USE OF TRAPS TO ESTIMATE *CRYPTOLESTES FERRUGINEUS* (COLEOPTERA: CUCUJIDAE) IN STORED WHEAT

By

MICHAEL D. TOEWS

Bachelor of Science
Fort Hays State University
Hays, Kansas
1995

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1998

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Thesis Approved:

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Thesis Advisor

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Dean of the Graduate College

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CHAPTER 1

INTRODUCTION

Overview

The United States is a global leader in annual wheat (*Triticum aestivum* L.) production. Grain production and storage is a multi-billion dollar industry and is the cornerstone of agribusiness. Following harvest in the U.S., wheat is often stored in commercial grain management facilities consisting of numerous concrete silos. Long-term bulk grain storage in these silos provides the ideal habitat for a number of insect species that attack stored-products including *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae), the rusty grain beetle. Stored-product insects, if improperly managed, continue to infest grain in storage and eventually result in reduced profit margins. Chemical inputs have traditionally been used to control infestations of storage insects; however, domestic users and importing countries are imposing greater restrictions on pesticide residues in raw grain and cereal products. These restrictions, coupled with existing insect infestations, threaten to destroy the ability of domestic producers to deliver clean, residue-free grain. Therefore, it is critical to develop new methodologies to manage insect infestations of stored product insects in concrete silos.

This project was designed to improve trap-based sampling methods for management of the rusty grain beetle, one of the most prolific insect pests in North American stored wheat. A trap-based monitoring system may provide grain managers with timely information using a minimal amount of effort. Current literature on insect sampling utilizing traps is limited to steel storage facilities; however, most storage facilities in the southern U.S. are constructed of concrete (Kenkel et al. 1994). Additional aspects of this project were to provide quantitative information about the ecology of *C. ferrugineus* in concrete silos as a basis for establishing effective sampling programs and to assess trap designs and electronic detection of trapped insects. New methodologies and improved sampling protocols resulting from this project will enable grain managers to effectively safeguard this important human food source.

Background and Significance

Grain Storage in the United States

Throughout the 1990's, U.S. wheat production was nearly 2.4 billion bushels annually (USDA 2000). From 1990 to 1999, an average of 1.6 billion bushels was still being stored each December of these years (USDA 2000). Insects are directly responsible for 10-20% of post-harvest losses to stored grain in the US. A survey of Oklahoma grain producers and elevator managers found that both groups ranked insects as their greatest storage problem (Cuperus et al. 1990, Kenkel et al. 1994). Aside from direct feeding, insects also damage grain by contaminating it with cast skins, fecal material, webbing, and body parts. Insect feeding in stored grain can also contribute to lower grain test weights (bulk density) and the presence of musty odors. In addition, heavily infested grains favor development of fungi, including some that produce mycotoxins. Insect feeding, grain contamination, lower test weights, and fungi each can suppress grain value at the point of sale.

Control of stored-product insects is usually addressed through pesticide application. Unfortunately, pesticides tend to leave residues. In 1996, 70.3% of all wheat sampled from domestic grain elevators had malathion residues, 73.2% had chlorpyrifos-methyl residues, and 14.4% had chlorpyrifos residues (USDA 1996). Elevator managers utilize phosphine for insect control but insecticide resistance to phosphine is an increasing problem (Halliday et al. 1983). Industry has a genuine need to find and implement effective new methodologies to manage constantly evolving insect populations.

Cryptolestes ferrugineus

The rusty grain beetle is well adapted to reproducing in stored grain. Females deposit two to three eggs per day in small crevices or cracks in grain kernels (Rilett 1949, Smith 1965). Eggs hatch in four to five days and the complete lifecycle takes about three weeks at optimum conditions of 35.0 °C and 70% RH (Smith 1965). Sex ratio is female (62-75%) biased resulting in many eggs over a short time period (Rilett 1949). Rusty grain beetles have an average lifespan of 6 to 9 months and adults are very cold tolerant compared to other stored-product insects (Fields and White 1997).

The rusty grain beetle is a secondary (external feeding) cosmopolitan stored grain pest (Rees 1996). It does not cause insect damaged kernels (IDK), an official U.S. grain grading factor. The insect does feed on broken kernels, flour, and perhaps mold in the grain mass. An insect population buildup can result in increased grain moisture and temperature. Increased temperature and moisture can increase the population growth rate of stored product insects. These conditions can lead to mold development, which increases the probability of a price discount at the point of sale. Additionally, any two or more live stored-product insects per sample result in grain that will be graded as "infested" (GIPSA 1997). In Kansas, delivering infested wheat resulted in a discount of $0 to $0.6 per bushel (Reed et al. 1989); a nationwide survey found the mean discount rate was $0.08 per bushel (Kenkel et al. 1994).

Wheat Storage in Concrete Silos

Many countries that import wheat request no pesticide residues (zero tolerance). In addition, foreign and domestic consumers, flour millers, and breakfast cereal manufacturers are moving toward more stringent standards for kernel uniformity, grain cleanliness, falling number, wet gluten, extraction, and other end-user characteristics (Kenkel 1997). To meet these demands, elevator operators currently manage insect populations in concrete silos with multiple, calendar-scheduled phosphine gas fumigations during a single storage year. Operators then fumigate again before sale to prevent the possibility of an insect-induced price discount. Numerous residual insecticide applications and fumigations occur throughout grain transportation and storage processes (Cuperus et al. 1990). Unfortunately, silos are not gas tight so effective fumigation is often unlikely. Additional problems with the U.S. grain storage system include repeated worker exposure hazards to fumigants and insecticide resistance.

A common strategy for insect control is to reduce grain temperature by aeration using ambient air. Large bulks of grain, such as those found in concrete silos, maintain temperatures conducive to insect activity well into the winter months. Only half of the concrete silos in the U.S. are equipped with aeration systems, so insect control by grain cooling may be unattainable in these structures (Kenkel et al. 1994). A common alternative to aeration is grain "turning." In this process, grain from one silo is emptied into the bottom of the elevator and then elevated into another silo. The grain cools as it falls to the bottom of the new silo. This process results in a slight temperature decrease and only limited insect mortality. However, turning also increases broken kernels. More broken kernels will reduce the official grade and the profit potential of the commodity.

There are always a few bushels of wheat left on the silo floor after grain has been removed. This residual grain is rarely removed because of accessibility problems and costs associated with labor. Residual grain acts as an insect refuge over the spring and summer until harvest. Over a span of years, this residual grain gets packed tightly and becomes so dense that phosphine gas penetration is limited. Because of these complications, insect populations within the silo can persist from year to year prior to grain fill in midsummer.

Importing countries and domestic flour millers desire high-quality grain from the U.S.(Cuperus and Krischik 1995). In order to meet the demand for this high quality grain, a pest control system must limit insect pests while providing a residue-free product for consumers and importing countries. New sampling protocols would enable industry to target judicious use of pesticides while preventing excess losses. Trap-based insect monitoring has proven successful in small steel grain bins and could be used in concrete silos. However, these techniques need to be optimized for use in silos. Accurate estimates of pest populations are needed immediately to reduce fumigant overuse.

Insect Sampling in Concrete Silos

Grain and insect samples are difficult to obtain in concrete silos due to limited means of entry. Grain can be accessed at two sites. A ½ m^2 door on top of the silo allows access to the top of the grain bulk if the silo is filled. Access to the rest of the grain mass only occurs when grain is being moved. Country elevator operators move grain among silos to segregate or mix lots of wheat with different properties such as high protein, test weight, moisture content, and insect damage prior to shipping. Samples can be obtained from the open conveyer belt that carries the commodity when it is being moved. Grain samples must then be placed in a bag, labeled, and brought to a laboratory prior to inspection. Laboratory processing includes passing grain over an inclined sieve to separate out the insects (White 1983). This type of data collection is seldom used in industry due to the enormous investment of both time and labor in addition to the expertise needed to count and identify the insects.

The deep bin cup and the vacuum sampler can be used to remove grain samples for absolute density estimation of insect populations (Hagstrum et al. 1995). The deep bin cup is a spear shaped brass cylinder attached to the end of a long extendable metal handle. The operator can control when grain enters the cup; thus samples can be obtained from the desired depth or location. Vacuum sampling utilizes a commercial suction device with removable hose sections. Samples can be obtained as far down as 10 m or more from the grain surface with all of the hose sections attached. Insects are usually sieved from the grain and then expressed as the number of live insects per kg of wheat. These methods require enormous inputs of time and skilled labor.

Two methods are available for sampling moving grain. The pelican sampler is a specialized leather pouch attached to a long pole that is used to catch a sample of falling grain. It is designed for use when unloading or loading railcars and trucks. Grain being moved on belts can be sampled using an Ellis cup. This sampling tool is a narrow aluminum cup that is lowered into the moving grain stream. Ellis cup samples are good for detecting insects but the operator must stand next to the conveyer belt and take samples for as long as the grain is moving. Large elevators may move grain every day and the process could take weeks to complete. Small operations run their equipment all day for weeks on end when selling grain.

Sampling methods in concrete silos are limited by Federal Occupational Safety and Health (OSHA) regulations specifying that the operator must stay out of the silo or obtain clearance for entry into a "confined space" (CFR 2000). Entry into a confined space entry requires a permit, atmospheric testing, a body harness with lifeline, on site rescue equipment, and additional personnel. In addition, any remaining mechanical equipment connected to that silo must be locked using a "lock-out/ tag-out" system (CFR 2000). Silos often share fans or augers, so portions of the elevator may be inoperable while the entry is in progress. Therefore, confined space entries are discouraged because they are impractical for quick sampling.

Numerous complications have eliminated past scientific endeavors for insect detection and estimation in concrete silos. Research in concrete silos is cumbersome, because the work must be conducted during the business day while not interfering with the normal business activities of the profit driven enterprise. This is often impractical, due to the time involved in extracting grain samples for research. In addition, corporate headquarters may demand that the grain be moved or sold even when the on-site manager has agreed to cooperate with the research project. There are no commercial size facilities built solely for research purposes.

Use of Traps for Stored-product Beetles

Currently, stored-product insect traps are commercially available and have been successfully used for sampling programs in steel bins (White et al. 1990). Advantages to insect sampling with traps include: ease of use, minimal requirements for time and manpower, increased sensitivity to detect adult insects (Barak and Harein 1982, Lippert and Hagstrum 1987), ability to sample continuously over an extended period of time, and traps can be used without physically entering the silo. Trapping is a better method of finding new infestations because insects are detected in traps earlier than in grain samples (White et al. 1990).

A stored-grain insect probe trap is essentially a perforated tube made of metal or plastic. Small holes are spaced evenly across the surface of the trap body. The top of the tube is sealed with a plastic cover. At the bottom of the tube is an escape proof collection reservoir that can be removed easily. Traps can be placed near the grain surface or pushed down several meters into the grain mass with a long pole.

Insects, such as the rusty grain beetle, actively move arou/nd in the grain mass foraging, seeking mates, and following pheromone trails. Insects also move in relation to changing temperature in the grain (Flinn and Hagstrum 1998). Traps positioned in the grain exploit natural movement as the insects encounter the trap during dispersal. Other species of insects are not as active as rusty grain beetles and are less likely to be captured in these traps.

A more recent development for trapping stored-product pests is an electronic probe trap called the Electronic Grain Probe Insect Counter (EGPIC) (Shuman et al. 1996). This system consists of a probe trap body modified with an infrared beam generator and infrared detecting sensor. Supporting electronics and a computer software program allow insects to be counted automatically as they fall through the probe. While falling, an insect breaks the plane of an infrared light beam and then the computer registers a count in electronic memory. Collected data are conveniently displayed on a computer located in the office. This system requires little maintenance once the traps are in place. The EGPIC system can accommodate multiple probes so that numerous bins can be monitored simultaneously.

Future domestic and foreign grain buyers will require tighter specifications for kernel uniformity, grain cleanliness, and pesticide residues (Kenkel 1997). A usable trap-based monitoring system would benefit the seller and provide the buyer with better quality grain. Further development of the EGPIC system may eliminate the time needed for servicing traps so that monitoring would be more economical than fumigation by the calendar. Trap-based sampling would eliminate the need for excessive grain fumigation, reduce the chance of worker exposure hazards, and decrease the time constraints imposed by traditional sampling. Grain suppliers could sell to any country in the world without the threat of rejection. Finally, end-point users and consumers would benefit from reduced or residue free grains free of insect cast skins, fecal material, body parts, and foul odors.

Objectives

This dissertation was written in separate publishable manuscripts. The following three chapters are formal manuscripts that will be submitted for publication in scientific journals. Each chapter was written to conform to the guidelines and policies for manuscript preparation with the Entomological Society of America. The second chapter includes probe trap results from laboratory studies in growth chambers. Objectives were to investigate how insect captures changed with grain temperature and starting insect density. Chapter 2 also includes a study about recovery of dead insects in probe traps. Chapter 3 includes all work with the EGPIC system in small steel bins on the experiment farm. Objectives were to: (1) compare rusty grain beetle captures among three different insect trap designs; (2) examine changes in rate of electronic counts following probe insertion into the grain; (3) determine if time of day affects rate of electronic counts; and (4) investigate the relationship between mean daily air temperature and quantity of electronic counts when probes are placed near the grain surface. Finally, Chapter 4 includes extensive results comparing grain samples to insect captures in probe traps. The study was conducted at two commercial elevators with concrete silos. Objectives were to: (1) document insect infestation immediately after harvest; (2) contrast captures in probe traps to insects sieved from grain samples; (3) model insect captures in probe traps with grain quality parameters and temperature. Each of these objectives was completed independently for the most common insect species: rusty grain beetle, lesser grain borer, rice weevil, and red flour beetle.

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Address correspondence to:

Michael D. Toews

127 Noble Research Center

Stillwater, OK 74078-3033

(405) 744-5099 or

FAX (405) 744-6039

e-mail: toewsm@okstate.edu

Factors affecting capture of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) in traps placed in stored wheat

MICHAEL D. TOEWS AND THOMAS W. PHILLIPS

Department of Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, OK 74078-3033.

Abstract

Cryptolestes ferrugineus (Stephens), the rusty grain beetle, infests grain externally and is a frequent pest of stored wheat throughout North America. Detection and population estimation of this insect are important in avoiding discounts at the point of sale. Laboratory experiments presented here show that WB II and PC pitfall traps capture adult rusty grain beetles; furthermore, capture rates were strongly related to insect densities in wheat. In a simultaneous test of insect density and temperature between 20° and 40°C, insect capture in WB II probe traps increased linearly with insect density in the grain but had a quadratic response to temperature. Further studies ascertained that hole density and diameter of the probe trap were unrelated to insect capture. Finally, another experiment revealed that dead rusty grain beetles could be recovered in probe traps. The incidence of dead insects increased with insect density when insects were found in an aggregated dispersal pattern, such as would be found following phosphine fumigation of grain. Experiments discussed here will help grain managers understand how probe traps may be used in *C. ferrugineus* population estimation.

Key Words: Stored products, rusty grain beetle, probe traps, population estimation, temperature.

Introduction

Cryptolestes ferrugineus (Stephens) (Coleoptera: Cucujidae), the rusty grain beetle, is a cosmopolitan pest of stored cereals and oilseeds. This insect is characterized as an external infesting insect and thus it does not cause a great deal of damage to the actual wheat kernel compared with those insects classified as internal feeders. However, regardless of species, a large insect population can result in increased grain temperature, moisture, and mold. Each of these conditions over time could warrant a price discount at the point of sale. The mere presence of insects in stored wheat is problematic since two or more live weevils or other live insects injurious to stored grain per sample result in grain that will be graded as "infested" in the United States (GIPSA 1997). In Kansas, delivering infested wheat resulted in a discount of $0 to $0.6 per bushel (Reed et al. 1989); a nationwide survey found the mean discount rate was $0.08 per bushel (Kenkel et al. 1994).

Rusty grain beetles are well adapted to reproducing in stored grain. Females deposit two to three eggs per day in small crevices or cracks in grain kernels (Rilett 1949, Smith 1965). Eggs hatch in four to five days while the complete life cycle takes about three weeks under optimum conditions of 35.0°C and 70% RH (Smith 1965). Sex ratio is biased (62-75%) in favor of females (Rilett 1949). Rusty grain beetles have an average lifespan of 6 to 9 months and adults are known to be very cold tolerant compared to other stored-product insects (Fields and White 1997).

Sampling of rusty grain beetles using modified pitfall traps is well documented (Loschiavo 1974, Loschiavo and Smith 1986, White and Loschiavo 1986, Lippert and Hagstrum 1987, Subramanyam and Harein 1990, Vela-Coiffier et al. 1997). The operation of pitfall traps in grain is based on moving insects contacting and entering the trap, falling through a void in the trap, and accumulating in the collection reservoir. Trapping is successful because rusty grain beetles readily move through grain. Factors affecting movement of rusty grain beetles include temperature, moisture, and gravity (Watters 1969, Loschiavo 1983). Advantages to insect sampling with traps include ease of use, increased sensitivity for adult insects (Barak and Harein 1982, Lippert and Hagstrum 1987), and ability to sample continuously over an extended time period. New insect infestations are detected in traps earlier than in grain samples (Lippert and Hagstrum 1987, Vela-Coiffier et al. 1997).

Several studies have elucidated some of the factors affecting trap captures of rusty grain beetles in stored wheat. These factors included: trap design (Subramanyam et al. 1993, Fargo et al. 1994, Weston and Barney 1998), trap position (White and Loschiavo 1986, Hagstrum 2000), grain temperature (Loschiavo and Smith 1986, White and Loschiavo 1986, Fargo et al. 1989, Hagstrum et al. 1998), population density (White and Loschiavo 1986), and shape of the storage container (Loschiavo 1974). Consideration of these factors in combination could help grain managers interpret trap catch data for pest management decisions (White et al. 1990).

Objectives of these laboratory studies included: 1) investigate the effects of rusty grain beetle density in grain on the number of insects trapped using two trap designs; 2) examine the simultaneous effects of temperature and insect density on captures of rusty grain beetles; 3) assess the effects of hole density and diameter of probe traps on number of rusty grain beetles trapped; 4) determine how aggregation pattern affects recovery of dead rusty grain beetles in probe traps.

Materials and Methods

General Methods.

Adult rusty grain beetles used for these studies originated from established laboratory colonies. The colony began with adults collected from stored wheat near Stillwater, OK during June 1998. Subsequent generations were raised in the laboratory and maintained on hard red winter wheat, *Triticum aestivum* L., cv AgSeco 7853, in environmental chambers set at 34.0°C and 70% RH under completely dark conditions. Wheat for rearing was cracked and adjusted to 13% moisture. Adult insects used during experiments were comprised of mixed ages and sexes to simulate natural conditions.

All experiments were conducted with AgSeco 7853 wheat obtained directly from a local grower during the week of harvest 1997. Wheat was frozen at –10.0°C for 7 d to eliminate possible insect infestation prior to experiments. Bulk grain was stored in a cold room at 10.0°C when not in use. Grain moisture contents were uniform among silos within a given experiment, and varied among experiments from 8.6% to 13.5% (method of American Society of Agricultural Engineers 1996).

The experimental unit in all experiments was a laboratory "silo" filled with wheat. Experiments 1 and 2 were conducted with 27.2 kg of grain placed in 61 cm tall round plastic "silos" made from PVC pipe with an inside diameter of 30.5 cm. Silos had 13 cm thick plexi-glass bottoms cemented into the cylinder. Experiments 3 through 6 were conducted with 17 kg wheat in 35.5 cm tall plastic "silos" composed of 19 liter plastic buckets with an inside diameter of 28 cm. Plastic garbage bags were stretched across the top of each silo and anchored in place with large rubber bands to prevent insect immigration or emigration.

Silos were filled with wheat and placed immediately in environmental chamber(s) for 24 h to allow homogenous grain temperatures prior to insect introduction. Insects were always released on the grain surface at a point midway between the center and silo outer wall. Insects were given 72 h to disperse without any traps in place. After the insect dispersal period, a trap was inserted into the center of the silo and left in place for 72 h, after which it was removed and the trapped insects counted.

Commercially available traps utilized in these studies were the Storgard WB II Probe (Trécé Inc., Salinas CA) and the PC trap (Agrisense BCS Ltd., Mid Glamorgan, UK). Traps did not contain attractants and no killing agents were used. The WB II is a probe style trap consisting of a 34 cm long perforated cylinder with a diameter of 33 mm (Burkholder 1988). A detachable collection tip holds insects following capture. The trapping region consists of a continuous series of holes positioned along the length of the trap. In contrast, the PC trap captures insects across a horizontal plane instead of on a vertical plane as found on the WB II trap. The trapping surface of the PC trap is on a lid snapped into place on top of a cone-shaped collection reservoir (Cogan et al. 1990). The cone opening measures 90 mm in diameter and the cone is 120 cm deep. The PC and WB II traps, as well as experimental traps fabricated in the laboratory, were used in the following experiments.

Experiment 1. Insect Captures in Probe Traps at Different Insect Densities.

Adult rusty grain beetles captured in WB II traps were investigated in densities of 0.4, 1.8, and 3.7 insects per kg grain. The centrally positioned trap was pushed to the bottom of the silo. This experiment used a randomized complete block design with six replicates of each treatment. A single walk-in growth chamber (Conviron model PGV

36, Winnipeg, Canada) set to 32.0° ± 0.5° C, 30 ± 5% RH, under completely dark conditions housed all experimental units simultaneously.

Experiment 2. Insect Captures in PC Traps at Different Insect Densities.

Capture of adult rusty grain beetles in PC traps was investigated in densities of one, two, and three insects per kg of grain. A single PC trap was inserted 17 cm below the center of the grain surface. An inverted 0.95 liter mason jar was used to push the centrally located trap into place and maintain the desired orientation with the trapping surface on a horizontal plane. This depth represents the middle of the trapping region when using a probe style trap. Experimental design was a randomized complete block design with 10 replicates of each treatment. A large walk-in growth chamber (Conviron model PGV 36, Winnipeg, Canada) set to 32.0 ± 0.5 C, 30 ± 5% RH, under completely dark conditions housed 15 experimental units for a single run. The experiment was conducted twice and the two runs combined to yield the 10 replications per treatment.

Experiment 3. Effects of Temperature and Insect Density.

This experiment was conducted to investigate simultaneous effects of temperature and insect density on capture of insects with WB II probe traps. Temperatures were 20, 25, 30, 35, and 40°C while insect densities were 1, 2, and 3 insects per kg of grain. Temperature loggers were maintained in all five chambers throughout the experiment to insure that experimental conditions in each replication were identical among weeks. Relative humidity was maintained at 65 ± 15% in all chambers by providing pans of water in the bottom of each chamber.

Since the experiment required multiple environmental chambers, a series of smaller upright chambers (model I-35 LVL, Percival Scientific, Boone, IA) was used and a smaller silo was adopted to accommodate more replications in the smaller area. The new silos were shorter than the total length of the probe taps; therefore, a hole was cut in the bottom of each silo so that the collection reservoir extended outside and below the silo (Fig. 1A). The entire trapping surface of each probe was exposed to the grain. Because traps were permanently anchored to the silo, a new methodology was devised to prevent insect capture in traps during the 72 h dispersal period. Legal size copier paper was tightly wrapped around each probe trap and taped with scotch tape to form a tube extending from the silo bottom to the headspace above the grain. One piece of tape was applied the length of the joint where the paper edge wrapped around itself. Following the 72 h insect dispersal, paper sleeves were removed by gently sliding them off the top of each probe, thus exposing the trapping surface.

Experimental design was a split plot arrangement of a randomized complete block design. The main plot effect was temperature, maintained in five separate environmental chambers, while the split plot consisted of the three insect densities established within each silo. One silo at each experimental density was present in all chambers. The experiment was blocked by week totaling 8 main plot replications.

Experiment 4. Probe Trap Hole Density Effect.

Probe traps were constructed in the laboratory to test the effect of variable hole density on adult rusty grain beetle captures. Thirty cm long probe traps, made of 22 mm diameter (o.d.) CPVC water pipe (2 mm thick walls), were drilled perpendicularly to the vertical plane with 3.175 mm diameter evenly spaced holes at densities of 40, 80, or 120 holes per individual trap. The holes were all concentrated in a 15 cm region starting 2 cm from the bottom end of each probe. The top of the trap was plugged with a number 2 rubber stopper and the bottom of the trap was cemented into a hole in the bottom of the silo using hot melt hobby glue (Adhesive Technologies, Inc., Hampton, NH). Below the silo, a small plastic funnel was cemented to the bottom so that it diverted all captured insects into a 10 ml glass collection vial (Fig. 1B). Stretched Parafilm-M Laboratory Film (American National Can, Neenah, WI) attached the collection vial to the plastic funnel. Previous research (Toews unpublished) determined that rusty grain beetles could not escape the collection vial. Since traps were cemented to the silo, the paper sleeve methodology described in experiment 3 was used. Insects were released in all experimental units at a rate of two beetles per kg of grain.

This experiment was designed as a randomized complete block with two replications per block. The experiment was conducted in four upright environmental chambers (model I-35 LVL, Percival Scientific, Boone, IA) set at 35.0° C, 70% RH, under completely dark conditions. The block effect for this experiment was defined as an individual growth chamber; each containing six silos (two each of the three hole density levels). In essence, each treatment was replicated eight times.

Experiment 5. Probe Trap Body Diameter Effect.

Probe traps of differing diameters using the same quantity of holes were examined to determine how diameter might influence insect capture. Probe traps were constructed in the laboratory from 30 cm long PVC pipe with outside diameters of 26, 42, and 60 mm. Wall thickness of the two smaller probes was 2 mm while thickness of the largest probe was 4 mm. All probes had 216 total holes spaced 1 cm apart within each row. Holes occupied a 28 cm stretch along the probe starting 2 cm from the bottom side. Rows of holes (same number of rows on all traps) were evenly spaced as calculated by probe trap diameter. The top end of each trap was plugged with a rubber stopper and the bottom of the trap was cemented to the silo bottom as described previously. A plastic funnel and collection vial were also attached as described previously. The paper sleeve method of exclusion was used as per experiments 3 and 4 to prevent trapping insects during acclimation. Insects were released in all silos at a rate of two beetles per kg of grain.

A randomized complete block design was used with multiple replications per block. The experiment was conducted in five upright environmental chambers set at 35.0° C, 70% RH, under dark conditions. The block effect for this experiment consisted of an individual growth chamber. All chambers contained six silos using two each of the three probe diameter levels. In essence, each treatment was replicated eight times.

Experiment 6. Recovery of Dead Rusty Grain Beetles in Probe Traps.

Adult rusty grain beetles were killed in a laboratory fumigation chamber using 223 ppm hydrogen phosphide gas (phosphine) at 24.0°C for 72 hr (methods of Phillips et al. 2000). Previous research (Toews unpublished) determined that no rusty grain beetles survived this treatment.

Fixed effects, including time period, insect density, and dispersion of insects within the experimental unit, were evaluated to determine how each affected recovery of dead adult insects in WB II probe traps. Two time period treatments included simply inserting the trap and then immediately removing it, contrasted with leaving it in the grain for 72 h. Other simultaneous factors included densities of 2, 5, and 10 insects per kg of grain, and insects placed in a uniform versus an aggregated dispersion pattern. All experiments were performed at ambient conditions.

Dispersion patterns with dead insects in silos were artificially manipulated to approximate opposite statistical distributions. For a uniform dispersion pattern, grain was poured through a funnel to give a uniform diameter grain stream while filling the silos and individual insects were dropped into the grain stream at evenly spaced intervals. Since grain flows all the way to the outer walls of the silo, it can be assumed that the introduced insects also flowed away from the falling grain stream and were distributed throughout the silo at approximately even intervals. The aggregated dispersion pattern was approximated by filling the silo with all but 350 g of uninfested wheat and then placing all insects in the center of each silo. The remaining grain was then added on top of the insects.

After filling each silo with grain, a single WB II probe trap was centrally inserted all the way to the bottom. Once probes were inserted, silos were intentionally left undisturbed so that vibrations would not bias the 72 h time treatment by knocking insects into traps. Care was taken to insert and remove traps at approximately the same speed. The experimental design was a 3 x 2 x 2 factorial arrangement of treatments in a completely randomized design with 8 replications per treatment combination.

Data Analysis.

Data from all experiments were analyzed using PC-SAS version 8.0 (SAS Institute 1999). All count data were transformed prior to analysis using a square root transformation (Zar 1984) to correct for heteroscedastic data associated with counts. Statistical inference was obtained using PROC MIXED (SAS Institute 1999) with Satterthwaite degree of freedom adjustments for the variance components. Significant interactions were further scrutinized using the SLICE option of the LSMEANS statement.

When differences ($P < 0.05$) occurred, numerically related treatments were analyzed further with trend analysis using orthogonal polynomials. Coefficients for unusually spaced data were calculated using the method of Wishart and Metakides (1953). PROC REG (SAS Institute 1999) was used to generate the equations and R^2 values.

Results

Insect captures increased with insect density using both trap types. Captures of adult rusty grain beetles in WB II probe traps were directly proportional to actual insect density in the silo during experiment 1 (Fig. 2A). Insect captures increased in a linear trend with increased insect density ($F = 34.97$; df = 1, 15; $P < 0.01$). As expected with count data, standard errors increased with increased insect capture. During experiment 2, captures of adult rusty grain beetles in PC traps were also directly related to insect density in the silos (Fig 2B). Moreover, there was a significant linear trend associated with increasing insect density ($F = 28.08$; df = 1, 26, $P < 0.01$).

Main effects for both temperature and insect density were independently significant for capture of rusty grain beetles in WB II traps during experiment 3 (Fig. 3A and 3B). Numbers of insects captured varied quadratically with temperature ($F = 34.92$; df = 1, 35; $P < 0.01$) while the effect for density had a strong linear trend ($F = 53.31$; df = 1, 69.7; $P < 0.01$). In comparing the quantity of captured insects at each density, the increase was not proportional to insect density. At the 1 insect per kg density, 6.1 rusty grain beetles were captured but the mean only increased by 2.6 insects in the 2 insects per kg treatment instead of doubling. However, at the 3 insects per kg density the mean capture increased by 4.3 additional insects.

Neither hole density or probe diameter affected number of insects captured. During experiment 4, quantity of insects captured in the hole density treatments did not vary significantly ($F = 0.04$; df = 2,21; $P = 0.96$). Mean captures of adult rusty grain beetles were: 3.4 ± 1.2 insects at 40 holes per trap; 3.5 ± 1.2 beetles at 80 holes per traps; and 3.5 ± 0.8 insects at 120 holes per trap. Likewise, insect captures in experiment 5 did not differ significantly among the probe trap diameters ($F = 2.42$; df = 2, 12.3; $P = 0.13$). Mean captures in 26 cm diameter traps were 5.5 ± 0.8 insects, compared to 8.9 ± 1.5 beetles in the 42 cm diameter probe, and 8.7 ± 1.3 insects in the 60 cm diameter trap body.

The insect density by dispersion pattern interaction for recovery of dead insects in WB II traps was significant during experiment 6 ($F = 15.20$; df = 2, 84, $P = 0.01$). When this two-way interaction was examined for simple effects (LSMEANS SLICE) while controlling for the interaction, it clearly demonstrated the majority of the variation contributing to this interaction was attributed to the aggregated dispersion pattern ($F = 31.55$; df = 2, 84; $P < 0.01$) as opposed to the uniform pattern by itself ($F = 0.01$; df = 2, 84; $P = 0.99$). In the uniform pattern treatment, only 1 insect was recovered in the 2 insects per kg treatment and 2 insects each in the 5 and 10 insects per kg treatments (Fig. 4). There were no differences in captures of rusty grain beetles between time period treatments (immediate = 3.5 ± 0.8, 72 h = 2.9 ± 0.7; $F = 0.72$; df = 1, 84; $P = 0.40$).

Discussion

Results from experiments 1, 2, and 5 clearly indicate that captures of rusty grain beetles accurately reflect actual insect densities within the grain. Under these controlled conditions, WB II probe traps appear to have great potential for estimating rusty grain beetle population density based on the linear trends that were strikingly evident. In addition, although this was a small-scale test, treatment levels were appropriate for approximating infestations at economically important numbers in actual storage conditions. In the US, any two or more live stored-product insect pests per sample of grain result in classification of "infested" grain (GIPSA 1997). All experiments reported here were conducted near the two insects per kg benchmark.

Even though the WB II and PC traps were not compared in the same experiment, the smaller error bars associated with the WB II probe trap certainly favor its suitability for density estimation studies. These differences are particularly evident when one considers that there are nearly twice as many replications in experiment 2 compared to experiment 1; increased replication should reduce the standard error if the variation is similar. Additionally, the larger R^2 value for the WB II probe trap indicates that trap captures associated with this trap type did a better job of explaining variations in insect density. More total insects were captured in PC traps under similar conditions, which may be advantageous for other situations such as mass trapping or insect detection at very low population levels.

Experiment 3 clearly reinforced prior findings (Loschiavo and Smith 1986; White and Loschiavo 1986) that temperature has a significant effect on captures of insects in probe traps. The range of temperatures in this study simulated typical conditions experienced in stored grain after harvest as well as the appropriate developmental temperatures for this species (Sinha and Watters 1985). Of particular interest, was the quadratic trend over the various temperatures. A basic assumption of this work is that capture of insects in traps is related to insect behavior, since probe traps simply assay insect movement. Decreased insect captures at 25 and 30°C indicate that rusty grain beetles are less active under these conditions and perhaps do not seek other conditions because these temperatures are optimal for settling. The 20°C temperature may represent sub-optimal conditions that were too cool, thus we hypothesize that the insects increased their movement in an effort to find a more suitable environment. The rate of increase in insect activity was especially noticeable from 30 to 40° C. Flinn and Hagstrum (1998) demonstrated that rusty grain beetles readily move from cooler (20° C) to warmer regions (42° C) of stored wheat. Hagstrum (1987) presented data documenting spatial variation in temperature of 7-10° per m in winter farm-stored wheat whereby insects could clearly move to warmer regions. Future rusty grain beetle population density models utilizing probe traps need to account for differences in the rate of movement based on temperature.

Results from experiment 3 further demonstrate that a positive density dependent relationship with insect capture in WB II probe traps was constant across temperatures. In the simplest scenario, an increase in insect density should result in a proportional increase in insect capture, but such a relationship was not observed. Data presented here suggest a more complex relationship unexplained by variations in temperature and density alone. Further studies could investigate the possibility that insect behavior around traps may change with increasing insect density.

Results from experiments on hole density and probe diameter provide important information pertaining to future trap design. Increasing the number of holes in the probe trap did not affect total insect capture but reduced slightly the variability associated with increased hole density. A smaller variance estimate could potentially reduce confidence interval size, which would be beneficial for estimation purposes. We initially assumed that a greater density of holes would result in more insects being captured due to increased potential for encountering holes. We also assumed that a trap with a large diameter would capture more insects than one with a small diameter simply because a larger "target" was presented. Large diameter traps would be unwieldy to use in commercial storage situations and our data indicate there is nothing to gain by using them. In addition, large diameter traps are difficult to insert into the grain due to the grain quantity that must be displaced. If fixed in the silo before filling, large diameter traps may break loose when the grain was moved.

These results clearly show that dead insects can be recovered in probe traps if they were present in an aggregated distribution pattern. Earlier work found that natural insect populations in full size silos tend be heavily concentrated in a few key areas of the grain mass and thus have a negative binomial distribution or aggregated pattern (Hagstrum et al. 1985). One area of the grain mass typically infested with insects is the peak of grain associated with the location where grain falls into the silo during loading. This area of the grain mass tends to have a warmer temperature, a higher moisture content, and a greater concentration of broken kernels and small debris (Noyes et al. 1995). Previous research has shown that populations of rusty grain beetles move toward and congregate in areas with higher temperatures (Flinn and Hagstrum 1998) and higher humidity (Loschiavo 1983). All of these conditions contribute to making the habitat more conducive to insect activity. The most common control tactic for insect infestation is phosphine fumigation. When grain was not moved, fumigation would leave dead adults in an aggregated dispersal pattern. Our data imply that dead insects will likely be recovered in probe traps following fumigation when the grain has not been moved and a significant infestation was present. In cases where grain is moved from one silo to another during application of fumigant, very few dead insects would be subsequently recovered in probe traps since movement of the grain would result in a more uniform dispersion pattern of dead insects.

An *a priori* assumption of using probe traps in stored grain has been that all dead insects found in a trap must have been alive to get into the trap. Since our experiment showed that previously dead insects could be recovered in probe traps, we recommend that history of fumigation and grain movement be considered when using probe traps to monitor grain insects.

Use of probe traps in stored wheat is an effective method to monitor and estimate insect populations; these data are needed to make informed integrated pest management decisions in stored grain management (Hagstrum and Flinn 1992). Grain managers should be aware that probe traps are very sensitive instruments for stored wheat insect detection, even at low population densities. Results of these experiments show that captures of rusty grain beetles from probe traps can be used to estimate population density across a range of temperatures and insect densities in clean wheat of similar moisture and grade. Other insect species are less active in the grain mass and may respond differently to probe traps (Southwood 1978; Wakefield and Cogan 1999). Once further research describes relationships between probe trap captures and densities of key pests in grain, these traps will be valuable tools for making integrated pest management decisions in stored grain.

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Figure Legends

Fig. 1A. Sketch of experimental silo retrofitted to maximize trapping surface of probe trap during experiment 3.

Fig. 1B. Sketch of experimental silo retrofitted with PVC probe trap body and collection reservoir used in experiments 4 and 5.

Fig. 2A. Mean ± SE captures of rusty grain beetles in WB II probe traps at 0.4, 1.8, and 3.7 insects per kg of wheat.

Fig. 2B. Mean ± SE captures of rusty grain beetles in PC traps at densities of 1, 2, and 3 insects per kg of wheat.

Fig. 3A. Mean ± SE captures of rusty grain beetles in WB II traps across densities at temperatures from 20 to 40°C.

Fig. 3B. Mean ± SE captures of rusty grain beetles in WB II traps across temperatures at densities of 1, 2, and 3 rusty grain beetles per kg of grain.

Fig. 4. Mean ± SE recovery of dead rusty grain beetles in WB II traps at aggregated and uniform dispersion patterns using densities of 2, 5, and 10 insects per kg of grain.

Fig. 1A

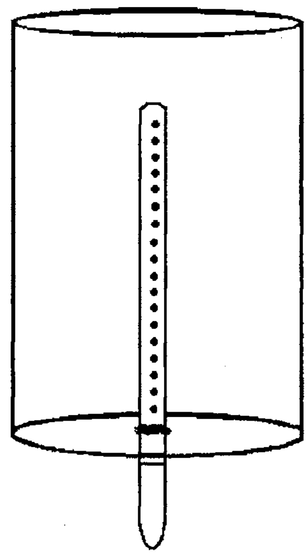


Fig. 1B

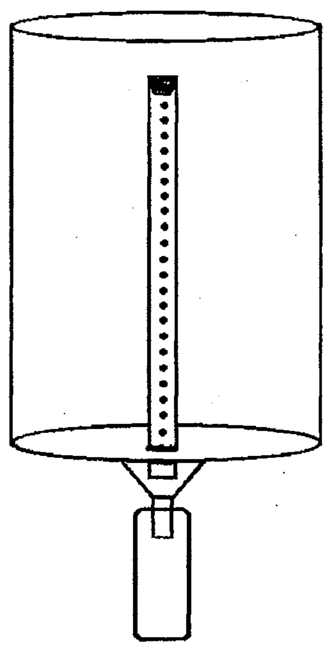


Fig. 2A

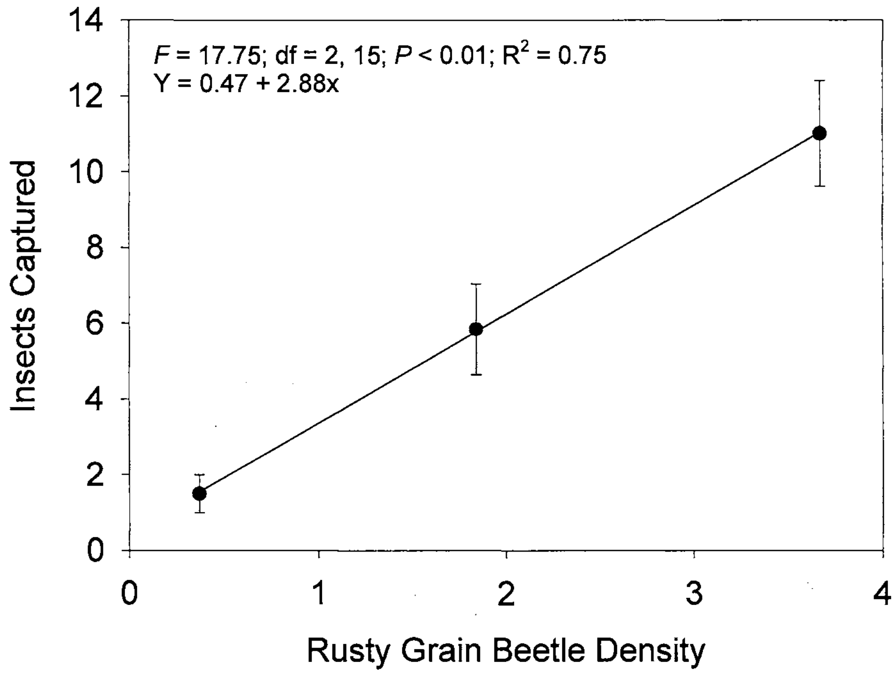

F = 17.75; df = 2, 15; P < 0.01; R2 = 0.75
Y = 0.47 + 2.88x
Insects Captured
Rusty Grain Beetle Density
0
2
4
6
8
10
12
14
0
1
2
3
4

Fig. 2B

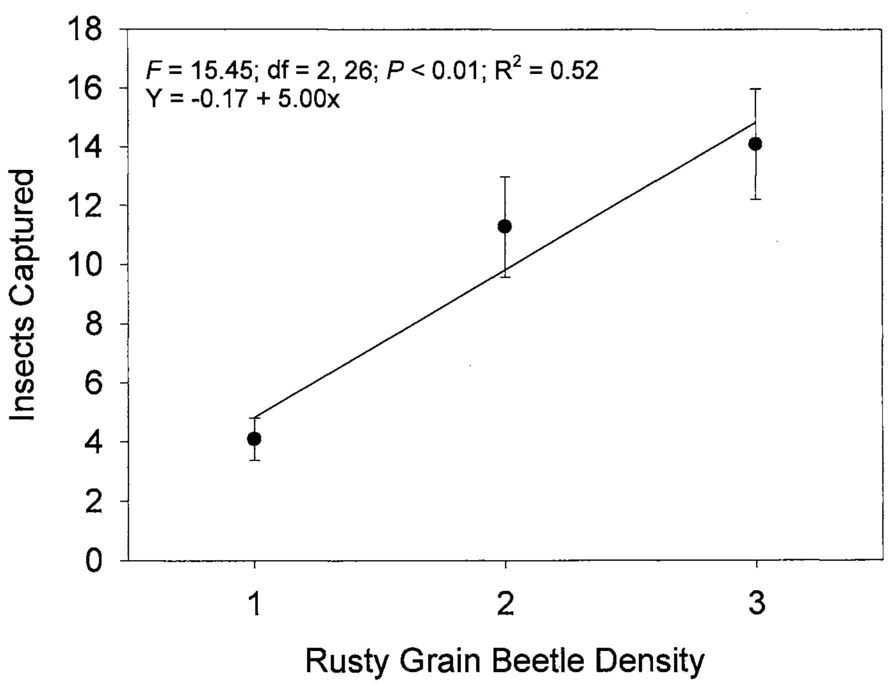

F = 15.45; df = 2, 26; P < 0.01; R2 = 0.52
Y = -0.17 + 5.00x
Insects Captured
Rusty Grain Beetle Density
0
2
4
6
8
10
12
14
16
18
1
2
3

Fig. 3A

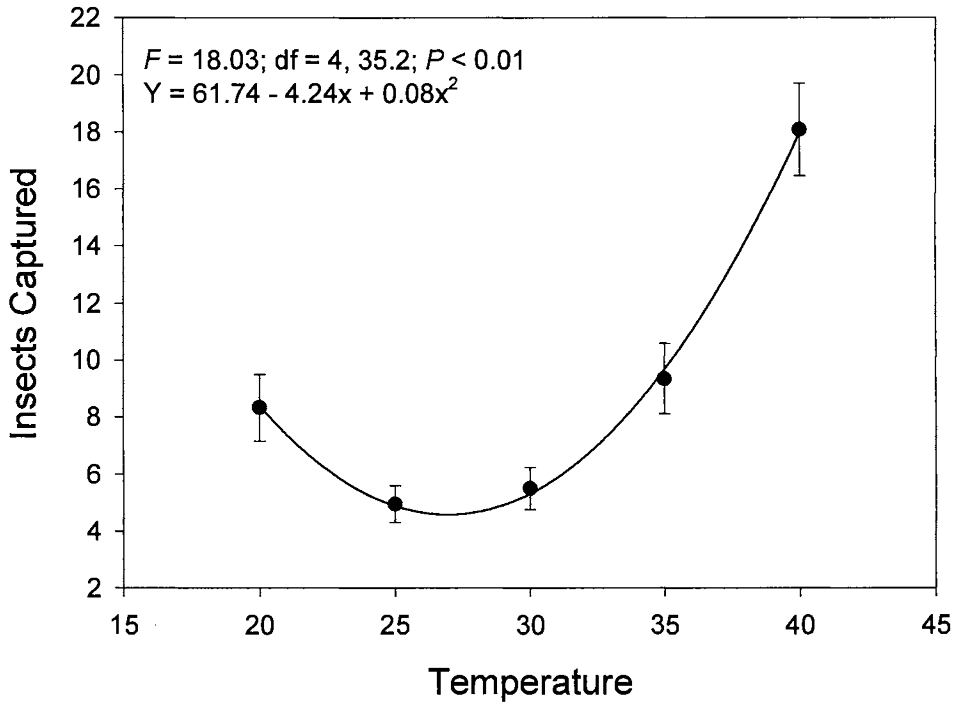

F = 18.03; df = 4, 35.2; P < 0.01
Y = 61.74 - 4.24x + 0.08x²
Insects Captured
22
20
18
16
14
12
10
8
6
4
2
15
20
25
30
35
40
45
Temperature

Fig. 3B

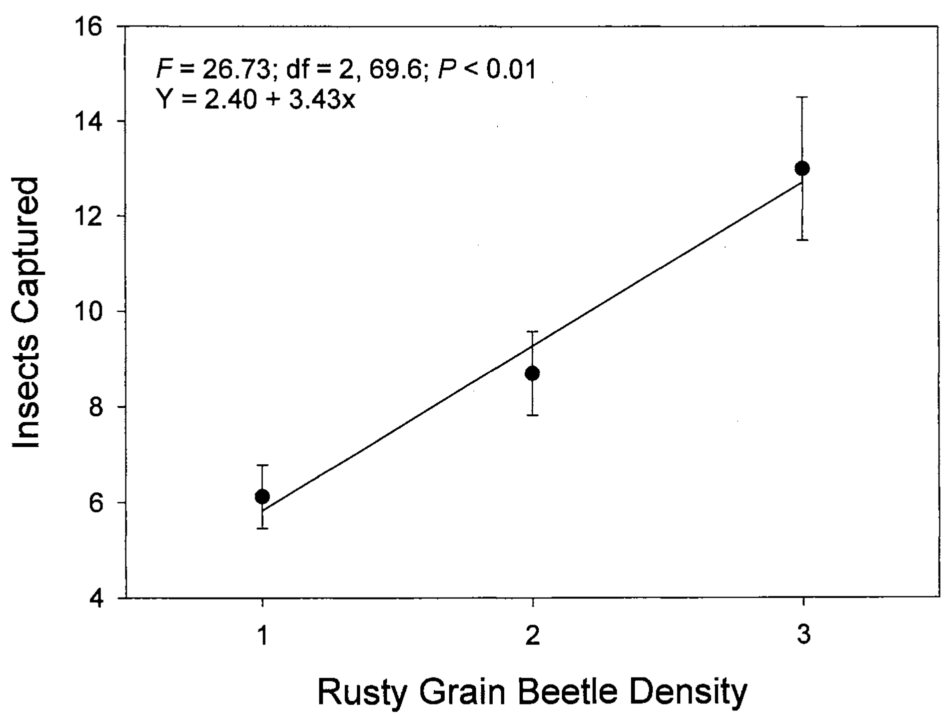
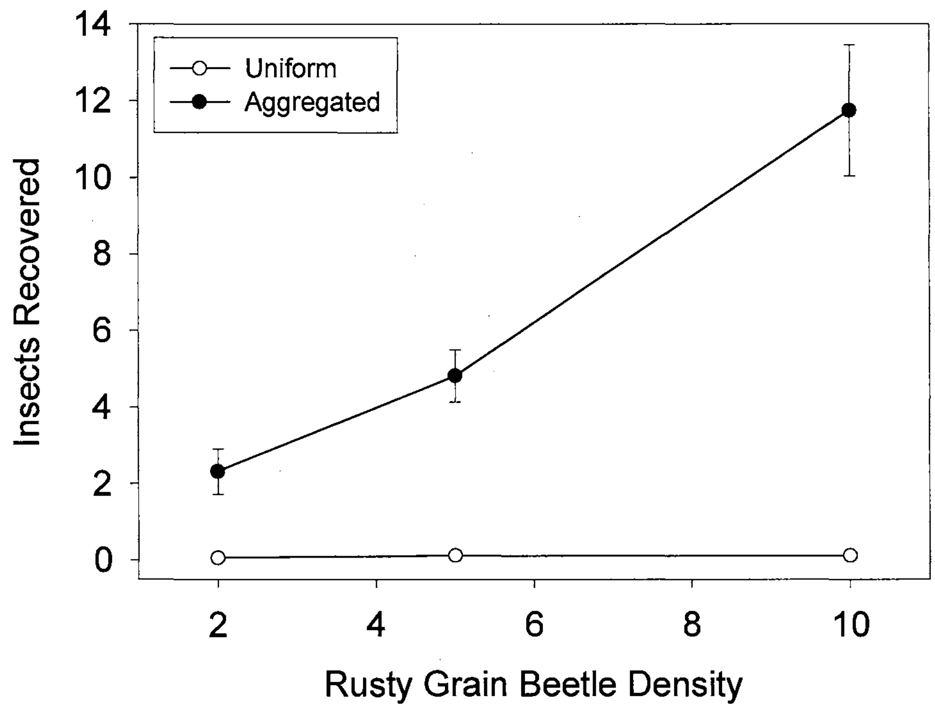

F = 26.73; df = 2, 69.6; P < 0.01
Y = 2.40 + 3.43x
Insects Captured
16
14
12
10
8
6
4
1
2
3
Rusty Grain Beetle Density

Fig. 4


Uniform
Aggregated
Insects Recovered
14
12
10
8
6
4
2
0
2
4
6
8
10
Rusty Grain Beetle Density

For: Journal of Stored Products Research

Address correspondence to:

Michael D. Toews

127 Noble Research Center

Stillwater, OK 74078-3033

(405) 744-5099 or

FAX (405) 744-6039

Email: toewsm@okstate.edu

Electronic and manual monitoring of *Cryptolestes ferrugineus* (Coleoptera: Cucujidae) in stored wheat

MICHAEL D. TOEWS[1], THOMAS W. PHILLIPS[1], AND DENNIS SHUMAN[2]

[1]Department of Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, OK 74078-3033.

[2]Center for Medical, Agricultural, and Veterinary Entomology, ARS-USDA, P. O. Box 14565, Gainesville, FL 32604.

Abstract

Field populations of *Cryptolestes ferrugineus* were captured in traps placed in small steel bins filled with stored wheat. Traps tested included two types of commercially available probe-pitfall traps and the electronic grain probe insect counter (EGPIC) system. Quantity of insects captured was compared among the three traps. Insect captures in PC pitfall traps exceeded those found in WB II probe and EGPIC probes. Number of rusty grain beetle captures was similar between the EGPIC probes and WB II probe traps. In a second study, insect counts generated by the EGPIC system were analyzed for changes in rate of capture after inserting the probe, and changes within a single 24 h period. Rusty grain beetle counts increased with increasing daily mean air temperature and decreased when air temperature decreased. There was a consistent increase in rate of counts during the early evening hours. Rate of counts did not vary consistently with days after the probe was inserted into the grain.

Key Words: Probe trap, rusty grain beetle, temperature, seasonal trend, diel periodicity

Introduction

Cryptolestes ferrugineus (Stephens) (Coleoptera: Cucujidae), the rusty grain beetle, is well adapted to reproducing in stored grain. Large populations can result in price discounts at the point of sale. Females deposit two to three eggs per day in small crevices or cracks in grain kernels (Rilett 1949, Smith 1965). Eggs hatch in four to five days and the complete life cycle takes about three weeks at optimum conditions of 35.0° C and 70% RH (Smith 1965). Sex ratio is female biased (62-75%), resulting in many eggs over a short time period (Rilett 1949). Rusty grain beetles have an average lifespan of 6 to 9 months and adults are very cold tolerant compared to other stored-product insects (Fields and White 1997). This species moves in relation to changing grain temperature (Flinn and Hagstrum 1998).

Sampling rusty grain beetles using modified pitfall traps is well documented (Loschiavo 1974, Loschiavo and Smith 1986, White and Loschiavo 1986, Lippert and Hagstrum 1987, Subramanyam and Harein 1990, Vela-Coiffier et al. 1997). The principle behind operation of pitfall traps placed in grain is that moving insects contact and enter the trap, fall through a void, and are collected in an escape proof reservoir below the trap. Advantages to insect sampling with traps include ease of use, minimal requirements for time and manpower, increased sensitivity in detecting adult insects (Barak and Harein 1982, Lippert and Hagstrum 1987) and the ability to sample continuously over an extended time period. Trapping is an effective method of finding new infestations as insects are detected in traps earlier than in grain samples (White et al. 1990). However, traps require bin entry and periodic servicing by an operator. Additional drawbacks include time required to count captured insects and expertise required to identify species. Grain bins are considered a confined space by the Occupational Safety and Health Administration. Therefore, the operator must follow established procedures to comply with federal laws and avoid known exposure hazards (CFR 2000).

The Electronic Grain Probe Insect Counter (EGPIC) (Shuman et al. 1996, Litzkow et al. 1997), an electronic insect monitoring system, has the potential to provide remote monitoring and a log of pest activity with reduced need for bin entry once a system is installed. EGPIC utilizes a cylindrical probe trap body that is equipped with an infrared beam generator and infrared detecting sensor at the base of the probe. Supporting electronics and a computer software program allow insects to be counted automatically as they fall through the probe; captured insects cross the infrared light beam and register an electronic count to a computer located inside an office. EGPIC represents a unique research tool for the study of grain insect ecology because it provides real-time data on insect activity at known locations in a grain bin. The EGPIC system has been used to count insects in the laboratory (Epsky and Shuman 2001) and in a stored oat facility (Arbogast et al. 2000). Research is needed on the use of EGPIC in field settings so that applications for pest management can be developed.

Specific objectives of this study were to: (1) compare rusty grain beetle captures among three different trap designs; (2) examine changes in rate of electronic counts following probe insertion into the grain; (3) determine if time of day affects rate of electronic counts; and (4) investigate the relationship between mean daily air temperature and quantity of electronic counts when probes are placed near the grain surface.

Materials and Methods

Study Site.

Studies took place on the Oklahoma State University Agricultural Experiment Station Farm located in Stillwater, Oklahoma. All experiments were conducted in steel bins, 3 m high by 1.8 m diameter, filled with 4.6 metric tons (180 bu) of hard red winter wheat, *Triticum aestivum* L., cv AgSeco 7853. Wheat in the bins was obtained the week of harvest (1997) and all insects were a result of immigration. *Cryptolestes ferrugineus* was by far the predominant species but *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) also inhabited these bins.

EGPIC System.

The prototype system (Arbogast et al. 2000) used in these experiments supports eight probes and creates a file with a time-stamp for each electronic count. Probes were modified from Agrisense lexan plastic probe traps (Trécé Inc., Salinas CA) (Burkholder 1984) with the holes sloping upwards (Subramanyam et al. 1989). The computer and electronic components of the EGPIC system were located on-site in a temperature-controlled outbuilding. Data were logged into the computer hard drive and transported to the laboratory on a floppy disk.

Trap Comparison Experiment.

Cryptolestes ferrugineus captures in three designs of stored-grain insect traps were compared to determine if the EGPIC probe body was similar in performance to other traps. Storegard WB II (Trécé Inc. Salinas, CA) (Burkholder 1988), 1997 EGPIC prototype (Arbogast et al. 2000), and PC pitfall traps (Agrisense BCS Ltd., Mid

Glamorgan, UK) (Cogan et al. 1990) were included in the study. The PC trap is an inverted cone in which the top surface has holes for insect entry, thus the trapping area is on a horizontal plane instead of on a vertical plane, as found with the EGPIC probe and WB II trap. PC traps were deployed at two locations in the grain; one just below the surface of the grain and the other located 17 cm from the grain surface. This depth represented the trapping midpoint for the probe traps. One of each of the four trap types: WB II, EGPIC, PC at the surface, and PC at 17 cm were placed in each of six bins. Bins were divided from the center of the bin into four equal quadrats. Individual traps were randomly assigned to an individual quadrat and placed 30 cm from the center of the bin. The EGPIC probe and WB II traps were positioned in the grain so that the top of the trap was 1 cm below the grain surface. All traps were left in place for 7 d, at which time the insects were removed and brought back to the laboratory for identification and counting. Trap position was then re-randomized and traps were placed back in the bins. This procedure was replicated each week over a four week period starting in September 1999. Although electronic counts were generated by the EGPIC system, only the actual physical counts of insects in the collection tip were analyzed.

Electronic Monitoring Study.

Electronic counts were analyzed to determine if counts increased after probe insertion and how time of day affected rate of counts. A single EGPIC probe was inserted into the center of each of eight bins starting in July and continuing through November 1998 when the insect captures fell to virtually zero. We experienced a series of technical difficulties throughout 1999 and did not use any of those data. Valid electronic monitoring started again in March 2000 and was conducted through June of the same year. Analyses are reported by month. The top of the probe was pushed into the grain until it was level with the grain surface. Every 7 d, insects were removed from the collection reservoir, infrared diodes were cleaned with compressed air, and probes were placed back in the grain and a new electronic file was started. Trapped insects were subsequently brought to the laboratory for identification and counting.

The database for this study was assembled by dividing a single probe's manual insect count for the week by the corresponding EGPIC electronic count. Count data were used only if this ratio was between 0.90 and 1.10, and if 95% of total insect captures in the probe were *C. ferrugineus*.

Temperatures were recorded throughout the studies. Mean daily air temperature was logged at a weather station located 450 m from the bins. Grain temperature 17 cm below the grain surface in the middle of the bin was obtained weekly during 1998 in eight bins. Grain temperature was obtained using type K thermocouple wire (Seedburo Equipment Co., Chicago IL) connected to an electronic thermocouple reader (Model 51, Fluke Corp., Everett, WA). From March through April 1999 small data loggers (HOBO TEMP, Onset Computer Corp, Bourne, MA) were placed in three bins to monitor and record real time temperature changes. One logger was placed on the top grain surface while a second was pushed into the grain to a depth of 17 cm. Loggers were set to record temperature for the entire week at five-minute intervals.

Statistical analyses were conducted using PROC MIXED (SAS Institute 1999) with degree of freedom adjustments for the variance components following the methods of Satterthwaite. The covariance structure was specified as compound symmetry. This procedure fit a mathematical model to the data set thereby eliminating the need for a data transformation to meet traditional analysis of variance assumptions. The compound symmetric covariance structure was chosen due to the nature of the data in which rate of electronic counts from similar times of day are correlated; meanwhile, there is less correlation between number of counts taken several hours apart. To determine if counts increased after probe insertion, comparisons were made among the 24 hour periods within individual weeks. Three hour time periods within a single day were compared to determine how time of day affected count rate. All analyses were presented by month and no general comparisons were conducted among months or between years due to the lack of repetition on these experimental units. Electronic counts from August and September were combined because the bins became heavily infested with *Liposcelis spp.* (Psocoptera: Liposcelidae) which interfered with electronic counts and made it difficult to find data sets meeting the assumptions discussed above.

Results

Manual Trap Comparison.

During the four-week study, 67,000 insects were recovered in traps. Rusty grain beetles comprised the majority of captures (96.8%) followed by *Cephalonomia waterstoni* (Gahan) (Hymenoptera: Bethylidae) (3.0%); and less than 1% each of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae; *Ahasverus advena* (Waltl) (Coleoptera: Cucujidae); *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae); *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae); *Typhaea stercorea* (L.) (Coleoptera: Mycetophagidae); and *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae). Only rusty grain beetle captures were used in the statistical analyses.

Stored grain insect traps captured different quantities of insects based on their principle of design. The WB II and EGPIC traps, which have their trapping surface on a horizontal plane, captured significantly fewer rusty grain beetles per week than did the PC traps at either depth in the grain (Fig. 1). Adult insect captures in PC traps were similar between locations in the grain.

Electronic Monitoring.

Insect species captured during this study were similar to results reported in the previous experiment. In general, mean rate of insect counts per hour started low in March (0.8 counts per hour) and gradually increased through April (1.8), May (2.2), and June (5.1), before reaching a maximum of 5.9 in July. Count rate then began receding through August (4.9), September (3.3), and October (1.8), until very few (0.5 counts per hour) were recorded in November. There were no significant interactions between day of capture and hours of capture during any of the analyses. Variation in counts among days after the probe was inserted (within a single week) showed no constant trends. Significant differences among days were detected in 4 of the 8 analyses, but the patterns were highly inconsistent.

Time of day had a significant effect on frequency of electronic counts. During 2000 there was a marked peak in electronic counts between 1500 and 1800 hours (Fig. 2). Although the analysis from April was not significant, the same pattern of higher counts between 1500 and 1800 hours was evident. The trend was also evident between 1800 and 2100 hours in July through September portion of the 1998 data (Fig. 3). This biological phenomenon was not present during the cooler months when very few counts were recorded such as during the months of October and November. Further inspection of the data from all months, showed a general decrease in electronic counts late in the morning (Figs. 2 and 3).

Temperature.

Mean daily air temperatures recorded at the weather station depict clear trends based on the season. Mean air temperature in 2000 was comparatively low in March (mean =10° C) and steadily increased through the month of June (mean = 24° C; Fig. 4A). In 1998, mean daily air temperature began quite warm in the month of July (mean = 30° C) and steadily decreased through the month of November (mean = 12° C; Fig. 4B). In 1998, grain temperatures show a slow warming trend from the start of the study on July 15 (Fig. 5). Grain temperature peaked on September 4 (week 8) and then steadily decreased. Temperature loggers from 1999, revealed that surface grain and air directly above the grain fluctuated an average of 25 °C from weekly minimum to weekly maximum. In contrast, grain temperature 17 cm below the surface only fluctuated an average of 2.7°C from the weekly minimum to weekly maximum. In addition, while surface grain temperature fluctuated daily, the temperature in the grain mass stayed constant on a daily basis and warmed or cooled little over the course of several days.

Correlation analysis between the mean daily air temperature and the mean electronic counts by week of occurrence were significant for the year 2000 data (F = 8.98; df = 1, 9; P = 0.02; $R^2 = 0.50$) and the 1998 data (F = 10.31; df = 1, 8; P = 0.01; $R^2 = 0.56$). During 2000, electronic counts increased as the mean daily temperature increased. Likewise, during 1998, counts fell with the falling mean daily temperatures.

Discussion

This study clearly depicted that insect captures in the prototype EGPIC probes were similar to WB II probe traps. Therefore, we feel it is a valid assumption that introspection gained into insect populations using the EGPIC system are applicable to similar insect populations when captured using WB II probe traps. Subramanyam et al. (1993) reported a similar finding when comparing the Agrisense lexan trap and the WB II probe trap. This finding means that thresholds devised using commercially available traps such as the WB II are applicable with the electronic monitoring system. Similar to our finding with the PC traps, Wakefield and Cogan (1999) captured more *Sitophilus granaries* (L.) (Coleoptera: Curculionidae) in PC traps than WB II probe traps.

Insect behavioral differences may play an important role explaining differences in insect captures among trap types. A possible explanation of these results is that rusty grain beetles in these bins move vertically in the grain mass depending on grain temperature near the surface. Under these conditions a device with the trapping surface spread out on a horizontal plane, such as the PC trap, would be contacted by more insects than one with the trapping surface on a vertical plane. Changes in temperature could initiate this daily movement. Large temperature differences exist daily in surface grain as we observed with the temperature logger on the grain surface. Insects may migrate down into the mass toward cooler grain during the hottest times of the day. Conversely, at cooler times during the day insect populations may migrate back to the surface.

Rusty grain beetle insect populations increase in time (Flinn et al. 1997) but captures in probe traps are related to both insect density and temperature (Toews Ch 2). Data interpretation reported here is based on electronic counts generated when insects fell through probes positioned near the grain surface. Rate of electronic counts per hour was reflective of general ambient temperature. Previous laboratory studies (Fargo et al. 1989; Toews Chapter 2) have shown that temperature significantly affects number of insects captured in traps. Correlation analyses have shown how mean electronic counts are correlated to mean air temperature. This finding leads us to believe that ambient temperature is also important in predicting trap captures of rusty grain beetles in the field. Populations of rusty grain beetles in November (1998) were likely large but cool grain temperatures (Fig. 5, weeks 17 through 19) resulted in a low rate of electronic counts.

We clearly demonstrated that differences in electronic counts can be dependent on time of day. Diel periodicity of an insect in the grain is a novel finding. While no direct evidence of insect diel periodicity in grain exists in the literature, the phenomenon has been demonstrated in flight behavior of other stored-product beetles including *Trogoderma variabile* (Coleoptera: Dermestidae), *Rhyzopertha dominica* (F.) (Wright and Morton 1995), and *Prostephanus truncatus* (Coleoptera: Bostrichidae) (Tigar et al. 1993). Since there were significant differences in rates of captures within a single day, a 24 hour period is the shortest trapping duration that should be prescribed if density estimation is the goal of monitoring.

Diel increases in locomotion could be the result of a circadian rhythm. This may be similar to the crepuscular flight patterns of *R. dominica* (Wright and Morton 1995). Alternatively, temperature could be driving the change. We documented daily high temperatures at midday on the grain surface. Perhaps the insects burrowed below the region where they could be captured in the traps during the middle of the day. Following this logic they would then emerge back near the surface of the grain toward evening when the grain had cooled. However, predictable daily temperature changes did not occur in the region of the grain where the insects encounter the traps.

We originally believed that EGPIC counts would be generally lower on the first day since insertion of the probe into the grain may cause disruption in the immediate area. We further hypothesized this disruption in the grain may break up insect pheromone trails resulting in relatively less activity in that general area. However, no consistent tends related to mean rate of counts per hour among days after the probe was inserted in the grain were observed.

From a practical standpoint we showed that quantity of rusty grain beetle captures in the EGPIC traps are similar to WB II probe traps. More insects were captured in PC pitfall traps both on the surface and 17 cm down in the grain mass than in probe style traps. Rusty grain beetles enumerated by the EGPIC system throughout the season increased with increasing daily mean air temperature and decreased as air temperature decreased. There was a definite increase in insect counts in the early evening hours during the warmest months. These finding suggest future trapping durations of at least 24 h and possibly several days to ensure a representative sample is collected.

The EGPIC monitoring system enabled us to examine diel changes in insect counts. Without the development of an electronic monitoring system we could not have made this discovery. Many additional ecological studies are now possible with the ability to log insect captures in time and space. Additionally, the system has potential to make integrated pest management a more attractive proposition for grain managers. The EGPIC system has the potential to reduce much of the time and labor constraints to insect monitoring. Use of an automated system should equip managers with the data needed to objectively determine when fumigation is warranted. Additionally, managers will not have to fumigate prior to sale as an insurance plan.

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Figure Legends

Fig. 1. Mean ± SE number of rusty grain beetles captured per week among four traps over a four-week period. $F = 10.51$; df = 3, 79.2; $P < 0.0001$; means with the same letter are not significantly different ($P > 0.05$; Fishers LSD Test).

Fig. 2. Mean ± SE rate of electronic counts per three-hour time period by month from March through June 2000.

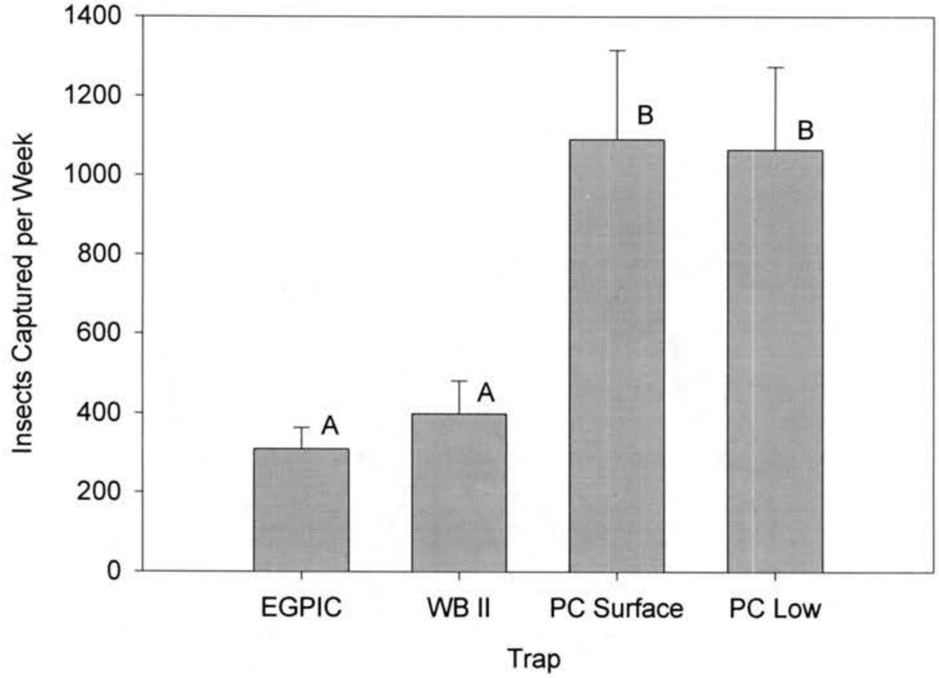
Fig. 3. Mean ± SE rate of electronic counts per three hour time period by month from July through November 1998.

Fig. 4A. Mean daily air temperature from March through June 2000.

Fig. 4B. Mean daily air temperature from July through November 1998.

Fig. 5. Mean ± standard deviation grain temperature (°C) 17 cm below the grain surface in the center of the bin during 1998. Data collection began July 15, 1998 (week 1).

Fig. 1



1400
1200
1000
800
600
400
200
0
Insects Captured per Week
A
A
B
B
EGPIC
WB II
PC Surface
PC Low
Trap

Fig. 2

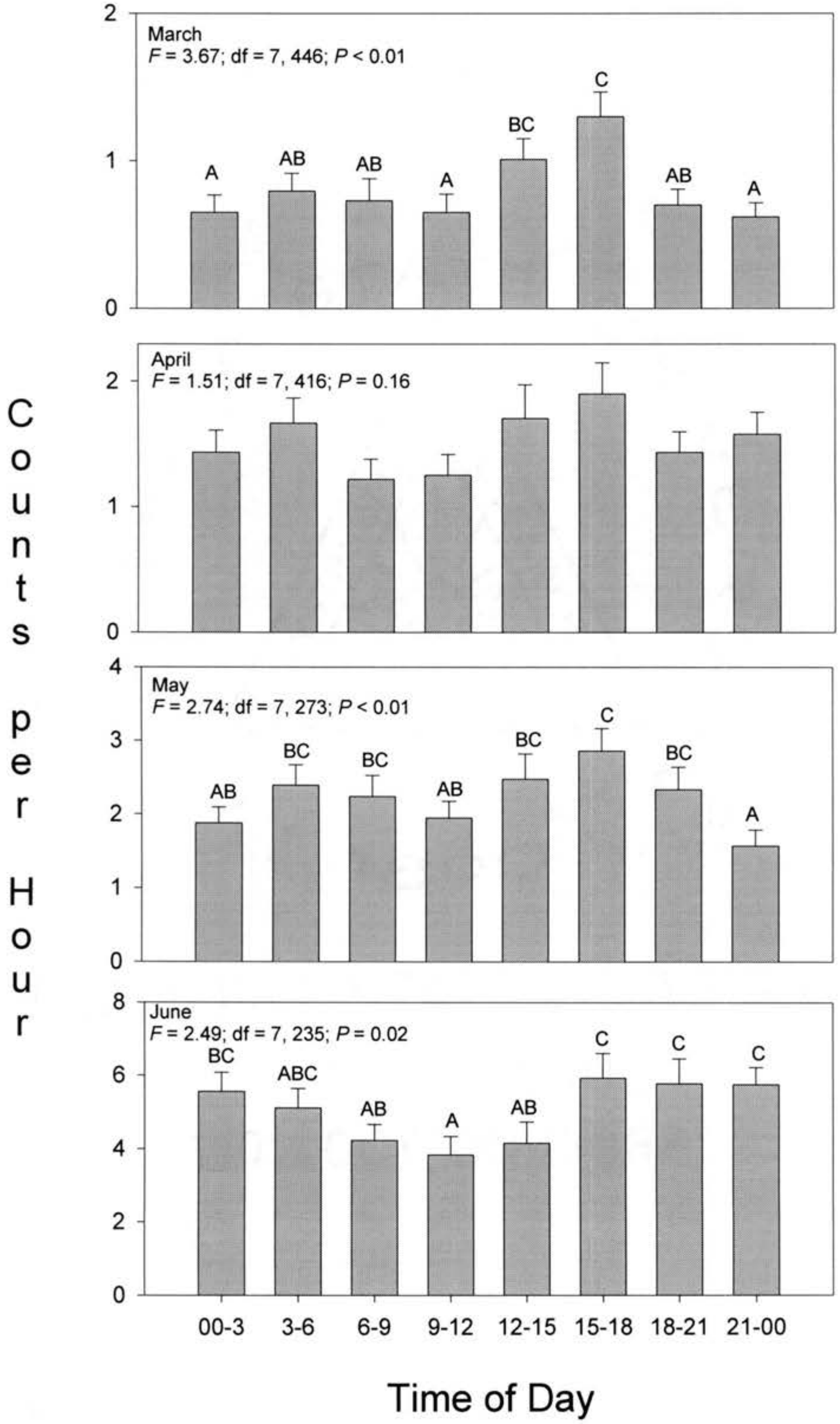
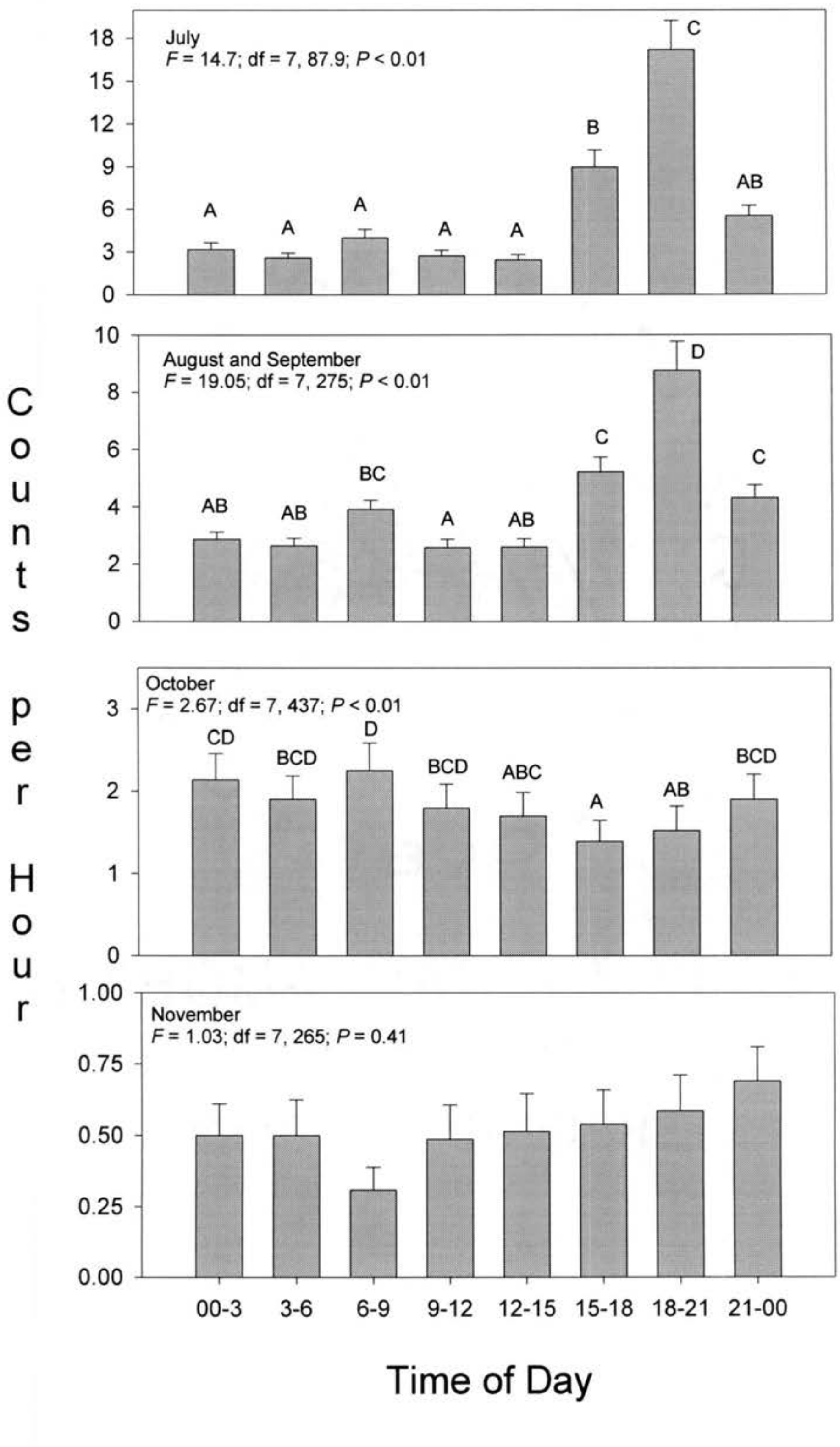
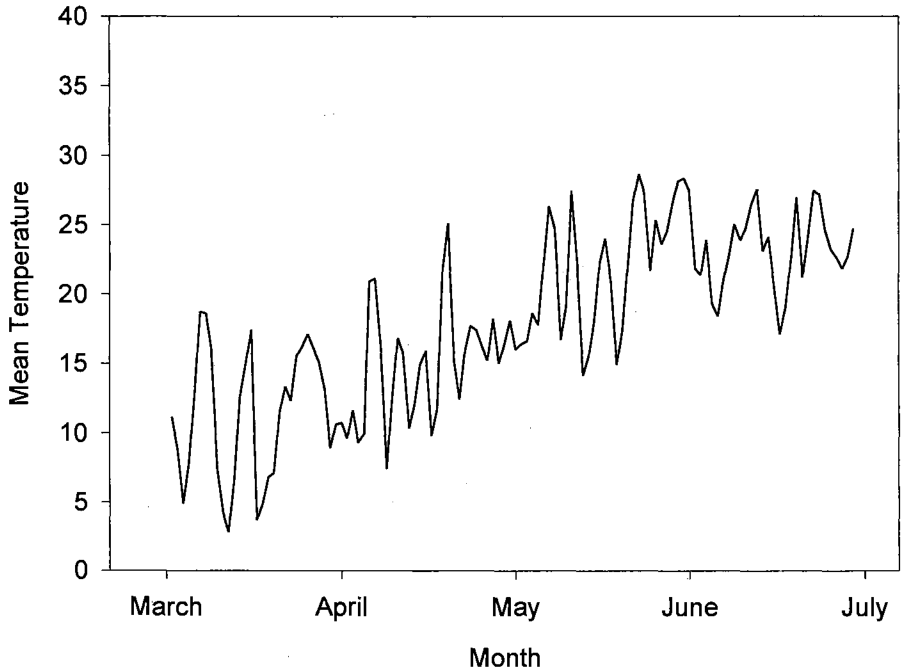

March
F = 3.67; df = 7, 446; P < 0.01
A
AB
AB
A
BC
C
AB
A
April
F = 1.51; df = 7, 416; P = 0.16
May
F = 2.74; df = 7, 273; P < 0.01
AB
BC
BC
AB
BC
C
BC
A
June
F = 2.49; df = 7, 235; P = 0.02
BC
ABC
AB
A
AB
C
C
C
Counts per Hour
00-3
3-6
6-9
9-12
12-15
15-18
18-21
21-00
Time of Day

Fig. 3



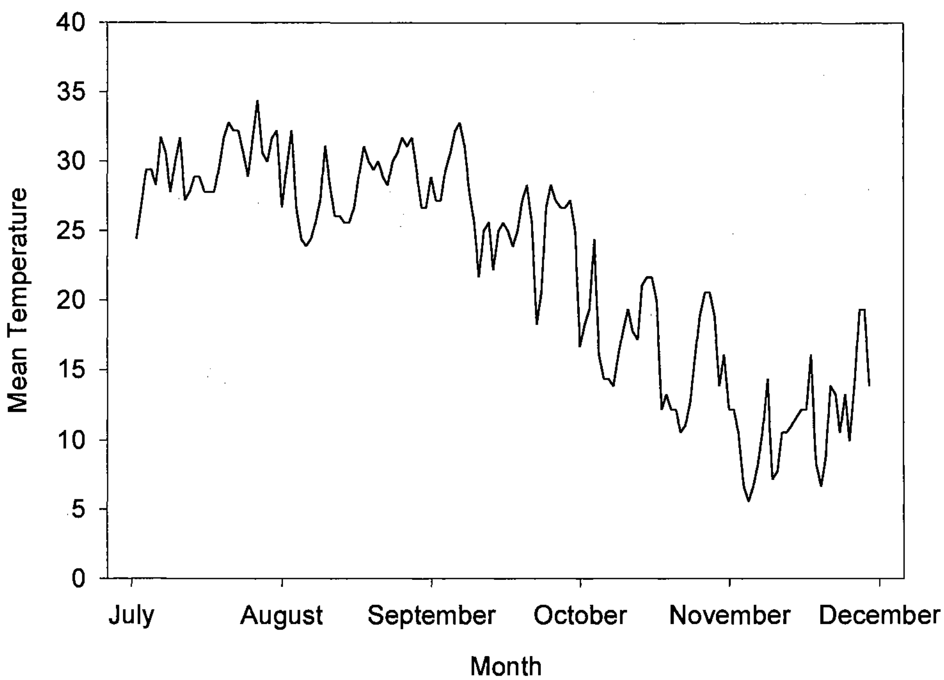
July
F = 14.7; df = 7, 87.9; P < 0.01
August and September
F = 19.05; df = 7, 275; P < 0.01
October
F = 2.67; df = 7, 437; P < 0.01
November
F = 1.03; df = 7, 265; P = 0.41
Counts per Hour
00-3
3-6
6-9
9-12
12-15
15-18
18-21
21-00
Time of Day

Fig. 4A



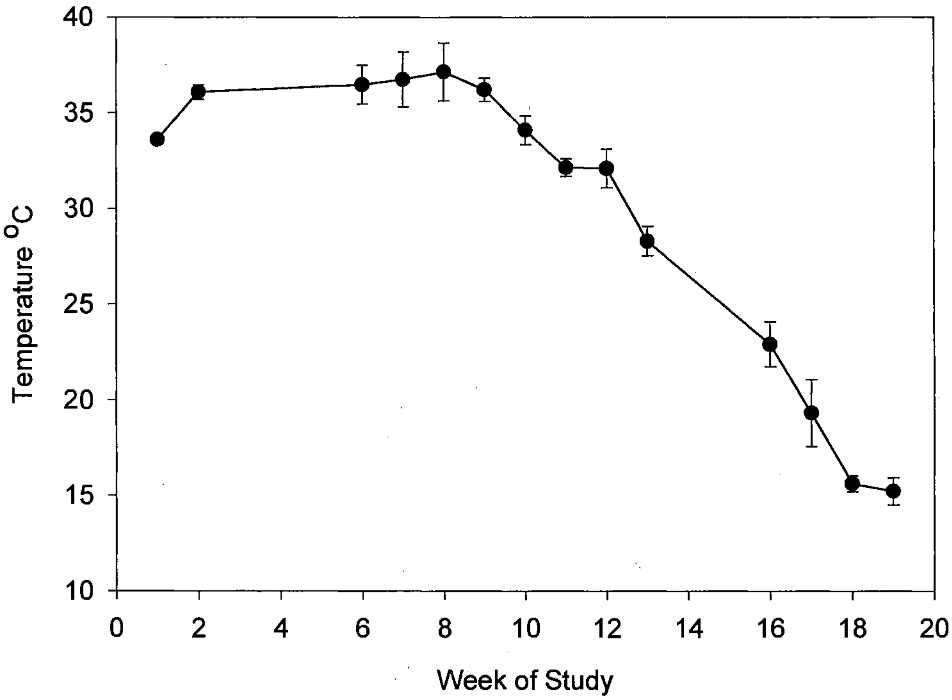
40
35
30
25
20
15
10
5
0
Mean Temperature
March
April
May
June
July
Month

Fig. 4B



40
35
30
25
20
15
10
5
0
Mean Temperature
July
August
September
October
November
December
Month

Fig. 5



40
35
30
25
20
15
10
Temperature °C
0
2
4
6
8
10
12
14
16
18
20
Week of Study

For: Environmental Entomology

Section: Pest Management and Sampling

Address correspondence to:

Michael D. Toews

127 Noble Research Center

Stillwater, OK 74078-3033

(405) 744-5099 or

Fax (405) 744-6039

e-mail: toewsm@okstate.edu

Toews et al.: Estimating grain beetles using probe traps

Estimating populations of grain beetles using probe traps in wheat-filled concrete silos

MICHAEL D. TOEWS[1], THOMAS W. PHILLIPS[1] and MARK E. PAYTON[2]

[1]Department of Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, OK 74078-3033

[2]Department of Statistics, Oklahoma State University, 301 Math Sciences, Stillwater, OK 74078-3033

Abstract

Harvested wheat in the central and southern United States is frequently transported to a central location and stored in concrete silos. Pest insect populations persist throughout the duration of storage at these facilities. Grain from two locations in north central Oklahoma was examined for insects immediately after harvest using probe traps and grain samples. Relationships between probe traps and actual insect densities were compared at the surface and 1 m below grain surface. Grain temperatures, officialU.S.grade, and grading parameters were collected throughout the study. Probe trap captures of key species in order of most common occurrence included *Cryptolestes ferrugineus* (Stephens), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.), and *Sitophilus oryzae* (L.). No differences in insect population densities between the grain surface and 1 m below the surface were shown when averaged across the storage season. Probe trap estimates of *C. ferrugineus* population density were more accurate when taken 1 m below the surface location and modeled with grain temperature and dockage content. Probe trap estimates of *R. dominica* populations were better on the surface than 1 m below the surface.

Key Words: *Cryptolestes ferrugineus, Tribolium castaneum, Rhyzopertha dominica, Sitophilus oryzae*, sampling, stored wheat

Introduction

The United States is a global leader in annual wheat (*Triticum aestivum* L.) production (Anderson et al. 1994). Grain production and storage is a multi-billion dollar industry and represents the cornerstone of agribusiness in the Midwest. Following harvest in the US, wheat is often stored in commercial grain management facilities referred to as country elevators. A country elevator typically serves farmers within a short driving distance. Commercial storage structures in the southernU.S.are predominately concrete (Kenkel et al. 1994). Long-term wheat storage in these structures provides the ideal habitat for a number of common stored-product insect species including: *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae), the rusty grain beetle; *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), the lesser grain borer; *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the rice weevil; and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle. These insects, if improperly managed, continue to infest grain throughout the storage period and eventually result in a reduced profit for the manager and seller.

Traditionally, chemical inputs have been used to control infestations of storage insects. However, millers have imposed a no pesticide residue standard when purchasing cereal products (Kenkel et al. 1994). In addition, foreign and domestic consumers, flour millers, and breakfast cereal manufacturers are moving toward more stringent standards for kernel uniformity, grain cleanliness, falling number, wet gluten, extraction, and other end-user characteristics (Kenkel et al. 1994). Consequently, it has become increasingly difficult to deliver insect free grain without leaving pesticide residues.

To deliver clean, residue-free grain, elevator operators currently manage insect populations in concrete silos with calendar-scheduled phosphine gas fumigation. Operators typically fumigate again before sale to prevent the possibility of an insect-induced price discount. Numerous residual insecticide applications and fumigations occur throughout grain transportation and storage processes (Cuperus et al. 1990). Unfortunately, few concrete silos are gas tight so an effective fumigation is often unlikely. Additional problems with the current system include repeated worker exposure hazards to the fumigant and insecticide resistance problems.

Stored-product insect traps have been available for many years and have been used to sample insects in farm-stored wheat (Lippert and Hagstrum 1987, Vela-Coiffier et al. 1997, Hagstrum et al. 1998, Wakefield and Cogan 1999). The principle of operation behind pitfall traps is that moving insects contact and enter the trap, fall through a void, and are accumulated in the collection reservoir. Probe traps (a specialized pitfall trap) for use in stored grain were originally developed by Loschiavo and Atkinson (1967); a thorough review of probe trap development is discussed in White et al. (1990). Advantages to insect sampling with traps include ease of use, minimal requirements for time and manpower, increased sensitivity to detect adult insects (Barak and Harein 1982, Lippert and Hagstrum 1987), and ability to sample continuously over an extended period. Trapping is a better method of finding new infestations as insects are detected in traps earlier than in grain samples (Vela-Coiffier et al. 1997, Hagstrum et al. 1998).

Effective integrated pest management requires that reliable data on the pest population be collected through sampling (Hagstrum and Flinn 1996), but sampling pests in concrete grain silos is very difficult. Access to place insect traps or take grain samples is a limiting factor in concrete silos. Entry into a silo requires a "confined space" entry permit. This entails atmospheric testing, a body harness with lifeline, on site rescue equipment, additional personnel, and "lock-out" of any electrical or mechanical equipment connected to that silo (CFR 2000). Silos often share mechanical equipment so entire portions of the elevator may be inoperable while entering a single silo. In addition, unfavorable weather can be problematic as silo access lids on top of the structure are often outside. Due to these limitations, we are unaware of any past projects utilizing traps in concrete silos.

Objectives of this study were: 1) assess the effects of trap location and temperature on number of insects collected; 2) compare how probe traps correlate with actual density at two locations in the grain; and 3) model insect capture using insect captures in probe traps, grain quality parameters, and temperature. Each of the objectives was completed independently for *Cryptolestes ferrugineus*, *Tribolium castaneum, Rhyzopertha dominica*, and *Sitophilus oryzae*.

Materials and Methods

Studies were conducted at two country elevators located in north-central Oklahoma during the 2000 storage season. Both elevators had two silo systems composed of 10 to 23 concrete silos with an average capacity of 408.7 metric tons per silo. Inside silo shape was generally round or hexagonal and silos ranged from 30.5 to 36.5 m tall. A bucket elevator moved grain from the pit area (where trucks dump grain) at ground level to a distributor located above the silos. Grain then flowed through the top of the appropriate silo through a downspout from the distributor. The cooperating businesses primarily stored hard red winter wheat and did not carryover grain from the previous storage season. No residual grain protectants were used in the elevators; however, periodic phosphine fumigations were conducted to control severe infestations of *R. dominica*. The wheat harvest progressed quickly due to favorable weather conditions and elevators were filled to capacity by early July. Studies reported here began immediately following harvest and continued for 17 weeks.

Probe Traps and Deep Bin Cup Samples

Unbaited WB-II probe traps (Trécé Inc., Salinas, CA) (Burkholder 1988) were placed in 12 silos during weeks 1, 4, and 8 for the first two months of the study and then every other week for the final eight weeks. Probe traps were left in the grain for 72 h on each trapping date. Two probe traps were inserted into each silo through the access port on top of the structure. One trap was positioned so the top of the trap was 2 cm below the grain surface while the second trap was positioned 1 m below the grain surface. Traps were pushed straight down into the grain with 1.3 cm steel pipe threaded to a 4.5 cm PVC pipe joint. Temperature data loggers (HOBO TEMP, Onset Computer Corp, Bourne, MA) were attached with 0.3 cm braided steel cable alongside the top of each probe trap. Loggers recorded grain temperature every two hours while traps were in place. Captured, live adult insects were identified to species and counted.

Grain samples were obtained on the same dates when probe traps were placed in the grain mass. Samples were obtained immediately before probe traps were placed using a 38.1 cm deep bin cup (each cup yielded ~ 250g of wheat) (Seedboro Equipment Co., Chicago IL). The cup was pushed into the grain a total of five times making a circle with a radius of 25 cm around each trap site. Grain samples were obtained at the same depth as the corresponding probe trap. The five samples from the same depth were combined and brought to the laboratory for processing. Insects were removed from the grain with a U.S. no. 14 standard testing sieve (Fisher Scientific Co., Pittsburgh, PA). Immediately after insect removal, all sifted material was reconstituted into each sample and the samples were submitted to a certified grain-grading laboratory for assignment of a numerical grade according to U.S. grain standards (GIPSA 1997). Grading factors include moisture content; dockage (material larger and smaller than wheat); test weight (bulk density in pounds per bushel); shrunken and broken kernels (matter that passes through a 0.064" X 3/8" oblong-hole sieve after removal of dockage); damaged kernels (weathered or diseased kernels, or frost, germ, heat, mold, and sprout damage); foreign material (all matter other than wheat after removal of dockage and shrunken and broken kernels); total defects (percentage of damaged kernels, foreign material, and shrunken and broken kernels); and insect damaged kernels (kernels bored by internal infesting species).

Quantities of the four most common insect species captured were compared at the two depths in the grain mass. Species were analyzed independently using PROC MIXED (SAS Institute 1999) with degree of freedom adjustments following the methods of Satterthwaite. A REPEATED statement was used in order to model the variance-covariance relationship within the individual traps within silos. An autoregressive with period 1 covariance structure proved to be the most effective in modeling the intra-trap correlation. Insect recovery from the grain samples was expressed as insects per kg and contrasted between the two locations in each silo. Finally, insect capture from probe traps was adjusted using temperature as a covariate of trap capture (analysis of covariance) and then compared between locations in the grain.

Correlation of Grain Samples and Trap Capture

Trapped insects and grain samples were compared at the two locations in the grain mass to examine how well probe traps estimate actual insect density. Data were modeled using PROC GENMOD (SAS Institute 1999) with actual insect densities from grain samples as a predictor of probe trap capture. The GENMOD procedure fits a generalized linear model to the data by maximum likelihood estimation of the set of parameters of interest. The probability distribution was set to POISSON and the link function was set to LOG. The resulting insect density versus trap captures slope was compared by trap location for each species using chi-square analysis. This procedure is also known as poisson regression, which is similar to normal regression except the response variable is poisson distributed instead of normally distributed. The scaled deviance statistic was used to assess goodness of fit using the model.

Estimating Insect Density

Corresponding data from probe trap capture, insect density from grain samples, mean grain temperature during the trapping period, numerical grade, and grain grading factors were combined into the same dataset. These data were modeled using multiple regression (PROC REG; SAS Institute 1999) where insect density from the grain samples was the predicted variable. This analysis was repeated independently for each of the four most common insect species.

Results

Probe Trap Captures and Grain Temperature

Adult insects in order of most common occurrence in probe traps over the course of the study included: *C. ferrugineus; T. castaneum*; *R. dominica; S. oryzae*; *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae), the saw-toothed grain beetle; *Cephalonomia waterstoni* (Gahan) (Hymenoptera: Bethylidae); *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae); *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae); *Cynaeus angustus* (LeConte) (Coleoptera: Tenebrionidae), the larger black flour beetle; *Ahasverus advena* (Waltl) (Coleoptera: Cucujidae), the foreign grain beetle; and *Typhaea stercorea* (L.) (Coleoptera: Mycetophagidae), the hairy fungus beetle. There were roughly 90 times more *C. ferrugineus* captured than *T. castaneum*. In a few very isolated instances, insects belonging to the families Nitidulidae and Anthocoridae were recovered but a more detailed taxonomic identification was not made.

No interactions were detected between week of the study and location of traps in the silo. Mean temperature across locations decreased in a linear trend through the study ($F = 109.94$; df = 1, 85.7; $P < 0.0001$). Significant differences in temperature between locations in the grain were detected on two occasions (Table 1). These differences are attributable to major cold fronts that moved through the area. The cool air affected grain temperature on top of the grain mass more than 1 m below the surface. There were no differences between numbers of adult insects captured in traps at the surface or 1 m below the surface for *C. ferrugineus* ($F = 0.08$; df = 1, 37.5; $P = 0.7770$), *T. castaneum* (F

= 0.20; df = 1, 45.1; P = 0.6591), *R. dominica* (F = 2.71; df = 1, 10.1; P = 0.1306), or *S. oryzae* (F = 0.24; df = 1, 50.4; P = 0.6233) (Table 2).

Insects in Grain Samples

Species of adult insects in order of common occurrence recovered in grain samples obtained from deep bin cups included: *C. ferrugineus*, *R. dominica*, *T. castaneum*, *S. oryzae*, *C. waterstoni*, and *T. elegans*. There were nearly twelve times more *C. ferrugineus* collected than *R. dominica*. Review of the grain sample data show there were no overall differences in numbers of captures between sampling locations for *C. ferrugineus* (F = 0.55, df = 1, 32.4; P = 0.4635), *R. dominica* (F = 0.70; df = 1, 51.3; P = 0.4078), *T. castaneum* (F = 0.05; df = 1, 44.3; P = 0.8262), or *S. oryzae* (F = 0.51; df = 1, 56.1; P = 0.4763) (Table 2).

Correlation of Grain Samples and Trap Capture

Poisson regression was used to relate insect captures to known insect densities derived from corresponding grain samples. The regression equation slope for insect density as a predictor of probe trap captures was different at the two locations for *C. ferrugineus* (Fig. 1A and B) and *R. dominica* (Fig. 2A and B). However, these slopes were not significantly different between collection locations for *S. oryzae* (Fig. 3A and B) and *T. castaneum* (Fig. 4). Scaled deviance statistics, shown on the figures, are rather high indicating that these models do not fit particularly well.

Modeling Insect Density

A summary of grain grading factors by location is presented in Table 3. Since the slopes of the regression equation density versus trap captures at the two locations in the grain mass were different for *C. ferrugineus* and *R. dominica*, these species were modeled separately by location of the traps. However, only one model was produced for *S. oryzae* and *T. castaneum* since the slopes for the regression of trap captures vs. density at the two locations in the grain mass were not significantly different. Significant effects for modeling *C. ferrugineus* density at the surface included probe trap capture, dockage, and a quadratic temperature effect (Table 4A). *Cryptolestes ferrugineus* captures 1 m below the surface were best fitted using only probe trap capture and temperature. Density of *R. dominica* at the upper location was best fitted using only probe trap captures, while temperature and probe trap captures were significant at the lower depth (Table 4B). *Sitophilus oryzae* density was best fit using a quadratic term for probe trap capture (Table 5). *Tribolium castaneum* density was best predicted with trap captures only.

Discussion

All insect species captured in probe traps during this study are known to occur in stored wheat in the Great Plains. The majority of probe trap captures (*C. ferrugineus* and *T. castaneum*) represent coleopteran pests that infest grain externally. This is important because only internal pests, such as *R. dominica* and *S. oryzae*, cause the type of kernel damage that triggers a price discount. Tunneling and boring officially contribute to insect damaged kernels (GIPSA 1997). The presence of the parasitoid species *C. waterstoni, T. elegans* and *A. calandrae* confirm the occurrence of substantial pest populations during the study. *Cephalonomia waterstoni* is a parasitoid of *Cryptolestes spp.* while *T. elegans* and *A. Calandrae* are parasitoids of beetle pests such as *R. dominica* and *S. oryzae*.

Comparison of slopes between actual densities and trap captures illustrated differences in insect behavior at different regions in the grain mass. While there was not a difference in general population size between locations (as evidenced by the deep bin cup samples), there were obvious differences in the relationship between insect density and probe trap captures by location for two insect species. These differences are likely due to increased dockage, damaged kernels, foreign material and shrunken or broken kernels at the surface location. Small insects like *C. ferrugineus* may not move through the grain as easily in this "dirty" grain and thus likely encountered traps less frequently. Based on the scatter plot this appeared to be more pronounced at high insect density. The scatter plot and forced regression statistic (scaled deviance) for *S. oryzae* were strong because very few insects were captured in either probe traps or grain samples. *Tribolium castaneum* was frequently captured in probe traps but not in grain samples. This was not surprising because traps are more sensitive than grain samples for locating insect infestations (Barak and Harein 1982, Lippert and Hagstrum 1987). Furthermore, traps permit earlier insect detection than grain samples (Lippert and Hagstrum 1987, Vela-Coiffier et al. 1997, Hagstrum et al. 1998).

General grain grading parameters for multiple regression were chosen because all elevators have the tools and knowledge to complete this task for any given silo grain sample. Probe trap captures did not correlate well with actual insect density for all species but we postulated that the addition of grain grading factors and temperature may help improve estimates. Consideration for multiple factors in combination could help grain managers interpret trap catch data for pest management decisions (White et al. 1990). Analysis of the data with multiple regression allowed us to model significant temperature effects, grain grading parameters, and the differences in location from data collection simultaneously. The *C. ferrugineus* multiple regression equation at the surface proved our hypothesis that extra dockage at the surface changes the rate of captures in probe traps. The large difference in R-squared values between the two locations likely indicates the complexity of trap-insect interactions at the surface where insects may be constantly immigrating and emigrating from the region. At the lower position there may be less variability in insect numbers thus the model explained much more of the variation. A more accurate estimation of *C. ferrugineus* populations can be made with probe traps at 1 m below the surface.

Rhyzopertha dominica multiple regression analysis yielded variable results pertaining to the value of grain temperature based on the location of the trap. Captures at the surface may have been less related to grain temperature than the air temperature above the grain (headspace). The headspace temperature fluctuates daily with air temperature while bulk grain temperature fluctuates little. Large fluctuations in the headspace could potentially influence insect activity in the immediate area. Populations captured at the lower trap position experience few changes in temperature and could be more static. Temperature became a significant term in the equation only when tested at the lower placement. A lack of *S. oryzae* and *T. castaneum* recoveries in the grain samples prevented detailed analyses for these species. A dataset with more recoveries is necessary to confirm the value of probe traps to evaluate population density for *S. oryzae* and *T. castaneum*. If the grain is actually infested, a possible way to recover more insects is to take much larger grain samples.

Sampling methods used for integrated pest management need to help the pest manager determine when a pest population reaches the economic threshold. Regardless of the storage environment, the relative population density estimate provided by the probe trap must be converted to an absolute estimate. Lippert and Hagstrum (1987), working in farm-stored wheat, recommend dividing trap captures by trap efficiency to complete this task. Trap efficiency is calculated by taking the number of insects in a trap divided by the number of insects in a grain sample. Vela-Coiffier et al. (1997) used simple linear regression between numbers of insects captured in probe traps and absolute density determined with a single 265 g deep bin cup sample or 650 g trier sample in farm-stored wheat. Their methods did not consider how grain temperature or grain quality parameters may affect insect movement. Hagstrum et al. (1998), also working in farm-stored wheat, developed methods to adjust insect captures obtained from probe traps to absolute density using linear equations. They based the models on groups of temperatures (< 14, 14 – 23, and > 23°C) and storage periods (0 – 90, 90 –135, and 135 to 190 days). Hagstrum et al. (1998) found that trap depth did not affect insect captures between traps placed on the surface and traps placed 7.6 cm below the grain surface. In a laboratory study, insect captures of adult rusty grain beetles in probe traps were dependent on grain temperature and insect density (Toews Chapter 2). Studies reported here validate application of the laboratory data because temperature was also an important variable in the field.

This project described how probe traps could be used to acquire insect sampling data in concrete silos. Here we used multiple regression to relate the relative population estimate to an absolute density estimate. Using our methods, the trap efficiency calculation changes based on interactions between insects and traps at the specific location the data were collected. Grain quality parameters and temperature were evaluated as continuous variables possibly affecting numbers of insects captured in probe traps. Models in this paper need to be validated using an independent data set.

The integrated pest management approach to grain storage offers promise of increased profit and marketability to the seller through cost-benefit analysis. The principle component of this approach is storing insect-free grain at appropriate temperature and moisture content to prevent loss of quality through insect infestation or microbial colonization. Monitoring, known as "scouting" in row crop agriculture, is the window whereby managers interpret needs once the commodity is in storage. Thresholds for pest management decisions in stored wheat need to be established based on the sampling method. Vela-Coiffier et al. (1997) reported simple estimated economic injury levels for probe traps. An appropriate economic injury level for stored grain insects is 2 insects per sample since wheat is officially considered "infested" at this level. The appropriate economic threshold exists somewhere under the economic injury level. Sampling is the missing link in the integrated pest management program at this time. This study is the first to show how traps can be used in concrete silo storage facilities to monitor and estimate insect population density in stored wheat.

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Table 1. Mean ± SE grain temperature (°C) by location in the grain silo.

Week[1]	Location			
	Surface	1 m below	*F*	*P*
1	30.3 ± 0.7	31.7 ± 0.8	0.45	0.5049
5	31.9 ± 0.4	32.5 ± 0.78	0.06	0.8016
9	23.1 ± 7.2	32.7 ± 1.9	8.11	0.0053
11	23.4 ± 0.7	24.8 ± 2.08	0.57	0.4508
13	21.3 ± 0.5	23.7 ± 1.2	0.51	0.4754
15	22.1 ± 0.7	24.5 ± 1.5	0.65	0.4211
17	11.8 ± 1.0	17.7 ± 2.1	5.43	0.0218

[1]Study began on July 21, 2000.

df for each comparison = 1, 102.

Table 2. Mean ± SE number of insects recovered in probe traps and corresponding grain samples at two depths. Grain sample densities are expressed in insects per kg grain. Number of samples are given in ().

Species	Insect Density by Location (no. samples)			
	Surface		1 m Below Surface	
	Probe Traps (62)	Grain Sample (64)	Probe Traps (60)	Grain Sample (65)
C. ferrugineus	275.8 ± 96.7	27.6 ± 8.7	357.1 ± 133.3	18.1 ± 10.8
R. dominica	3.0 ± 1.0	2.4 ± 1.0	3.9 ± 1.8	1.4 ± 0.5
S. oryzae	0.2 ± 0.1	0.2 ±0.2	0.3 ± 0.1	0.04 ± 0.03
T. castaneum	5.1 ± 2.2	0.3 ± 0.2	1.8 ± 0.8	0.2 ± 0.2

Table 3. Mean ± SE grain grade and grading parameters on the surface and at 1m below the grain surface.

Variable[1]	Location	
	Surface	1 m Down
Grain Grade	2.7 ± 0.2	2.4 ± 0.2
Dockage (%)	4.3 ± 0.5	3.5 ± 0.5
Test Weight (lbs/bu.)	58.0 ± 0.2	58.8 ± 0.2
Moisture Content (%)	11.4 ± 0.1	11.8 ± 0.1
Damaged Kernels (%)	1.0 ± 0.3	0.7 ± 0.2
Foreign Material (%)	0.5 ± 0.1	0.3 ± 0.0
Shrunken and Broken Kernels (%)	1.6 ± 0.1	1.4 ± 0.1
Total Defects (%)	3.0 ± 0.3	2.5 ± 0.2
Insect Damaged Kernels (#/100g)	4.8 ± 1.1	3.9 ± 0.8

[1]n = 56 to 66 per location

Table 4A. Model fitting for *C. ferrugineus* at two locations in the grain.

Location	Variable	Est ± SE	F	P
Surface[1]	Intercept	42.54 ± 22.99	8.10	0.0064
	Trap captures	0.04 ± 0.01	15.57	0.0003
	Dockage	4.70 ± 2.21	7.33	0.0093
	Temperature**2	-0.06 ± 0.03	6.27	0.0157
1 m Down[2]	Intercept	3.47 ± 14.91	62.08	< 0.0001
	Trap captures	0.08 ± 0.00	480.07	< 0.0001
	Temperature	-0.46 ± 0.52	41.53	< 0.0001

[1] $F = 12.98$; df = 3, 49; $P < 0.0001$; $R^2 = 0.44$

[2] $F = 300.15$; df = 2, 53; $P < 0.0001$; $R^2 = 0.92$

Table 4B. Model fitting for *R. dominica* at 2 location in the grain.

Location	Variable	Est ± SE	F	P
Surface[1]	Intercept	-0.36 ± 0.59	65.12	< 0.0001
	Trap captures	1.25 ± 0.10	186.01	<0.0001
1 m down[2]	Intercept	5.38 ± 1.46	30.93	< 0.0001
	Temperature	-0.17 ± 0.05	10.22	< 0.0023
	Trap Captures	0.24 ± 0.03	53.31	<0.0001

[1] $F = 186.01$; df = 1, 51; $P < 0.0001$; $R^2 = 0.78$

[2] $F = 28.53$; df = 2, 53; $P < 0.0001$; $R^2 = 0.52$

Table 5. Model fitting for *S. oryzae* and *T. castaneum* with combined locations.

Species	Variable	Est ± SE	F	P
S. oryzae[1]	Intercept	-0.03 ± 0.05	1741.42	< 0.0001
	Trap captures**2	0.20 ± 0.01	297.30	<0.0001
T. castaneum[2]	Intercept	0.21 ± 0.14	340.75	< 0.0001
	Trap Captures	0.03 ± 0.01	7.19	<0.0084

[1]$F = 297.30$; df = 1, 121; $P < 0.0001$; $R^2 = 0.71$

[2]$F = 7.19$; df = 1, 121; $P < 0.0084$; $R^2 = 0.06$

Figure Legends

Fig 1A. Relationship of *C. ferrugineus* density per kg to captures in probe traps at the grain surface in concrete silos.

Fig 1B. Relationship of *C. ferrugineus* density per kg to captures in probe traps located 1 m below the grain surface in concrete silos. Difference in slopes between locations in the grain: $\chi^2 = 117.17$; df = 119; $P < 0.01$.

Fig 2A. Relationship of *R. dominica* density per kg to captures in probe traps at the grain surface in concrete silos.

Fig 2B. Relationship of *R. dominica* density per kg to captures in probe traps located 1 m below the grain surface in concrete silos. Difference in slopes between locations in the grain: $\chi^2 = 241.58$; df = 119; $P < 0.01$.

Fig 3A. Relationship of *S. oryzae* density per kg to captures in probe traps at the grain surface in concrete silos.

Fig 3B. Relationship of *S. oryzae* density per kg to captures in probe traps located 1 m below the grain surface in concrete silos. Difference in slopes between locations in the grain: $\chi^2 = 0.05$; df = 119; $P < 0.82$.

Fig 4A. Relationship of *T. castaneum* density per kg to captures in probe traps at the grain surface in concrete silos.

Fig 4B. Relationship of *T. castaneum* density per kg to captures in probe traps located 1 m below the grain surface in concrete silos. Difference in slopes between locations in the grain: $\chi^2 = 2.68$; df = 119; $P < 0.10$.

Fig. 1A

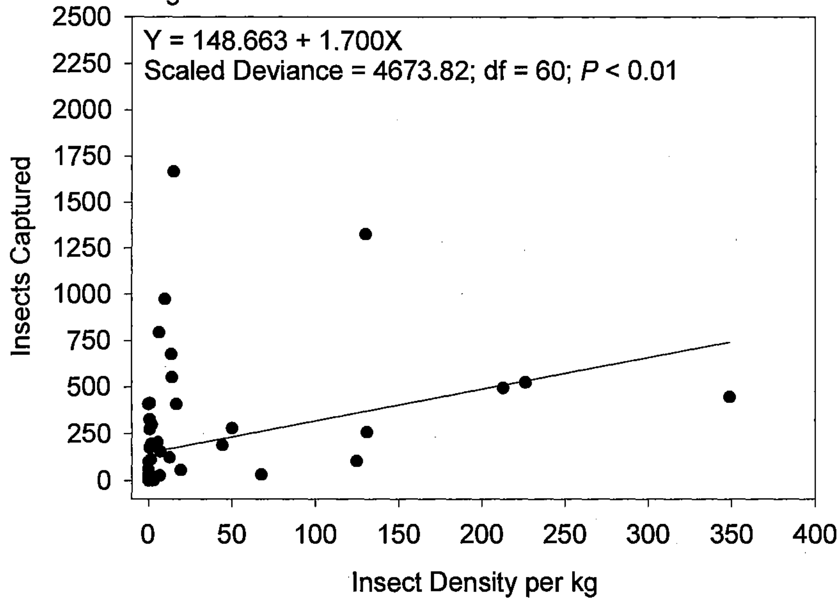

Y = 148.663 + 1.700X
Scaled Deviance = 4673.82; df = 60; P < 0.01
Insects Captured
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
0
50
100
150
200
250
300
350
400
Insect Density per kg

Fig. 1B

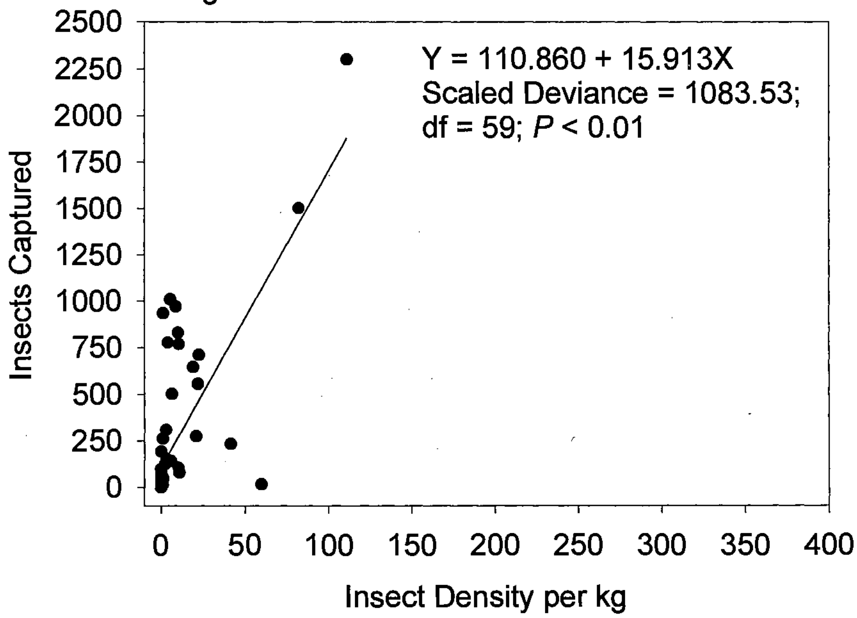
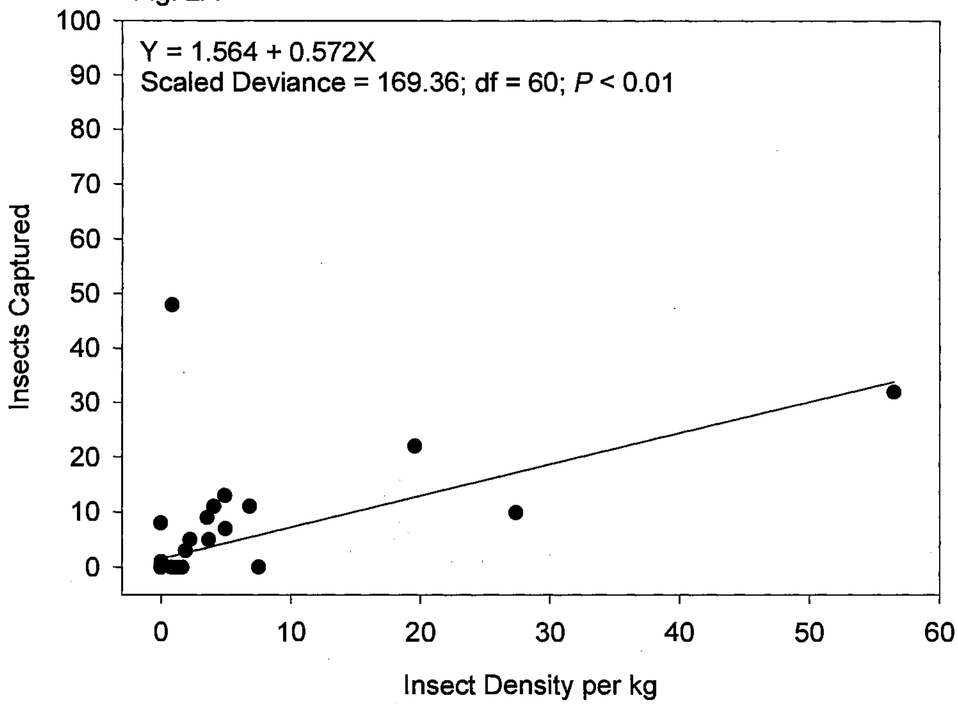
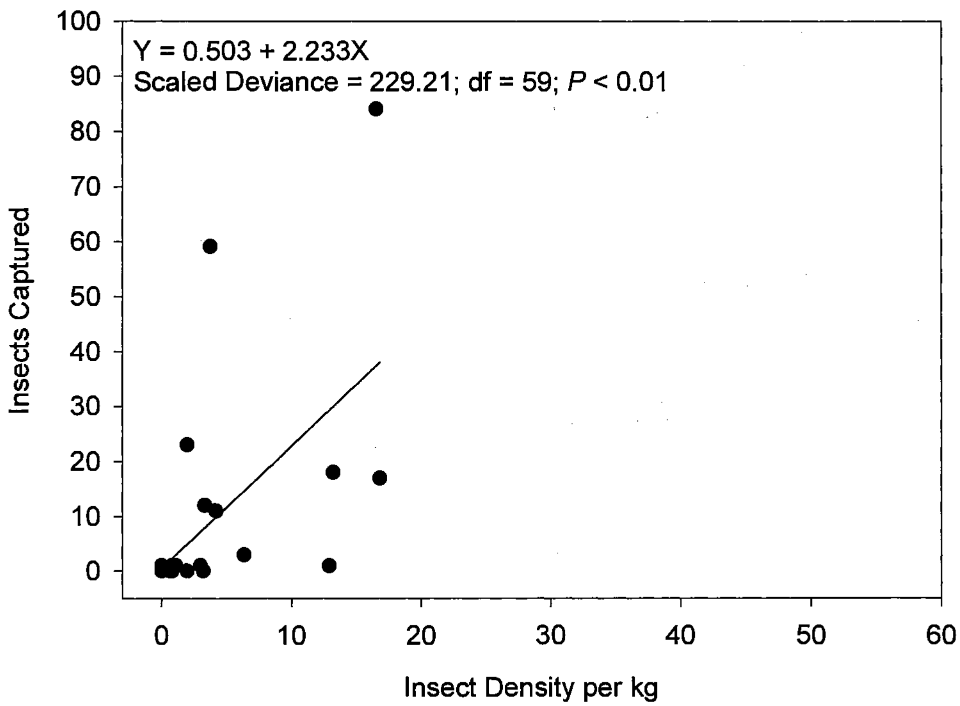

Y = 110.860 + 15.913X
Scaled Deviance = 1083.53;
df = 59; P < 0.01
Insects Captured
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
0
50
100
150
200
250
300
350
400
Insect Density per kg

Fig. 2A



Y = 1.564 + 0.572X
Scaled Deviance = 169.36; df = 60; P < 0.01
Insects Captured
Insect Density per kg

Fig. 2B



Y = 0.503 + 2.233X
Scaled Deviance = 229.21; df = 59; P < 0.01
Insects Captured
Insect Density per kg

Fig. 3A

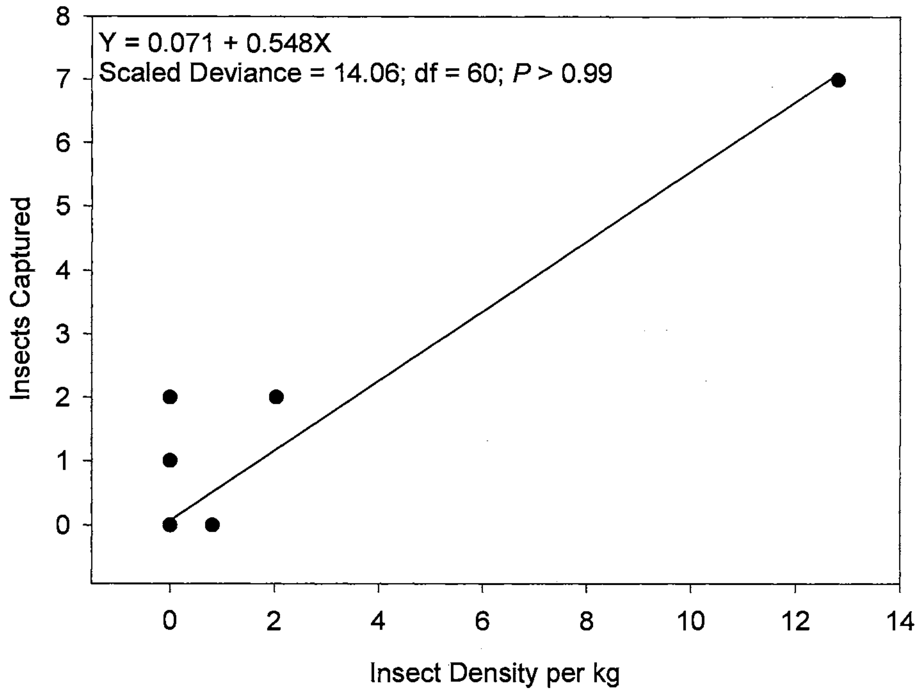

Y = 0.071 + 0.548X
Scaled Deviance = 14.06; df = 60; P > 0.99
Insects Captured
Insect Density per kg
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14

Fig. 3B

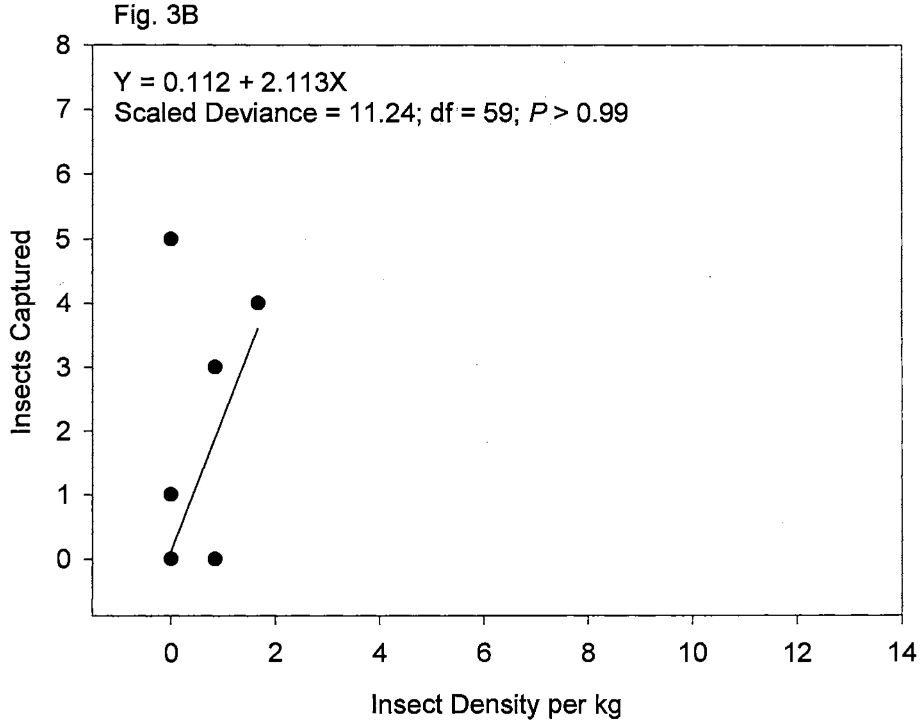

Y = 0.112 + 2.113X
Scaled Deviance = 11.24; df = 59; P > 0.99
Insects Captured
Insect Density per kg
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14

Fig. 4A

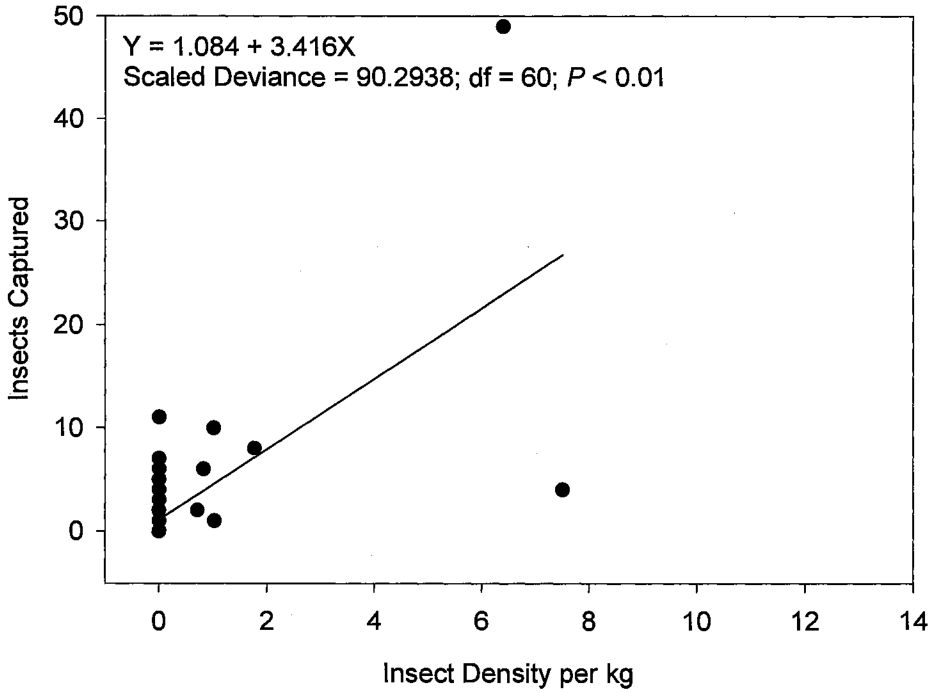
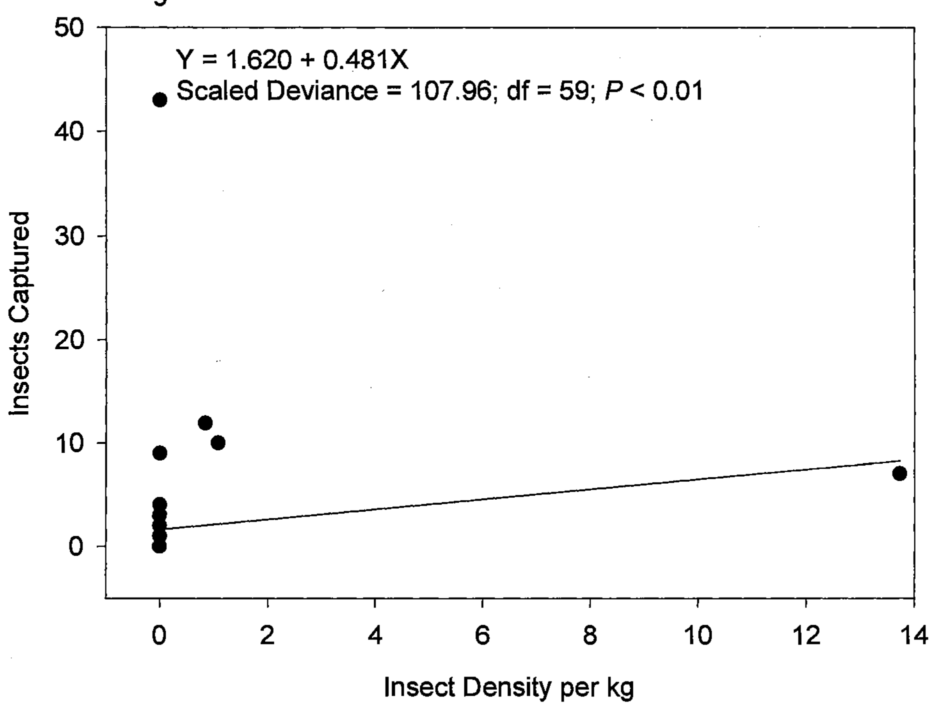

Y = 1.084 + 3.416X
Scaled Deviance = 90.2938; df = 60; P < 0.01
Insects Captured
Insect Density per kg
50
40
30
20
10
0
0
2
4
6
8
10
12
14

Fig. 4B


Y = 1.620 + 0.481X
Scaled Deviance = 107.96; df = 59; P < 0.01
Insects Captured
Insect Density per kg
50
40
30
20
10
0
0
2
4
6
8
10
12
14

APPENDIX A

Quantification of Insects Immediately After Harvest

Abstract

Twenty-four concrete silos were probed using vacuum sampling immediately after harvest. Samples were obtained on the surface and then proceeded down in 1.2 m intervals to a depth of 10.8 m. In addition, bottom samples were obtained by emptying grain from the bottom of 26 silos. Results demonstrated that few insects were present in the upper half of the silos immediately after harvest. More insects were found on the surface than the average of the other depths. In contrast, bottom samples were heavily infested with key pest species including *Sitophilus oryzae* and *Rhyzopertha dominica.*

Introduction

Previous studies have evaluated abundance or dispersion of insect pests in stored wheat in small steel storage facilities (Lippert and Hagstrum 1987, Vela-Coiffier et al. 1997, Hagstrum et al. 1998, Hagstrum 2000). However, only one published study has been conducted in concrete silos. Mahmood et al. (1996) showed that maximum insect activity was found on the surface of a single wheat-filled concrete silo in Pakistan. They started sampling the silo, using vacuum sampling, about one month after the silo was filled with wheat. No studies are available that evaluate insect dispersion in concrete silos immediately after harvest. Objectives of the current study were to: 1) Document the presence of insects at the time of initial silo filling; and 2) compare the number of insects found in on the surface against the average of all other depths.

Materials and Methods

A series of grain samples (2400 ± 400 g) were obtained in 24 silos using a gasoline powered sampling device (GVS, Ltd., Prairie Village, KS). Samples were all obtained immediately after the entire elevator was filled to capacity following harvest. Three-weeks passed between the first load of wheat from farmers and when the elevator was filled. Vacuum probing is a method of grain acquisition in which a commercial "vacuum cleaner" is attached to sections of metal pipe that are pushed into the grain mass. The sections of pipe were pushed down through the top opening of each silo. The experimenter, standing on the roof above the silos, obtained grain samples from a collection chamber also located on the roof. Grain samples were obtained on the surface and then between the depths of 0 - 1.2, 1.2 - 2.4, 2.4 - 3.6, 3.6 - 4.8, 4.8 - 6.0, 6.0 -7.2, 7.2 - 8.4, 8.4 - 9.6 and 9.6 - 10.8 m from the top grain surface. Surface samples were obtained with the vacuum probe by only collecting grain in contact with air above the grain mass. Grain samples were returned to the laboratory and sifted twice over an inclined sieve (White 1983) to separate adult insects from the grain. The sieve was set 25° from horizontal with a 6 mm gate opening and had a 2 mm mesh screen. Beetles were identified to species and counted; density of each species was computed on an insects per kilogram basis for each sample.

Bottom samples were obtained in 26 total silos from two silo systems at one elevator. These samples were obtained by opening the spout at the bottom of each silo and collecting the first 700 ± 100 g of wheat that poured out. Bottom samples were processed similar to the vacuum samples. In cases where bottom samples were too dirty to flow down the sieve, they were first hand sieved using a 0.07" by 0.5" slotted commercial grain sieve (Seedburo Equipment Co., Chicago, IL).

Results

Numbers of insects per kg present in the initial grain samples derived with vacuum probing was very low (Table 1). In 9 of the 24 silos no insects were recovered at any depth. Furthermore, exclusive of the bottom samples, there were between 1 and 5 total insects in 10 additional silos. There were differences between insect density on the surface and an average of the remaining depths for *C. ferrugineus* ($F = 4.52$; df = 1, 142; $P = 0.0352$), *R. dominica* ($F = 7.62$; df = 1, 142; $P = 0.0065$) and all insects combined ($F = 4.79$; df = 1, 142; $P = 0.0303$). Linear trends, decreasing from the density at the surface, were present for *C. ferrugineus* ($F = 4.74$; df = 1, 142; $P = 0.0311$) and *R. dominica* ($F = 5.33$; df = 1, 142; $P = 0.0224$).

Bottom samples, which were not statistically compared to the vacuum samples, were highly infested. No bottom samples were completely free of insects. The predominant insect found in bottom samples was *S. oryzae* (mean = 66.8 ± 27.2 adults per kg) in contrast to the surface samples where the *C. ferrugineus* was the most common (Table 1).

Discussion

High insect densities in bottom samples were surprising to the elevator operator as a commercial pest controller had sprayed inside all silos prior to harvest with an insecticide. We postulated that the high insect counts in bottom samples were due to a thick layer of infested residual grain that was unaffected by the chemical treatment. Residual grain is likely a sanitation problem that could be fixed by removing all residual grain after emptying the silo. A few bushels of wheat are frequently left on the silo floor unless someone physically enters the silo and cleans it. Residual grain is rarely removed because of accessibility problems and high labor input. This grain acts as an insect refuge over the spring and summer until harvest. Because of these complications, insect problems can persist from year to year.

We expected that all depths in the grain would be insect free since these pests have not been found in truck samples from harvested wheat in Oklahoma (Phillips et al. 2000). However, rather than assuming the insects infested the grain before storage, readers should realize that the entire elevator facility required three weeks to fill. Insects likely immigrated into the structure throughout harvest as the silos were filled. *Cryptolestes ferrugineus*, a very active species, was located at all but one depth examined; however, *R. dominica*, a less active species, was present at just three locations in the vacuum samples. *Sitophilus oryzae* is a good wall climber and may have emigrated upward from the floor to infest the new grain when the silos were filled.

This project showed grain in silo bottoms was heavily infested immediately after harvest. It appears much of this problem could be corrected by cleaning and sanitizing empty silos. The recovery of numerous *S. oryzae* and *R. dominica* in the bottom samples indicate that great potential exists for residual insect populations to infest new crop wheat. We confirmed that few insects, especially serious pests, are present in the top half of the grain immediately after harvest. Stored-product insect infestations do not originate in the field but likely immigrate into the wheat after storage or survive in residual grain.

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Table 1. Mean ± SE number of beetles per kg by depths starting on the top.

Depth in m (# samples)	Density of Given Species				
	C. ferrugineus[1]	*S. oryzae*[2]	*R. dominica*[3]	*T. castaneum*[4]	All Insects
Surface (17)	0.24 ± 0.10	0.18 ± 0.13	0.10 ± 0.08	0.02 ± 0.02	0.54 ± 0.21
0.0-1.2 (20)	0.13 ± 0.05	0.07 ± 0.05	0.04 ± 0.03	0.00 ± 0.00	0.24 ± 0.11
1.2-2.4 (23)	0.07 ± 0.06	0.07 ± 0.04	0.00 ± 0.00	0.12 ± 0.10	0.26 ± 0.15
2.4-3.6 (23)	0.18 ± 0.09	0.07 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.24 ± 0.11
3.6-4.8 (21)	0.07 ± 0.05	0.09 ± 0.09	0.04 ± 0.04	0.03 ± 0.03	0.22 ± 0.13
4.8-6.0 (23)	0.04 ± 0.04	0.15 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.19 ± 0.14
6.0-7.2 (18)	0.07 ± 0.05	0.15 ± 0.08	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.11
7.2-8.4 (17)	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04
8.4-9.6 (12)	0.06 ± 0.04	0.14 ± 0.14	0.00 ± 0.00	0.03 ± 0.03	0.24 ± 0.15
Bottom (24)	43.4 ± 10.5	66.8 ± 27.1	33.5 ± 18.2	11.0 ± 4.5	162.3 ± 44.4

Overall vacuum sample analysis of variance by species:

[1] $F = 1.50$; df = 8, 142; $P = 0.1625$

[2] $F = 0.43$; df = 8, 142; $P = 0.9006$

[3] $F = 1.42$; df = 8, 142; $P = 0.1919$

[4] $F = 0.95$; df = 8, 142; $P = 0.3394$

γ

VITA

Michael D. Toews

Candidate for the Degree of

Doctor of Philosophy

Thesis: USE OF TRAPS TO ESTIMATE *CRYPTOLESTES FERRUGINEUS* (COLEOPTERA: CUCUJIDAE) IN STORED WHEAT

Major Field: Entomology

Biographical:

Personal Data: Born in Salina, Kansas, on 17 November 1972, the son of Dennis and Renée Toews.

Education: Graduated from Salina South High School, Salina, Kansas in May 1991; received Bachelor of Science degree in Biology from Fort Hays State University, Hays, Kansas in May 1995; received Master of Science degree with a major in Entomology at Oklahoma State University, Stillwater, Oklahoma in May 1998. Completed the requirements for the Doctor of Philosophy degree with a major in Entomology at Oklahoma State University in May, 2001.

Experience: Worked as an Interpretive Naturalist for the Kansas Department of Wildlife and Parks during the summers of 1993 and 1994; worked as a crop scout for Collingwood Grain Inc. at Oakley, Kansas during the summer of 1995; employed by Oklahoma State University as a graduate research/teaching assistant from 1995 through 1998; employed as a graduate research associate from 1998 through May 2001.

Professional Memberships: Entomological Society of America, Southwestern Entomological Society, Sigma Xi., Phi Kappa Phi.

Professional Award: Awarded the 2000 John Henry Comstock Graduate Award by the Entomological Society of America.