

Role of Food and Structural Complexity on Capture of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in Simulated Warehouses

M. D. TOEWS,¹ F. H. ARTHUR, AND J. F. CAMPBELL

USDA-ARS Grain Marketing and Production Research Center, Manhattan, KS 66502

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ABSTRACT *Tribolium castaneum* (Herbst) is a cosmopolitan pest of stored products, grain processing, and food warehouses. This study was initiated to more fully characterize the role of structure, sanitation, and trap location on capture of *T. castaneum* in pheromone-baited pitfall traps commonly used in insect monitoring programs. Food patches, spatial structure, and trap position were manipulated in pilot scale warehouses with a known density of insects. Significantly greater quantities of insects were captured in traps placed in warehouses without food patches than those with food patches. Insects tended to be captured with greater frequency in the corners and underneath shelves than in the middle of warehouses. Correlation between actual density and trap captures was stronger in warehouses without food patches, suggesting that sanitation is an important part of pest monitoring. Finally, placement of concrete blocks in the middle of the warehouse did not increase the number of beetles captured in that area compared with warehouses without concrete blocks. Results from this study should be considered when developing guidelines for trap interpretation.

KEY WORDS red flour beetle, sampling, trapping, monitoring, insect behavior

THE BASIS OF STORED PRODUCTS integrated pest management (IPM) is a reliable insect sampling program that is able to detect insects, estimate changes in populations, and locate sources of infestation (Burkholder 1990). Absolute and indirect sampling methods are available for sampling and monitoring insect populations in mills and warehouses (Good 1937, Salama and Salem 1973, Buchelos 1980, Lindgren et al. 1985, Ho et al. 1997, Arbogast et al. 2002, Campbell et al. 2002, Arbogast and Chini 2003). Absolute estimates, obtained by counting insects in a known quantity of product, should provide the best estimate of pest density. However, counting insects in packages, building voids, or inside equipment can be expensive because sampling may be destructive and highly labor intensive. Indirect sampling methods, those where insect density cannot be directly expressed, are generally less time consuming and do not require destructive sampling. Pheromones and food-baited traps are examples of indirect methods (Burkholder 1984, Barak 1995). Interpretation of pheromone/food bait trapping programs is complicated because the relationship between trap capture and product infestation has not been established.

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a cosmopolitan pest associated with stored grain, flour and feed mills, food processing facilities, warehouses, and retail stores. The species can complete development between 20.0 and 37.5°C and in ≈19–20 d under optimum conditions (35.0°C and >70% RH) (Howe 1956). *T. castaneum* infests a variety of commodities (Good 1936) and milled products but also can feed and develop on seed-borne fungi (Sinha 1966). Adults may live up to 3 yr or more, and adult females can lay eggs for >1 yr (Good 1936), although food resource and temperature strongly affect adult longevity. This species readily moves among resource (flour) patches, can distribute eggs among multiple patches, and can complete development in very small patches (Campbell and Hagstrum 2002, Campbell and Runnion 2003). Product contamination by whole insects, eggs, fragments, frass, benzoquinones, and cast skins is possible in *T. castaneum*-infested processing plants and warehouses (Baur 1984).

Pheromone traps are widely available, but few research-based guidelines exist to establish monitoring programs based on insect behavior and ecology (Campbell et al. 2002). This study was initiated to more fully characterize the role of structure, sanitation, and trap location on capture of the red flour beetle, *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae), in pheromone baited pitfall traps. This insect is widely regarded as the most important insect pest of

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¹ Corresponding author: USDA-ARS GMPRC, 1515 College Ave., Manhattan, KS 66502 (e-mail: mtoews@gmprc.ksu.edu).

the grain processing industry. Pilot scale studies in small warehouses were conducted so that treatments could be replicated, adequate controls could be used, and accurate estimations of true population density could be obtained. Our objectives were to (1) examine the influence of food patches on insect captures, (2) investigate the role of structure (objects placed to intersect insect movement) and how it may influence the captures, and (3) evaluate how trap position influences the number of *T. castaneum* adults captured in traps.

Materials and Methods

Insects. *Tribolium castaneum* adults used in experiments were reared in 0.94-liter glass jars containing 300 g of wheat flour fortified with 5% (by weight) brewer's yeast (ICN Biomedicals, Aurora, OH). The original colony was established from ≈ 100 late-instar larvae collected in November 2001, at an operating flour mill in Kansas. The colony was maintained at $27.0 \pm 0.5^\circ\text{C}$ and $65 \pm 5\%$ RH in environmental growth chambers. Rearing density was ≈ 100 adults per 300 g of flour.

Pilot Scale Warehouses. Research was conducted in five climate-controlled pilot scale warehouses measuring 5.9 m long by 2.8 m wide by 2.2 m tall. Warehouses were framed with wood and covered with Masonite exterior siding and an asphalt shingled roof. Insulation was installed underneath the floors, walls, and ceilings before covering with plywood. Interior walls were sealed with caulk and coated with industrial food production grade primer (PR-14 WB Kwick Prime; Valspar Flooring, Chicago, IL) and epoxy (EC-7 Epoxy; Valspar Flooring) to seal cracks and crevices, eliminating or minimizing refugia for insects. Warehouses were cleaned between experiments by removing all items in the warehouse, thoroughly sweeping floors, and vacuuming all interior surfaces. A single data logger (HOBO TEMP; Onset Computer, Bourne, MA) was placed on the floor at the center of each warehouse to record temperature and relative humidity during experiments. A 24:0 (L:D) photoperiod was used in each warehouse. Two 100-W incandescent light bulbs provided lighting at 42.0 ± 2.6 lx ($n = 20$), measured at ground level with a digital light meter (model 401025; Extech Instruments, Waltham, MA).

Pilot Scale Shelves. Each warehouse was equipped with three custom-made pilot scale shelving units to simulate large equipment, provide structure, and provide shelter to conceal food patches. Shelves were constructed of a 2.5-cm-square tubular steel frame, a galvanized steel shelf, and detachable kickplates with a small gap to permit insect movement under the shelf. The sealed framework measured 44.3 cm wide by 106.7 cm long by 11.6 cm tall, whereas the outside dimensions of the shelf were 53.3 cm wide by 119.4 cm long.

Insect Traps. All experiments were conducted using Storgard Dome traps (Trécé, Adair, OK). These are floor-placed pitfall traps developed for use in food processing facilities; the trap includes a dome-shaped

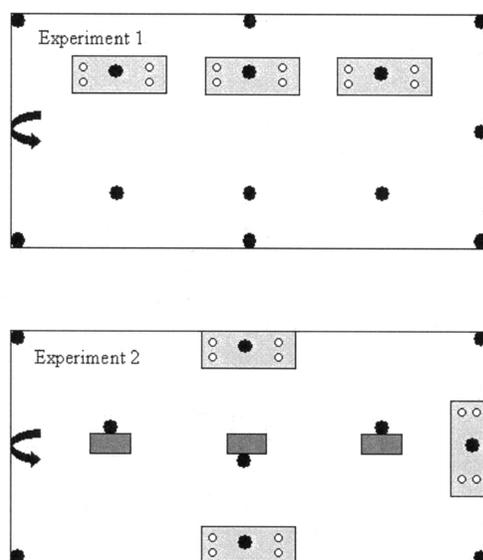


Fig. 1. Position of the door, shelves, traps, and concrete blocks in pilot scale warehouses during experiments (A) 1 and (B) 2. The arrow represents the entry door, light gray rectangles represent pilot scale shelves, black circles represent pitfall traps, white circles represent food patches, and dark gray rectangles represent concrete blocks. Drawings are not to scale.

dust cover with a diameter of 115 mm. Traps were baited with a commercial pheromone lure (Trécé) specific for *Tribolium* spp. (Obeng-Ofori 1991) and 15 drops of food oil (Trécé). Every 3 wk traps were cleaned with warm soapy water, the food oil was replaced, and new pheromone lures were inserted. Traps were generally checked in the early afternoon (1300–1400 hours).

Experiment 1. This test was designed to investigate whether the presence of food patches would affect the total number of insects captured in traps. We were also interested in determining if the food patches would concentrate the insects, and subsequent captures, in the immediate area. Two warehouses were arranged with three shelves each, positioned equidistant from each other and in a single row parallel to one of the long sides of the building (Fig. 1A). A trap was not placed at the midpoint of the wall containing the door. There was a 30.5-cm sanitation aisle between the floor wall junction and the outermost edge of the shelves. A sanitation aisle allows pest managers to clean between the wall and product in a working warehouse. Here, the sanitation aisle was intended to provide a buffer zone between insects moving along the wall and those inhabiting the area under the shelves. In one of the warehouses, we placed four food patches under each shelf unit. Food patches consisted of 10 g of wheat flour on 110-mm filter paper disks (Whatman International, Maidstone, UK). No food patches were provided in the second warehouse.

Fifty 1- to 2-wk-old and 50 2- to 3-wk-old unsexed adults were released in the center of each of the two

warehouses. One week after insect release, we positioned pitfall traps with one trap in each of the four corners (corner trap), one trap at the midpoints along each wall (wall trap), one trap under each of the three shelves (shelf trap), and three additional traps in the open space opposite the traps under the shelves (middle trap). Middle traps were placed the same distance from the closest wall as the shelf traps (Fig. 1A). Insects captured in traps were removed and counted weekly for a period of 3 wk. While visiting to service the traps, we released 10 additional adults of mixed age and sex into each warehouse to simulate immigration. Because no immature insects were seeded into the warehouses and there was not enough time to complete a new generation, the total number of insects in each warehouse could be calculated by subtracting the number of insects captured in traps from those added to the warehouse, assuming no mortality, immigration, or emigration. After a thorough cleaning of the warehouses, the experiment was conducted a second time with the assignment of food patches switched between the two warehouses. A total of two replications with food patches and two additional replications without food patches were conducted.

Experiment 2. This test was designed to investigate the hypothesis that providing objects to intersect insect movement (structure) in the center of the room would increase the likelihood of insect captures in those traps. For example, placing traps near I-beams or along machinery in the middle of the warehouse may increase captures because we assumed that an insect would encounter the object and then move parallel to the object. Five warehouses were arranged with the shelves positioned adjacent to the wall midway between the corners of the two long sides and one short side (Fig. 1B). Four food patches, consisting of 9 g of wheat flour, were evenly spaced under each of the three shelves in each warehouse. Three concrete blocks (38 by 19 by 19 cm) were placed equidistant from each other in the center of three of the five total warehouses. One hundred fifty 2- to 3-wk-old *T. castaneum* adults were released in the center of each warehouse. One week later, one trap was positioned in each of the four corners (corner trap), under each of the three shelves (shelf trap), and alongside each of the concrete blocks (middle trap) or in the same location as the blocks in the warehouses not receiving concrete blocks (Fig. 1B). A trap was not placed at the midpoint of the wall containing the door. Adults captured in traps were removed and counted weekly for a period of 3 wk. During the sampling visits, we released 10 additional adults of mixed age and sex into each warehouse to simulate immigration. After a thorough cleaning of the warehouses, the experiment was repeated with the assignment of concrete blocks reversed between warehouses and new pheromones in the traps. A total of five replications with concrete blocks and five replications without concrete blocks was conducted.

Experimental Design and Data Analysis. A problem inherent to all experiments using pheromone traps in limited spaces is the potential for trap positions to be

autocorrelated. This lack of independence among positions can compromise the use of parametric statistics for comparisons among trap positions. To address this concern, we attempted to first model the data using a semivariogram (Isaaks and Srivastava 1989, Rossi et al. 1992, Liebhold et al. 1993, Brenner et al. 1998, Nansen et al. 2003). However, meaningful semivariogram analyses require an average of at least 30–50 pairs of observations for each lag spacing (Journal and Huijbregts 1978, Liebhold et al. 1993) and a minimum of four points on the semivariogram (Nansen et al. 2003). Our studies were conducted in pilot scale warehouses where it would be inappropriate to use a sufficient quantity of traps to meet these assumptions. Therefore, to investigate spatial autocorrelation in our data sets, we used the OUTPAIR option in the PROC VARIOGRAM procedure in PC SAS 8.0 (SAS Institute 1999) to calculate the lag-distance intervals between all possible pairs of traps within a warehouse and paired these data with the absolute value of the difference in trap captures between those same two traps. We then used linear regression (PROC REG; SAS Institute 1999) to see if the difference between each pair of trap captures could be predicted using the distance between those traps. In short, a significant regression with a negative slope would indicate autocorrelation.

Each experiment was designed as split plot arrangement of a randomized complete block design. We analyzed mean number of insect adults captured under treatment regimens consisting of a main plot factor (presence/absence of food or presence/absence of concrete blocks) and a subplot factor (trap position). The response variables were averaged by trap position within each warehouse and averaged across the 3-wk period each replication was conducted. A square-root transformation was applied before statistical analyses to meet the assumption of equal variance (Zar 1984). Interactions, main plot comparisons, subplot comparisons, and single degree of freedom contrasts were performed with PROC MIXED (SAS Institute 1999) with the NOBOUND option and degree of freedom adjustments for the variance components following the methods of Kenward and Roger (1997). Actual treatment means and SEMs are presented in the figures. Pearson correlation coefficients (Pearson 1920) were generated for experiment 2 using weekly warehouse trap captures by location and calculated insect densities (assuming no immigration or emigration).

Results

Environmental Conditions. Data loggers showed that conditions during experiment 1 were $25.6 \pm 0.03^{\circ}\text{C}$ and 30.6 ± 0.15 RH (SEM: $n = 2,016$). Temperature and RH during experiment 2 were $24.5 \pm 0.05^{\circ}\text{C}$ and 44.1 ± 0.16 RH ($n = 5,160$) over the 3-wk period.

Experiment 1. The regression analyses in which we modeled the absolute value of the difference in trap captures by the linear distance separating these two traps showed that there was not a detectable relation-

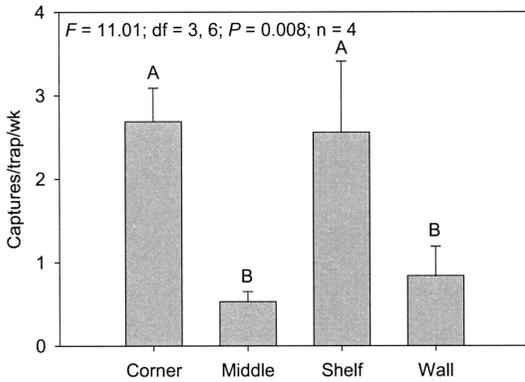


Fig. 2. Mean \pm SEM number of *T. castaneum* collected in pitfall traps by trap position across weeks and food patch presence/absence during experiment 1.

ship ($F = 0.13\text{--}3.40$; $df = 1,76$; $P = 0.07\text{--}0.72$) in eight of the main plot experimental units. In cases where the regression was statistically significant, the r^2 values ranged from 0.06 to 0.10. We concluded that, in the majority of the cases, there was not evidence for autocorrelated data and proceeded to analyze the data using parametric tests.

There was no interaction between the presence/absence of food patches and the position of traps in the warehouse ($F = 0.76$; $df = 3,6$; $P = 0.556$). Across all trap positions, there were fewer mean insect captures in warehouses containing food patches (1.23 ± 0.23) compared with warehouses without food patches (2.09 ± 0.37 ; $F = 346.1$; $df = 1,1$; $P = 0.034$). Directly contrasting the number of captures under shelves with food patches (3.7 ± 1.3) and number of captures under shelves without food patches (1.4 ± 0.3) showed that there was a 2.6-fold difference, but it was not statistically different ($F = 5.2$; $df = 1,6.1$; $P = 0.062$). There was a significant difference among captures of adult insects among the three trap positions ($F = 11.01$; $df = 3,6$; $P = 0.008$), because insects were more likely to be found in traps positioned under the shelves and in corners. Less than one-third as many insects were recovered in traps placed along the wall or in the middle of the warehouse (Fig. 2).

Separate Pearson correlation coefficients were generated for warehouses with food and without food because this factor significantly affected trap captures. A strong correlation ($r = 0.96$; $P = 0.002$; $n = 6$) between the mean number of captures in all traps and the actual number of insects in each warehouse was observed for warehouses without food patches. When looking at correlations between individual position means and the actual number of insects in each warehouse, the strongest relationship was for the shelf trap ($r = 0.88$; $P = 0.020$; $n = 6$), followed by the wall traps ($r = 0.72$; $P = 0.106$; $n = 6$), corner traps ($r = 0.66$; $P = 0.150$; $n = 6$), and finally, the middle traps ($r = -0.05$; $P = 0.919$; $n = 6$). A weaker overall correlation between mean number of captures in all traps and the actual number of insects was observed for warehouses provisioned with food patches ($r = 0.50$; $P = 0.311$; $n =$

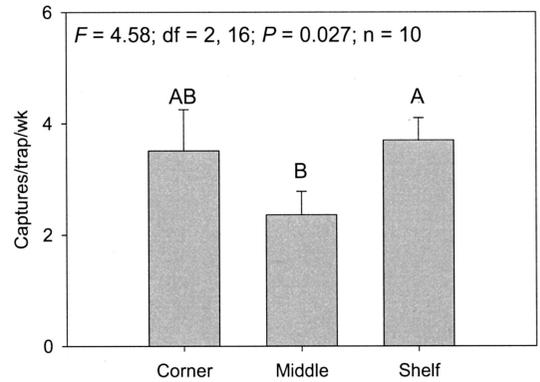


Fig. 3. Mean \pm SEM number of *T. castaneum* collected in pitfall traps by trap position across weeks and presence/absence of concrete blocks across weeks during experiment 2.

6). Correlations by trap position with the actual insect population were strongest for the shelf trap ($r = 0.640$; $P = 0.171$; $n = 6$), followed by the corner trap ($r = 0.54$; $P = 0.271$; $n = 6$), wall trap ($r = -0.08$; $P = 0.88$; $n = 6$) and, finally, the middle trap ($r = -0.21$; $P = 0.695$; $n = 6$).

Experiment 2. The regression analyses again showed that the regression equations were nonsignificant ($F = 0.01\text{--}3.83$; $df = 1,43$; $P = 0.06\text{--}0.93$) in 27 of the 30 main plot experimental units. In the three cases where the regression was statistically significant, the r^2 values ranged from 0.09 to 0.11. We concluded that in the majority of the cases, there was not evidence for autocorrelated data and proceeded to analyze the data using standard parametric tests.

There were no detectable interactions between presence/absence of the concrete blocks and effects attributed to trap position ($F = 0.31$; $df = 1,4$; $P = 0.607$). Likewise, there was no difference in the mean number of trap captures in warehouses containing concrete blocks (3.5 ± 0.4) compared with trap captures in warehouses without blocks (3.8 ± 0.4 ; $F = 0.31$; $df = 1,4$; $P = 0.607$). Traps positioned in the middle of the warehouses with concrete blocks (2.6 ± 0.6) captured a similar number of insects as traps in the middle of warehouses without concrete blocks (2.3 ± 0.5 ; $F = 0.02$; $df = 1,7.5$; $P = 0.899$). A strong trap position effect was identified in which ≈ 1.8 times more insects were captured in traps located under shelves than traps positioned in the middle of the warehouse (Fig. 3).

Discussion

The presence of food patches affected the total number of insects captured across trap positions. A plausible hypothesis for the observed suppression of captures is that insects tend to aggregate in the vicinity of the food patches and therefore are less likely to leave the food patches where they could be captured. Stejskal (1995) showed greater captures of *T. castaneum* in small glass arenas containing a pheromone

baited sticky trap only compared with arenas containing shelter only, shelter + food, or trap + shelter + food. Campbell and Hagstrum (2002) observed *T. castaneum* spent much more time in flour patches than outside of flour patches in small-scale experiments. In our study, the foraging range of the insects may have increased when no food patches were provided, thereby increasing the probability of encountering a trap. Interestingly, experiment 1 showed that correlation coefficients for most trap positions were greater when no food patches were provided. Warehouse managers and pest management professionals need to be aware that sanitation level influences the representativeness of trap-based insect monitoring programs. Likewise, an increase in insect captures after a thorough cleaning may not indicate an increase in population size.

We assumed that the concrete blocks would increase the number of insect captures in the middle of the warehouses, because dispersing insects would intersect the blocks, move parallel to the blocks, and intersect the trap. Our results suggest that traps placed along objects away from walls and corners may not capture insects unless a food patch is also present. Shelves in experiment 2 were placed away from the periphery, yet they still attracted insects assumedly because of the food patches and shelter.

Both experiments showed that the traps positioned in a corner or under a shelf captured a greater number of insects than traps positioned in the center of the room. In practice, this suggests that traps should be placed in corners and in hard to reach areas (i.e., under large equipment) to garner meaningful data about the presence of insects in those areas. In a detailed study of *T. castaneum* behavior in small arenas, Campbell and Hagstrum (2002) reported individuals outside of flour patches tended to be observed along the edge (within 0.12 m) of the arenas as opposed to the middle. Stored product insects may prefer to remain in sheltered areas in corners and along walls regardless of arena or warehouse size. The study by Campbell and Hagstrum (2002) also showed that the number of beetles observed in corners exceeded observations along walls. Arbogast et al. (2002) showed that traps along the walls and in corners detected insects when others did not in an empty botanical warehouse. Campbell et al. (2002) showed greater trap captures at wall locations compared with pillars in a food production facility. Although not quantified in any of these studies, the absence of direct light under shelves and along the outside periphery may also be a factor leading to a favorable place for inactivity.

From a practical standpoint, current research using traps to monitor insect populations generally recommend a loose grid of traps across the entire facility. Our results suggest that, for red flour beetle, concentrating trapping efforts under refugia, in corners, along walls, and most importantly, near food sources will yield more information about insect populations in the warehouse environment. A notable exception to this rule would be if the trap captures would be used for

spatial analysis for precision targeting using contour mapping (Liebhold et al. 1993, Brenner et al. 1998). Contour mapping requires a large number of traps arranged in a grid for the map to have any predictive abilities. Results from this study show that the overall level of sanitation in a facility, food resource availability, and general placement of traps should be carefully considered when developing guidelines for trap interpretation of *T. castaneum*.

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