ORIENTATION AND DISPERSAL OF *CRYPTOLESTES FERRUGINEUS* (STEPHENS) (COLEOPTERA: LAEMOPHLOEIDAE) IN RESPONSE TO VARIOUS SEMIOCHEMICALS

by

STEPHEN M. LOSEY

B.S., University of Nebraska-Lincoln, 2012

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2015

Approved by:

Major Professor
Dr. Thomas W. Phillips

Abstract

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), is a very common worldwide pest of stored grains. The orientation of *C. ferrugineus* beetles to various semiochemicals was studied in both the laboratory and field. In laboratory experiments glass two-choice pitfall bioassay dishes were used. Mixed-sex populations of beetles responded positively and significantly to cucujolide I and II separately and in combination compared to controls. Bioassays using the two aggregation pheromones of the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Dominicalure 1 and 2 together elicited unexpected significant attractive responses by *C. ferrugineus* as did assays with the synthetic pheromones from other species. Bioassays showed that *C. ferrugineus* were not attracted to either corn meal or rolled oats, but they were attracted to commercial grain-based lures and also to ethanol, a natural fermentation product from grains. Several bioassays were conducted to test the attractiveness of naturally produced beetle volatiles to *C. ferrugineus*, but these showed no orientation to volatiles from either the beetles or their food only. Field tests were conducted in Kansas to test attractiveness of the synthetic pheromones cucujolide I and II in lures together with wheat versus wheat only as a control using Lindgren funnel traps. Field tests in 2014 with cucujolide II pheromone lures showed a significant response by feral *C. ferrugineus* when tested against wheat. Other field tests to examine dispersal of *C. ferrugineus* relative to grain storages showed that more *C. ferrugineus* were caught on the western side of grain bins compared to other cardinal directions at two different locations. Results from field and laboratory studies suggest that semiochemical-based tools can be developed to study dispersal behavior in field populations of *C. ferrugineus.*

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to thank a number of people that helped me along the way. First, I am thankful and grateful to my advisor, Dr. Thomas Phillips, for taking me under his wing and teaching me about the world of stored-product entomology. He spent countless hours teaching, tutoring and helping me along the path to this degree. Next, I would like to thank my committee members, Dr. Greg Daglish and Dr. Bhadriraju Subramanyam for their help, advice, and encouragement along the way. Another person I would like to thank is Dr. John Ruberson for countless meetings, inquiries, and advice; he has helped me with immensely over these last two and a half years. He did it all with a smile on his face. Members of Dr. Phillips's laboratory cannot go unrecognized. I would like to thank Jaime Aikins for his help with chemicals and for bringing hilarious newspaper articles in for a good laugh; Barbara Amoah and Salehe Abbar for their advice on many different occasions and introducing me to their cultures, and Drs. Mukti Ghimire, Zhaorigetu Chen, and Ozgür Saglam, who not only introduced me to other cultures, but helped me grow as a laboratory member as well. I would like to especially thank Edwin Afful, Aaron Cato, and Eric Cordeiro who put up with so much of my terrible jokes and pranks that I'm surprised they stayed sane. They all helped me with data collection, interpretation and statistical analysis as well. I'm grateful to the many local farmers, farmer cooperatives and businesses who allowed me to trap and collect data on their land. This degree happened because of them. Food and pheromone lures were graciously supplied by Trece Inc., Alpha-Scents and Research Directions. Drs. Raymond Cloyd and James Campbell provided key infrastructural assistance. I would like to extend a special thanks to my sources of funding for this project, the Plant Biosecurity CRC and the Kansas State University Department of Entomology. Lots of people inside this department helped me with forms, procedures, and ideas. To these people I am eternally thankful for their help. Last, but certainly not least, I am thankful for my family. Without their help, kind words, tough love, ideas and examples I wouldn’t be where I am today. Specifically my mother, father, brother, step-father and fiancée. Kathy and Jeff Hollifield pushed me to keep on tasks, to not give up, and provided very useful chemical information, Mike Losey and Jerry Losey taught me that I get what I earn, and made sure I was always on my toes asking countless times if I was done with my thesis yet. Dr. Tiffany Mainini helped me with so much throughout my degree program that I feel lucky to have met her. Without her I would be taking two steps backward and one step forward. For these family members and others I am thankful, they are my backbone.

Dedication

I would like to dedicate this Masters of Entomology thesis to my late grandmother, Donna Jean Mahaffey. She was the person who really encouraged me to grow my interest in plants and animals from a very young age. When she knew that I was leaning more towards insects she insisted on teaching me that insects need plants and to learn the important plants where I would find the insects I liked most. She bought and planted these plants on her property so that I could go out and search through them to find the insects I liked. I learned a lot from her and the countless field guides she gave me. I am eternally thankful for her and her knowledge, the countless things she taught me, and her unwavering patience.

Chapter 1 - Introduction and Literature Review

Introduction

Stored-product insects pose a serious threat to stored, post-harvest grains. A 1992 article estimated annual losses that stored-product insects were responsible for at nearly $500 million in damages (Harein and Meronuck 1992). Insect pests can cause damage to stored grain in different ways, such as raising the temperature of stored grains, directly consuming the grain, contaminating grains with insect fragments and waste and also by raising the moisture content in the grain, thus allowing mites and fungi to proliferate (Sinha and Wallace 1985, Gorham 1991). It is important to control these pests because of the amount of damage they can inflict to stored commodities. Additionally, insect contamination in raw commodities can result in price discounts at the time of sale (Reed et al. 1989). The presence of live stored-product insects in processed food makes the food adulterated, and there are standards for the presence of insect fragments in wheat flour. These problems are a constant challenge to farmers, elevator managers, and grain-processing companies.

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), is the most common secondary grain pest in the United States (Cuperus et al. 1986). This small cosmopolitan pest infests 69 commodities around the world (Hagstrum and Subramanyam 2009). In addition to stored wheat, *C. ferrugineus* has been recorded infesting other primary agricultural products including, but not limited to, black pepper, chili, cocoa, coffee bean, cotton seed, dried fruits, hemp, licorice, oilseeds, peanuts, rice, dried tomatoes, and yams. *Cryptolestes ferrugineus* has been shown to cause heating when infesting stored grain (Sinha 1961), and can damage wheat by consuming the germ. Both adults and larvae feed mostly on damaged kernels, grain dust, and fungi in stored grain. Throne and Culik (1989) reported that *C. ferrugineus* developed faster and adults deposited more eggs when the size of cracked corn pieces were increased.

Since *C. ferrugineus* are found worldwide and in such a large number of commodities, there are control measures put into place to keep populations in check. Some of these control measures include sanitation, fumigation, and residual insecticide applications. However, before a control measure is to be deployed, the insect must be detected.

Various methods have been studied for detecting *C. ferrugineus* adults in stored-grain or in stored-grain structures. Perforated probe traps (Subramanyam et al. 1993) inserted below the grain surface are effective in capturing *C. ferrugineus* adults (Reed et al. 1991, Phillips et al. 2000). Adults of *C. ferrugineus* walking through the grain mass encounter a probe trap and fall into it. There are several factors that affect the capture of *C. ferrugineus* adults in traps. These include the temperature of the grain mass which can affect insect activity, density of insects, and trap location within the grain mass (White and Loschiavo 1986, Fargo et al. 1989, Subramanyam et al. 1993, Fargo et al. 1994, Hagstrum 2000). Toews et al. (2005) showed that the number of *C. ferrugineus* captured in probe traps at the grain surface and one meter below the surface were similar.

Biology of the Rusty Grain Beetle, *Cryptolestes ferrugineus*, (Stephens)

The adult rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) is an approximately 3 mm long, reddish-brown dorsoventrally flattened beetle. The life cycle of the rusty grain beetle, from hatching of the egg to the emergence of the adult, can take as little as 19 days under ideal conditions of 35°C and 75 % relative humidity (Rilett 1949). Development will cease to occur if the grain moisture drops below 12% and/or the relative humidity drops to 40% or below (Canadian Grain Commission 2013). Female *C.*

ferrugineus are able to lay 2-3 eggs daily, usually between kernels of grain or in splits and cracks within the grain's surface (Rilett 1949, Hagstrum et al. 2012). As female *C. ferrugineus* age they lay fewer and fewer eggs, something that is uncommon among the stored product insects (Arbogast 1991). The average fecundity per female is 242 eggs (Davies 1949). Eggs are somewhat large, compared to the total length of the adult females. Each egg averages 0.76 mm in length while adults average only 2 mm in length (Rilett 1949). Eggs are white and shaped as a stretched, oblong oval.

After 4-5 days a small, light white larva, slightly longer than the egg, emerges. Larvae immediately begin to search for food, preferring the wheat endosperm, especially if it is already being attacked by fungi (Hagstrum et al. 2012). If conditions are right, one wheat kernel can carry one larva through development to the pupal stage (Rilett 1949). If there is a lack of food, the beetles will become cannibalistic and feed on pupae and eggs (Sheppard 1936).

There are four instars, and all instars can be very mobile when searching for new food. Fully grown fourth-instar larvae are robust, hairy, and can be up to 4 mm long (Canada). Pupae are naked and can only move their abdomens back and forth and will do so readily when disturbed. The insect stays in the pupal stage for around four days, but starts to turn to a reddish brown color on the third day. The adult insect emerges with tan colored elytra and can take close to 24 hours to become its reddish brown adult color.

Adults copulate within the first 24 to 48 hours of emergence and females oviposit shortly thereafter. Copulation in *C. ferrugineus* has been recorded taking place for up to 235 minutes, with a 20-minute break during the process, and then the same the following day. Copulation occurs with the male and female pairing end to end (Rilett 1949).

Adult *C. ferrugineus* are long-lived with some having been shown to live up to 1.5 years in the laboratory (White and Bell 1993), but most average eight months (Rilett 1949, Hagstrum et al. 2012). This pest has been shown to be a good flier when the temperature is above 30°C (Rilett 1949). A combination of adults being able to withstand temperatures as low as -20.4°C inside grain, being a good flier and being able to withstand absence of food for up to 8 days allow for infestations of *C. ferrugineus* to spread and persist (Hagstrum et al. 2012).

Management Tactics

Many management tactics are used to keep *C. ferrugineus* populations in check. One of the most popular management tactics is to fumigate with phosphine gas (Hagstrum et al. 2012). However, due to the widespread presence of *C. ferrugineus* and the widespread use of phosphine, *C. ferrugineus* has evolved resistance to this compound (Konemann et al. 2014).

Phosphine can be applied in different ways and is extremely effective when used correctly. Grain elevator operators use phosphine as a fumigant to kill stored grain pests, including *C. ferrugineus*, on a calendar schedule so that they can meet the demand of milling companies for insect-free grain (Toews et al. 2005). When measures are not taken to properly seal fumigated grain storage areas, low concentrations of the gas will allow insects to survive and resistance can be selected if resistant genes are present in the pest population. Resistant insects can spread phosphine resistance genes to other grain bins and to distant geographic regions through flight and the grain trade. Therefore, information on flight and dispersal of *C. ferrugineus* can help in understanding the dynamics of phosphine resistance spread.

Pheromones

Borden et al. (1979) were the first to report on the aggregation pheromones produced by male *C. ferrugineus* while feeding that are attractive to both sexes. These pheromones act as intraspecific signals, and were first isolated and identified by Wong et al. (1983). The aggregation pheromone consists of two macrolide lactones, and these were identified as ferrulactone I and II, (E, E)-4, 8-decadien-10-olide and (S)-(Z)-3-dodecen-11-olide. These chemicals are commonly referred to as cucujolide I and II, respectively. A 9:1 mixture of cucujolide I: cucujolide II was determined to generate the highest response in a laboratory study compared with other mixture ratios (Wong et al. 1983). Loschiavo et al. (1986) conducted the first field study using synthetic versions of the male-produced aggregation pheromone against adults in a grain bin in Canada and found that the trap containing synthetic pheromone did not catch more adults than the control trap.

Pheromone Traps

Pheromone-baited traps have been used to detect stored-product insects since the black carpet beetle, *Attagenus unicolor* (Brahm) (Coleoptera: Dermestidae), pheromone was first described by Silverstein et al. (1967). Many of the pheromones for stored-product pests are known and some are commercially available for use in population monitoring and detection (Phillips 1997; Phillips et al 2000).

There are several trap designs to capture *C. ferrugineus* adults within and outside grain storage bins. In stored commodities, *C. ferrugineus* adults can be captured using perforated probe traps placed inside grain masses and with sticky traps placed outside of grain bins (Nansen et al. 2004). Lindgren multiple funnel traps have been used to collect many types of flying insects, in warehouses (Martinez and Granovsky 1986) or outdoors (Lindgren 1983). The

Lindgren funnel trap is the standard for bark beetle and wood-boring beetle trapping. A rubber septum that has been impregnated with synthetic aggregation pheromone is hung by wire inside the trap. As insects respond to the pheromone they encounter a funnel and fall down through one or more funnels into the collection cup on the bottom. Insects trying to escape the trap will typically take flight from the inside funnel surface at a perpendicular aspect and encounter the funnel immediately above and fall back down into the collection cup.

Inside stored-grain there are many microenvironments that need to be accounted for. Getting an accurate representation of which and how many insects are in the grain mass is possible with a few sampling methods. Pheromone traps are not used inside of grain masses, but are used frequently outside of storage facilities. Once those levels are determined, a plan can be put together to treat the grain on an "as needed" basis.

Objectives

The first objective of this thesis was to examine the flight response of wild *C. ferrugineus* to various semiochemicals. This objective might determine if flying beetles could be caught and perhaps what semiochemicals might elicit orientation of wild *C. ferrugineus* outside of grain bins. The second objective of this thesis was to determine the efficacy of synthetic pheromone lures for attracting *C. ferrugineus* in field tests. This objective would help us to understand how well synthetic lures, for this species, work in the field for detection or monitoring. The third objective of this thesis was to determine the dispersal pattern of *C. ferrugineus* in relation to stored grain bins. This objective would help us to better understand where and how these beetles disperse to and from stored grain. The fourth and final objective of this thesis was to determine what semiochemicals *C. ferrugineus* might be attracted to in lab bioassays. This objective could help us to better understand the range of semiochemicals affecting orientation of *C. ferrugineus*.

Materials & Methods

Field trapping:

Bucket and Lindgren multiple funnel traps were placed at two locations in north-central Kansas.

1. Dispersal near grain bins assessed with bucket traps

Bucket traps with grain were used to assess in-flight orientation of *C. ferrugineus* near infested grain bins. Bucket traps were designed by suspending an 18.9 liter plastic bucket from a squirrel baffle that was hung on a trap stand (Figure 1). The bucket then had approximately 670 g of soft white wheat. After adding wheat a 1.9 cm hardware cloth clipped down over the opening at the top of the bucket to prevent disturbance by vertebrate pests. The bucket trap assembly was suspended from its trap stand approximately 12.7 cm off the ground. This design allowed the bucket trap to move with the wind, keep the majority of rain water out with the squirrel baffle, but also allow responding beetles go inside the bucket. Bucket traps contained wheat berries to retain responding *C. ferrugineus* and possibly serve as a natural attractant. Two locations were used for bucket trap experiments in north-central Kansas. At one location four bucket traps were placed surrounding a grain bin, approximately 18 m in diameter and 15 meters tall, that contained wheat and infested with many species of stored-product insects including *C. ferrugineus*. Bucket traps were placed singly at each of the four cardinal directions around the bin, approximately 6 m from the bin side wall. At the second location, spacing only allowed for two bucket traps to be placed around the bin (approximately 15 m diameter x 22 m tall) which contained rice, one on the west side of the bin and one on the north side of the bin. Bucket traps were checked on a weekly basis for 7 weeks, which represented replicates by time, at both locations with the bucket contents being poured into a plastic bag (Ziploc® bag, S.C. Johnson, Racine, WI) and brought to the laboratory to be sieved out and beetles counted. New wheat was added to the bucket traps, these traps were checked weekly from 31 July 2014 through 29 September 2014. Numbers of *C. ferrugineus* trapped in a week at a given bin were converted to proportion of beetles trapped at a given direction for that week, proportion data were subjected to the angular transformation (the arcsin of the square-root of the proportion) and then analyzed using one, two, or three-way analysis of variance (ANOVA) to determine significant differences among what independent variables (SAS).

2. Flight responses to various semiochemicals:

Three screening experiments were conducted to determine if *C. ferrugineus* would respond in flight to traps baited with a variety of semiochemicals. Sixteen, four-unit Lindgren funnel traps were hung using a rebar trap stand 1.5 m tall. Distance between any two traps was 10 m (Figures 2 and 3). The traps were placed at four locations in the North Farm are of the campus of Kansas State University north of Kimball Avenue with four traps at each location serving as four experimental blocks. Lures containing known semiochemicals were either purchased or donated by commercial sources or prepared by me for field-testing. Attractants tested were wheat, 75% ethanol, Russell food lure of unknown grain-based extracts (Russell IPM Ltd., Deeside, Flintshire, United Kingdom), *Sitophilus oryzae* (L.) pheromone and *C. ferrugineus* pheromone (Alpha Scents Inc., West Linn, Oregon, United States), Xlure food attractant (Russell), chemical food lures, (Sigma-Aldrich) loaded at 25 mg on rubber septa (Thermo Fisher Scientific). Two of the attractants, 3-methyl-1-butanol and 4-ethylbenzaldehyde, were used because they had shown by Collins et al. (2005) to have some attractiveness in laboratory studies. Approximately 20 g of soft white wheat was placed in all trap collection cups to maintain live insects and to serve as a common source of host-odor attractants. Rubber septa lures were added to traps using wire to suspend the lure inside the trap without it touching the sides of the trap. A 75% solution of ethanol in water was evaporated from a slow release container was made from a 40-ml plastic Falcon tube (Fisher Scientific, Waltham, MA) with a 7 mm hole drilled through the screw-on lid and a piece of cotton wick (114 mm long x 3 mm wide) inserted to within 1 mm of the bottom of the tube and extending out through the hole in the lid. Experimental treatments were randomly assigned to each trap in a four-trap block to achieve a randomized complete block design. Experiments were conducted from mid-June through early September 2013. Traps stayed in their respective locations for 4 d before being checked, their contents collected, and then being re-positioned randomly for an additional period of four days.

3. Field responses to cucujolide-II

Four-unit Lindgren funnel traps were used in 2014 to assess the activity of synthetic cucujolide II provided by the Alpha Scents Inc. (West Linn, OR, USA). Blocks of two traps each were established with traps separated by 20 m with at least 30 m away from any other block. Each trap contained a small, hand-made mesh bag that contained approximately 10 g of semi-crushed soft, white winter wheat berries. One trap within each block was designated that control and had wheat only, while the second trap was the treatment with a cucujolide-II lure + wheat. Each trap contained a piece of crumpled paper towel to provide a harborage for trapped beetles and a 1 square cm piece of a “no-pest strip” insecticide (active ingredient Dichlorvos)in the bottom of the trap as a killing agent. Traps at each location were checked approximately every 1-2 days. After checking each trap in a block, a coin would be flipped to determine the next location of each trap in each replication. This method was used at both locations to add as much randomization as possible to the experiment. Data for each replicate from this funnel trap experiment were converted into proportions and then transformed using the arcsine transformation (arcsine square root proportion or angular transformation (Zar 1980)) prior to analysis at each site with a t-test using the R statistical program (R Core team 2014).

Laboratory assays of semiochemicals

Field experiments yielded very low numbers of *C. ferrugineus* and were highly labor-intensive. Laboratory experiments were therefore conducted to confirm some findings from field work and further examine semiochemical based orientation. All laboratory experiments were conducted using established colonies of *C. ferrugineus* obtained from the USDA Center for Grain and Animal Health Research in Manhattan, KS. These insects were reared on a diet consisting of rolled oats and brewer's yeast (95:5) in glass pint jars (each pint jar had an approximate volume of 508 ml) covered using filter paper to ensure air and moisture diffusion. Colonies were maintained in a growth chamber at 28°C and 50-70% RH. Adult insects used in bioassays were 3-4 weeks old and were separated from their food substrate using a US number 12 sieve (Fisher Scientific). Beetles were then held in groups of 20 for 2-3 h before being used in bioassays to ensure uniform starvation and to possibly enhance their olfactory response after being deprived of food.

A two-choice pitfall bioassay design, similar to that used by Pierce et al. (1991), was used in these experiments to determine the orientation of walking adult beetles relative to a given semiochemical stimulus (Figure 4). This design was made from the following: two shell vials (2 dram (Fisher Scientific, Waltham, MA) each vial was 19 mm in diameter x 50 mm tall and with a volume of 11 ml), a Petri dish lid of standard glassware (100 mm in diameter x 20 mm height) and one Petri dish bottom (90 mm diameter x 20 mm height) with two custom-made holes in the floor 10 mm in diameter and placed 40 mm apart and 15 mm from the side walls positioned along a mid-line passing through the center of the floor. A wooden block had 40 holes (each hole approximately 21 mm in diameter) drilled to hold shell vials for 20 each 2-choice assays for each of the experiments (Figure 5). A set of 10 bioassay dishes per treatment was evaluated in a small room maintained at 22-24 ^{o}C and 30% RH, and experiments were conducted in complete darkness. All glassware was washed using soap and water, rinsed with deionized water, and then rinsed with acetone and dried in an oven at 65.5^{o} C for a minimum of 2 h prior to use in all assays. Laboratory bioassays were conducted to determine the response of *C. ferrugineus* to different food volatiles, some of which are commonly used in commercial stored product insect traps. Trecé Company supplied four of their commonly used insect trap baits. These baits were labeled as follows, DA, TRE-0093, TRE-0088, and Storgard Oil. The compounds DA, TRE-0088 and TRE-0093 were anonymous commercial samples of natural or synthetic plant-based volatiles. Storgard Oil is a commercial lure with grain-based food odors used in stored-product insect traps.

Materials to be evaluated for beetle orientation were placed in the bottom of one shell vial that was designated as the treatment, while the second vial was either empty or contained a presumably neutral material and served as the control. Treatment and control were assigned at random to a given vial in each two-choice bioassay. Liquid Teflon was applied to the top side-wall of each vial to prevent escape of responding insects. Materials to be tested were classified as host/food-related or insect-related and were either whole materials such as crushed grain, extracts or mixtures of whole natural material, or synthetic compounds (i.e. synthetic pheromones used in lures, food volatiles, and beetle odors). Most of the synthetic pheromones tested were commercially available to me only as formulated on rubber septum lures for use in commercial traps. These included the following species' pheromone lures *Lasioderma serricorne* (Fabricius), *Rhyzopertha dominica* (Fabricius), *Prostephanus truncatus* (Horn), *Plodia interpunctella* (Hübner), and *Tribolium castaneum* (Herbst).

Preliminary experiments suggested that rubber septa alone, not formulated with pheromones, could elicit positive orientation by beetles. One preliminary experiment found that beetles responded more to a hexane extract of a rubber septum compared to hexane alone. Thus, all studies with pheromone septa had a blank septum without pheromone as a control to assure that any non-pheromone attractants were present in both sides of the bioassay.

Each replicate two-choice assay evaluated the orientation of 20 adult *C. ferrugineus* of mixed sex and age as to the number falling into a given vial or remaining on the arena floor. For each bioassay unit the Petri dish cover was removed, the 20 beetles were gently poured out of the vial onto the center of the bioassay floor and the cover was replaced. The bioassay was allowed to run for 18 hours, which was based on preliminary testing at different exposure times. Each bioassay was stopped after 18 h by turning on the lights in the bioassay room and dishes being removed from the vials. Each dish was observed to make sure any beetles still in the arena were recorded. After the 18 h exposure period the number of beetles in the main arena, treatment vial and control vial were recorded for each bioassay unit. Orientation to a given stimulus was usually evaluated with 20 bioassay replicates, though limited testing material in some cases necessitated fewer replicates with a minimum of 10.

After all data were collected for each test, the data were statistically compared using a paired t-test in the statistical software "R" to see if there were any treatment effects on beetle choice (R Core Team 2014).

Results

Field Studies

Orientation to Bucket Traps

Feral *C. ferrugineus* flying near grain bins at two locations in north-central Kansas were found inside bucket traps. At the Junction City location buckets could be placed only on two sides of the bin. On the western side of a rice-filled bin the bucket trap collected on average 29.4 ± 5.5 (n=7 one-week periods) beetles over the course of the 49-d experiment. On the northern side of the same rice-filled bin the bucket trap caught on average 21.9 ± 5.7 beetles over the whole 49 d. Numbers of beetles captured over time varied widely with many zeros, so captures by week were analyzed by the proportion of beetles in a given week that went to each bucket. Figure 6 shows analysis of the proportion of *C. ferrugineus* collected at the north and west directions at this site, and these were significantly different form each other.

At the Abilene location a bucket trap was placed at each of the four cardinal directions around a wheat-filled bin. Here the mean ± SE catch in the trap on the northern side was 0.8 ± 0.3 (n=7) beetles over the course of the 49-d experiment. The bucket trap on the eastern side of the bin collected 1.0 ± 0.6 beetles, the southern bucket trap collected 0.6 ± 0.2 beetles and the western bucket trap collected 7.0 ± 2.0 beetles on average. Figure 7 reports the analysis of the proportion of *C. ferrugineus* collected at each of the four directions at this site and shows that highest proportion of beetles trapped was in the bucket trap at the northern aspect of the bin.

Orientation of flying beetles to traps with various semiochemicals

A variety of insects were trapped in the pheromone screening studies and most were identified just to family, while *Cryptolestes* beetles were identified to the genus level (Table 1).

The number of *Cryptolestes spp.* trapped over the three studies was only15 beetles. Although the target insect species for these studies was *C. ferrugineus*, several other species were trapped and are reported here to provide information on the variety of insects responding to these traps. Numbers were quite variable for all the insects captured across trapping periods so that statistical analyses were not conducted. Raw data are reported for the various insect groups trapped in these experiments (Table 1). Experiment A found that *Cryptolestes spp.* were attracted to traps containing an ethanol lure but not to the other test compounds. In all, twelve *Cryptolestes spp.* were collected during the 10-d experiment only in the traps containing ethanol as the sole attractant. Experiment B was conducted using ethanol and different lures, but no *Cryptolestes spp.* were collected in the ethanol-baited traps or in any other traps. Mostly Pscocidae were collected in groups of 60 to 95 in Experiment B. Dermestidae trapped in this experiment were only in the trap baited with X-Lure. Experiment C was conducted for 13 4-d periods and assessed activity of the rice weevil pheromone and *C. ferrugineus* pheromone Cucujolide-II, which caught one and two *Cryptolestes spp.*, respectively. An interesting note about this experiment is the ethanol treatment; while it caught zero *Cryptolestes spp.*, it did catch 277 green June beetles, *Cotinis nitida* (Linneaus) (Coleoptera: Scarabaeidae) (Table 1 experiment C).

Orientation to funnel traps with synthetic C-II

Experiments were run at the Junction City and Abilene locations used for previous experiments. Of the total 201 *C. ferrugineus* trapped at the Junction City location, Lindgren multiple funnel traps with just wheat as an attractant caught on average 30.9% ± 5.0 % of all beetles compared to an average of 69.1% ± 5.0 % of all beetles from traps containing synthetic C-II rubber septa and wheat (Figure 8a). The same treatments were compared at the Abilene location with only 54 beetles trapped. Traps with wheat alone caught a mean percent ± SE of

7.4% ± 6.5% beetles while traps containing synthetic C-II rubber septa and wheat caught 92.3% ± 6.5% beetles (Figure 8b). Traps were checked following trapping periods of 2-3 days daily for a total of 31 trapping periods. There were very low numbers caught in these experiments, with some replicates not catching any beetles, which prevented application of statistical analyses directly on the raw data. Therefore, any experimental block of two traps that captured one or no beetles was not included in the analysis. For the remaining trapping blocks in a given trapping period the proportions of collected *C. ferrugineus* in each trap were calculated and the treatment effect was evaluated with a t-test following an arcsine transformation of the proportion data. Figures 8a and 8b show that at both locations there was a significantly higher proportion of *C. ferrugineus* captured in traps baited with the synthetic C-II with wheat compared to those having wheat only.

Two-Choice Laboratory Bioassays

Results for two-choice bioassay experiments are group by the types of volatiles that were being tested against mixed-sex adults of *Cryptolestes ferrugineus*. In tests with grains and grain products beetles showed positive responses to both wheat and corn meal, but there was no significant response to rolled oats or crushed dog food (Figure 9). For commercial food lures *C. ferrugineus* did not respond significantly to the synthetic compounds "DA" and "TRE-0093", oriented to control vials in comparison to "TRE-0088" (Figure 10), suggesting this material may be repellent to *C. ferrugineus*. "Storgard Oil", a material used as host volatiles for grain beetles in Trecé trap products, elicited a significant positive response, as did the Russell food lure (Figure 10). Ethanol was included in these studies because initial field trials suggested its attractive activity and it is a common fermentation product from recently killed plant material as well as from moist, aging grain, such as grain infested by *C. ferrugineus*. Ethanol at 15 µl on filter paper elicited a positive response from responding beetles, but at the higher dose of 25 µl there was no effect (Figure 10). In summary, these experiments with food-based stimuli showed that Storgard Oil elicited, by far, the highest positive response.

Three experiments were conducted to determine if semiochemicals from *C. ferrugineus* adults feeding on grain would elicit a positive response from beetles (Figure 11). In the two experiments using oats as a food odor source, beetles responded positively only when the control vial contained neither oats nor beetles. In the other two experiments that compared responses to mixed-sex adults on oats vs oats and to males only on wheat vs wheat, found no significant response to the vials containing beetles (Figure 11).

Assays were conducted to test synthetic cucujolide I, cucujolide II, and a mixture of both loaded on to rubber septa (Research Directions, Brisbane, Australia) versus blank septa elicited strong positive responses from adult *C. ferrugineus* (Figure 12). Bioassays with synthetic cucujolide II dissolved in hexane in three different doses revealed significant positive orientation at 2500 ng and 5000 ng applied to filter paper, but not at 25 ng (Figure 12). Interestingly, *C. ferrugineus* showed significant positive responses to synthetic pheromones from 4 out of the 5 insect species tested (Figure 13). Rubber septa containing the synthetic pheromone of the cigarette beetle (CB septa), *Lasioderma serricorne*, elicited the strongest positive responses and lures for *Rhyzopertha dominica* (DL-1+2), *Prostephanus truncatus* (PT Bullet Lure) and *Plodia interpunctella* (IMM Septa) elicited lower but significantly positive responses. Lures for the red flour beetle (RFB Septa) *Tribolium castaneum*, did not elicit a significant response compared to unbaited septa.

Discussion

Results suggest that *C. ferrugineus* adults are attracted to various semiochemicals from other *C. ferrugineus* as well as some foods, grain oils and pheromones of other species of stored product insects and decaying plant breakdown chemicals. This is a wide variety of semiochemicals from a wide variety of sources.

Some grain-based foods, such as cracked wheat and corn meal, were attractive to *C. ferrugineus* while odors from rolled oats or dog food were not. Volatiles developed by commercial suppliers elicited mixed responses from *C. ferrugineus*. *C. ferrugineus* preferred food lures containing some grain odors and plant decomposition compounds as compared to odors of fruit trees (e.g., TRE and DA compounds). Surprisingly, *C. ferrugineus* showed little response to semiochemicals directly from other mixed-sex or male-only *C. ferrugineus*. Another surprising result is that 5000 ng was the amount of synthetic cucujolide II needed to produce a statistically significant response in *C. ferrugineus*. These beetles did, however, show highly significant responses to cucujolide I, II and a mixture of I and II as well as to four other species' pheromone lures.

Many insects, including *Cryptolestes spp.* were caught during field experiments responding to funnel traps baited with different semiochemicals in my preliminary studies. One insect group collected in every experiment, and in most treatments, were the psocids. These insects are common in grain (Hagstrum et al. 2012) and come in winged and wingless species, but information about psocid flight behavior is limited. Even in unbaited traps, the winged Psocoptera were well represented. Ethanol as a treatment seemed to attract the biggest variety of insects captured across all three experiments conducted in 2013. Five different insect groups were collected using ethanol as the treatment. Hundreds of specimens of psocidae, elateridae, and scarabaeidae were collected in response to ethanol. *Cryptolestes spp*. were also found in these traps, but in much lower numbers. Ethanol is a known attractant for various insects associated with decomposing plant materials, particularly some forest beetles (Schlyter and Birgersson 1999). Species of Laemophloeidae are common insects in decomposing trees and *Cryptolestes* may respond to similar semiochemicals, perhaps also associated with decomposing grain. Two chemicals that had previously shown some attractiveness to *Cryptolestes spp.* in laboratory experiments, 3-methyl-1-butanol and 4-ethylbenzaldehyde (Collins et al. 2005), apparently did not attract feral *Cryptolestes spp.* in this study. However, over 60 psocids responded to traps baited with these two chemicals combined. Sawtoothed grain beetle lure and Xlure, two attractants used to monitor for stored-product insects, were tested but neither one attracted *Cryptolestes.* Both were able to attract over eighty psocids and Xlure attracted over twenty dermestids as well. When *S. oryzae* and *C. ferrugineus* pheromones were tested, both attracted some *Cryptolestes* and elaterids in very small numbers.

When bucket traps were placed outside grain bins it was hypothesized that there would be a clear directional effect since wind in this region comes from the south southwest during the summer months. This was statistically significant at one location, but not at the other. At both locations bucket traps placed on the western side of the grain bins caught more beetles than any other side tested. In previous studies the amount of feral *C. ferrugineus* caught flying around the outside of stored wheat bins increased in July and peaked in August (Nansen et al. 2004). The same study showed that there can be considerable variation, by year, in the number of *C. ferrugineus* caught at the four cardinal directions In this same study, it was found that there was a significant capture of *C. ferrugineus* on the western side of grain bins containing stored wheat.

In the years with most *C. ferrugineus* being caught on the western side of grain bins, the wind was recorded as coming from the south. Therefore my results are consistent with those of Nansen et al. (2004), but reasons for beetles flying to or away from grain bins at their western edge remain unclear.

Using synthetic pheromones to trap feral *C. ferrugineus* had never been done before this study. Synthetic cucujolide II on rubber septa was used in addition to a bag of semi-crushed wheat as the attractant inside Lindgren multiple funnel traps. These two materials were used in this way to replicate what the conditions might be inside of or just outside of grain bins that have the odors of stored wheat and feeding male *Cryptoleste*s producing pheromone. These results suggest that feral *Cryptolestes* will respond to synthetic versions of their pheromones, these pheromones can be used outside, in field conditions, as lures in detection surveys. Wong et al. (1983) were the first to report the identities of male-produced pheromones in *C. ferrugineus*, and Loschiavo et al. (1986) were unable to demonstrate activity for beetles in grain bins responding to baited traps in the grain. The results in this thesis may be the first to document in-field flight of *C. ferrugineus* to synthetic cucujolides.

Responses of *C. ferrugineus* in laboratory bioassays to food and beetle volatiles were minimal and much different than expected. Based on the field data using cucujolide II and wheat to attract feral *Cryptolestes*, it was expected that wheat and beetles feeding on food would elicit a positive response. Only one food test showed a highly positive and statistically significant response from *C. ferrugineus*- this was wheat versus a blank control. The oats versus blank control bioassay unexpectedly showed that beetles preferred the blank control no more than the oats with an average of 9.95 ± 0.8 beetles in the oats and 9.45 ± 0.7 beetles in the blank control.

This result was unexpected because all beetles used in all laboratory bioassays in this study were raised on a rolled-oats diet.

However, since my other laboratory bioassays showed food odors were attractive compared to an empty control vial (Figure 9), it is possible that responses of beetles to beetles on oats were actually to the presence of oats in that treatment compared to an empty vial control. The results of this bioassay showed that an average of 10.6 ± 0.5 beetles responded to the adults on rolled oats while only an average of 8.9 ± 0.4 beetles responded to the blank. However, the subsequent bioassay comparing adults on oats to oats only revealed no statistically significant orientation of beetles to either of the two treatments.

When TRE-0088 dissolved in methanol was tested in a bioassay the results showed that it acted as a repellent, with the majority of beetles responding to the methanol control. TRE-0088 was an unknown plant odor, but it was the only semiochemical among all tested that elicited a negative response. The commercial Russell food lure is made of grain-based food odors similar to those in Storgard Oil and it also elicited positive response in our beetles. Pure ethanol was then tested in two different amounts, 15µl and 25µl. Ethanol was tested because the pheromone screening work suggested it may be an attractant and, as noted above, because Laemophloeidae are a type of bark beetle and bark beetles tend to be attracted to decaying wood. One of the main degradation compounds from plants is ethanol. This result was consistent with the preliminary trapping studies and also suggests ethanol may be a useful semiochemical for studying or monitoring *C. ferrugineus*.

It isn't unheard of for one species to be attracted to or to use the pheromone(s) of another species as clues to food or potential reproduction success (Phelan 1986). Having highly significant responses to the majority of interspecific lures tested might be explained by this natural behavior. Bioassays that assessed orientation of *C. ferrugineus* to synthetic pheromones of other stored product insects indicated positive responses in four out of five cases. Since these other species are found in the same habitats as *C. ferrugineus* it is possible that interspecific attraction could occur as part of host location behavior. Interspecific attraction has been reported in other habitats for moths (Baker 1989) and beetles (Byers 1992), so such phenomena in stored product beetle suggests interesting biology and potential applications. Future work on interspecific attraction for *C. ferrugineus* may elaborate these interactions and provide additional semiochemicals for use in IPM applications.

Conclusion

These bioassays showed that *C. ferrugineus* will respond to commercial trap lures, interspecific insect pheromones, plant degradation chemicals, food, beetles on food and intraspecific synthetic pheromones. Some of the results from bioassays were surprising, but may be explained by the natural behavior of the beetles or by conditions that are different in the laboratory than in the beetle's natural environment.

Field experiments proved for the first time that feral *C. ferrugineus* could be captured using the synthetic cucujolide II pheromone impregnated on rubber septa. This system may prove to be the best, cheapest and easiest way to monitor for these stored-grain pests. Bucket traps showed that more beetles were caught on the western side of grain bins, but it's not known if these beetles were leaving the bin or coming to the bin when they encountered the traps.

Table 1. Captures of insects in Lindgren multiple funnel traps baited with various semiochemicals in Manhattan, KS, during summer 2013*

Experiment	Date Range	Treatments	Most common families collected	Total specimens from each taxon
A[1]	6-18-13 to 6-28-13	Blank	Elateridae	11
			Psocidae	51
		Ethanol	Anthicidae	41
			Laemophloeidae, *Cryptolestes* spp.	12
			Elateridae	82
			Psocidae	66
			Scarabaeidae	5
		3-Methyl-1-butanol	Psocidae	31
		4-EthylBenzaldehyde	Psocidae	32

Experiment	Date Range	Treatments	Most common families collected	Total specimens from each taxon
B[1]	6-28-13 to 7-10-13	Blank	Psocidae	63
		Ethanol	Psocidae	57
			Elateridae	57
		SGB	Psocidae	84
		Xlure	Psocidae	94
			Dermestidae	25

Table 1. (cont.)

Experiment	Date Range	Treatments	Most common families collected	Total specimens from each taxon
C[2]	7-10-13 to 9-2-13	Blank	Elateridae	40
			Scarabaeidae	50
		Ethanol	Psocidae	6
			Scarabaeidae *Cotinis nitida*	277
			Elateridae	86
		Sitophilus oryzae Pheromone	Elateridae	10
			Scarabaeidae	21
			Cucujidae *Cryptolestes* spp.	1
		C. ferrugineus Pheromone Cucujolide-II	Elateridae	21
			Cucujidae *Cryptolestes* spp	2

1 Totals for two repetitions.

2 Totals for thirteen repetitions.

*Traps checked and contents collected every 4 days or as needed due to weather. Positions of traps in each 4-trap block were re-randomized after each trapping period.

Figure 1 Bucket trap in field showing trap stand, squirrel baffle and white bucket for capturing *C. ferrugineus* flying near grain bins.

Figure 2 A Lindgren multiple funnel trap used in experiments.

Figure 3 Lindgren multiple funnel traps deployed in a line for a randomized bock of traps at North Farm, Kansas State University, Manhattan, KS.

Figure 4 Drawing of two-choice pitfall bioassay dishes for reference.

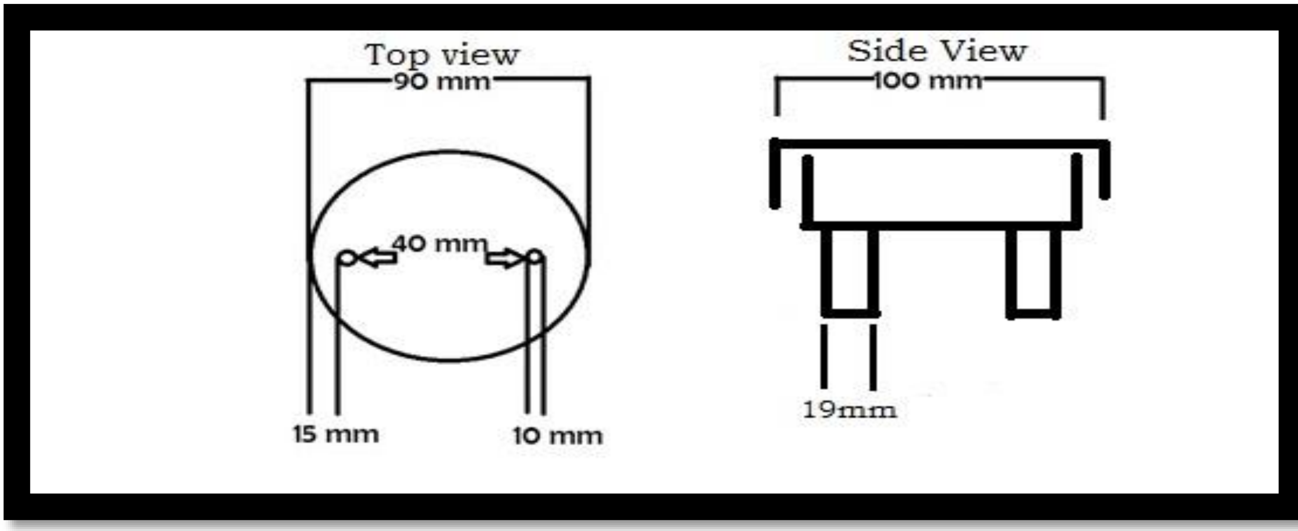


Figure 5 A group of ten two-choice pitfall bioassay dishes over response vials in bioassay block. Each dish equals one repetition.

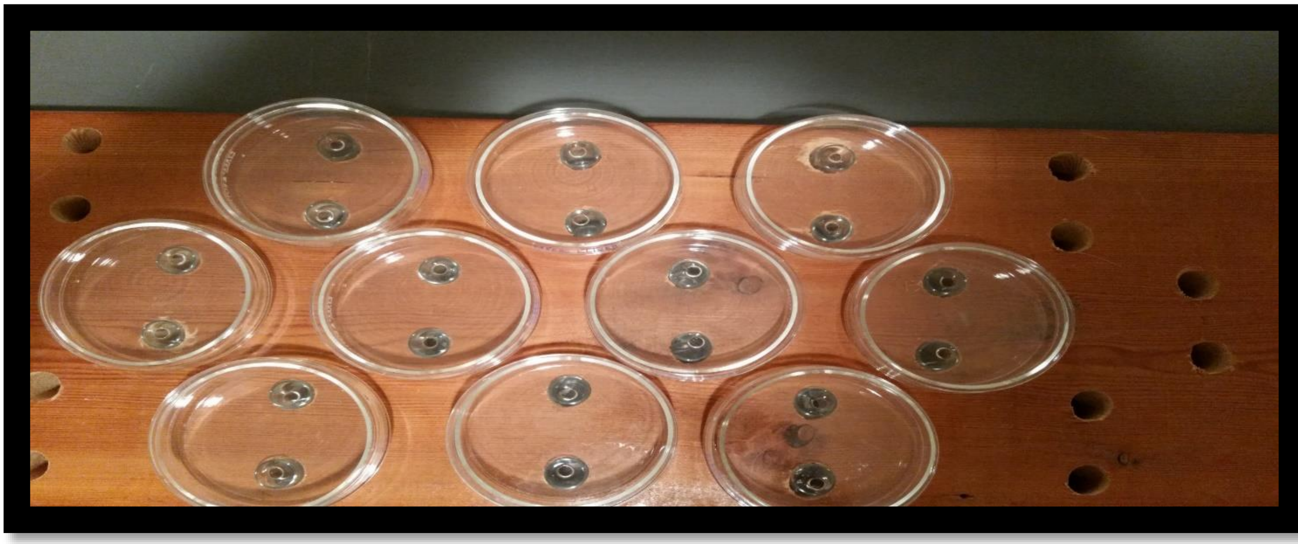
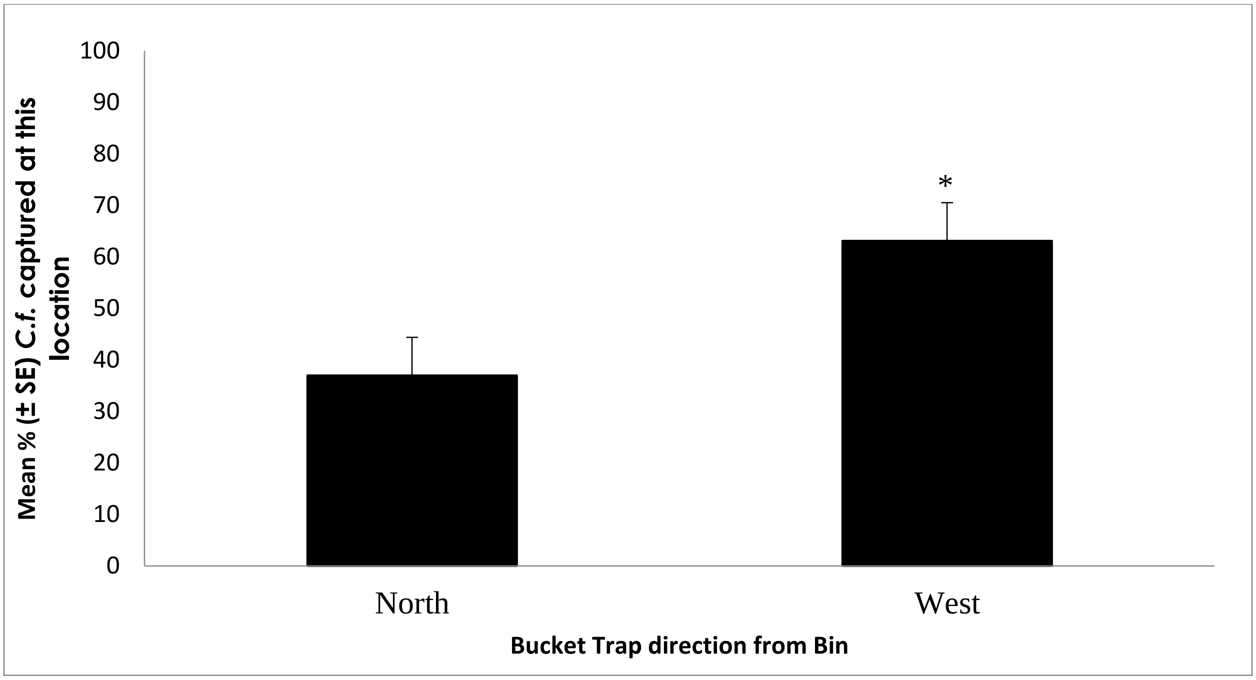
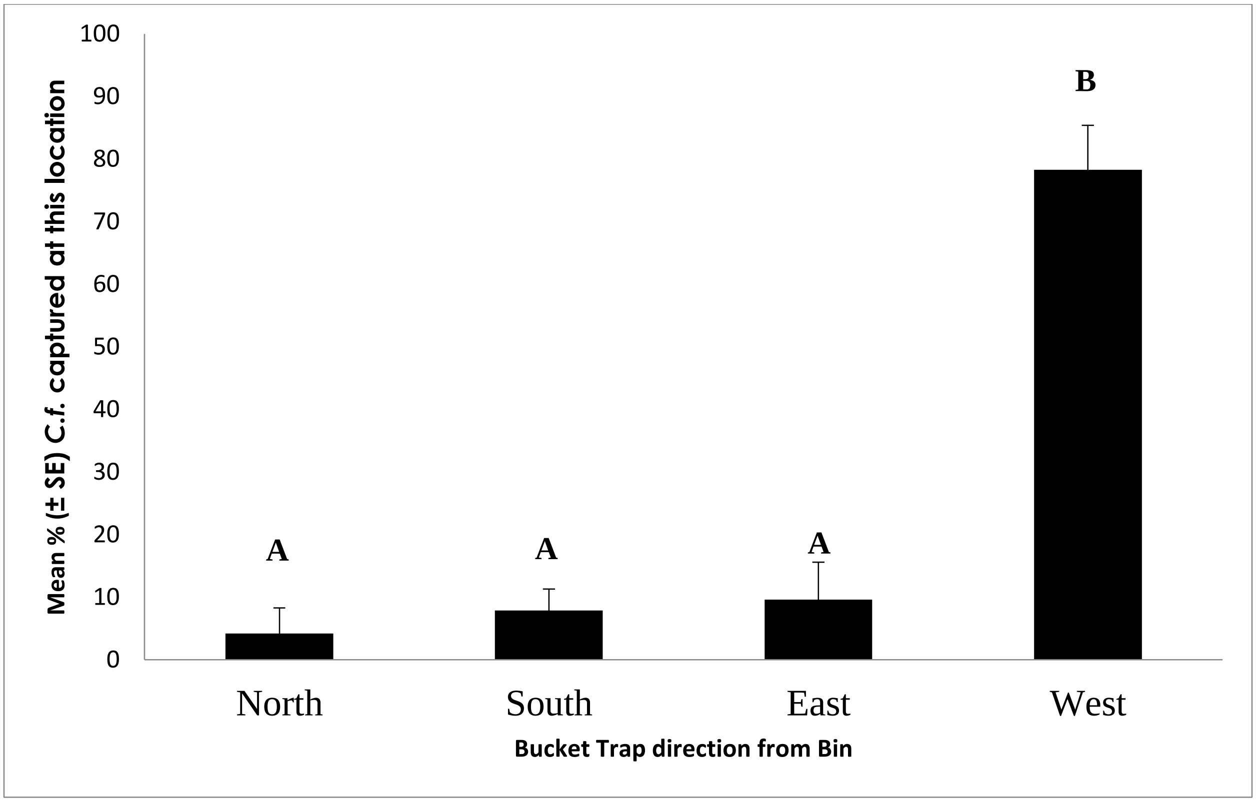


Figure 6 Average percentage of feral *C. ferrugineus* caught at two cardinal directions around a grain bin at the Junction City location.



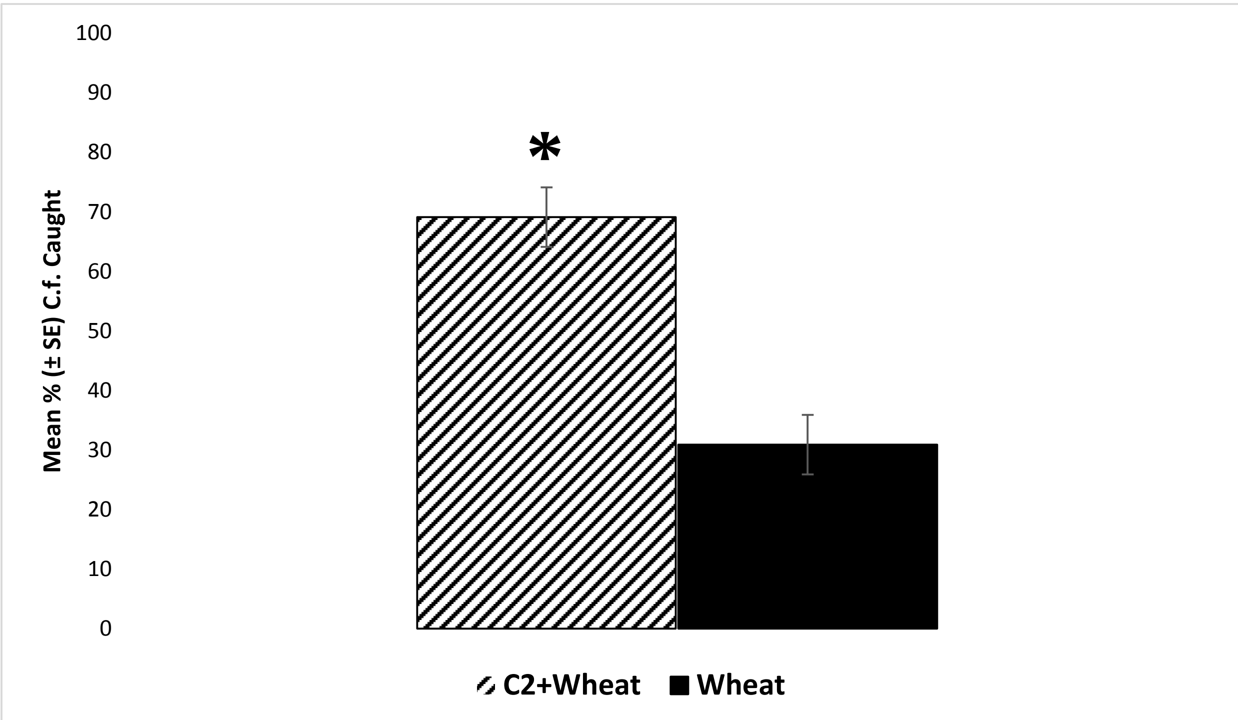
Data analyzed using paired t-test in R statistical program.

Figure 7 Average percentage of feral *C. ferrugineus* caught at four cardinal directions around a grain bin at the Abilene location.



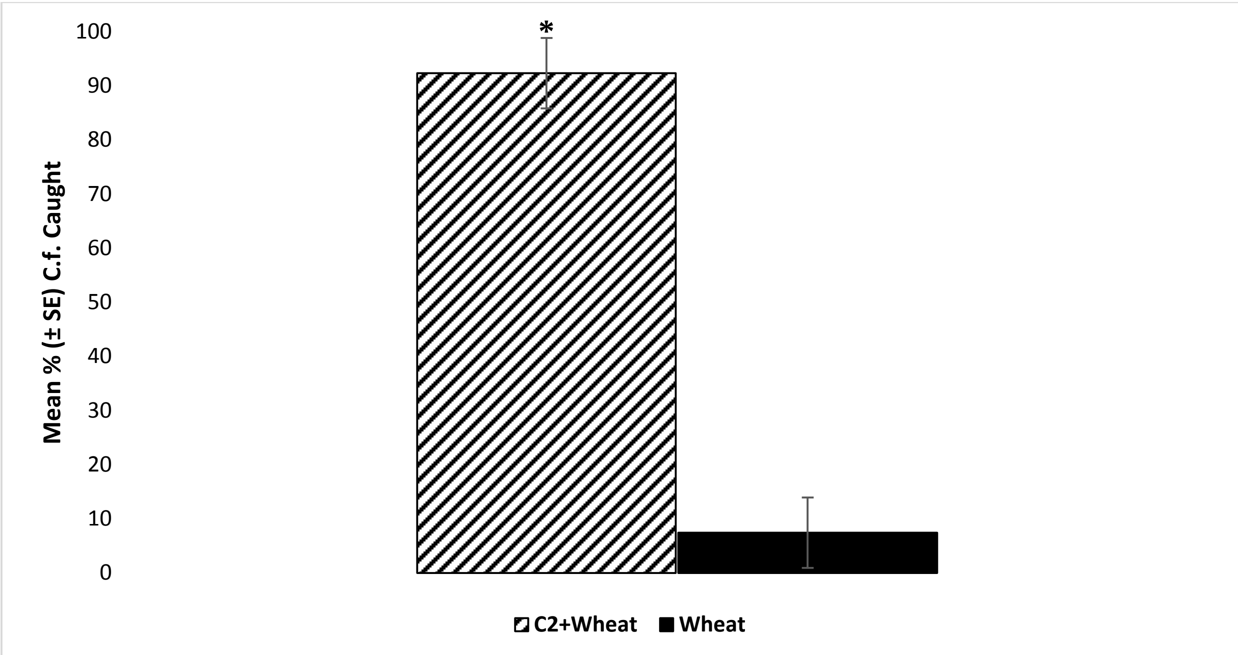
Data analyzed using ANOVA on SAS statistical program. LS means test used. All directions with same letter above error bars are considered not statistically different from each other.

Figure 8a Average percent feral *C. ferrugineus* caught in Lindgren multiple funnel traps baited with synthetic cucujolide-II at Junction City location.



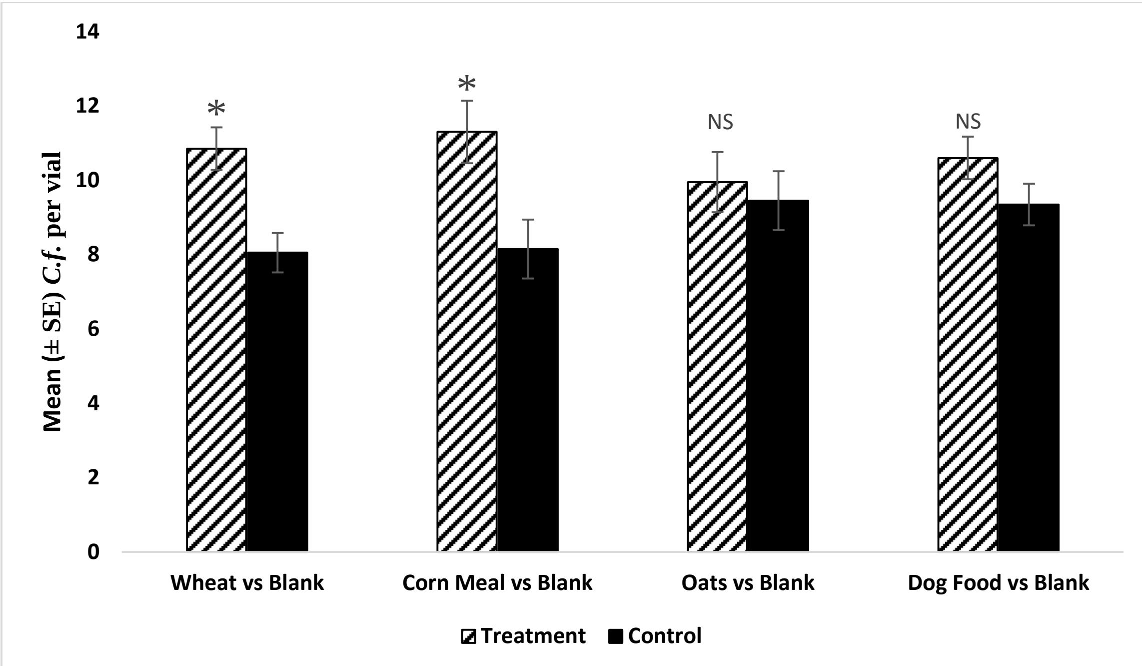
Data analyzed using t-test. P-value= 0.0015. n= 18.

Figure 8b Average percent feral *C. ferrugineus* caught in Lindgren multiple funnel traps baited with synthetic cucujolide-II at Abilene location.



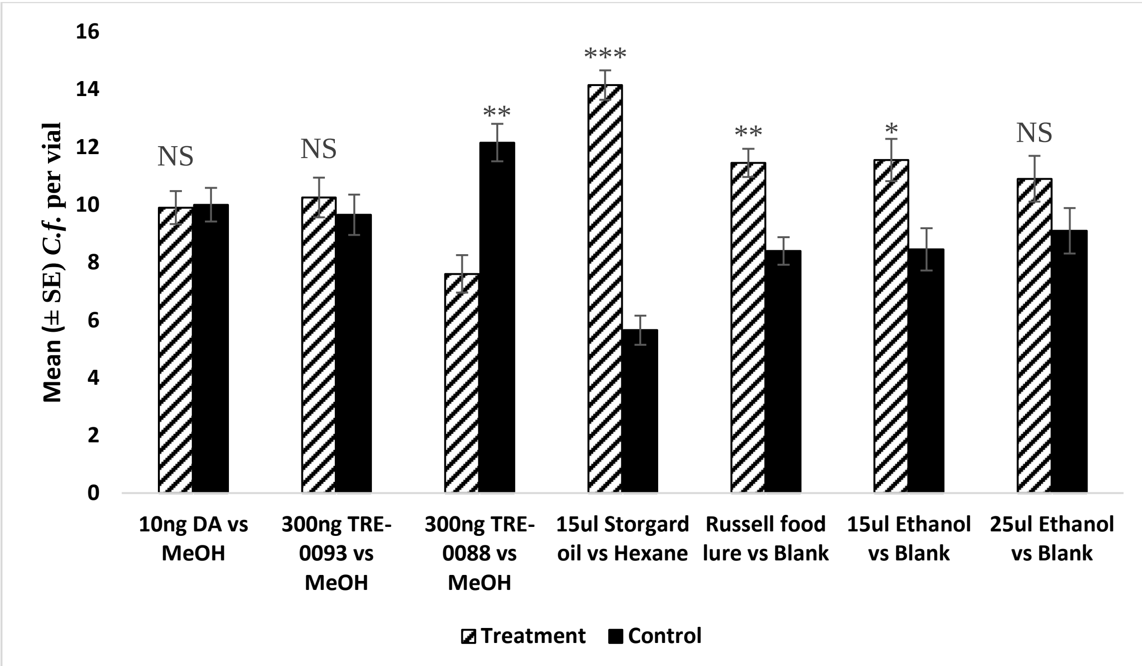
Data analyzed using t-test. P-Value= 0.0013. n=6.

Figure 9 Responses of *Cryptolestes ferrugineus* to cracked wheat kernels, corn meal, rolled oats and commercial dog food using a two-choice pitfall bioassay.



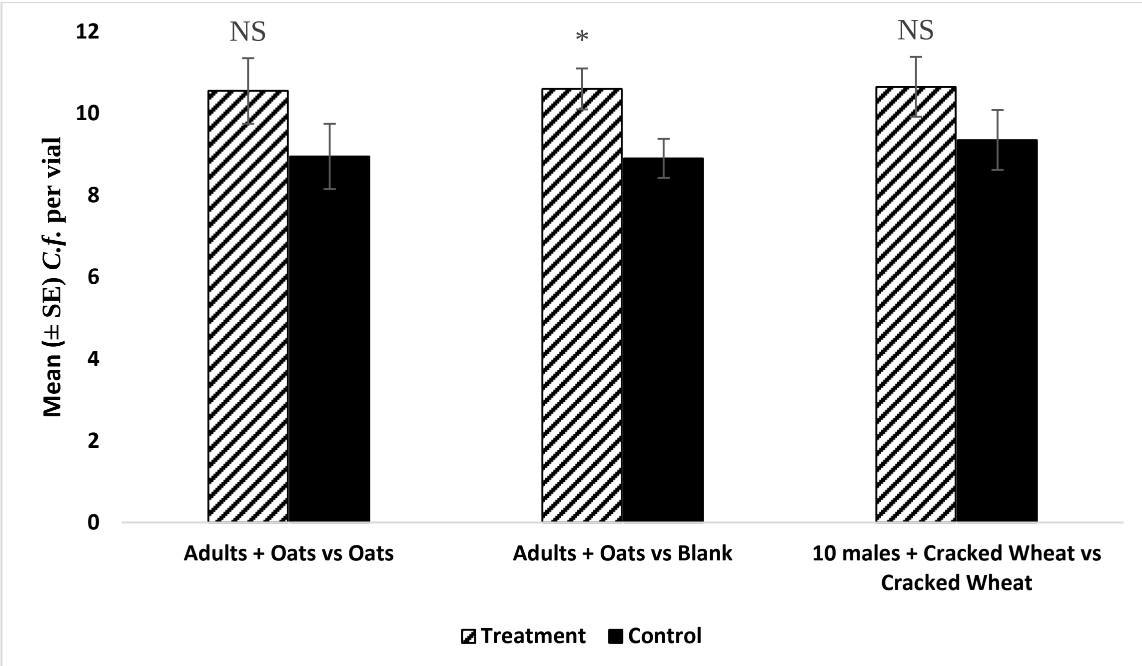
Data were analyzed using paired t-test; *, P< 0.1; NS, P> 0.1; n=20.

Figure 10 Response of *Cryptolestes ferrugineus* to various synthetic or naturally derived food volatiles using a two-choice pitfall bioassay.



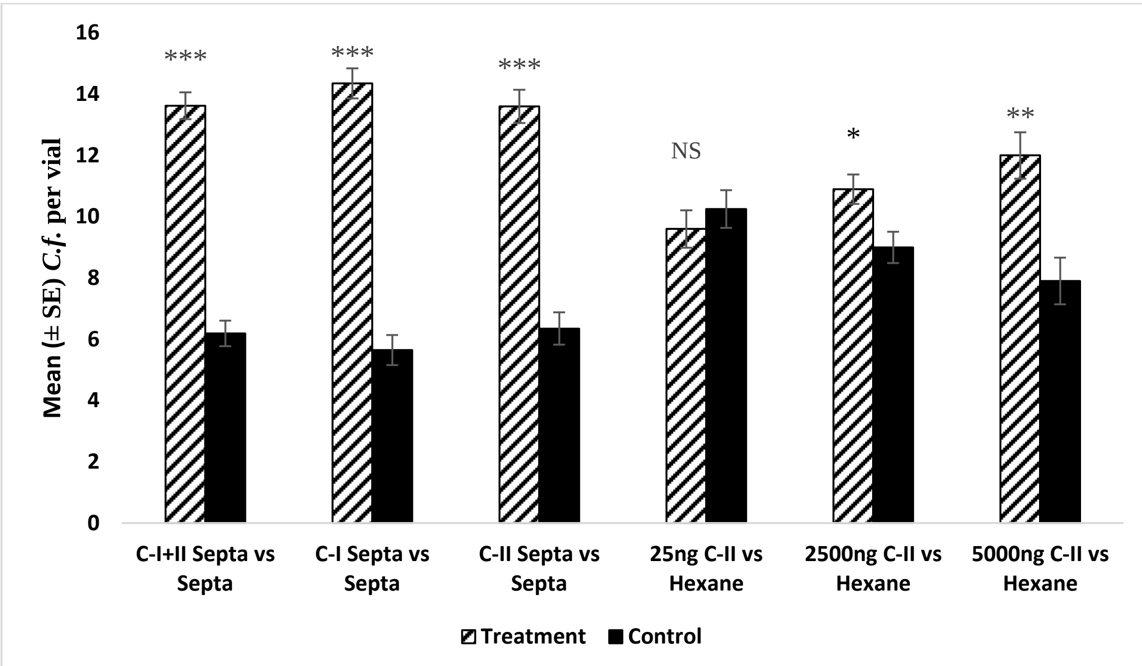
All data were analyzed using paired t-test; NS, P> 0.1; *, P< 0.1; **, P< 0.01; ***, P< 0.001; n=20.

Figure 11 Responses of *Cryptolestes ferrugineus* to volatiles from conspecific adults feeding on grains using a two-choice pitfall bioassay.



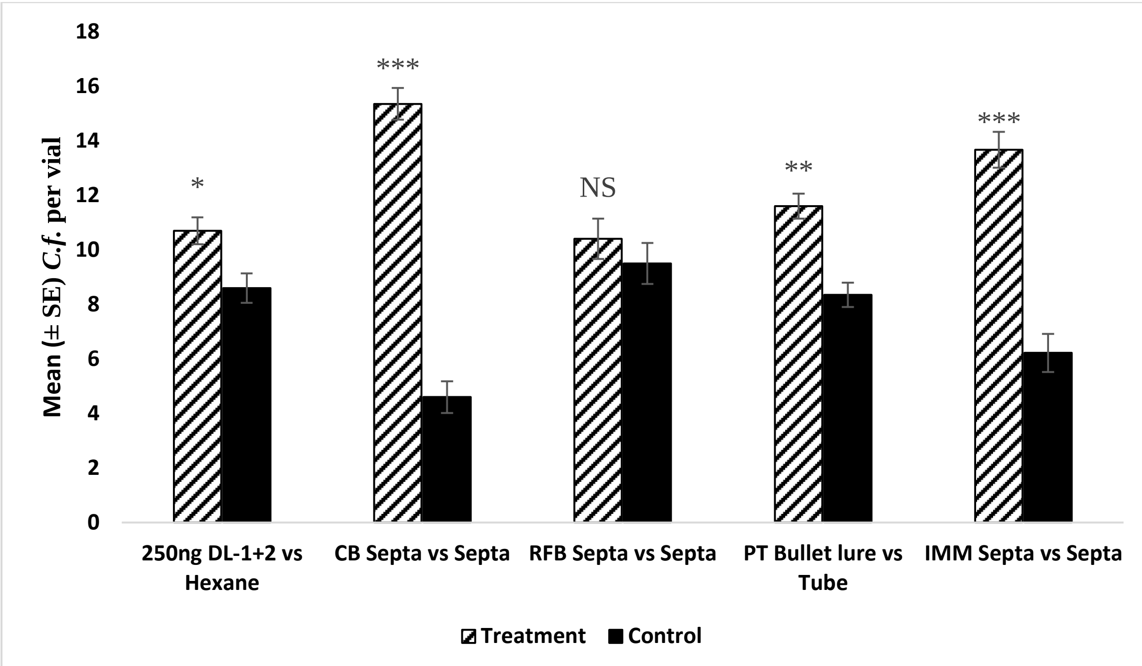
All data were analyzed using paired t-test; NS, P> 0.1; *, P< 0.1; n=20.

Figure 12 Response of *Cryptolestes ferrugineus* to rubber septa loaded with the synthetic *Cryptolestes* pheromones “cucujolide I” or “cucujolide II” (C-I, -II), and synthetic C-II applied in hexane to filter paper in a two-choice pitfall bioassay.



All data were analyzed using paired t-test; NS, P> 0.1; *, P< 0.1; **, P< 0.01; ***, P< 0.001; n=20.

Figure 13 Responses of *Cryptolestes ferrugineus* to rubber septum lures loaded with synthetic pheromones of five species of stored product pests using a two-choice pitfall bioassay.



Data were analyzed using paired t-test; NS, P>0.1; *, P< 0.1; **, P< 0.01; ***, P< 0.001; n=20. DL= Dominicalure 1 and 2, CB= Cigarette beetle (*Lasioderma serricorne*), RFB= Red Flour Beetle (*Tribolium castaneum*), PT= Larger grain borer (*Prostephanus truncatus*), IMM= Indian meal moth (*Plodia interpunctella*).

References

Arbogast, R.T. 1991. Beetles: Coleoptera, pp. 131-176. In: J.R. Gorham (Ed.), Ecology and Management of Food-Industry Pests. FDA Technical Bulletin 4. VA: Association of Analytical Chemists.

Baker, T. C. 1989. Sex pheromone communication in Lepidoptera: new research progress. Experientia 45: 248-262.

Borden, J. H., M. G. Dolinski, L. Chong, V. Verigin, H. D. Pierce, Jr., and A. C. Oehlschlager. 1979. Aggregation pheromone in the rusty grain beetle, *Cryptolestes ferrugineus* (Coleoptera: Cucujidae). *Can. Ent.* 111: 681-88.

Byers, J. A. 1992. Attraction of bark beetles *Tomicus piniperda*, *Hylurgus palliates,* and *Trypodendron domesticus* and other insects to short-chain alcohols monoterpenes. J. Chem. Ecol. 18: 2385-2402

Canadian Grain Commission. 2013. Rusty Grain Beetle *Cryptolestes ferrugineus* (Steph.). https://www.grainscanada.gc.ca/storage-entrepose/pip-irp/rgb-cr-eng.htm.

Collins et al. 2007. Progress towards a multi-species lure: Identification of components of food volatiles as attractants for three storage beetles. J. Stored Prod. Res. 43: 53-63.

Cuperus, G. W., Prickett, C. K., Bloome, P. D., and Pitts, J. T. 1986. Insect populations in aerated and unaerated stored wheat in Oklahoma. J. Kans. Entomol. Soc. 59.4: 620-627.

Davies, R.G. 1949. The biology of *Laemophloeus minutus* Oliv. (Col. Cucujidae). Bull. Entomol. Res. 40: 63-82.

Fargo, W.S., D. Epperly, G. W. Cuperus, B. Clary, and R. Noyes. 1989. Effects of temperature and duration of trapping on four stored grain insect species. J. Econ. Entomol. 82: 970-973.

Fargo, W. S., G. W. Cuperus, E. L. Bonjour, W. E. Burkholder, B. L. Clary, and M.E. Payton. 1994. Influence of probe trap type and attractants on the capture of four stored-grain Coleoptera. J. Stored Prod. Res. 30: 237-241.

Gorham, J. R. 1991. Insect and Mite Pests in Food: An Illustrated Key. Vol. 1 and 2. USDA Agric. Handbk. 655.

Hagstrum, D. W. 2000. Using five sampling methods to measure insect distribution and abundance in bins storing wheat. J. Stored Prod. Res. 36: 253-262.

Hagstrum, D. W. and B. Subramanyam. 2009. Table 1.1 Insects and their natural enemies associated with stored products, p 183. *In* Hagstrum, D. W. and B. Subramanyam (eds.), Stored-product insect resource. AACC International Inc. St. Paul, MN.

Hagstrum, D.W, T.W. Phillips and G. Cuperus (Eds.). 2012. Stored Product Protection. Kansas State University. Manhattan, KS. 352 p. (http://ksre.ksu.edu/library/p.aspx?tabid=70).

Harein, P. and Meronuck, R. 1992. Stored grain losses due to insects and molds and the importance of proper grain management. In: Krischik, V., Cuperus, G. W. and Galliart, D. (eds) Stored Product Management, Oklahoma Cooperative Extension Service Publication, E-912, Oklahoma, pp. 29-31.

Konemann, C. E., Opit, G. E., Gautam, S. G., Bajracharya, N. S., Shakya, K. 2014. Levels of phosphine resistance in rusty grain beetles, Cryptolestes ferrugineus (Stephens) (Coleoptera: Laemophloeidae), from stored wheat in Oklahoma. DOI: 10.14455/DOA.res.2014.75. 11th International working conference on stored product protection.

Lindgren, B.S. 1983. A multiple-funnel trap for Scolytid beetles (Coleoptera). Can. Entomol. 115: 299-302

Loschiavo, S. R., J. Wong, N. D. G. White, H. D. Pierce Jr., J. H. Borden and A. C. Oehlschlager. 1986. Field-Evaluation of a pheromone to detect adult rusty grain beetles, *Cryptolestes ferrugineus* (COLEOPTERA, Cucujidae), in stored grain. *Can. Ent.* 118.1: 1-8.

Martinez, J. L., Granovsky, T. A., Williams, H. J., Vinson, B., and Burkholder, W. E. 1986. Estimation of aerial density of the lesser grain borer (Coleoptera: Bostrichidae) in a warehouse using Dominica lure traps. DOI: http://dx.doi.org/10.1093/jee/79.4.1134. 1134-1138.

Nansen, C. et al. 2004. Relationship between flight activity outside grain bins and probe trap catches inside grain bins of *Cryptolestes ferrugineus*. Environ. Entomol. 33(5): 1465-1470.

Phelan, P. L., and Baker, T. C. 1986. Cross-attraction of five species of stored-product Phycitinae (Lepidoptera: Pyralidae) in a wind tunnel. Environ. Entomol. 15: 369-372

Phillips, T. W. 1997. Semiochemicals of stored-product insects: research and applications. *J Stored Prod Res* 33.1: 17-30.

Phillips, T. W., P. M. Cogan and H. Y. Fadamiro. 2000. Pheromones. Pages 273-302, In Subramanyam, Bh. And D. W. Hagstrum, eds. Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers. Boston.

Pierce, A. M, Borden, J. H., Oehlschlager, A. C. 1991. Olfactory response to beetle-produced volatiles and host-food attractants by *Oryzaephilus surinamensis* and *O. mercator*. Can. J. Zool. 59: 1980-1990.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rilett, Omar R. 1949. The Biology of *Laemophloeus ferrugineus* (steph.). *Can. J. Res.* 27: 112-148.

Schlyter, F. and G. A. Birgersson. 1999. Forest Beetles. Pages 113-148, In Hardie, J. and A. K. Minks, eds. Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants. CABI Publishing.

Sheppard, E.H. 1936. Note on *Cryptolestes ferrugineus* Steph., a cucujid occurring in the *Trichogramma minutum* parasite laboratory in Colorado State College. Colo., Agr. Exp. Stn., Tech. Bull. 17, 20p.

Silverstein, R. M., Rodin, J. O., Burkholder, W. E., Gorman, J. E. 1967. Sex attractant of the black carpet beetle. Science. 157: 85-87

Sinha, R. N. 1961. Insects and mites associated with hot spots in farm stored grain. Can. Entomol. Issue 8: Vol. 93, 609-621.

Subramanyam, Bh., D. W. Hagstrum, and T. C. Schenk. 1993. Sampling adult beetles (Coleoptera) associated with stored grain: comparing detection and mean trap catch efficiency of two types of probe traps. Environ. Entomol. 22: 33-42.

Throne, J. and M.P. Culik. 1989. Progeny development and duration of development of rusty grain beetles, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae), on cracked and whole corn. J. Entomol. Sci. 24: 150-155.

Toews, M.D., T.W. Phillips and M.E. Payton. 2005. Estimating populations of grain beetles using probe traps in wheat-filled concrete silos. Environ. Entomol. 34(3): 712-718.

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

White, N. D. G. and Bell, R. J. 1993. Variable longevity in the rusty grain beetle, *Cryptolestes ferrugineus*. Proceedings of the 6th International Working Conference on Stored-product Protection. Vol. 1. Pp. 608-612.

Wong, J. W., V. Verigin, A. C. Oehlschlager, J. H. Borden, H. D. Pierce, A. M. Pierce, and L. Chong. 1983. Isolation and identification of two macrolide pheromones from the frass of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae). J. Chem. Ecol. 9.4: 451-74.