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How varying pest and trap densities affect *Tribolium castaneum* capture in pheromone traps

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Abstract

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is an important insect pest in food processing facilities. Pheromone trapping is frequently used to monitor red flour beetle populations in structures; however, the optimal trap density and the relationship between trap captures and beetle density is not known. Two experiments were performed concurrently in environmentally controlled 30-m^2 walk-in chambers to determine the relationship between aggregation pheromone trap captures of red flour beetles and beetle and trap number. In one experiment, beetle density was kept constant at 200 individuals per chamber while trap number was varied from 1 to 8, and in the other experiment trap number remained constant at one per chamber while beetle density varied from 20 to 800 individuals. Results indicated that approximately one out of 23 red flour beetles were captured in a trap. Number of beetles captured in traps increased significantly as beetle density increased; however, the proportion of beetles captured remained consistent across beetle densities with a mean of 4.7 $\pm$ 0.6% of individuals captured. Trap captures varied significantly with trap placement within experimental chambers, indicating that subtle differences in the trapping environment can influence trap captures. Data suggested that trap densities of 0.07–0.10 m^{-2} (2–3 traps per chamber) would maximize trap capture, whereas a trap density of 0.13 m^{-2} (four traps per chamber) would maximize the predictive ability of a trapping equation estimating beetle density from trap captures. Results provide information needed to more thoroughly explore how environmental factors might influence red flour beetle trap capture in the absence of changes in beetle density. Further understanding of these relationships will allow for more accurate assessments of absolute beetle density from pheromone trap capture data.

Introduction

Understanding the spatial and temporal dynamics of insect pest populations is a critical component of effective pest management; however, absolute sampling of some pest populations can be difficult, especially for insects occurring at low densities or in cryptic habitats. One solution is the use of traps which actively attract pest insects, thereby improving detection in spite of a population being sparse. When compared to absolute sampling methods, monitoring with traps requires less sampling effort (Borges et al., 2011) and improves pest detection efficiency and consistency (Barak & Harein, 1982; Obeng-Ofori & Coaker, 1990). Furthermore, by adding appropriate visual, chemical, aural, or physical attractants, traps can be tailored to monitor a specific arthropod species or life stage (Cohnstaedt et al., 2012). Monitoring insects with traps not only indicates whether a pest is present but can also indicate the extent of a pest's distribution (Arbogast et al., 2005; Sciarretta & Trematerra, 2006), when management tactics are necessary (Reddy & Guerrero, 2001), and whether such management tactics have been successful (Campbell et al., 2010a,b). Given these advantages, the food processing industry relies on trapping with pheromone and kairomone attractants to characterize pest infestations in facilities that store and process foodstuffs. However, to utilize trapping data for quantitative assessments of pest pressure, relationships between trap capture and factors which may alter trap captures must be well understood (Hagstrum et al., 1990; Campbell, 2012; Semeao et al., 2012).

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Attractive traps are designed to take advantage of pests' natural behavior and attempt to invoke a specific response in the target insect species and/or life stage to increase the chance of capture. However, this means that any factor which alters or interferes with a pest's desired response can influence trap capture rates and obscure the relationship between trap capture and actual pest density (Cuperus et al., 1990). Many such confounding factors have been shown to significantly affect trap capture rates (Obeng-Ofori & Coaker, 1990; Mankin et al., 1999; Mullen & Dowdy, 2001; Arbogast et al., 2005; Toews et al., 2005, 2006; Fedina & Lewis, 2007; Hawkin et al., 2011; Campbell, 2012; Semeao et al., 2012). Considering these confounding factors, it is not surprising that few studies have successfully associated captures with traps and actual pest density (Allen et al., 1986; Thorpe et al., 1993; Reddy & Guerrero, 2001; Miller et al., 2010). Furthermore, with the exception of Miller et al. (2010), the applicability of these studies is restricted to a narrow window of time and/or space or to a single species and/or life stage.

The optimal trap density for monitoring *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is not well understood. General guidelines recommend a trap density of approximately 0.01 traps m^{-2} when initiating a monitoring program and although it is recommended that trapping intensity be reduced and concentrated in areas with identified infestations, optimal trap density for the reduced trapping intensity is not included in the recommendations (Trécé, 1999). Furthermore, a wide range of trap densities have been used in published studies. *Tribolium castaneum* monitoring in operational flour mills has ranged from 0.01 up to 0.06 traps m^{-2} (Campbell & Arbogast, 2004; Toews et al., 2006; Campbell et al., 2010a,b). In smaller, simulated warehouse studies, trap densities used were more variable across studies, from 0.03 up to 0.8 traps m^{-2} (Toews et al., 2005; Hawkin et al., 2011; Limonta et al., 2011). Thus, optimal trap density remains difficult to determine, but will rely on a balance between the fundamental biology of *T. castaneum* and practical and economic considerations including facility type, economic costs, and concurrent pest management practices.

On the basis of previous research on mating disruption (Miller et al., 2006), Miller et al. (2010) described the mechanisms underlying mating disruption of the codling moth *Cydia pomonella* (L.) with a competitive attraction model and validated the model with field experiments. The mechanistic framework was based on the concepts of enzyme-substrate interactions in biochemical kinetics in which male moths must proceed through a series of steps for successful mating disruption to occur. Pests proceed through a similar series of steps as part of a successful trapping system. Miller et al. (2010) described the results of these steps with a 'trapping equation' which stated that cumulative capture of (male) moths in a trap was equal to the product of the findability of the trap, the efficiency of the trap, the retention time of the trap, and the density of male moths. Trap findability is the probability that an individual senses the pheromone, contacts the pheromone cloud, responds to the stimulus, and follows the pheromone cloud to its source. Trap efficiency is the proportion of visiting individuals which are captured, and trap retention time is the proportion of an individual's remaining life span spent in/at the trap. Here, the concepts developed to improve mating disruption of the codling moth are applied to pheromone trap capture data of the red flour beetle to estimate the parameters from Miller et al.'s (2010) trapping equation, thereby providing a method for estimating absolute *T. castaneum* density.

We performed two experiments to examine how trap captures of the red flour beetle related to absolute beetle density. We performed experiments in a simplified, controlled environment to minimize the influences of confounding factors. In one experiment, pheromone trap number was varied while keeping beetle density constant, which will enable estimations of optimal trap density to be developed. In the second experiment, beetle density was varied while keeping pheromone trap number constant, which will provide the information needed to parameterize Miller et al.'s (2010) trapping equation. Data obtained from these experiments can also help expand existing population models (Flinn et al., 2010), which attempt to describe the dynamics of red flour beetle populations in food processing facilities.

Materials and methods

Experimental chambers

Two experiments were performed concurrently in walk-in environmental chambers (6.1 × 4.9 m), with gray insulated metal floors and white insulated walls (Figure 1). Both chambers were kept at 22.1 ± 0.3 °C, 46.6 ± 1.8% r.h., and continuous light. Chamber conditions were selected to prevent beetle flight while maintaining a realistic environment. Temperature was monitored once per hour by two data loggers (HOBO® U12 Temp/RH; Onset Computer, Bourne, MA, USA), one near the floor and one near the ceiling of each chamber. Chambers were sealed by applying tape along wall seams and around the door. To prevent beetle escape, liquid teflon (polytetrafluoroethylene 60; Sigma-Aldrich, St. Louis, MO, USA) was applied to the tape along the perimeter of the chamber and insect trap coating (Tangle-Trap; Tanglefoot, Grand Rapids, MI, USA) was applied to tape around the door.

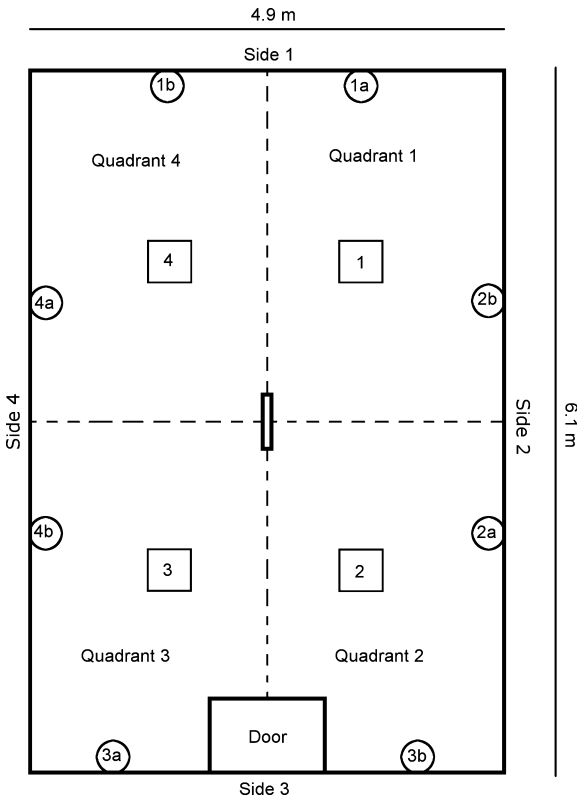

Figure 1 Experimental chamber details (not to scale). Circles represent the eight trap locations in each experimental chamber. The four squares represent the refugia in the chambers. The center rectangle is the location where *Tribolium castaneum* adults were released for each experimental replication. Dashed lines indicate the four quadrants established in each chamber.

To simulate shelter and food availability under real-world conditions, four cardboard refugia were placed within each chamber. Refugia were 22 × 22 cm pieces of 3.2-mm-thick corrugated cardboard containing 2.5 g of diet that were placed in the same locations in chambers (Figure 1) and were replaced for all experimental replicates.

Experimental design

A complete block design was used with eight blocks replicated over time. Each experiment consisted of eight treatments and each treatment was represented once per block. Within an experimental block, treatments proceeded sequentially, increasing trap number (from 1 to 8), or beetle density (from 20 to 800), as the block progressed. For the trap number experiment, 200 *T. castaneum* individuals were released per 24-h replication. For the beetle density experiment, trap number remained constant at one per chamber and beetle density was varied at 20, 40, 60, 100, 200, 400, or 800 individuals released per 24-h replication. For both experiments, trap(s) were randomly assigned to a location within the chamber (Figure 1) and each trap location was represented equally for each treatment.

Pheromone traps

Traps (Storgard® The Dome™ Trap; Trécé, Adair, OK, USA) were baited with pheromone and kairomone lures and consisted of a top and bottom made of molded plastic. The top was dome-shaped and could accommodate the addition of up to three pheromone lures. At the center of the bottom was a pitfall trap surrounded by a circular ramp allowing insects to climb the ramp and fall into the trap. Once captured in the trap, insects were unable to escape.

A rubber septum impregnated with *Tribolium* spp. aggregation pheromone (Trécé) was attached to the top of the trap. A 3.5-cm filter paper pad was placed in the bottom of the trap and saturated with 0.4 g of an oil-based kairomone food attractant (Storgard® Oil; Trécé). Pheromone lures for all experimental traps to be used for a block were opened on the day prior to initiation of the block. Two sets of traps were used for each experiment, allowing traps to be cleaned between blocks. After each 24-h experimental replication, the filter paper pad and excess food attractant were removed from the trap and fresh materials were added. Between blocks, each of which took ca. 2 weeks to complete, traps were thoroughly cleaned and new pheromone lures were added. Monitoring methods utilized in this study correspond to industry recommendations and are those most frequently employed to monitor infestations of crawling insects in food processing facilities (Trécé, 1999).

Insects

Adult *T. castaneum* individuals used in the experiments were from a laboratory colony collected from a food facility in KS in November 2001. The colony was maintained following the procedures outlined in Toews et al. (2005) and was kept in an environmental chamber at 28.9 ± 0.3 °C, 63.2 ± 2.1% r.h., and L16:D8. Adults had eclosed between 4 and 30 days prior to use in the experiments and individuals were used only once. Males and females each composed approximately 50% of the population (KA Buckman, pers. obs.).

Beetle release and recovery

Experimental individuals were added to square plastic Petri dishes (23 × 23 × 2 cm) containing one or more pieces of 3.2-mm-thick corrugated cardboard. The width of the cardboard was constant at 1 cm but the length and number of cardboard strips was adjusted so that beetle density was constant at 1 per 0.23 cm^2. For releases of 100

beetles or less, a single piece of varying length was used and for releases of 200, 400, 600, and 800 beetles, the required length of cardboard could not be accommodated by the plastic dishes so the number of pieces increased to 2, 4, 6, and 8 pieces of cardboard (each 23 cm long), respectively. Beetles readily entered the cardboard and settled inside the space between corrugations. Plastic dishes were moved to a reach-in environmental chamber at 21.8 ± 0.6 °C, 42.8 ± 7.2% r.h., and L16:D8 the day before experiment initiation. The following day, beetles were released into experimental chambers by placing the cardboard with the settled insects on the center of the floor. This release method was developed to attenuate any disruptive impacts of the release on beetle behavior.

Once released, beetles were allowed to disperse and encounter refugia and traps for 24 h. After 24 h, the release cardboard strips, refugia, and traps were removed from the chamber. The approximate location of the remaining beetles dispersing in the chamber, which had not entered refugia or traps, was noted. These individuals were collected with an insect vacuum (Bioquip Products, Rancho Dominguez, CA, USA) and returned to the laboratory. Beetles at each location, refugium, trap, release cardboard, or chamber floor, were counted. If all individuals were not initially recovered, the experimental chamber was checked again for any overlooked beetles. If all the beetles still had not been recovered, the number of missing individuals was recorded.

Estimating absolute *Tribolium castaneum* density

We applied Miller et al.'s (2010) trapping equation to pheromone trapping of *T. castaneum* to estimate the density of adult beetles. In the case of red flour beetles, both males and females respond to the aggregation pheromone and food-based kairomone used in traps, so the resulting estimate of beetle density encompasses both sexes. Trap retention time is assumed to be 1, as once an individual falls into the trap, it cannot escape. Trap capture data from the two experiments were used to estimate the trap findability × trap efficiency parameter, which describes the probability of an individual beetle locating, entering, and falling into a trap.

We adapted Miller et al.'s (2010) trapping equation to the red flour beetle pheromone and kairomone trapping system:

$$\text{Density}_{\text{RFB}} = \text{C}/(\text{T}_{\text{findability}} \times \text{T}_{\text{efficiency}}), \qquad (1)$$

where C is the number of individuals captured, $\text{T}_{\text{findability}}$ is the findability of the trap, $\text{T}_{\text{efficiency}}$ is the efficiency of the trap, and $\text{Density}_{\text{RFB}}$ is the density of red flour beetles. Thus, trap capture data for red flour beetle can be used to estimate density once the $\text{T}_{\text{findability}} \times \text{T}_{\text{efficiency}}$ parameter is estimated.

The $\text{T}_{\text{findability}} \times \text{T}_{\text{efficiency}}$ parameter can be determined from the slope of the regression of number of beetles trapped per experimental run on the beetle density (the beetle density experiment) based on Miller et al. (2010). The intercept of the regression line was assumed to be zero when performing regression because when no traps are present, no beetles can be captured. The product of the inverse of the estimated slope and number of beetles captured in traps yields an estimate of beetle density. Using this methodology and trap capture data from the trap number experiment, we calculated the predicted beetle density for each replication of the beetle density experiment:

$$\text{Density}_{\text{RFB}} = (1/\text{slope}) \times (\text{C}/\text{T}_{\text{density}}), \qquad (2)$$

where 'slope' is the estimated slope of the regression line, C is the cumulative capture of red flour beetles per experimental run, and $\text{T}_{\text{density}}$ is the number of traps per chamber. We then compared the predicted density to the actual density of 200 individuals to briefly explore the predictive value of this model. We compared the mean predicted beetle densities among trap densities with ANOVA (Proc GLM, SAS version 9.2; SAS Institute, Cary, NC, USA) to determine whether trap number affected the accuracy of model predictions. We also regressed the predicted beetle densities on trap number to determine whether there was a relationship between trap number and accuracy of model predictions (Proc REG). We used a ln-transformation to normalize predicted beetle densities to comply with the assumptions of the statistical models.

Statistical analysis

To determine whether the fate of released beetles varied among treatments for each experiment, number (trap number experiment) or proportion (beetle density experiment) of individuals found in each location within experimental chambers was compared with ANOVA (Proc GLM). Possible locations included for both experiments were as follows: traps, refugia combined, release cardboard strips (i.e., those not dispersing), and chamber floor (i.e., dispersing, but not settled in a refuge or trap). For the trap number experiment, individuals per trap was also included. When treatment was a significant factor, least-squares means (ls-means) were compared and a Tukey adjustment was applied to compensate for multiple comparisons. Square-root transformations were required for the total beetles in traps and beetles per trap (trap number experiment) and for the beetles per trap and total beetles in refugia (beetle density experiment).

Simple linear regression was utilized to determine the relationship between treatments, that is number of traps or number of individuals released, and the percentage of individuals dispersing from the release site (Proc REG).

To determine whether trap location within the chamber affected beetle capture, capture data from the trap number experiment were compared among treatments and chamber side along which the trap was located (Figure 1) using a Kruskal–Wallis test (Proc NPAR1WAY). For the beetle density experiment, data conformed to the assumptions of normality and trap capture among treatments and chamber side were compared with ANOVA (Proc GLM). The potential interaction between treatment and chamber side was also included in the model. For each significant factor in the model, the ls-means were separated using a Tukey–Kramer adjustment to compensate for multiple comparisons. Trap capture data from the beetle density experiment required a ln-transformation to conform to the assumptions of the model.

To establish whether beetle movement was random, each chamber was divided into four quadrants (Figure 1) and the number of insects found in each quadrant, either in a trap, refuge, or actively dispersing, was compared to a uniform distribution. Insects which did not disperse from the release site were not included. Contingency tables were calculated and significant differences were determined with χ^2 tests to determine whether beetle distribution significantly varied from a uniform distribution, in which 25% of dispersing beetles would be expected to be found in each chamber quadrant.

Pearson correlation coefficients were calculated for proportion of individuals dispersing and trap number and proportion of individuals dispersing and beetle density (Proc CORR) to determine whether beetle dispersal behavior might be related to trap number or population density.

Results

Varying trap number experiment

Trap number significantly impacted the number of individuals caught in traps, with increasing cumulative capture as the number of traps in the chamber increased ($F_{7,63} = 5.95$, $P<0.0001$; Figure 2A). With only a single trap present, significantly fewer beetles were captured than when three to eight traps were present. When two traps were present, significantly fewer beetles were captured than when seven or eight traps were present. However, trap number did not significantly impact the number of individuals caught per trap, which was similar across all trap densities ($F_{7,63} = 1.17$, $P = 0.34$; Figure 2A).

The impact of trap number on the total number of individuals entering refugia was significant ($F_{7,63} = 3.18$, $P = 0.0075$) with significantly more individuals entering refugia when two traps were present compared to when eight traps were present. Although other comparisons were not significant, there was a trend of fewer individuals being found in refugia as trap number increased (Table 1). Trap number did not have a significant impact on the number of individuals remaining at the release site ($F_{7,63} = 1.86$, $P = 0.097$) or the number of individuals actively dispersing (i.e., those individuals which had left the release site, but not settled in a trap or refuge) at the conclusion of 24-h experimental replications ($F_{7,63} = 2.09$, $P = 0.062$).

The proportion of individuals which dispersed from the release site (i.e., any individual found in traps, refugia, or actively moving in the chamber) was similar across trap densities ($F_{1,63} = 1.42$, $P = 0.24$; Figure 3A). Even though

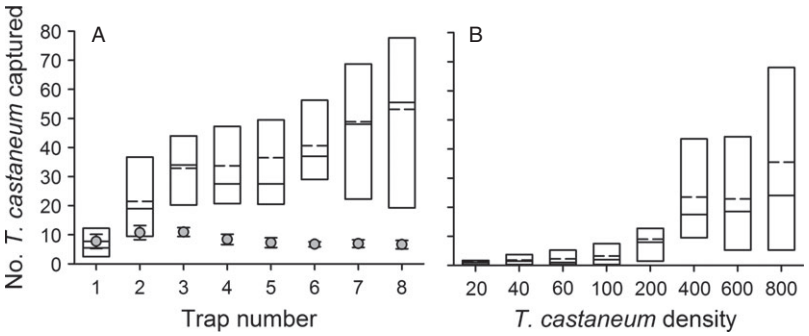

Figure 2 Mean (± SE) number of *Tribolium castaneum* captured in traps (A) as pheromone trap number increased, and (B) as *T. castaneum* density increased. Boxes show the distribution of the central 50% of total trap captures, with the central solid and broken lines indicating the median and mean number of individuals captured in traps, respectively. Gray circles indicate the mean number of individuals per trap.

Table 1 Mean (± SE) number of *Tribolium castaneum* beetles per location within chambers [i.e., in traps, refugia, or release sites, or observed to be actively dispersing in quadrants 1–4 (Q1–Q4) of the chambers; see also Figure 1]

					Actively dispersing			
Experiment	Treatment	Trap(s)	Refugia	Release site	Q1	Q2	Q3	Q4
Varying trap number	1	8 ± 2	48 ± 5	97 ± 7	15 ± 2	12 ± 3	7 ± 2	12 ± 2
	2	22 ± 5	52 ± 4	67 ± 9	14 ± 3	15 ± 2	10 ± 2	17 ± 2
	3	33 ± 6	39 ± 4	83 ± 8	16 ± 3	10 ± 2	6 ± 1	12 ± 2
	4	34 ± 7	46 ± 5	69 ± 8	16 ± 3	12 ± 2	8 ± 2	14 ± 3
	5	37 ± 8	35 ± 3	85 ± 8	13 ± 2	11 ± 2	7 ± 2	11 ± 3
	6	41 ± 5	36 ± 3	75 ± 8	17 ± 3	8 ± 1	6 ± 2	13 ± 2
	7	49 ± 9	36 ± 4	76 ± 6	12 ± 2	9 ± 2	4 ± 1	10 ± 2
	8	53 ± 12	34 ± 3	73 ± 9	12 ± 3	8 ± 2	7 ± 1	11 ± 2
Varying beetle density	20	2 ± 1	6 ± 1	6 ± 1	2 ± 1	2 ± 0	1 ± 0	2 ± 1
	40	2 ± 1	11 ± 2	12 ± 2	5 ± 1	3 ± 1	2 ± 1	4 ± 0
	60	2 ± 1	15 ± 1	18 ± 3	9 ± 1	5 ± 1	3 ± 1	5 ± 1
	100	3 ± 1	25 ± 3	40 ± 4	9 ± 1	8 ± 1	5 ± 2	8 ± 1
	200	9 ± 3	40 ± 4	96 ± 9	16 ± 2	13 ± 3	8 ± 2	12 ± 2
	400	24 ± 6	105 ± 11	158 ± 16	34 ± 3	26 ± 4	19 ± 3	24 ± 2
	600	23 ± 7	125 ± 10	318 ± 23	39 ± 4	30 ± 5	23 ± 2	31 ± 3
	800	36 ± 13	181 ± 14	412 ± 29	49 ± 4	40 ± 8	30 ± 4	38 ± 4

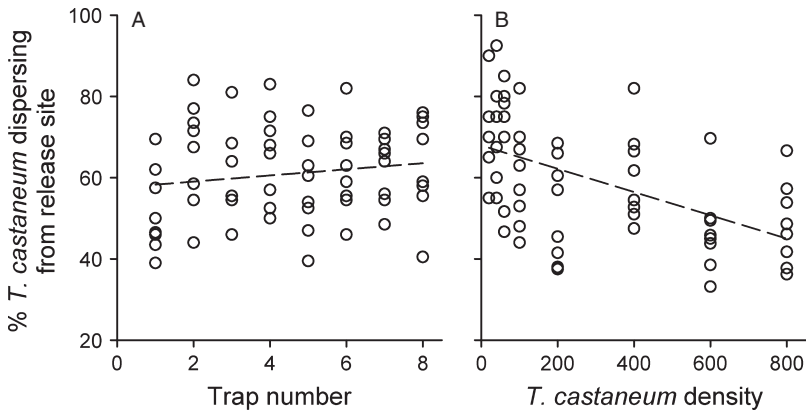

Figure 3 The effect of (A) trap number and (B) beetle density on the percentage of *Tribolium castaneum* dispersing from the release site. Dashed lines are the linear regression lines. Only *T. castaneum* density was significantly related to the % individuals dispersing from the release site.

there appears to be a slight positive relationship between trap number and proportion of individuals dispersing, the slope estimate (0.75 ± 0.63) was not significantly different from zero (t = 1.19, d.f. = 1, P = 0.24). Thus, it is unlikely that increasing trap number attracted beetles from the release site.

Trap capture was not consistent among trap locations during the trap number experiment (χ^2 = 167.9, d.f. = 3, P<0.0001). The most individuals (16 ± 1 beetles per trap) were captured on side 2, fewer were captured on side 4 (10 ± 1), even fewer on side 1 (3 ± 0) and the fewest (1 ± 0) on side 3.

Varying *Tribolium castaneum* density experiment

Beetle density did significantly impact the number of beetles entering traps ($F_{7,63}$ = 18.89, P<0.0001; Figure 2B). Significantly fewer beetles were captured when beetle density was 20 compared to densities of 200-800. At densities of 40, 60, or 100 beetles, significantly fewer individuals were captured than at densities of 400, 600, and 800. At a density of 200 beetles, significantly fewer were captured compared to the highest beetle density of 800 (Figure 2B). However, red flour beetle density did not have a significant impact on the proportion of individuals caught in traps ($F_{7,63}$ = 0.51, P = 0.83). Similarly, the proportion of

individuals entering refugia was consistent across all beetle densities ($F_{7,63} = 1.34$, $P = 0.25$; Table 1).

Beetle density significantly impacted the proportion of individuals remaining at the release site ($F_{7,63} = 8.24$, $P<0.0001$), even though the area of the release site per beetle was held constant. When 20, 40, or 60 individuals were present, a significantly smaller proportion remained at the release site compared to when 200, 600, or 800 individuals were present (Table 1). At the beetle density of 400, the proportion of beetles remaining at the release site was similar to all other treatments. Similarly, increasing beetle density led to a significantly smaller proportion of individuals actively dispersing in the chamber ($F_{7,63} = 6.01$, $P<0.0001$). A significantly smaller proportion of beetles was actively dispersing at densities of 600 or 800 when compared to densities of 40 or 60 (Table 1).

Unlike in the trap number experiment, there was a significant relationship between beetle density and the percentage of individuals found outside of the release site ($F_{1,63} = 25.62$, $P<0.0001$). The regression line had a slope estimate which was significantly smaller than zero, -0.03 ± 0.01 ($t = -5.06$, d.f. = 1, $P<0.0001$), implying that as red flour beetle density increased, the percentage of individuals remaining at the release site decreased (Figure 3B).

Consistent with the trap number experiment, trap locations had significantly different trap captures during the beetle density experiment ($F_{3,63} = 16.79$, $P<0.0001$). Significantly more beetles were captured in traps located on side 2 (21 ± 7 beetles per trap), than on sides 1 (6 ± 2) or 3 (3 ± 1). Side 4 (20 ± 5 beetles per trap) also had traps which captured significantly more individuals than traps on sides 1 and 3.

Trap findability × trap efficiency parameter estimate

There was a significant, positive linear relationship between beetle density and the mean number of individuals captured in traps ($F_{1,63} = 80.58$, $P<0.0001$; $r^2 = 0.977$). The slope estimate of the regression line was 0.0443 ± 0.0024. Using the slope as an estimate of $T_{findability} \times T_{efficiency}$, the resulting formula is as follows:

$$\text{Density}_{RFB} = 22.57 \times (C/T_{density}). \quad (3)$$

We used this formula to estimate beetle density using independent data from the trap number experiment. We found that the beetle density estimates and trap number exhibited a negative trend (Figure 4). Predicted beetle densities did not significantly vary among trap densities ($F_{1,63} = 0.95$, $P = 0.48$; Figure 4). These results do not signify that the model lacks predictive value as we also observed that the 95% confidence intervals of predicted beetle densities for all trap densities contain the actual beetle density of 200, except when six traps were present (95% CI: 9–199), which was close to containing the actual beetle density.

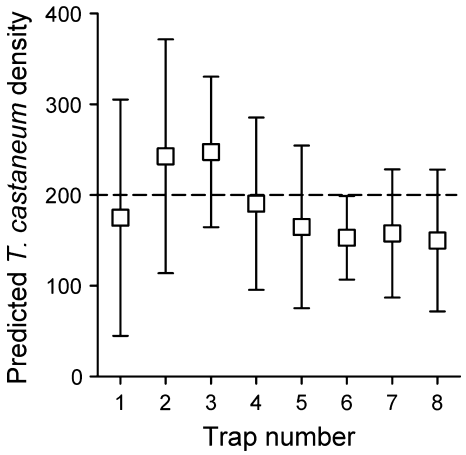

Figure 4 Mean predicted *Tribolium castaneum* densities (± 95% confidence interval) calculated from equation 3 (see text). The dashed line indicates the actual beetle density.

Tribolium castaneum distribution in chamber

Red flour beetles did not disperse randomly in either experiment or in either chamber. During the trap number experiment, individuals found in refugia, traps, and actively dispersing within the chamber (n = 7 629) were 36 and 15% more likely to be located in quadrant 1 or 4, respectively, at the conclusion of a 24-h replication than would be expected if beetles were moving randomly ($\chi^2 = 589.73$, d.f. = 3, $P<0.0001$). Individuals were 12% less likely to be located in quadrant 2 and 38% less likely to be located in quadrant 3 than would be expected if individuals were randomly dispersing. During the beetle density experiment, a similar trend was observed with individuals (n = 8 901) being 46 and 13% more likely to be located in quadrants 1 and 4, respectively, and 19 and 40% less likely to be located in quadrants 2 and 3, respectively, than would be expected by random movement ($\chi^2 = 929.96$, d.f. = 3, $P<0.0001$).

Discussion

Regression analysis indicated that the $T_{findability} \times T_{efficiency}$ parameter for pheromone trapping of the red flour beetle was 0.0443, implying that about one out of 23 beetles successfully sensed, located, and entered pheromone traps. This parameter estimate was lower than estimated for male *C. pomonella*, which was 0.5, indicating that approximately one out of two male moths completed

the steps of the pheromone trapping/mating disruption model (Miller et al., 2010). This disparity between the attractiveness of a moth sex pheromone and beetle aggregation pheromone was expected as the *Tribolium* spp. aggregation pheromone is not attractive at long distances (Obeng-Ofori & Coaker, 1990; Hawkin et al., 2011; Campbell, 2012). However, we were able to demonstrate a significant relationship between beetle density and beetle capture in traps (Figure 2B).

When we used the $T_{findability} \times T_{efficiency}$ estimate to calculate the 'expected' number of beetle adults present in the chamber during the trap number experiment, captures per trap at trap densities of 1, 5, 6, 7, and 8 underestimated the number of beetles present by an average of 13–26%, while trap densities of 2 and 3 overestimated the number of beetles present by an average of 21 and 24%, respectively (Figure 4). At a trap density of 4, beetle density was underestimated by less than 5%, on average, suggesting that this trap density, 0.13 traps m^{-2} (four traps per chamber), might yield the most accurate density estimates using the trapping equation (3) (Figure 4).

Trap density that appeared to best balance trap efficacy, that is number of beetles captured per trap and trapping effort, was 0.07–0.10 traps m^{-2} (2–3 traps per chamber). This density appears to provide useful information about red flour beetle populations while minimizing costs of monitoring. The information gained by increasing traps to >0.10 m^{-2} may not compensate for the increased cost of such a dense trapping scheme. The trap density supported by our data, 0.07–0.10 m^{-2}, is 7–10 times higher than the general guidelines for establishing *Tribolium* spp. monitoring programs in food processing and storage facilities (Trécé, 1999). However, trap densities of 0.01–0.06 m^{-2} have been successfully used in long-term monitoring studies at operating USA flour mills (Campbell & Arbogast, 2004; Toews et al., 2006; Campbell et al., 2010a,b), suggesting that increasing trap density may be feasible in some facilities.

We did not expect to find such a large variation in trap capture among the locations within the chamber (Table 1). However, such spatial variation in highly controlled environments has also been observed with *Plodia interpunctella* (Hübner) males (Nansen et al., 2006). We hypothesize that spatial variation in air movement within chambers may have altered the size and shape of pheromone plumes, thereby affecting beetle capture in traps. Campbell (2012) showed that under still air conditions there was essentially no active space around traps, whereas with airflow beetles responded out to 90 cm. Although air speed measurements inside chambers indicated only minimal air movement along both short and long walls (0.06–0.49 and 0.10–0.64 m s^{-1}, respectively; KA Buckman, unpubl.), these small differences could have contributed to the observed variation. Beetle behavior may also have contributed to the increased captures on long walls as more beetles would be expected to encounter long walls than short walls, and once moving along the long walls, beetles would essentially be funneled into those traps.

Another unexpected observation was the significant, negative relationship between beetle density and dispersal from the release site (Figure 3). Beetle density in the cardboard used to release individuals was constant for all beetle densities (1 beetle per 0.23 cm^2); thus, overcrowding within the release area was likely not the cause. Furthermore, males would not have produced aggregation pheromone at the release point because no food was present. Thus, it is not clear why the reduction in dispersal was observed. Further study of this phenomenon is needed to determine whether this is a real relationship or simply an artifact of the release method.

These results indicate that a higher trap density than would be usually utilized in the field is needed to most accurately estimate beetle densities. However, the highest trap densities tested (5–8 per chamber) did not increase the accuracy of beetle density estimates. Thus, a reasonable starting point for further evaluation might be to determine if similar experiments on a larger, more realistic spatial scale also indicate that high trap density is needed to obtain efficacious population estimates. Careful analysis of environmental differences among the independent data sets, coupled with an assessment of the validity of model predictions may highlight the factors providing the greatest contribution to changes in red flour beetle trap captures. Finally, given the multitude of potential sources of sampling error in pheromone trapping programs, an important next step is to determine how large a margin of error in population estimates can be tolerated while maintaining the utility of such estimates.

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