modified pitfall traps during the 1998 studies. Spatial variation was analyzed by plotting capture of the three most commonly trapped beetles for each mill. Captures of beetles per trap per trapping period at each designated location within the mills were computed and plotted on a biweekly (1997) or weekly (1998) basis. An analysis of variance using PROC MIXED in SAS (SAS Inst. 1996) was performed

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followed by a mean separation (LSD) to determine significant difference in capture of each of the beetles by location within the mill. Seasonal activity was plotted by summing beetle capture from all traps within the mills during a trapping period, and were expressed as the mill-wide number of beetles per trap per day. Seasonal activity was plotted for *T. castaneum* in mill 1, and for *T. castaneum*, *Typhaea stercorea* (L.) and *Cryptolestes ferrugineus* (Stephens) in mill 2. These additional two species were plotted for mill 2 because they were trapped more abundantly than *T. castaneum* in this mill. *T. castaneum* seasonal activity from tail-over samples was observed in mill 1 by consolidating the daily counts into biweekly (1997) or weekly (1998) counts that corresponded to the same intervals used for trapping. Values are plotted as live beetles per system per day. The daily beetle per system counts from the six systems were consolidated into biweekly (1997) or weekly (1998) beetle per system counts. In order to estimate the effects of phosphine and methyl bromide fumigation on beetle populations, a paired t-test was performed using PROC UNIVARIATE on square root-transformed beetle counts from traps and tail-over samples immediately before and after MB and phosphine fumigations with the null hypothesis that the difference between the two captures was not different from zero. The relationship between numbers of *T. castaneum* trapped to those sampled directly from the bulk flour bin tailings was assessed by correlating the biweekly or weekly beetles per trap capture to the mean number of beetles per system sampled from tailings for the same interval of time using PROC CORR. The confidence level for all analyses was set at  $\alpha = 0.05$ .

## Results

During the 1997 study in mill 1, *T. castaneum* was the most commonly captured pest, other species captured, in order of decreasing abundance, include: *Typhaea stercorea*, *Ahasverus advena* (Waltl), *Sitophilus oryzae* (L.), *Oryzaephilus surinamensis* (L.), and *Cryptolestes ferrugineus* (Stephens) (Fig. 3.2). During the 1998 study in mill 1, *T. castaneum* was again the most abundantly captured beetle pest, other species captured, in order of decreasing number, include: *T. stercorea*, *O. surinamensis*, *C. ferrugineus*, and *A. advena* (Fig. 3.3). Capture of all beetles increased during 1998 in mill 1 over 1997 captures. In mill 2 (1998 only), *T. stercorea* was the most abundant pest species captured throughout the study, followed by *C. ferrugineus*, *T. castaneum*, *Trogoderma spp.*, *O. surinamensis*, and *A. advena* (Fig. 3.4).

Capture of *T. stercorea* was significantly different by location within mill 1 during 1997, being highest in the feed area of warehouse. Capture of this beetle among all other locations was not significantly different (Fig. 3.5). The capture of *T. castaneum* and *C. ferrugineus* revealed no significant differences by location. During 1998 in mill 1, capture of *T. stercorea* was again significantly different by location with the highest capture being within the first floor. The capture of *T. castaneum* and *O. surinamensis* was not different among locations (Fig. 3.6). In mill 2, the capture of *T. castaneum*, *C. ferrugineus*, and *T. stercorea* was not different among locations (Fig. 3.7).

The seasonal capture of *T. castaneum* in mill 1 during 1997 was initially lower at the beginning of the study and was highest toward the end, regardless of MB fumigation (Fig. 3.8). Capture of the beetle was significantly lower following the 31 May MB fumigation, and increased after the 30 August fumigation, though not significantly over

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pre-fumigation capture ( $t = 1.74$ ;  $df = 50$ ;  $P = 0.0900$ ). Seasonal activity of *T. castaneum* from bulk load-out tailing samples in mill 1 during 1997 were generally higher at the beginning of the study (Fig. 3.8). Live beetles sampled from the load-out raised slightly following the 31 May phosphine fumigation, but not significantly higher than pre-fumigation capture ( $t = 2.49$ ;  $df = 5$ ;  $P = 0.2434$ ). Captures were significantly lower following the 30 August fumigation (Fig. 3.8). The test of the correlation coefficient of *T. castaneum* trapping data to data from tailing samples revealed no significant relationship during the 1997 study ( $r = 0.3973$ ;  $P = 0.1789$ ). *T. castaneum* capture was highest during the first week of the 1998 study in mill 1, and dropped significantly following the 4 July MB fumigation (Fig. 3.9). Observations of *T. castaneum* sampled in bulk load-out tailings during 1998 also dropped significantly following the 4 July fumigation (Fig. 3.9). Beetles in load-out samples remained relatively low until 10 September, followed by a steady increase throughout the remainder of the study. The test for the correlation coefficient comparing the mill 1 1998 *T. castaneum* trapping data throughout the entire mill to beetles sampled from tail-over revealed a nearly significant positive correlation (Fig. 3.10). Correlation of beetle counts from tail-over samples to the mean beetles per trap per day capture of the area below the bulk stored product bins was similarly nearly significant (Fig. 3.10).

The highest capture of *T. stercorea* in mill 2 during 1998 was observed during the second week of the study and declined over the following three weeks. Capture rose slightly following the 1 August MB fumigation, but not significantly from pre-fumigation capture ( $t = 1.58$ ;  $df = 16$ ;  $P = 0.1761$ ), and remained relatively steady throughout the remainder of the study (Fig. 3.11). Capture of *C. ferrugineus* was cyclic for the first five

weeks of the study followed by steady lower captures throughout the remainder (Fig. 3.11). Capture fell immediately preceding MB fumigation and was not significantly different following treatment ( $t = 1.81$ ;  $df = 16$ ,  $P = 0.0947$ ). Capture of *T. castaneum* was highest during the first week of the study, and decreased significantly following MB fumigation. Following fumigation, weekly beetle capture remained relatively low and steady throughout the remainder of the study.

## Discussion

Trap catches were lower than expected during the 1997 study in mill 1, particularly on the mill processing floors, despite evidence of a substantial beetle population observed from visual observations while at the mill. Low capture of *T. castaneum* was likely attributed to low efficiency of traps due to dusty conditions in the mill. Flour dust, particularly abundant among the mill processing floors, can offer a food source to beetle populations and appears to also interfere with the efficacy of traps that rely on sticky or oily surfaces to capture insects. It was commonly observed that the traps in the warehouse area were still effective after the two-week interval between trap servicing, whereas the trapping oil reservoir of traps in other areas of the mill had been saturated with dust to the point that they could not have killed any insects falling into them. Furthermore, the amount of dust covering the inner sides of the pitfall trap was often sufficient that any trapped insect could have likely crawled out of the trap; further reducing trap efficiency. Evidence of trap interference from dust was observed from the mill dust study reported in Chapter II. Traps placed in the warehouse area experienced little to no increase in weight due to dust, whereas the third floor of the mill experienced the highest dust increase. Tingle & Mitchell (1975) during moth trapping studies demonstrated that adhesive traps could be detrimentally affected by dust.

It was discovered that the method of cleaning the various mill areas played an important role in trap interference as well. An observation from the Chapter II dust accumulation study was that traps within some areas of the mill (i. e. the area below the bulk stored four bins and the feed area of warehouse 1), unlike the third floor processing area, were adequately protected from dust by the trap dust cover. This may explain why



dusty areas such as the feed area of warehouse 1 and the area below the bulk storage bins experienced relatively high beetle capture compared with the milling floors. The notable difference between these areas (below bulk bins and feed area) and the milling floors is that the feed area and the area below the bulk bins were only cleaned weekly with compressed air blow-downs, whereas the milling floors (floors 3 to 8) were cleaned daily with compressed air. These compressed air blow-downs proved rather detrimental to both the sticky and pitfall traps. The high pressure air currents created from this cleaning technique forced dust under even the PVC end-cap cover modification used to protect the pitfall trap (Chapter II). The standard cardboard covers for the pitfall traps used during 1997 likely offered even less protection from this cleaning technique. Therefore, trap efficacy in the mill seemed to be a function both of the amount of dust in the particular mill location, as well as the method and frequency of cleaning within that area. Thus, an accurate estimation of beetle spatial distribution in mill 1 was likely not recorded during either year, although possibly more accurate during 1998 over 1997 due to the dust cover modified traps used in the 1998 study. The suspected lower efficacy of traps used during 1997 may explain the lack of correlation observed between data from *T. castaneum* trap capture and those sampled from bulk load-out tailings. Trap efficiency was likely higher in mill 2 compared with mill 1, as mill 2 did not perform compressed air blow-downs, but rather cleaned with a vacuum system and sweeping.

Capture of *T. castaneum* in mill 1 was increased during the 1998 study, perhaps due to factors in addition to use of the modified trap. The decreased interval of trap servicing interval from biweekly to one-week likely played a role in higher trap capture. Many of the traps on the mill floors were likely rendered ineffective after one or two of



the daily compressed-air floor cleanings described above. Therefore, more frequent trap servicing would allow more time overall to capture insects before they would be inundated with dust from cleaning. This idea is further suggested by the fact that all other beetle pests captured during the 1998 study, with the exception of *S. oryzae*, increased over 1997 captures as well. The overall *T. castaneum* populations may have been higher in the mill during 1998. Based on the only independent measure of beetle abundance available, namely, the number of beetles sampled from tailings of the bulk load-out, the total number of beetles observed for the 1997 study was 410.55 (30 wk), compared with 1,100.00 (20 wk) for the 1998 study.

Mill 2 was different than mill 1 in relative abundance of beetle pests captured. *T. stercorea* was the most abundantly captured pest in mill 2. The location of *T. stercorea* capture is likely explained by this species' fungivorous feeding habits (Sinha & Watters 1985), and therefore it is not surprising that this beetle was commonly trapped in the musty basement areas. *C. ferrugineus* was the second most abundantly captured beetle in mill 2 and was largely concentrated in space and time to the fourth floor during the first and fourth weeks of the study. These data indicate a "hot spot" of activity, which was apparently temporal, as capture of this beetle was relatively low following the initial peaks of activity. *T. castaneum* was the third most abundantly captured species in mill 2. Capture of this beetle both spatially and temporally was less variable than the other two species and likely reflects a steady and relatively evenly dispersed population throughout the mill. It is interesting to note that *T. stercorea* and *C. ferrugineus* were captured more abundantly than *T. castaneum*, despite using pheromone-baited traps specifically for *Tribolium*. This observation, as well as the low number of *Tribolium* sampled from the

bulk product, are evidence that *T. castaneum* was not as abundant as other pests in this mill. Another difference of mill 2 beetle capture over mill 1 was that a notable amount of *Trogoderma* were captured in mill 2, where they were rarely captured in mill 1. These differences between the two mills probably reflect varying beetle populations among the two locations.

One striking difference between the two mills was the apparent lack of large beetle populations in mill 2 bulk flour bins compared with mill 1 as evidenced from tail-over samples. This can most likely be explained by the high turn-over in mill 2—each bin was emptied twice a day according to the mill manager. Mill 2 had much less storage capacity than mill 1, furthermore all load-out systems were bulk to rail or truck, which facilitated fast turn-over compared with bagging systems used in mill 1. Therefore, flour was cycled through these bins, apparently before a beetle infestation could take hold.

It should be understood that not every pest species captured in the mill is a direct threat to the product. For instance, the large size, length, and amount of setae on *T. stercorea*, which give it its hairy appearance also make it unable to maneuver through bulk flour. This fact, along with the beetles preference for fungi make it a minor economic pest to the mill; however, its presence should be of concern as harbouring large populations of any pest insect is undesirable. Another beetle, *C. ferrugeneus* prefers the germ portion of the wheat kernel (Sinha & Watters 1985). Because flour does not contain wheat germ, this beetle is more often a pest to whole wheat or intermediate flour products that still contain germ.

Varying effects on *T. castaneum* populations of fumigations are indicated according to the particular treatments and methods of sampling. According to the tail-

over counts, the 31 May 1997 fumigation in mill 1 seemed to have had no effect on the beetle population, whereas the beetle trap captures decreased following this same fumigation. The lack of effect observed from tail-over data following the 31 May phosphine fumigation is most likely due to the fact that many of the bins contained too much flour to be treated. Conversely, the 30 August fumigation does seem to have been effective according to the tailing counts; not surprisingly, most of the bins were fumigated. Trap captures, however, increased slightly following the 30 August MB treatment. The 4 July 1998 fumigation in mill 1 was followed by significant reductions in both the numbers of beetles sampled from tailings, and beetles trapped.

Conclusions regarding MB effectiveness are difficult to make from these data. Because phosphine was used in the flour bins, trapping data are the only estimate available of MB effect. Furthermore, the mill is not a closed system, so trapping data may have included immigrating beetles from outside following fumigation, and therefore may confound results. Better attempts should be made to prevent the possible access of beetles into the mill from outside in order to isolate any fumigation effect. This is obviously a challenge in a functioning mill where bay doors must be opened to ship the product. Nevertheless, fumigation effects were likely reflected by significant reductions in beetle captures following treatment, although captures were never eliminated completely.

Results from 1998 in mill 1 show a positive relationship between numbers of *T. castaneum* caught in traps and those sampled in bulk flour load-outs. Both the comparisons of beetles captured mill-wide, as well as that of beetles captured directly below the bulk flour bins were similarly nearly significantly correlated to tailings. The

majority of *T. castaneum* capture occurred in the area below the bulk stored product bins, therefore, the overall mill capture was largely made up of capture within this area, and partly explains the similarities. Also, capture throughout the mill locations was relatively consistent; thus, isolating capture below the bins did not reveal any capture patterns that were not expressed in the overall mill capture.

An important consideration regarding the comparison of trap capture to tailings counts is that although the counts were both taken within the same facility, and counted the same beetle species, the bulk stored product bins and various areas of the mill are quite different environments. The bulk stored bins offer a large, relatively undisturbed and unlimited food source, whereas beetles occurring throughout other areas of the mill (i. e. in machinery, floors, and walls) are likely limited in food supply, shelter, and are more likely to be frequently disturbed from sanitation procedures. These differences, no doubt, affect the beetle population dynamics. Therefore, it may not be surprising that the two data sets in neither 1997 nor 1998 correlated significantly to each other.

Although mill personnel were already actively monitoring insect activity via tail-over counts, trapping offered important additional information on insect activity for a number of reasons. First, trapping allowed a look at insect activity outside the product line. This technique could potentially be used to identify "hot spots" of pest activity allowing for more localised control measures, and thereby avoiding or reducing the need for costly fumigations (Vick et al. 1990). Pheromone trapping can also provide data on the dynamics of a pest population that would allow for better timing of control measures (Chambers 1990, Phillips 1994). Additionally, trapping allows an observation of insect activity before and after chemical treatment, thereby offering an estimation of

effectiveness (Levinson & Buchelos 1979). Estimates of insect activity outside the facility and immigration/emigration can be done through trapping (Vick et al. 1990, Chapter IV). Additionally, the activity of more insect pests can be monitored from trapping data than was available from tailings where primarily only *Tribolium* occurred (Faustini et al. 1990).

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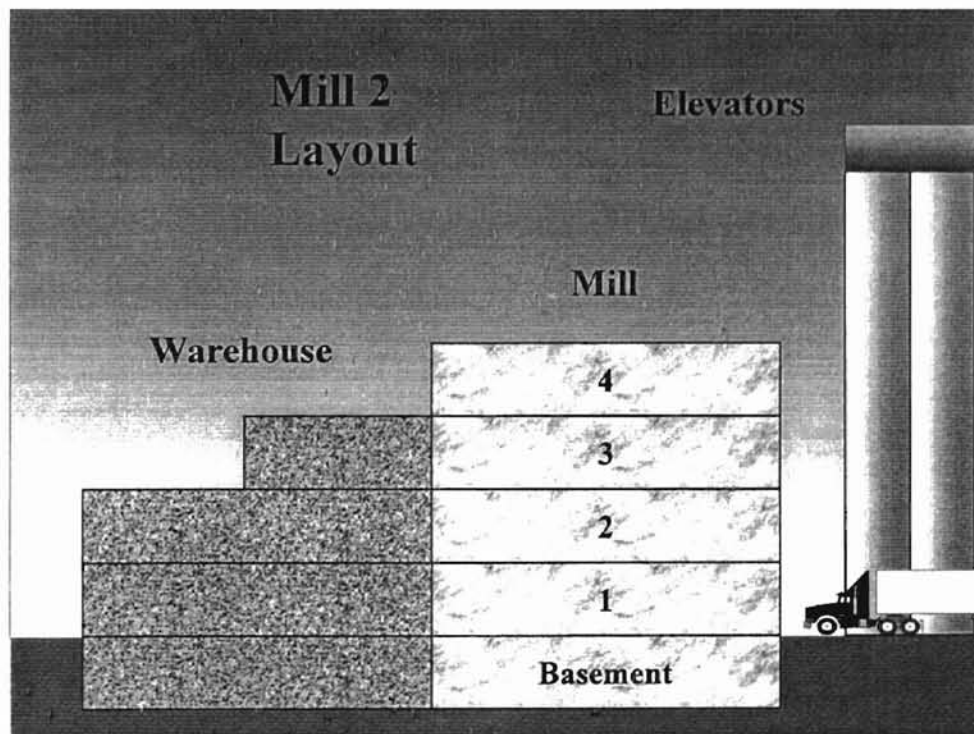
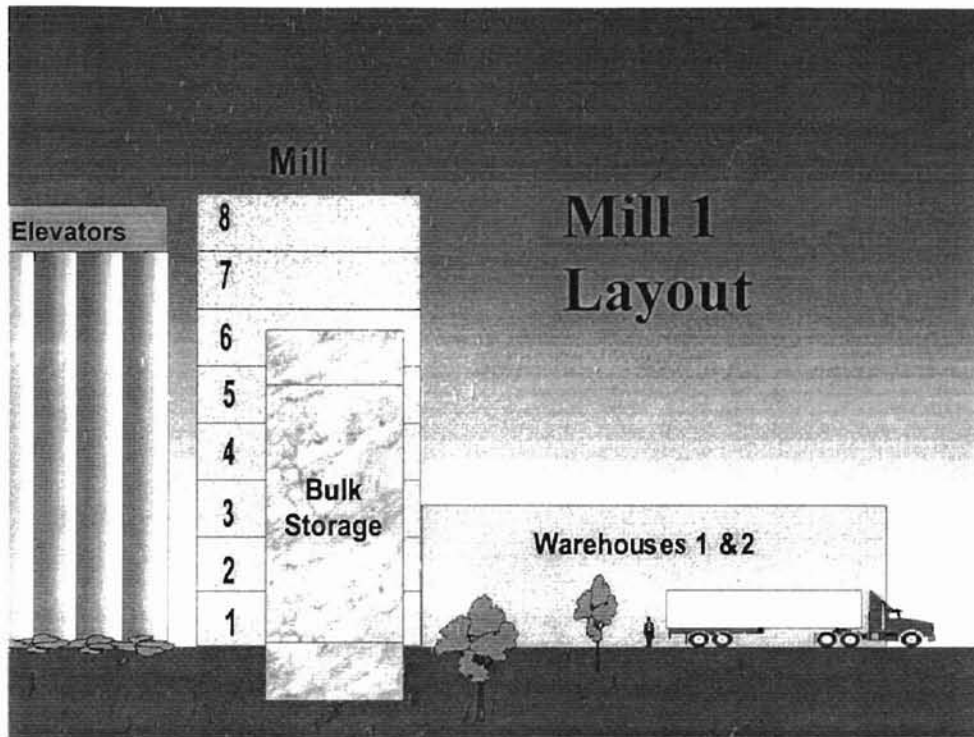
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**Fig. 3.1.** Layout of mills 1 & 2. Traps were deployed on floors three to eight of mill 1, above and below the bulk flour bins, and in warehouse 1. Traps were deployed among all mill 2 floors (basement to fourth floor) and corresponding warehouse areas of each floor (basement to third floor).



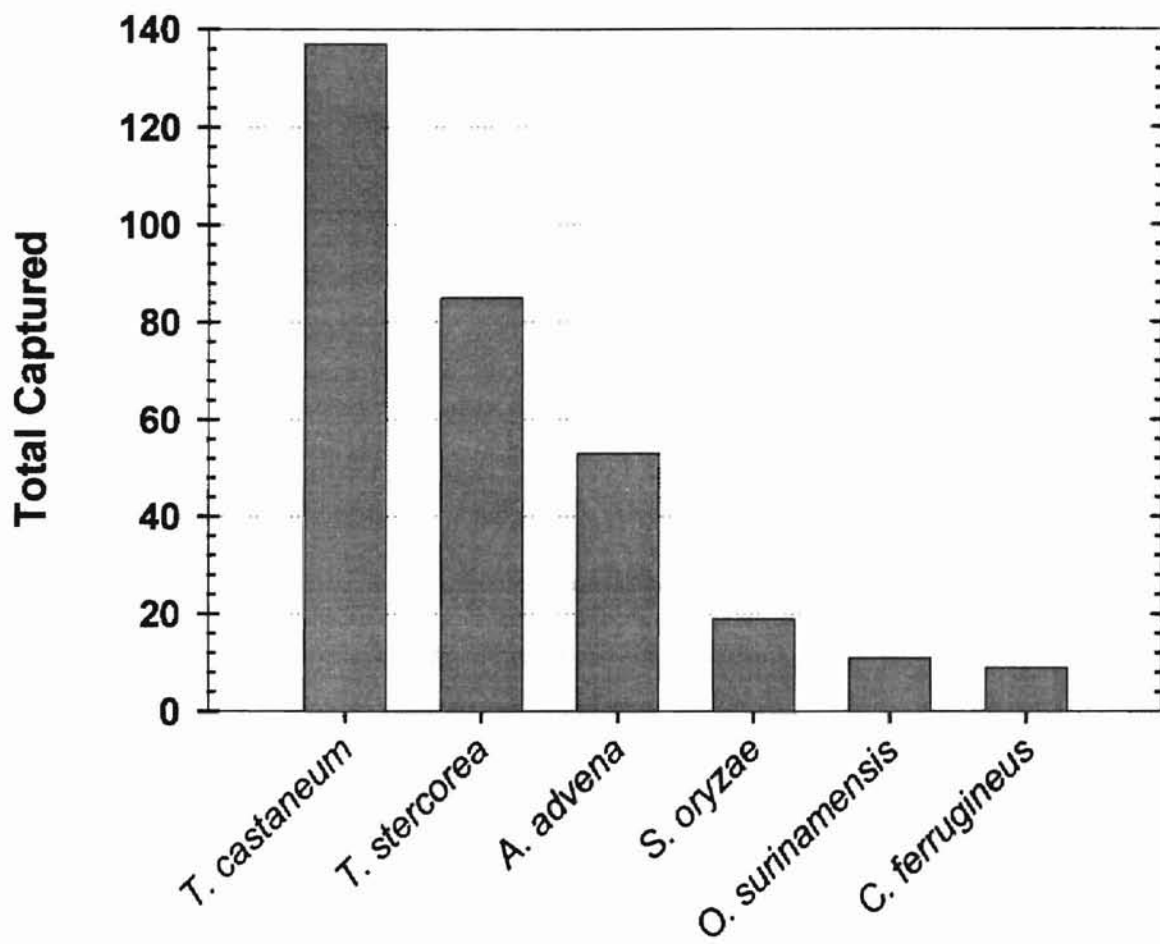


Mill 1 - Flour mill, 1900s  
 Mill 2 - Flour mill, 1900s

**Fig. 3.2.** Total capture of stored-product beetle pests throughout mill 1 during 1997.

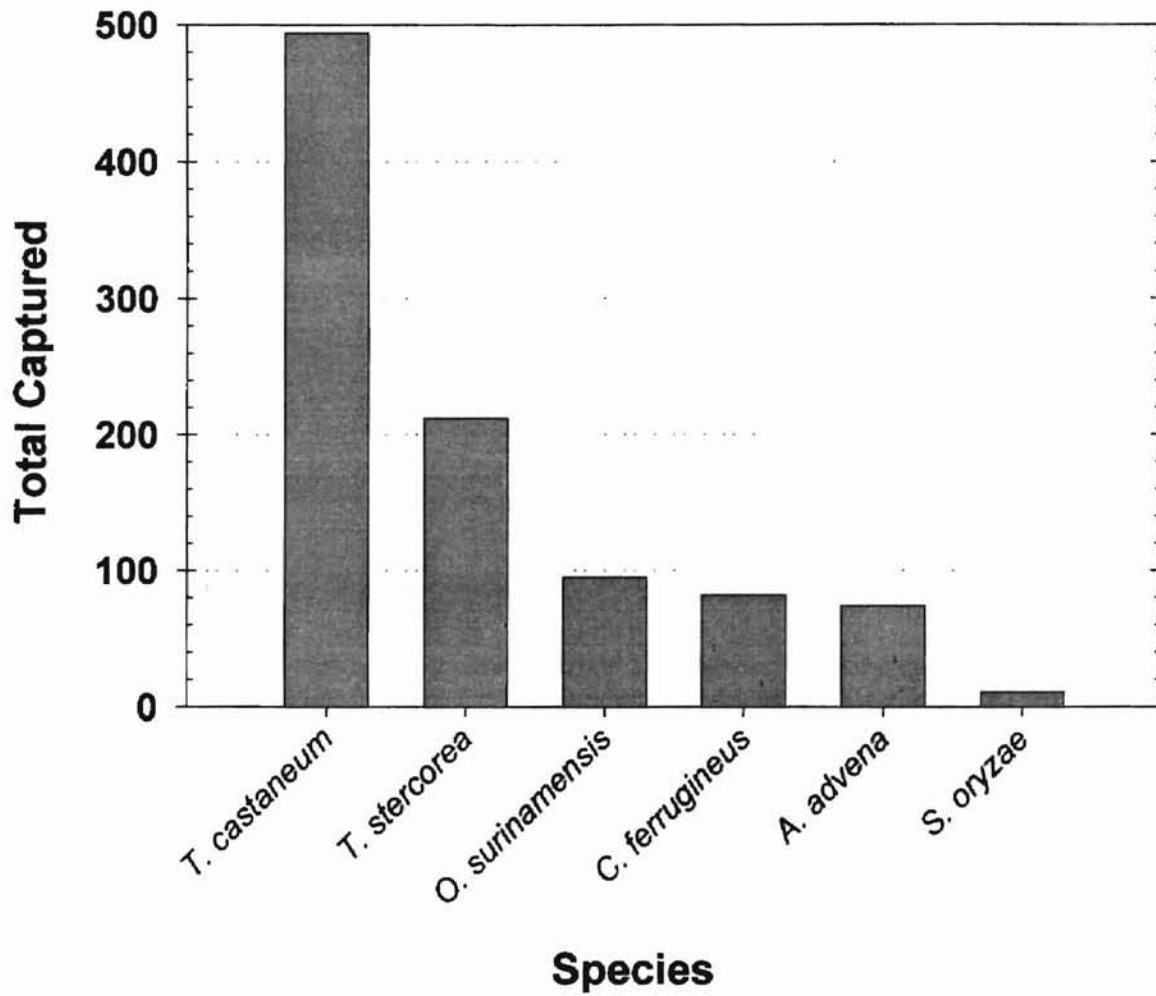
Results are from 13 biweekly observations from 34 pitfall and 28 sticky traps.

Mill 1 - Total - 1997



**Fig. 3.3.** Total capture of stored-product beetle pests throughout mill 1 during 1998.

Results are from 18 weekly observations from 16 modified pitfall traps.



**Fig. 3.4.** Total capture of stored-product beetle pests throughout mill 2 during 1998.  
Results are from 18 weekly observations from 17 modified pitfall traps.

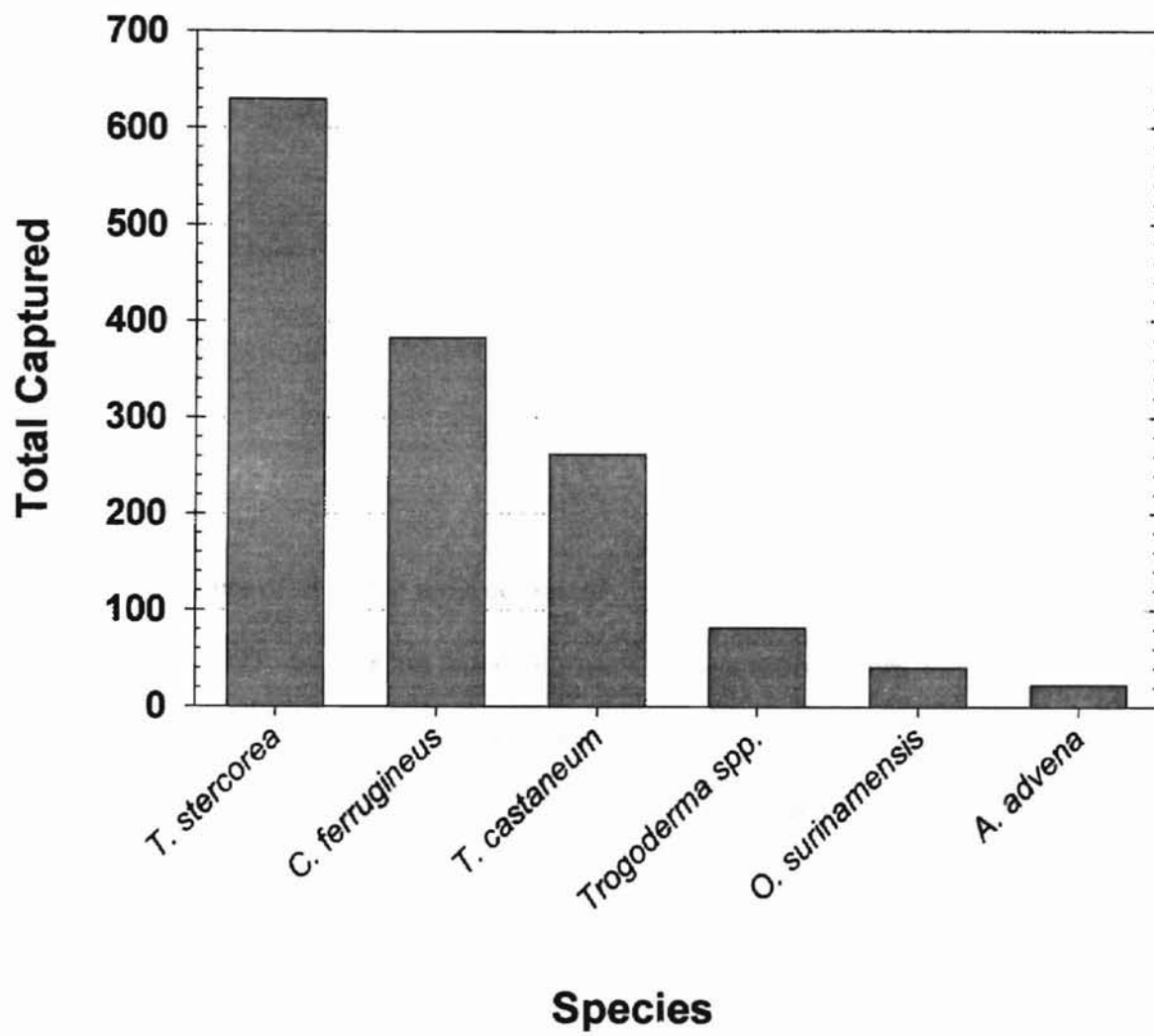
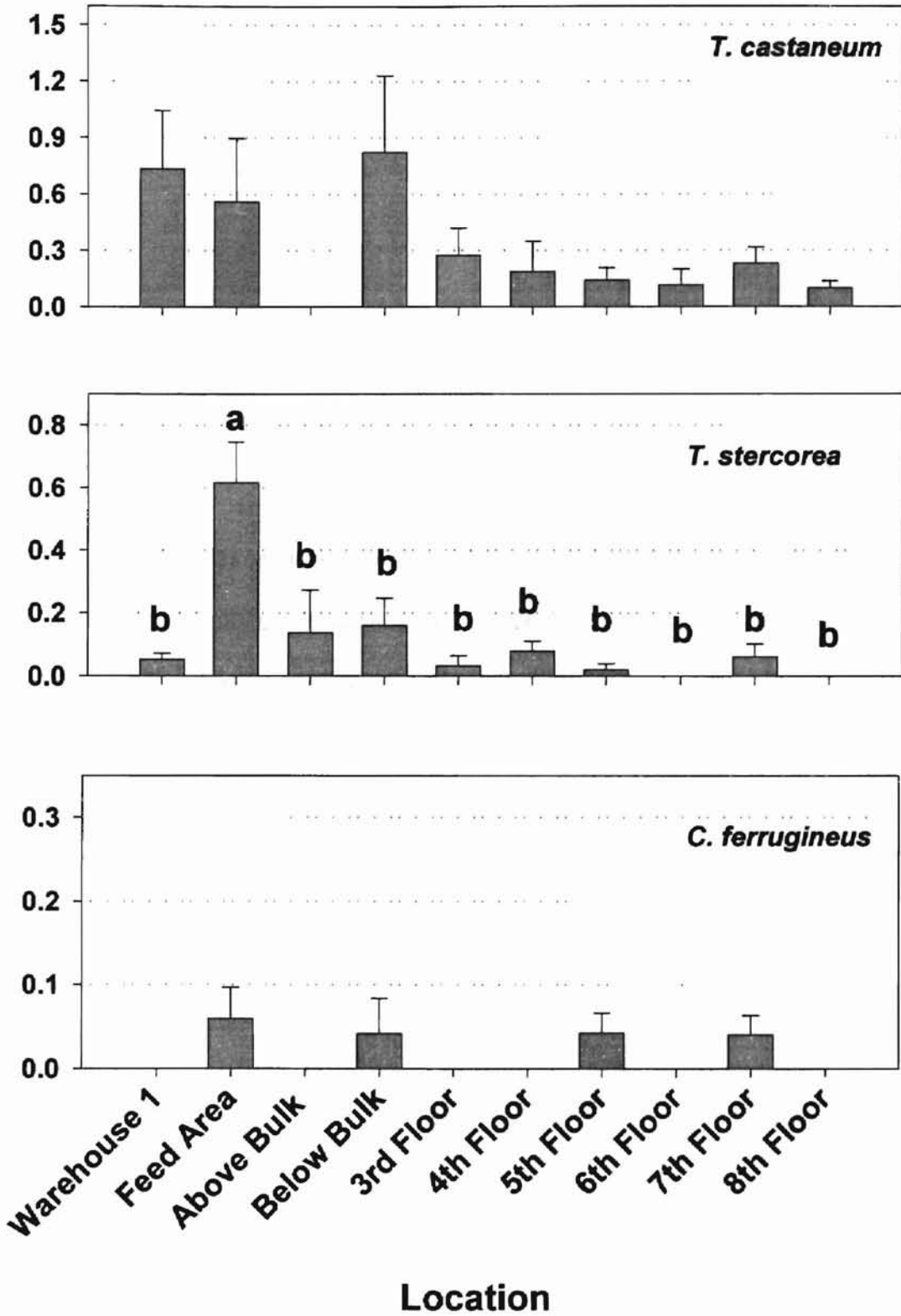


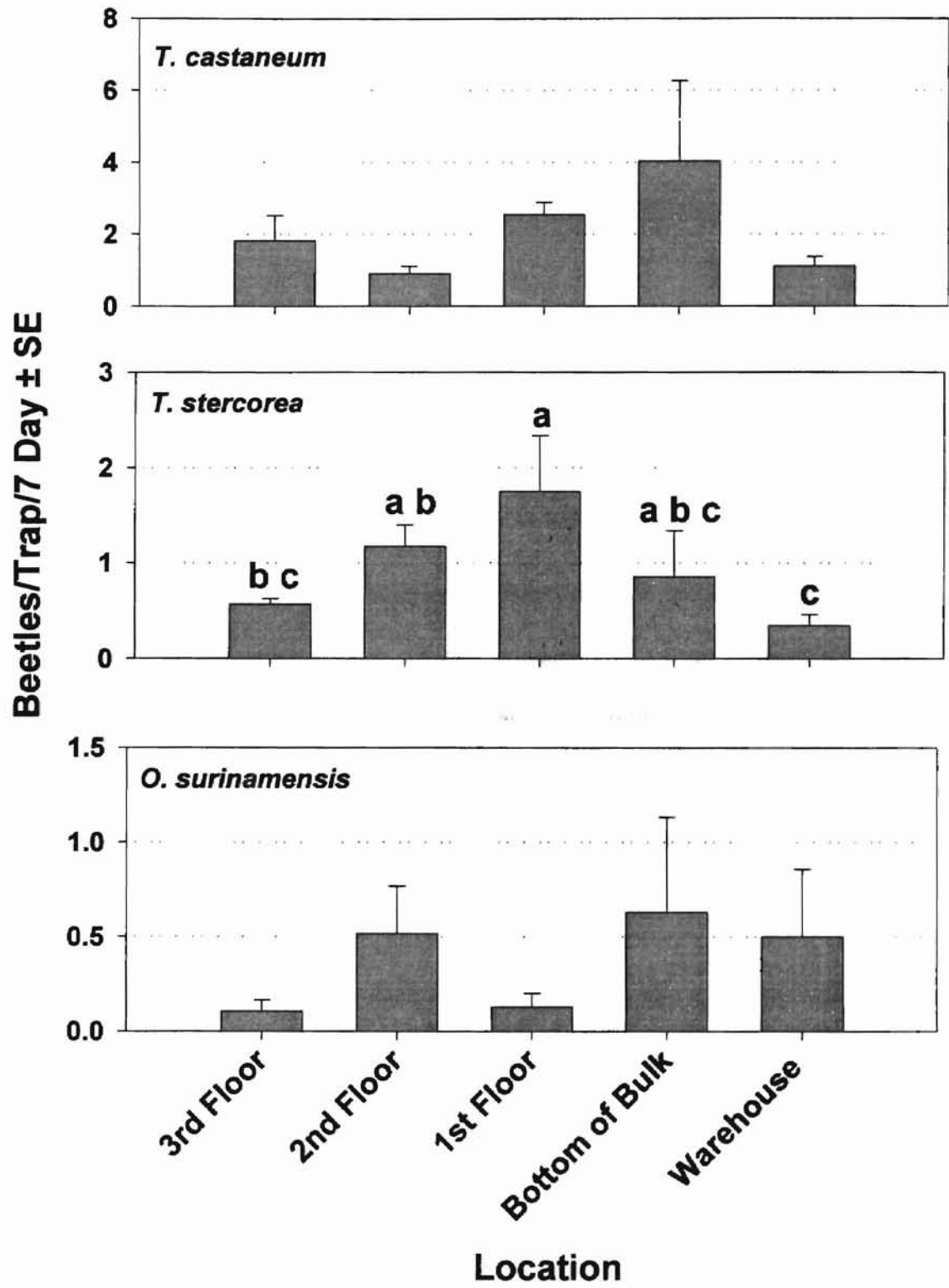
Fig. 3.5. Capture of the three most commonly trapped beetle pests by locations within mill 1 during 1997. Results are from 13 biweekly observations from 34 pitfall traps. Capture of *T. castaneum* was not significantly different by location ( $F = 1.77$ ;  $df = 9, 25$ ;  $P = 0.1241$ ). There was a significant difference in capture of *T. stercorea* ( $F = 10.90$ ;  $df = 9, 25$ ;  $P = 0.0001$ ). Capture of *C. ferrugineus* was not significantly different by location ( $F = 1.57$ ;  $df = 9, 25$ ;  $P = 0.1791$ ). Means with the same letter are not significantly different.



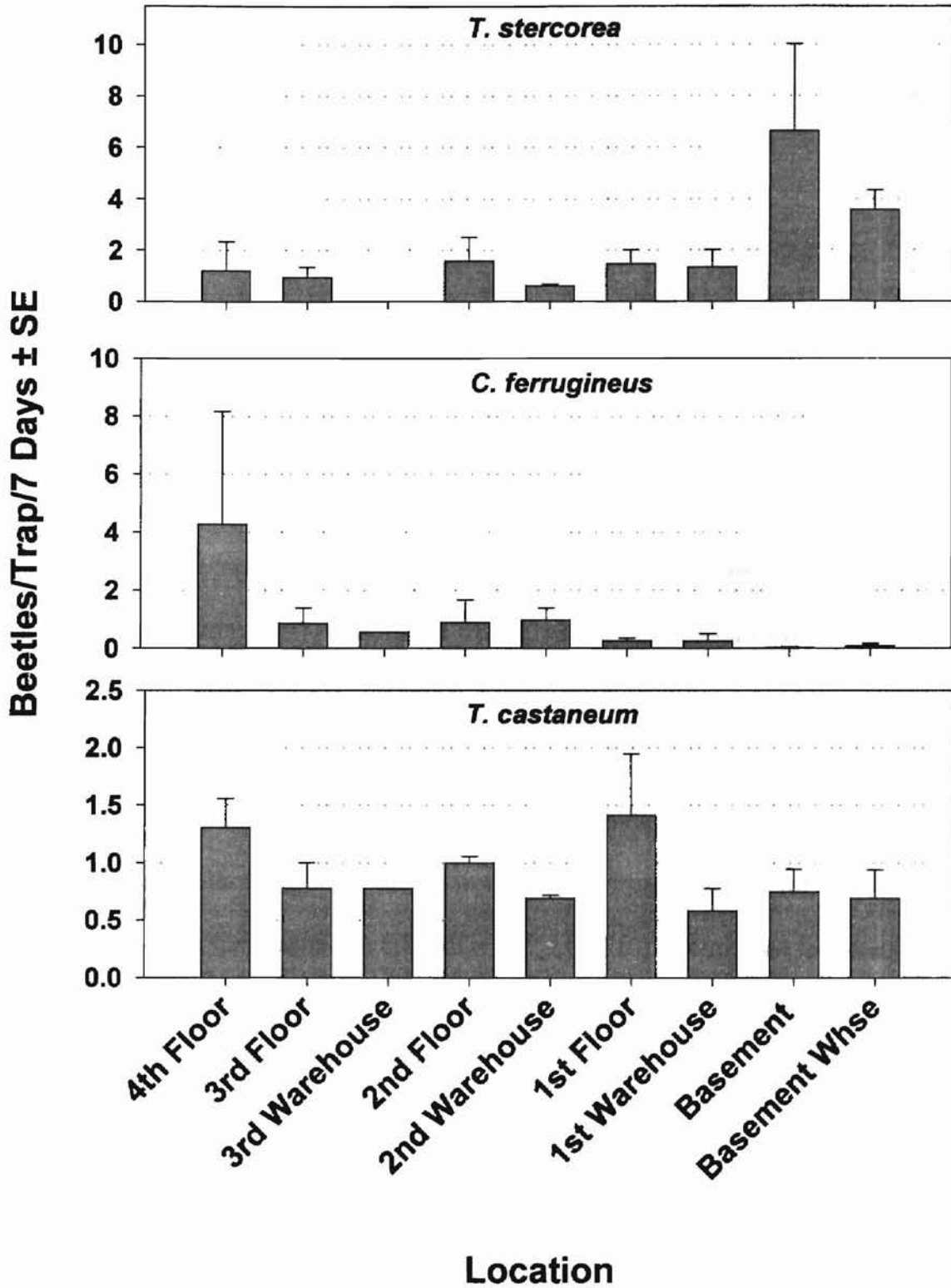
Beetles/Trap/14 Day  $\pm$  SE



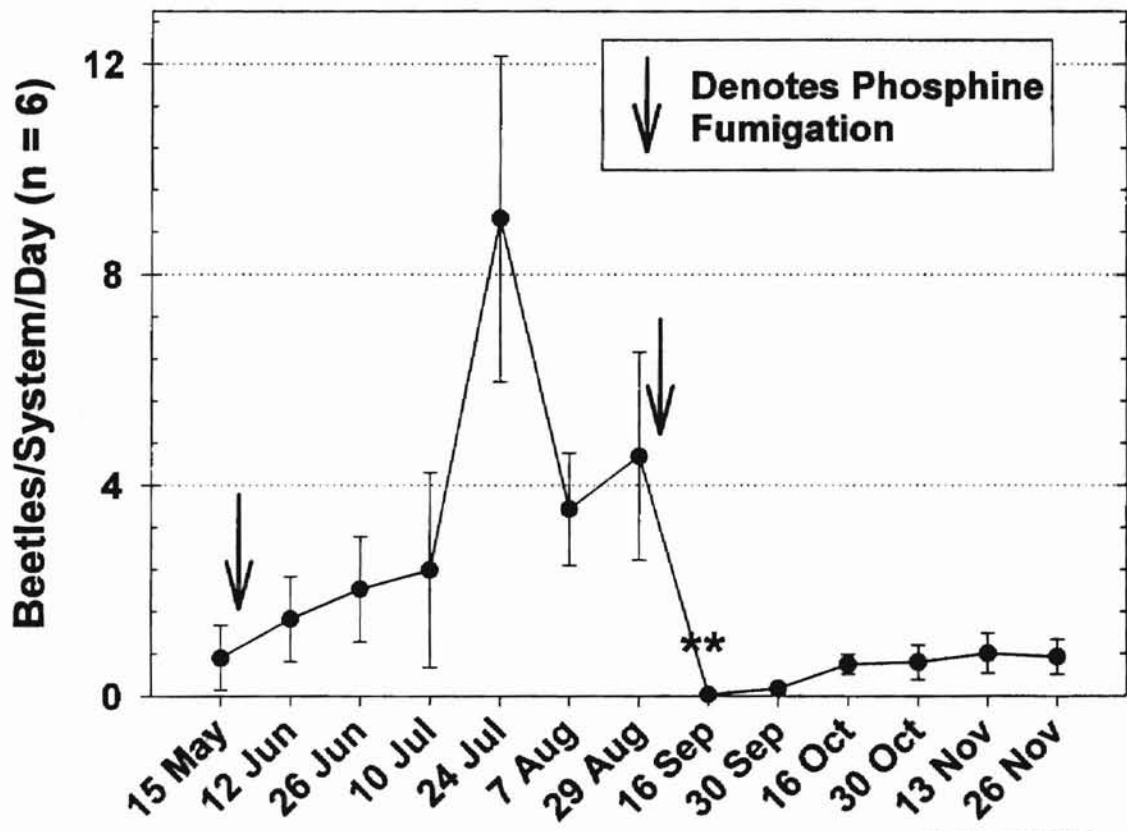
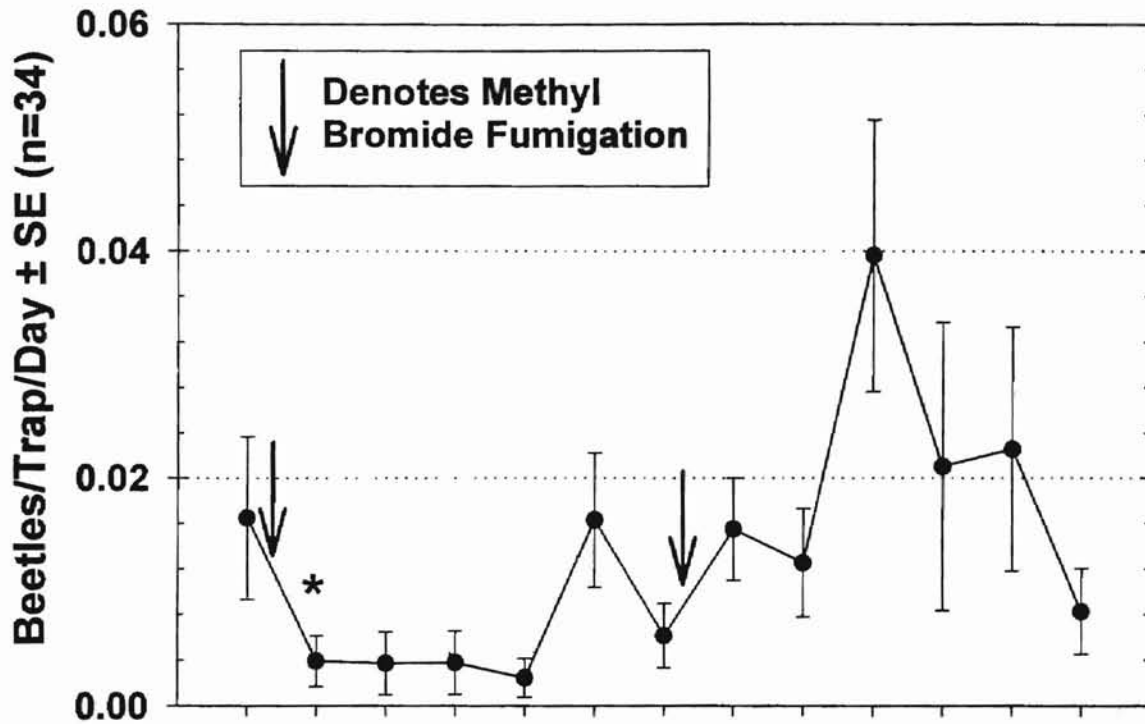
**Fig. 3.6.** Capture of the three most commonly trapped beetle pests by location within mill 1 during 1998. Results are from 18 weekly observations from 16 modified pitfall traps. Capture of *T. castaneum* was not significantly different by location ( $F = 2.59$ ;  $df = 4, 11$ ;  $P = 0.0952$ ). There was a significant difference in capture of *T. stercorea* ( $F = 4.79$ ;  $df = 4, 11$ ;  $P = 0.0176$ ). Capture of *O. surinamensis* was not significantly different by location ( $F = 0.66$ ;  $df = 4, 11$ ;  $P = 0.6315$ ). Means with the same letter are not significantly different.



**Fig. 3.7.** Capture of the three most commonly trapped beetle pests by location within mill 2 during 1998. Results are from 18 weekly observations from 17 modified pitfall traps. Capture of *T. stercorea* was not significantly different by location ( $F = 2.28$ ;  $df = 8, 8$ ;  $P = 0.1326$ ). There was not a significant difference in capture of *C. ferrugineus* ( $F = 0.84$ ;  $df = 8, 8$ ;  $P = 0.5917$ ). Capture of *T. castaneum* was not significantly different by location ( $F = 1.25$ ;  $df = 8, 8$ ;  $P = 0.3804$ ).



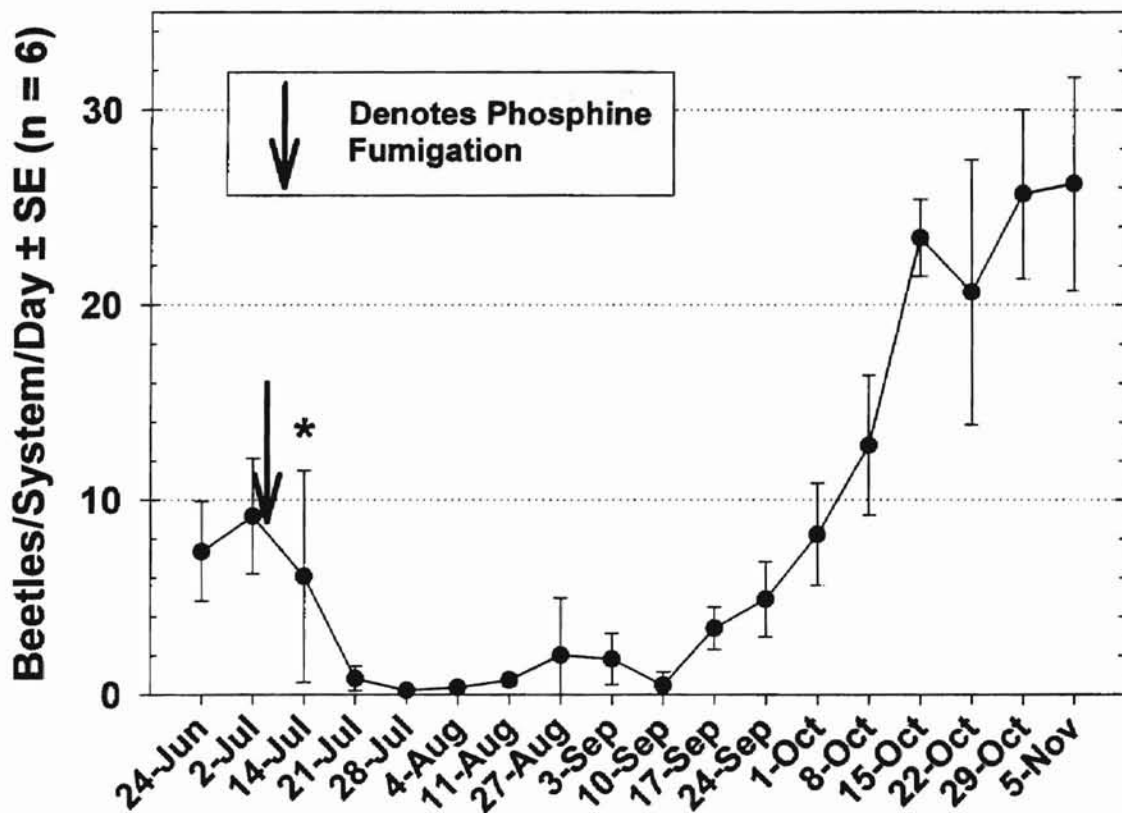
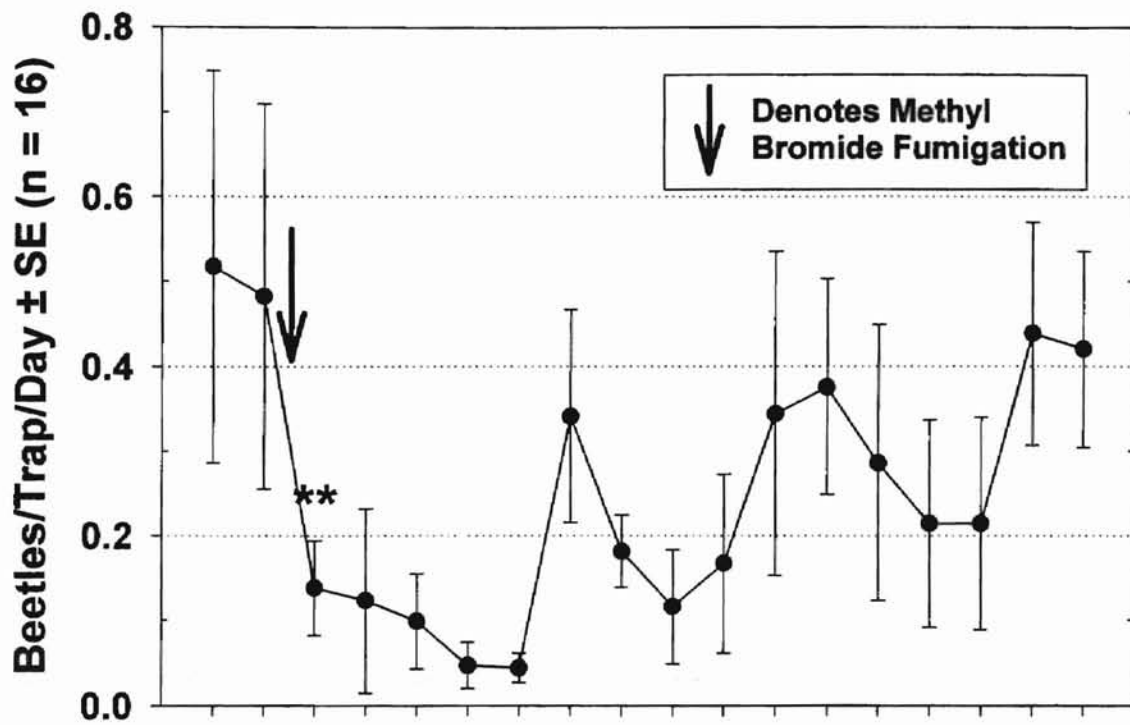
**Fig. 3.8.** Trap capture throughout mill 1 and counts of *T. castaneum* from six load-out system tailings during 1997. Results are from biweekly observations from 34 pitfall traps. Load-out counts are consolidated to correspond with trapping times and intervals. Arrows denote fumigations on 31 May and 30 August. Significantly fewer numbers of beetles were trapped following the 31 May methyl bromide fumigation ( $t = 2.13$ ;  $df = 23$ ;  $P = 0.0455$ ). Significantly fewer beetles were sampled from tail-overs following the 30 August phosphine fumigation ( $t = 6.83$ ;  $df = 5$ ;  $P = 0.0010$ ). Count data for t-tests were square root transformed.



\*  $P < 0.05$   
 \*\*  $P < 0.01$

Fig. 3.9. Trap capture throughout mill 1 and counts of *T. castaneum* from six load-out system tailings during 1998. Trapping results are from weekly observations from 16 modified pitfall traps. Load-out counts are consolidated to correspond with trapping times and intervals. Arrows denote fumigation on 4 July. Beetle trap capture was significantly lower following methyl bromide fumigation ( $t = 3.72$ ;  $df = 11$ ;  $P = 0.0047$ ). Beetles sampled from tail-over samples were significantly lower following phosphine fumigation ( $t = 4.10$ ;  $df = 4$ ;  $P = 0.0262$ ). Count data for t-tests were square root transformed.

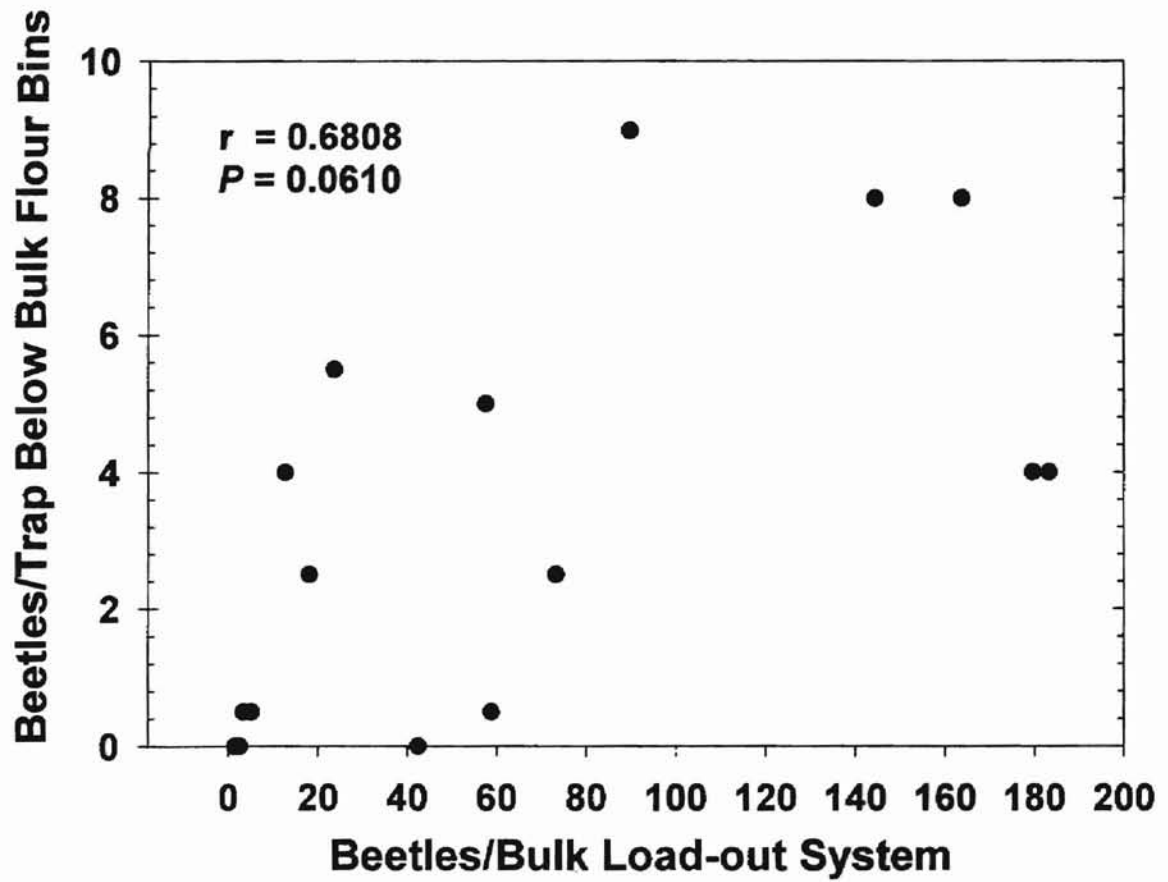
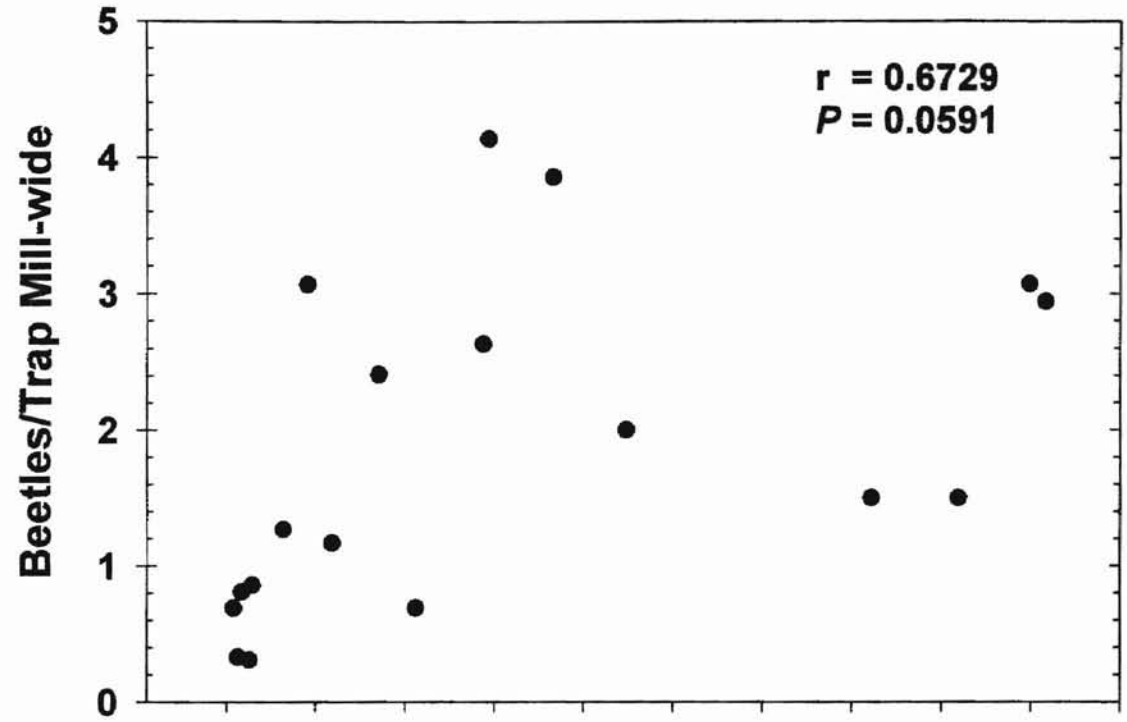




\*  $P < 0.05$   
 \*\*  $P < 0.01$

$r = 0.5178$   
 $P < 0.0001$

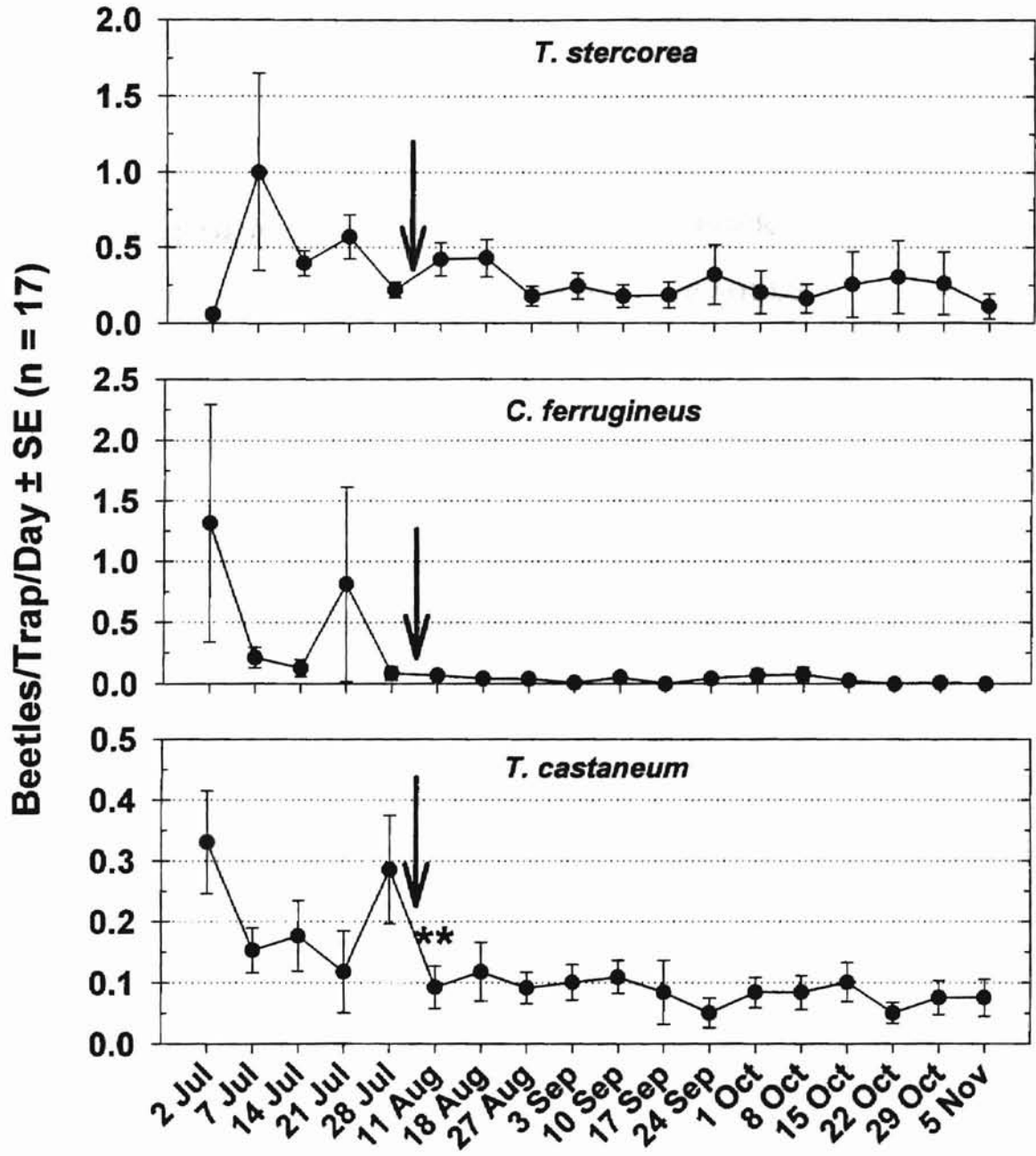
**Fig. 3.10.** Correlation of *T. castaneum* trapped in mill 1 to those sampled from bulk load-out tailovers during 1998. Results are from 18 weekly observations from 16 modified pitfall traps (mill-wide data) and 2 modified pitfall traps (below bulk flour bins data). Load-out data are from six load-out systems.



**Fig. 3.11.** Capture of *T. stercorea*, *C. ferrugineus*, and *T. castaneum* throughout mill 2 during 1998. Results are from weekly observations from 17 modified pitfall traps.

Arrows denote methyl bromide fumigation on 1 August. Capture of *T. castaneum* was significantly lower following methyl bromide fumigation ( $t = 3.88$ ;  $df = 16$ ;  $P = 0.0015$ ).

Count data for t-tests were square root transformed.



↓ Denotes Methyl Bromide Fumigation

\*\*  $P < 0.01$

Abstract

Introduction

Materials

## CHAPTER IV

### **CAPTURE OF *PLODIA INTERPUCTELLA* WITH PHEROMONE-BAITED TRAPS IN AND AROUND FLOUR MILLS**

## Abstract

Studies were conducted at two flour mills where males of the Indianmeal moth, *Plodia interpunctella*, were captured using pheromone-baited traps. Objectives were: 1) to determine male *P. interpunctella* distribution in space and time through the use of pheromone-baited traps, and 2) to monitor insect activity before and after methyl bromide fumigation to assess efficacy of treatment. Commercially available sticky traps baited with the *P. interpunctella* sex pheromone were placed at various locations outside and within the larger mill. Moths were captured in substantial numbers following methyl bromide fumigations. The highest numbers of *P. interpunctella* were caught outside the facility and at ground floor locations near outside openings. During the second year of this study, additional traps were placed in gallery areas located above the mill's concrete stored-wheat silos and these traps captured more moths than those traps within the mill. An additional study attempted to determine the outdoor dispersion of moths relative to the mill, by trapping moths at various distances from the structure. Results revealed a negative correlation between moth capture and increasing distance from the facility, suggesting that the flour mill is the focal point of moth activity. The effectiveness of the methyl bromide fumigations could not be assessed since moths captured after fumigation may have immigrated from outdoors. This study documents high levels of *P. interpunctella* outdoors relative to those recorded inside a food processing facility. Therefore, potential for immigration of *P. interpunctella* into flour mills and other stored product facilities from outdoor habitats may be greater than previously recognized.

immediately outside the structure. These early studies suggest that outdoor occurrences of moths such as *P. interpunctella* and *C. cautella* can be attributed to emigration from nearby storages, and that these species do not breed in wild habitats. Some researchers have concluded that moth infestation inside a food processing facility is mainly attributed to the introduction of infested product (Levinson & Buchelos 1979), rather than by immigration of adults from outdoor locations.

The present study monitored *P. interpunctella* in and around two flour mills in both 1997 and 1998. Specific objectives of the study were: 1) to observe variation in moth activity in space and time both inside and outside the building, 2) determine *P. interpunctella* activity before and after methyl bromide fumigations to obtain a relative measure of fumigation impact on the moth population, and 3) evaluate the outdoor dispersion of the moth relative to a flour mill.



### Traps

Two types of sticky traps designed for flying insects, both manufactured by Trécé Inc. (Salinas, CA), were used in these studies. The Pherocon III D delta trap was used in both 1997 and 1998 mill trapping studies. The reduced size of the openings of this trap type makes it more effective for use in dusty environments such as flour mills. This trap measures 18 cm wide by 11 cm in height. The other model, Pherocon II, was used in the outdoor dispersion study. It forms a diamond shape when viewed end-on, is smaller than the Pherocon III D (15 cm X 18 cm), and lacks the end features to reduce dust interference. Moths and other flying insects are captured by entering the trap from the lateral opening and then become stuck when contacting the sticky inner surface.

All traps were baited with pheromone lures provided by the manufacturer for use with these traps. Lures consisted of a rubber septum impregnated with ZETA, and were placed on the trap's bottom inner sticky surface. Mullen et al. (1991) tested the longevity of these lures and found that they were effective in capturing *P. interpunctella* for up to 40 wk. However, in these studies lures were replaced every four wk in order to maintain relatively constant pheromone release.

### Mill Trapping Studies

Traps were deployed in and around a commercial wheat flour mill (mill 1 of Chapter III). Four main areas inside the mill buildings were monitored for *P. interpunctella* during either one or both years' studies. Traps were deployed on floors three through eight which consisted of milling areas where wheat was processed into

flour (Fig. 3.1), and measured  $\approx 25 \times 50$  m. Areas above and below the 28 bins containing the bulk stored flour were also monitored for moths. These areas measured  $\approx 20 \times 20$  m. Traps were also deployed in each of the mill warehouses where the packaged product was stored before shipping by rail or truck. Warehouse 1 was divided into two separate areas, the main warehouse ( $\approx 65 \times 85$  m), where the packaged product was stored before shipping, and the feed area ( $\approx 10 \times 15$  m) that contained systems for processing animal feeds. Warehouse 2 measured  $\approx 65 \times 85$  m and was adjacent to warehouse 1. Finally, traps were placed in the gallery (enclosed top area) above the concrete silos that stored whole wheat in the grain elevator section of the mill which consisted of three main rooms, each  $\approx 30 \times 100$  m. These gallery areas were only used during part of the 1998 study.

Traps were positioned approximately 2 m from the floor, from 3 to 30 m apart, depending on size of the trapping area. When two traps were deployed within a given area, they were placed at opposing ends of the area. During both years, four traps were placed in warehouse 2 attached to support beams located in the center of the warehouse, approximately 10 m apart. Traps were replaced as needed when either more than half the sticky surface was covered with moths, or the trap had accumulated enough dust that its effectiveness was likely reduced.

During the 1997 study (1 May - 26 November), traps were checked biweekly and all *P. interpunctella* counted. Two traps were placed on floors three through eight, in areas above and below the bulk-stored product, and in warehouse 1. Four traps were positioned in warehouse 2. Four traps were added outside the mill on 29 August, two were placed 30 m apart in a field 50 m south of the mill, and two others were positioned

next to the south side of the building, one near the rail car mill entrance, and another on the south loading dock.

During the 1998 study (16 June - 5 November), traps were checked weekly and placed similarly inside the mill to the 1997 study placement, although no traps were placed on floors four through eight and in the area above the bulk stored used due to low capture of moths in 1997. Traps were placed outside the mill at the beginning of the 1998 study; one in a field 50 m south of the mill, another 50 m north of the mill, a third two m south of the mill on the south loading dock, and the fourth trap was attached to the outside wall of the mill on the west loading dock. In addition, one trap was placed in each of the three gallery areas above the wheat silos on 10 September.

Fumigations with methyl bromide were done on 31 May and 30 August 1997, and on 4 July 1998 by a contracted fumigator. All areas of the mill were fumigated with the exception of warehouse 2 and the wheat silos. Flour inside the bulk storage bins was fumigated with magnesium phosphide on the same dates. All traps were removed 1-3 d prior to fumigations and replaced as soon after treatment as possible in order to obtain an accurate assessment of the impact of fumigation on moth populations.

### **Outdoor Moth Dispersion Study**

This experiment utilized mill 2 described in Chapter III. This location was preferable to mill 1 because the objective was to trap moths at different distances from a single large food source (the flour mill and its grain elevators). Mill 1 was surrounded by several neighboring grain structures that likely harbored *P. interpunctella* populations that may have confounded the results. Traps were positioned throughout the residential area surrounding mill 2 at distances from 6 to 440 m in various directions (Fig. 4.1). Efforts

were made to distribute the traps in a manner that provided good representation of both distance and direction from the mill. Twenty eight new traps and lures were attached  $\approx$  3 m high on utility poles, collected after one wk, and the number of *P. interpunctella* males captured was recorded. The distance of each trap from the mill was determined using a survey laser instrument (model Criterion 400, Laser Inc. Technology, Englewood, CO), sited from the trap location to the top of the mill's grain elevator. Traps were deployed on 24 September and collected on 1 October 1998. The mean outside temperature during the study was 25.3° C, ranging from 11.7 to 35.3° C.

### Data Analysis

Captures of moths per trap per trapping period at each designated location at mill 1 were computed and plotted on a biweekly (1997) or weekly (1998) basis. An analysis of variance was then performed in SAS (SAS Inst. 1996) using PROC MIXED followed by a means separation (LSD) if ANOVA was significant to test for differences in capture by location within the mill. Seasonal moth activity was plotted by summing male moth capture across all locations within the mill during a trapping period and are reported as the number of male moths per trap per day  $\pm$  SE. In order to estimate the effects of phosphine and methyl bromide fumigation on moth populations, a paired t-test was performed using PROC UNIVARIATE on square root-transformed beetle counts from traps and tail-over samples immediately before and after MB and phosphine fumigations with the null hypothesis that the difference between the two captures was not different from zero. Moth captures from the outdoor moth dispersion study were analyzed through a linear regression of moth capture (square root transformed) against distance from the facility using PROC REG. The trapping area for this study was divided into halves four

different ways (north versus south, east versus west, northwest versus southeast and northeast versus southwest) in order to identify a directional effect in moth captures. An ANOVA using PROC GLM was performed on trap captures within the respective halves to test for differences. Trap captures were adjusted for distance as a covariate for analysis. The confidence level for all analyses was set at  $\alpha = 0.05$ .

## Results

The occurrence of *P. interpunctella* at various locations within mill 1 during 1997 was significantly different with the highest recovery occurring within warehouse 2, followed by warehouse 1, and the area below the bulk flour bins (Fig. 4.2). Moth capture among the other mill locations was not significantly different. The two warehouse areas and the area below the bulk flour bins, which had the highest trap captures, were all located on the ground floor close to large outside openings. Moth capture in 1998 had a similar trend by capture among locations to that in 1997 although not significantly different by location (Fig. 4.3). The highest capture occurred within warehouse 2, followed by warehouse 1, and the area below the bulk flour bins, and was lowest within the third floor.

Biweekly moth capture during the 1997 study is shown in Fig. 4.4. There was an overall rise for the first eight wk of the study to the season high on 7-29 August, followed by a gradual decline until the end of the study. A significant decline in the number of moths captured was observed following both MB fumigations during 1997. Although moth capture following the 30 August 1997 fumigation was highly significantly lower, a similar decline also occurred near the end of September during 1998, which was not associated with a fumigation (Fig. 4.5). Therefore, the decline in capture observed following the 30 August 1997 fumigation might have merely coincided with a seasonal decline in the overall moth population or activity. Weekly moth capture during 1998 rose significantly following the 4 July fumigation, and continued to increase until the eleventh week of the study (10-17 September), followed by a decline to the end of the study (Fig. 4.5).

Traps placed outside the facility during the 1997 study captured more moths than even the areas of highest capture (ground floors) within the mill (Fig. 4.6). The first comparison of the study (2-16 September), revealed more than a 36-fold higher moth capture outside the facility compared with capture within the mill. Moth capture both within and outside the mill steadily decreased in subsequent weeks, likely due to a population decrease associated with seasonal cool temperatures and diapause (Tzanakakis 1959). During the 1998 study, captures of moths were again highest outside the mill (Fig. 4.7), and seasonal capture within the mill followed a pattern similar to that observed outside the mill. Captures in the gallery areas, above the wheat silos, was initially lower than that outside the mill, but was followed by higher moth capture than that observed both within, and outside the mill, for the remainder of the study.

The numbers of *P. interpunctella* trapped at various distances from mill 2 ranged from 2 to 120 during a one-wk trapping period (Fig. 4.8). Regression of the moth capture data against increasing distance from the mill revealed a significant negative relationship. A comparison of the mean number of moths per trap (adjusted for distance) north versus south of the mill was nearly significant ( $F = 3.90$ ;  $df = 1$ ;  $P = 0.0623$ ). No other divisions of the trapping area revealed differences closer to being significantly different; east versus west ( $F = 1.55$ ;  $df = 1$ ;  $P = 0.2279$ ), northeast versus southwest ( $F = 1.07$ ;  $df = 1$ ;  $P = 0.3139$ ), and northwest versus southeast ( $F = 2.15$ ;  $df = 1$ ;  $P = 0.1578$ ).

## Discussion

There were three general observations made during these studies: 1) *P. interpunctella* trapped inside the mill seemed to be coming from outdoors, 2) The mill's wheat storage silos were likely a predominant source of moths captured, and 3) *P. interpunctella* can be captured regularly in high numbers outside during the summer and early fall moths, and are concentrated around the flour mill/wheat silos.

An *a priori* assumption of this research was that *P. interpunctella* populations perpetuate inside the mill facility as a closed system. There was no expectation that moth activity outside the facility would be important to the spatial or population dynamics of moths in the mill. Thus, traps were initially deployed only inside the mill. However, it was soon observed that substantial moth activity occurred immediately following fumigations, and that capture was consistently higher in areas of the mill that were near large outside openings. Based on these observations, additional traps were placed outside the mill in late August 1997. From the subsequent high capture observed outside, it seemed probable that a significant contribution of moths inside the mill was a consequence of direct immigration into the structure from outside, via large opened truck and rail-loading doors. Based on these observations, captures of moths immediately following methyl bromide fumigation likely reflects more on the immigration of the moth, rather than the relative effectiveness of the fumigant. Therefore, unless better measures are taken to prevent moth entry into the mill, suppressing this pest species with fumigation will be difficult.

Failure to suppress moth populations in a flour mill following fumigation has been noted in the past. Levinson & Buchelos (1979) trapped storage moths in a flour mill



in southern Greece and found that *P. interpunctella* and *C. cautella* were only slightly suppressed following phosphine fumigation, but that *Sitotroga cerealella* (Olivier) and *E. kuehniella* were reduced to below economically damaging levels. It is possible that *P. interpunctella* and *C. cautella*, which are ecologically similar species (Hinton 1943), are more inclined to occur in outdoor habitats than other storage pests, and hence may readily re-invade structures after fumigation.

In the present study, many more moths were captured in the indoor gallery sections above the grain silos than were caught inside the flour mill building. Furthermore, numbers of moths captured above the silos were similar to the high numbers captured just outside the facility, and moths were active in the silo gallery longer in the season (into the period of cool weather) than those outdoors. Therefore, it seems likely that many of the moths captured outdoors originated in the grain silos, which contained an abundant food supply. Moths dispersing from the grain silos could then easily invade the warehouse and other indoor areas through openings. Further study using mark-recapture methodology is required to investigate this hypothesis.

The outdoor moth dispersion study around mill 2 found a significant negative correlation of trap catch with distance from the mill, but the relatively low regression value suggests that additional sources of moths, in addition to the mill, existed. This mill was within a residential location, with many homes that could harbor *P. interpunctella*. In addition, the sampling radius contained another single wheat silo in the northeastern quadrant, and there were two commercial wheat storage facilities within a kilometer of the area studied (one to the northeast and one southeast). The wheat storages north of the study mill may have accounted for the greater number of moths trapped in the north

versus the south quadrants. *P. interpunctella* males can disperse up to 1.6 km in 24 h (Ganyard 1971), thus moths captured in this study could have easily dispersed from any of the potential breeding sites within and outside the study area. Another consideration, common to all pheromone-trapping studies that use sex pheromones, is that only the activity of male moths was assessed. Therefore, no data are available concerning the movement of females, the only individuals responsible for introducing the damaging larval stage of the insect.


In summary, these studies document high levels of outdoor activity of *P. interpunctella*, particularly in the proximity of the flour mills, and point to the potential for immigration into food processing facilities from outdoors. These data suggest that the risk of introducing the moth to a facility with contaminated products is not the only source of infestation to be considered. In addition, an assumption throughout this study, and most stored-product research, is that the insects reproduce on human-stored food products. If wild populations do occur, apparently living on some non-human stored food materials, it only increases the potential of moth infestation via immigration. A practical suggestion to managers of flour mills and other stored-product facilities is to limit access of moths to the facility from outdoors. Two obvious methods of limiting moth immigration are the use and maintenance of effective screens on doors and windows, as well as limiting the time that large loading bay doors are open.

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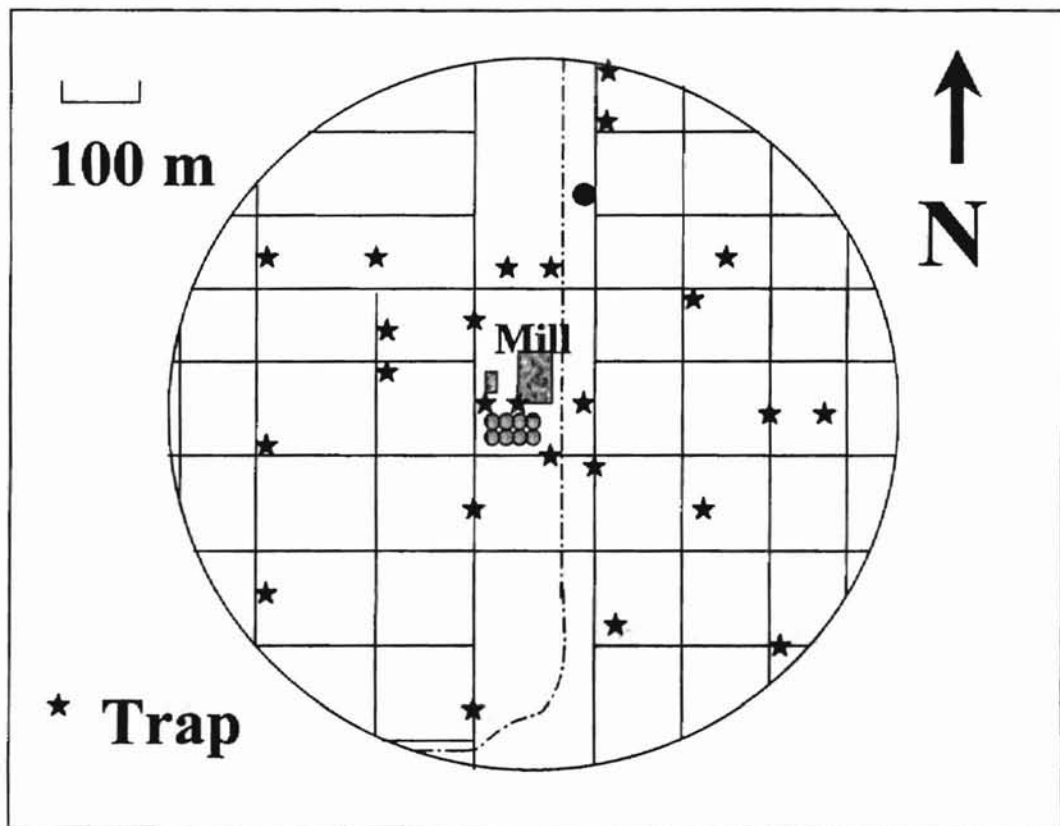
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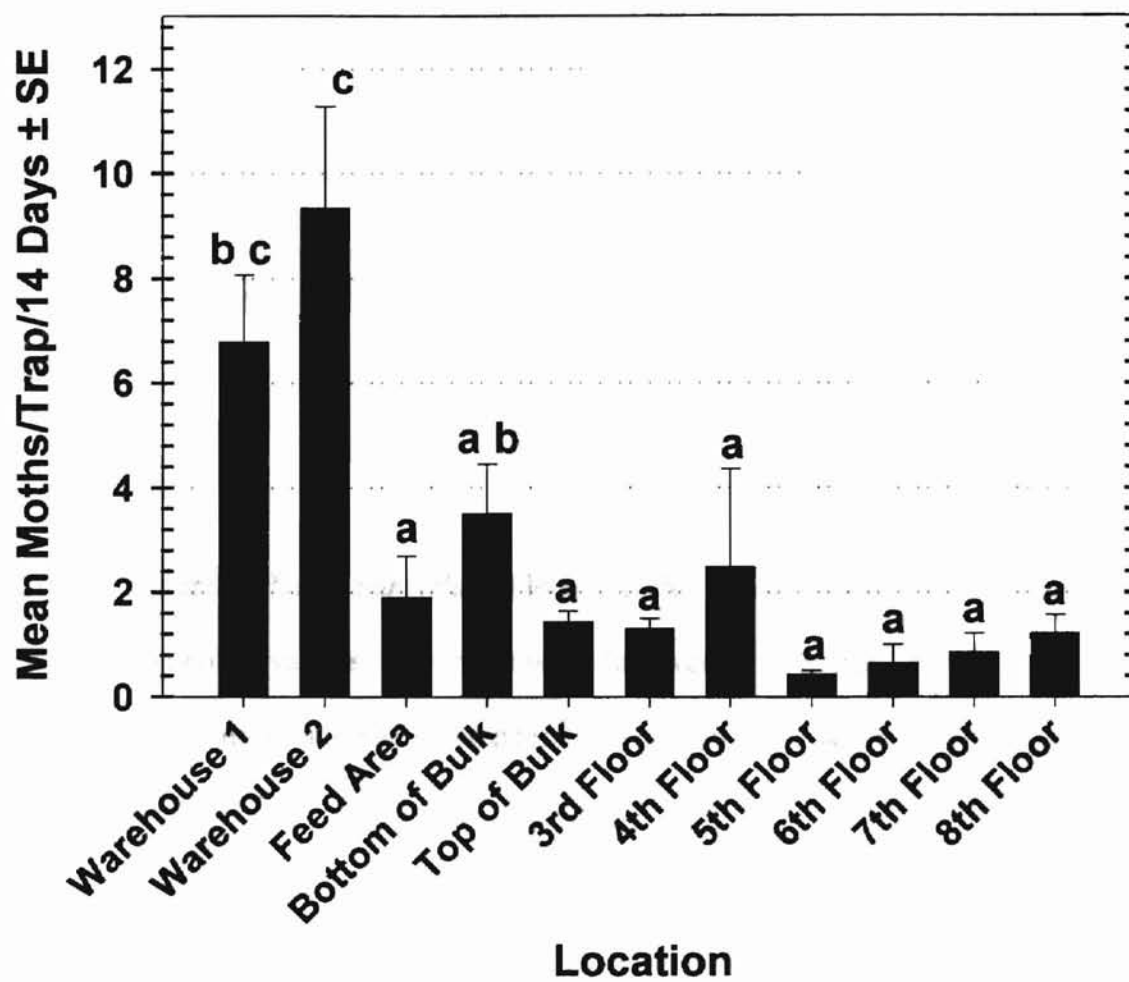
**Fig. 4.1.** Placement of traps around mill 2 for the moth outdoor moth dispersion study. Stars denote approximate placement of traps. Lines denote residential streets, dotted line denotes train tracks, and the separate stored wheat bin north of the mill is denoted by a single circle.



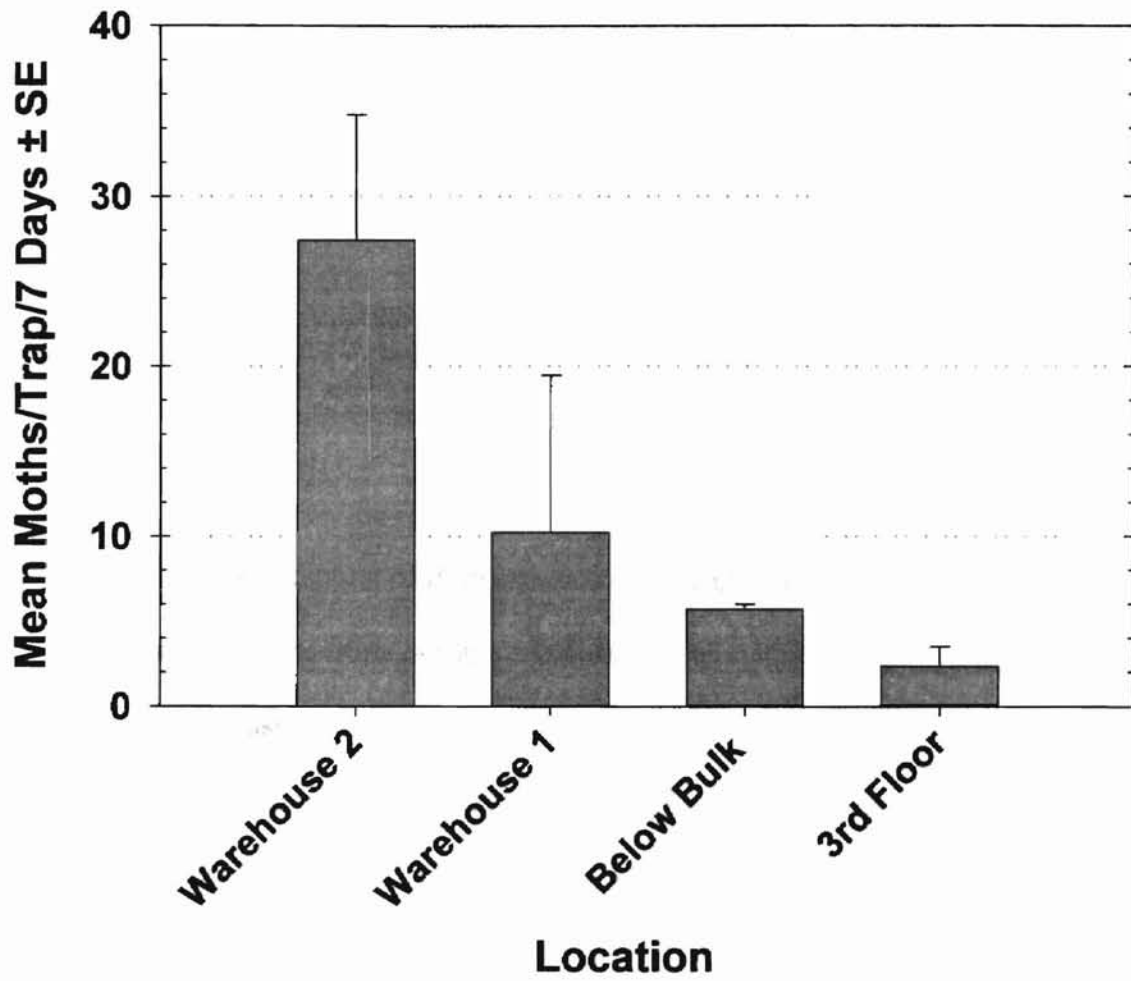



**Fig. 4.2.** Capture of *P. interpunctella* by location within mill 1 during 1997. Results are from 13 biweekly observations. Four traps were deployed in warehouse 2; two traps were deployed in all other locations. Analysis of variance revealed significant difference in capture by mill location ( $F = 5.28$ ;  $df = 10, 15$ ;  $P = 0.0021$ ). Means with the same letter are not significantly different.



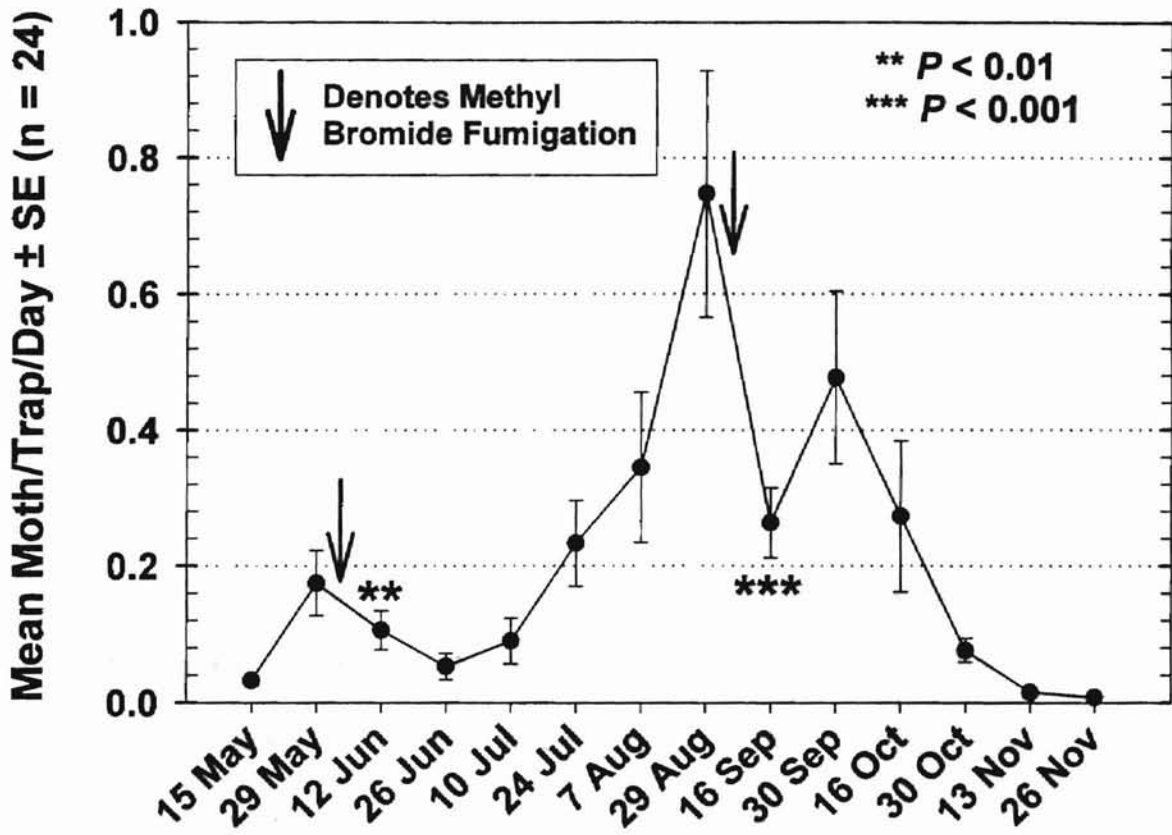



**Fig. 4.3.** Capture of *P. interpunctella* by location within mill 1 during 1998. Results are from 18 weekly observations. Two traps were deployed in each area. Analysis of variance revealed no significant differences in moth capture across various mill locations ( $F = 3.54$ ;  $df = 3, 4$ ;  $P = 0.1269$ ).



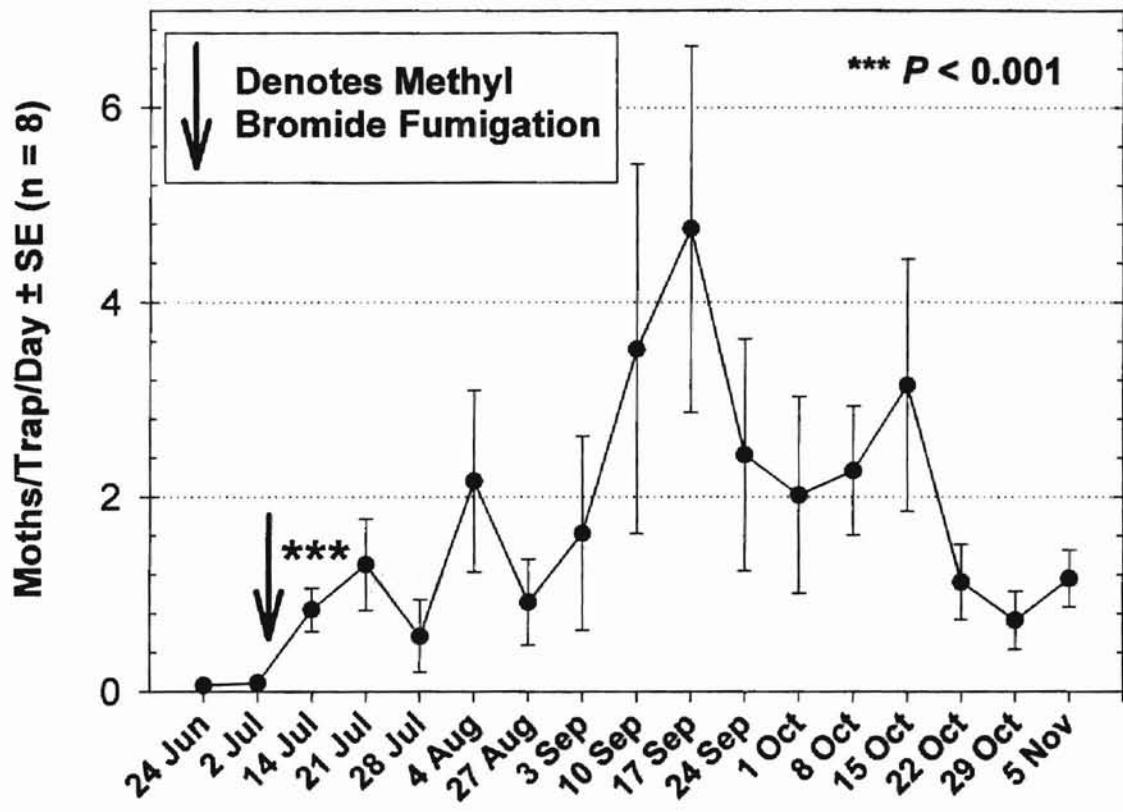


**Fig. 4.4.** Biweekly capture of *P. interpunctella* throughout mill 1 during 1997. Results are mean observations from 24 traps. Arrows denote methyl bromide fumigations on 31 May and 30 August. Significantly fewer numbers of moths were trapped following the 31 May ( $t = 3.82$ ;  $df = 17$ ;  $P = 0.0018$ ), and the 30 August ( $t = 7.51$ ;  $df = 22$ ;  $P = 0.0001$ ), methyl bromide fumigations. Count data for t-tests were square root transformed.





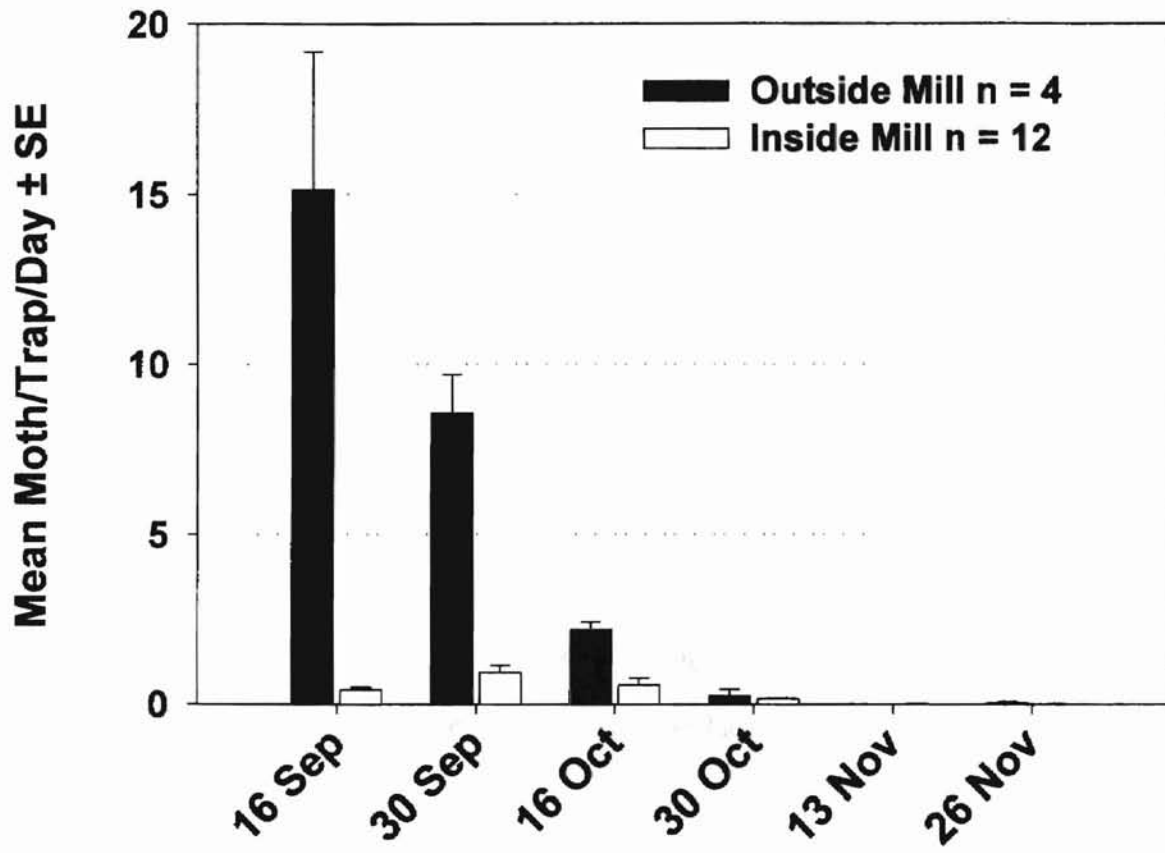
**Fig. 4.5.** Weekly capture of *P. interpunctella* throughout mill 1 during 1998. Results are mean observations from 8 traps. Arrow denotes methyl bromide fumigation on 4 July. Significantly more moths were trapped following fumigation ( $t = 8.44$ ;  $df = 6$ ;  $P = 0.0002$ ). Count data for t-tests were square root transformed.



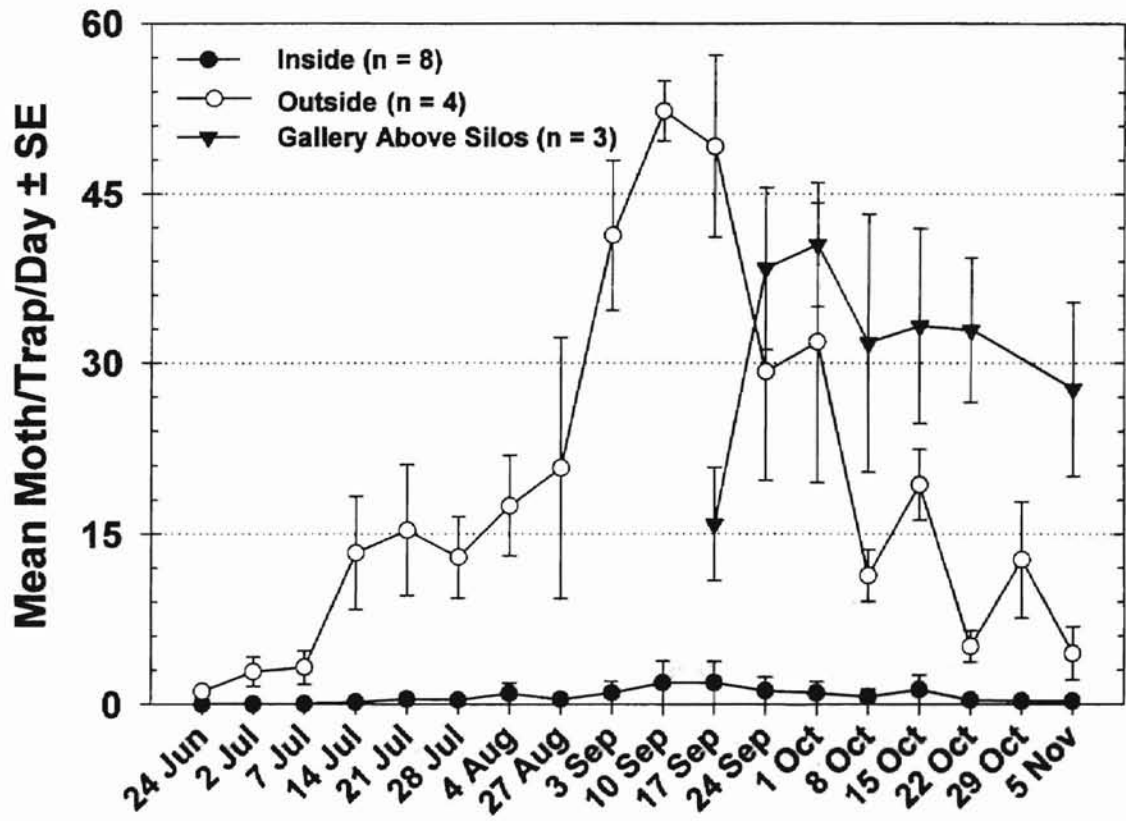


**Fig. 4.6.** Comparison of biweekly capture of *P. interpunctella* in traps outside, and on ground floors within mill 1 during 1997.

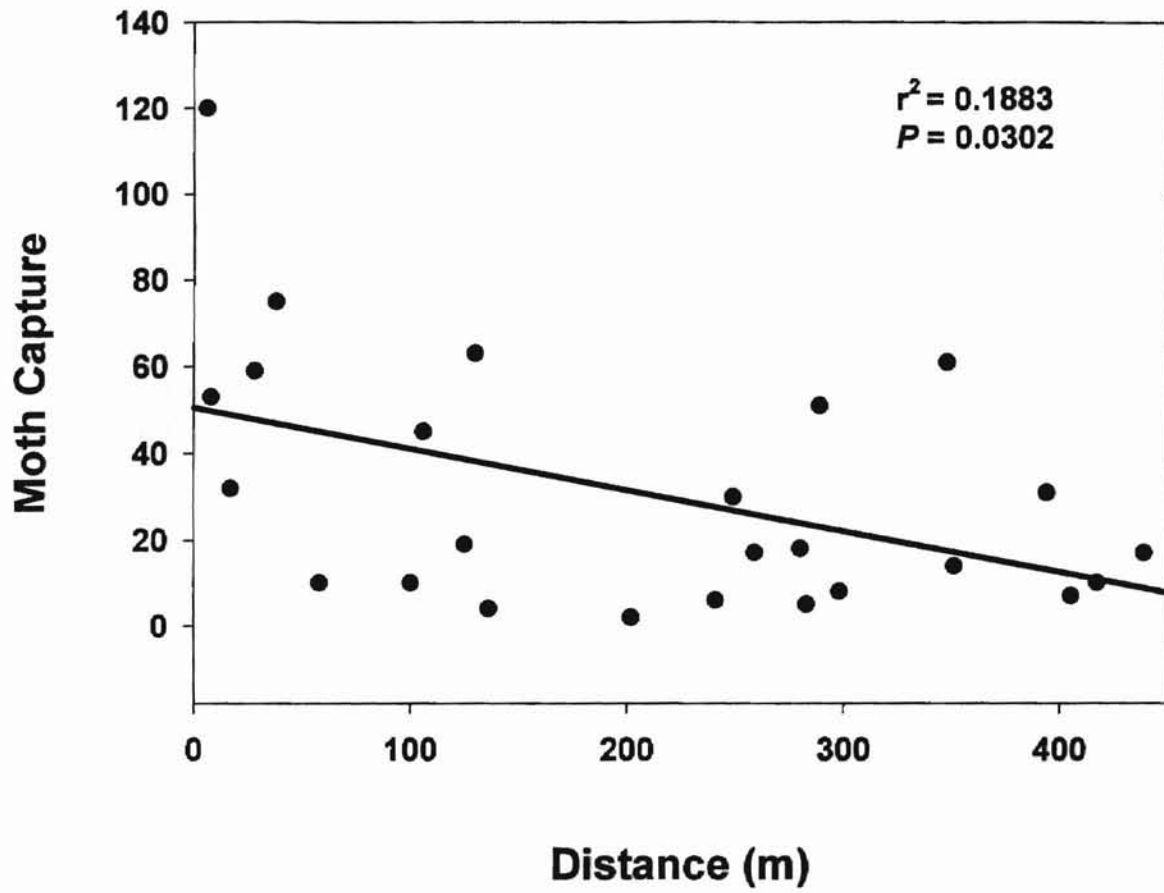




**Fig. 4.7.** Weekly capture of *P. interpunctella* throughout 1998. Results from 8 traps within the mill, 4 traps outside the mill, and 3 traps placed in the galleries above the bulk-stored wheat silos.



**Fig. 4.8.** Regression of *P. interpunctella* captures at various distances from a flour mill. Results are number of moths captured (square root transformed) during a seven day interval from 25 traps positioned from 6 to 440 m from the mill. A significantly negative relationship was revealed ( $b = -0.0076$ ,  $r^2 = 0.1883$ ,  $P = 0.0302$ ). Data were square root transformed for regression.



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