SPATIAL DISTRIBUTION, DISPERSAL BEHAVIOR AND POPULATION STRUCTURE OF *TRIBOLIUM CASTANEUM* HERBST (COLEOPTERA: TENEBRIONIDAE)

by

ALTAIR ARLINDO SEMEAO

B.S., Federal University of Viçosa, Brazil, 2004
M.S., Federal University of Viçosa, Brazil, 2006

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2011

Abstract

Knowledge of factors influencing the establishment, persistence and distribution of stored-product pests aids the development of effective Integrated Pest Management (IPM) programs in food storage and processing facilities. This research focused primarily on *Tribolium castaneum,* which is one of the most important pests of mills. Populations of *T. castaneum* from different food facilities can potentially be interconnected by either their own dispersal behavior or by human transportation. Population genetic structure analyses based on microsatellites and other insertion-deletion polymorphisms ("indels") showed that populations from different mills around the US are genetically distinct from each other, but the level of differentiation was not correlated with the geographic distance. A potential source of insect infestation within a food facility is spillage that accumulates outside or movement from bulk storage facilities on site. Results from three facilities showed that most stored-product species were captured both inside and outside buildings, but *T. castaneum* was rarely captured outside of the facilities. Spatial distribution of all species outside was associated with the proximity of buildings, not necessarily with areas with accumulated spillage. *T. castaneum* populations inside facilities are potentially exposed to frequent genetic bottlenecks resulting from structural fumigations. Changes in allele frequencies through time, based on the analysis of microsatellites and other indels in individuals collected in a mill, confirmed bottleneck effects. To understand how spatial distribution of *T. castaneum* within a mill could be influenced by environmental and physical factors, a range of variables were measured at each trap location. There was significant variation among trap locations regarding beetle captures and the variables measured, but increase in beetle captures correlated only with increase in temperature and spillage production. *Tribolium castaneum* response to visual cues could influence attraction to pheromone and kairomone olfactory cues used in traps. Results of laboratory experiments showed that adults respond to tall narrow black shapes and placing traps in front of these shapes can increase captures. This research provides new insights into factors influencing the spatial distribution of *T. castaneum* and could help in improving monitoring programs for this important pest of the food industry.

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Co-Major Professor James F. Campbell	Co-Major Professor Robert J. Whitworth	Co-Major Professor Phillip E. Sloderbeck

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Acknowledgements

First I would like to express my deep appreciation to my co-major advisor Dr. James F. Campbell who has, during the last four years, provided great support. Also, I would like to recognize and appreciate his patience, and guidance on developing, conducting and analyzing the results of the experiments. I also want to thank my co-major advisors Dr. Robert (Jeff) Whitworth and Phillip E. Sloderbeck for their advice and helpful comments during my course at Kansas State University. The committee members, Dr. Richard Beeman and Dr. Shawn Hutchinson were of great help in guiding the research in their area of expertise. I cannot forget to thank Dr. Marcê Lorenzen who was of great support on the primers design and helping to choose microsatellites for part of this research, and the great technical assistance of Tereza O'Leary during my time in Dr. Beeman's lab. I thank Richard Hammel (Rich) for his technical assistance and his great humor which made my days more enjoyable in the lab and during the long field trips. I would like to recognize the help of all undergraduate students in our lab, that through the last four years, had helped with the hard work. I would like to thank all my friends living in Manhattan and those that were here at some point. Special thanks to David, Ana Paula, Thais, Iliano, Patricia, Luciano and Lessando. I would like to thank all graduate students at the Department of Entomology, who shared their research findings during seminars and informal conversations, and also contributed to my growth as an Entomologist. Finally, I would like to thank my family for the love and support when I decided to leave my home town in Brazil to come to US, and my lovely wife Flávia who has been on my side for the last five years and deserves my deep appreciation.

Dedication

I dedicate this dissertation to my father (José Semeão) and to the loving memory of my mother (Maria das Graças Fagundes Semeão). They deserve all my appreciation.

Chapter 1 - Introduction

This dissertation is focused on providing additional understanding of aspects of ecology and behavior of stored-product pests associated with food processing and storage facilities that contribute to their spatial and temporal distribution. Although Chapter III evaluates the distribution of all stored-product pests, the major focus of this dissertation is the important stored-product pest, the red flour beetle (*Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)). For this species, research focused on elucidating at a large scale, how populations might be interacting either by active movement or human transport of infested material and how the genetic composition of populations can experience changes through the course of time. For that, population genetic approaches were applied and molecular markers were used to study populations distributed throughout the continental United States and in different time periods within one facility. At a small spatial scale, this research describes the distribution of stored-product pests inside and in the proximity of food processing facilities and identifies characteristics of the landscape that might influence their distribution. For this analysis, geostatistical tools and behavioral experiments were used.

More than 1,660 insect species are reported to be associated with stored-products including species that are granivores, fungivores, omnivores, and natural enemies and distributed in the orders Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera, Lepidoptera and Psocoptera (Hagstrum and Subramanyam 2009). Among the arthropods associated with food processing facilities, stored-product pests require major attention because of the damage that can occur due to their presence. It has been estimated that cereal production needs to increase by 50% between 2000 and 2030 to meet the world's demand (FAO 2009). This need for increased food production will require, among other strategies, the maintenance of food quantity and quality after harvest and processing. There is also increasing demand by the public for decreased use of pesticides. This situation highlights the need for a better understanding of the ecology and behavior of stored-product pests in order to develop more effective Integrated Pest Management (IPM) programs.

Stored-product pests can cause infestations in grain before harvest, and post-harvest during transportation, storage, processing and marketing (Tigar et al. 1994, Hagstrum and Flinn

1995, Arbogast and Throne 1997, Roesli et al. 2003, Perez-Mendoza et al. 2004). Problems resulting from their presence can range from loss of quantity and quality due to direct feeding on grain or processed products to contamination or infestation of foods resulting in consumer complaints and lawsuits for manufacturers. During post-harvest, losses of stored grain during storage can be up to 90%, depending on the type of food stored, pest species involved, and location and type of the storage system (Cao et al. 2002). In developed countries, the presence of insects even at low densities can trigger the need to fumigate or cause serious loss in its market value. Depending on the level of the problem, grain can be downgraded or rejected (Pinniger et al. 1984). For processed commodities, costs due to stored-product insects are typically not due to direct damage, but can still result in an increase of the need for sanitation and chemical treatments, loss of customer good will, health hazards associated with allergens in food, and consequences of unsatisfactory food safety inspections (Campbell et al. 2002). The need for more effective IPM programs is also a result of the phasing out of methyl bromide for structural fumigations under the 1987 Montreal Protocol on Substances that Deplete the Ozone Layer (Fields and White 2002).

Stored-product species and other arthropods are often found associated with different type of facilities utilized during storage, processing and marketing of grain and food for human and animal consumption (e.g., farm bins, flat storage facilities, feed mills, flour mills, and retail stores) (Dowdy and McGaughey 1994, Arbogast et al. 2000, Athanassiou et al. 2003, Roesli et al. 2003, Campbell and Arbogast 2004, Nansen et al. 2004, Larson et al. 2008). These species can be detected inside and outside these facilities, with the number and diversity of species depending on different factors such as geographic location, type of food produced, degree of sanitation, and pest management practiced. The distribution of some major pest species inside food processing facilities has been well studied and it has given insight into improving monitoring and management programs. With the support of baited traps and geostatistical techniques, trends in temporal changes in the distribution and spatial location of foci of infestation can be detected making possible the use of more targeted management in space and time (Arbogast et al. 2000, Strother and Steelman 2001, Campbell et al. 2002, Trematerra and Sciarretta 2004, Trematerra et al. 2007). It has also been possible to evaluate the effectiveness of management strategies such as fumigation, by comparing trends in captures before and after the implementation of this management strategy (Phillips et al. 2000, Campbell and Arbogast 2004,

Campbell et al. 2010a,b). Studies of insects outside food processing facilities have focused more on the detection and temporal dynamics of stored-product species in the areas surrounding facilities, and to a lesser extent the spatial distribution in these areas (e.g., Likhayo and Hodges 2000, Dowdy and McGaughey 1998, Kučerová et al. 2005). The distribution outside food processing facilities can differ by species. For example, Campbell and Mullen (2004) found that the warehouse beetle *Trogoderma variabile* (Ballion) was more close associated with the proximity of buildings at a processing facility site than the Indianmeal moth *Plodia interpunctella* (Hübner). Other species such as the larger grain borer *Prostephanus truncatus* (Horn) and the lesser grain borer *Rhyzopertha dominica* (F.) have been shown to be distributed at large scale agricultural and nonagricultural areas and they can be associated with the presence of forested areas (Nansen et al. 2004, Campbell et al. 2006, Mahroof et al. 2010).

The red flour beetle is a major worldwide pest of stored grain, cereal products, and many other stored commodities used for human and animal consumption and it is often found in environments where humans process and store grain and grain-based products (Sinclair and Haddrell 1985, Arbogast et al. 2000, Campbell and Arbogast 2004). *T. castaneum* is proposed to be of Indo-Australian origin and it is less able to tolerate cold temperatures and survive outdoors than the closely related species *Tribolium confusum* du Val (Hedges and Lacey 1996, Robinson 2005). However, *T. castaneum* can survive and reproduce inside climate controlled structures year round. *Tribolium castaneum*'s world-wide geographic distribution suggests dispersal by human movement of infested materials, but since it is capable of flight, some dispersal may be due to the beetles own behavior (Sinclair and Haddrell 1985, Ho and Boon 1995, Robinson 2005, Daglish et al. 2010). How much dispersal among food processing facilities is present, how far *T. castaneum* can actively disperse, and the relative importance of active dispersal behavior or human movement of infested materials, are not well understood for this pest. While it is difficult to evaluate the relative contribution of these two types of dispersal experimentally, it is possible to utilize other strategies that can indirectly estimate levels of movement to evaluate the interconnection among distinct populations.

Advances in molecular methodologies have provided tools to measure the interconnection of species populations from different locations throughout the world and the intra- and inter-population levels of genetic variation have been widely used as discriminating criterion for estimating isolation and gene flow among populations. Many different types of

molecular markers have been developed and can be used for this purpose (Avise 2004), with microsatellites being one of the most widely used markers. Microsatellites are repetitive sequences of 1 to 6 nucleotides (typically 2 to 4) that are typically distributed at high frequencies throughout the genome of eukaryotes (Ramel 1997). Because of their high level of polymorphism and wide distribution within the genome, microsatellites are ideal markers for a variety of applications such as paternity determination and evolutionary genetic analyses including population genetics (Pai et al. 2003). Population genetics is the study of allele frequency distribution and change under the influence of different evolutionary processes such as natural selection, genetic drift, mutation and gene flow. Population genetic analysis also can be used to evaluate population subdivision and structure. Many different factors can prevent panmixia and lead to the genetic differentiation of populations; including ecological (e.g., mating system, social structure, dispersal and spatial distribution), genetic (e.g., rate of mutation, genetic drift, and natural selection) and environmental factors (e.g., population bottleneck, landscape fragmentation, and physical barriers).

Stored-product insects that occur in food processing facilities such as flour mills inhabit resource patches that are isolated and discontinuous, both within and among food processing facilities within the broader urban and/or rural landscape (Campbell 2005). The spatial isolation of food processing facilities suggests that individual populations of stored-product insects within a facility may develop unique genetic fingerprints. These populations within a facility may also regularly experience severe reductions in size as a result of periodic treatments such as fumigation or heat (Fields and White 2002) and the consequent genetic drift can lead to increased differentiation if gene flow among these populations is relatively low. This scenario has been studied in other systems showing how population size reduction and isolation can have strong effects on population differentiation (e.g., Slatkin 1977, Jordan and Snell 2008).

Tribolium castaneum is to date the only beetle with a completely sequenced genome and it is an important model for study of molecular and developmental genetics (Lorenzen et al. 2005, Tribolium Genome Sequencing Consortium 2008). For *T. castaneum*, unique and polymorphic molecular markers, including an abundance of microsatellite loci, have been identified and some assessment of the genetic structure of populations has been undertaken (Pai et al. 2003, Demuth et al. 2007, Demuth and Wade 2007, Drury et al. 2009). These studies have found significant differentiation among spatially distinct populations of *T. castaneum*, but with

unexpectedly low levels in some cases where populations are separated even by very large geographic distances. Understanding the population genetic structure of *T. castaneum* can be of great importance since it can define at which spatial scale different populations are interacting and may help decide how management strategies and monitoring programs should be implemented. For example, if amount of gene flow is greater than predicted it might indicate immigration into facilities is occuring and management should focus on reducing the entry of new individuals after control interventions have been implemented.

The widespread geographic distribution of most stored-product pests is possible because food storage and processing facilities are environments that provide favorable habitats and resources for the survival and reproduction of these pests. Favorable resources for stored-product insects are spatially patchy in distribution not only inside the structures, but also outside. Favorable resources outside include spillage and blown out materials that can accumulate on surfaces. For example, spillage during the processes of loading and unloading and fine food material emitted from exhaust systems of structures can accumulate outside and create resource patches that could be exploited by stored-product pests. Levels of outside stored-product insect activity can be quite high (Dowdy and McGaughey 1994, Dowdy and McGaughey 1998, Likhayo and Hodges 2000, Campbell and Mullen 2004) and this suggests the potential for immigration into the facilities and provides an important source of infestation that needs to be considered during implementation of IPM programs. There are many studies showing the spatial distribution of stored-product pests inside food facilities (e.g., Campbell et al. 2002, Nansen et al. 2004, Trematerra and Sciarretta 2004, Lazzari et al. 2010), but less focus has been given to the spatial distribution outside. Campbell and Mullen (2004) evaluated the spatial distribution and movement patterns of *P. interpunctella* and *T. variabile* outside a food plant, and found that individuals of *T. variabile* marked outside were captured inside. At another location, movement of *P. interpunctella* into a flour mill was demonstrated (Campbell and Arbogast 2004).

Although outside flight activity and movement into facilities has been shown, the outside sources of insects are not well understood. As mentioned before, one potential source is spillage patches that can accumulate outdoors around structures. Even though this has been hypothesized, there is a lack of published studies that have focused on evaluating this hypothesis. This research attempts to fill this gap by taking a novel approach that uses a landscape perspective to evaluate outside distribution and the factors that might influence this distribution.

The actual spatial scale in which populations are spread throughout the landscape is not well understood either. Distribution of stored-product pests will not be restricted to the limits of the property of a food processing facility and they can potentially invade agricultural, nonagricultural, and urban areas. The spatial and temporal distribution of stored-product species outside storage and processing facilities can be affected by the landscape structure surrounding these facilities and how organisms interact with the spatial and temporal landscape heterogeneity (Campbell et al. 2006). Infestations of feral individuals can be present in households, forested areas and continuously interact with the infestation in the facilities. However for this research objective, the focus will be on the potential sources that are located on site at a food processing facility.

Identifying reasons for the continuous presence of stored-product pests inside food storage and processing facilities is critical for evaluating the effectiveness of management strategies and it is the first step for improving management strategies. A number of research studies have shown that stored-product insects can be recovered consistently over long periods of time in food facilities (Campbell et al. 2002, Ryne et al. 2007, Campbell et al 2010a). Even structural fumigations that have good penetration into hidden refugia exploited by stored-product insects do not always completely eliminate populations (Toews et al. 2006, Small 2007, Campbell et al. 2010a). Persistence of *T. castaneum* in mills after fumigation may be due to survival in areas with insufficient fumigant penetration or because of recolonization by individuals from outside the treated area. Changes in population genetic profile can be an indication of the efficacy of management strategies, and potentially of the different mechanisms of rebound after treatment, if comparisons can be made among groups of individuals present before and after the implementation of a management tactic. Understanding how genetic profiles within a population change over time is also important for evaluation of the genetic structure of populations since this approach typically relies on measurements of allele frequency heterogeneity from spatially distinct populations for inferring relationships (Holsinger and Weir 2009). If the genetic composition of a population is not stable at ecological time scales, this variation can make it more difficult to elucidate processes involved in the spatial genetic structure of populations that were collected in different time periods (Heath et al. 2002, Østergaard et al. 2003).

As discussed earlier, stored-product insects tend to be patchy in distribution within food facilities. The landscape inside a food processing facility can be characterized as a mosaic of favorable and unfavorable habitat patches for these insects. The maintenance and spatial arrangement of these patches are subject to changes through time and these changes will consequently affect the stored-product pests' distribution since the ability to find favorable and avoid unfavorable habitat patches is a component of any organism's biology (Campbell et al. 2002, Campbell 2005). Distribution of stored-product pests inside facilities can potentially be predicted if information on the spatial variation and the conditions of different habitat patches is known. Traps for capturing stored-product pests and gaining information on their distribution have been widely used in the pest management industry and have been the focus of research. Trapping can also evaluate the effects of management strategies, including how treatment changes spatial distribution (Phillips et al. 2000). Because traps used in food processing facilities capture primarily dispersing individuals, the relationship between spatial pattern of insect distribution and spatial pattern in capture in traps could be impacted by landscape patterns. Although pheromone based trapping programs have been used for pest monitoring, limited efforts have been made to gather information on the unique microhabitats at each trap location and how these may impact capture of insects in traps. The size and design of the facilities result in spatial variation in environmental and physical factors and this variation has not been well studied *in situ*.

Focusing on *T. castaneum*, it is known that environmental factors such as temperature and relative humidity, physical features of the landscape such as food availability and trap location, trap features such as physical design, and the internal state of the insect can impact the spatial distribution pattern of trap captures. For example, temperature can influence active movement, flight initiation, developmental time, fecundity and population growth (Sokoloff 1974, Trematerra and Sciarretta 2002, Jian et al. 2005, Cox et al. 2007). Temperature can greatly vary across different locations in a food processing facility depending on the presence of milling machinery that can produce heat, location of heating and cooling equipment, and building orientation. Presence of food patches and the physical environment can influence *T. castaneum* movement (Romero et al. 2009) and potentially trap capture. Toews et al. (2005) found that more *T. castaneum* were captured in traps placed in locations without food patches, in the corners and underneath shelves. In order to improve the use of information on the distribution of

stored-product pests in management programs, it is important to identify and understand causal factors influencing distribution. Identifying the levels of spatial variation and the characteristics of favorable habitats can help with the evaluation of captures in traps since better interpretation of the spatial variation in number being captured throughout the facility will be possible.

Integrated pest management of stored-product insects in food processing facilities relies on monitoring data to guide management decisions and to evaluate the effectiveness of the program (Barak et al. 1990, Burkholder 1990). Often this monitoring data is generated using pheromone and/or kairomone baited traps that rely on insect olfaction to facilitate captures, but typically don't exploit other sensory modalities such as vision (Chambers 1990). Response of *T. castaneum* to traps is not considered very strong and therefore any feature improving this response will be desired. *T. castaneum* may also use visual cues for interacting with the environment in food processing facilities and finding trap locations. *T. castaneum* has reduced color vision and enhanced UV and long wavelength light reception, suggesting cryptozoic origins (Jackowska et al. 2007). These combined factors may lead beetles to be responsive to dark shapes since evolutionarily and currently they may indicate favorable habitats. Although response to visual cues by *T. castaneum* is relatively poorly understood, this is a potential field of study that could be investigated for improvement of monitoring and management. For example, trap design could be enhanced by designing traps with attracting colors. Another possibility would be to explore the presence of equipment or other features with attractive colors for placing traps near them.

Objectives

There is a need for more information about sources of *T. castaneum* infestation, their spatial distribution pattern, both inside and outside processing facilities, and the factors that generate this distribution. Thus, this research aim is to fill some of these data gaps at both large and small spatial scales for the major stored-product species *T. castaneum* at food processing facilities. The following specific objectives will be addressed:

1. Determine population structure of *T. castaneum* among different food processing facilities distributed throughout the continental United States.

2. Determine spatial and temporal distribution patterns of stored-product pests in the landscape of three grain processing and storage facilities and determine which environmental and landscape factors influence the outside distribution patterns.

3. Determine changes in genetic diversity of populations of *T. castaneum* through time as a result of bottlenecks caused by structural fumigations.

4. Determine potential factors involved in the variation of trap captures of *T. castaneum* inside a commercial flour mill.

5. Evaluate *T. castaneum* response to black color and the potential of combining with olfactory cues to improve monitoring in food processing facilities.

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Chapter 2 - Genetic structure of *Tribolium castaneum* populations in mills

Abstract

The red flour beetle, *Tribolium castaneum* (Herbst), is primarily found associated with human structures such as wheat and rice mills, which are spatially isolated resource patches with apparently limited immigration and frequent population bottlenecks. These two factors can strongly influence the genetic structure of *T. castaneum* populations. Genetic diversity and differentiation were investigated among nine populations of *T. castaneum* collected from wheat and rice mills ranging from 0.3 to 5,700 km apart, using eight polymorphic loci (microsatellites and other insertion-deletion polymorphisms), each with 3 to 14 alleles. Seventy two locus-by-population combinations were evaluated, of which 31 deviated significantly from Hardy-Weinberg equilibrium, all due to a deficiency of heterozygotes. AMOVA analysis indicated that 8.3% of the variation in allele frequency resulted from comparisons among populations but comparison among different commodities or geographic regions did not find significant differences. Genetic differentiation among populations was significant, with F_{ST} values varying from 0.018 to 0.149, but genetic distance was not significantly correlated with geographic distance. Recent genetic bottlenecks were confirmed in five out of nine populations. Correct assignment to the source population was successful for 56% of individuals collected. These results provide evidence that populations of *T. castaneum* show spatial genetic structure, with 97% of pairwise comparisons being significant. However, the levels of genetic differentiation and the lack of isolation by distance found here require further refinement of techniques for understanding forces driving the spatial structure of *T. castaneum* populations.

Introduction

Genetic variation at the intra- and inter-population level has been widely used as discriminating criterion for estimating levels of isolation and gene flow among populations of organisms. Ecological (e.g., mating system, social structure, dispersal and spatial distribution), genetic (e.g., rate of mutation, genetic drift, and natural selection) and environmental factors (e.g., population bottleneck, landscape fragmentation, and physical barriers) are among those factors that can prevent panmixia and lead to the genetic differentiation of populations. Stored-product insects occur in food processing facilities such as mills and as a result inhabit resource patches that are isolated and discontinuous, potentially both within facilities and among food facilities within the broader urban and/or rural landscape (Campbell 2005). The spatial isolation of food processing facilities suggests that individual populations of stored-product insects may develop unique genetic fingerprints. However, this tendency toward genetic isolation and differentiation may be countered by the poorly understood process of long-range dispersal by behavioral mechanisms or passive transport during human commerce. Moreover, populations in some food facilities frequently experience severe population reductions or extinctions as a result of periodic treatments such as fumigation or heat (Fields and White 2002), and populations recover through re-infestation by survivors and/or new immigration (Campbell and Arbogast 2004, Campbell et al. 2010a,b) and this process can impact population structure. These and other determinants of population genetic structure have been examined in several species of stored-product insects (e.g., Dowdy and McGaughey 1996, Fleurat-Lessard and Pronier 2006, Demuth and Wade 2007).

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is a major pest in wheat and rice mills worldwide (Sokoloff 1974). The global distribution of this insect and its long association with stored food suggest that passive movement via human dispersal and commerce was an important determinant of its current distribution and population structure. However, the extent to which current populations in individual food processing or storage facilities are interconnected by passive or active movement is not well understood. *T. castaneum* can fly and has been captured far from human-made grain storages, but is not regarded as a strong flier (Sinclair and Haddrell 1985, Daglish et al. 2010). Monitoring studies in flour mills suggest that *T. castaneum* populations are often relatively self-contained within

individual mills, and that these populations go through frequent genetic bottlenecks due to regular fumigations of the building (Campbell and Arbogast 2004, Toews et al. 2006; Campbell et al. 2010a,b). Therefore, there is evidence suggesting that *T. castaneum* populations within mills may be spatially and genetically structured. If genetic structure could be confirmed by genetic analysis of *T. castaneum* populations, genetic fingerprinting of populations could be a useful tool for the discovery of infestation sources. This is a significant issue because flour and products made from flour can become infested at different points in the food distribution channel, starting with the mill itself. If particular mills could be implicated or eliminated as possible sources of downstream infestations, control efforts could be better targeted.

Tribolium castaneum is an excellent subject for study of population structure and has a long history of use in population biology research. As the only beetle with a completely sequenced genome, it is also an important model for study of molecular and developmental genetics (Lorenzen et al. 2005, Wang et al. 2006, Denell 2008, Tribolium Genome Sequencing Consortium 2008). The genetic characterization of this species has facilitated the identification of unique and polymorphic molecular markers, including an abundance of microsatellite loci, and has triggered several recent studies aimed at using such markers for assessment of the genetic structure of populations (Pai et al. 2003, Demuth et al. 2007, Demuth and Wade 2007, Drury et al. 2009).

The present study focuses on understanding the genetic structure of *T. castaneum* populations found in commercial mills, with the objective that this analysis could provide insight into mechanisms by which populations within mills are initiated and maintained. Unlike previous studies, beetles were collected directly from the mills, rather than laboratory colonies initiated from different locations, and to control for temporal variation beetles were collected at equivalent time points. Beetles were collected from nine sites within the continental United States and Puerto Rico, and were genotyped using microsatellites and other insertion-deletion (indel) polymorphisms as markers. Specifically, this research sought to determine: 1) the genetic structure of these populations; 2) the correlation between levels of differentiation and geographic separation of populations; and 3) the ability to accurately assign individuals to their source population.

One source of beetles within a mill is immigration into the structure using their own flight or walking behavior from the surrounding area. Based on this mechanism, it was

hypothesized that isolation by distance (IBD) would be one of the major forces driving levels of genetic differentiation among populations since IBD results from the tendency of individuals to find mates from nearby populations rather than distant populations. To evaluate this, mills with different distances from each other were selected. However, given the behavior and habitats exploited by these beetles, human aided movement of individuals is highly likely and in the case of mills could be expressed in two general ways. First, movement of infested grain onto the mill location could be a source, with beetles then moving from the the bulk storage and transportation equipment into the mill structure. Second, infested food material such as packaged flour and other processed commodities can be moved around the country in food distribution channels and thus beetles could be moved into the landscape around a mill by human aided dispersal. Beetles can then move from the surrounding environment using their own behavior onto a mill site and eventually into the mill. If the first mechanism of human aided movement is operating, then IBD might still be expected, although perhaps relationship may be less clear cut. If the second mechanism of human aided dispersal is operating then IBD would not necessarily be expected. Wheat and rice mills do not share the same sources of grain, so they would be predicted based on individual movement and human aided movement in bulk grain to have *T. castaneum* populations that are more genetically isolated between these commodities than within the same type of mill. The geographic area over which grain is harvested and transported to be used in a mill could also impact population structure if movement of beetles in bulk grain is important, and this study includes mills of varying size, including one small mill that has a relatively local source of grain, and mills in the southeastern portion of the US and in Puerto Rico that use grain produced primarily in the central plains and shipped to them through grain distribution channels. If a stepping stone pattern of *T. castaneum* human aided movement throughout the US in infested material and beetle dispersal behavior then a limited population structure could should be expected within mills, with isolation of the mills from surrounding sources of dispersing beetles being more important than distance among mills.

Materials and methods

Sample collection

Individuals of *Tribolium castaneum* were collected from rice and wheat mills across the United States and the territory of Puerto Rico using Storgard Dome traps baited with *Tribolium* spp. pheromone lure and food oil (Trécé, Adair, OK, USA). Samples from each location (≥30 beetles) were obtained from multiple traps distributed throughout each facility over a ~2 wk monitoring period. Samples were collected between July and August, 2007, except for one location (KS1, collected in March, 2005). Locations NE1, NE2, KS1, and KS2 were in Nebraska and Kansas (Midwest), CA1 and CA2 were in California (West Coast), and LA1, FL1, and PR1 were in Louisiana, Florida, and Puerto Rico (Southeast) portions of the U.S. and its territories. Locations LA1, CA1 and CA2 were rice mills and the others were wheat flour mills. After collection and prior to DNA extraction, individual beetles were placed in 1.5 mL centrifuge tubes with 75% ethanol and frozen at -80°C. Reference specimes were deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research (KSU-MEPAR) under the voucher #214.

DNA extraction and fragment analysis

Food oil derived from the traps was cleaned from the beetles prior to DNA extraction by rinsing beetles with the solvent Histo-Clear (National Diagnostics, Atlanta, GA, USA) for five minutes and then washing them with double distilled water. Genomic DNA from individual beetles was extracted using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). Because beetles collected in traps could have been dead for periods up to 2 wks or more and exposed to high temperature conditions, DNA degradation was expected in many of the individual beetles collected. The following DNA amplification protocol proved effective for these trap-collected insects: PCRs were conducted in a final volume of 12 µl containing 0.5 µl genomic DNA, 0.12 µl (5U/µl) Taq DNA polymerase (Promega, Madison, WI, USA), 0.12 µl dNTP (25 mM), 1.68 µl $MgCl_2$ (25 mM), 2.4 µl 5x PCR buffer, 0.12 µl forward tailed primer, 0.24 µl reverse primer and 0.12 µl M13 labelled primer (primers from IDT, Coralville, IA, USA). The cycling program included 95°C, 5 min for initial denaturation; 95°C, 45 sec; 58°C, 1

min; 72°C, 1 min for 15 cycles; 95°C, 45 sec; 50°C, 2 min; 72°C, 1 min for 24 cycles; and a final extension at 72°C for 5 min.

The selection of loci started with a list of microsatellites and indels with potential for population genetics analysis, with these molecular markers identified either by screening of the *T. castaneum* genome or from Demuth et al. (2007). A total of 31 markers were included in the initial analysis, but many were ultimately excluded based on several criteria. Some markers were polymorphic, but were considered poor in terms of quality of amplified fragments or uniqueness, so new primers were designed for the same locus to improve the amplification. Markers were confirmed to be unique by similarity searches to the *T. castaneum* genome (http://www.beetlebase.org) using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1997). Markers were also selected to be distributed on as many chromosomes as feasible (Table 2.1) and were tested for heterozygosity in preliminary tests with at least 5 beetles from each of the nine populations. Ultimately eight unique polymorphic loci were selected, including six microsatellites (MS1 through MS6) and two other non-microsatellite insertion-deletion polymorphisms (ID1 and ID2) (Table 2.1).

For high-throughput genotyping, fluorescent-labelled PCR fragments were produced using a M13 oligonucleotide adaptor sequence attached to the 5' end of the forward primers that allowed the incorporation of the fluorescent dye into the amplicons (Schuelke 2000). PCR products were analyzed (Sequencing and Genotyping Facility, Plant Pathology, Kansas State University) and allele sizes scored using an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA) and GeneMarker version 1.85 software (SoftGenetics, State College, PA, USA).

Analysis

For each population-by-locus combination, the expected and observed heterozygosities were calculated using GDA v. 1.1 (Lewis and Zaykin 2001). Linkage disequilibrium and deviation from Hardy-Weinberg equilibrium were tested using GENEPOP v. 4.0 (Raymond and Rousset 1995). For multiple comparisons, the sequential Bonferroni procedure was applied to determine the significance level (Rice 1989). The DNA amplification procedure that was used to deal with varying levels of DNA degradation, helped reduce the possibility of allele dropout, but still occurrence of other factors such as null alleles could affect the results. MICRO-CHECKER

program (van Oosterhout et al. 2004) was used to determine if deviations from HWE were due to the presence of null alleles or genotyping errors. Presence of null alleles and the failure to account for their presence can underestimate the within-population genetic diversity and overestimate differentiation among populations (Dakin and Avise 2004). Since MICRO-CHECKER indicated that the presence of null alleles was the most probable explanation for the deviations from HWE, the software Freena was used to estimate corrected F_{ST} values (Chapuis and Estoup 2007). The genetic structure of the populations was evaluated using the analysis of molecular variance (AMOVA) procedure in Arlequin v. 3.11 (Schneider et al. 2000) with different levels of hierarchy (i.e., commodity processed in facility (rice and wheat), and geographic region (west coast, midwest and southeast)). Isolation by distance was investigated with the Mantel test using semi-matrices of genetic distance (F_{ST}/1-F_{ST}) and of geographic distance (natural log (km)) as implemented in GENEPOP.

Bottlenecks were inferred for each population under the assumption of mutation-drift equilibrium by infinite allele (IAM) and two-phase (TPM) models using the program BOTTLENECK v. 1.2.02 (Cornuet and Luikart 1996). The program measures the temporary excess of heterozygosity that results from a reduced population size (Cornuet and Luikart 1996, Luikart et al. 1998a,b). Significance ($\alpha = 0.05$) of observed heterozygosity excess or heterozygosity deficiency relative to that expected at mutation-drift equilibrium was tested using the Wilcoxon sign-rank test (Luikart et al. 1998a, Luikart and Cornuet 1998). The IAM and TPM were used with 1000 iterations each. An assignment approach was used to verify the likelihood of correctly assigning individuals to their population of origin. The assignment test was carried out using the software GENECLASS 2 (Piry et al. 2004).

Results

Genetic diversity within populations

No significant linkage disequilibrium was observed in any of the pairwise comparisons using GENEPOP ($P > 0.05$), indicating that all loci included in this study assorted independently or recombined freely. The mean number of alleles per locus varied from 4.5 to 7.6, and within a population the number of alleles per locus varied from 3 to 14 (Figure 2.1, Table 2.2). The population from Puerto Rico (PR1) had the highest number of alleles when considering all loci.

Allele frequencies, regardless of allele size or locus, were highly variable among populations: ranging from 0.00 to 0.87. Most alleles were not unique to one population, but at least one private (i.e., only occurring in one population) allele was present in each population. Private allele frequencies within populations ranged from 0.02 to 0.39, with PR1 having the most private alleles (total of 8). Presence of alleles at low frequencies (i.e., 0.02) might reflect sampling error for detection of an allele present globally at low frequency. Null alleles (i.e., undetectable alleles due to factors such as mutations at the primer sites) were estimated to be present in all loci tested (mean frequency in each population ranged from 0.08 to 0.16).

There was considerable individual genetic variability within populations for most loci, with observed heterozygosity ranging from 0.06 to 0.84. In many cases observed heterozygosity was lower than the expected (Table 2.2), which has also been observed in other studies of *T. castaneum* (Demuth et al. 2007, Drury et al. 2009) and in other beetle species (Brouat et al. 2003, Schrey et al. 2008). Expected heterozygosities varied among populations, with values ranging from 0.22 to 0.86 (Table 2.2). Almost half of the locus-by-population combinations (31 of 72) showed significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction ($P < 0.01$), manifested by a deficit in heterozygotes (Table 2.2).

Genetic differentiation among populations

Significant levels of genetic differentiation were detected among populations ($P < 0.001$) (Table 2.3). However, permutation tests between two different commodities or among different geographic regions did not find significant levels of genetic differentiation (Table 2.3). After correcting for the presence of null alleles, F_{ST} values ranged from 0.018 to 0.149 (Table 2.3, upper diagonal), with the Global pairwise F_{ST} for all loci and population pairs being 0.082. Average F_{ST} value for the rice mills (3 mills) was 0.063 and for wheat mills (6 mills) was 0.091. The average genetic differentiation between the two groups was 0.082. Midwest mills (4 mills) showed an average F_{ST} value of 0.097 and southeast mills (Gulf Coast and Puerto Rico) showed an average F_{ST} value of 0.047. The average genetic differentiation between these two groups was 0.087. There was as much or more variation within wheat mill populations as between rice and wheat mill populations, and there was as much or more variation within midwest populations as between midwest and southeast populations.

To evaluate how much population variance could be explained by the distances between mills, the correlation between genetic and geographic distance was evaluated. There was no significant correlation between geographic distance (Table 2.3, lower diagonal) and genetic distance ($F_{ST}/1\text{-}F_{ST}$) using the Mantel test ($P = 0.61$) (Figure 2.2). For example, the F_{ST} value for the pairwise comparisons between NE1 and KS1 (distance = 247 km), LA1 and FL1 (distance = 1072 km), and CA1 and NE1 (distance = 2148 km) were similar; 0.069, 0.064, and 0.059, respectively.

Population bottlenecks

Bottleneck analysis revealed the existence of a bottleneck in the recent past for five out of nine populations, with results tending to be consistent under the two models. For NE1, the probability values for Wilcoxon rank test under IAM (p = 0.01) and TPM (p = 0.04) were significantly different from a mutation-drift equilibrium indicating excess of heterozygotes. Similarly, excess of heterozygotes were detected for NE2 under IAM (p = 0.01) and TPM (p = 0.03), FL1 under IAM (p = 0.01) and TPM (p = 0.01), CA2 under IAM (p = 0.01) and TPM (p = 0.03), and LA1 under IAM (p = 0.03). No excess of heterozygotes, based on Wilcoxon rank test under, were detected for KS1 under IAM (p = 0.10) and TPM (p = 0.16), CA1 under IAM (p = 0.16) and TPM (p = 0.27), PR1 under IAM (p = 0.37) and TPM (p = 0.37), and KS2 under IAM (p = 0.16) and TPM (p = 0.19).

Assignment of individuals to their population of origin

To determine how well individual beetles could be assigned to their population of origin, GENECLASS-based assignment test was used to compute the probability of correctly assigning each multilocus genotype to a given population. Only 56% of the individuals were correctly assigned to their population of origin. When the Bayesian probability of correct assignment was averaged among all individuals within a population, the values ranged from 0.43±0.06 to 0.60±0.06. The population LA1 had the lowest average Bayesian probability, followed by KS2, NE1, PR1, KS1, NE2, CA1, CA2, and FL1.

Discussion

Results of this study provide evidence of genetic differentiation among populations of *T. castaneum* in individual mills, but did not find IBD which would be expected given the distances

between facilities tested. Another recently published study has also shown genetic differentiation of populations of *T. castaneum* (Drury et al. 2009). Drury and colleagues (2009) analyzed microsatellites from *T. castaneum* populations originating from different continents and found F_{ST} values that ranged from 0.03 to 0.35, with a global pairwise F_{ST} of 0.18. This global pairwise F_{ST}, a measure of genetic distance, was more than twice what was obtained among the populations in the current study; high versus moderate levels of differentiation (Balloux and Lugon−Moulin 2001). For only populations originating within the U.S., Drury et al. (2009) obtained a global pairwise F_{ST} value of 0.127, which was more similar to what was obtained in in this research and in both cases indicates a moderate level of differentiation. In both, Drury et al. (2009) and the present study, there was a lack of relationship between geographic distance and genetic distance, despite the greater geographic distances in Drury et al. (2009). Reports of measures of the levels of inter-population differentiation, that are lower than hypothesized using microsatellite analysis, have been published for other insect species (e.g., Paupy et al. 2004, Roos and Markow 2006). For example, Lehmann et al. (1996) found that *Anopheles gambiae* (Diptera: Culicidae) showed low estimates of differentiation between populations that were as much as 6,000 km apart despite mark-release-recapture experiments that suggested that active dispersal of this species is restricted to a few kilometers.

Lack of IBD for *T. castaneum* is not consistent with mills primarily being connected by active dispersal and the lack of structure due to geographic location or commodity processed in the mill also suggests that movement of infested grain into the mill may not be a likely mechanism either. It was hypothesized that direct mixing most likely would occur among only some of the locations (e.g., CA1 and CA2), and be reduced or inexistent among others (e.g., between rice and wheat mills). It was predicted that rice and wheat mills, compared to other food facilities types (e.g., processing plants, warehouses, distribution centers, retail stores, consumer pantries), would have the greatest genetic differentiation since they have little inbound processed materials that might be infested, other than the grain brought on site for milling. Different of what was expected; comparisons among groups of different commodities or geographic regions using AMOVA did not find significant levels of genetic differentiation. Once grain is harvest, it can be shipped to local country elevators or directly to the mill and then to different terminals or port elevators for storage or export and the transport can be done by truck, rail or barge. Each of these storage facilities and transportation modes can be infested

with individuals that compose a mixed gene pool. The gene pool present in the grain can later be transferred to processing facilities when the grain is processed. Additionally, it is possible that beetles brought onsite in grain could migrate from the bulk storage into the mills and some mills do obtain grain harvested over a large geographic area. If the hypothesis of mixed gene pool transferred to the processing facility is possible then southeast mills should have the least amount of genetic structure since these mills should have grain coming from several locations and transportation modes and therefore great diversity and more unique alleles. Midwest mills in general should have less diversity, and the KS2 mill which is the smallest and most local should be the most isolated. Based on the results, the hypothesis is, in part, confirmed. Two of the southeast populations were among the ones with the highest number of alleles (LA1 and PR1) and highest H_{obs} (LA1 and FL1). Also, one population (PR1) had the highest number of private alleles. Moreover, the population KS2 had an overall F_{ST} of 0.10 which is the second highest among all populations in this study.

Beetles may also be able to migrate into facilities from the surrounding environment, especially if residential and retail facilities are in the proximity. Given the potential movement of infested material from a large number of sources, the cumulative impact of this could be that there is a large amount of gene flow throughout the US and limited population structure. In this particular way, mixing can be happening through a stepping-stone model. In this model of movement, gene flow can occur in ways that it is difficult to estimate. Individuals could infest different commodities, contribute to the gene pool at one location and move to other locations. Human-aided dispersal via transport of commodities such as flour has been shown to play an important role in mixing populations of stored-product species (Ryne and Bensch 2008). In this scenario, proximity to urban areas would be an important factor. In this case, populations KS2 and LA1 are the most isolated and would figure among those with the lowest gene pool and therefore least genetic diversity. Based on the results, it is not possible to confirm these predictions since neither of these populations were among the ones with the lowest levels of genetic diversity.

There are several other factors not directly related to gene flow among mills that could explain the observed population structure (e.g., occurrence of null alleles, intrinsic characteristics of the markers, and presence of genetic bottlenecks caused by fumigations that result in genetic drift). Clear distinction among these factors is difficult, but it is possible to reduce the effects of

some confounding factors. Based on the analysis conducted here, null alleles were present, but it was possible to account for them and reduce their potential affects on the results. Nonetheless, it is important to note that there is some controversy regarding the presence of null alleles and their impact on population genetic structure studies: noticeably affecting F-statistics (Dakin and Avise 2004), while having less of an effect on Bayesian analysis (Orsini et al. 2008). Null alleles may also reduce the power of assignment tests, having a stronger effect if the total number of loci is low (Carlsson 2008). There are simulation studies suggesting that the bias introduced by null alleles is negligible when their frequency is less than 0.2 (Dakin and Avise 2004) and in this study the frequency of null alleles was never greater than 0.2.

Other characteristics of microsatellites need to also be considered when interpreting results in population genetic studies. Microsatellites are known for rapid evolution, with mutation rates of approximately 10^{-3} per locus per generation (Weber and Wong 1993, Jarne and Lagoda 1996), which can make them useful for detecting differentiation among populations over relatively short periods of time, but they also can show high within-population variance (Carbonnelle et al. 2007), which can exaggerate the perceived genetic diversity within populations and reduce power to differentiate among populations. Additionally, microsatellites are more likely to be neutral than other markers, but reduction in the levels of differentiation among populations can occur if there are constraints on microsatellite evolution, such as biased mutation rates (Garza et al. 1995) and/or selection for certain allele sizes (Epplen et al. 1993) even in populations that are geographically distant. Thus, some of the limited population differentiation could be due to the limitations of the markers used. Differentiation indices across loci were not consistent; indicating that heterogeneity among loci was large (Table 2.1).

Processes occurring within each food processing facility can also impact *T. castaneum* population structure. Frequent structural fumigations can create genetic bottlenecks due to genetic drift and small numbers of beetles contributing to population rebound after treatment (Campbell et al., 2010 a,b), which can reduce genetic variation or result in a non-random sample of the original population. Genetic bottlenecks were detected in five of the populations using Wilcoxon rank test, confirming that most of the populations are generally going through reduction in size causing genetic drift that can influence the measurements of genetic structure. Since genetic bottlenecks were not confirmed for some of the population, it is possible that the bottleneck effects were not evident in those populations for the particular period in which

samples were collected or that the used method was able to detect a recent bottleneck even though it might have occurred.

Beetles used in this research were collected directly from mills, as opposed to other studies with *T. castaneum* that used populations that had been lab-cultured for varying periods of time. In general, the levels of heterozygosity in the populations of this research collected directly from the field, were higher than those found in Drury et al. (2009) populations obtained from laboratory colonies, which might reflect some loss of genetic diversity in the laboratory. The Drury et al. (2009) populations held the longest in the laboratory, in some cases more than 20 years, tended to have relatively high pairwise F_{ST} values compared with other populations maintained for shorter periods of time in the laboratory. For some insect species, differences between laboratory colonies and field populations have been demonstrated (Norris et al. 2001, Gomez-Sucerquia et al. 2009). For example, long-term maintenance of laboratory colonies can result in a reduction of alleles per locus and overall heterozygosity levels.

The results obtained in this study and others dealing with *T. castaneum* populations (Beeman 2003, Demuth and Wade 2007, Drury et al. 2009) provide evidence of population structure within the United States, but at this point there is insufficient information to explain the levels of differentiation found among some of the populations that, based on geography and other factors, would be predicted to show greater differentiation. To this point, the observed genetic differentiation among populations of *T. castaneum*, at the scales studied suggest sufficient gene flow to prevent higher genetic differentiation and the occurrence of genetic bottlenecks expected in the wake of fumigation. Given the genetic information available for this species, a better understanding of population structure and gene flow should be possible, and this could lead to improved pest management programs. Moreover, continued refinement of polymorphism-based analysis of population structure will aid evaluation of IPM program effectiveness and resistance management by enabling the identification of infestation sources.

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Figures and Tables

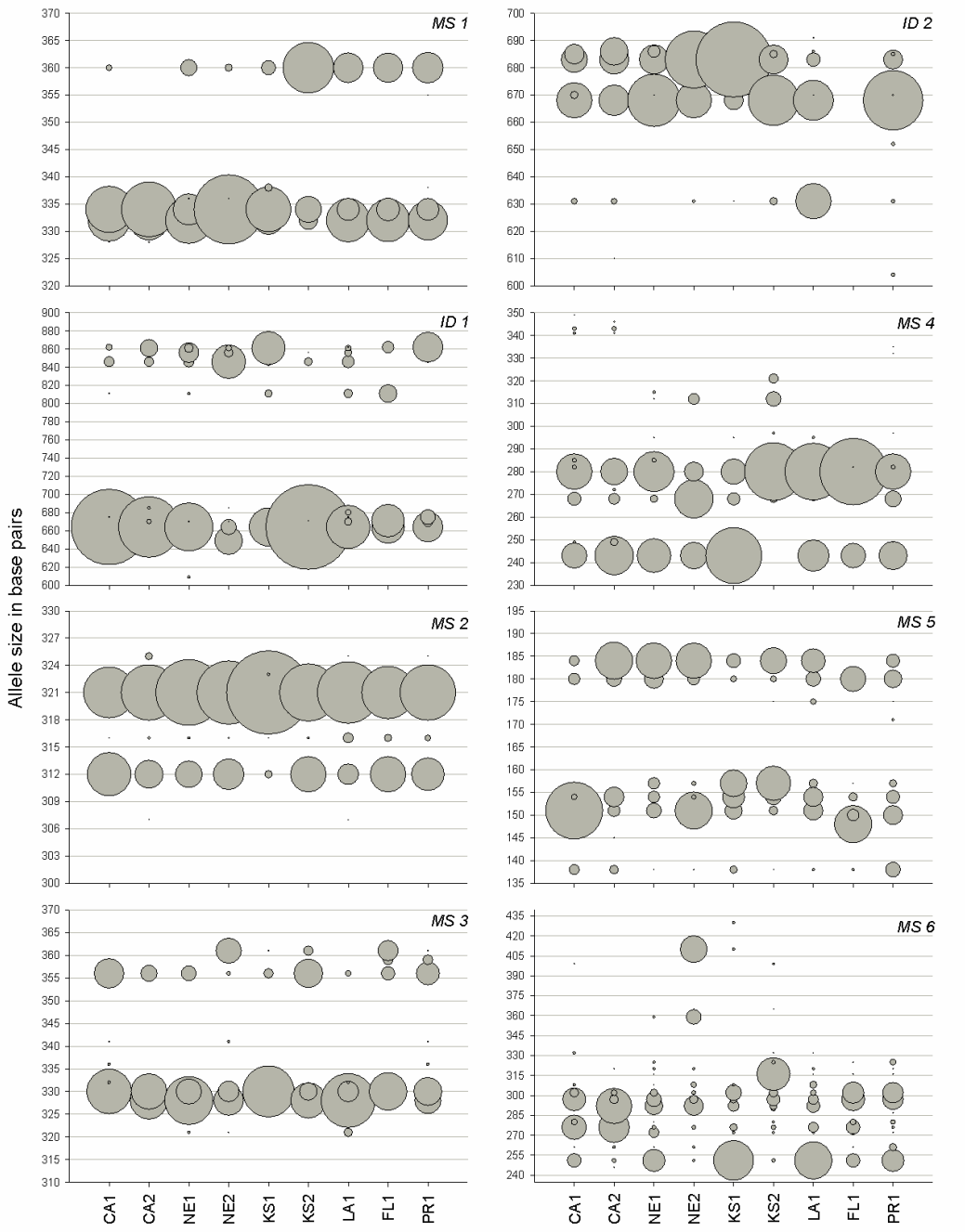

Figure 2.1 Distribution of allele sizes and frequencies for eight molecular markers (MS1-MS6, ID1-ID2) in nine populations of *Tribolium castaneum* (x-axis). Y-axis values correspond to the allele size in base pairs and the diameter of circles corresponds to the relative frequency of the respective allele in each population.

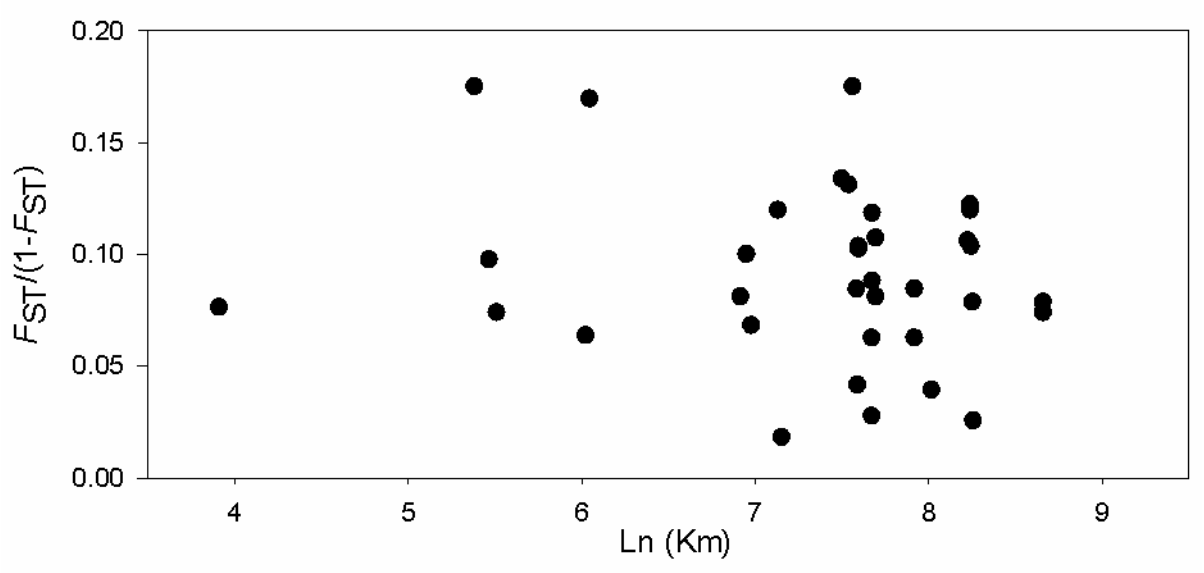

Figure 2.2 Isolation by distance of *Tribolium castaneum* populations based upon the relationship between genetic distance ($F_{ST}/1-F_{ST}$) and the natural logarithm of geographic distances (Ln (Km)).

Table 2.1 Marker code, linkage group (LG), motif, primer sequences (forward and reverse) and locus-specific F_{ST} values for each of the eight molecular markers (microsatellite or other indels) used for evaluation of *Tribolium castaneum* population structure. Primer sequences for MS6 were obtained, and primer sequences for MS4 were modified, from Demuth et al. (2007). In the reserve primer for ID2, W and Y are used as nomenclature for incompletely specified bases (W was used to specify that adenine or thymine can be used in that location and Y is for thymine or cytosine).

Code	LG	Motif	Primer sequences (5'-3')	Locus-specific F_{ST} values
MS1	2	GTT(10)	CGACGACGAGAAGGGAGGTA	0.074
			GCAAGGAGGCCATGAATAAAA	
ID1	3	-	AACTTTAAACCCATCTCACTCAA	0.132
			ATCATACTTTCAGACCCAGACAC	
MS2	3	ATAA(5)	GTAAACAGGAGGACAGGCTAAAAGTG	0.025
			CATCGAACGAGGCTGTGAATAAAC	
MS3	3	AAT(7)	TATCCGAAATTTTATCTACTCAT	0.052
			AGGACCCTTTTTACTTTTTCAG	
ID2	3	-	CCGCTTTCGTCTCRCAGTTGC	0.119
			CTAWYGTAAGACTTATTAGGCACGTTC	
MS4	9	AAT(3)-AAT(5)-AAT(8)	AGCCGCAACAAAGTAAGCAA	0.079
			TTCTGACTACCACCGACAGATTT	
MS5	9	TAA(16)	AAGTGCTGCTGTATTTTATT	0.105
			TCAGACTCCGTATCCTTTATT	
MS6	10	AAT(19)	AAATTCTCGGCTTTTTGGGT	0.070
			GAGCTGGCGGTTATATTGGA	

Table 2.2 Summary of genetic information for nine populations of *Tribolium castaneum* (columns 3-11) and eight molecular markers: number of individuals (*N*), number of alleles (*Na*), number of private alleles (*Npa*), observed heterozygosity (H_{obs}), unbiased expected heterozygosity (H_{exp}), non-significant (NS) Hardy-Weinberg disequilibrium level (HW) at 5% adjusted by Bonferroni's correction.

Marker	Variable	CA1	CA2	NE1	NE2	KS1	KS2	LA1	FL1	PR1
MS1	*N*	32	28	32	32	30	36	32	29	28
	Na	4	3	4	5	4	3	3	3	5
	Npa	0	0	0	1	0	0	0	0	1
	H_{obs}	0.281	0.179	0.250	0.188	0.400	0.111	0.281	0.793	0.464
	H_{exp}	0.579	0.514	0.638	0.454	0.674	0.615	0.652	0.656	0.686
	HW					NS			NS	NS
ID1	*N*	28	30	31	31	30	34	31	16	19
	Na	5	5	7	7	5	4	9	4	7
	Npa	0	0	1	1	0	0	1	0	0
	H_{obs}	0.143	0.367	0.484	0.355	0.433	0.147	0.387	0.063	0.158
	H_{exp}	0.372	0.561	0.693	0.762	0.708	0.216	0.760	0.736	0.781
	HW			NS		NS	NS			
MS2	*N*	32	32	32	31	30	36	32	32	32
	Na	3	5	3	3	4	3	5	3	4
	Npa	0	0	0	0	1	0	0	0	0
	H_{obs}	0.531	0.688	0.500	0.484	0.200	0.444	0.625	0.563	0.594
	H_{exp}	0.520	0.579	0.454	0.466	0.245	0.509	0.538	0.563	0.552
	HW	NS	NS	NS	NS	NS	NS	NS	NS	NS
MS3	*N*	29	32	32	32	30	35	32	14	29
	Na	6	4	5	7	5	6	6	5	8
	Npa	1	1	1	0	0	1	0	0	2
	H_{obs}	0.448	0.313	0.438	0.500	0.633	0.314	0.313	0.071	0.379
	H_{exp}	0.677	0.684	0.662	0.781	0.651	0.737	0.631	0.775	0.780
	HW	NS		NS	NS	NS				

Table 2.2 Continued

Marker	Variable	CA1	CA2	NE1	NE2	KS1	KS2	LA1	FL1	PR1
ID2	*N*	31	31	31	31	30	36	31	31	25
	Na	5	5	4	3	3	4	6	0	7
	Npa	0	1	0	0	0	0	1	0	2
	H_{obs}	0.839	0.645	0.419	0.516	0.300	0.361	0.677	0.000	0.560
	H_{exp}	0.745	0.725	0.598	0.513	0.352	0.623	0.675	0.000	0.580
	HW	NS	NS	NS	NS	NS		NS		NS
MS4	*N*	32	30	31	30	30	35	32	31	32
	Na	9	8	8	4	4	6	4	4	9
	Npa	1	1	1	0	0	1	0	0	2
	H_{obs}	0.563	0.333	0.194	0.133	0.333	0.400	0.250	0.452	0.563
	H_{exp}	0.772	0.747	0.698	0.718	0.580	0.605	0.545	0.458	0.748
	HW								NS	NS
MS5	*N*	32	31	32	31	30	36	32	32	32
	Na	5	6	6	6	6	7	7	7	9
	Npa	0	1	0	0	0	0	0	1	1
	H_{obs}	0.594	0.516	0.375	0.484	0.833	0.417	0.625	0.750	0.563
	H_{exp}	0.614	0.766	0.774	0.702	0.811	0.761	0.830	0.757	0.864
	HW	NS			NS	NS		NS	NS	NS
MS6	*N*	31	31	32	32	30	36	32	31	30
	Na	10	9	14	11	9	14	11	10	12
	Npa	0	0	0	0	1	2	0	0	0
	H_{obs}	0.839	0.742	0.719	0.563	0.567	0.611	0.781	0.774	0.433
	H_{exp}	0.842	0.752	0.874	0.848	0.780	0.842	0.804	0.848	0.849
	HW	NS	NS	NS		NS	NS	NS	NS	
All loci	*N*	32	32	32	32	30	36	32	32	32
	Na	47	45	51	46	40	47	51	36	61
	Npa	2	4	3	2	2	4	1	1	8
	H_{obs}	0.530	0.473	0.422	0.403	0.463	0.351	0.492	0.495	0.464
	H_{exp}	0.640	0.666	0.674	0.656	0.600	0.614	0.679	0.685	0.730

Table 2.3 AMOVA analyses for different hierarchical levels. First, analysis was used for comparison among all individual populations. Second, besides individual populations, analysis also compared group of commodities (rice and wheat). Finally, three different geographic region (western, midwest and southeast) were compared. Significant differentiation (P < 0.001) was based on permutation tests (10000 permutations).

Source of variation	d.f.	Sum of Squares	Variance component	Percentage of variation	*P*-value
Among populations	8	88.36	0.15	8.32	< 0.001
Within populations	571	921.26	1.61	91.68	< 0.001
Among commodity groups	1	9.29	-0.01	-0.44	0.630
Among populations within commodities	7	79.07	0.15	8.56	< 0.001
Within populations	571	921.26	1.61	91.88	< 0.001
Among geographic regions	2	28.51	0.02	1.31	0.156
Among populations within regions	6	59.85	0.13	7.35	< 0.001
Within populations	571	921.26	1.61	91.35	< 0.001

Table 2.4 Pairwise F_{ST} values (upper diagonal) and geographic distances in km (lower diagonal) for populations of *Tribolium castaneum* with a global F_{ST} of 0.082. F_{ST}s were corrected for the presence of null alleles using Freena. Non-significant (NS) *P*-value for the test of genotypic differentiation after Bonferroni's correction (*P* = 0.00139).

	CA1	CA2	NE1	NE2	KS1	KS2	LA1	FL1	PR1
CA1		0.051	0.059	0.097	0.106	0.094	0.078	0.107	0.073
CA2	< 0.3		0.027	0.075	0.081	0.093	0.059	0.109	0.069
NE1	2148	2148		0.071	0.069	0.060	0.018^{NS}	0.078	0.025
NE2	2195	2195	50		0.089	0.145	0.107	0.149	0.094
KS1	2153	2153	247	236		0.149	0.091	0.118	0.096
KS2	19901	1990	413	422	217		0.075	0.116	0.073
LA1	2747	2747	1277	1250	1043	1007		0.064	0.038
FL1	3787	3787	1967	1922	1807	1880	1072		0.040
PR1	5763	5763	3852	3804	3732	3834	3030	1976	

Chapter 3 - Spatial-temporal distribution of stored-product pests around food processing and storage facilities

Abstract

The presence and distribution of stored-product insects outside food storage and processing facilities can potentially influence the risk of immigration into the facility. The spatial and temporal distribution of stored-product pests was assessed in the proximity of three food processing facilities using two types of traps and the potential effects of the landscape features on their distribution were evaluated. Important stored-product pests and fungus feeding species were found outside all three facilities. The most abundant species captured in corrugated traps located outside were *Cryptolestes* spp. (32.6%), *Typhaea stercorea* (20.4%) and *Oryzaephilus surinamensis* (19.4%). In the Lindgren traps, the most abundant species captured were *T. stercorea* (42.1%), *Cryptolestes* spp. (17.2%) and *Ahasverus advena* (13.9%). No correlation was observed between total captures inside and outside the structures at the facilities ($P > 0.05$). Insect captures in outside traps tended to be lower than captures inside structures, and higher captures in outside traps were associated with proximity of buildings, but not the presence of spillage. The stored-product pest species composition at each site tended to be similar over time, although relative frequency of different species varied, with at least two areas of high capture observed at each site; inside the food processing facility and near the bulk storage structures. These results support the hypothesis that emigration from different structures at each facility may be primary source of outside insect activity at these locations.

Introduction

Food processing facilities typically consist of a landscape of multiple buildings and storage structures, which vary in type, size and shape among locations, and can be populated by a diverse community of arthropods. According to Hagstrum and Subramanyam (2009), approximately 1,660 insect species are associated with stored-products including species that are granivores, fungivores, omnivores, and natural enemies and which are distributed in the orders Coleoptera, Diptera, Hemiptera, Homoptera, Hymenoptera, Lepidoptera and Psocoptera. In general, monitoring and management of stored-product pests is focused on populations inside the structure of the buildings and storage bins. However, stored-product insects have been readily captured outside of structures (Throne and Cline 1989, 1991, Fields et al. 1993, Dowdy and McGaughey 1994, Dowdy and McGaughey 1997, Dowdy and McGaughey 1998, Doud and Phillips 2000, Likhayo and Hodges 2000, Campbell et al. 2002, Campbell and Arbogast 2004, Campbell and Mullen, 2004, Kučerová et al. 2005, Campbell et al. 2006), and movement from outside into facilities has been documented (e.g., Campbell and Arbogast, 2004). Thus, the presence and the distribution of stored-product pests beyond the limits of the structure of the facilities should also be considered when implementing integrated pest management (IPM) programs. However, outside monitoring of stored-product insects has tended to rely on traps that capture flying individuals, so the sources of these insects are not well understood.

Sources of stored-product insects captured outside can be individuals dispersing from other structures - either short-range dispersal from other structures on site or long-range dispersal from other locations. However, food material that can be exploited by stored-product insects can accumulate in the landscape surrounding structures. For example, whole grain spillage can accumulate in unloading areas, dust and other material from processing can blow out of facilities and accumulate in areas outside, trash containers and excess equipment stored outside can contain food that can be exploited by insects. The persistence of these outside food accumulations and their quality as resources, is likely to be highly variable. These outside food patches can be exploited by stored-product insects as locations for oviposition and development and/or as stepping stones which can attract dispersing adults into the proximity of structures and provide food and shelter for these dispersing individuals. Although the potential importance of these food accumulations has been widely acknowledged in the food industry, there is a lack of

research evaluating their importance as sources of infestation. However, spillage accumulations inside grain elevators have been shown to be exploited by stored-product pests (Arthur et al. 2006).

Basic structural characteristics of the landscape can affect species abundance and distribution (Turner 1989, Wiens 1997, French et al. 2004), and the abundance and spatial and temporal distribution of stored-product insects outside food facilities is likely to be affected by the composition and structure of the landscape surrounding these facilities. Spatial distribution of stored-product insects in the landscape outside of food facilities can potentially be influenced by a wide range of factors. The most intuitive hypothesis is that the distribution of food spillage is a major factor that impacts the number and distribution of insects outside. However, where the spillage accumulates and how degraded the spillage has become are likely to influence its suitability as a resource, with this impact will likely varying among species. For example, degraded food material may favor exploitation by fungivore stored-product insects. Different environmental factors such as temperature may also be important. Higher temperatures and amount of food material accumulating have been shown to be associated with *Tribolium castaneum* distribution inside a flour mill (Chapter 5). In addition, the nature of the surface (e.g. gravel, grass, concrete) can affect accumulation of spillage and also movement of stored-product insects. A practical benefit from knowing the distribution of the community of stored-product pests outside storage and processing facilities, and the relationship between distribution and features in the landscape is that it can help managers target outside monitoring and pest management tactics in order to reduce the risk of immigration into facilities.

One of the major pests in wheat and rice mills worldwide is the red flour beetle, *T. castaneum* (Sokoloff 1974). Its distribution inside structures such as flour mills, warehouses, and retail stores has been well studied (Ho and Boon 1995, Campbell et al. 2002, Trematerra and Sciarretta 2004). Response of *T. castaneum* populations to structural fumigations has suggested that populations are relatively self-contained within individual structures (Campbell and Arbogast 2004, Toews et al. 2006, Campbell et al. 2010a,b), although the potential for movement of beetles from either outside sources or from other structures in the proximity of the facility are unknown. *T. castaneum* has been captured outside both in the proximity of, and far from, food facilities (e.g., Sinclair and Haddrell 1985, Dowdy and McGaughey 1994, Subramanyam and Nelson 1999).

The objectives of this study were: 1) evaluate the spatial and temporal distribution patterns of stored-product pests in the landscape of three grain processing and storage facilities, and 2) determine which environmental and landscape factors are associated with outside insect distribution patterns.

Materials and Methods

Study sites

This study was conducted at three sites (herein coded as site A, B, and C) located in the central USA (Fig. 3.2A, 3.3A, 3.4A). Site A is a commercial storage and processing facility and contains multiple buildings including a five-floor flour mill (~4,531 m^3) with attached elevator with concrete silos, warehouse and packaging building, small metal three-floor feed mill, variety of office and storage shed buildings, a second grain elevator with concrete silos, one large metal bin, and two ground bunker storage locations with moveable wood bulkheads. Surrounding these structures the landscape primarily consisted of areas of gravel and mowed grass within the property line of the facility. Accumulation of food spillage was observed in areas near the mill and grain elevators. The property is bordered by residential areas, a paved road and agricultural fields. Site B is small feed mill (~280 m^2) composed of one metal building used for processing animal feed which has large doors that are often open allowing for easy movement into and out of the facility. In the proximity of the feed mill, there are 20 small bins in which either grain (primarily corn) or processed feed are stored. The landscape around the feed mill is primarily gravel and grass, with the site bordered by paved roads and an open field. Site C is a relatively new concrete pilot-scale flour mill (2,044 m^2) composed of five floors (Kansas State University, Hall Ross Flour Mill). In the proximity of the mill, there are eight small storage bins. Facility is designed for research and education purposes and does not operate continuously. The area immediately surrounding the building is composed primarily of mowed grass, brush, and paved areas.

Stored-product insect monitoring

Monitoring was conducted using two types of traps (see Appendix A). The first trap was a modified design of a corrugated cardboard trap (Likhayo and Hodges 2000, Daglish 2006) that captures walking insects and was predicted to primarily detect localized insect activity. The

corrugated traps consisted of two layers of corrugated plastic held between two metal plates. Each layer consisted of four pieces corrugated plastic (9 cm x 3 cm and 2 mm thick) arranged to form a square 9 x 9 cm. For the bottom layer, pieces were glued to a square piece of metal (9 x 9 cm) leaving square space in the middle (3 x 3 cm). For the top layer, pieces were glued in the same arrangement to a square piece of clear plastic (9 x 9 cm) with a circular hole in the center (~ 2 cm diameter). One *Tribolium* spp. pheromone lure (Trécé, Adair OK), and ~3 g of cracked wheat were placed in the space in the center of the corrugated plastic pieces. After adding pheromone and cracked wheat, a second piece of metal was added to the top of the stack. Both top and bottom metal plates had a hole in the center, through which a machine bolt inserted from underneath and the trap layers held together by tightening a wing nut on the portion of the bolt projecting from the top of the trap. The hole in the metal bottom piece was countersunk on the underside so that trap could lay flat with the bolt inserted. The other trap type was the Lindgren funnel trap (Phero Tech Inc., Delta, BC, Canada) (Lindgren 1983) and it was used to capture flying individuals. The collection reservoir of the traps contained cracked wheat (~200 g) on top of a piece of window screen inserted into the reservoir to elevate the wheat 2 cm from the bottom of the trap to avoid accumulation of water that can lead to grain spoilage.

The corrugated traps were distributed in an irregular grid pattern at each site, and most of the traps were placed outside the buildings in the surrounding area within limits of the property line (Figure 3.2A, 3.3A, and 3.4A). Trap positions were also selected in order to get adequate representation of the different habitat types in the landscape at each site. The number of traps in each site was defined according to the evaluation of the complexity of the landscape (i.e., number of different habitats) and the size of the property. The geographic coordinates of the traps location were recorded using a Global Positioning System (GPS) receiver (Garmin GPS Map 76, Olathe, KS). Monitoring with corrugated traps was conducted between July and October in 2007 and 2008 for sites A and B. At these two sites, monitoring took place during six monitoring periods (three in 2007 and three in 2008). Site C was monitored only in 2007 (three monitoring periods) since there were few captures outside at this site (Table 3.1). In each monitoring period, corrugated traps were in place for 48 h, then collected and placed individually in plastic bags (Ziploc®, Johnson & Son, Inc., WI) and returned to the laboratory where individuals were identified to species when possible or at least to genus, and total number in each group determined.

Lindgren funnel traps were distributed in the landscape surrounding the facilities. Because there was a smaller number of Lindgren traps, these were distributed in order to have at least one trap in each different habitat type in the landscape at each site. Monitoring with Lindgren traps was conducted in 2008 and 2009 and traps were kept continuously at the sites from August to October of both years, with contents of the traps collected every two weeks. Contents of each trap were placed individually in plastic bags and returned to the laboratory. All captured individuals were identified to species when possible or at least to genus, and total number of each group counted. Reference specimes were deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research (KSU-MEPAR) under the voucher #214.

Analysis of spatial and temporal distribution of stored-product insects

For analysis of spatial distribution, only data collected with the corrugated traps were used, while analysis of temporal distribution was assessed using both corrugated and Lindgren traps. For the spatial distribution analysis, the geographic coordinates of trap locations and the capture data was imported into ArcMap 9.3 computer software (ESRI®, Redlands, CA). Average Nearest Neighbor analysis was used to assess if the pattern of distribution of traps at each site could potentially be clustered since they were distributed in an irregular fashion. Different methods of interpolation were tested including inverse distance weighting (IDW), radial basis function (RBF) and kriging. RBF is only considered useful when working with data sets that have attributes that gently vary (e.g., elevation) and also when a large number of data points are included. For kriging, the assumption is that there is spatial autocorrelation of that needs to be determined prior to use. IDW has the advantage of considering that weight of sampled points decrease as distance increases from the interpolated points. Prelimary evaluation of the three methods indicated that although all three gave similar results, given the constraints on the other methods, IDW was the most appropriate. Contour maps of stored-product pest spatial distribution were developed using the Spatial Analyst extension in ArcGIS 9.3. Maps were made using Inverse Squared Distance Weighting (ID^2W) method for each monitoring period in each site. The search radius for site A was set for 40 meters and 10 meters for sites B and C. The larger radius was used at site A because of its larger size and greater distance between trap locations.

Spatial autocorrelation of stored-product insect captures was assessed at each site using two spatial statistical approaches. All species considered as stored-product pests (Table 3.1 and 3.2) were pooled together for analysis because captures of individual species were often too low to be considered individually. First, Global Moran's Index was computed (Moran 1948) for each site and monitoring period to evaluate whether the pattern of distribution was clustered, dispersed or random. Moran's I values range from + 1 (strong positive autocorrelation) to – 1 (strong negative autocorrelation), with a value of 0 indicating random distribution. Second, Anselin Local Moran's Index was computed (Anselin 1995), which unlike Global Moran's I, computes spatial autocorrelation at each sampling location based on neighbouring values within a local neighborhood search. A high positive Z score for a trap location indicates that the surrounding traps have similar values (either high or low).

The evaluation of temporal dynamics of stored-product insects, for the captures with corrugated and Lindgren traps, was conducted by calculating the average number of insects/trap/monitoring period at each site. Also, the proportion of each species or species groups were plotted for each monitoring period at each site using the sum of individuals caught during each monitoring period. Seasonal variation was also compared visually considering each consecutive monitoring period.

Environmental data and landscape features at trap locations

Environmental conditions such as temperature, relative humidity (rh), and wind speed were collected at the beginning and end of each monitoring period at each corrugated trap location using a handheld weather meter (Kestrel® 3000, Nielsen-Kellerman, Boothwyn, PA) held ~50 cm above trap location. Also, for selected traps at each location, temperature and rh were recorded over the entire course of the trapping period using HOBO data loggers (Onset Computer, Bourne, MA). Data loggers were placed near traps (~10 cm) and they were covered with a dome shaped cover to protect from rain and covered with a wire cage staked to the ground to keep them in place. Both sources of data (handheld weather meter and data loggers) were used to determine averages at each trap location.

Landscape features around each trap were assessed by taking digital photographs of each trap location and based on these images calculating the proportion of each landscape type in the proximity of the trap (see Appendix B). The digital camera (Nikon D100, Nikon Inc., Melville,

NY) was held 1 m above the trap and held horizontally to the ground when taking the picture. From the digital pictures, the percent coverage of different landscape types (grass, gravel, and concrete) within a 1 m^2 area around the traps was determined by tracing outlines and calculating area of shapes using Image J software (Abramoff et al. 2004).

The proximity of vertical surfaces, associated with the sides of building or storage structures, was indicated as either present (vertical surface inside the 1 m^2 square area around the trap) or absent. Shade over the trap also had two classes: (1) no shade over the trap and (2) presence of shade over the trap. Shade was determined by directly observing the traps in the field and by analyzing pictures using Image J software as described above. Shade levels will obviously change over the course of the day, so time was standardized between 10:00 a.m. and 12:00 p.m. The presence of spillage accumulating at each trap location was determined at the surface of 1 m^2 area around traps. Spillage was defined as any material that was observed such as grain, flour or any other derived material obtained from grain processing. The presence of spillage was defined as 0: no spillage present, 1: up to 50% of the area covered with spillage, and 2: between 50 and 100% of the area covered with spillage. These indices were determined by visually assessing each trap location during site visits and by analyzing digital pictures with Image J software.

Distance to closest storage structure (e.g., metal bin, elevator) and distance to mill building (i.e., flour mill in site A and C, feed mill in site B) were determined by measuring the straight line distance from each trap location to the closest outside wall of the facility using a metric tape measure and/or a hand-held laser meter (Disto™ classic, Leica Geosystems AG, Heerbrugg, Switzerland).

Data analysis

In order to compare the community of stored-product insects associated with traps at these facilities, Simpson's Index of diversity was calculated (Simpson 1949) as applied to trapping data in Larson et al. (2008). Values of Simpson's Index of diversity can vary from 0 to 1 in which values close to 0 indicate low species diversity and values close to 1 indicate high species diversity.

To explore the relationship between environmental and physical factors and capture of stored-product pests outside the food processing and storage facilities, two different approaches

were used. First, measured variables were compared between trap locations with and without captures by applying Student's t-test or contingency table analysis (Sigmaplot v. 11 (Systat Software Inc., Chicago IL)). Second, stepwise regression (SAS software, version 9.2 (SAS Institute, Cary, NC)) was used to determine the most significant variables associated with stored-product insect captures. For stepwise regression, only variables with significance $P < 0.15$ were entered into the analysis.

Results

Species associated with food storage and processing facilities

Across the facilities and monitoring years and in both trap types, a total of 3,678 stored-product insects from 13 species or species groups were captured, with a total of 1,098 individuals from species that are primarily fungus feeders (Table 3.1 and 3.2). The fungus feeders present at the three sites were the hairy fungus beetles *Typhaea stercorea* (L.) and *Litargus balteatus* LeConte, and the silken fungus beetle *Cryptophagus* sp. Considering the relative number of traps of each type and the length of each monitoring period, corrugated traps captured an average of 1.9±0.2 individuals/trap/day, while Lindgren traps captured 0.5±0.1 individuals/trap/day. Corrugated traps inside the structures captured an average of 4.4±1.1 individuals/trap/day while corrugated traps outside captured 1.1±0.7 individuals/trap/day. There was not a significant correlation between the total number of individuals captured inside and outside ($r = 0.26$; $P = 0.36$) when including all sites and monitoring periods. For the year that corrugated traps were placed at the three sites (2007), site C had the highest absolute capture (765 individuals), followed by site B (678 individuals), and site A (269 individuals). In 2008, site A had the highest absolute capture with 414 individuals, and site B had 222 individuals captured. In Lindgren traps, site A had the highest absolute capture (1084 individuals), followed by site B (171 individuals) and site C (75 individuals). However the number captured in an individual monitoring period varied over time (Table 3.3).

In corrugated traps (inside and outside), *Cryptolestes* spp. (29.7%) was most abundant, followed by *Oryzaephilus surinamensis* (L.) (15.9%) and *Sitophilus* sp. (13.7%). In the Lindgren traps, the most abundant species captured was *T. stercorea* (42.1%), followed by *Cryptolestes* spp. (17.2%) and *Ahasverus advena* (Waltl) (13.9%). Considering only the corrugated traps

located outside, the most abundant species captured was still *Cryptolestes* spp. (32.6%), followed by *T. stercorea* (20.4%) and *O. surinamensis* (19.4%). Considering just the inside traps, *Cryptolestes* spp. (27.2%) was again the most abundant species followed by *Sitophilus* sp. (22.4%).

In general, all three sites shared the same species or species groups, but the relative frequency was different among sites. At site A, the predominant species captured in corrugated and Lindgren traps was *T. stercorea* (42.5% and 49.1%, respectively), followed by *Cryptophagus* sp. (20.2% and 13.1%, respectively). The third most abundant species was *A. advena* (14.9%), in the corrugated traps, and *Cryptolestes* spp. (12.7%) in the Lindgren traps. At site B, the predominant species captured was *Cryptolestes* spp.: 77.9% of captures in corrugated traps and 47.4% of captures in Lindgren traps. Lindgren traps also captured *A. advena* (15.2%) and *Litargus balteatus* (12.3%). At site C, the predominant species captured in corrugated traps was *O. surinamensis* (88.9%) and in Lindgren traps was *A. advena* (48.0%), followed by *T. stercorea* (16.0%) and *Cryptolestes* spp. (13.3%). Considering captures in both types of traps, only *Palorus subdepressus* (Wollaston) and *Tenebrio* sp. were never caught in one of the sites (site C). All the other insects were either captured in the corrugated or Lindgren traps at each site. The Simpson's index of species diversity in captures was low for all three facilities and was similar across years and trap types (Figure 3.1). Similar to total number captured in traps, the species diversity outside was not significantly correlated with species diversity inside ($r = 0.02$; $P = 0.94$).

The stored-product pest *T. castaneum* was captured at the three facilities and in both types of traps (total of 220 individuals were captured). Most *T. castaneum* individuals were captured inside structures with corrugated traps (80.5%). Corrugated traps located outside captured 12.7% and Lindgren traps captured 6.8% of the individuals. Considering all species, captures for *T. castaneum* corresponded to 14.1% of the total captures inside the structures, 2.2% of the captures outside with corrugated traps, and 1.2% of the captures with Lindgren traps. The number captured in each facility was relatively low compared to the most predominant species. At site A, the capture of *T. castaneum* corresponded to 3.6% and 1.0% of the total captured outside on the corrugated and Lindgren traps, respectively. At site B, the capture of *T. castaneum* corresponded to 1.7% and 0.6% of the total number captured outside in the

corrugated and Lindgren traps, respectively. At site C, the capture of *T. castaneum* corresponded to 2.0% and 4.0% of the total captured outside in the corrugated and Lindgren traps, respectively.

Spatial distribution of stored-product pests

Location of traps had a dispersed distribution at all three locations: site A (nearest neighbor ratio = 1.48, Z score = 6.42, $P < 0.001$), site B (nearest neighbor ratio = 1.61, Z score = 7.42, $P < 0.001$), and site C (nearest neighbor ratio = 1.74, Z score = 6.92, $P < 0.001$). The accuracy of the IDW interpolation method was evaluated using the cross validation tables in which Geostatistical Wizard tool in ArcGIS uses the semivariogram to predict a value at each measured location as if that measured location was not part of the dataset. By doing so, the model produces estimates at each measured location providing an idea of the model's accuracy. Evaluation of the cross validation report and the prediction errors (Table 3.4) showed that prediction at low capture points tended to be overestimated and predictions at high capture points tended to be underestimated.

In most cases, global Moran's I indicated that the distribution of total stored-product insects captured in corrugated traps did not have positive spatial autocorrelation (i.e., stored-product insect captures were not clustered in distribution). Moran's I values ranged from -0.25 to 0.22, and only the last period of monitoring at site A had a significant positive autocorrelation (Table 3.4). Although Moran's I values did not support a positive autocorrelation of stored-product pests captures, Anselin Local Moran's I indicated that some trap locations with high values had traps in the proximity with similar high values. This pattern in the distribution can also be seen in the contour maps which indicate that captures do not appear evenly distributed across the landscape (Figures 3.2-3.4).

At site A, stored-product insect captures tended to be higher in the area around the two elevators (outside or in tunnel under elevator) and in the open space between these two structures (Figure 3.2). In the first monitoring period of 2008, one focus of capture was identified near the bunker storage location where corn was being temporarily stored. In this case, *Sitophilus* sp. represented 71% of the total captures. At site B the focus of insect captures was centered at the feed mill, specifically in the area designated for receiving grain. High levels of spillage were observed to accumulate in this area. However, in the second monitoring period of 2007, a high infestation of *Cryptolestes* spp. was detected in the traps near one of the outside bins. In this

monitoring period, *Cryptolestes* spp. captures represented 89% of the total capture. The second period of 2008 was unusual in that it had the most atypical composition of species, with none of the major species commonly captured represented. At site C, two major foci of insect capture could be identified; one located inside the flour mill and one located near one of the bulk grain storage bins (Figure 3.4). Inside the mill, two locations accounted for most of the captures and *O. surinamensis* was the major species captured. The trend of captures was similar across monitoring periods, although *Cryptolestes* spp. captures increased in the last monitoring period of 2007.

Environmental/physical factors and trap captures

Most of the environmental and physical factors measured at trap locations were not significantly different between locations with and without captures ($P > 0.05$). Significant differences were found in terms of number of individuals captured in presence or absence of vertical surfaces associated with edges of structures ($\chi^2 = 20.6$, df = 1, $P < 0.0001$). For traps that captured stored-product insects, 77.1% were in locations that had vertical surfaces within the 1 m^2 area surround the trap, compared to 22.9% of the traps without captures.

Significant difference was found between trap capture and presence or absence of shade ($\chi^2 = 14.5$, df = 1, $P < 0.001$). Trap locations with stored-product insect captures had equal likelihood of having shade (49%) or no shade (51%), but locations without insect captures were more likely to not have shade (69%) than to have shade (31%). Presence of shade at these locations was typically associated with presence of structures, but there was not a significant interaction between presence of vertical surfaces in the proximity of the trap and presence of shade ($\chi^2 = 0.07$, df = 1, $P = 0.80$). This suggests the impact of structures on recovery of insects in traps even when traps were far enough away from the structure that they were not included in the vertical edge groupings.

Spillage accumulation outside the structures of the facilities was observed in all three sites. Twenty four percent of the trap locations outside had some level of spillage accumulation. Considering all species, trap locations with spillage captured on average 1.58±0.20 individuals/trap and traps without captured 2.50±0.88 individuals/trap ($\chi^2 = 0.12$, df = 1, $P = 0.38$). Considering only the fungus feeders, trap locations with spillage captured on average

0.60±0.08 individuals/trap and traps without captured 0.78±0.28 individuals/trap (χ^2 = 0.09, df = 1, *P* = 0.60).

Stepwise regression was performed on the data from traps located outside the facilities. When the number of captured stored-product insects was regressed against the environmental and physical variables measured at the three locations; three variables were included in the model (F = 3.09; df = 3; *P* = 0.03). The variables entered and the model R^2 were: distance to closest storage structure (R^2 = 0.007, *P* = 0.076), distance to mill (R^2 = 0.013, *P* = 0.078), and wind speed (R^2 = 0.020, *P* = 0.087). Considering some predominant species individually using stepwise regression, some differences in the significant variables were found. For *O. surinamensis*, four variables were included in the model (F = 8.31; df = 4; *P* < 0.0001): temperature (R^2 = 0.028, *P* = 0.0003), distance to closest storage structure (R^2 = 0.045, *P* = 0.004), distance to mill (R^2 = 0.062, *P* = 0.004) and rh (R^2 = 0.067, *P* = 0.13). For *Cryptolestes* spp., only one variable was included in the model: rh (F = 2.41; df = 1; *P* = 0.12; R^2 = 0.005). For *T. stercorea*, two variables were included in the model (F = 3.78; df = 2; *P* = 0.02): temperature (R^2 = 0.008, *P* = 0.005) and grass area (R^2 = 0.016, *P* = 0.06).

Discussion

The landscape of food processing and storage facility site is a complex arrangement of structures and open areas in which the occurrence of favorable conditions may allow the development and reproduction of stored-product insect species. The results of this research support the idea that there is a potential pool of insects outside of food processing facilities, such as mills, that could move into these structures and contribute to infestation, with analysis of distribution suggesting that sources may be both other locations on site, such as bulk storage, and locations offsite since many species were captured both walking on ground and in flight. Although captures inside tended to be higher in most periods, there were some periods in which captures outside were greater than or similar to those inside the facilities. In addition, the species composition found both inside and outside the food facilities was similar. These results suggest that the dynamics inside and outside could potentially be linked by the process of dispersal.

Fungus feeders at the three sites were captured primarily outside the facilities: only *T. stercorea* had captures inside (5% of the total) as well as outside, while *L. balteatus* and *Cryptophagus* sp. were only captured outside. These species can be captured inside facilities

(Doud, 1999, Campbell and Arbogast 2004, Toews et al. 2006), with all three species having been recovered in inside traps at Site A (J. F. Campbell, unpublished data). The species of stored-product pests captured outside the three processing and storage facilities represent a variety of feeding habits. For example, internal feeders such as *Sitophilus* sp., external feeders such as *T. castaneum, O. surinamensis* and *Cryptolestes* spp., and fungal feeders such as *T. stercorea, L. balteatus* and *Cryptophagus* sp., were found. Large numbers of the internal feeders *Sitophilus* sp. were found near bulk storage areas both inside (i.e., elevators) and outside (ground storage). Arthur et al. (2006) also reported *Sitophilus* spp. and *Cryptolestes* spp., as being commonly recovered in spillage residue inside grain elevators. The fungus feeding group was more likely to be outside and this is probably due to their ability to exploit degrading food spillage. Species that are primarily secondary feeders can be associated with whole grain, broken kernels and grain dust as well as processed grain-based products (Sinha and Watters 1985, Arbogast 1991), and in this study were more variable in terms of locations where they were found.

All sites had foci of captures (i.e., locations with highest captures in the same monitoring period) located inside structures at a facility, but all also had some foci located outside as well. However, the species composition of these foci was not always the same between inside and outside. Only for site A, species that have been shown in previous studies using pheromone baited flight traps to have a high correlation with inside and outside captures at food processing and storage facilities were frequently captured in traps used in this study. For example, Doud and Phillips (2000) found similarity in the dynamics of *P. interpunctella* inside and outside flour mills and the number captured tended to reduce as the distance to the flour mills increased. Campbell and Mullen (2004) found that *T. variabile* captures outside a food plant correlated with captures in a warehouse thus indicating potential movement between these locations. Campbell and Arbogast (2004) in another study at site A, found significant correlation between captures inside and outside for *P. interpunctella* and *T. variabile* and mark-recapture data indicated that *P. interpunctella* was capable of entering the building from outside. The lack of similarity in the species composition inside and outside for some of the sites probably resulted from the fact that only corrugated traps were placed inside and therefore species that tend to fly more than walk (e.g. moths) were less likely to be captured inside, but could eventually be captured outside in the Lindgren traps. The potential of outside populations to influence inside populations has

implications for the effectiveness of different management and monitoring tools (Campbell and Mullen 2004).

In this study, the spatial distribution of stored-product pests captures was not significantly explained by many of the measured environmental and physical factors. In the case where regressions were significant, low R^2 values indicated poor explanatory value to the relationship between the factors and trap captures. It is reasonable to assume that the spatial distribution of species will follow the distribution of resources that are favourable to their development and reproduction. As the resources are reduced in some locations, the individuals should change their distribution by emigration and immigration within the limits of the population distribution (MacLeod and Donnelly 1963, Taylor 1981). The sources of insects outside could be the movement from infestation in other structures (e.g., elevators) or outside spillage. The results presented here more strongly support the idea of movement from infestations in other structures since the outside foci based on the contour maps were located near different structures. Also, the analysis of outside traps found that presence of vertical surfaces and shade were associated with higher insect captures, while presence of spillage did not increase captures in traps. Presence of vertical surfaces in most situations was a result of traps being located near the walls of the facilities. Besides indicating that the insects could have originated from inside the structures, more captures near the buildings can also be an indication that buildings by themselves can influence the effectiveness of traps (e.g. beetles tending to follow edges of walls). Finally, the lack of association between captures and spillage also supports the hypothesis that sources of insects captured is primarily other structures.

In two of the three sites it was possible to observe different levels of food material that had accumulated. Although no relationship was found between presence of spillage and abundance of captures or species diversity, more research will be necessary to evaluate how material can accumulate outside and how it can support these different species. Moreover, presence of spillage and spillage quality outside may be very transitory since it will be degraded more rapidly outside and this may create conditions where it is a favorable resource for only a short period of time. Even being a transitory resource, spillage can still be exploited by stored-product insects, but it may be that small spillage accumulations do not produce sufficient numbers of individuals to impact the pattern of captures in traps. Spillage material from outside one of the facilities was collected and immature stages of some of the species (including

Cryptolestes spp, *A. advena*, and *T. variabile*) associated with stored-products and other non stored-product species were found in 7 out of 33 samples located near the flour and feed mills. It is also possible that presence of spillage may interfere with insect response to traps, so lack of correlation with spillage could be due to reduced capture efficiency due to factors such as competing attractants. These findings support the rationale for a more elaborate study that focuses on measuring how well different species of stored-product insects can develop in spillage with varying levels of degradation and how they impact captures in traps and insect movement patterns.

The similarity in species composition between Lindgren and corrugated traps could indicate that ground traps are not necessarily detecting local infestations, but reflecting the level of general outside flight activity detected by the Lindgren traps. This might explain why no strong associations between environmental and landscape factors and captures of walking insects were found, since captures might not be related to local conditions. In this scenario, it is still not clear if stored-product insects captured outside originated from locations at the facility or from other locations off site, since association with structures on site could be because they were attracted to the buildings and other storage structures.

This current study and the study by Campbell and Mullen (2004) are among the few evaluating the spatial distribution of stored-product insects in the proximity of food processing facilities. Contour analysis obtained from traps placed in the different habitat types provided maps of infestation which are easy to interpret and are of potential practical use in that they identify target areas for pest management. Different patterns of distribution are likely to occur in other landscapes and therefore other locations should be investigated to determine how generalizable the pattern is among sites. Other studies should extend the temporal scale of monitoring through the seasons and in this way it may be possible to better detect locations with early signs of outside activity which will give a better idea of possible primary foci. Understanding these distributions can help in the implementation and interpretation of monitoring programs that include the outside portion of the facility and also the selection and targeting of pest management tactics for more effectives IPM programs.

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Figures and Tables

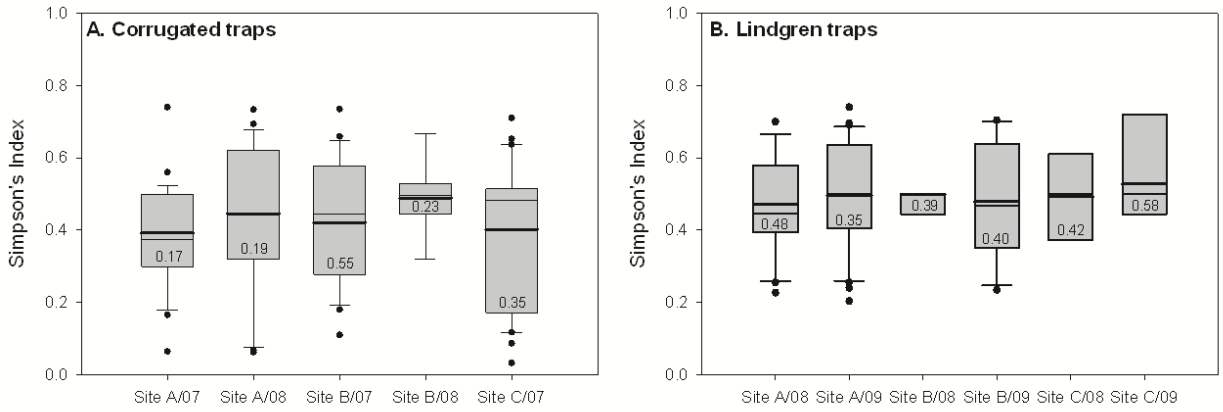

Figure 3.1 Simpson's index of species diversity for stored-product insects captured at each of three sites (A, B, and C) in 2007 (07), 2008 (08), and 2009 (09) as a function of trap type (corrugated trap and Lindgren trap). Grey boxes represent 50% of the data, bars represent 95% of the data, and individual points represent individual trap locations outside of the 95% distribution. Traps that had no capture and traps with only one species captured were excluded, and number inside the boxes indicates the proportion of the total trap locations that were included for the estimation of the index. Indices were estimated for each year by grouping data of all monitoring periods in that year.

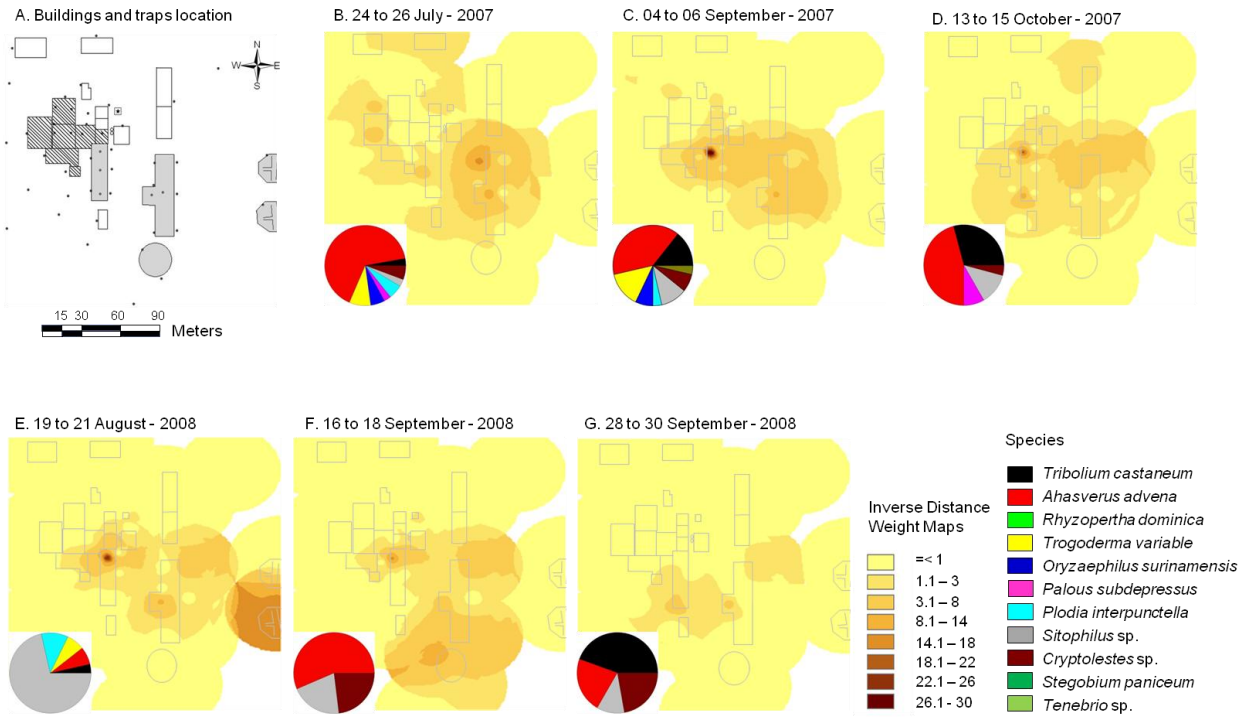

Figure 3.2 Map of the landscape at Site A indicating structures, with buildings in gray represent structures for storage of grain and hatched buildings represent structures for processing and storage of products, and corrugated trap locations (black dots) (A.) and Inverse Distance Weighting contour maps for total number of stored-product insects captured with corrugated traps during each sampling interval (B-G). Pie charts indicate the proportion of each species captured in each monitoring period. Larger individual maps are in Appendices C-I.

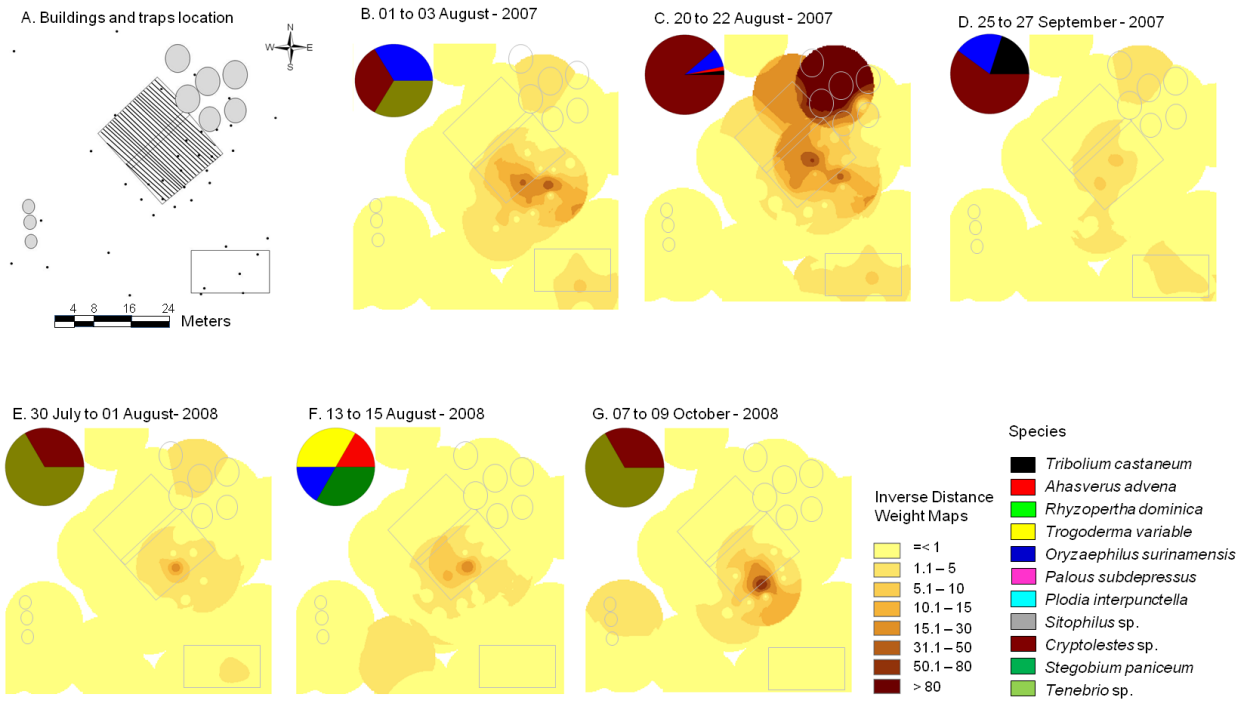

Figure 3.3 Map of the landscape at Site B indicating structures, with buildings in gray represent structures for storage of grain and hatched buildings represent structures for processing and storage of products, and corrugated trap locations (black dots) (A.) and Inverse Distance Weighting contour maps for total number of stored-product insects captured with corrugated traps during each sampling interval (B-G). Pie charts indicate the proportion of each species captured in each monitoring period. Larger individual maps are in Appendices J-P.

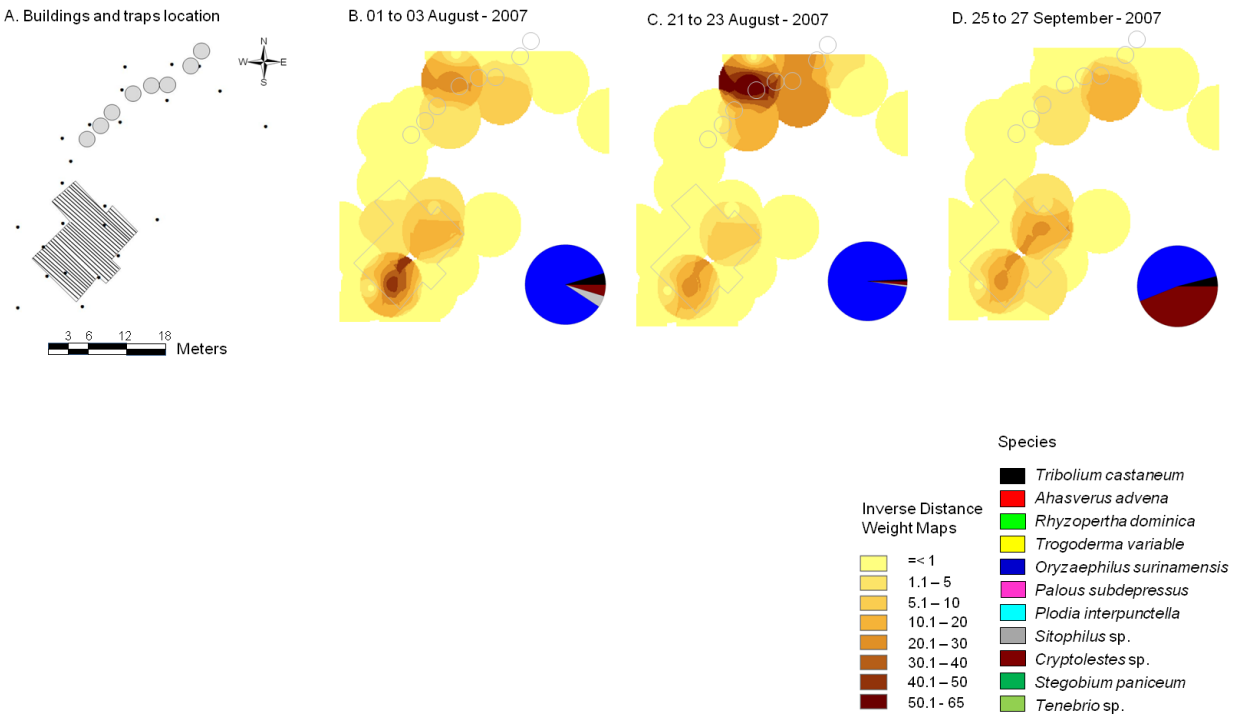

Figure 3.4 Map of the landscape at Site C indicating structures, with buildings in gray represent structures for storage of grain and hatched buildings represent structures for processing and storage of products, and corrugated trap locations (black dots) (A.) and Inverse Distance Weighting contour maps for total number of stored-product insects captured with corrugated traps during each sampling interval (B-G). Pie charts indicate the proportion of each species captured in each monitoring period. Larger individual maps are in AppendicesQ-T.

Table 3.1 Total number of individuals of all stored-product insect species or species groups captured each year (2007 and 2008) with corrugated traps placed inside (I) and outside (O) structures at three different food processing facility sites (A, B, and C). Site C was only monitored in 2007.

Species	Site A				Site B				Site C			
	2007		2008		2007		2008		2007		2008	
	I	O	I	O	I	O	I	O	I	O	I	O
Tribolium castaneum	26	12	2	5	3	7	1	0	145	4	-	-
Ahasverus advena	0	45	1	26	5	6	0	1	1	0	-	-
Rhyzopertha dominica	0	0	0	0	0	0	0	0	8	0	-	-
Trogoderma variable	4	7	1	2	6	0	11	2	0	0	-	-
Oryzaephilus surinamensis	0	4	7	0	32	30	10	1	113	176	-	-
Palorus subdepressus	0	3	3	0	0	0	0	0	0	0	-	-
Plodia interpunctella	8	3	1	3	2	0	0	0	0	0	-	-
Sitophilus sp.	67	7	50	29	97	0	57	0	11	3	-	-
Cryptolestes spp.	18	5	15	11	19	321	3	3	287	15	-	-
Stegobium paniceum	0	0	0	0	36	0	6	2	0	0	-	-
Tenebrio sp.	0	1	0	0	93	1	98	6	0	0	-	-
Typhaea stercorea	2	10	3	192	0	20	5	0	2	0	-	-
Litargus balteatus	0	0	0	14	0	0	0	0	0	0	-	-
Cryptophagus sp.	0	47	0	49	0	0	0	16	0	0	-	-

Table 3.2 Total number of individuals of all stored-product insect species or species groups captured each year (2008 and 2009) with Lindgren traps placed outside at three different food processing facility sites (A, B, and C).

Species	Site A		Site B		Site C	
	2008	2009	2008	2009	2008	2009
Tribolium castaneum	0	11	1	0	1	2
Ahasverus advena	28	95	10	16	18	18
Rhyzopertha dominica	2	1	6	2	1	0
Trogoderma variable	67	31	4	5	2	0
Oryzaephilus surinamensis	0	4	0	0	0	0
Palorus subdepressus	0	0	1	0	0	0
Plodia interpunctella	1	7	0	0	0	2
Sitophilus sp.	15	8	0	0	0	0
Cryptolestes spp.	51	87	15	66	6	4
Stegobium paniceum	0	2	0	2	0	0
Tenebrio sp.	0	0	0	0	0	0
Typhaea stercorea	193	339	7	9	7	5
Litargus balteatus	0	0	0	21	0	6
Cryptophagus sp.	28	114	0	6	0	3

Table 3.3 Mean and standard error of the mean of the total number of stored-product insect species captured per day in two different trap types (Corrugated and Lindgren) within each monitoring period at three food processing facility sites (A, B, and C). Corrugated traps were not placed at site C during 2008.

Location ID	Trap type	Monitoring period	Start date	End Date	Inside Mean ± SE (n)	Outside Mean ± SE (n)
Site A	Corrugated	1	07/24/07	07/26/07	1.68 ± 0.54 (11)	0.47 ± 0.22 (38)
		2	09/04/07	09/06/07	2.45 ± 1.38 (11)	0.41 ± 0.14 (39)
		3	10/09/07	10/11/07	1.55 ± 0.91 (11)	0.97 ± 0.21 (39)
		4	08/06/08	08/08/08	2.00 ± 1.14 (11)	1.01 ± 0.44 (39)
		5	09/14/08	09/16/08	1.05 ± 0.68 (11)	2.38 ± 1.21 (39)
		6	09/30/08	10/02/08	0.73 ± 0.42 (11)	0.85 ± 0.40 (39)
	Lindgren	1	08/06/08	08/19/08		0.08 ± 0.04 (10)
		2	08/19/08	09/04/08		1.21 ± 0.30 (10)
		3	09/04/08	09/14/08		0.64 ± 0.20 (10)
		4	09/14/08	09/30/08		0.39 ± 0.28 (10)
		5	09/30/08	10/09/08		0.62 ± 0.30 (10)
		6	08/13/09	08/20/09		2.84 ± 0.99 (10)
		7	08/20/09	09/03/09		1.69 ± 0.60 (10)
		8	09/03/09	09/17/09		0.74 ± 0.37 (10)
		9	09/17/09	10/01/09		1.10 ± 0.23 (10)
		10	10/01/09	10/15/09		0.01 ± 0.01 (10)
		11	10/15/09	10/29/09		0.04 ± 0.02 (10)
Site B	Corrugated	1	08/01/07	08/03/07	7.28 ± 2.58 (9)	0.21 ± 0.15 (31)
		2	08/20/07	08/22/07	7.67 ± 3.03 (9)	5.79 ± 4.78 (31)
		3	09/25/07	09/27/07	1.33 ± 0.54 (9)	0.21 ± 0.13 (31)
		4	07/30/08	08/01/08	1.94 ± 1.06 (9)	0.10 ± 0.07 (31)
		5	08/13/08	08/15/08	2.56 ± 1.36 (9)	0.10 ± 0.05 (31)
		6	10/07/08	10/09/08	6.11 ± 4.28 (9)	0.31 ± 0.12 (31)

Table 3.3 Continued

Location ID	Trap type	Monitoring period	Start date	End Date	Inside Mean ± SE (n)	Outside Mean ± SE (n)
Site B	Lindgren	1	07/25/08	08/11/08		0.25 ± 0.17 (6)
		2	08/11/08	09/02/08		0.06 ± 0.02 (6)
		3	09/02/08	09/23/08		0.08 ± 0.04 (6)
		4	08/04/09	08/17/09		0.68 ± 0.29 (6)
		5	08/17/09	09/03/09		0.25 ± 0.10 (6)
		6	09/03/09	09/18/09		0.26 ± 0.16 (6)
		7	09/18/09	10/09/09		0.17 ± 0.10 (6)
		8	10/09/09	10/26/09		0.03 ± 0.02 (6)
Site C	Corrugated[b]	1	08/01/07	08/03/07	15.25 ±9.72 (8)	1.26 ± 0.74 (17)
		2	08/21/07	08/23/07	8.69 ± 1.86 (8)	2.95 ± 1.54 (22)
		3	09/25/07	09/27/07	11.50 ± 2.45 (8)	0.57 ± 0.30 (22)
	Lindgren	1	07/28/08	08/11/08		0.14 ± 0.04 (3)
		2	08/11/08	08/25/08		0.31 ± 0.09 (3)
		3	08/25/08	09/18/08		0.13 ± 0.13 (3)
		4	09/18/08	09/30/08		0.19 ± 0.19 (3)
		5	08/04/09	08/21/09		0.18 ± 0.14 (4)
		6	08/21/09	09/10/09		0.18 ± 0.09 (4)
		7	09/10/09	10/01/09		0.17 ± 0.04 (4)

Table 3.4 Mean and standard deviation of prediction error in total stored-product insect captures from IDW interpolation for each trap location in the landscapes of three food processing facility sites (A, B, and C) and the Global Moran's I spatial autocorrelation measures for the distribution of insect captures with associated Z scores and *P* values, with significant nonrandom distributions indicated with an asterisk (*).

Trapping Periods	Prediction Error (Standard Deviation)	Moran's I	Z score	*P*-value
Site A				
24 to 26 July, 2007	0.33 (3.15)	0.07	1.10	0.27
04 to 06 September, 2007	0.64 (5.16)	0.03	0.72	0.47
09 to 11 October, 2007	0.39 (3.66)	-0.11	-1.29	0.20
06 to 08 August, 2008	0.25 (4.65)	0.04	0.84	0.40
14 to 16 September, 2008	0.27 (2.69)	0.10	1.44	0.15
30 September to 02 October, 2008	0.16 (1.34)	0.22	2.97	0.003*
Site B				
01 to 03 August, 2007	1.33 (9.95)	0.07	1.03	0.30
20 to 22 August, 2007	-1.51 (48.37)	0.01	0.61	0.54
25 to 27 September, 2007	0.16 (1.97)	-0.01	0.22	0.83
30 July to 01 August, 2008	0.30 (3.48)	0.03	0.8	0.42
13 to 15 August, 2008	0.31 (4.29)	0.004	0.40	0.69
07 to 09 October, 2008	0.91 (13.61)	-0.02	0.12	0.91
Site C				
01 to 03 August 2007	1.69 (12.53)	-0.25	-1.63	0.10
21 to 23 August 2007	1.63 (15.90)	0.001	0.33	0.74
25 to 27 September, 2007	1.00 (8.06)	-0.23	-1.32	0.19

Chapter 4 - Temporal analysis of genetic diversity changes and bottlenecks in a population of the stored-product pest *Tribolium castaneum*

Abstract

Temporal analysis of genetic stability is an important approach that has been often neglected in population genetic studies. Lack of stability in genetic composition can limit the analysis of genetic structure and the interpretation of these analyses will require a cautious approach, but on the other hand, it can provide insights about processes involved in population dynamics. *Tribolium castaneum* is a worldwide pest in food storage and processing facilities where populations exhibit long-term persistence with periodic reductions in abundance (i.e., potential genetic bottlenecks) due to structural fumigations. Here, eight insertion-deletion polymorphisms, including six microsatellites, were used to investigate temporal changes in genetic diversity and the effect of structural fumigations in five sampling periods over a period of eight years. There was an erosion of genetic diversity over the sampling period. Significant differences were observed in allele frequencies among sampling periods and the relative differences were lower compared to results from spatially distinct populations using the same set of molecular markers. Bottleneck analysis showed a decline in population size in four out of five sampling periods. These results show that characteristics that make *T. castaneum* self-contained inside anthropogenic environments such as food processing facilities and the genetic drift caused by interventions such as fumigations determine temporal genetic changes of populations.

Introduction

Genetic structure studies typically rely on measurements of allele frequency heterogeneity from spatially distinct populations for inferring relationships (Holsinger and Weir 2009). These studies assume that the observed genetic pattern of each population is stable over time and samples are typically collected at one time period. However, the genetic composition of populations even at ecological time-scales may not be stable since they can be subject to processes that can generate changes in allele frequencies (Slatkin 1985, Jorde and Ryman 1995, Skaala et al. 2006, Carroll et al. 2007). This variation can make more difficult the elucidation of past processes, current relationships, and future patterns from molecular data. It is required that a state of equilibrium has developed between genetic drift and gene flow in the population of interest otherwise the estimates of population differentiation will be single "snapshots" (Hoffman et al. 2004). Studies of temporal change in genetic diversity have been scarce in the literature, but this topic has recently gained more attention by conservation biology researchers (e.g. Cristescu et al. 2010). Temporal analysis of genetic diversity is also potentially important in understanding pest populations and the impact of pest management programs, which has the converse goals of conservation biology.

Tribolium castaneum is an important pest of food processing and storage facilities, and is distributed throughout the world. Conditions inside many of these facilities allow *T. castaneum* populations to survive and grow all year long under relatively constant conditions. Major fluctuations in abundance of this species inside structures is driven by management tactics such as structural fumigation with insecticides which can reduce estimated population size by an average of 85%, and sanitation programs that eliminate patches of resource as well as local populations of the beetle (Fields and White 2002, Fields 2006, Campbell et al. 2010a). *T. castaneum* populations are thought to be relatively self-contained within individual mills, and therefore population rebound after treatment is likely due to survival of individuals and, to a lesser extent, the immigration of new individuals (Campbell and Arbogast 2004, Toews et al. 2006, Campbell et al. 2010a,b). Both processes may have significant impacts on the genetic structure of the *T. castaneum* population within the mill.

Advances in molecular methodologies provide good opportunities for evaluating short and long-term genetic changes in populations due to management, and they can complement

current methods used to evaluate trends in population dynamics and effectiveness of management strategies. Spatial genetic structure for populations of *T. castaneum* has been shown both worldwide and within the US, but a significant relationship between geographic and genetic distance has not been identified and most populations have limited allele variation (genetic diversity) (Drury et al. 2009, Chapter 2). One potential reason behind this limited genetic diversity might be the bottlenecks frequently experienced by populations in food processing facilities such as flour mills. Fumigation treatments cause significant reductions in population size inside mills and consequently could increase genetic drift. Population genetic theory predicts that, as consequence of genetic drift, small populations should show lower levels of genetic variability than larger populations (Hartl and Clark 1997). Alternatively, if the population at a location extends over a larger area than an individual mill and recovery in abundance results from movement of individuals from untreated areas then might not see a change in genetic variability. Finally, if populations after a fumigation result from immigration of individuals from some other location than we might predict that genetic variation would be unchanged but that there would be higher levels of genetic differentiation between fumigation events.

In this study, results from a long-term monitoring project and the use of molecular markers were combined to better understand the processes involved in fluctuations of *T. castaneum* inside a commercial flour mill, and to test the hypothesis of erosion of genetic diversity caused by fumigations. To better understand the population genetic characteristics of *T. castaneum*, it is important to evaluate genetic diversity of temporal samples from the same location that are separated from each other by not only time but also by potential bottlenecks such as structural fumigations. This evaluation can also provide insights into the efficacy of management strategies and factors involved in the rebound of populations following treatment. In this context, the genotypes of individuals based on eight insertion-deletion polymorphisms markers from five inter-fumigation sampling periods were compared, spanning a period of 8 years.

Materials and methods

Samples collection

Tribolium castaneum samples were collected from a wheat flour mill in the midwest USA. A long-term monitoring program has been conducted in the mill (Mill #1) and detailed information on the facility and the dynamics of trap capture are provided in Campbell et al. (2010a,b). Figure 4.1 shows the mean trap capture variation, fumigation dates, and when samples for genetic analysis were collected: November 2002 (M1NOV02), March 2003 (M1MAR03), August 2003 (M1AUG03), February 2005 (M1FEB05), and June 2009 (M1JUN09). Sample M1JUN09 was collected using Storgard Dome traps (Trécé, Adair, OK, USA) baited with *Tribolium* spp. pheromone lure and 15 drops of food oil in a filter paper in the pitfall container. All the other samples were collected using the same type of trap and pheromone lure, but instead of food oil traps were baited with a mix of flour and cracked wheat. At each time point, samples consisting of between 36 and 39 individual beetles were derived from the total number of beetles captured over a two-week monitoring period. Sample dates were chosen to represent a range in the number of days (130 to 2394 days) and number of fumigations (1, 3, or 5 fumigations) between sample collections. After collection, beetles were placed in 1.5 mL centrifuge tubes with 75% ethanol, and frozen at -80°C until the DNA could be extracted.

DNA extraction and PCR

Genomic DNA was extracted from a total of 190 *T. castaneum* using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA). PCRs were conducted in a final volume of 12 µl containing 0.5 µl genomic DNA, 0.12 µl (5U/µl) Taq DNA polymerase (Promega, Madison, WI, USA), 0.12 µl dNTP (25 mM), 1.68 µl $MgCl_2$ (25 mM), 2.4 µl 5x PCR buffer, 0.12 µl forward tailed primer, 0.24 µl reverse primer and 0.12 µl M13 labelled primer (primers from IDT, Coralville, IA, USA). The cycling program included 95°C, 5 min for initial denaturation; 95°C, 45 sec; 58°C, 1 min; 72°C, 1 min for 15 cycles; 95°C, 45 sec; 50°C, 2 min; 72°C, 1 min for 24 cycles; and a final extension at 72°C for 5 min.

Genotyping

Genotypes were determined based on eight unique polymorphic loci including six microsatellites (MS1 through MS6) and two other insertion-deletion polymorphisms (ID1 and ID2) (Table 4.1). These molecular markers were either a result of screening of the *T. castaneum* genome or based on the literature (Demuth et al. 2007). Markers were confirmed to be unique by similarity searches to the *T. castaneum* genome (http://www.beetlebase.org) using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1997).

For high-throughput genotyping, fluorescent-labelled PCR fragments were produced using a M13 oligonucleotide adaptor sequence attached to the 5' end of the forward primers that allowed the incorporation of fluorescent dyes into the amplicons (Schuelke 2000). PCR products were analyzed using an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA) at the Sequencing and Genotyping Facility (Plant Pathology Department, Kansas State University) and allele sizes scored using GeneMarker version 1.85 software (SoftGenetics, State College, PA, USA).

Data analysis

For each temporal sample, the number of alleles per locus (A), allelic richness (R) and allele frequencies were calculated using FSTAT v. 3.9.5 (Goudet 1995). The expected and observed heterozygosities were calculated using GDA v. 1.1 (Lewis and Zaykin 2001). Tests for deviation from Hardy-Weinberg equilibrium at each locus were performed using GENEPOP v. 4.0 (Raymond and Rousset 1995). For multiple comparisons, sequential Bonferroni procedure was applied to determine the significance level (Rice 1989). Pairwise Wright's fixation indices, F_{ST}, following Weir and Cockerham (1984) were first calculated using FSTAT, and the same package was used to evaluate the significance of pairwise F_{ST} values by randomly permuting multilocus genotypes among samples (10,000 permutations) and not assuming Hardy-Weinberg equilibrium. For the latter, the pairwise significance was given after standard Bonferroni corrections. Because all loci had different frequencies of null alleles, pairwise F_{ST}s were corrected by applying the ENA correction in the Freena package (Chapuis and Estoup 2007). The significant differences in number of alleles per locus, allelic richness and expected heterozygosity among samples were tested using a nonparametric Wilcoxon signed-ranks test. Correlations between genetic distance [$F_{ST}/(1-F_{ST})$] and temporal distance (days) among

pairwise comparisons of sampling periods were performed by Mantel test (Mantel 1967) using the program Isolde as implemented in GENEPOP.

Based on the long- term monitoring data, drastic reductions caused by the implementation of fumigations in the facility were evident. Therefore, the occurrence of bottlenecks in the population were expected. Bottlenecks were inferred for each sampling period under the assumption of mutation-drift equilibrium by infinite allele (IAM) and two-phase (TPM) models using the program BOTTLENECK v. 1.2.02 (Cornuet and Luikart 1996). The program measures the temporary excess of heterozygosity that results from a reduced population size (Cornuet and Luikart 1996, Luikart et al. 1998a,b). Significance ($\alpha = 0.05$) of observed heterozygosity excess or heterozygosity deficiency relative to that expected at mutation-drift equilibrium was tested using the Wilcoxon sign-rank test (Luikart et al. 1998a, Luikart and Cornuet 1998). The IAM and TPM were used with 1000 iterations each. For the two-phase model, generalized stepwise mutation was assumed, in which a proportion of the stepwise mutation model was set to 0 with a variance in mutation lengths of 0.36 (Estoup et al. 2001). Also, a qualitative descriptor of the allele frequency distribution (mode-shift indicator) was used which can discriminate bottlenecked populations from stable populations (Luikart and Cornuet 1998).

To further evaluate the effects of fumigations, the relationship of variation in genetic diversity and level of genetic differentiation between sampling periods with number of fumigations, fumigation efficacy, and time for average capture in traps to rebound to the threshold of 2.5 beetles/trap/monitoring period were compared (see Campbell et al. 2010b for definition of the threshold).

Results

Genetic diversity among sampling periods

Genetic diversity varied among sampling periods, and tended to be low to moderate in most cases. A total of 63 alleles across eight loci were observed from the five sampling periods. Seven of the eight loci were polymorphic in all sampling periods (Table 4.2, Figure 4.2). Locus ID1 was monomorphic for M1MAR03, but had at least four distinct alleles in the other sampling periods. The maximum number of alleles (18 alleles) for a given sampling period was observed

in the locus MS6 for M1NOV02. Allele richness ranged from 1.00 in M1MAR03 to 14.87 in M1NOV02 (Table 4.2). There were two private alleles found in the sample M1NOV02, one private allele found in the sample M1MAR03, five private alleles found in the sample M1AUG03, and three private alleles found in the sample M1JUN09. Observed and expected heterozygosities of each sample ranged from 0.000 to 0.816 (mean 0.380) and from 0.000 to 0.916 (mean 0.619), respectively (Table 4.2). Exact tests for deviations from HWE across all loci showed that 22 out of 40 (55%) significantly deviated after Bonferroni corrections (Table 4.2). They were scattered in six out of eight loci of all five sampling periods without any detectable pattern. Null allele frequencies using the EM algorithim as implemented in Freena were detected with a mean value of 0.146.

There were no overall significant differences in allelic richness ($\chi^2 = 1.15$, df = 4, $P = 0.89$), expected heterozygosity ($\chi^2 = 1.28$, df = 4, $P = 0.86$), and number of alleles ($\chi^2 = 1.11$, df = 4, $P = 0.89$) among sampling periods (Figure 4.3A,B). Significant differences in expected heterozygosities, however, were found between M1FEB05 and M1JUN09 (Signed Rank test, S = -15, $P = 0.039$). Different levels of fumigation efficacy were found for the fumigations performed between sampling periods. The levels of efficacy were: 97% M1NOV02-M1MAR03 (one fumigation); 84% M1MAR03-M1AUG03 (one fumigation); 96, 98, and 95% M1AUG03-M1FEB05 (three fumigations); 96, 88, 100, 95, and 77% M1FEB05-M1JUN09 (five fumigations). Although not significant, a tendency for mean number of alleles, observed and expected heterozygosities to increase was observed between M1MAR03 and M1AUG03 (Figure 4.3A and 4.3B). The fumigation efficacy between these two periods was estimated as being only 84% (Figure 4.3C) and the time necessary to rebound to the threshold was lower than all other sampling intervals (Figure 4.3D). Therefore, it is likely that a large gene pool was present for this period and contributed to the apparent in crease in the genetic diversity.

Genetic differentiation among sampling periods

Overall genetic differentiation among samples was significant ($F_{ST} = 0.052$, $P < 0.001$). Pairwise F_{ST} comparisons were significant in all ten possible tests with F_{ST} values ranging from 0.014 to 0.096 (Table 4.3). A temporal Mantel test did not reveal a significant positive correlation between genetic distance [$F_{ST}/(1-F_{ST})$] and time between sampling periods ($R^2 = 0.122$, $P = 0.323$) (Figure 4.4). Although not significant, the data suggest a trend of increase in

genetic distance with increase in time between sampling periods. The number of fumigations between sampling periods (Figure 4.1) did not appear to affect the level of differentiation between temporal samples. For example, between M1MAR03 and M1AUG03 there was one fumigation performed with F_{ST} of 0.046, but between M1AUG03 and M1FEB05 there were three fumigations performed with F_{ST} of 0.026.

Genetic bottlenecks

Evidence of a recent population decline in *T. castaneum* was detected in 4 out of 5 sampling periods in the facility. Since the results using IAM and TPM were similar, only TPM results are presented. Wilcoxon sign-rank tests detected a significant excess of observed heterozygosity in population M1NOV02 ($P = 0.006$), M1MAR03 ($P = 0.004$), M1AUG03 ($P = 0.020$), and M1JUN09 ($P = 0.027$), but not for M1FEB05 ($P>0.05$). In general time points with significant bottlenecks occurred earlier in the monitoring period when populations were larger and fumigations more frequent. The exception is the M1JUN09 which also indicated bottlenecks but also occurred after many fumigations and when populations level had been low for several years (Fig. 1). It is unclear why the M1FEB05 population did not have a signficant bottle neck in the analysis. However, the allele frequency distribution test (mode-shift indicator) showed that no shift in the distribution could be detected for any of the samples, which remained in a normal L-shape characteristic of populations in mutation-drift equilibrium.

Discussion

Significant differences, using Wilcoxon sign-rank test as implemented in BOTTLENECK v. 1.2.02, were observed among the five inter-fumigation sampling periods showing that there were changes in the genetic composition of the population resulting in a lack of temporal stability. *Tribolium castaneum* adults can live up to three years (Walter 1990), and a single population may have several overlapping generations with adult generations consisting of various cohorts. The offspring of different adult generations may interbreed randomly, and therefore there is low chance of great genetic variation among consecutive generations (time periods). For temporal variation to occur, it is necessary that some strong force act to produce temporal variability, such as the predicted impact from bottlenecks due to structural fumigations. When populations have their size reduced, genetic drift proportionally increases causing changes

in allele frequency (Waples and Teel 1990, Waples 1990a,b). The overall level of temporal genetic differentiation among sampling periods (F_{ST} = 0.052) was lower than the overall F_{ST} values previously found in studies of spatial genetic differentiation of populations of *T. castaneum* (Drury et al. 2009, Chapter 2). This is consistent with other species, where typically processes resulting in genetic differentiation between populations separated in space have stronger effects than processes producing genetic differentiation within a population through time (e.g., Tessier and Bernatchez 1999, Arnaud and Laval 2004, Hoffman et al. 2004).

In populations with temporal stability of genetic diversity, a consistent pattern of frequency of allele distribution and genetic distances should be observed. This stability can be achieved in different ways. For example, gene flow can increase temporal genetic stability in populations if in equilibrium with genetic drift. Also random mating is expected to occur in most natural populations and should maintain temporal genetic stability. For *T. castaneum* populations, reduced inflow of individuals from external sources that could provide gene flow may affect the genetic stability of a population and also the patchy distribution inside food processing facilities. Even though there are not studies providing exact levels of inflow of individuals from outside sources, monitoring studies in flour mills suggest that *T. castaneum* populations are often relatively self contained within individual flour mills (Campbell and Arbogast 2004, Toews et al. 2006, Campbell et al. 2010 a,b). Therefore, this suggests that population persistence and rebound is mainly driven by individuals surviving the bottleneck events and consequent gene flow could not counteract the decline in genetic diversity. At the study study site, a mill has been present at this location for more than 100 years (J.F. Campbell, personal communication), but there has been considerable change to the facility over this time, so the total length time that a population could have persisted at this location is unknown as is the number of bottlenecks and colonization events that might have occurred during this time. During the time period genetic structure was analyzed, changes in the frequency of alleles through time was observed (see Figure 4.2, and 4.3). These changes were more evident for some loci than others which suggest that the effect on each locus was different. The lack of significant positive correlation between genetic distance [$F_{ST}/(1-F_{ST})$] and temporal distance (days) suggest that samples more distant from each other (temporal distance) have not always the highest levels of differentiation. This result may indicate that despite some samples having been collected in shorter intervals, the changes in the genetic composition due to bottlenecks were stronger.

However, the trend seen in the relationship of genetic distance and temporal distance (Figure 4.4) suggests a potential positive relationship. Therefore, it is possible that the inclusion of other samples with longer time distances would provide significant positive correlation providing evidence that differentiation will increase with the increase in time distance.

The analysis using BOTTLENECK detected bottlenecks in 4 out of 5 sampling periods using the Wilcoxon sign-rank test, which is in accordance to predictions for this site. Nonetheless, the mode-shift test was unable to provide the same result and showed no shift in allele frequencies. The latter, which is a qualitative method, is not a proper statistical test in that the type I error rate varies with sample size. Cristescu et al. (2010) testing the efficiency of the same software to detect bottlenecks in two koala populations having a known history of being through bottlenecks, found results relatively similar to the results in this research. The mode-shift test falsely indicated that the two populations were at mutation-drift equilibrium. They argue that the power of the test and consequent lack of mode-shift distortion can result from different reasons such as severity of the bottlenecks, the number of individuals genotyped, number of neutral loci used, etc. Thus, some limitations are present, and conclusions should not be drawn only with these results about changes in genetic diversity. However, as other results also indicated the occurrence of bottlenecks, it is likely that the results with Wilcoxon rank-rank test are supported.

There are still limitations in correlating the number of beetles that are captured in traps through monitoring programs and the population size in the facility. The individuals genotyped for each sampling period should be representative of the current population which is, however, difficult to assess even if samples were evenly collected throughout the entire facility. This limitation should be taken in consideration because it can lead to false conclusions if sampling is not representative. For example, individuals that survive after fumigation and potentially represent most of the gene pool after this bottleneck event may be coming from two subpopulations within the facility. If two distinct subpopulations are not equally sampled in two distinct sampling periods this can potentially create variation among sampling periods. Based on these results and the data on trap capture after fumigations, it can be assumed that indeed the population size was reduced to a very small proportion of the population, and in all sampling periods, all potential subpopulations were randomly sampled and genotyped.

In conclusion, these results indicate unstable genetic composition of *T.castaneum* in a single facility over time, with the hypothesis that fumigations have a major impact on population genetic structure inside the facility due to genetic drift being supported. There are still some gaps that need to be filled regarding the sources of rebounding individuals. Although these are likely survivals from the fumigation, they can potentially be moving in from other structures or even from outside sources. Further research addressing this question could, for example, measure levels of genetic structure at the scale of a food facility including different rooms in the main structure of the food facility, and individuals collected at the vicinity of the buildings.

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Figures and Tables

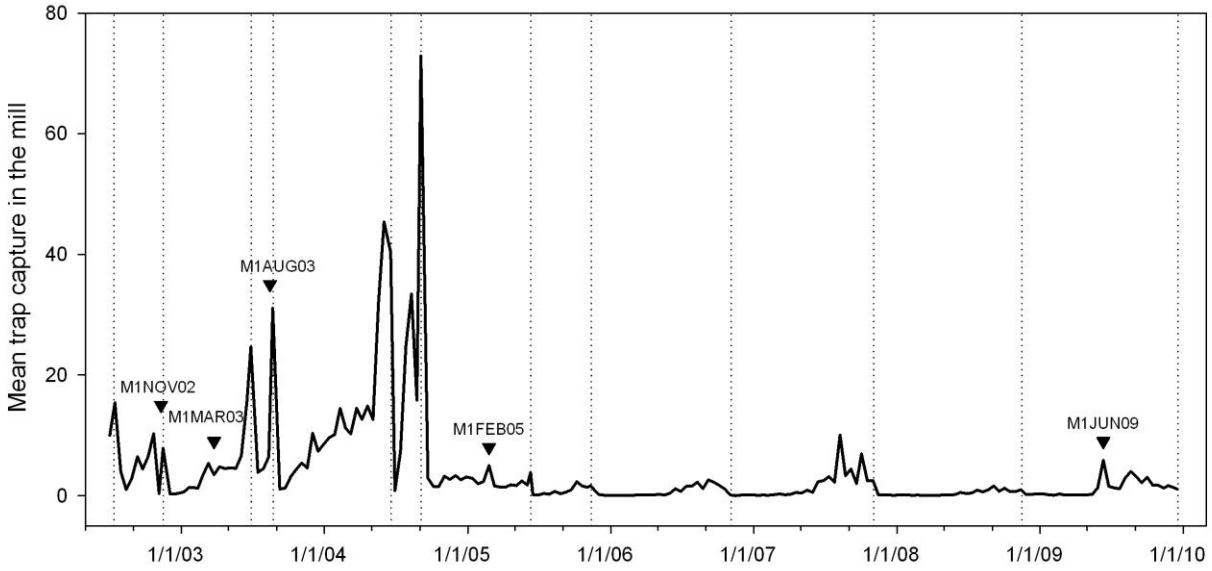

Figure 4.1 Mean capture of *Tribolium castaneum* adults per trap per two week monitoring period within the mill over time, with vertical dotted lines indicating dates of structural fumigations, and labeled triangles indicate the dates when samples for temporal genetic analysis were collected: November 2002 (M1NOV02), March 2003 (M1MAR03), August 2003 (M1AUG03), February 2005 (M1FEB05), and June 2009 (M1JUN09).

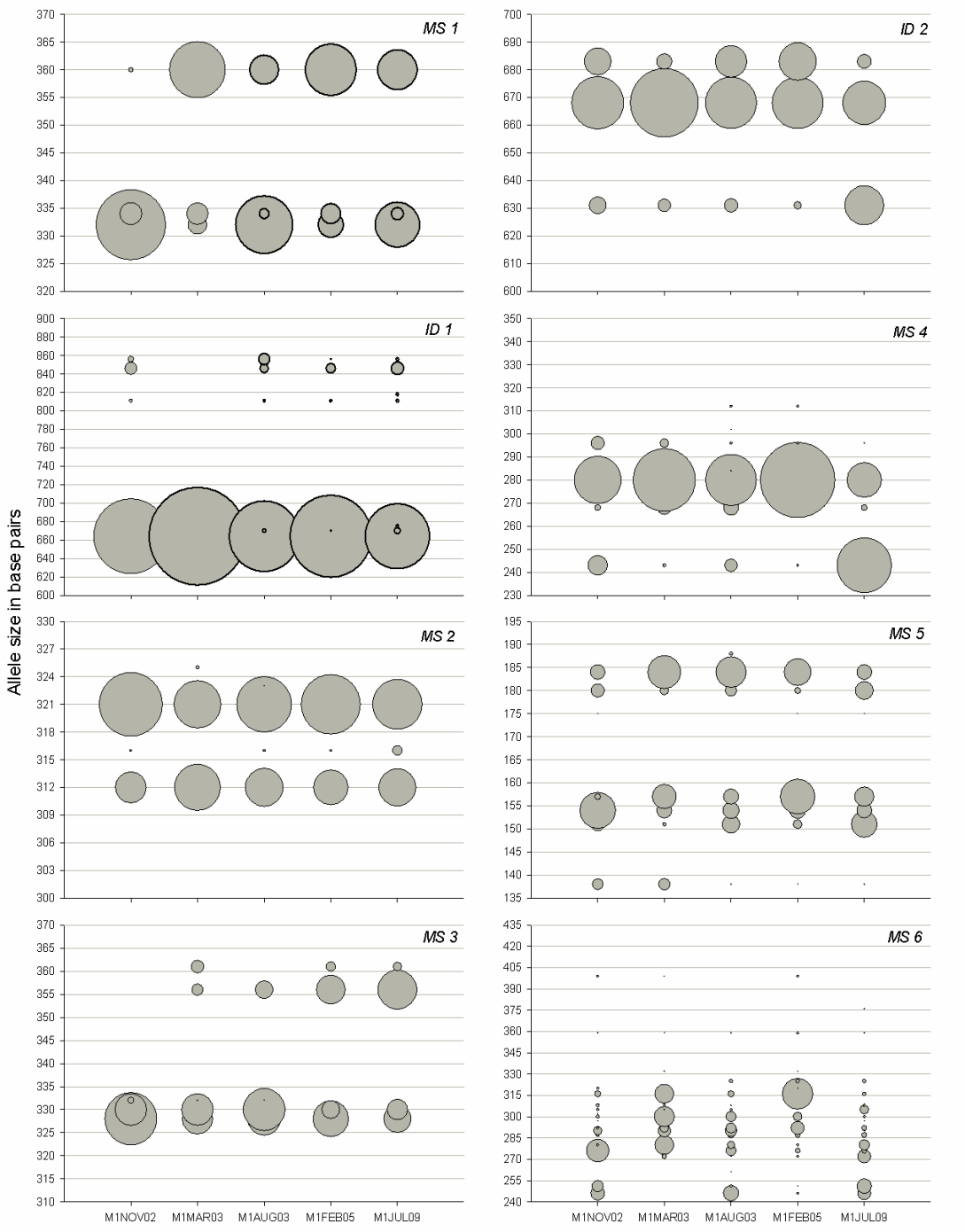

Figure 4.2 Distribution of allele sizes and frequencies across eight molecular markers (MS1-MS6, ID1-ID2) and five *Tribolium castaneum* population (x-axis)sampling periods, with y-axis values corresponding to the allele size in base pairs and the relative diameter of circles corresponding to the relative frequency of the respective allele in each population.

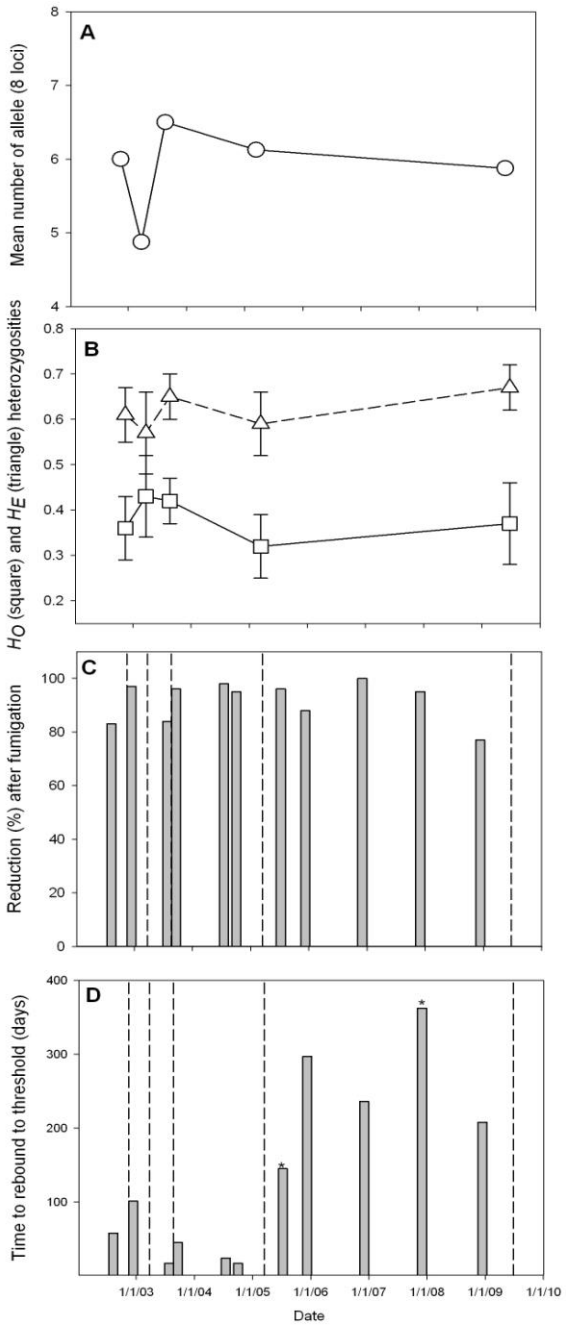

Figure 4.3 Temporal pattern in (A) mean number of alleles; (B) observed (squares) and expected (triangles) heterozygosity; (C) reduction in the number of *T. castaneum* captured immediately after fumigation; and (D) time to rebound to the threshold of 2.5 beetles/trap/monitoring period. Vertical dotted lines indicate periods in which samples were collected in the mill. Asterisks on the bars in (D) indicate periods that mean trap capture did not reach 2.5 beetles before the following fumigation.

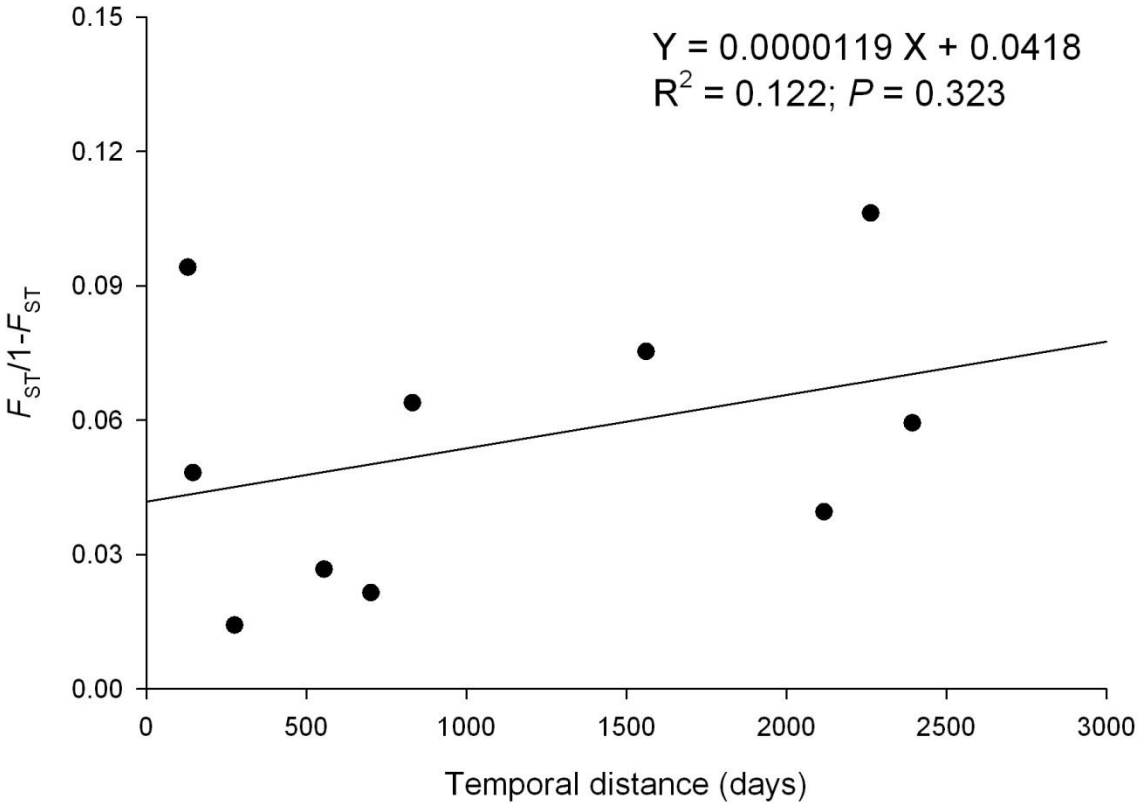

Figure 4.4 Relationship between genetic distance (F_{ST}/1-F_{ST}) and temporal distance (days) between sampling periods of a *Tribolium castaneum* population in a flour mill.

Table 4.1 Marker code, linkage group (LG), motif, primer sequences (forward and reverse) and locus-specific F_{ST} values for each of the eight molecular markers (microsatellite or other indels) used for evaluation of *Tribolium castaneum* populations. In the reverse primer for ID2, W and Y are used as nomenclature for incompletely specified bases (W was used to specify that adenine or thymine can be used in that location and Y is for thymine or cytosine).

Name	LG	Motif	Primer sequences (5'-3')*	Number of alleles	H_{exp}
MS1	2	GTT(10)	CGACGACGAGAAGGGAGGTA GCAAGGAGGCCATGAATAAAA	3	0.560
ID1	3	-	AACTTTAAACCCATCTCACTCAA ATCATACTTTCAGACCCAGACAC	7	0.334
MS2	3	ATAA(5)	GTAAACAGGAGGACAGGCTAAAAGTG CATCGAACGAGGCTGTGAATAAAC	5	0.528
MS3	3	AAT(7)	TATCCGAAATTTTATCTACTCAT AGGACCCTTTTTACTTTTTCAG	6	0.697
ID2	3	-	CCGCTTTCGTCTCRCAGTTGC CTAWYGTAAGACTTATTAGGCACGTTC	3	0.574
MS4	9	AAT(3)-AAT(5)-AAT(8)	AGCCGCAACAAAGTAAGCAA TGACTACCACCGACAGATTT	9	0.578
MS5	9	TAA(16)	AAGTGCTGCTGTATTTTATT TCAGACTCCGTATCCTTTATT	8	0.791
MS6	10	AAT(19)	AAATTCTCGGCTTTTTGGGT GAGCTGGCGGTTATATTGGA	22	0.890

* Primer sequences for MS6 were obtained (Tca-10.1), and primer sequences for MS4 were modified (Tca-9.1), from Demuth *et al.* (2007)

Table 4.2 Summary statistics for eight loci and five sampling dates for *Tribolium castaneum* including allele number (A), sample size (N), allele richness (R), observed and expected heterozygosities (H_{obs} and H_{exp}), with significant departures from Hardy-Weinberg equilibrium designated by *.

		Sample date				
Marker	Variable	M1NOV02	M1MAR03	M1AUG03	M1FEB05	M1JUN09
MS1						
	N	39	38	38	36	39
	A	3	3	3	3	3
	R	2.96	3.00	3.00	3.00	3.00
	H_{obs}	0.128	0.237	0.263	0.111	0.205
	H_{exp}	0.434*	0.584*	0.554*	0.617*	0.610*
ID1						
	N	39	36	38	35	30
	A	4	1	5	5	7
	R	3.89	1.00	4.72	4.04	6.65
	H_{obs}	0.205	0.000	0.289	0.171	0.067
	H_{exp}	0.391*	0.000^{NS}	0.458^{NS}	0.282^{NS}	0.538*
MS2						
	N	39	38	37	36	39
	A	3	3	4	3	3
	R	2.79	2.92	3.38	2.83	3.00
	H_{obs}	0.410	0.500	0.324	0.472	0.590
	H_{exp}	0.475^{NS}	0.544^{NS}	0.531^{NS}	0.502^{NS}	0.586^{NS}
MS3						
	N	38	38	38	35	21
	A	4	6	5	5	4
	R	3.97	5.55	4.36	4.94	4.00
	H_{obs}	0.316	0.526	0.421	0.343	0.048
	H_{exp}	0.600*	0.762^{NS}	0.668^{NS}	0.736*	0.717*
ID2						
	N	39	37	35	36	37
	A	3	3	3	3	3
	R	3.00	3.00	3.00	3.00	3.00
	H_{obs}	0.564	0.459	0.543	0.361	0.595
	H_{exp}	0.606^{NS}	0.468^{NS}	0.601^{NS}	0.571^{NS}	0.623^{NS}

Table 4.2 Continued

Marker	Variable	M1NOV02	M1MAR03	M1AUG03	M1FEB05	M1JUN09
MS4						
	N	39	38	38	35	39
	A	6	5	9	7	4
	R	5.52	4.91	7.64	6.22	3.52
	H_{obs}	0.256	0.316	0.316	0.057	0.385
	H_{exp}	0.697*	0.554*	0.682*	0.399*	0.556 NS
MS5						
	N	39	38	38	36	35
	A	7	6	7	7	7
	R	6.52	5.91	6.47	6.16	6.20
	H_{obs}	0.333	0.816	0.632	0.417	0.657
	H_{exp}	0.791*	0.782 NS	0.807 NS	0.761*	0.814 NS
MS6						
	N	38	38	37	36	36
	A	18	12	16	16	16
	R	14.87	9.89	13.61	13.60	13.78
	H_{obs}	0.684	0.579	0.595	0.639	0.389
	H_{exp}	0.898*	0.858*	0.916*	0.861*	0.914*

Table 4.3 Estimates of population differentiation (F_{ST}) between different sampling periods within a flour mill shown in the lower diagonal and in the upper diagonal, ** and * indicates significant differences between pairs at the 0.01 and 0.05 level, respectively, after Bonferroni correction.

	M1NOV02	M1MAR03	M1AUG03	M1FEB05	M1JUN09
M1NOV02		**	**	**	**
M1MAR03	0.086		**	*	**
M1AUG03	0.014	0.046		*	*
M1FEB05	0.060	0.021	0.026		**
M1JUN09	0.056	0.096	0.038	0.070	

Chapter 5 - Trap-based evaluation of *Tribolium castaneum* capture in a flour mill and the influence of environmental and physical factors

Abstract

Environmental and physical variables can be important factors involved in the distribution of stored-product pests in food processing facilities, but there is a lack of research evaluating these factors in situ. Data from a long-term *Tribolium castaneum* monitoring program was used to evaluate spatial variation in captures among trap locations and to determine possible correlations with environmental and physical factors. Four data sets (complete data set, cool season, warm season, and 2009/2010) were created and analyzed, with all data sets showing significant differences among trap locations in terms of beetle captures and proportion of time that traps exceeded the threshold of 2.5 beetles per trap per period ($P < 0.0001$). Among all the environmental and physical factors measured, mean temperature and spillage accumulation at trap locations were the factors that showed significant relationships with variation of beetle captures: locations with greater temperatures and spillage accumulation tended to capture more beetles in traps. Traps with equipment in close proximity tended to capture more beetles, but there was no significant correlation between the number of pieces of equipment and the mean number of beetles captured. Presence of equipment near traps was also related to an increase in spillage accumulation and mean temperature. Traps on the upper floors tended to have more captures, which could also be associated with presence of equipment and greater spillage accumulation and mean temperature.

Introduction

Understanding the spatial distribution of stored-product pest species in food storage and processing facilities is an important step for a successful Integrated Pest Management (IPM) program (Campbell et al. 2002). This type of information can lead to improved targeting of intervention tactics, and consequently reduced use of pesticides (Brenner et al. 1998). Based on pheromone based monitoring data, insects are typically not uniformly distributed inside food facilities (Arbogast et al. 2000, Campbell et al. 2002, Trematerra and Sciarretta 2004, Trematerra et al. 2007). This distribution in captures can be generated by the distribution of infested food patches inside the facilities, but could also be impacted by insect movement patterns and insect responses to spatial patterns in cues that influence behavior (Campbell 2005). In order to improve the use of the information on the distribution of stored-product pests as a management tool, we should identify and understand causal factors influencing distribution (Trematerra and Sciarretta 2002).

Tribolium castaneum is a major pest of flour mills and is considered difficult to monitor and manage because individuals can exploit hidden refugia where food material can accumulate such as equipment, ledges, cracks and crevices (Campbell et al. 2010a) and because traps used to capture dispersing individuals are baited with aggregation pheromone and kairomones that are not strongly attractive. Several studies have shown that *T. castaneum* and other stored-product species are spatially patchy in distribution inside food storage and processing facilities (Rees 1999, Arbogast et al. 2000, Doud and Phillips 2000, Campbell et al. 2002, Trematerra and Sciarretta 2004, Trematerra and Gentile 2006), suggesting that sub-populations can exist in different habitats each experiencing different population dynamics. Structural fumigation is an important management tool for reducing infestations in food processing and storage facilities (Phillips et al. 2000). Studies have shown that *T. castaneum* can be captured even a few days after fumigation, which suggests some level of survival of treatment or immigration from outside (Phillips et al. 2000, Toews et al. 2006, Small 2007, Campbell et al. 2010a,b). Beetles may be able to survive treatment by persisting in areas where the penetration of the fumigant is insufficient, but the spatial pattern of fumigation impact has not been previously investigated. If survival within a structure is occurring then it is possible that traps near locations with higher levels of survival will be the first locations to rebound and thus provide information about the

spatial pattern in fumigation efficacy. If beetles are moving in from other untreated areas then pattern of recovery in beetle captures in traps may be related to routes of entry into facility.

The locations where traps are placed inside food facilities for the monitoring of stored-product pests can have considerable variation in environmental and physical conditions that could potentially influence captures of insects in traps. For example, trap locations can vary in terms of temperature, relative humidity, air flow, food spillage accumulation, light intensity, angles of approach to trap, proximity to equipment and routes of entry. The relative importance of these different factors in terms of influence on capture in traps is not well known, especially in operating food facilities. Campbell et al. (2002) reported that captures of *Trogoderma variabile* Ballion was impacted by whether the trap was along a wall or next to a pillar. Nansen et al. (2004) found that *Plodia interpunctella* (Hübner) captures in traps was influenced by trap position and the presence of landing surfaces. This species was also reported to respond to the presence of light preferring to rest in illuminated areas, but presence of light did not improve capture in traps baited with pheromone or food lures (Sambaraju and Phillips 2008).

The relationship between trap captures and level of infestation of *T. castaneum* is not particularly strong (Toews et al. 2009), and the relationship between dispersing individuals and captures in traps may be further complicated if capture efficiency of traps is impacted by the landscape in which the trap is placed. Little is known about the impact of these factors on *T. castaneum* behavior in commercial facilities, but some laboratory studies have shown influences on its behavior. Temperature can have major effects on the distribution of *T. castaneum* since it can influence active movement, flight initiation, developmental time, fecundity and population growth (Sokoloff 1974, Trematerra and Sciarretta 2002, Jian et al. 2005, Cox et al. 2007). Accumulation of flour and presence of shelter on surfaces can impact movement behavior and oviposition (Campbell and Runnion 2003, Toews et al. 2005, Romero et al., 2010). *Tribolium castaneum* is attracted to dark tall and narrow shapes and this can increase trap capture in traps placed near these objects (Chapter 6). *Tribolium castaneum* is considered a cryptozoic species (Jackowska et al. 2007), thus it should choose locations with low light intensity even though lighting can attract some stored-product pest species (Phillips and Throne 2010). *Tribolium castaneum* tend to move along edges and to move more slowly when moving along them (Campbell and Hagstrum 2002). Therefore, all these factors and others can potentially have

implications for the monitoring of this species since these factors can vary in the landscape around different traps and influence the probability of capture differently among locations.

Here, a case study is presented in which the influence of environmental and physical factors on captures of *T. castaneum* in a food processing facility was investigated. In this study, the objective was to carry out a trap-based evaluation of spatial variation of beetle captures of *T. castaneum* inside a flour mill, and assess possible relationships that might exist between the spatial variation in captures with environmental and physical factors surrounding the traps. Even though spatial statistical approaches seem to be the most appropriate to study spatial variation, other approaches were used since there was an interest in the variation of trap captures in two (i.e. within floor) and three dimensions (i.e. across floors). Analyses were applied to monitoring data included in Campbell et al. (2010a,b), but here additional monitoring data were used and the focus was on the variation among individual trap locations rather than the mean capture reported in earlier publications.

Materials and Methods

Flour Mill and Monitoring Program

The mill was a commercial wheat flour mill with five floors, with more detailed information about the mill (Mill #1) and its management program described in Campbell et al. (2010a,b). The long-term monitoring of *T. castaneum* in the mill began in July 2002 and the mill was continuously monitored until March 2010. After November 2004, the IPM program was improved by adding regular aerosol treatments with 1% or 3% synergized pyrethrins (Entech Fog-10 or Entech Fog-30, Entech Systems, Kenner, LA) (29.6 ml/28.3 m3) and methoprene (Diacon II, Wellmark International, Schaumburg, IL) (3.0 ml/283.2 m3). Additionally, management tactics involved enhanced and targeted sanitation and residual insecticide applications in areas with elevated trap captures (Campbell et al. 2010a,b).

The focus of the present research was the period after the improvement of the IPM program because average captures more closely resemble the means obtained in monitoring programs in other food processing facilities (J.F. Campbell, unpublished data). Preliminary analyses also showed that trap capture before and after IPM improvement were correlated (Pearson correlation: $r = 0.62$, $P < 0.0001$). *T. castaneum* adults were monitored using Dome

pitfall traps placed on the floor, and containing a pheromone lure for *Tribolium* spp. (*T. castaneum* and *T. confusum*) and kairomone oil soaked piece of paper (Trécé Inc., Adair, OK). Fifty-five traps were distributed across the mill with eleven traps placed on each floor. Traps were maintained at approximately the same locations during the entire monitoring program. Traps were typically serviced every two weeks, but whenever it was not possible, trap capture was standardized to a 2-wk trapping interval. During the target period, six structural fumigations were performed (five with methyl bromide and one with sulfuryl fluoride (ProFume; Dow AgroSciences, Indianapolis IN), with five inter fumigation intervals.

Tribolium castaneum monitoring data sets

Different data sets related to beetle captures were created for analysis. The complete data set, which includes 142 monitoring periods, represents all beetle capture data from November 2004 until March 2010. Because seasonal differences may exist in terms of factors such as temperature, beetle captures were grouped into warm and cool seasons. The cool season data set includes 78 monitoring periods with beetle capture data collected from October through March of each year, and the warm season data set which includes 64 monitoring periods with beetle capture data collected from April through September of each year. Since physical and environmental conditions were measured only in the summer/fall 2009 and winter 2010, beetle capture data just during the periods of time when data on physical and environmental conditions were collected (2009/2010 data set) were also evaluated, which includes eight monitoring periods.

The mean number of beetles captured within each of the four data sets for each of the 55 trap locations was calculated. To evaluate variation among traps vs. the impact of fumigation, the mean number of beetles in the last period before fumigation, mean number of beetles in the first period after fumigation, and fumigation efficacy, which was defined as the ratio between the mean number captured after and the mean number captured before fumigation, were also calculated. The mean trap capture threshold of 2.5 beetles/trap/monitoring period was developed in Campbell et al. (2010b), and was proposed to be related to increased risk of large increases in beetle captures over time. Here, the proportion of time that a trap location exceeded this threshold considering the complete data set and also grouping by cool and warm season was calculated. As a measure of rebound, the average number of days after fumigation until beetle

captures reached or exceeded the threshold was calculated for each trap location for the complete data set.

Environmental and Physical Factors Associated with Trap Locations

At each trap location, ten factors were measured that were hypothesized to potentially affect *T. castaneum* capture in traps: temperature, relative humidity (rh), light intensity, spillage accumulation, shortest distance from trap to wall, shortest distance from trap to window, wall length adjacent to trap, total of approach angles to trap, total length of vertical edges near trap, and presence of milling equipment near trap (all equipment and specific types of equipment).

Data on temperature, rh, light intensity and spillage accumulation were collected during four two-week monitoring periods during summer and fall of 2009 and four two-week monitoring periods during winter of 2010. Temperature and rh were recorded hourly over the total two week monitoring periods using data loggers (HOBO® H8 family, Onset Computer Corp., Pocasset, MA) attached to the top of each trap using a piece of Velcro tape (Stick Pack™, Velcro USA Inc., Manchester, NH). For each monitoring period, the mean hourly temperature and rh was calculated. Light intensity was measured at the beginning of each monitoring period, between 10:00 am and 12:00 pm, using a light meter (Model EA33, Extech Instruments Corporation, Waltham, MA) placed at the top of each trap. To evaluate spatial patterns in the accumulation of flour dust (i.e., spillage), a Petri dish bottom (6 cm diameter) was attached to the top of the data logger using a piece of Velcro tape attached in turn to the top of the Dome traps. Petri dishes were in place during the entire two-week monitoring periods. Petri dishes were weighed before and after placing them in the mill, and the amount of accumulated flour spillage was determined from the difference of weights.

The physical landscape factors that are relatively stable over time were measured on September 9th, 2009 either directly on that day or from digital photographs taken on that date. Distance to window was determined by measuring the straight line from the top of the trap to the lower corner of the closest window with a metric tape measure. Most of the traps were located along walls or at the corner of walls. A metric tape measure was also used to determine the length of the closest wall and the distance from the trap to the closest walls. Since milling equipment is a likely source of insects, circumferences with a radius of 3 and 5 meters were drawn at each trap location and the number and type of pieces of equipment within each of these

circles were determined. Total of approach angles to traps and the total length of vertical edges were determined within a 1 m^2 area around each trap location. These two variables were determined using digital pictures taken at each trap location. For each trap location, digital photographs were taken from ~1 m above and based on these images the factors were calculated using the software Image J (Abramoff et al. 2004). Before calculating the factors, the software was calibrated using the size of the trap as a reference. For each trap location, approach angle was calculated by measuring each open angle using the "Angle" tool in Image J and then they were added to have the total approach angle. For the total length of vertical edges similar approach was used with the "Straight Line Selections" tool.

Statistical Analysis

Nonparametric (PROC NPAR1WAY) and parametric (PROC GLM) analyses using SAS v. 9 software (SAS Institute, Cary, NC) were used to identify differences among individual trap locations and among trap locations grouped by floor. When applicable, a Wilcoxon test was performed using SAS software and Bonferroni correction of 0.05 divided by the number of tests to correct for the multiplicity of comparisons. Data was analyzed for normality using PROC UNIVARIATE (SAS software), and either log (x+1) or arcsin square root transformed as necessary. To evaluate the relationships between environmental and physical factors and the different measures of beetle capture data sets, Pearson correlation (PROC CORR), stepwise regression (PROC REG), and linear regression (PROC REG) (SAS software) were used. For further analysis, ten trap locations with the highest or lowest mean beetle captures were selected and differences in environmental and physical features were compared using pairwise test (PROC N1PARWAY Wilcoxon). Data in graphs and text is presented as untransformed mean + sem.

Results

Spatial variation in capture of Tribolium castaneum among trap locations

The first step in analysis of impact of different environmental factors on trap captures was to determine if there were differences among the individual traps in their captures of *T. castaneum*. For this analysis, trap locations were considered as independent. In the complete data set, differences in average beetle capture occurred among trap locations (Kruskal-Wallis

test: χ^2 = 516.2; df = 54; $P < 0.0001$), with mean values ranging from 0.2±0.1 to 4.5±0.6 beetles/monitoring period (Figure 5.1A). Differences in mean beetle capture among trap locations also occurred when data were sorted into warm (range: 0.2±0.0 to 6.85±1.8 beetles/monitoring period) (Kruskal-Wallis test: χ^2 = 418.3; df = 54; $P < 0.0001$) (Figure 5.1C), and cool (range: 0.2±0.1 to 4.0±0.9 beetles/monitoring period) (Kruskal-Wallis test: χ^2 = 199.8; df = 54; $P < 0.0001$) (Figure 5.1E) seasons. On average, more beetles were captured in the warm (1.4±0.1 beetles/trap/monitoring period) compared to the cool (1.0±0.1 beetles/trap/monitoring period) season (Wilcoxon test: χ^2 = 11.2; df = 1; $P < 0.0001$). For the 2009/2010 data set, 98% of beetle captures occurred in the summer/fall collection period and therefore only this set of data was analyzed. Significant differences occurred among trap locations (Kruskal-Wallis test: χ^2 = 115.3; df = 54; $P < 0.0001$), with beetle captures ranging from 0.0±0.0 to 10.5±2.3 beetles/monitoring period (Figure 5.1G).

Significant differences were found when grouping all traps located on the same floor. Beetle captures in the complete data set ranged from 0.7±0.1 to 1.7±0.2 beetles/trap/monitoring period (Kruskal-Wallis test: χ^2 = 15.31; df = 4; $P = 0.004$), with fourth floor having the highest captures and basement the lowest (Figure 5.1B). In the warm season, beetle captures were highest on the third and fourth floors and lowest in the basement (Kruskal-Wallis test: χ^2 = 28.3; df = 4; $P < 0.0001$) (Figure 5.1D), but beetle captures did not differ among floors in the cool season (Kruskal-Wallis test: χ^2 = 2.02; df = 4; $P = 0.73$) (Figure 5.1F). Even though there were significant differences among floors for the 2009/2010 data set (Kruskal-Wallis test: χ^2 = 14.72; df = 4; $P = 0.005$), no separation of the means was possible after Bonferroni correction (range: 1.1±0.2 to 5.4±0.8 beetles/trap/monitoring period) (Figure 5.1H).

Spatial variation in effect of fumigation on Tribolium castaneum capture among trap locations

Mean beetle captures in the last period before fumigation ranged from 0.0±0.0 to 4.6±1.3 beetles/monitoring period, but trap locations were not significantly different from each other (Kruskal-Wallis test: χ^2 = 61.78; df = 54; $P = 0.22$) (Figure 5.2A). In 52% of the pre-fumigation periods, no beetles were captured in the last period before fumigation, with pre-fumigation periods without capture evenly distributed among all trap locations. No significant differences occurred in beetle captures in the first period immediately after fumigation (range: 0.0±0.0 to

1.6±1.6 beetles/monitoring period) (Kruskal-Wallis test: $\chi^2 = 52.71$; df = 54; $P = 0.52$) (Figure 5.2C). In 92% of the cases, no beetles were captured in the first period after fumigation. The remaining 8% had captures with captures occurring in 19 out of 55 trap locations, but with only three trap locations capturing beetles after more than one fumigation. There was no correlation in mean beetle capture among trap locations before and after fumigation (Pearson correlation: r = 0.09, $P = 0.49$). Percent reduction in beetle capture after fumigation ranged from 0 to 100%, with an average of 95.0±1.6%. There were many occasions in which no beetles were captured in the last period before fumigation. This made it difficult to obtain enough replication in percent reduction after treatment for trap locations in order to perform statistical comparisons among trap locations.

Another approach used to evaluate the variation in the effect of fumigation on *T. castaneum* capture among trap locations was to consider the threshold value of 2.5 beetles/trap/monitoring period and evaluate variation among trap locations. Considering each fumigation individually, and only using trap locations with captures of at least one beetle prior to treatment (48% of cases), 33% of trap locations had captures greater than the threshold. The percentage of fumigations resulting in 100% reduction did not differ above or below the threshold value (Wilcoxon test: Z = -1.55; df = 1; $P = 0.12$): 16% did not reach 100% reduction after fumigation compared to 6% not reaching 100% reduction when lower than the threshold. Rebound in beetle captures, at individual trap locations after fumigation, was evaluated using two different measures: proportion of monitoring periods between fumigations that captures of beetles were equal to or greater than the threshold of 2.5 beetles and the number of days after fumigation until the threshold was reached or exceeded. For the complete data set, the proportion of monitoring periods with trap capture equal or above the threshold was significantly different among trap locations (GLM procedure: F = 2.76; df = 54; $P < 0.0001$) (Figure 5.2E) ranging from 0.01±0.01 to 0.42±0.11. However, time to initially reach or exceed threshold did not differ among trap locations (GLM procedure: F = 0.74; df = 54,275; $P = 0.91$) (Figure 5.2G). The proportion of monitoring periods with trap capture equal or above the threshold was significantly different among trap locations when grouping the data into warm (range: 0.0±0.0 to 0.58±0.1) (GLM procedure: F = 2.95; df = 54; $P < 0.0001$) and cool season (range: 0.0±0.0 to 0.34±0.2) (GLM procedure: F = 1.51; df = 54; $P = 0.018$). The average proportion of monitoring

periods with capture equal or above the threshold was slightly but significantly higher in the warm (0.17±0.0) than the cool (0.14±0.0) seasons (t-test: t = -2.18; $P = 0.03$).

It is possible that some of the different data sets and measures of beetle activity are collinearly correlated and therefore subsequent analysis could be applied to a subset of them. Mean number of beetles captured in the complete data set was correlated with mean number captured in the warm season (n = 55; r = 0.931; $P < 0.001$), cool season (n = 55; r = 0.860; $P < 0.001$), and 2009/2010 (n = 55; r = 0.400; $P = 0.002$) data sets. Mean number of beetles captured in the complete data set was also correlated with proportion of time equal or above threshold (n = 55; r = 0.927; $P < 0.001$), and the number captured in the last period before fumigation (n = 55; r = 0.550; $P < 0.001$). Mean number of beetles captured in the complete data set was negatively correlated with time to initially reach or exceed threshold (n = 55; r = -0.676; $P < 0.001$). The only non-significant correlation was for comparing mean number of beetles captured in the complete data set with the number of beetles captured after fumigation (n = 55; r = 0.040; $P = 0.77$).

No significant differences were detected among floors in any of the measurements related to fumigation efficacy: beetle captures in the last period before fumigation (Kruskal-Wallis test: $\chi^2 = 2.13$; df = 4; $P = 0.71$) (Figure 5.2B), beetle captures in the first period immediately after fumigation (Kruskal-Wallis test: $\chi^2 = 1.09$; df = 4; $P = 0.90$) (Figure 5.2D), and fumigation efficacy (Kruskal-Wallis test: $\chi^2 = 4.29$; df = 4; $P = 0.37$). Similarly, no significant differences were detected among floors in the proportion of time that mean trap capture reached or exceeded the threshold in the complete (range: 0.14±0.09 to 0.52±0.09) (GLM procedure: F = 1.73; df = 4; $P = 0.18$) (Figure 5.2F), in the warm (range: 0.07±0.05 to 0.28±0.1) (GLM procedure: F = 1.28; df = 54; $P = 0.30$), and cool season (range: 0.05±0.04 to 0.31±0.13) (GLM procedure: F = 1.19; df = 4; $P = 0.34$) data sets.

Spatial variation in environmental and physical factors among trap locations

The second step in analysis was to determine if there were differences among the individual trap locations in the selected environmental and physical factors. Differences in mean temperature occurred among trap locations (GLM procedure: F = 1.87; df = 54; $P = 0.0005$), with mean temperature ranging from 19.6±2.0 to 26.6±1.6ºC (Figure 5.3A). When sorted by seasons, the warm season had no differences in the mean temperature among trap locations

(GLM procedure: F = 0.86; df = 54; *P* = 0.74) with mean temperature ranging from 22.3±2.8 to 30.3±1.7ºC. In the cool season, differences in mean temperature occurred among trap locations (GLM procedure: F = 26.46; df = 54; *P* < 0.0001) with mean temperature ranging from 15.0±0.5 to 26.9±1.4ºC. Mean temperature in the mill was higher during warm (25.2±1.7 ºC) than during cool (20.3±0.2 ºC) season (pairwise t-test: t = 3.24, *P* = 0.02).

Differences in rh occurred among trap locations (GLM procedure: F = 8.56; df = 54; *P* < 0.0001), with rh ranging from 23.7±0.2 to 47.1±1.4% (Figure 5.3C). In the warm season, differences in rh occurred among trap locations (GLM procedure: F = 4.68; df = 48; *P* < 0.0001) with rh ranging from 24.1±0.2 to 48.0±1.2%. In the cool season, differences in rh also occurred among trap locations (GLM procedure: F = 59.07; df = 34; *P* < 0.0001) with rh ranging from 23.4±0.0 to 49.1±1.5%. Relative humidity in the mill was higher during warm (34.0±0.4) than during cool (30.43±0.4) season (pairwise t-test: t = 5.88, *P* = 0.002).

Differences in light intensity occurred among trap locations (GLM procedure: F = 33.8; df = 54; *P* < 0.0001) with light intensity ranging from 2.40±1.3 to 179.9±26.6 lux (Figure 5.3E). In the warm season, differences in light intensity occurred among trap locations (GLM procedure: F = 4.95; df = 54; *P* < 0.0001) with light intensity ranging from 1.38±0.5 to 152.3±50.9 lux. In the cool season, differences in light intensity also occurred among trap locations (GLM procedure: F = 21.82; df = 54; *P* < 0.0001) with light intensity ranging from 1.70±0.4 to 207.6±14.0 lux. Light intensity in the mill was not different between warm and cool season (pair-wise t-test, t = -0.16, *P* = 0.88).

Differences in spillage accumulation occurred among trap locations (GLM procedure: F = 8.55; df = 54; *P* < 0.0001) with spillage accumulation ranging from 0.02±0.01 to 0.11±0.02 g (Figure 5.3G). In the warm season, differences in spillage accumulation occurred among trap locations (GLM procedure: F = 5.45; df = 54; *P* < 0.0001) with spillage accumulation ranging from 0.00±0.00 to 0.53±0.20 g. In the cool season, differences in spillage accumulation also occurred among trap locations (GLM procedure: F = 3.00; df = 54; *P* < 0.0001) with spillage accumulation ranging from 0.00±0.00 to 0.34±0.02 g. Spillage accumulation was not different between warm and cool season (pairwise t-test, t = 0.38, *P* = 0.72).

Significant differences were found among the different floors regarding the environmental variables. Considering all monitoring data, temperature (GLM procedure: F = 0.51; df = 4; *P* = 0.73) (Figure 5.3B) did not differ among floors. When grouped into warm and

cool seasons, temperature in the warm season (F = 0.50; df = 4; *P* = 0.73) did not differ significantly among floors, but temperature in the cool season showed statistical differences among floors (range: 18.6±0.4 to 22.8±0.7 ^{o}C) (GLM: F = 14.34; df = 4,15; *P* < 0.0001). Relative humidity (GLM procedure: F = 82; df = 4; *P* = 0.52) (Figure 5.3D) did not differ among floors when considering all monitoring data, but when grouped by seasons both warm (range: 31.5±1.1 to 38.0±1.0%) (GLM: F = 10.63; df = 4; *P* =0.0013) and cool (range: 25.0±0.6 to 34.3±0.2%) (GLM: F = 39.32; df = 4; *P* < 0.0001) seasons showed statistical differences among floors. Differences were observed in light intensity among floors considering all monitoring periods (GLM procedure: F = 33.8; df = 4; *P* < 0.0001) (Figure 5.3F) and when sorted by warm (range: 10.6±1.1 to 46.9±4.1 lux) (GLM: F = 9.57; df = 4; *P* = 0.0005) and cool (range: 9.9±0.3 to 49.8±6.0 lux) (GLM: F = 26.57; df = 4; *P* < 0.0001) seasons. Finally differences were observed in spillage accumulation among floors considering all monitoring periods (GLM procedure: F = 8.55; df = 4; *P* < 0.0001) (Figure 5.3H) and when sorted by warm (range: 0.03±0.02 to 0.11±0.01 g) (GLM: F = 5.01; df = 4; *P* = 0.009) and cool (range: 0.01±0.00 to 0.12±0.03 g) (GLM: F = 3.75; df = 4; *P* = 0.026) seasons.

All the physical factors measured showed variation among trap locations. Distance to windows ranged from less than 1 meter to approximately 10 meters. Averaging among traps within a floor, the basement (480.4±95.5 cm) and first floor (527.5±97.4 cm) traps had the longest average distance to windows (GLM procedure, F = 2.67, df = 4, *P* = 0.04). Traps had distances to walls that ranged from 0 to 210 cm, but in most cases (80%) they were located within 10 cm from the walls. Traps with a distance greater than 10 cm to the closest wall were distributed across all floors and no differences in this measure were detected among floors (GLM procedure, F = 0.48, df = 4, *P* = 0.75). Total length of vertical edges and total approach angle to traps were highly variable among trap locations. Total angle for approaching the trap ranged from 70 to 360 degrees. Most of the traps were located very close to walls or pieces of equipment which limited the approaching angle to a maximum of 180 degrees. Traps located in the basement had a larger angle for approaching traps (230.7±26.9 degrees on average) (GLM procedure, F = 4.27, df = 4, *P* = 0.005) than any of the other floors which did not differ from each other. Total length of vertical edges ranged from 10 to 357 cm within the 1 m^2 area around traps, with no differences detected among floors (GLM procedure, F = 0.59, df = 4, *P* = 0.67).

Relationship between environmental and physical factors and capture of Tribolium castaneum in traps

Two approaches were utilized to evaluate the relationships between environmental and physical features at each trap location and mean beetle capture at that location: stepwise regression and comparison of highest and lowest beetle capture locations. Stepwise regression was performed between each of the different measures of beetle capture and the environmental and physical factors, with significant models reported in Table 5.1. Significant factors included in the stepwise regression model were also evaluated separately using linear regression (Table 5.1). Mean temperature, spillage and distance to windows were factors included in different stepwise regression models that were also significant in the individual linear regressions. Considering all the different measures of beetle capture, temperature was the factor that entered the stepwise regression model most frequently (six times) and most frequently had a significant linear regression (five times). The relationship with temperature was strongest when mean temperature and beetle captures were compared for the data set that they were collected concurrently (2009/2010 data set) ($r = 0.31$, $P < 0.0001$) (Table 5.1). Although this was only a snapshot of the trends in the temperature in the mill, it highlights that temperature appears to be an important factor influencing pattern of beetle capture.

The second approach was to create two groups of traps for each measure of beetle capture: 'high' which included the ten trap locations with the highest means (top 18%) and 'low' which included the ten traps with the lowest means (bottom 18%). Environmental and physical factors were then compared between these two groups using pairwise statistical tests. All four data sets (complete, warm, cool, and 2009/2010) had significant differences between the groups with high and low captures (Figure 5.4A). In the complete data set, the group 'high' captured on average 2.61±0.29 beetles/trap/monitoring period and the group 'low' captured 0.40±0.02 beetles/trap/monitoring period (Wilcoxon test, $Z = 3.74$, $P < 0.001$). In the warm season data set, the group 'high' captured on average 3.19±0.50 beetles/trap/monitoring period and the group 'low' captured 0.37±0.03 beetles/trap/monitoring period (Wilcoxon test, $Z = 3.74$, $P < 0.001$). In the cool season data set, the group 'high' captured on average 2.25±0.28 beetles/trap/monitoring period and the group 'low' captured 0.35±0.03 beetles/trap/monitoring period (Wilcoxon test, $Z = 3.74$, $P < 0.001$). In the 2009/2010 data set, the group 'high' captured on average 6.75±0.74 beetles/trap/monitoring period and the group 'low' captured 0.48±0.10 beetles/trap/monitoring

period (Wilcoxon test, Z = 3.78, $P < 0.001$). Only mean temperature and spillage accumulation were statistically different between the groups with 'high' and 'low' capture. Mean temperature was higher at locations with high beetle capture than in low beetle capture locations in both the warm season and the 2009/2010 data sets (Figure 5.4B): 27.2±0.6ºC compared to 25.1±0.4ºC (Wilcoxon test: Z = 2.53; $P = 0.01$) and 27.4±0.6ºC compared to 24.6±0.3ºC (Wilcoxon test: Z = 2.61; $P = 0.01$) for the warm season and 2009/2010 data sets (which was primarily warm season as well), respectively. Mean temperature did not differ among groups for the complete data set (Wilcoxon test: Z =1.70; $P = 0.08$) or cool data set (Wilcoxon test: Z = 1.78; P =0.08). Spillage accumulation in trap locations with a high mean beetle capture in the complete data set was greater (0.09±0.03 g) than trap locations with a low mean capture (0.03±0.01 g) (Wilcoxon test: Z = 2.08; $P = 0.04$) (Figure 5.4C). Spillage accumulation in trap locations with a high mean beetle capture in the cool season was greater (0.11±0.03 g) than trap locations with a low mean capture (0.03±0.01 g) (Wilcoxon test: Z = 2.04; P =0.04). Spillage accumulation was not significantly different in the warm season data set (Wilcoxon test: Z = 1.85; $P = 0.06$) or 2009/2010 (Wilcoxon test: Z = 0.08, $P = 0.94$) data set.

Relationship between beetle captures in traps and the presence of milling equipment

At three meter radius, pairwise comparison between groups of traps with and without equipment found significant differences in mean number of beetles captured (Wilcoxon test: Z = -3.66; $P < 0.001$). Average beetle captures in traps with equipment was 1.54±0.18 beetles/monitoring period and in traps without equipment was 0.78±0.07 beetles/monitoring period. At five meters radius, pairwise comparison between groups of traps with and without equipment also found significant differences in terms of mean beetle capture (Wilcoxon test: Z = -4.49; $P < 0.001$). Average beetle captures in traps with equipment was 1.46±0.15 beetles/monitoring period and in traps without equipment was 0.64±0.08 beetles/monitoring period. Even though the presence of equipment nearby influenced trap captures, the relative number of equipment was not significantly correlated with the average capture at either three (n = 30, r = -0.13; $P = 0.48$) or five (n = 37, r = -0.004; $P = 0.98$) meter radius.

Since mean temperature and spillage accumulation were significantly related with mean beetle capture and are also likely associated with the presence of equipment, these data were compared among trap locations with and without equipment. At 3 m radius, only temperature

was different between the groups with and without equipment. Temperature in traps near equipment was on average 1^{o}C higher (23.5±0.3) than traps without equipment (22.4±0.3) (Wilcoxon test: Z = -1.99; P = 0.04).

It is possible that specific types of milling equipment are more likely to have *T. castaneum* associated with them due to the presence of suitable refugia within them or the amount of spillage they produce, so additional comparisons were performed just on specific floors that contained certain types of milling equipment to evaluated this hypothesis. On the third floor, the mean number of beetles captured was not correlated with the number of purifiers present at three (n = 11, r = 0.10, P = 0.76) and five (n = 11, r = 0.18, P = 0.60) meters radius. In the fourth floor, the mean number of beetles captured was not correlated with the number of sifters present at three (n = 11, r = 0.21, P = 0.54) and five (n = 11, r = 0.10, P = 0.78) meters radius. In the second floor, the mean number of beetles captured was not correlated with the number of roller mills present at three (n = 11, r = 0.43, P = 0.18) and five (n = 11, r = 0.48, P = 0.13) meters radius. Thus, the number of each specific equipment did not affect the number of individuals captured.

Discussion

Spatially complex environments such as food processing facilities where resource patch size and quality vary temporally and spatially, can contribute to a spatially patchy distribution to the populations of species inhabiting these facilities, and in turn generate variation in beetle captures in traps. The results showed that for *T. castaneum*, captures across 55 trap locations in a food processing facility varied considerably. There are many studies showing that captures of *T. castaneum* inside food storage and processing facilities has a spatial patchy distribution (Arbogast et al. 2000, Campbell et al. 2002, Trematerra and Sciarretta 2004, Trematerra and Gentile 2006). Typically this is evaluated using contour maps and has focused on single floor locations, but many food processing facilities have multiple floors which requires consideration of the three-dimensional distribution of stored-product pests. Spatial variation in captures in traps should be related to the distribution of beetles, but beetle capture in traps can also be influenced by other factors. This study focused not only on how captures vary among individual trap locations, but also how much of this variation might be attributable to factors associated

with the environment. This type of information might help in identifying where to place traps, and for target management strategies.

Campbell et al. (2010a), when analyzing the combined data for two flour mills, found that mean beetle captures was significantly correlated between the last period before and the first period after fumigation. This indicates a consistent percent reduction in beetle captures following treatment, and highlights the benefits of avoiding letting populations build to high levels prior to fumigation. However, when analyzing this pattern at the individual trap level at one of the two mills included in the previous study, there was no significant correlation between before and after fumigation mean trap captures. This could be either because only a portion of the dataset used in the previous study was analyzed or because this relationship does not occur at the individual trap level. Campbell et al. (2010a) included in their analyses the combined data for two facilities and the period before the implementation of the IPM for this particular mill (Mill #1), when trap captures were very high and captures immediately after fumigation were frequent. Comparing beetle capture before and after fumigation considering only the period before implementation of IPM, there was significant correlation ($n = 55$, $r = 0.64$, $P < 0.001$), which was determined by the presence of high captures. When looking at individual trap locations, after improvement of IPM, most locations had zero captures after treatment (92% of the time) and the locations with captures immediately after fumigation were highly variable among fumigations. This suggests either that locations of persistence after fumigation are not consistently in the same locations, with foci of capture after treatment tending to be scattered around the facility, or that there is poor spatial association between capture in traps and local sources of infestation. Presence of adult beetles in traps within the mill immediately after fumigation can result from two mechanisms: survival of treatment within structure or movement into structure after treatment (Campbell and Arbogast 2004, Campbell et al. 2010a). Campbell et al. (2010) discussed about the variation in the distribution of *T. castaneum* and suggested a link with the presence of different microhabitats (e.g., spatial variation in temperature) and the spatial variation in gas concentration and time (CT) during fumigation which could consequently provide conditions where survival could occur. Capture of *T. castaneum* immediately after fumigation is not a new finding and studies at other locations have found similar results (Phillips et al. 2000, Toews et al. 2006, Small 2007), but this analysis does not indicate specific areas

where beetles are more likely to be recovered after treatment and further evaluation is needed to determine the sources of these beetles.

The spatial variation among trap locations in different environmental and physical factors could influence *T. castaneum* behavior or population distribution. Average mill temperature was higher in the warm season and temperature in this season was less variable among trap locations and within monitoring periods at each trap location. The fact that temperature in the cool season showed significant variation among traps suggests that temperature could have a greater influence on capture during the cool season. However, for captures in the cool season, spillage was a significant covariant in the model, not temperature. Even though temperature measurements were not statistically different among trap locations in the warm season, traps with highest captures had a warmer average temperature than traps with the lowest captures. At this point it is not clear what role temperature plays on the variation of capture. Higher temperature could lead to greater beetle captures due to multiple mechanisms: higher temperatures could be associated with more rapid pheromone release from the lures making them more attractive, beetle rate of movement increases with temperature which could increase frequency of trap encounter, beetles may actively select and/or develop more rapidly in spillage patches in warmer areas thus making it more likely to find infested patches in these areas, and/or higher temperatures might be correlated with other sources of beetles such as proximity of milling equipment.

Equipment (i.e., processing machinery) and conveying systems have dead-spaces where food products accumulate, do not move, and can be cleaned only when machines are stopped and disassembled (Phillips and Throne 2010). The milling process also produces spillage of food material outside of equipment either through materials spilling or fine materials settling on surfaces. This spillage material can accumulate in protected areas and also provide food patches for insect development. Generation and accumulation of spillage and debris in different areas can influence population structure in a facility and consequently trap capture since areas with accumulation can offer food odor and harborage to insects (Campbell et al. 2002, Trematerra and Sciarretta 2004, Campbell 2005, Phillips and Throne 2010). It is difficult to identify and measure these accumulations, but a novel strategy was developed of measuring the settling of fine materials as an indirect measure of how likely an area was to have spillage accumulations. Ideally higher beetle captures at trap locations should be related to localized infestation and

milling equipment is a likely location for infestation and also a critical location to detect infestation since these locations could be more likely to lead to beetles entering the milling stream. Presence of equipment near traps was associated with higher mean beetle captures, although not correlated with the number of pieces of equipment. As expected, presence of milling equipment in the proximity of a trap was also associated with greater production of spillage and higher mean temperature. *Tribolium castaneum* was recovered from product samples taken from mill equipment at this location (Campbell and Arbogast 2004), so higher captures in traps near equipment could be because these are sources or because the higher temperature and spillage near these equipment increase captures. The relative importance of these different factors needs further investigation.

The division of mill into different floors presents clearly observable zones, but measurable differences in beetle captures or landscape features were more difficult to observe. Only mean capture in the complete data set, during the warm season and summer/fall 2009 showed significant differences among floors. Trematerra and Gentile (2006) found that *T. castaneum* and *T. confusum* were only observed on the upper floors of a seven floor industrial semolina mill in Italy. They suggested that the presence and spatial distribution of insect pests in the mill was influenced by the position of key areas of interest, such as the entry and access points and areas with large amounts of food resources and environmentally favorable conditions. The observation that *T. castaneum* and *T. confusum* were more frequently found on the upper floors is pertinent considering that higher temperatures should be observed in these areas since hot air rises. Trematerra and Gentile (2006) found that a corner where the most *Tribolium* spp. were caught was exposed to the south, thus being the warmer zone of the mill, and also contained machinery which generated dust that was difficult to remove. However, in this study although beetle captures sometimes were greater on the upper floors, mean temperature at traps was not significantly different among floors.

In conclusion, the main objective of this research was to identify factors that could potentially have important influences on trap captures in food processing facilities. The overall results described here show that it is possible to improve monitoring in food processing facilities by locating areas that maximize the possibility of detecting beetles. Thus, locations with higher temperature and higher spillage accumulation will increase the likelihood to capture beetles. However, it is important to highlight that beetles were captured in all trap locations and not

having traps in these other less obvious areas could lead to missing activity. Apparently, this is the first time that variables such as reported here have been measured at the same time in a food processing facility for the study of the relationship with *T. castaneum* capture. Managers of processing facilities historically relied on calendar-based fumigations and other tactics for insect management, but with the phaseout of methyl bromide and the costs associated with other fumigants and heat treatment, they need to rely more on other IPM strategies.

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Figures and Tables

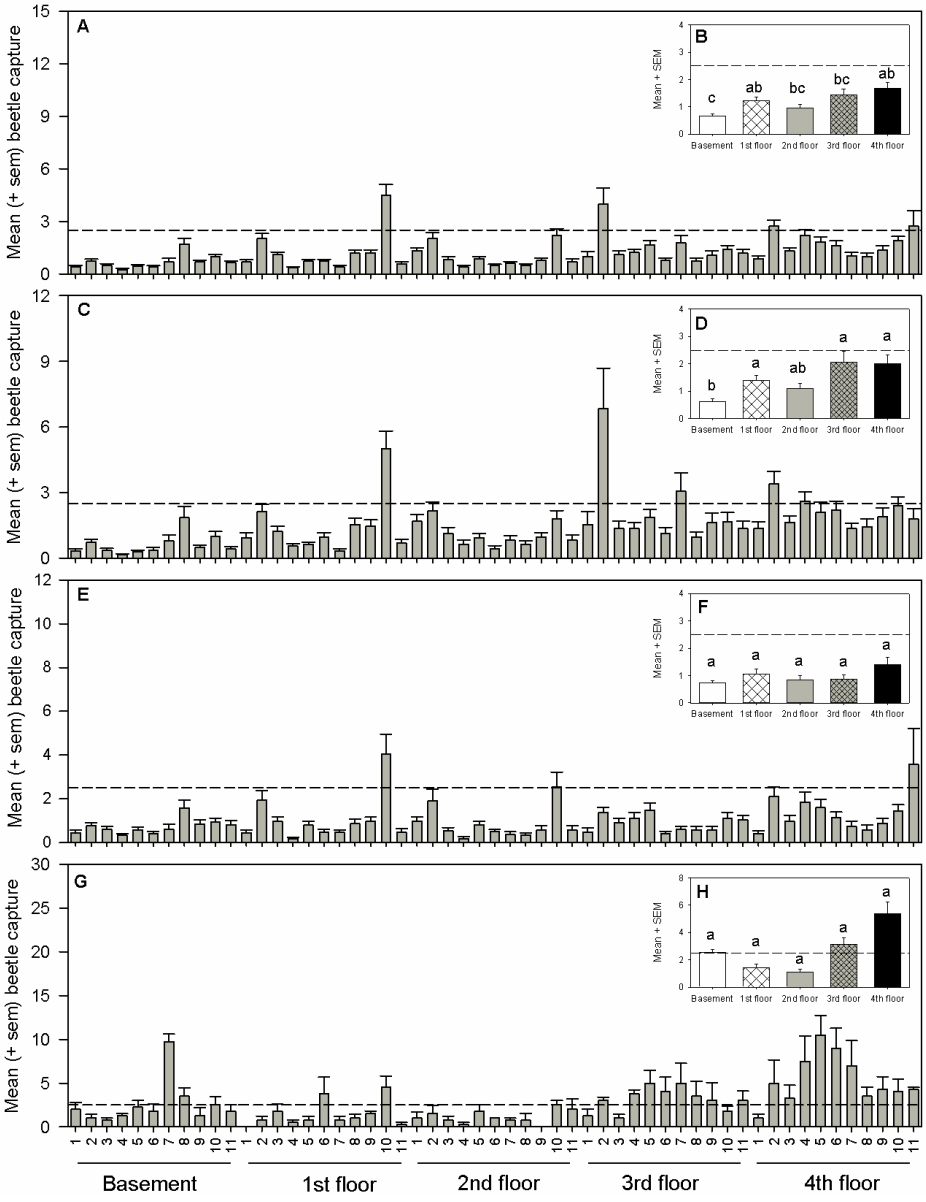

Figure 5.1 Mean (+sem) capture of *Tribolium castaneum* adults in traps within flour mill for (A) complete monitoring period, (C) warm season periods only, (E) cool season periods only, and (G) just during periods in 2009/2010 when environmental data was collected. Smaller graph (B, D, F, and H) within each larger graph represents the mean of the same data for traps on each floor of the mill. Different letters above bars in the smaller graphs represent means that are significantly different based on Wilcoxon sign-rank test. Dashed lines represent the threshold level.

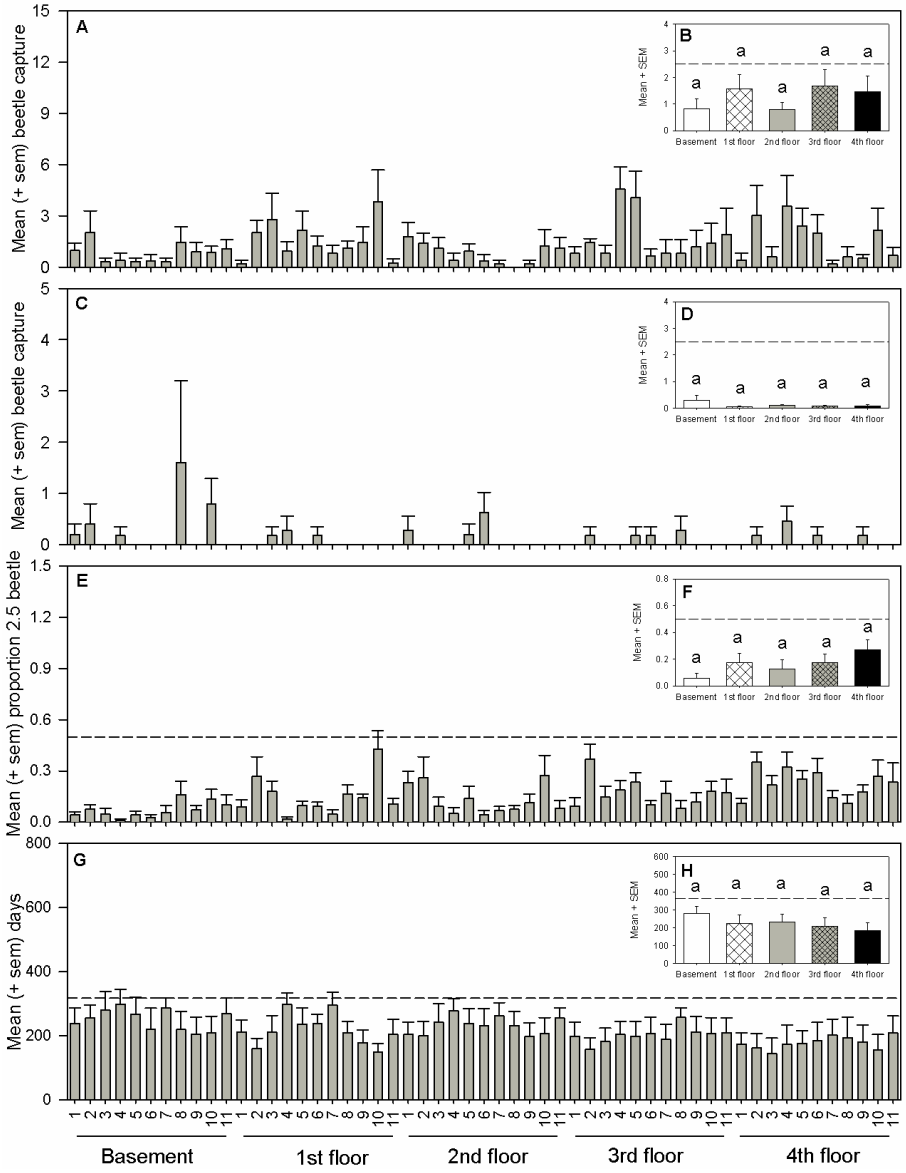

Figure 5.2 Mean (+sem) across trap locations for (A) *Tribolium castaneum* capture in the last monitoring period before structural fumigation of mill, (C) capture in the first period after fumigations, (E) proportion of monitoring periods that capture of *T. castaneum* in a trap reached or exceeded threshold, and (G) number of days after fumigation until capture of *T. castaneum* reached or exceeded threshold. Smaller graph (B, D, F, and H) within each larger graph represents the mean of the same data for traps on each floor of the mill. Different letters above bars in the smaller graphs represent means that are significantly different based on Wilcoxon sign-rank test. Dashed lines in E represent the median proportion of traps with capture level in G the average interfumigation interval.

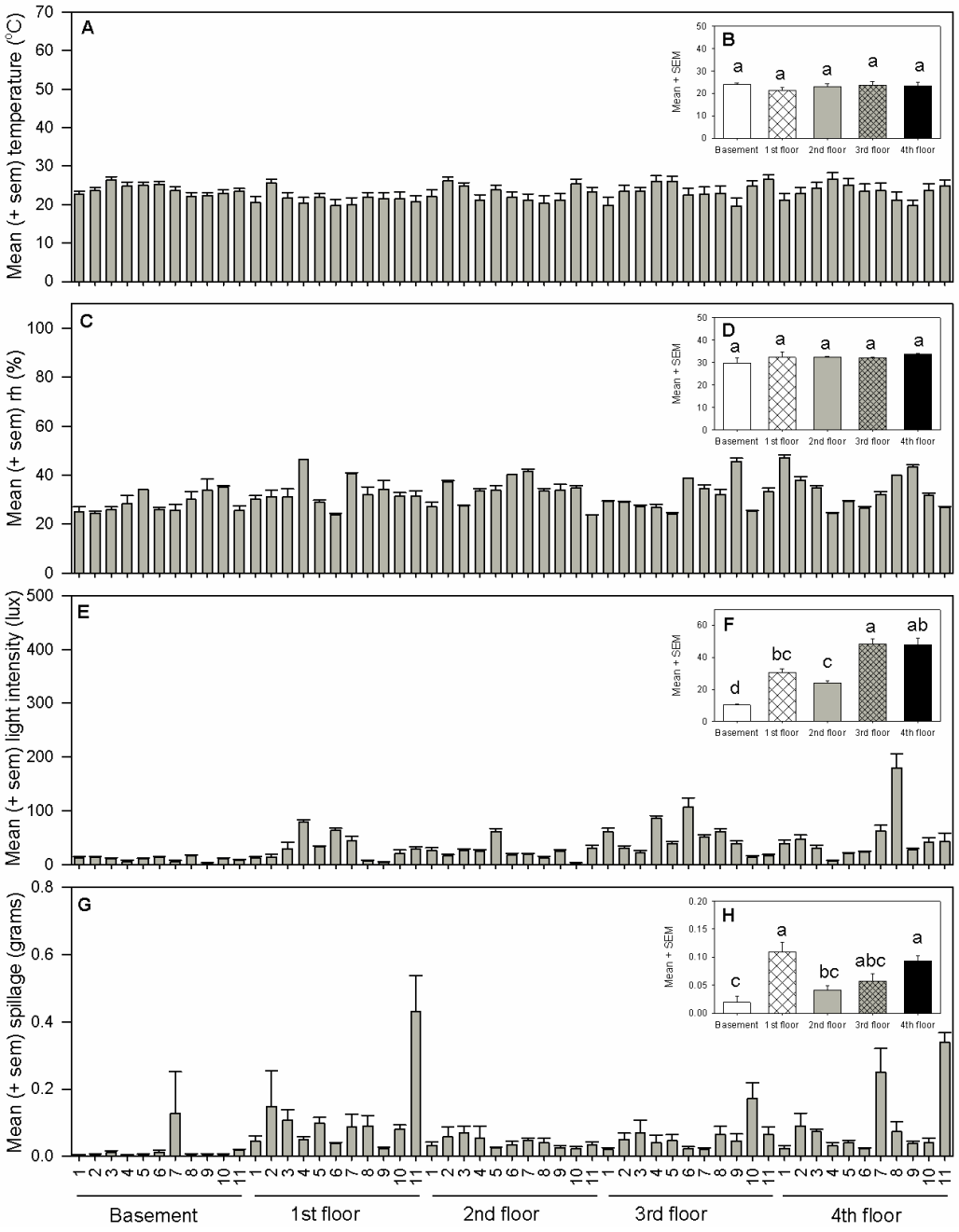

Figure 5.3 Mean (+sem) values across trap locations within flour mill for (A) mean temperature, (C) mean relative humidity, (E) light intensity, and (G) spillage accumulation relative humidity. Smaller graph (B, D, F, and H) within each larger graph represents the mean of the same data for trap locations on each floor of the mill. Different letters above bars in the smaller graphs represent means that are significantly different based on Wilcoxon sign-rank test.

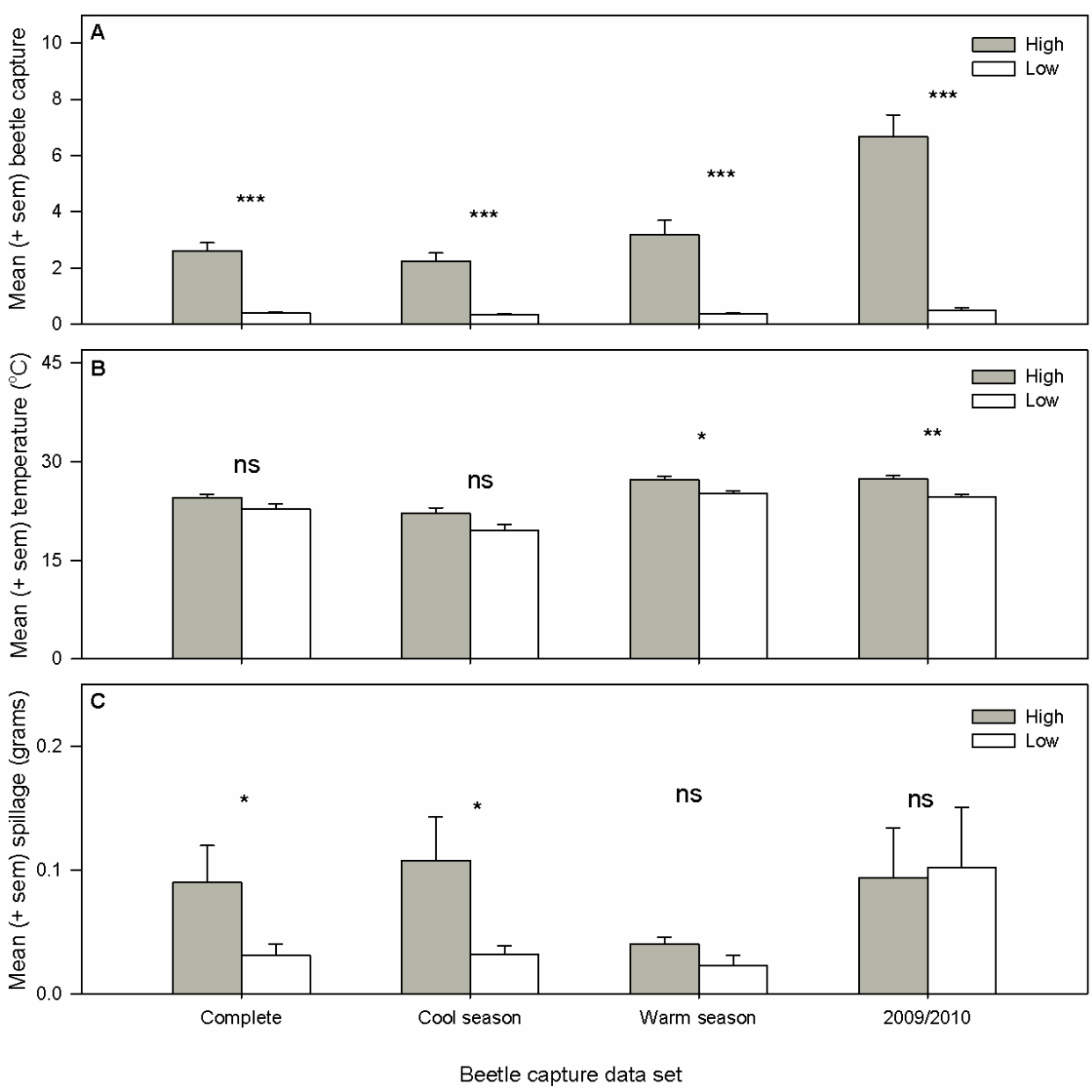

Figure 5.4 Pairwise comparisons between traps with the ten highest mean and ten lowest mean captures of *Tribolium castaneum* within the mill in (A) mean number of beetles captured, (B) mean temperature, and (C) spillage accumulation. Symbols above bars indicate statistical differences between high and low traps within a dataset, with ns indicating P>0.05, * indicating P<0.05, ** P<0.01, *** P<0.001.

Table 5.1 Stepwise and linear regressions between measures of beetle captures, effects of fumigation and environmental and physical factors. Here are presented five out of ten data sets. Other data sets were excluded since they showed high correlation with the ones presented here.

Data set	Factor	Stepwise regression				Linear regression	
		F	*P*-value	Partial R^2	Model R^2	R^2	*P*-value
Mean beetle capture - complete data set	Mean temperature	3.37	0.07	0.07	0.07	0.06	0.08
Mean beetle capture - cool season	Spillage	8.17	0.007	0.20	0.20	0.16	0.002
	Angle for approaching	5.63	0.008	0.06	0.26	0.01	0.20
Mean beetle capture - last period before fumigation	Mean temperature	8.06	0.007	0.15	0.15	0.12	0.008
	Perimeter of edges	8.48	0.0007	0.12	0.27	0.05	0.10
Mean beetle capture - summer/fall 2009	Mean temperature	3.87	0.06	0.08	0.08	0.31	< 0.0001
	Total angle	4.44	0.02	0.09	0.17	0.04	0.16
	Perimeter of edges	5.08	0.004	0.09	0.26	0.03	0.22
Days with capture equal or above threshold within fumigations intervals	Distance to windows	5.35	0.03	0.10	0.10	0.11	0.02

Chapter 6 - Response of *Tribolium castaneum* and *T. confusum* adults to vertical black shapes and its potential to improve trap capture

Abstract

Tribolium castaneum and *T. confusum* can be monitored in food processing facilities using traps baited with pheromones and kairomones, but beetle response to traps might be enhanced by adding visual cues. Against a white background, *T. castaneum* adults were more likely to visit black pillars than white pillars when presented with a choice (e.g., 73% of beetles visited black and 17% visiting white pillar), and visits to black pillars increased with pillar height. When tested against a black background, beetles did not show a significant preference for either color pillar regardless of height. When comparing beetle's captures in pheromone/kairomone baited traps placed in front of a white or black panel in a white arena under high, low, or dark light conditions, more beetles were captured in traps in front of black panels under both high and low light conditions, but not under dark conditions. A similar pattern of capture under low light and dark conditions was also found for the closely related species *T. confusum*. In a larger scale choice test, the same pattern of greater *T. castaneum* captures in traps in front of black panels than white panels was obtained, whether traps were placed in corners or along walls. These results suggest that captures in monitoring traps could be increased by adding dark vertical shapes behind trap locations or placing traps near dark structures.

Introduction

Integrated pest management of stored-product insects in food processing facilities relies on monitoring data to guide management decisions and to evaluate the effectiveness of the program (Barak et al. 1990, Burkholder 1990). Often this monitoring data is generated using pheromone and/or kairomone baited traps that rely on insect olfaction to facilitate captures, but typically don't exploit other sensory modalities such as vision (Chambers 1990). However, in other systems color preferences by economically important insects have been demonstrated (Southwood 1978, Prokopy and Owens 1983), and visual cues, typically attractive colors and trap shapes, have been exploited to monitor and manage insect pests (e.g., Hoback et al. 1999, Strom and Goyer 2001, Athanassiou et al. 2004). Although response to color and shape by stored-product insects is relatively poorly understood, traps that combine olfactory cues such as pheromones and kairomones with visual cues may offer the potential to increase captures of stored-product insects.

Exploitation of visual signals in insect monitoring programs fall into three general categories: lights that attract insects, colored objects that are attractive because of their specific reflectance and shapes or silhouettes that stand out against a contrasting background. Pinero et al. (2006) found that female melon flies (*Bactrocera cucurbitae* (Coquillett)) were primarily attracted to objects of hemispherical shape associated with either yellow, white, or orange pigments. Mainali and Lim (2010) found that western flower thrips (*Frankliniella occidentalis* (Pergande)) were more attracted to flat yellow sticky traps on a black background and suggested that the black background may help the insect to perceive reflectance with minimal interception from other sources of reflectance. There is considerable variation among insect species in their color preference, but attractive colors typically resemble suitable habitats for mating, oviposition, or feeding (e.g., Hoback et al. 1999, Cilek 2003, Döring et al. 2004, López-Guillén et al. 2009). For example, many herbivorous insects respond to the color yellow or white, which corresponds to the peak color reflectance of plants (Prokopy and Owens, 1983). Insect species that attack trees such as bark beetles respond to vertical trunk silhouettes for landing and short range orientation, and traps that provide appropriate visual silhouettes are usually more efficient (Lanier 1983, Finch and Collier 2000).

Tribolium castaneum (Herbst), red flour beetle, and *T. confusum* Jacquelin du Val, confused flour beetle, (Coleoptera: Tenebrionidae) are major pests of food processing facilities, especially wheat and rice mills, and walking beetles can be monitored using traps based on a pitfall design (e.g., Mullen 1992) and baited with pheromone and kairomone attractants. These traps have been used successfully to monitor pest populations of *T. castaneum* (e.g., Campbell et al. 2010), but the level of attraction by flour beetles to these baited traps is widely, although anecdotally, reported as relatively weak. Reza and Parween (2006) showed that *T. castaneum* larvae and adults differed in their tendency to aggregate on different colors when presented with a choice between different color surfaces. Of the colors tested in this study, adults of *T. castaneum* exhibited a tendency to aggregate only on black surfaces. This study evaluated if adult *T. castaneum* are actually attracted to the color black and if the height of the black shape impacts the level of attraction. Black shapes were combined with a commercially available pheromone/kairomone baited trap to determine if this can increase the effectiveness of these traps at capturing beetles. Finally, it was confirmed that *T. confusum* exhibited a similar increase in trap captures with black shapes.

Materials and Methods

Experimental conditions

Tribolium castaneum and *T. confusum* adults used in the experiments were obtained from laboratory colonies maintained in 0.94 l glass jars containing wheat flour fortified with 5% (by weight) brewer's yeast (ICN Biomedicals, Aurora, OH). Both colonies were established within 10 months before experiments were conducted by mixing ~100 adults collected from food facilities in Kansas, USA. Reference specimes were deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research (KSU-MEPAR) under the voucher #214. The colonies were maintained at 28.0 ± 0.5°C, 60.0 ± 5.0% r.h. and 14:10 light:dark cycle (mean ± SE). Only adults less than two months old were used in experiments and collected the same day as experiments were conducted. Beetles were held for at least 30 min in Petri dishes without food inside the experimental chambers prior to the start of the experiment. Individual beetles were only used once in an experiment.

Experiment 1 was conducted inside a chamber (4.7 m long by 4.7 m wide by 2.4 m tall) located in a room without controlled temperature and r.h. and where lighting resulted primarily from natural sunlight. To reduce environmental variability all replicates were performed between 1300 h and 1600 h. The mean ± SE environmental conditions in chamber during experiments were 21.3 ± 0.4°C air temperature, 22.7 ± 0.8% r.h., and 380.5 ± 2.3 lux light intensity. All subsequent experiments were conducted in an environmental chamber (6.1 m long by 4.9 m wide by 2.4 m tall) held at 25.0 ± 0.0°C, 66.5 ± 0.0% r.h. and 24 h light (214.9 ± 0.3 lux). Temperature and rh were measured using either data loggers (HOBO® H8 family, Onset Computer Corp., Pocasset, MA) or handheld meter (Kestrel® 3000, Nielsen-Kellerman, Boothwyn, PA), and light intensity was monitored using light meter (Model EA33, Extech Instruments Corporation, Waltham, MA).

Experiment 1: Are T. castaneum adults attracted to black shapes?

Beetle response when presented with a choice between black or white pillars of different heights, when the background color was either black or white, was evaluated. This enabled to determine if beetle response was due to the dark shape or just due to contrast with the background. An experimental arena was constructed inside chamber described above from four foam core boards (1 x 1 m squares) taped together to form a cube, with the top open and the floor of the chamber forming the floor of the box. Depending on the treatment conditions, the inside of the box was covered with either white or black paper (Art Kraft Duo-Finish paper, C2F Inc., Beaverton, OR). Three different types of cuboids (pillars) were constructed out of corrugated cardboard with dimensions of 14 x 14 cm and height of either: 2 cm (short), 14 cm (medium), or 33 cm (tall). The heights of the pillars were selected to give a range of heights, with the tallest pillar height predicted to be perceived by the beetle as an extremely tall shape given the size of the beetle and the release distance used in the first experiment. The pillars were covered with the same type of paper used inside the box, either black or white depending on the treatment conditions.

At the center of the arena floor, a square (50 x 50 cm) was marked off with a pencil and this was the observation zone for recording beetle behavior. In each replicate, one black pillar and one white pillar of the same height was placed at opposite sides of the square marked on the floor (adjacent to the line but outside the observation zone). Individual *T. castaneum* adults were

placed inside 1.5 mL centrifuge tubes, acclimated under conditions in arena prior to release, and then released by placing an opened and inverted tube at the center of the observation zone and removing the tube after beetle inside was observed to upright and walking. After release, the time it took for either of the following events to occur was recorded: reach the edge of the observation zone, reach the black pillar, or reach the white pillar. Observations were terminated after any of these three events occurred or if 300 seconds had elapsed. Replications consisted of individual beetles exposed to pairs of pillars of the same height against either a black or a white background. Within first the white and then the black arena color, the order of the pillar height pairs was completely randomized, and within each height the orientation of the pillars was alternated (North-South and East-West orientations). A total of 25 replicates of each treatment were performed against the black background, and a total of 30 replicates of each treatment, except for one treatment that had 31 replicates, were performed against the white background.

Data were analyzed in SAS version 9.1.2 (SAS Institute, Cary NC) using contingency table analysis (PROC FREQ). For each color background, the contingency tables had 3 rows (pillar height) and three columns (beetles reach edge, reach black pillar, reach white pillar). From a total of 166 beetles tested, only 3 beetles remained in the observation zone for the 300 seconds and they were excluded from analysis. Chi-square test was used for testing the null hypothesis that the size of the pillars did not affect the events and to evaluate differences in the number of beetles reaching black and white pillars.

Experiment 2: How is the movement behavior of T. castaneum impacted by black pillars of different heights?

Movement pathways of individual beetles when exposed to pillars of different colors and heights were evaluated in a no-choice experiment. Individual beetles were observed in a wood arena (120 cm long by 120 cm wide and 23.5 cm tall) painted white (Zinsser Bulls Eye 1-2-3 primer, Rustoleum, Vernon Hills, IL) and held in chamber and under environmental conditions described above. The wood arena was covered by a box made of white paper (Art Kraft Duo-Finish paper) (120 cm long by 120 cm wide by 130 cm tall) with an observation hole cut in the center of the top through which a video camera could be inserted. This paper box enabled a video camera to be placed at the necessary height above the floor of the arena to record beetles and also provided a monochromatic white environment in which was possible to observe

response to black shape. The observation zone inside the wood arena in which activity of individual beetles was recorded was 50 x 50 cm.

Beetle behavior in each replicate was recorded over a period of 180 s using a portable digital video camera recorder (Model HDR-XR520V, Sony Electronics Inc., San Diego, CA) placed 130 cm above the floor of the arena. Digital video files were transferred to a computer and beetle positions determined and movement pathways analysed using EthoVision 3.0 (Noldus Information Technology, Wageningen, The Netherlands). The following pathway metrics were calculated and analyzed: heading (degrees), distance moved (cm), velocity (cm/s), turn angle (degrees), angular velocity (degrees/s) and meander (degrees/cm). The time it took to reach the pillar or the edge of the observation zone was measured.

In each replication, one pillar (short white, short black, tall white, tall black as described in experiment 1) was placed along the edge of the observation zone, and a total of twenty two beetles were tested in each treatment. The order of the four pillar heights was completely randomized and the orientation of the pillars within a given height was rotated in a clockwise direction (at least 5 replications in each direction). Between replicates the arena was sprayed with water and cleaned with paper towel.

Survival or time-to-event analysis followed by pairwise multiple comparison procedures (Holm-Sidak method) in SigmaPlot (SigmaPlot version 11, Systat Software, Chicago, IL), and general linear models (PROC GLM) procedure and contingency table analysis in SAS were used to evaluate differences among treatments (pillar height and color). Contingency table analysis was performed using a four column (pillar height) and two row (visit or not visit pillar) table. Additionally, heading (direction of movement relative to a reference line) data was analyzed for deviation from uniform distribution using circular statistics and mean heading compared among treatments using Watson-Williams test (Zar 1999). Because the location of the pillar rotated among replications, to facilitate comparisons heading values calculated by EthoVision software were transposed so that all headings were relative to a reference line from release point to pillar. Relative heading angles (0° to 180°) were used rather than absolute heading angles (0° to 360°).

Experiment 3: Does the use of black shape increase Tribolium spp. captures in pheromone and kairomone baited traps in simple landscape?

To determine if increased attraction to black shapes could increase captures in traps, the number of beetles captured in pheromone/kairomone baited traps was measured when placed against black or white panels under different light intensity conditions. The pheromone trap used was the Dome trap baited with pheromone lure for *Tribolium* spp. and 15 drops of food oil (Trécé Inc., Adair, OK, USA). Two traps were used in each replicate, with traps placed against two opposite interior walls of the arena described in Experiment #2. Behind each trap, a piece of 0.03 mm galvanized metal (7.5 cm wide by 18 cm tall) painted either flat black or flat white (ColorPlace® - Fast dry spray paint, Wal-Mart Stores, Inc., Bentonville, AR) was placed. Three light intensities – high, low and dark – were tested and these corresponded to 2,160.0 ± 12.6, 98.1 ± 0.6, and 0.0 ± 0.0 lux, respectively. Light intensity was measured at arena floor level at the midpoint of each wall. Different light intensities were obtained by placing four 120 cm long fluorescent light bulbs 80 cm above the arena and covering the arena with a sheet of translucent plastic. For dark treatment all lights in the environmental chamber were turned off, for the high light intensity treatment lights in the chamber were turned on and arena only covered with the plastic sheet, but for low light conditions three layers of paper (2 layers of white color and 1 layer of brown color Kraft paper) were placed on the top of the plastic. Temperature in the chamber and inside the arena at different light intensities was measured. The temperature in the chamber when the lights were turned on was 25.1°C ± 0.0°C, and when the lights were turned off was 24.9°C ± 0.0°C. The temperature inside the arena when the lights in the chamber were turned off but the four fluorescent light bulbs were turned on in the high light intensity treatment was 25.4°C ± 0.0°C.

At the beginning of each replicate, *T. castaneum* adults were released by placing a plastic Petri dish (9 cm diameter) containing 100 unsexed beetles at the center of the arena and using four pieces of folded paper (1.2 cm wide by 7.5 cm long) to create ramps that beetles could use to leave the dish. Twenty four hours after release, the number of beetles in each trap and in the arena was counted. Between replicates all beetles were removed, arena cleaned with 70% ethanol, and left uncovered for about 30 min. A new set of Dome traps and pheromone lures (aerated for 24 hr prior to start of experiment) was used for each replication. Eight replications (at each light intensity) were performed and the order of the treatments was completely

randomized. Within a light intensity and between replications, the walls that the traps and metal plates were placed against were rotated.

In a similar experimental protocol, a study was initiated to determine if the closely related species *T. confusum* would have similar responses to the traps with different color panels. The only difference in the design for this species was that based on the results with *T. castaneum*, the high light intensity treatment was excluded.

To determine if the number captured differed between black and white panels, pairwise comparisons between the two colors at each light intensity, were performed using t-tests or the nonparametric Mann-Whitney Rank Sum tests using SigmaPlot software. Nonparametric test was used when data did not always fit a normal distribution or pass the equal variance test. For comparisons among traps with same panel color at the different light intensities, the general linear models (GLM) procedure and Tukey mean comparison test (alpha = 0.05) using SAS software was utilized. Treatment means and SEMs are presented in the text and figures.

Experiment 4: Does the use of black shape increase T. castaneum captures in pheromone and kairomone baited traps in a more complex landscape?

Impact of black shapes behind traps on beetle captures in a larger and more complex landscape was investigated using the complete extent (29.7 m^2) of the environmental chamber with a table (86 cm wide by 152 cm long by 78 cm tall) and wind tunnel (86 cm wide by 223 cm long by 169 cm tall) placed next to each other in center of chamber. The chamber interior walls were white and experiments were run with lights on 24 h (484.6 ± 50.5 lux light intensity at floor level in corners and midpoints of walls). In the first set of experiments (four replicates), four Dome traps with pheromone lures and food oil, as described above, were placed in each corner of the chamber. In the second set of experiments (four replicates), Dome traps were placed at the midpoint along each of the walls. Pieces of 18 cm wide by 100 cm tall (0.5 mm thick) foam board covered with either black or white paper were placed behind each trap. Two opposite traps had black backgrounds and two opposite traps had white backgrounds, and their positions were rotated between replicates.

For each replicate, 300 *T. castaneum* adults were released using a Petri dish with ramps, as described above, placed on floor in the center of the chamber. Twenty-four hours after release, number of beetles in each trap and remaining in the chamber were counted. For

analysis, number of beetles in traps with the same color shape was combined and total number captured compared using paired t-tests (SigmaPlot). Traps placed in corners or along walls were analyzed separately. Between replicates all visible beetles were removed and chamber cleaned using an insect vacuum (Model 2820GA, BioQuip Products, Rancho Dominguez, CA, USA) and a broom.

Results

Experiment 1: Are T. castaneum adults attracted to black shapes?

In the arena with a white background, the number of beetles visiting a pillar was significantly affected by pillar color and height (3 x 3 contingency table analysis, $\chi^2 = 11.8$, df = 4, P = 0.019). When given a choice between short pillars, the number of beetles visiting each pillar color was not statistically different (2 x 2 contingency table analysis, $\chi^2 = 1.35$, df = 1, P = 0.246): 35.7% of the beetles visited the black and 14.3% visited the white pillar. When given a choice between medium pillars, significantly more beetles visited black pillars (58.1%) compared to white pillars (12.9%) (2 x 2 contingency table analysis, $\chi^2 = 4.96$, df = 1, P = 0.026). When given a choice between tall pillars, significantly more beetles visited black pillars (73.3%) than visited white pillars (16.7%) (2 x 2 contingency table analysis, $\chi^2 = 5.94$, df = 1, P = 0.015). However, when choice tests were performed in arenas with black background, the height and color of the pillars did not significantly affect the number of beetles visiting the two pillar colors (3 x 3 contingency table analysis, $\chi^2 = 1.98$, df = 4, P = 0.74).

Experiment 2: How is the movement behavior of T. castaneum impacted by black pillars of different heights?

The number of beetles visiting the pillars of different height and color was statistically different among the four treatments (4 x 2 contingency table analysis, $\chi^2 = 15.19$, df = 3, *P* = 0.002): 0 out of 15 (0.0%) visited the short white pillar, 1 out of 15 (6.7%) visited the tall white, 5 out of 21 (23.8%) visited the short black pillar, and 10 out of 20 (50.0%) visited the tall black pillar. Some of the beetles released tried to fly during the observation period, and these individuals were excluded from analysis. Interestingly, more beetles tended to fly in the white pillar treatments compared to the black pillar treatments: 7 beetles with short white pillar, 7

beetles with tall white pillar, 1 beetle with short black pillar, and 2 beetles with tall black pillars, out of a total of 22 beetles released in each treatment.

The only calculated movement parameters that showed statistical differences among treatments ($P < 0.05$) were time spent in the arena and heading. Using survival analysis to evaluate time spent in observation zone, there were significant differences among treatments (survival analysis log-rank test, $\chi^2 = 8.92$, df = 3, $P = 0.03$) (Figure 6.1). Comparing individual treatments, time spent in observation zone was only significantly different between short white and tall black pillar treatments (log-rank test with Holm-Sidak method, $\chi^2 = 8.74$, df = 1, $P = 0.003$); beetles reached the edge more quickly when the tall black pillar was present than when the short white pillar was present.

When evaluating the heading of released beetles, only the tall black pillar treatment had a circular distribution that was significantly different from uniform (circular analysis, z = 3.019, df = 20, $P < 0.05$). Comparison of mean heading across the four treatments showed significant differences among treatments (Williams Watson test, F = 8.34, df = 3, $P < 0.05$) (Figure 6.2A). Pairwise comparisons showed that mean heading of beetles in black pillar treatments were statistically lower than beetles in white pillar treatments, indicating a more directed movement toward pillar, but heading did not differ among pillar heights within a pillar color (Figure 6.2A). Pairwise comparisons performed with Williams Watson test showed differences between: tall black and tall white (F = 10.56, df = 1, $P<0.05$); tall black and short white (F = 11.38, df = 1, $P<0.05$); short black and short white (F = 6.77, df = 1, $P<0.05$); short black and tall white (F = 10.29, df = 1, $P<0.05$).

Distance moved (GLM, F = 1.95, df = 3, $P = 0.131$) (Figure 6.2B), velocity (GLM, F = 0.15, df = 3, $P = 0.927$) (Figure 6.2C), turn angle (GLM, F = 0.73, df = 3, $P = 0.537$) (Figure 6.2D), angular velocity (GLM, F = 0.44, df = 3, $P = 0.727$) (Figure 6.2E) and meander (GLM, F = 0.35, df = 3, $P = 0.787$) (Figure 6.2F) were not significantly different among treatments. There was an interesting trend for movement path metrics to have less variance in the tall black pillar treatment compared to treatments with white pillars (Figure 6.2).

Experiment 3: Does the use of black shape increase Tribolium spp. captures in pheromone and kairomone baited traps in simple landscape?

Pairwise comparisons of *T. castaneum* captures between traps with black and white shapes, showed that under high and low light intensity conditions traps with black shapes had greater captures than traps with white shapes (high light intensity: paired t-test, t = 2.61, df = 14, $P = 0.035$; low light intensity: Mann-Whitney test, U = 9.00, df = 14, $P = 0.015$). Under dark conditions, there was no difference in captures between traps with white or black shapes (paired t-test, t = -0.82, df = 14, $P = 0.44$) (Figure 6.3A).

The total number of *T. castaneum* captured in both traps under low light intensity (77.9 ± 4.6) was greater than the number captured under dark conditions (49.3 ± 8.5), but total number captured in the high light intensity treatment (65.4 ± 8.8) did not differ from either of the other treatments (GLM, F = 3.61, df = 2, $P = 0.045$). Comparing beetle captures just in traps with black shapes among the different light intensities revealed that more beetles were captured under the low light intensity (45.8 ± 5.2) compared to dark (23.1 ± 5.0) treatments, but number captured under high light intensity (40.6 ± 6.0) did not differ from other light intensities (GLM, F = 4.83, df = 2, $P = 0.019$) (Figure 6.3B). No differences were detected in beetle captures in traps with white shapes among the different light intensities (GLM, F = 0.95, df = 2, $P = 0.402$) (Figure 6.3C).

A similar pattern of black shapes increasing trap captures was observed for *T. confusum*, with beetle captures in traps with black shapes (25.9 ± 5.7) being greater than captures in traps with white shapes (11.1 ± 1.7) under low light intensity conditions (Mann-Whitney test, U = 13.00, df = 14, $P = 0.05$). Under dark conditions, beetle captures in traps with black shapes (11.5 ± 1.6) and captures in traps with white shapes (17.5 ± 3.5) were not different from each other (paired t-test, t = 25.00, df = 14, $P = 0.50$). There was no significant difference in total number captured in both traps between low light intensity (37.0 ± 5.6) and dark conditions (29.0 ± 4.8) (paired t-test, t = 1.13, df = 14, $P = 0.28$).

Experiment 4: Does the use of black shape increase T. castaneum captures in pheromone and kairomone baited traps in a more complex landscape?

More beetles were captured in traps with black shapes than in traps with white shapes in the more complex landscapes, whether traps were placed in corners (paired t-test, t = 10.94, df =

3, $P = 0.0016$) or along walls (paired t-test, t = 11.98, df = 3, $P = 0.036$) (Figure 6.4). Although there was a trend for captures in traps with black shapes to be lower when placed along walls than in corners, this trend was not statistically significant (paired t-test, t = 3.02, df = 3, $P = 0.057$).

Discussion

Additive effects of different sensory modes being used together as a tool for insect pest management has been shown for many species of insects (e.g., Strom and Goyer, 2001; Knight and Fisher 2006, Reddy et al. 2009). However, the potential of combining sensory modes to increase trap captures is still relatively unknown for many stored-product pest species. Results of experiments described here show that *T. castaneum* adults are attracted to tall dark shapes and their presence behind traps can increase the number of adults captured. This combination approach might be useful in improving the effectiveness of these traps in food processing and storage facilities, although factors such as light intensity and contrast with color background may impact the level of increase. An advantage of this type of strategy is that it would be relatively inexpensive and easy to implement this combination; e.g., painting a black shape behind trap locations along a wall or placing traps near dark objects already present in facility.

Results of experiments 1 and 2 showed that against a white background, *T. castaneum* adults were more likely to respond to black shapes and that response increased with the height of the shape. That this response did not occur to white shapes when the background was black, suggests that beetles are not just responding to contrast with the background. Analysis of movement patterns in Experiment 2 also showed that *T. castaneum* adults appear to be attracted toward tall black shapes and not just arrested at them, since in the tall black shape treatments beetles reached the edge more quickly, moved in a non-random direction, and had a heading directed toward the shape. Reza and Parween (2006) showed that adult *T. castaneum* have a preference for black, but actually only measured arrestment on this color. This is apparentlythe first study showing that beetles actively orient toward the color black and also that the response is greater for taller black shapes. It is also interesting that fewer beetles attempted to fly when black pillars were present compared to white pillars against a white background, suggesting that absence of any color may increase flight initiation.

Placing dark tall shapes behind pheromone and kairomone baited traps increased beetle captures in both simple and complex landscapes (Experiments 3 and 4). The fact that increased response to traps with black color shapes was lost when experiments were performed in the dark also supports the hypothesis that it is visual cues that are enhancing trap response. Visual cues appear to attract more beetles to the vicinity of the traps and thus increasing their potential of being captured. Response to traps in front of dark shapes was also strong in experiment 4 where there were other competing darker shapes in the environment (e.g., table and wind tunnel) and beetles started off farther from the traps. The experiment with *T. confusum* supports the hypothesis that both of these species respond similarly to dark shapes, which could be important since both of these species are pests in the same types of facilities, and can co-occur in the same structures (Small 2007).

Lighting in food processing facilities can be highly variable both temporally (e.g., lights on 24 hrs per day, lights off at night, lights on motion sensors) and spatially due to light fixture placement, window locations, and obstructions to light. For example, at one commercial food processing facility mean light intensity at 55 locations ranged from 1.4 ± 0.5 to 152.3 ± 50.8 lux, but most locations were below 50 lux (unpublished data). Insects have light thresholds necessary to perceive and discriminate different colors (Cammaerts and Cammaerts 2009). In these experiments, *T. castaneum* could perceive and respond to the black shapes under low light intensity conditions (~100 lux). Interestingly, total trap captures were lowest under dark conditions, and while the mechanism needs further evaluation it suggests that either activity in general and/or attraction was also reduced under these conditions or that this represents the baseline level of response to olfactory cues alone. There was also a non-significant reduction in trap captures under high light intensity conditions, and the observations suggest that beetles were finding refugia and moving less under these conditions. Lighting patterns in food facilities could therefore have a significant impact on the pattern of trap captures and the potential benefits of adding dark shapes to traps. It will be necessary to evaluate impact of black backgrounds on beetle response to traps under a wider range of light intensities in order to determine if this behavioral response will increase trap captures in commercial facilities.

The reason for *T. castaneum* adult response to black shapes, either in terms of current fitness or evolutionary origin, is not known. Response to dark shapes might be related to current fitness benefits of finding covered areas for refugia that would be in shadow. These areas for

refugia would have a greater probability of containing resources in terms of food, shelter, and finding mates. It may also be an evolutionary holdover and be related to ancestral response associated with exploiting trees. Sokoloff (1974) summarized the information available on natural habitats exploited by *Tribolium* spp. and concluded that this genus likely contained generalist feeders that exploited trees. *Tribolium castaneum* are occasionally found under the bark of trees and in acorns with longitudinally split seed coats (Subramanyam and Nelson 1999, Bonneton 2008). Some species of bark beetles (Coleoptera: Scolytidae) have been shown to respond to dark vertical shapes that may represent the silhouette of trees (Campbell and Borden 2006). The lesser grain borer *Rhyzopertha dominica*, a stored-product pest in the family Bostrichidae which contains primarily wood boring feeders, has been shown to respond to vertical silhouettes such as grain bins while flying (Mahrrof et al. 2010). *Tribolium castaneum* is thought to be more of a scavenger or predator under tree bark than feeding directly on trees, but it may still have been selected to respond to cues that indicate trees. *Tribolium castaneum* has reduced color vision and enhanced UV and long wavelength light reception, suggesting cryptozoic origins (Jackowska et al. 2007). These combined factors may lead beetles to be responsive to these tall dark shapes since evolutionarily and currently they may indicate favourable habitats.

In conclusion, the results presented here demonstrated that *T. castaneum* and *T. confusum* adults respond strongly to tall and narrow black shapes and that these shapes can increase the capture of beetles in pheromone/kairomone baited traps when placed in front of them. This combination of olfactory and visual cues offers the potential to increase trap capture efficiency in monitoring programs. Further research will be needed to evaluate how much of an increase can be obtained in more complex environments such as those found inside food processing and storage facilities.

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Figures and Tables

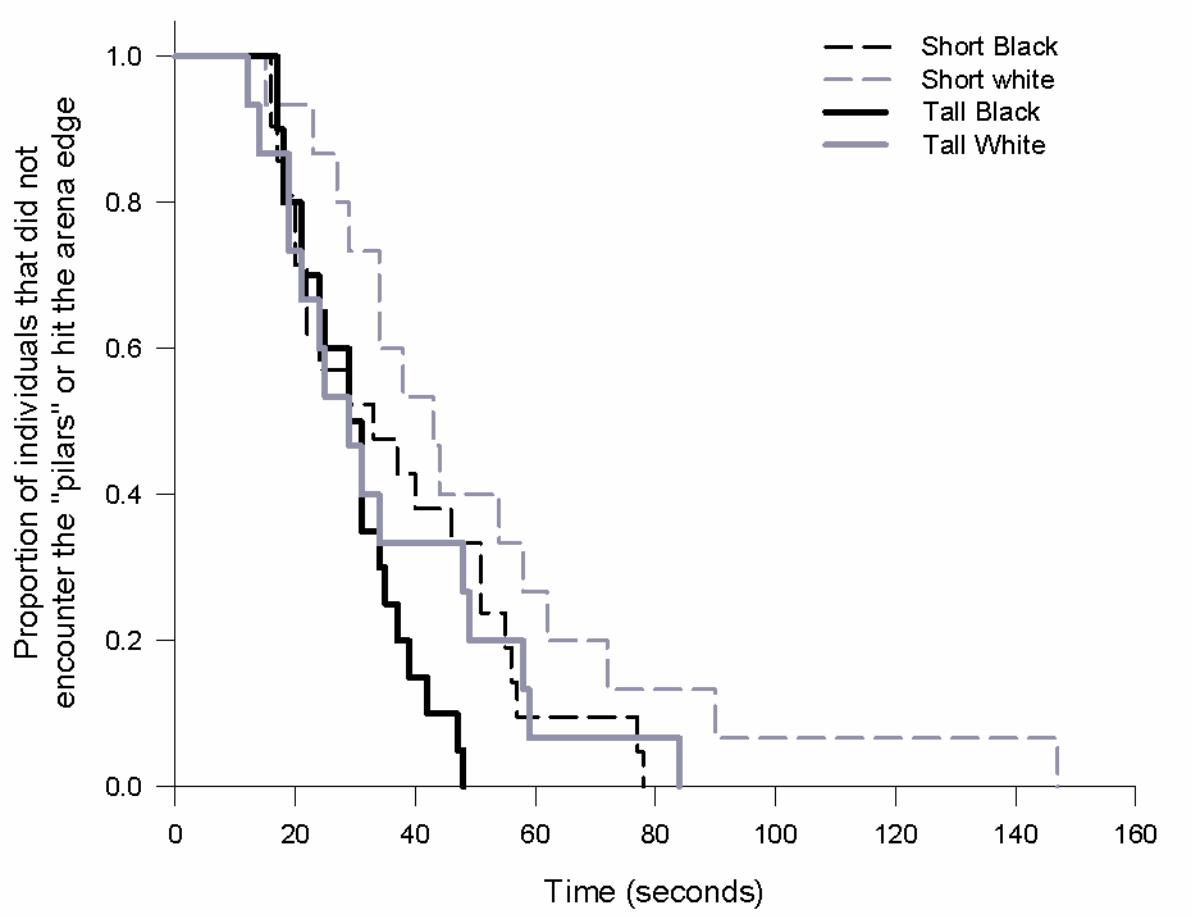


Figure 6.1 Time-to-event (survival) curves for the time after individual *Tribolium castaneum* adults are released until they reach the edge of the observation zone, including encountering the pillars aligned with the edge when exposed to black or white pillars of different heights.

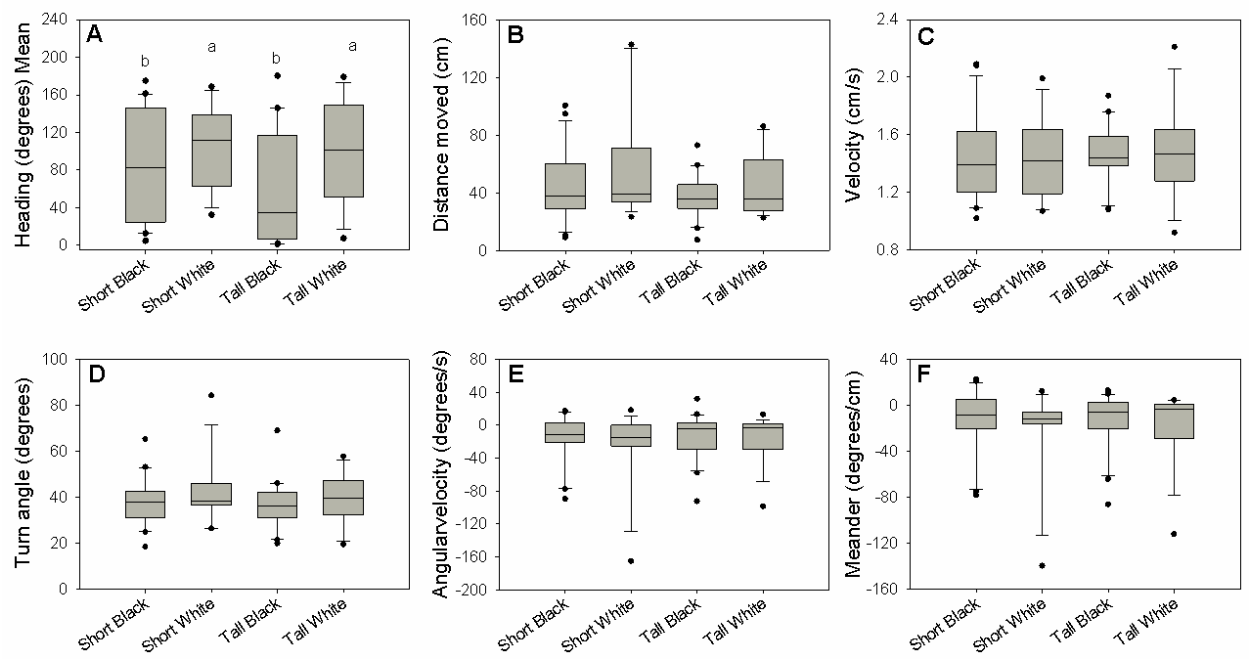


Figure 6.2 Pathway metrics for individual *Tribolium castaneum* subjected to different combinations of heights and color of cuboid shapes. Circular analysis showed that mean heading had significant differences among treatments. Pairwise comparisons performed with Williams Watson test showed differences between: tall black and tall white (F = 10.56, df = 1, *P*<0.05); tall black and short white (F = 11.38, df = 1, *P*<0.05); short black and short white (F = 6.77, df = 1, *P*<0.05); short black and tall white (F = 10.29, df = 1, *P*<0.05). The other pathway metrics did not show significant differences among the treatments when using GLM at a significance level of *P* = 0.05.

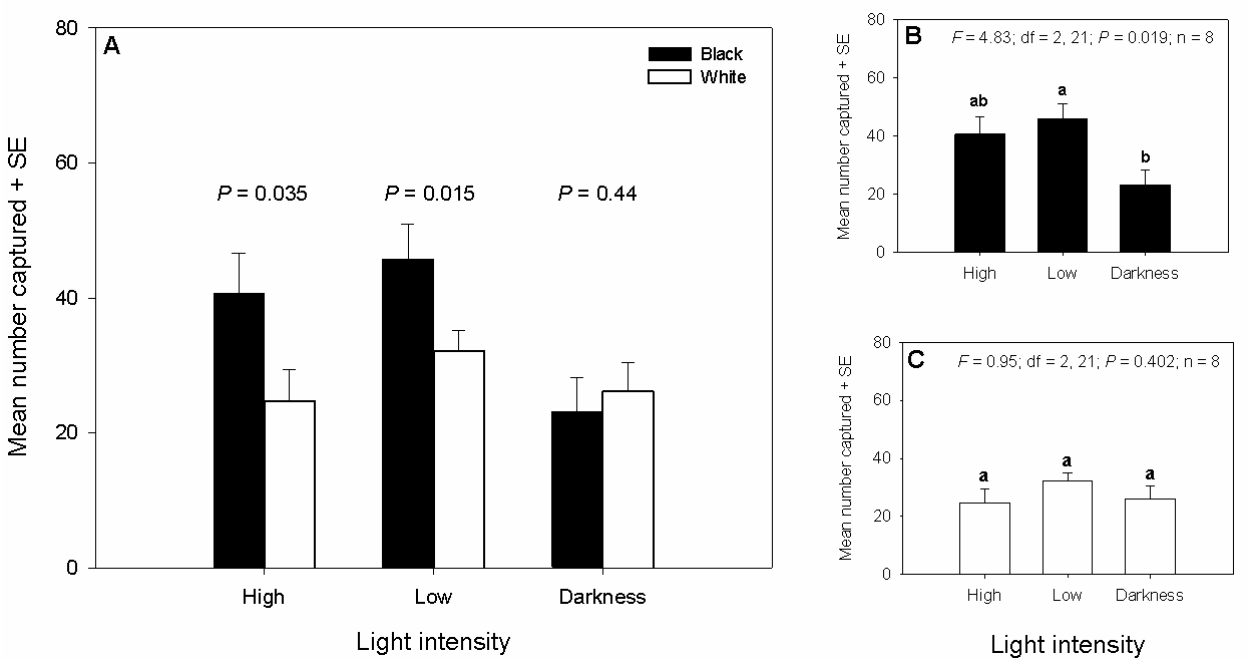


Figure 6.3 Mean + SEM number of *Tribolium castaneum* captured in pitfall traps with different black or white shapes and exposed to different light intensities in a simple environment. (A) Pairwise Mann-Whitney test and t-tests between traps with black and white background at different light intensities; (B) General linear models analysis among number of *T. castaneum* captured in traps with black background at different light intensities; and (C) General linear models analysis among number of *T. castaneum* captured in traps with white background at different light intensities.

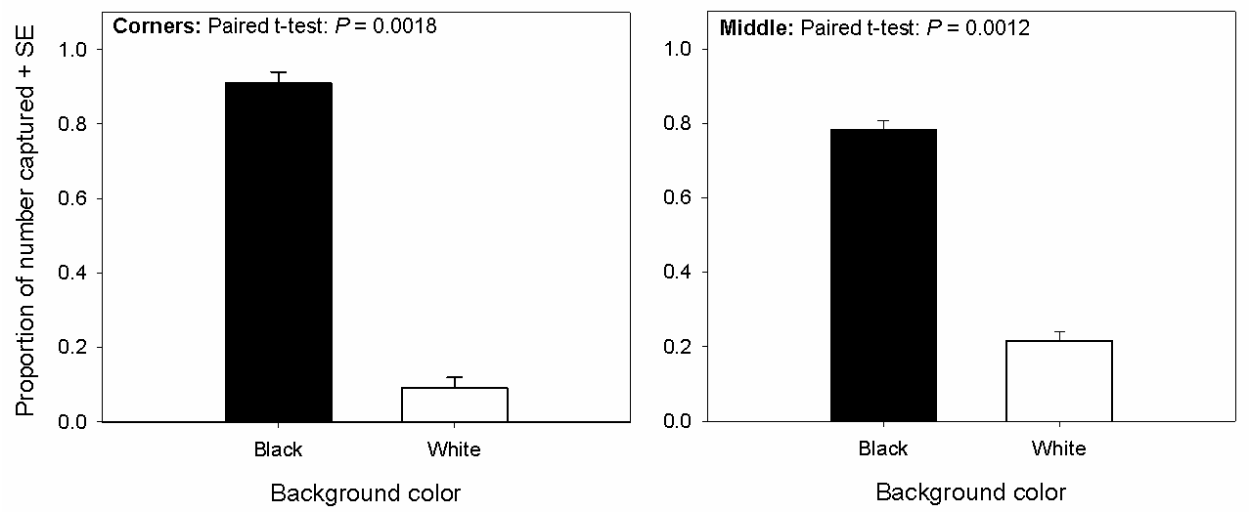


Figure 6.4 Mean proportion + SEM of *Tribolium castaneum* adults captured in pitfall traps with black and white background in a complex environment. Paired t-tests were used to evaluate differences between traps located at the corners ("Corners" graph) and at the middle of two opposite walls ("Middle" graph).

Conclusions

The worldwide phaseout of the fumigant insecticide methyl bromide, an effective compound for killing post-harvest insect pests of food processing and storage facilities, and the high costs associated with management strategies such as heat treatment and other fumigants, requires alternatives that are at the same time efficient and low cost. These alternative tactics are based on Integrated Pest Management (IPM) programs which in turn require better information on the ecology and behavior of stored-product pests to optimize their effectiveness. Also, it is necessary to develop monitoring tools that are effective in detecting these pests before they cause damage. Targeting pest monitoring and management programs can make them more effective, but our understanding of pest distribution patterns and what causes these patterns is still limited in food processing and storage facilities. This dissertation research addressed important knowledge gaps related to the ecology and behavior of stored-product pests, particularly *Tribolium castaneum*, and results can be used directly to guide IPM programs, but also identify important questions for further research.

At a large scale encompassing multiple food storage and processing facilities (Chapter 2), this research showed that there are genetically structured populations of *T. castaneum* with different levels of differentiation which were not correlated with the geographic distance among the populations. The levels of differentiation confirm the population structure, but suggest that gene flow among populations is possible. Populations within a location were not sufficiently unique that individuals could be reliably assigned to given locations based on the microsatellite and other indel loci tested: only 56% of the individuals tested could be correctly assigned to their source population. This reduced level of correct assignment to the source population, limits our ability to create genetic fingerprinting that could be a useful tool for the discovery of infestation sources during the production channel. Because the levels of gene flow from isolated food processing facilities are expected to be reduced, the molecular markers included in this research might not be as adequate as they should be for the population structure analysis and fingerprinting. However, because of the complete sequencing of the genome of this species, an extensive amount of microsatellites are available and could be further exploited.

In the local spatial scale of a food processing facility (Chapter 3), this research evaluated the distribution patterns of stored-product pests present inside and outside the structures of the

facilities. Their outside spatial distribution was mainly associated with factors correlated with proximity to buildings where grain is stored and food is processed, than with the presence of food spillage. Evaluation of data collected using two different types of traps suggest that there is considerable flight activity and that presence of insects on the ground was probably less associated with microhabitat at these locations and more likely due to settling of flying individuals and/or proximity to dispersal sources inside structures. While the potential exists for stored-product insects to exploit these outside spillage accumulations, at the sites evaluated no strong evidence was found to support this having an important role. Major species of stored-product insects such as *Sitophilus* sp., *Oryzaephilus surinamensis*, and *Cryptolestes* spp. were present in all facilities investigated both inside and outside the structures of the buildings. The major species, *T. castaneum*, also occurred at all locations, but most were captured inside suggesting that populations of this species are mostly self-contained inside structures with limited movement from outside. Results of this research showed that the spatial pattern to distribution outside varied among sites and over time at a site, but did not identify specific landscape features, other than proximity to structures, that should be targeted in management programs. However, more research will be necessary at additional locations before making firm conclusions about habitat patterns in the landscape and their use as a resource by stored-product pests.

Focusing a specific food processing facility, this research evaluated patterns in *T. castaneum* genetic diversity over time and spatial distribution of captures by traps. There was evidence for an erosion of genetic diversity occurring inside the food the processing facility (Chapter 4). This erosion suggests strong bottlenecks likely due to decline in population size associated with structural fumigations. That implementation of fumigation has contributed to reduced population levels and caused genetic changes in the population profile, shows that this management strategy is impacting populations. Results of Chapters 2 and 3, and unpublished data not included in the dissertation, support the hypothesis that gene flow of *T. castaneum* at a large (Chapter 2) and small scale is possibly occurring. However, the level of movement may be having a limited effect on the population structure inside the facilities. Therefore, rebounding seems to be the major source for re-infestation resulting from remaining individuals that survive implementation of management strategies and inhabit locations where disturbance is reduced and a continuous source of infestation can be present. Some unpublished data not included in the

dissertation showed that *T. castaneum* can move readily among floors of a flour mill and a small feed facility, but did not directly detect movement outside and into other structures. However, higher outside captures near buildings with high inside densities suggests this might be occurring at low levels that could not be detected.

In Chapter 5, the research addressed some of the factors that can determine the distribution inside food processing facilities and help to elucidate potential spots where foci can be occurring. Also, understating the environment inside and the interactions with captures can aid in implementing and interpreting *T. castaneum* monitoring programs and elucidating processes related to rebound after fumigation described in the previous paragraph. Temperature and spillage accumulation were two important factors associated with distribution of captures inside and these factors are linked in part with the presence of equipment near traps. Therefore, monitoring should consider evaluating variation at these factors in food processing facilities before choosing locations to place traps. This can help to identify sites of infestations even at low levels and therefore determine the use of target management or at least increase the awareness that more attention should be given to the dynamics at these locations.

Finally, this research provided information that *T. castaneum* responds to tall black shapes and this can increase captures when combined with commercial traps that are already available, but that only exploit the response of this species to olfactory cues (Chapter 6). The presence of dark structures in food processing facilities can also be explored with no addition to the cost of monitoring, but with the advantage of improving the levels of capture. It is important, however, to evaluate how this response to black color will be influenced by the response to temperature and spillage variation and all this information should be combined for the improvement of management of *T. castaneum*.

In summary, this research has contributed to a better understanding of the ecology and behavior of *T. castaneum* at different scales of distribution. This work provides fundamental information that contributes to the further understanding of interactions that can be used for improving integrated pest management programs in food processing facilities.

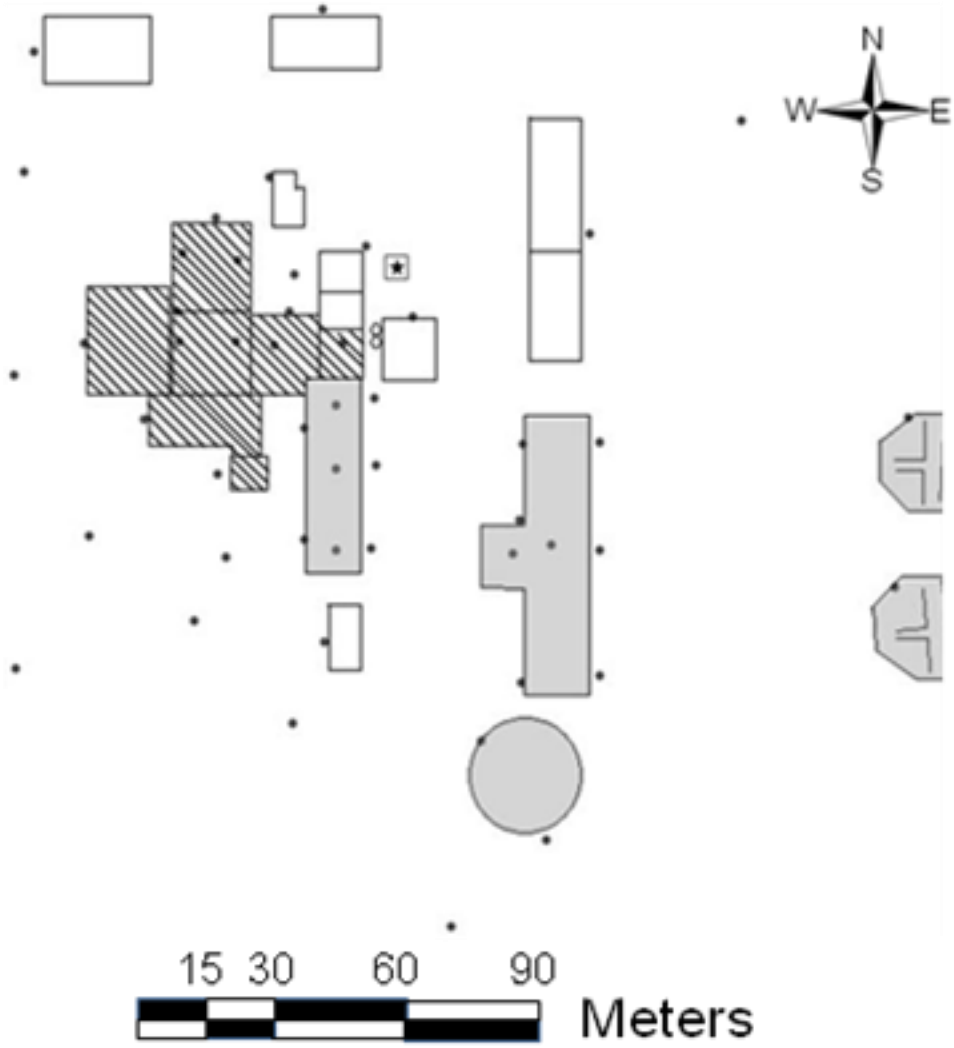
Appendix A - Chapter III: Types of traps used in the spatial and temporal distribution study

Corrugated trap

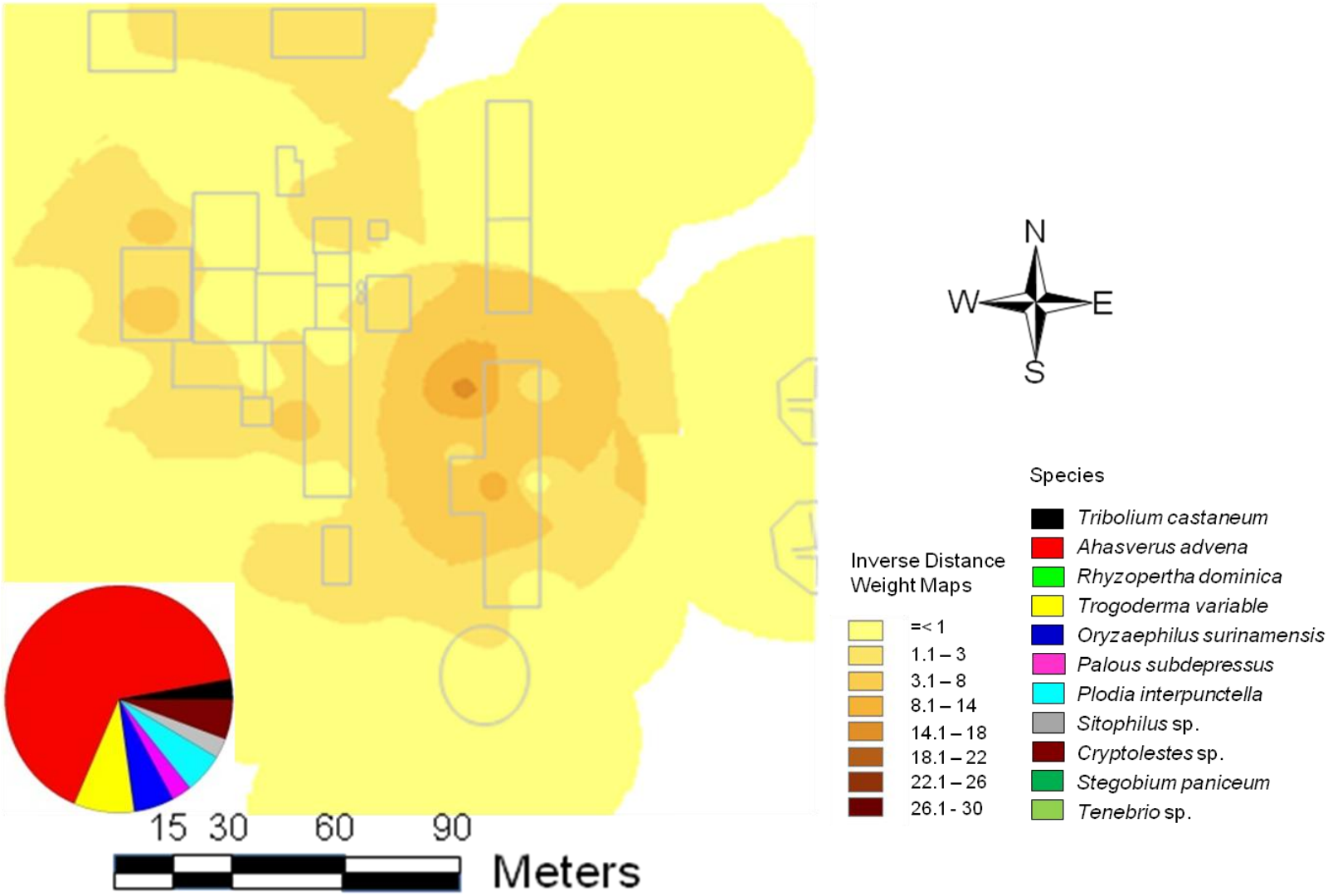
Lindgren trap

Appendix B - Chapter III: Examples of locations where corrugated traps were placed

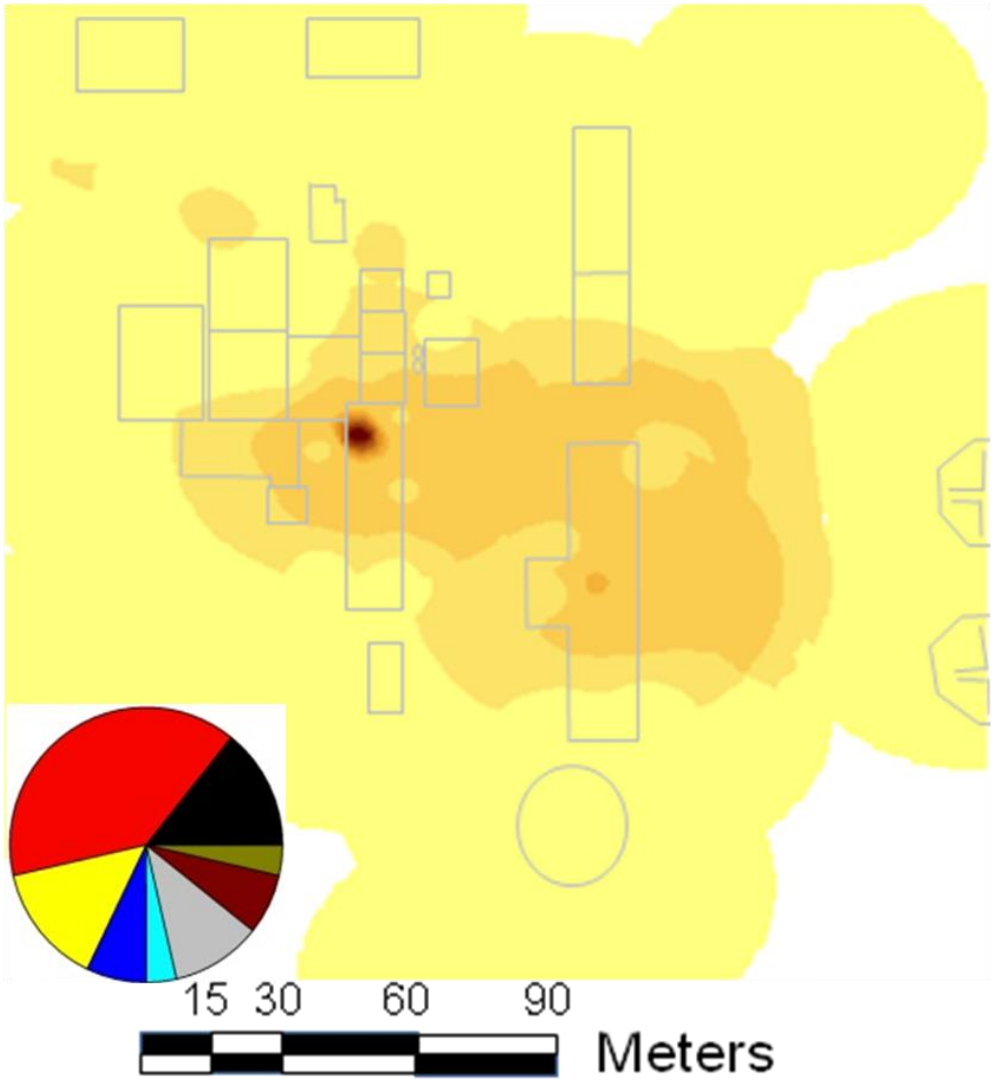
Appendix C - Chapter III: Site A: buildings and trap locations



Appendix D - Chapter III: Site A: 24 to 26 July, 2007



Appendix E - Chapter III: Site A: 04 to 06 September, 2007



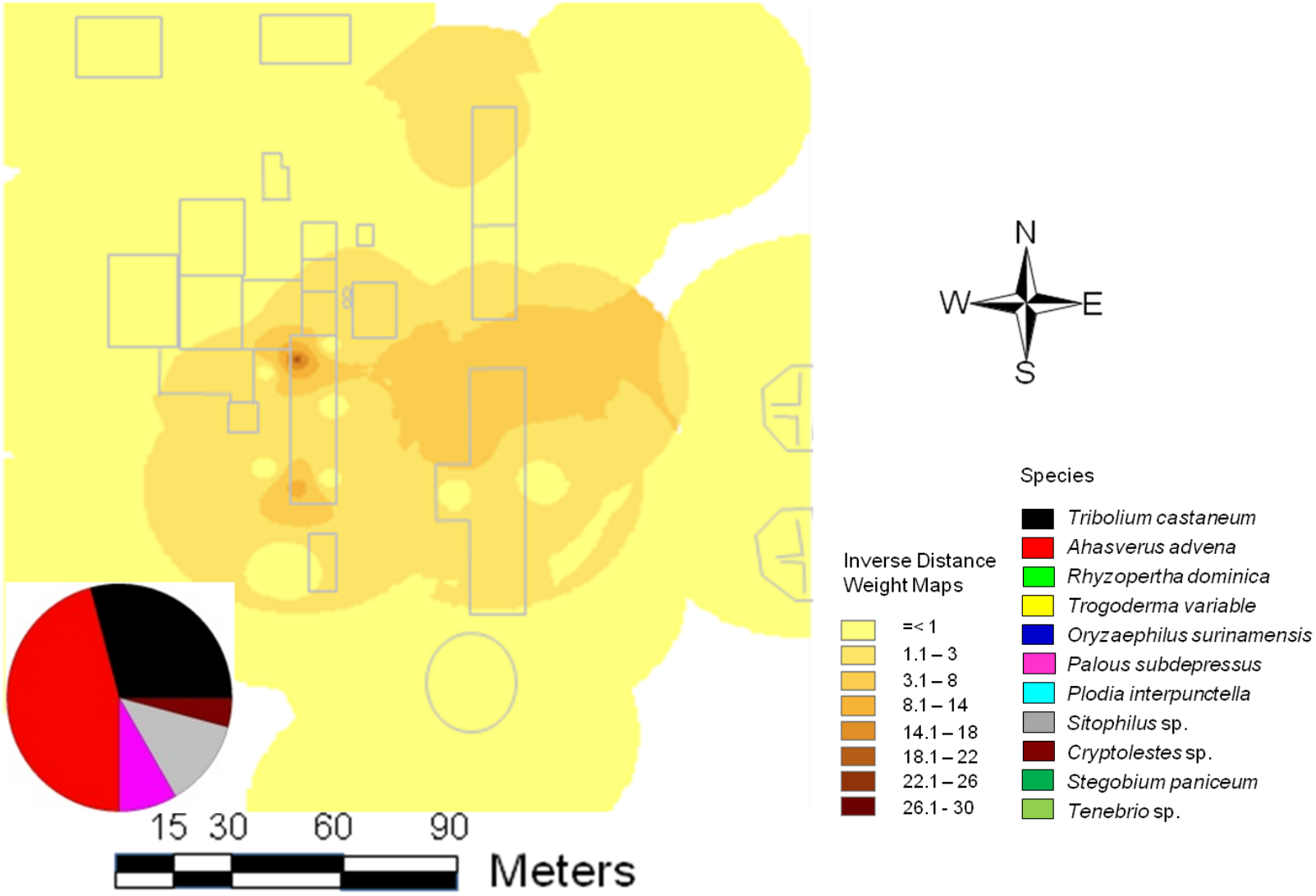
Inverse Distance Weight Maps

- =< 1
- 1.1 – 3
- 3.1 – 8
- 8.1 – 14
- 14.1 – 18
- 18.1 – 22
- 22.1 – 26
- 26.1 - 30

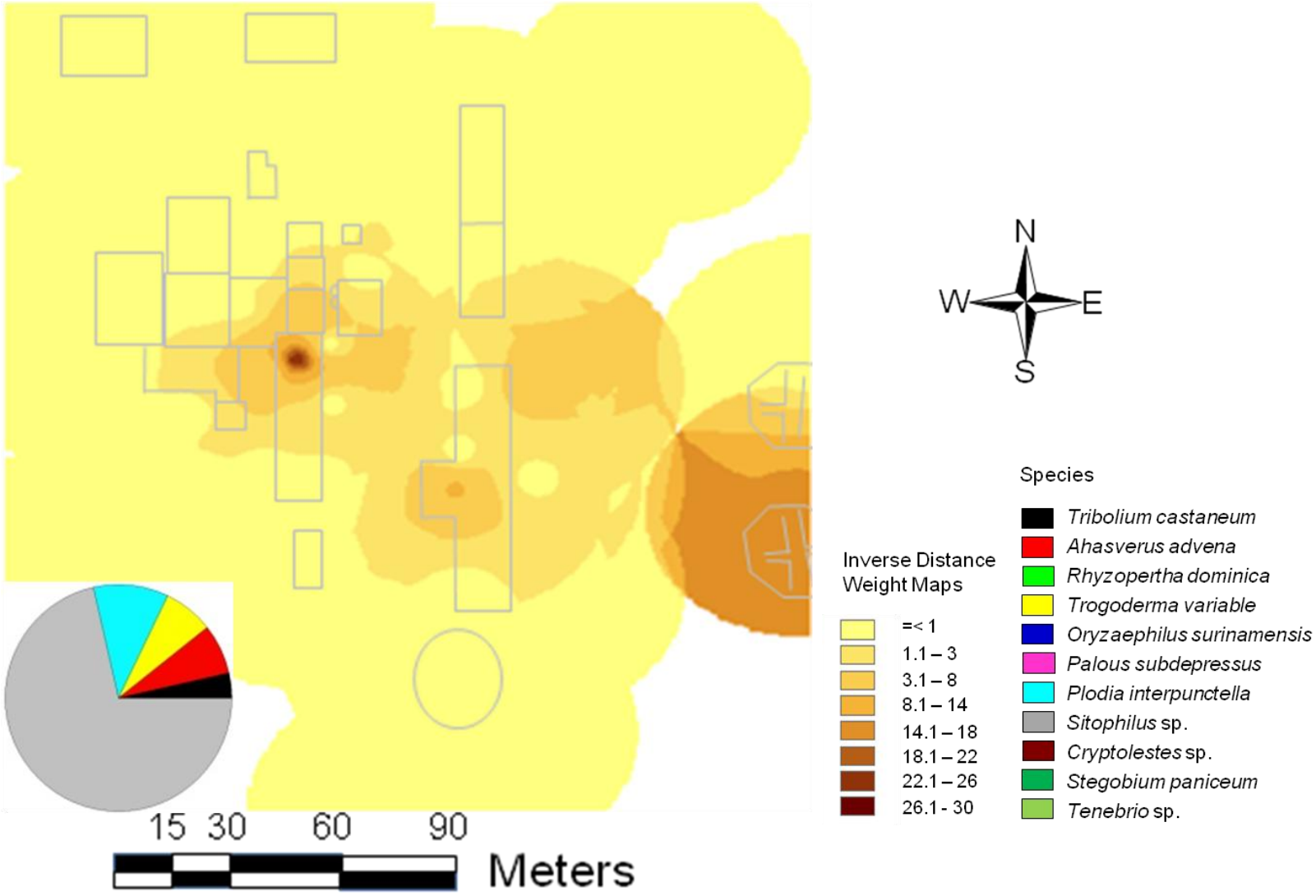
Species

- *Tribolium castaneum*
- *Ahasverus advena*
- *Rhyzopertha dominica*
- *Trogoderma variable*
- *Oryzaephilus surinamensis*
- *Palous subdepressus*
- *Plodia interpunctella*
- *Sitophilus* sp.
- *Cryptolestes* sp.
- *Stegobium paniceum*
- *Tenebrio* sp.

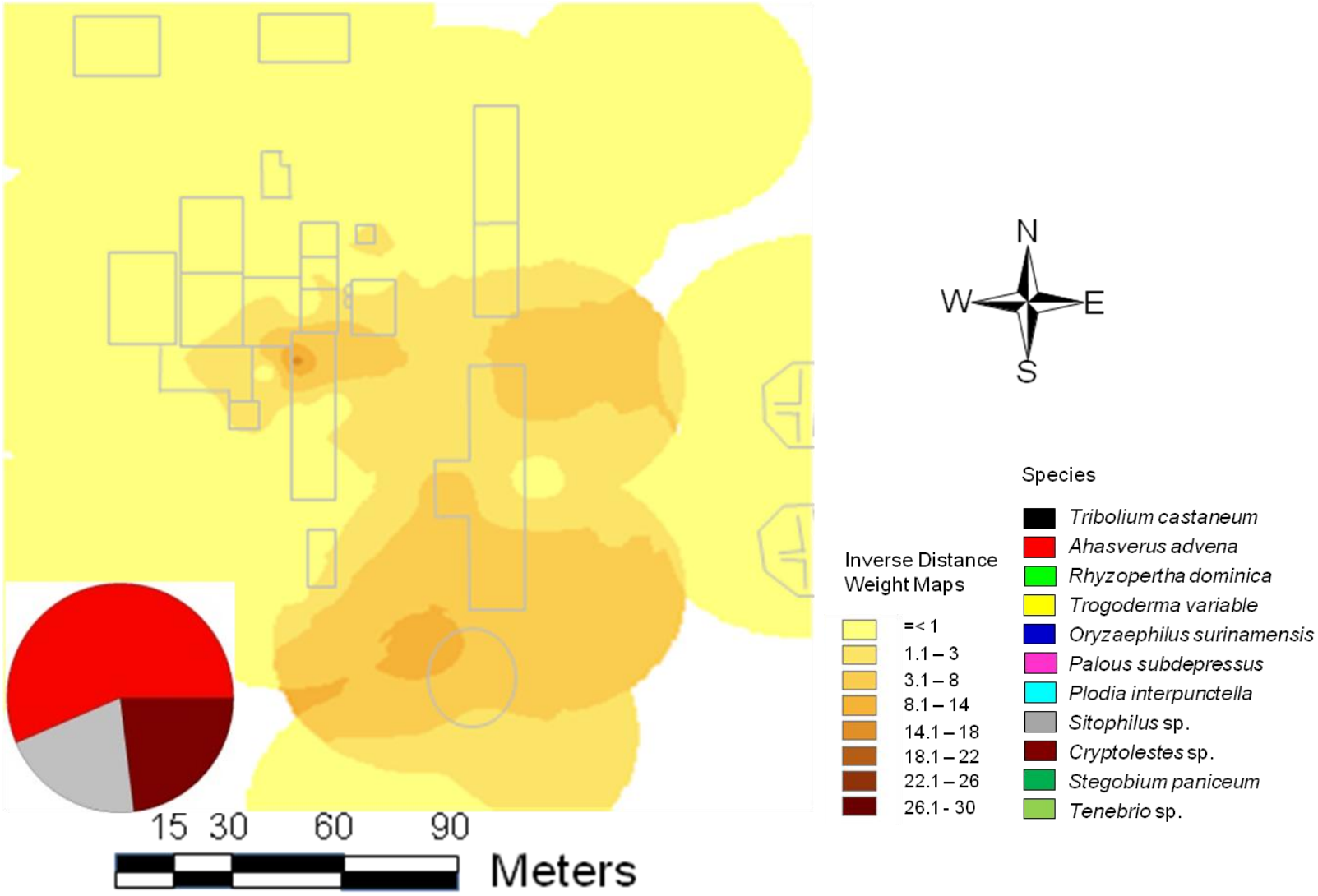
Appendix F - Chapter III: Site A: 13 to 15 October, 2007



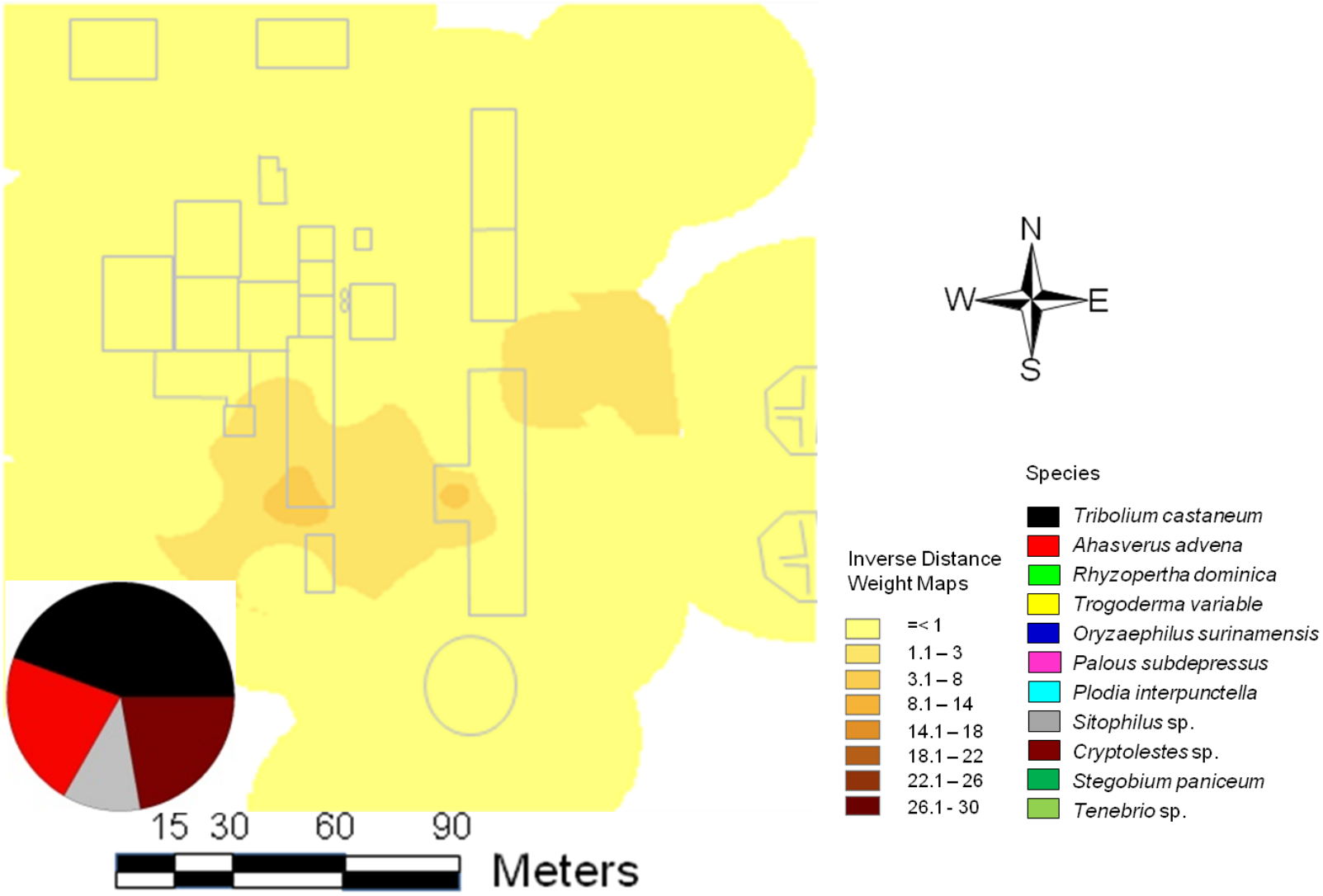
Appendix G - Chapter III: Site A: 19 to 21 August, 2008



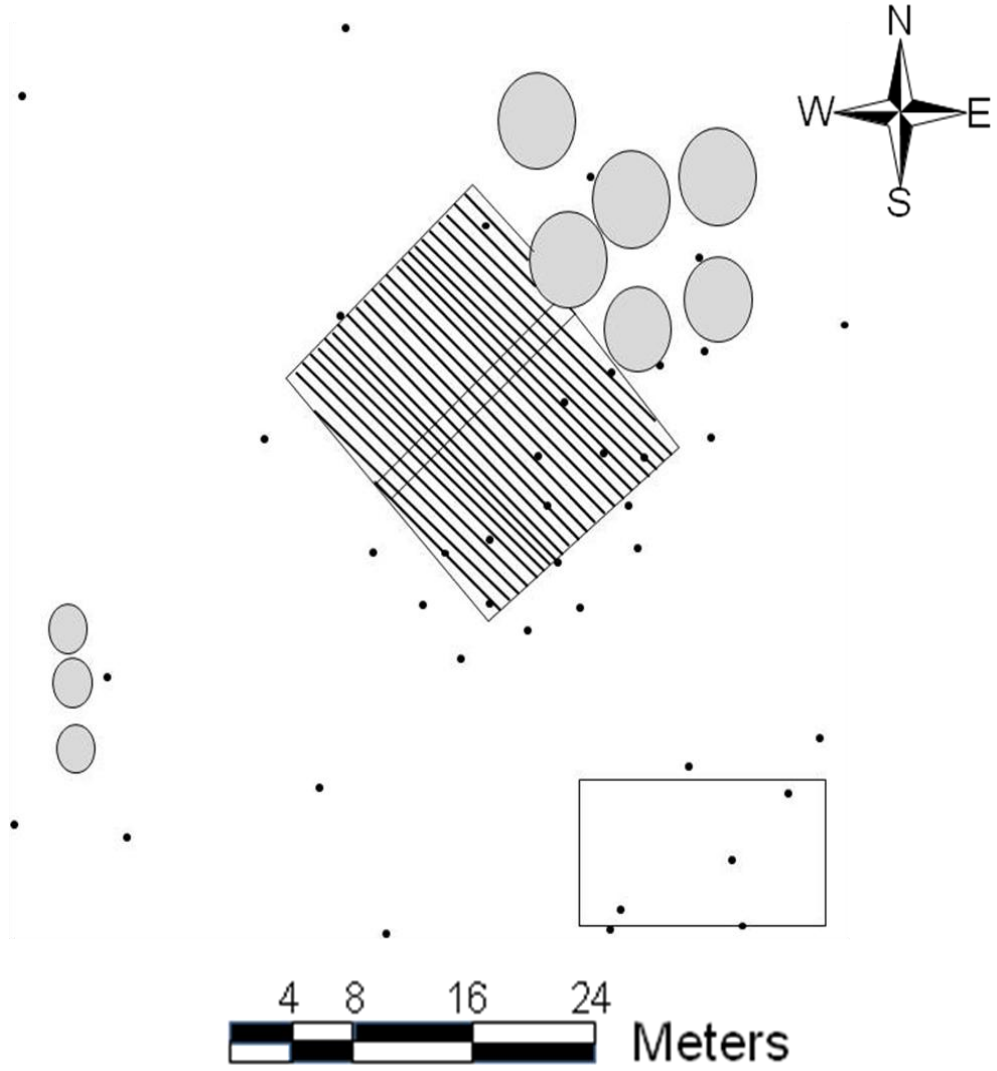
Appendix H - Chapter III: Site A: 16 to 18 September, 2008



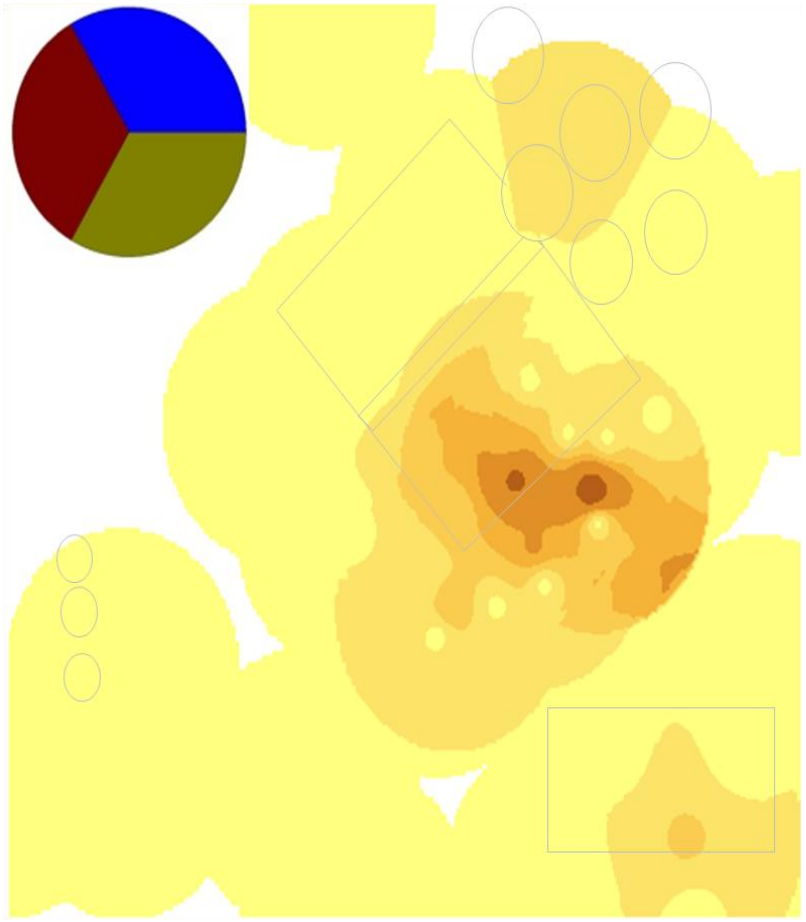
Appendix I - Chapter III: Site A: 28 to 30 September, 2008



Appendix J - Chapter III: Site B: buildings and trap location



Appendix K - Chapter III: Site B: 01 to 03 August, 2007



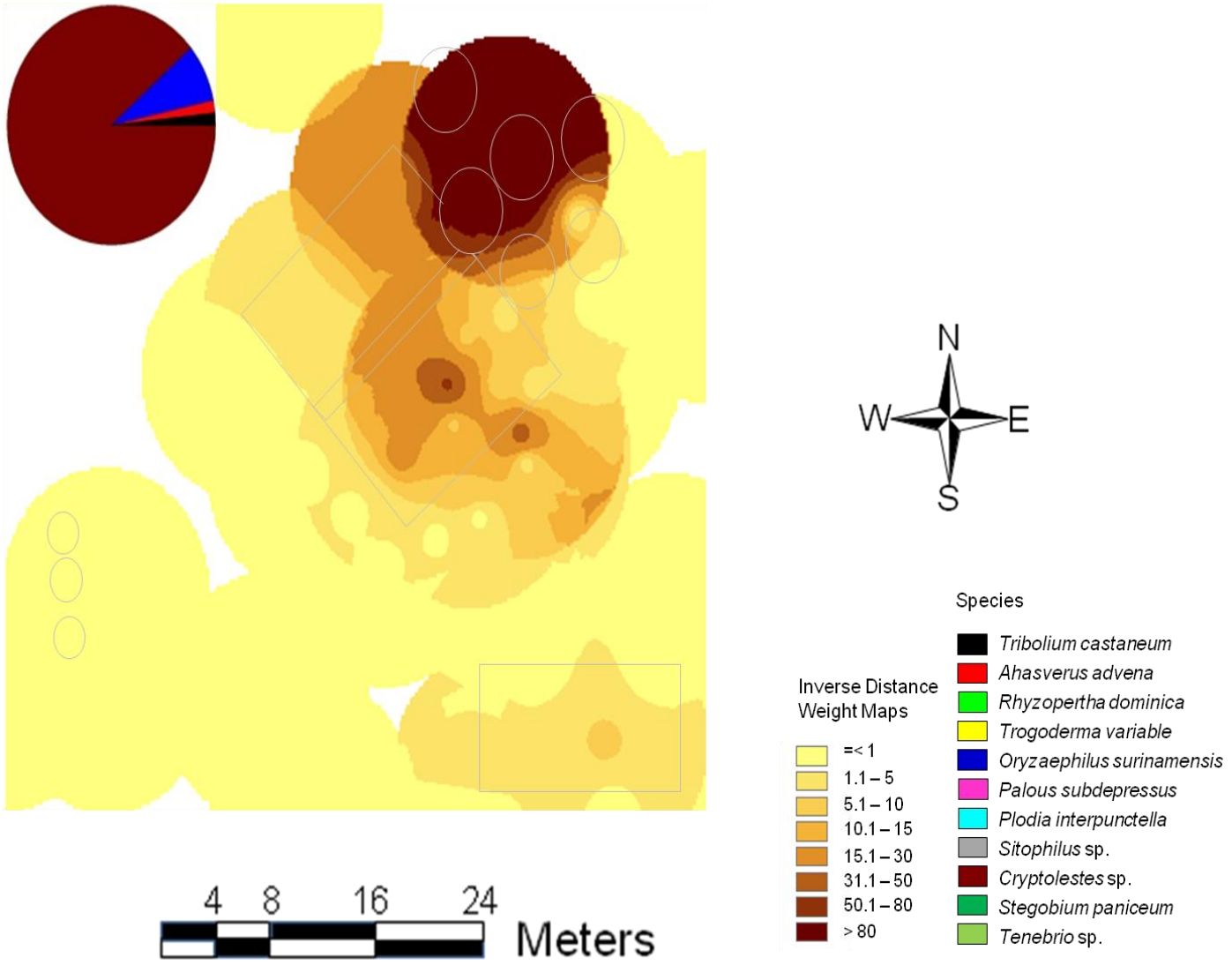
Species

- *Tribolium castaneum*
- *Ahasverus advena*
- *Rhyzopertha dominica*
- *Trogoderma variable*
- *Oryzaephilus surinamensis*
- *Palous subdepressus*
- *Plodia interpunctella*
- *Sitophilus* sp.
- *Cryptolestes* sp.
- *Stegobium paniceum*
- *Tenebrio* sp.

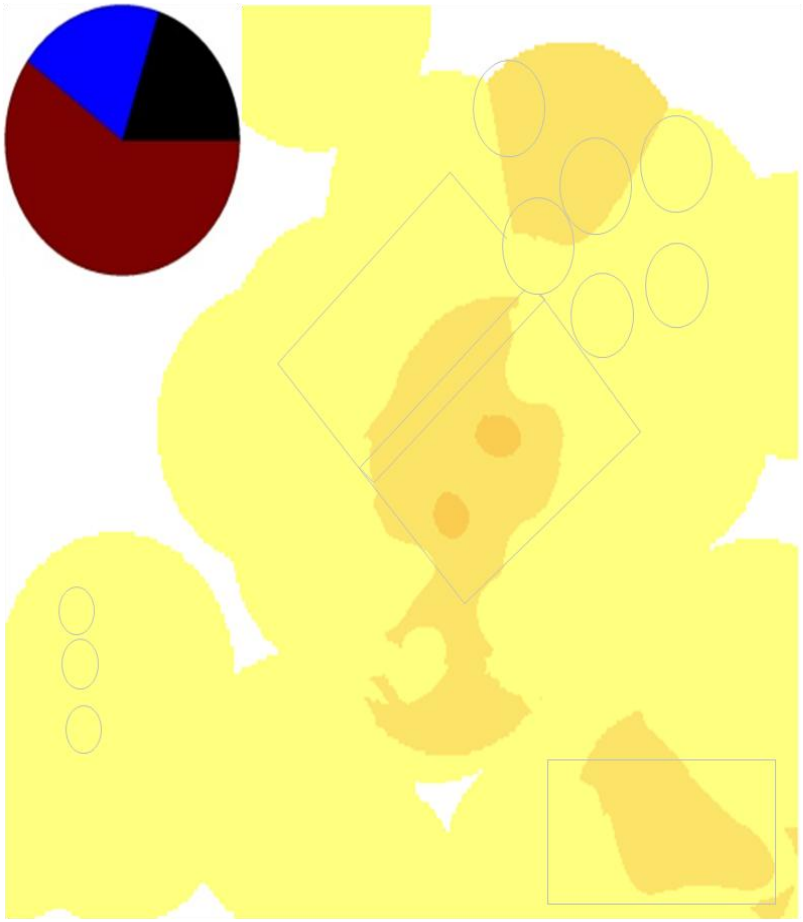
Inverse Distance Weight Maps

- =< 1
- 1.1 – 5
- 5.1 – 10
- 10.1 – 15
- 15.1 – 30
- 31.1 – 50
- 50.1 – 80
- > 80

Appendix L - Chapter III: Site B: 20 to 22 August, 2007



Appendix M - Chapter III: Site B: 25 to 17 September, 2007



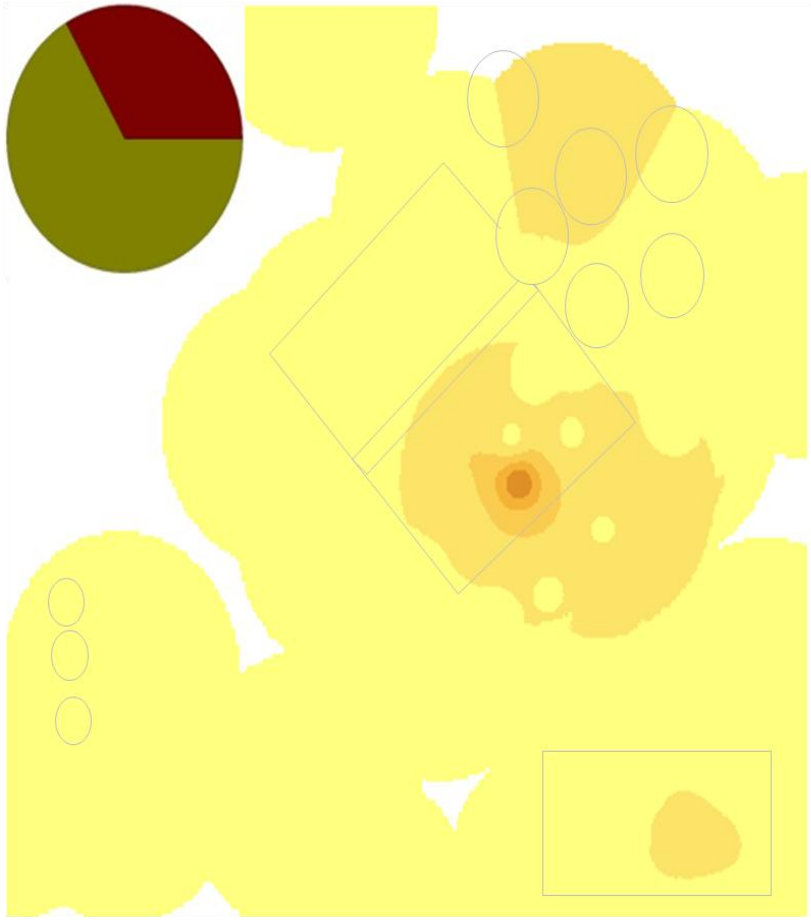
Species

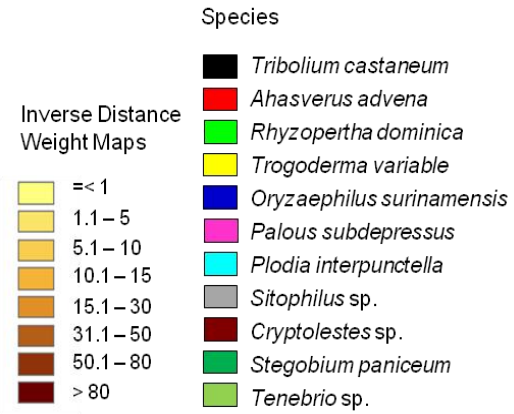
- *Tribolium castaneum*
- *Ahasverus advena*
- *Rhyzopertha dominica*
- *Trogoderma variable*
- *Oryzaephilus surinamensis*
- *Palous subdepressus*
- *Plodia interpunctella*
- *Sitophilus* sp.
- *Cryptolestes* sp.
- *Stegobium paniceum*
- *Tenebrio* sp.

Inverse Distance Weight Maps

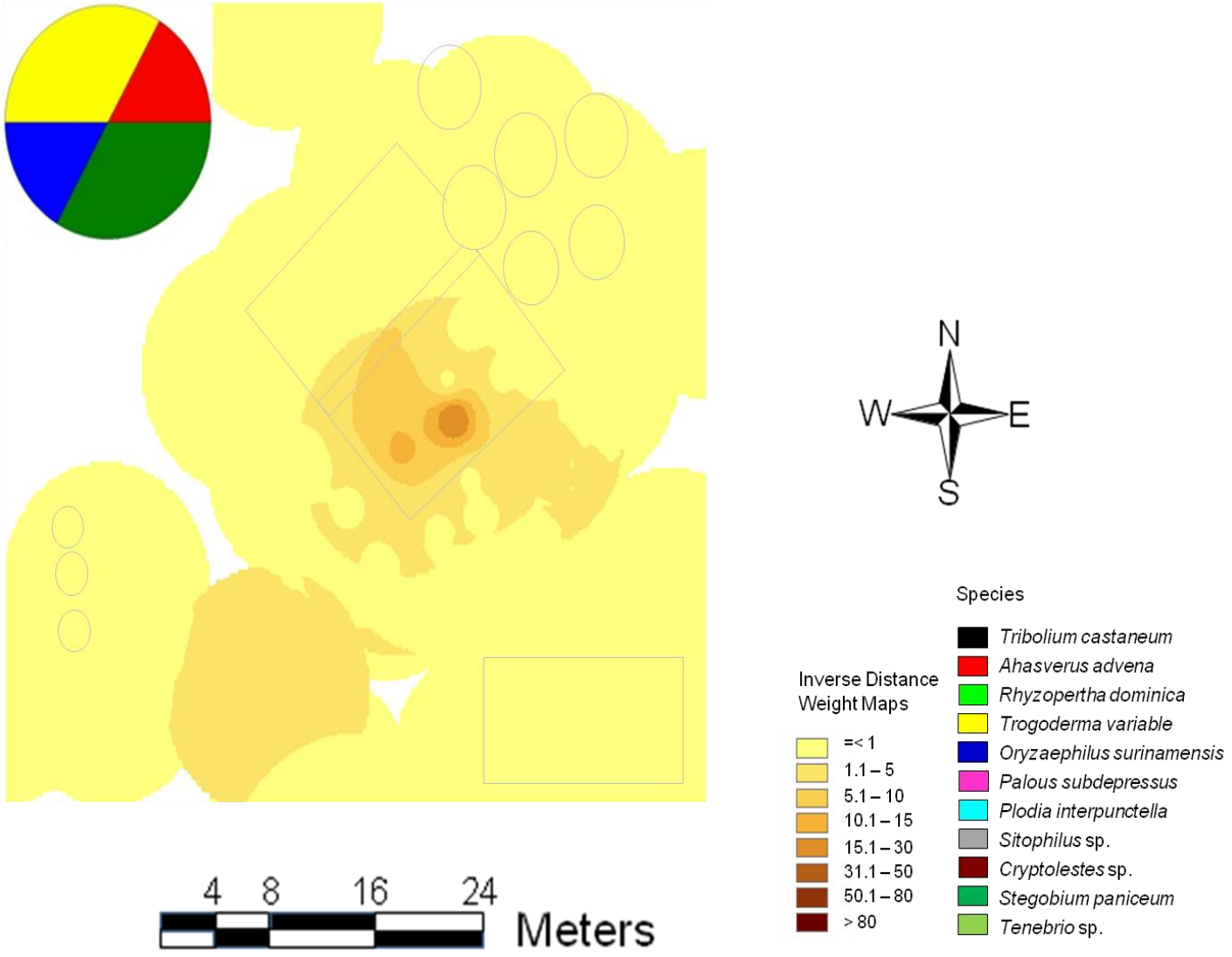
- =< 1
- 1.1 – 5
- 5.1 – 10
- 10.1 – 15
- 15.1 – 30
- 31.1 – 50
- 50.1 – 80
- > 80

Appendix N - Chapter III: Site B: 30 July to 01 August, 2008

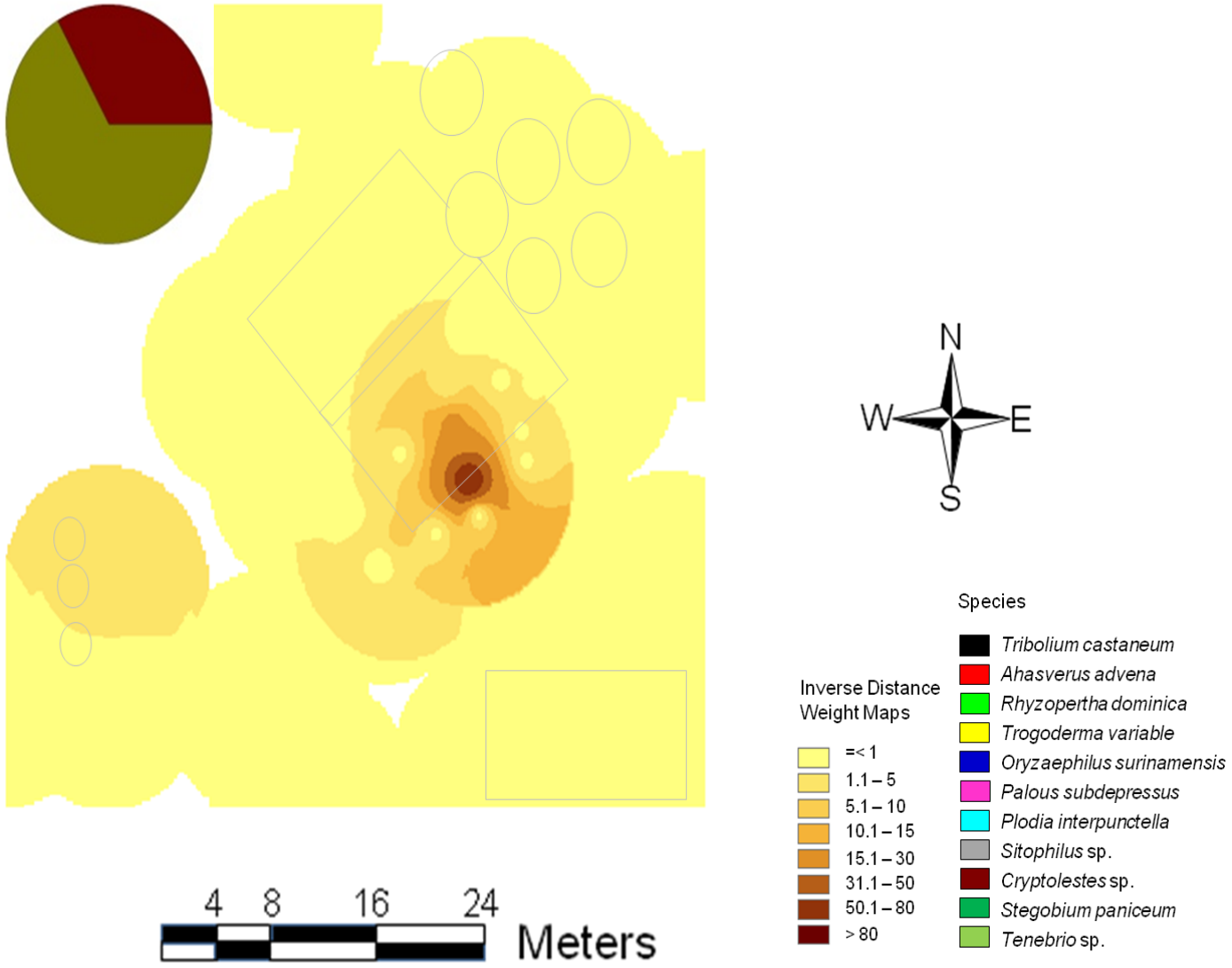




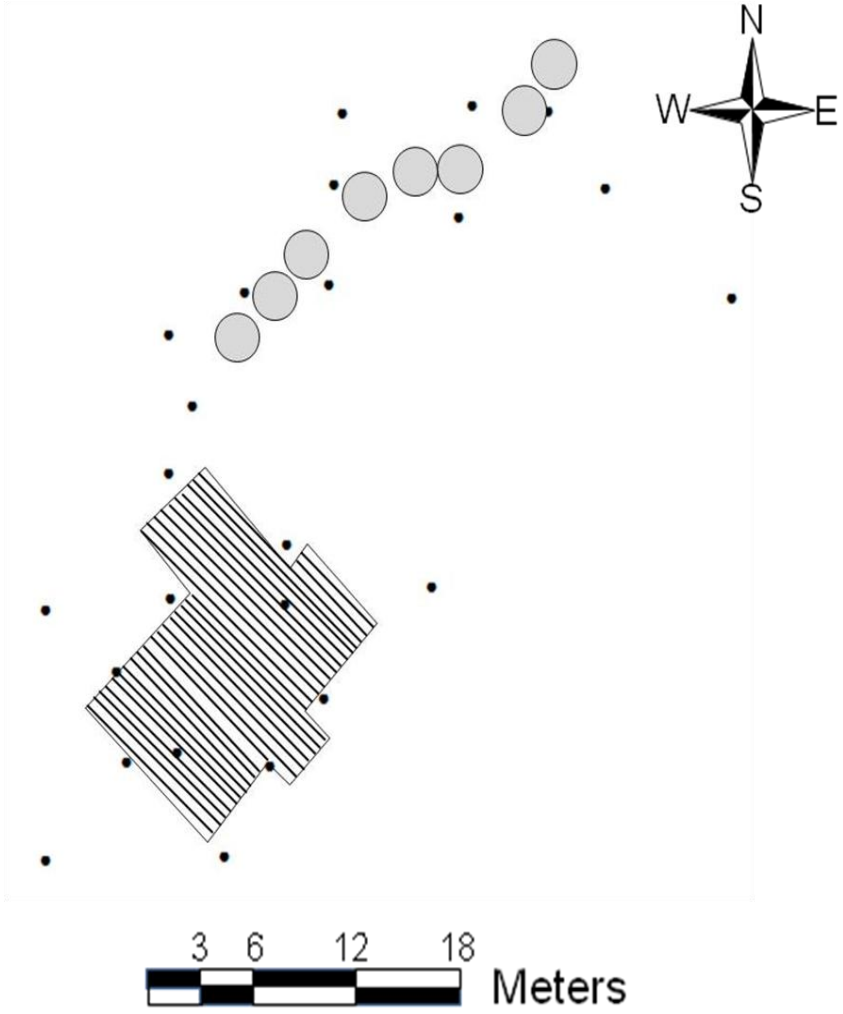
Appendix O - Chapter III: Site B: 13 to 15 August, 2008



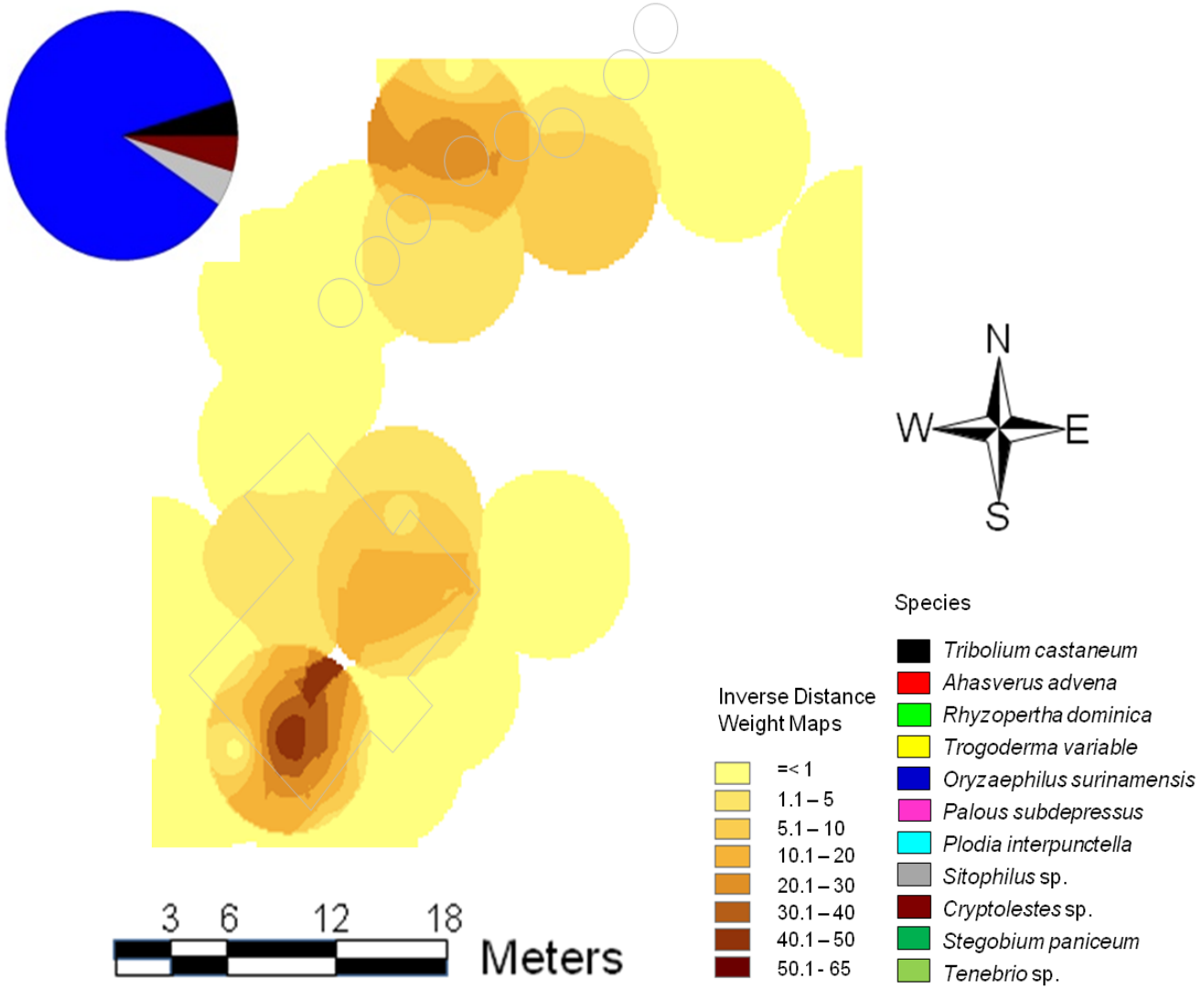
Appendix P - Chapter III: Site B: 07 to 09 October, 2008



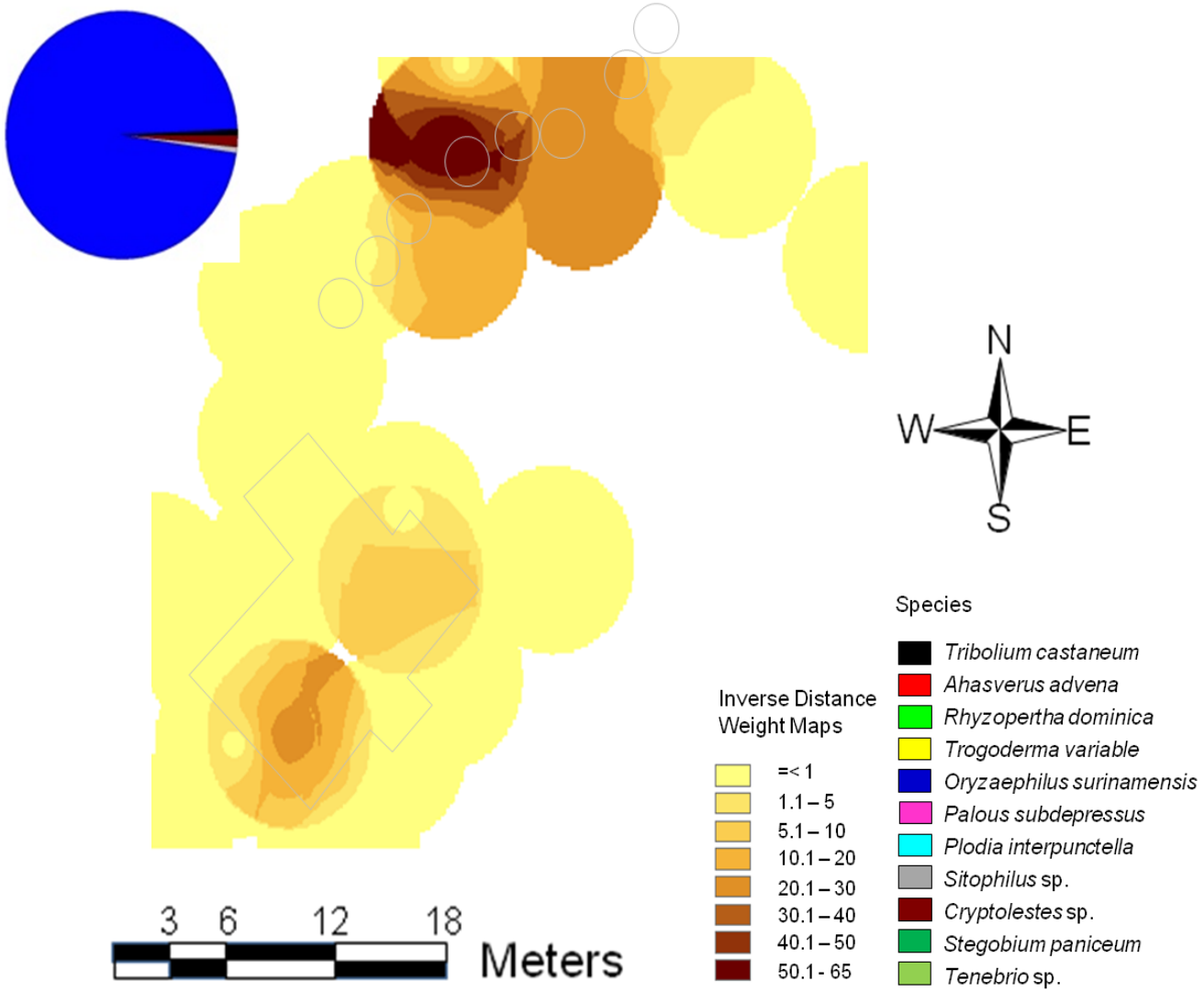
Appendix Q - Chapter III: Site C: buildings and trap locations



Appendix R - Chapter III: Site C: 01 to 03 August, 2007



Appendix S - Chapter III: Site C: 21 to 23 August, 2007



Appendix T - Chapter III: Site C: 21 to 23 August, 2007