Estimating Populations of Grain Beetles Using Probe Traps in Wheat-Filled Concrete Silos

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ABSTRACT  Probe traps are sensitive tools for detecting populations of beetles in stored grain, but their use for estimating insect density in concrete silos has not been established. Populations of grain beetles infesting wheat in concrete silos at two commercial storage facilities in north central Oklahoma were sampled using probe traps and grain probe samples over a 17-wk period. Grain temperature and quality parameters were collected during the study. Thirteen insect species were detected using probe traps, whereas eight species were detected with the grain samples; Cryptolestes ferrugineus and Rhyzopertha dominica were the most common insects collected in the grain samples. Across dates, there were no differences in mean number of insects recovered by species near the grain surface and 1 m below in either probe traps or grain samples. Comparison of slopes (insects recovered in grain samples regressed on insects in probe traps) showed that there were significant differences by trap position for C. ferrugineus but not R. dominica. Multiple regression models, developed to predict insect population estimates using number of insects captured in probe traps and a temperature component, indicated that more variability in the data were explained using traps positioned 1 m below the grain surface ($R^2 = 0.70$) than near the surface ($R^2 = 0.21$) for C. ferrugineus. About one-half ($R^2 = 0.53$) of the variability in insect density was described for R. dominica. These regression models show the potential for methodical use of grain probe traps in pest management decision-making.

KEY WORDS  Cryptolestes ferrugineus, Rhyzopertha dominica, sampling, monitoring stored wheat.

THE UNITED STATES is the world’s third largest wheat ($Triticum aestivum$) producer, with >56 million metric tons of wheat harvested in 2004 (WORC 2002, NASS 2004). After harvest, U.S. wheat is often stored in commercial grain management facilities referred to as country elevators. A country elevator serves farmers within a shipping distance of ~30 km. Commercial storage structures in the southern United States are predominately constructed of concrete (Kenkel et al. 1994). Long-term wheat storage in these structures provides the ideal habitat for population development of common stored-product insect pests such as Cryptolestes ferrugineus (Stephens) (Coleoptera: Laemophloeiidae), rusty grain beetle, and Rhyzopertha dominica (F.) (Coleoptera: Bostrichidae), lesser grain borer. Presence and feeding of these insects, if improperly managed, may lead to a reduction in value of the commodity.

Historically, chemical inputs (e.g., protectants) have been used to manage stored-product insect infestations. However, millers have voluntarily 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). It has become increasingly difficult to deliver high-quality insect-free grain under these management practices.

Elevator operators generally manage insect populations in concrete silos with calendar-scheduled phosphine gas fumigation to meet this demand for insect- and pesticide residue–free grain. However, concrete silos are not gas tight, and gas distribution within a silo during fumigation is heterogeneous, so an effective fumigation is not guaranteed (Noyes et al. 2001). Additional problems with the current system include repeated worker exposure hazards to the fumigant (Reed 2001) and insect resistance to phosphine gas (Champ and Dyte 1976).

Stored-product insect traps have been available for many years to detect insects in farm-stored wheat (Loschiavo and Atkinson 1967, 1973, Loschiavo and Smith 1986, Lippert and Hagstrum 1987, Vela-Coffier et al. 1997, Hagstrum et al. 1998, Wakefield and Cogan 1999). The principle of operation behind pitfall traps is that insects contact and enter the trap, fall through a void, and are accumulated in an escape-proof col-
lecion reservoir. Loschiavo and Atkinson (1967) originally developed probe traps, a specialized pitfall trap for use in stored grain; a thorough review of probe trap development is discussed in White et al. (1990). Cuperus et al. (1990) provide a general discussion of variables affecting insect capture in probe traps. 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 because insects are detected in traps earlier than in grain samples (Lippert and Hagstrum 1987, Vela-Coiffier et al. 1997, Hagstrum et al. 1998). However, interpretation of trap captures can be difficult compared with interpretation of data derived through absolute sampling methods.

Effective integrated pest management (IPM) requires that reliable population density estimates be collected through sampling (Serber 1982, Hagstrum and Flinn 1996), but sampling insects in concrete grain silos is difficult. For example, access to the grain in a silo is a limiting factor because a “confined space” entry permit is required in the United States. The permit requires 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 (Code of Federal Regulations 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 because silo access to the grain from the top of the silo is often located on the exterior of the building. Flinn et al. (2003) described using a vacuum probe sampler to obtain grain samples for pest management decision making in this environment; although data interpretation is simpler, this method requires specialized equipment and is considerably more labor intensive than using traps.

Objectives of this study were (1) to assess how placing traps near the surface of the grain versus 1 m below the surface affects the mean number of captured insects; (2) to examine the relationship between number of insects captured in probe traps and actual insect density by trap position in the grain; and (3) to develop simple models to estimate insect density using insect captures in probe traps, grain quality parameters, and grain temperature. Each of the objectives was completed independently for Cryptolestes ferrugineus and Rhyzopertha dominica.

Materials and Methods

Studies were conducted at two country elevators located in north central Oklahoma during the 2000 storage season. Each elevator had two freestanding silo systems containing 10–23 concrete silos with an average capacity of 408.7 metric tons per silo. Silo shape was generally round or hexagonal and silos ranged from 30.5 to 36.5 m tall with a mean diameter of 6 m. A bucket elevator moved grain from the pit area at ground level (where trucks unload grain) to a distributor located above the silos. Grain flowed by gravity into the appropriate silo through a downsput originating at the distributor. The cooperating elevator primarily stored hard red winter wheat and did not carry over grain from the previous storage season. No residual grain protectants were used in the elevators; however, managers turned (moved grain from one silo to another) and fumigated every silo at least once during the study to control severe infestations of R. dominica. Individual silo fumigations were not necessarily coordinated by date among or within elevators, and we had no ability to control the timing of such interventions. Obviously, fumigation affected insect population growth and dynamics, but our samples were obtained at discrete times when the grain was not moving or under fumigation. The 2000 wheat harvest progressed quickly because of favorable weather conditions, and elevators were filled to capacity in only 2 wk starting in early July. Studies reported here began immediately after harvest and continued for 17 wk.

Probe Traps and Deep Bin Cup Samples. Unbaited WB-II probe traps (Trécé, Salinas, CA) (Burkholder 1988) were placed in an average of nine silos during the study. We sampled monthly for the first 9 wk of the study and then every other week for the final 8 wk. On each sampling occasion, probe traps were inserted from the top of the silo and left in the grain for 72 h. Probe traps were placed in the grain through the 0.6-m² access port on top of the silo where grain enters during silo filling. Silo access ports were generally located over the center of the silo. In each silo, one trap was positioned so the top of the trap was 2 cm below the grain surface, whereas the top of the second trap was positioned 1 m below the grain surface. Traps were pushed into the grain with a 1.3-cm steel pipe threaded to a 4.5-cm PVC pipe joint, which fit loosely around the end of the probe traps. Cotton rope (0.95 cm diameter) was tied to each trap for retrieval purposes. By standing on top of the silo and using multiple sections of threaded pipe, the researchers never physically entered the silos. A temperature data logger (HOBO TEMP; Onset Computer, Bourne, MA) was affixed to each probe trap using 0.3-cm braided steel cable. Loggers recorded grain temperature every 2 h while traps were in place, and we used the mean temperature during that trapping interval for the modeling work. Adult insects captured in the probe traps were identified to species and enumerated. Grain samples were obtained immediately before probe traps were placed in the grain mass. Samples were procured using a cylindrical (3.5 cm diameter by 38.1 cm long) deep bin cup sampler (Seedburo Equipment, Chicago, IL). The deep bin cup sampler (each cup yielded 250 g of wheat) was pushed into the grain five separate times, making a ring with an ~25-cm radius around each trap position. These samples, obtained at the same depth as the corresponding probe trap, were pooled by depth and brought to the laboratory for processing. Insects were removed from
the grain using a standard testing sieve (Fisher Co., Pittsburgh, PA) with 1,410-μm sieve openings. We did not incubate the grain. All material (except insects) was reconstituted into each sample and submitted to a certified grain-grading laboratory for assignment of a numerical grade according to U.S. grain standards (GIPSA 1997). Quality analysis included moisture content; dockage (material larger and smaller than wheat); test weight (bulk density expressed as kg/hl); shrunken and broken kernels (material that passes through a 0.064 by 0.375-in 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 (sum of percentage of damaged kernels, foreign material, and shrunken and broken kernels); and insect-damaged kernels (kernels with exit holes bored by internal infesting species).

Data Processing. Quantities of C. ferrugineus and R. dominica were compared at the two sampling depths in each silo. Data were transformed using the square-root transformation (Zar 1984) to correct for heteroscedastic data commonly observed with counts. Comparisons were made independently by species using repeated-measures analysis of variance (ANOVA). We used the REPEATED statement of the procedure PROC MIXED (SAS Institute 1999), with degree of freedom adjustments following the methods of Kenward and Roger (1997), to model the variance–covariance relationship of the sampling depth response variables within the elevator and silo; random effects were considered to account for the variability within the elevator and silo within each silo. An autoregressive with period one covariance structure (Littell et al. 2002) proved to be effective to model the intraplate correlation. Insect recovery from the grain samples was normalized to insects per kilogram grain and was contrasted between the two sampling depths in each silo.

Correlation of Grain Samples and Trap Capture. Insects captured in traps and insects recovered in grain samples were modeled with poisson regression to examine how insects captured in traps related to actual insect density. Data were analyzed using PROC GENMOD (SAS Institute 1999), in which actual insect density from grain samples was the independent variable and probe trap capture was the dependent variable. Observations were removed from the data set if there were zero captures in both the grain samples and probe traps. The slope of these regression models was compared between sampling depths for each species using $\chi^2$ analysis, whereas the scaled deviance statistic was used to assess goodness-of-fit using the specified model. 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. This procedure is similar to conventional regression except the response variable is poisson distributed and the process does not produce an $r^2$ value.

Estimating Insect Density. Insects captured in probe traps, corresponding insect density from grain samples, mean grain temperature during the trapping interval, quadratic and cubic temperature polynomials, and all grain-grading factors were combined into the same dataset. These data were modeled using multiple regression (PROC REG; SAS Institute 1999), where insect density determined from the grain samples was the predicted variable. The resulting regression equations are presented as simple models that described insect density in wheat-filled concrete silos. These analyses were conducted separately for C. ferrugineus and R. dominica.

Results

In the preliminary data analyses, we looked at correlations of all variables except insects in the dataset. A significant correlation ($r = -0.76; P < 0.01; n = 117$) between mean grain temperature (surface and 1 m deep) and sampling date was observed (Table 1); therefore, only one of these variables should be used for modeling the insect data. When analyzing for differences in grain temperature between sampling depths in each silo, there was a significant interaction between sampling depth and collection date ($F = 4.54; df = 1.54.2; P = 0.04$). Significant temperature differences between sampling depths were detected during all sampling periods except the first two (Table 1); grain at 1 m from the surface was generally warmer than grain at the surface. The remaining grain-grading parameters (i.e., moisture content, test weight, dockage) are summarized in Table 2.

Probe Trap Captures and Grain Temperature. During the study, we collected nearly 25,000 insects in probe traps. C. ferrugineus was the most abundant species, followed by Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), red flour beetle; Oryzaephilus surinamensis L. (Coleoptera: Silvanidae), saw-toothed grain beetle; R. dominica; Cephalonoma waterstoni (Gahan) (Hymenoptera: Bethylidae); Sitophilus oryzae L. (Coleoptera: Curculionidae), rice weevil; Theocalus elegans (Westwood) (Hymenoptera: Pteromalidae); Anisopteromalus calandrae (Howard) (Hymenoptera: Pteromalidae); Ahaestus advena (Walsh) (Coleoptera: Silvanidae), foreign grain beetle; and Typhnaea stercorea L. (Coleoptera: Mycetoph-
agidae), hairy fungus beetle. We also recovered Liposcelis spp. (Psocoptera: Liposcelididae), sap beetles (Coleoptera: Nitidulidae), and predatory bugs (Hemiptera: Anthocoridae), but further taxonomic identification (or enumeration in the case of Liposcelis spp.) was not pursued. C. ferrugineus, T. castaneum, O. surinamensis, and R. dominica accounted for 96.2, 1.4, 1.2, and 0.8% of the total insect yield from probe traps, respectively. The remaining species each comprised ≤0.1% of the total captures on a per species basis. Probe trap insect recoveries included five species that were not detected using grain samples. C. ferrugineus was detected in 108/126 probe trap sampling events and 61/126 grain sampling events. R. dominica was detected in 36/126 probe trap sampling events and 36/126 grain sampling events. There were no significant differences in mean number of insect captures across time between the two sampling depths for C. ferrugineus (F = 0.41; df = 1,336; P = 0.53), R. dominica (F = 0.13; df = 1,454; P = 0.72), T. castaneum (F = 1.02; df = 1,421; P = 0.32), or S. oryzae (F = 0.0; df = 1,699.5; P = 0.92; Table 3).

Insects in Grain Samples. We recovered 1,745 insects in the grain samples. C. ferrugineus was the most abundant species, followed by R. dominica, O. surinamensis, T. castaneum, C. waterstoni, S. oryzae, T. elegans, and A. calandrae. C. ferrugineus, R. dominica, O. surinamensis, and T. castaneum accounted for 82.3, 11.6, 2.7, and 2.7% of the total insect yield from grain samples, respectively. Analyses of the grain sample data showed that there were no significant overall differences in numbers of insects between sampling depths for C. ferrugineus (F = 0.74; df = 1,282; P = 0.40), R. dominica (F = 0.14; df = 1,473; P = 0.71), T. castaneum (F = 3.93; df = 1,621; P = 0.09), or S. oryzae (F = 1.29; df = 1,729; P = 0.26; Table 3).

Correlation of Grain Samples and Trap Captures. Poisson regression was used to relate absolute insect densities obtained with grain samples to relative estimates of insect density acquired with the probe traps. The regression slopes were different between the two sampling depths for C. ferrugineus (χ² = 17.11; df = 96; P < 0.01) but not R. dominica (χ² = 0.21; df = 33; P = 0.65). Therefore, the data were modeled independently by sampling depth for C. ferrugineus but not R. dominica. Scaled deviance values for both C. ferrugineus sampling locations (top = 1.757; df = 47; P < 0.01; bottom = 688; df = 49; P < 0.01) and R. dominica (scaled deviance = 204.9; df = 33; P < 0.01) were rather high, indicating a lot of variability between the observed and predicted values. This lack of fit suggested that other factors could improve the model fitting, so we studied the data using multiple regression.

Modeling Insect Density. All available data including temperature and grain-grading parameters were included to show the strongest possible population density estimates. Because temperature can influence insect movement, we first plotted insect capture in probe traps as a linear function of temperature. At temperatures <15°C, there were no R. dominica captured in probe traps even though this species was recovered in the corresponding grain samples. Therefore, we eliminated observations of R. dominica if the temperature was <15°C to avoid biasing the equations. Similar findings were not observed with the C. ferrugineus plots. Multiple regression equations were modeled separately by sampling depth for C. ferrugineus but not R. dominica as a result of the slope comparison procedure above. Significant effects for modeling C. ferrugineus density at both sampling depths in the grain included probe trap capture and mean grain temperature (Table 4). The amount of variation explained by the models was much better at the lower sampling depth. R. dominica population density was also best fitted using probe trap captures and a linear temperature effect (Table 5).
Table 5. Multiple regression estimates for predicting *R. dominica* density from probe trap samples (3-d trapping interval) and temperature (°C) in wheat-filled concrete silos

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate ± SEM</th>
<th>Partial $R^2$</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>7.5 ± 2.5</td>
<td>—</td>
<td>8.59</td>
<td>0.01</td>
</tr>
<tr>
<td>Trap captures</td>
<td>0.5 ± 0.08</td>
<td>0.44</td>
<td>32.13</td>
<td>0.01</td>
</tr>
<tr>
<td>Temperature</td>
<td>−0.2 ± 0.1</td>
<td>0.09</td>
<td>6.67</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$F = 19.46; df = 2,34; P < 0.01; R^2 = 0.53.$

**Discussion**

All insect species captured in probe traps during this study are previously known to occur in stored wheat in Oklahoma. The most abundant species captured in probe traps, *C. ferrugineus*, is an externally infesting species that does not cause insect-damaged kernels (IDKs). This is an important distinction from the internal infesting species, *R. dominica* and *S. oryzae*, which do cause the tunneling and boring that, by definition (GIPSA 1997), cause IDKs and the resulting price discount or rejection in wheat intended for milling applications. However, presence of any two or more living insects injurious to grain will result in grain being classified as infested, which may also trigger a price discount (Reed et al. 1989). Recovery of *C. waterstoni*, *T. elegans*, and *A. calandrae* confirm the natural occurrence of substantial natural enemy populations in commercial grain storage. *C. waterstoni* is a parasitoid of *Cryptolestes* spp., whereas *T. elegans* and *A. calandrae* are parasitoids of internally infesting beetle pests such as *R. dominica* and *S. oryzae*.

A lack of *O. surinamensis*, *S. oryzae*, and *T. castaneum* recoveries in a substantial proportion of probe trap and grain samples prevented meaningful analyses and model building for these species. A dataset with more observations is necessary to evaluate the value of probe traps for making population density estimates for these species. A possible way to recover more insects would be to take much larger grain samples and place probe traps in grain for >3 d. Five species were captured in probe traps but not detected in the grain samples, suggesting that the probe traps are more sensitive than grain samples for detecting certain species of insects.

Temperature was a significant factor in increasing the amount of variation the multiple regression models explained. However, we were surprised that the temperature coefficients were negative in each case. Subsequent plotting of the field-collected data showed that they were not balanced with respect to range of temperature across collection dates. In broad terms, grain temperatures were greater during the first three sampling intervals when insect pressure was low. Later in the study, grain temperature predictably decreased with cooler weather, but insect populations increased because of reproduction. Fargo et al. (1989) showed a positive temperature effect for *C. ferrugineus* but not *R. dominica* in a tightly controlled laboratory study conducted at temperatures of 10, 21.1, and 32.2°C. Additional laboratory studies (Flinn and Hagstrum 1998, Toews and Phillips 2002, Jian et al. 2003) also showed that *C. ferrugineus* locomotion or capture in probe traps increased with grain, increasing temperature in at least parts of the temperature continuum. Arbogast et al. (2004) plotted weekly temperature and probe trap catch of *T. stercorea* and *T. castaneum* against time and fitted cubic polynomials to describe seasonal changes; they then used residual variation to make the trap capture variable independent of time. This procedure may work equally well with *C. ferrugineus*.

General grain-grading parameters and temperature were chosen for multiple regression because most elevator managers have the tools and knowledge to complete this task for any given grain sample. Previous research has shown that probe trap captures did not correlate with actual insect density for all species; however, consideration of multiple factors in combination could help grain managers interpret trap catch data for pest management decisions (White et al. 1990). The underlying assumption was that the addition of grain-grading factors and a specific effect for temperature might help improve population estimates. We considered using time as a factor in the regression equations, but this was impractical for commercial storage because the grain manager does not know the harvest date and fumigation history of the grain. Additionally, blending grain of different harvest dates into the same silo makes adoption of time as a known variable unfeasible.

Slope comparisons showed clear differences in rates of capture between the two sampling depths studied for *C. ferrugineus*. While there was not a quantitative difference in population size between sampling depths, as evidenced by the deep bin cup samples, there were obvious differences in the slope. We hypothesize that insects enter the grain from the headspace and may remain inactive for some time when transitioning between flight, feeding, and reproduction. Jian et al. (2003) determined by factorial experiment that speed and direction of *C. ferrugineus* movement were affected by factors including temperature gradients, geotaxis, and an interaction between these two. Toews et al. (2002) showed daily peaks in *C. ferrugineus* captures in electronic probe traps placed near the grain surface in small steel bins. We are unsure if similar temperature gradients and diel rhythms occurred deeper in the grain mass, which may have influenced insect capture in probe traps.

Sampling methods used for IPM need to help the pest manager determine when a pest population reaches the economic threshold. A relative population density estimate, such as that provided by a probe trap, must be converted to an absolute estimate with reasonable precision. Lippert and Hagstrum (1987) recommend regressing the ratio of average trap catch divided by the average number of insects per grain sample (0.2165 kg) against the number of insects per trap; this model explained 57% of the variation in their dataset. Vela-Coiffer et al. (1997) used linear regression between numbers of insects captured in probe traps and absolute density determined with a single 0.265-kg-deep bin cup sample or 0.650-kg trier sample;
their methods did not consider temperature but explained 25–34% of variation for C. ferrugineus. Hagstrum et al. (1998), also working in steel bins, developed models to adjust insect captures obtained from probe traps to absolute density (~1-kg sample) using linear equations. They based their models on groups of temperatures (<14, 14–23, and >23°C) that corresponded with storage periods (0–90, 90–135, and 135–190 d) in the four bins monitored during the study. This method explained 9, 57, and 30% of the variation in C. ferrugineus populations, respectively. They also found that two similar sampling depths, just below the surface and 7.6 cm below the grain surface, generally did not affect insect captures averaged across the storage season.

Significant difficulties have been shown when using probe trap captures to make pest management decisions. Athanassiou and Buchelos (2001) concluded that probe traps placed just below the grain surface and left for a period of 14 d were poor indicators of insect population density, determined using individual 0.750-kg grain samples, because the accuracy was poor and the number of traps required for a reasonable accuracy was excessively large. Subramanayam and Harein (1990) showed that a trapping duration of 2 instead of 7 d improved the accuracy of Cryptolestes spp. trap captures in stored barley because the variance of trap catch decreased three-fold; however, they also concluded that many traps were needed for estimating mean densities of most species. White and Loschiavo (1986) showed nearly twice as many C. ferrugineus captures in traps baited with live adults and hypothesized that naturally produced aggregation pheromones were responsible for the additional captures, a concept also mentioned by other authors (Loschiavo 1974, Barak and Harein 1982).

This paper is the first to show how probe traps could be used to estimate insect density in large wheat-filled concrete silos at commercial facilities. Grain quality parameters and temperature were evaluated as continuous variables potentially affecting the number of insects captured in probe traps. Here we used multiple regressions to relate the relative population estimate to an absolute density estimate over a range of environmental conditions in the field. Important findings include the observation that much more variation in C. ferrugineus activity could be explained by placing traps 1 m below the grain surface and how temperature can be used to directly adjust trap captures over changing conditions.

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