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Attraction of walking *Tribolium castaneum* adults to traps

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ABSTRACT

The red flour beetle, *Tribolium castaneum* (Herbst), is a major pest of food processing facilities and can be monitored using pitfall type traps. To determine how beetles interact with these traps under field situations, the behavior of individual beetles released in the vicinity of traps was observed in a large arena. Specifically, the response of adults to traps baited with combinations of commercially available pheromone and kairomone attractants was measured, as was the influence of beetle sex and strain, airflow presence or absence, and distance from trap. The beetle's response to traps was strongest (e.g., more encountered trap, more remained in observation zone, more time was spent on treatment side, and decreased speed and increased turn angle) to pheromone/kairomone or pheromone baited traps when there was air movement, while kairomone alone and all attractants under still air conditions generated no significant response by the beetles. Even with the best combination of attractants and with airflow, average number encountering trap was only 40%. With airflow, beetles were successful at locating a pheromone/kairomone baited trap out to 90 cm, the maximum distance tested, but under still air conditions even at 10 cm there was no difference between traps with and without attractants. Since airflow at trap locations within commercial food facilities can vary considerably, these patterns of response to traps could significantly impact insect detection.

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1. Introduction

Tribolium castaneum (Herbst), the red flour beetle, is a major pest of food processing facilities, such as mills, processing plants, warehouses and retail stores (Campbell et al., 2010). A variety of traps have been developed to capture walking stored-product pests and they are used to monitor *T. castaneum* inside food facilities (Burkholder, 1990; Chambers, 1990; Phillips, 1997; Phillips et al., 2000). These traps are typically placed on the floor inside facilities, but outside of cryptic resource patches where pest populations develop, hence they target dispersing individuals moving between resource patches. Commercially available traps for walking *T. castaneum* are typically baited with aggregation pheromone and food based kairomone lures and are based on a pitfall type of design (Mullen, 1992). Although, as discussed below, there has been extensive research on stored-product beetle response to each of these types of attractants, little information is available on how *T. castaneum* respond to commercially available traps and attractants under conditions similar to those found inside commercial food facilities. Anecdotal reports from users in the food and pest management industry suggest that response to these commercial traps by *T. castaneum* is limited. As a result, a better understanding of how beetles interact with baited traps is needed so that their use as a monitoring tool can be evaluated and improved.

Tribolium castaneum has an aggregation pheromone produced by feeding males that is attractive to both sexes and which has been identified as 4,8-dimethyldecanal (Suzuki, 1980; Suzuki et al., 1984). The pheromone occurs in four different forms, with males releasing all four at a 4:4:1:1 [(4*R*,8*R*):(4*R*,8*S*):(4*S*,8*R*):(4*S*,8*S*)] ratio, although commercial pheromone lures use a mixture of synthetic (4*R*,8*R*)- and (4*R*,8*S*)-isomers at a 1:1 ratio (Lu et al., 2011). A large number of studies have evaluated how *T. castaneum*, and the related species *Tribolium confusum* DuVal which shares the same pheromone (Suzuki and Sugawara, 1979), respond to this pheromone and how different factors influence the strength of response (Barak and Burkholder, 1985; Boake and Wade, 1984; Duehl et al., 2011a; Levinson and Mori, 1983; Lewis and Austad, 1994; O'Ceallachain and Ryan, 1977; Obeng-Ofori, 1991; Obeng-Ofori and Coaker, 1990b; Olsson et al., 2006; Ryan and O'Ceallachain, 1976; Sokoloff, 1974; Verheggen et al., 2007). Differences between sexes in pheromone response strength appear to vary with the experiment and/or species, with females responding more strongly (Levinson and Mori, 1983; Olsson et al., 2006), equally (Duehl et al., 2011a; Verheggen et al., 2007), or less strongly (Obeng-Ofori and

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Coaker, 1990a) than males. Strains have also been reported to differ in their response to pheromone concentration (Boake and Wade, 1984). Most of these studies have not measured long range attraction, although some experiments using windtunnel or olfactometer type designs have shown that beetles do move upwind to pheromone (Lu et al., 2011; Obeng-Ofori, 1991; Obeng-Ofori and Coaker, 1990b; Olsson et al., 2006).

Volatile chemical cues from foods can be important attractants, and a variety of materials have been evaluated as kairomones for stored-product insects (Barak and Burkholder, 1985; Hodges et al., 1985; Mahroof and Phillips, 2007; McFarlane and Warui, 1973; Nansen and Phillips, 2003; Obeng-Ofori, 1993; Pierce et al., 1990; Pinniger, 1975; Subramanyam et al., 1992). The granary weevil (*Sitophilus granarius* (L.)) is arguably the most extensively studied stored-product pest species in terms of its response to kairomones, with its response to whole or crushed seeds (Levinson and Kanaujia, 1981; Rietdorf and Steidle, 2002), crude extracts (Collins et al., 2007, 2004; Levinson and Kanaujia, 1982; Wakefield et al., 2005), as well as to specific compounds present in these foods (Collins et al., 2007, 2008; Germinara et al., 2008), evaluated and a trap using food based attractants developed (Collins and Chambers, 2003). Combining food odors with pheromone can increase *Sitophilus* spp. captures in pitfall bioassays or traps (Likhayo and Hodges, 2000; Phillips et al., 1993; Trematerra and Girgenti, 1989; Wakefield et al., 2005; Walgenbach et al., 1987), but increased response to combined attractants has sometimes been less or nonexistent in field monitoring situations (Hodges et al., 1998; Likhayo and Hodges, 2000).

Evaluation of *T. castaneum* attraction of food volatiles has been more limited, although most commercial monitoring uses a combination of pheromone and kairomone. Response of *T. castaneum* or *T. confusum* to food odors has been demonstrated, although it is typically not possible to determine if beetles are actually attracted or arrested by the odors (Phillips et al., 1993; Willis and Roth, 1950). *Tribolium* spp. attraction to flour appears to be negligible (Hughes, 1982; Romero et al., 2010). However, beetles are attracted to conditioned flour (i.e., flour that beetles have inhabited) if males are present and presumably producing pheromone (Hughes, 1982), but highly conditioned flour can become repellent to *T. castaneum* (Duehl et al., 2011b). Recent analysis of the genome sequence indicates that *T. castaneum* has a relatively large number of odorant receptor genes compared to other sequenced species, although how many of these genes have current functional value remains to be determined (Engsontia et al., 2008). Identification of specific compounds that are attractive to *Tribolium* spp. has not been published, and commercial lures appear based on crude food extracts or oils. Although the response to pheromones by *T. castaneum* can be increased by the addition of food odors (Phillips et al., 1993), how much this combination benefits pheromone trapping programs has not been reported.

A wide range of experimental techniques have been used to evaluate the response of walking stored-product insect species to different cues under choice and no choice conditions. These procedures generally fall into one of four categories, although there is considerable variation in experimental design within each category: (1) electroantennography (EAG) experiments where stimulation of antenna is used to determine potential attractants (Arnaud et al., 2002; Collins et al., 2007; Levinson and Mori, 1983; Verheggen et al., 2007); (2) behavioral bioassays in small spatial scale arenas with capture of insects used as response variable (e.g., pitfall bioassays) (Barak and Burkholder, 1985; Blotch Qazi et al., 1998; Boake and Wade, 1984; O'Ceallachain and Ryan, 1977; Ryan and O'Ceallachain, 1976; Trematerra et al., 2000); (3) behavioral bioassays in small spatial scale arenas without capture, but with insect position determined at a set time (Blotch Qazi et al., 1998; Ryan and O'Ceallachain, 1976; Suzuki and Sugawara, 1979; Willis and Roth, 1950) or at regular time intervals (Arthur et al., 2011; Blotch Qazi et al., 1998; Duehl et al., 2011a; Levinson and Mori, 1983; Seifelnasr et al., 1982); and (4) windtunnel/olfactometer bioassays where insect movement upwind to source is evaluated (Barak and Burkholder, 1985; Lu et al., 2011; Obeng-Ofori, 1991; Obeng-Ofori and Coaker, 1990b; Olsson et al., 2006; Romero et al., 2010; Verheggen et al., 2007).

Each of these experimental approaches has advantages as a tool to identify behaviorally active materials, but all have limitations in terms of extrapolating from them to how insects will interact with traps baited with these attractants in food facilities. Strength of response to a potential attractant can be inflated by confining beetles in small arenas, since this can lead to multiple encounters with sources and increase the likelihood of a positive response. Experiments done with groups of insects can also lead to interactions among individuals that can confound the detection of response to attractant (Trematerra et al., 1996). Use of either still air or moving air in experiments can generate different levels of response, but typically bioassays are conducted using only one or the other. More real world conditions can be generated by increasing the size of the arena and measuring insect captures (Arbogast et al., 2003, 2005; Mullen, 1992; Stejskal, 1995; Toews et al., 2005), but these experiments are time consuming, replication is difficult, and they can still generate conditions where insects interact multiple times with a trap. Commercial food facilities are even more difficult to use for trap comparisons given the spatial and temporal variation of population abundance and distribution (Arbogast et al., 2000; Campbell et al., 2002).

Detailed studies of beetle behavior around traps in a large arena could provide a more accurate picture of the effectiveness of different traps and attractants under food facility conditions. The protocol used here simulates a scenario where a trap is placed along a wall within a food facility, a typical location used in monitoring programs, and a beetle dispersing from a hidden refugia has an opportunity to either respond to the trap or leave the area. This is potentially a more stringent and realistic test of the strength of a response than can be obtained in behaviorally constraining containers. Because individual beetles are evaluated, confounding factors due to interaction among individuals are limited. Additionally, because the movement pattern of the beetle is observed, the role of attraction versus random encounter and arrestment at the trap can be evaluated. In addition, this approach enables the influence of different environmental factors on attraction to be tested. Here, the response of *T. castaneum* adults to Dome traps (Trece, Adair, OK) baited with commercially available attractants was measured, and the influence of beetle sex and strain, attractants in trap, airflow presence or absence, and beetle release distance from trap on the strength of response were assessed.

2. Materials and methods

2.1. Experimental arena

All experiments were conducted in a 5 by 5 m room inside a warehouse building (12 × 12 m, 4–6 m height) with heating and lighting, but no cooling capability. The room consisted of plywood floor and ~0.5 m tall plywood walls which were covered with linoleum flooring. The top and the sides of the chamber above the plywood wall were enclosed with plastic sheeting on a wooden framework (2.4 m height) and along one wall was a door. Near the ceiling on one wall were three exhaust fans that were run between trials to exchange the air in the chamber, but not during observations. An underfloor heating system was used to control the temperature of the room. Linoleum on the floor of

the chamber was roughened using sandpaper to facilitate traction by the beetles and grids of 5 by 5 cm squares were made using a permanent marker to facilitate the placement of traps, release of beetles, and the tracking of beetle movement as described below. Grids were 100 cm long and 50 cm wide, except when effect of release distance was tested, and placed with the long edge of the grid along each of two room walls.

2.2. Experimental protocol

Tribolium castaneum adults 2–4 weeks post-eclosion were selected from colony jars containing wheat flour and held at 25 °C, 65% rh, and 14:10 light:dark cycle. The sex of each individual was determined based on male leg characteristics (Good, 1936) and beetles were held individually in 30 ml clear plastic cups (Jet Plastica Industries, Hatfield, PA) without food overnight on the floor inside the experimental room prior to being tested. Beetles were released individually in all experiments, with at least one individual beetle tested from all the treatment combinations in an experiment on a given day in order to block the replications over time. Beetles were released by inverting a cup over the centerline of the long axis of the observation zone (within a four grid cell square) and adjacent to the wall (Fig. 1). Cup was moved back and forth within the release zone until beetle was observed to be upright and walking and then the cup was left in place for 30 s before lifting it to begin the experiment. In all experiments a Dome trap with different combinations of supplied attractants (kairomone oil and pheromone lure for *T. castaneum*) (Trece Inc, Adair, OK) was used to evaluate insect response. Attractants were added to traps the day before the trial was to be conducted with separate traps for each combination of attractants, but the same pheromone lure and kairomone oil used in multiple trials over a one week period and then replaced. A trap was placed within a square created by four 5 × 5 cm grids, with the midline of the trap 25 cm from the center of the release zone, except when effect of trap distance was tested, and the edge of the trap flush with the vertical wall (Fig. 1). Traps were held outside of the arena until ready to be used in an experiment and left in place 5 min before starting a trial.

To evaluate the influence of airflow on beetle response to traps, two fans were aligned along the wall and placed 1 m from the centerline of the observation zone where beetles were released. The fans were custom made for this experiment and consisted of a centrifugal fan within a metal housing with an output opening 12.3 cm wide by 5 cm tall. The two fans were aligned side-by-side and adjacent to the wall, with the output openings flush with the floor and facing so that air was blown across the trap toward the beetle release point. To control for any potential visual response by the beetles to the fans, they were left in place, but not turned on, for treatments without airflow. To evaluate air velocity across the observation area, a thermal anemometer (model 414, Testo AG, Lenzkirch, Germany) was used and air speed was measured at 1.5 cm above the floor surface at the center of each cell within the observation area, with sensor facing the direction of the fan and perpendicular to the wall. Airflow varied across the observation zone (Fig. 1) with an overall average and standard error of the mean (sem) of 0.82 ± 0.04 m/s. The average airflow in the observation zone adjacent to the wall and directly downwind from the fans, which including the trap and release point, was 1.15 ± 0.02 m/s. Airflow was 1.04 ± 0.05 m/s in the four grid cells forming the release zone. When fans were not turned on, the average airflow in the observation area was 0.03 ± 0.00 m/s.

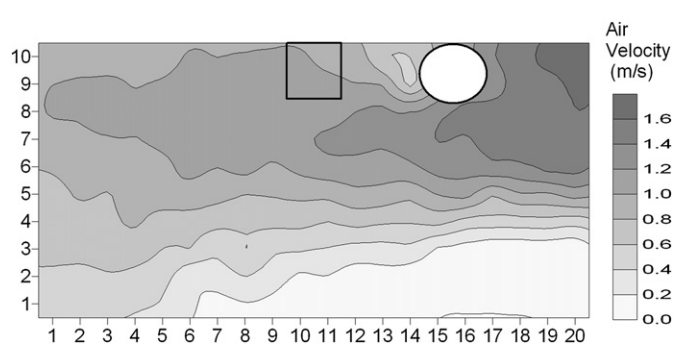

Fig. 1. Observation zone used for examining *T. castaneum* behavior, with *x* and *y* axis ticks indicating the position of 5 by 5 cm squares, the unfilled square indicating the release zone for the beetles (encompassing four 5 by 5 cm squares), the circle indicating trap position, and gray scale contours representing air speed (m/s) measurements at the center of each 5 by 5 cm square (no airflow treatment without fans running, had air velocity readings insufficient to create contour map).

After lifting the cup, beetles were observed for 5 min or until they left the observation zone or were captured within the trap. During the observation period the beetle's position was recorded every 5 s on a datasheet that contained a smaller scale grid that matched the grid pattern on the floor of the arena. Timing of observations was maintained using a metronome that emitted a sound at the appropriate time interval. To control for position effects on beetle behavior, the side of the chamber on which the trial was performed and the side of the observation zone in which the trap was placed were alternated. However, all the replicates were realigned to the same orientation for presentation and analysis. It was assumed that beetles leaving the observation zone would be unlikely to return, this assumption was evaluated using data from Experiment 1.

Before the first replicate in each block of the experiment, environmental conditions were measured at six locations within the observation zone: at each end and at the center of the long axis next to the vertical wall edge and at the opposite side of the observation zone. Air temperature and relative humidity were measured 1 cm and 4 cm above the floor surface, respectively, using a handheld weather meter (Kestrel 3000, Nielsen-Kellerman, Boothwyn, PA USA). Surface temperature was measured using an Atkins Series 396K thermometer (Atkins Technical, Gainesville, FL USA). The six measurements were averaged for each block, and during these experiments the mean (±sem) for surface temperature, air temperature, and relative humidity were 25.2 ± 0.2 °C ($n = 94$), 23.6 ± 0.2 °C ($n = 94$), and 28 ± 2% ($n = 93$), respectively.

2.3. Statistical analysis

The following metrics were determined for each replicate in each of the experiments described below: proportion of individuals that encountered the trap, proportion that left the observation zone, and proportion that remained in the observation zone until the observation was terminated; time to encounter trap, for those individuals that visited the trap; and proportion of observations points in the half of the observation zone containing the trap (from the midpoint of the release zone to the edge of the observation zone past the trap). Trap encounters included beetles that contacted the trap but did not remain at the trap and were either timed out or left the observation zone; beetles that encountered the trap and were still on the trap at the end of the observation period; and beetles that encountered trap and were captured in trap prior to end of observation period. For movement path analysis, a raster-based dataset was created by assigning each grid cell an *x* and *y* coordinate and recording the cell in which the beetle was located at each successive time point. From this data, the following path metrics were calculated and reported: average step length, which indicates rate of movement (measured as number of cells visited during a time interval); average turn angle, which indicates how

linearly beetles moved (measured from the center point of the cells visited); and fractal dimension, which is an overall measure of path tortuosity. These various metrics provide slightly different perspectives on the beetle response that can provide insight into the strength and mechanism of the response.

Dichotomous data for individual beetles such as encounter with trap or leaving observation zone was analyzed with the PROC GLIMMIX procedure in SAS v. 9.3 software (SAS Institute, 2011). Presence of treatments with zero responses causes errors with this analysis, so data was transformed by adding a positive response to one individual in each combination of treatments that had not previously responded positively (untransformed data is presented). Multiple comparisons were evaluated with Tukey–Kramer method using the Least Squares Means. Proportion of time on the positive side of observation zone and path metrics were analyzed using General Linear Models (GLM) procedure in SAS software. Proportion of time data distribution was normalized prior to analysis using an arcsine square root transformation. All comparisons were evaluated using an alpha value of 0.05. Untransformed data is presented in the text and figures as mean ± standard error of the mean (sem).

2.4. Experiment 1: influence of strain, sex, and presence or absence of air movement on response to Dome traps with different combinations of attractants

Two *T. castaneum* strains both of Kansas, USA origin were tested: one a laboratory strain which has been maintained in culture at the USDA laboratory since 1958, and the other a field strain collected from a flour mill within one year of the start of the experiments. Males and females were collected for each strain using methods described above. Experiments were conducted with and without fans running, as described above, to created airflow and no airflow treatment groups. The attractant combinations tested were those supplied with the Dome trap: the food oil kairomone and the pheromone lure. Dome traps were tested with no attractants added, kairomone oil only (~10 drops onto supplied piece of filter paper), pheromone lure only, and kairomone oil and pheromone lure combination. An additional control treatment consisted of no trap placed in the observation zone. To facilitate observation of the beetles on the traps, the lids of the Dome trap were not used in this experiment. The pheromone lure was mounted on a folded piece of wire placed in the bottom of the trap so that the lure was suspended above the trap in the same position that it would have been if mounted in the lid.

Treatments were blocked in the following manner due to limitations on the number of replicates that could be performed within a day, time needed to perform the observations, and temperature conditions in the chamber: beetle sex, airflow, and attractant type were all nested within beetle strain and blocked over time, with one replicate of all treatment combinations within a strain performed on a given day. The order of treatment combinations within a day was randomized. A minimum of 12 replications were performed for each treatment combination.

2.5. Experiment 2: influence of release distance on response to Dome traps

Experimental protocol was the same as with the other experiments, except that the beetles were released in zones whose centerlines were 10, 30, 50, 70 or 90 cm from centerline of the trap location. This resulted in a change in the size of the observation zone – which in this experiment extended the distance between the release zone and the trap position, plus an additional five grid cells (total of 25 cm) beyond on each end. The width of the observation zone was the same in all the treatments. In addition, in this experiment a normal nontransparent Dome lid was used on the traps, so observations of whether beetle was captured could only be made at the end of the observation. The factors tested in this experiment were trap distance, presence or absence of airflow and presence or absence of pheromone/kairomone attractant. The experiment was performed using the laboratory *T. castaneum* strain, with 10 replicates of males and 10 replicates of females (sexes combined for analysis to give an $n = 20$). The experiment was blocked by distance, with all different sex, attractant and airflow treatment replications conducted within a day. The order of the different distances was randomized and the orientation of the fans, trap and release point was alternated with equal numbers of replications in both directions.

3. Results

3.1. Experiment 1

For illustration, individual movement paths for the *T. castaneum* laboratory strain males under the different airflow and trap/attractant combinations are shown in Fig. 2, individual releases and different orientations are aligned in a single diagram for each treatment combination. Visual observation of the pathways for both strains indicated an increase in positive movement upwind when airflow was present compared to no airflow treatments, this movement was increased when traps were baited with pheromone alone or in combination with kairomone.

Initial analysis using a glimmix model for binomial data indicated that for trap encounters strain ($F = 1.55$, d.f. = 1, 454, $P = 0.2130$) and sex ($F = 1.54$, d.f. = 1, 454, $P = 0.2147$) were not significant, airflow ($F = 3.82$, d.f. = 1, 454, $P = 0.0513$) was marginally not significant, and none of the interactions were significant ($P > 0.05$), but trap/attractant type was highly significant ($F = 8.32$, d.f. = 1, 454, $P < 0.0001$). These differences resulted from beetle encounters with trap being more frequent when trap had pheromone or pheromone/kairomone, while encounters with traps with kairomone, empty traps and no trap controls were lower and not significantly different from each other, except that kairomone baited traps were also not different from the pheromone/kairomone baited traps. Since strain and sex were not significant factors in subsequent analyses they were combined. For glimmix analysis of the combined data, airflow was not significant ($F = 3.40$, d.f. = 1, 484, $P = 0.0657$), trap/attractant was significant ($F = 9.35$, d.f. = 4, 484, $P < 0.0001$), and the interaction was not significant ($F = 1.19$, d.f. = 4, 484, $P = 0.3145$). These differences were due to more beetles encountering traps with pheromone or pheromone/kairomone than those with kairomone alone, empty traps, or when no trap was present.

Although airflow was not a significant factor in the overall models, observation of data suggests that this might be due to the wide variation in level of response among different tested factors (Fig. 3A–F). Therefore, further analysis was performed sorting the data into the airflow treatments. Analyzing just the no airflow data, there was a significant effect of trap/attractant on beetle encounters with the traps ($F = 3.42$, d.f. = 4, 234, $P = 0.0097$), but the only pairwise difference was between the pheromone baited trap and the no trap control (Fig. 3G). With airflow present, there were also a significant effect of trap/attractant ($F = 6.99$, d.f. = 4, 220, $P < 0.0001$), with the pheromone/kairomone baited trap having more encounters than all other treatments, except the pheromone only treatment (Fig. 3H).

Two additional measures related to encounters with traps were also evaluated since they can provide additional insight into the strength of the response. First, beetles that remained on trap until

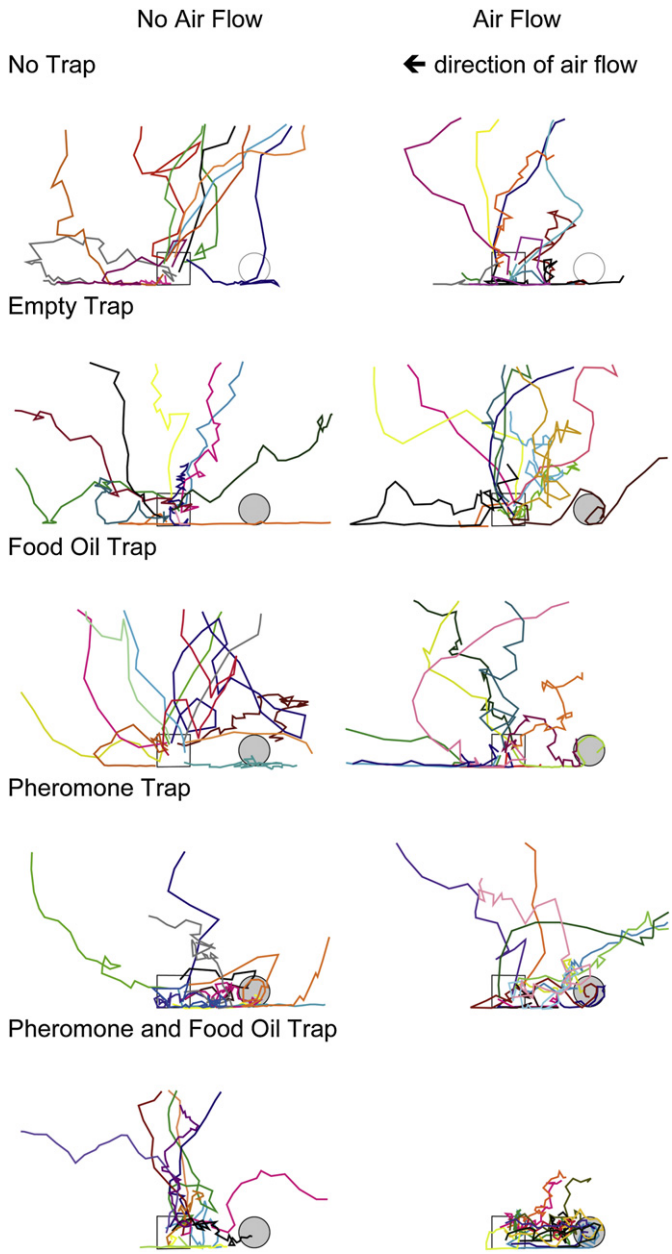

Fig. 2. Diagram of the movement pathways of individual *T. castaneum* (laboratory strain) male beetles under the different experimental conditions of no airflow or airflow with the following trap/attractant treatments: no trap, empty trap, kairomone (food oil) baited trap, pheromone baited trap, and pheromone/kairomone baited trap. Each line represents the path of an individual beetle, but are presented as a composite of replicates with orientation of the sources standardized to the right hand side, even though the orientation in actual replicates alternated. The square indicates the release zone for the beetles and the circle indicates the location of the trap, with both placed adjacent to a vertical edge along the lower portion of each figure.

the end of the observation period, including those that were captured, was determined since this provides a measure of arrestment at the trap as well as the probability of encountering the trap. With airflow there was a significant effect of attractant on the percent response ($F = 2.89$, d.f. $= 3, 188$, $P = 0.0368$), but Tukey–Kramer test did not indicate differences among specific treatments. Without airflow there was not a significant effect of attractant ($F = 1.89$, d.f. $= 3, 199$, $P = 0.1319$). Even under the combination of variables with the highest response, airflow with pheromone/kairomone baited trap, only 23% of beetles were retained either in or on the trap at the end of the observation period. The average time until a trap is encountered was also measured since this provides insight into whether beetles are responding more directionally or quickly to a trap. However, time to encounter trap was not significantly different among treatments ($F = 1.18$, d.f. $= 7, 82$, $P = 0.3214$), with both airflow and attractant type included as main factors in the model.

Since beetles may respond positively to different treatments, but within the timeframe of the experiment not encounter the trap, the proportion of observations where the beetle was in the half of the observation zone with the trap/attractant (i.e., positive response) was analyzed. The overall GLM model, which included strain, sex, airflow, and trap/attractant type as factors, was significant ($F = 3.51$, d.f. $= 39, 469$, $P < 0.0001$). Of the factors and their interactions included in the model, strain ($P = 0.0334$), airflow ($P < 0.0001$), trap/attractant ($P < 0.0001$), and airflow and attractant interaction ($P = 0.0026$) were significant. The field strain had a slightly stronger positive response than the laboratory strain (0.68 ± 0.02 ($n = 243$) and 0.61 ± 0.03 ($n = 266$), respectively), but the level of difference does not appear biologically meaningful. Airflow resulted in a stronger positive response than without airflow (0.74 ± 0.02 ($n = 240$) and 0.56 ± 0.02 ($n = 269$), respectively). And traps with pheromone and pheromone/kairomone (0.77 ± 0.03 ($n = 100$) and 0.74 ± 0.04 ($n = 104$), respectively) had a significantly stronger positive response than kairomone only, empty, and no trap treatments (0.60 ± 0.04 ($n = 100$), 0.56 ± 0.03 ($n = 103$) and 0.55 ± 0.04 ($n = 102$), respectively). The latter group did not differ from each other and were close to a neutral response, which would be 50% of time on the attractant side of observation zone. Combining the strains and sexes, airflow increased the time beetles were on the positive side of the observation zone. With no airflow, beetles spent approximately 50% of their time on positive side, with no differences among the different trap/attractant treatments (Fig. 4A). When airflow was present, there was a strong positive response to the two treatments with pheromone (>90%), while kairomone was not different from empty trap or no trap control (Fig. 4B).

Since attractants might elicit an area concentrated search or arrestment response that might ultimately lead to an increased capture probability, but not be detected within the short observation period, the tendency to leave the observation zone was evaluated as a measure of arrestment. In the overall glimmix model with strain, sex, airflow, and trap/attractant as factors, only airflow ($F = 9.69$, d.f. $= 1, 454$, $P = 0.0018$) and attractant ($F = 6.73$, d.f. $= 4, 454$, $P < 0.0001$) were significant main factors and none of the interactions were significant. Fewer beetles left the observation zone when airflow was present (49%) than when it was absent (65%). Among the attractants, more beetles left the observation zone in the no trap (70%), empty trap (68%), and kairomone (64%) trap treatments, than in the pheromone (48%) and pheromone/kairomone (35%) trap treatments, although the pheromone only treatment was not different from the kairomone only and empty trap treatments. Combining the strains and sexes, both airflow ($F = 8.86$, d.f. $= 1, 484$, $P = 0.0031$) and attractant ($F = 6.37$, d.f. $= 4, 484$, $P < 0.0001$) were significant factors, and the interaction was not significant ($F = 1.95$, d.f. $= 4, 484$, $P = 0.1012$). In the absence of airflow, there was no difference among trap/attractants ($F = 1.18$, d.f. $= 4, 249$, $P = 0.3218$) (Fig. 4C). However, in the presence of airflow there was a significant difference among trap/attractants ($F = 6.33$, d.f. $= 4, 235$, $P < 0.0001$) (Fig. 4D), and as in the combined airflow treatments the pheromone/kairomone combination had a lower leaving rate than all the other treatments, except the pheromone only trap.

An assumption of this experimental approach is that beetles that leave the observation zone (which triggers a terminated observation) are unlikely to ultimately encounter the trap. To

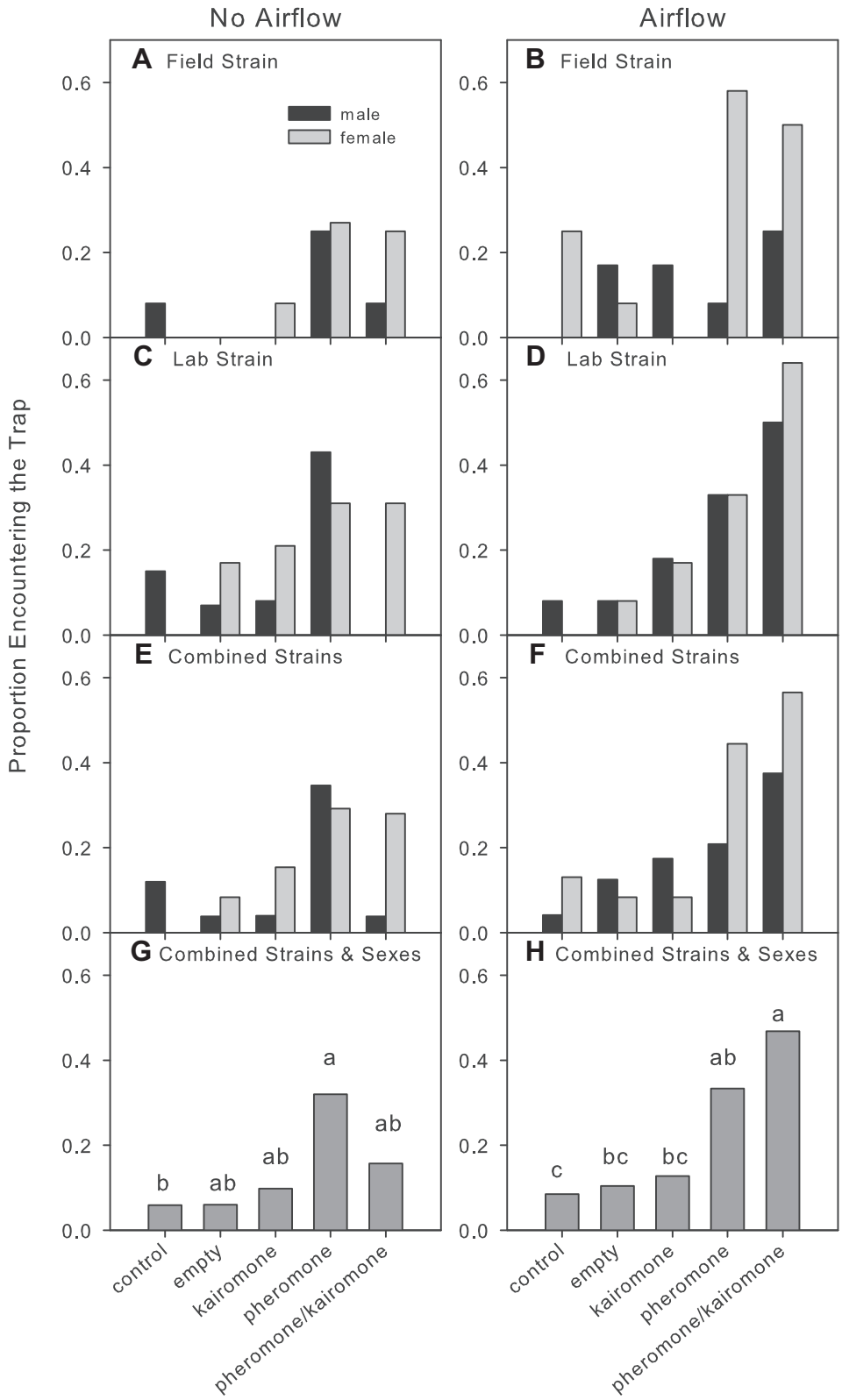

Fig. 3. Proportion of released *T. castaneum* encountering a Dome trap baited either with nothing (empty), kairomone (food oil), pheromone, or pheromone/kairomone combination, or to a no trap control within a 5 min observation period. Data is sorted into responses with and without airflow and then sorted by field (A) or laboratory (B) strain results, followed by strains combined (E,F), and then with strains and sexes combined (G,H). Letters above bars in graphs G and H indicate statistical differences, with bars that share a letter not being significantly different from each other within a graph ($P > 0.05$).

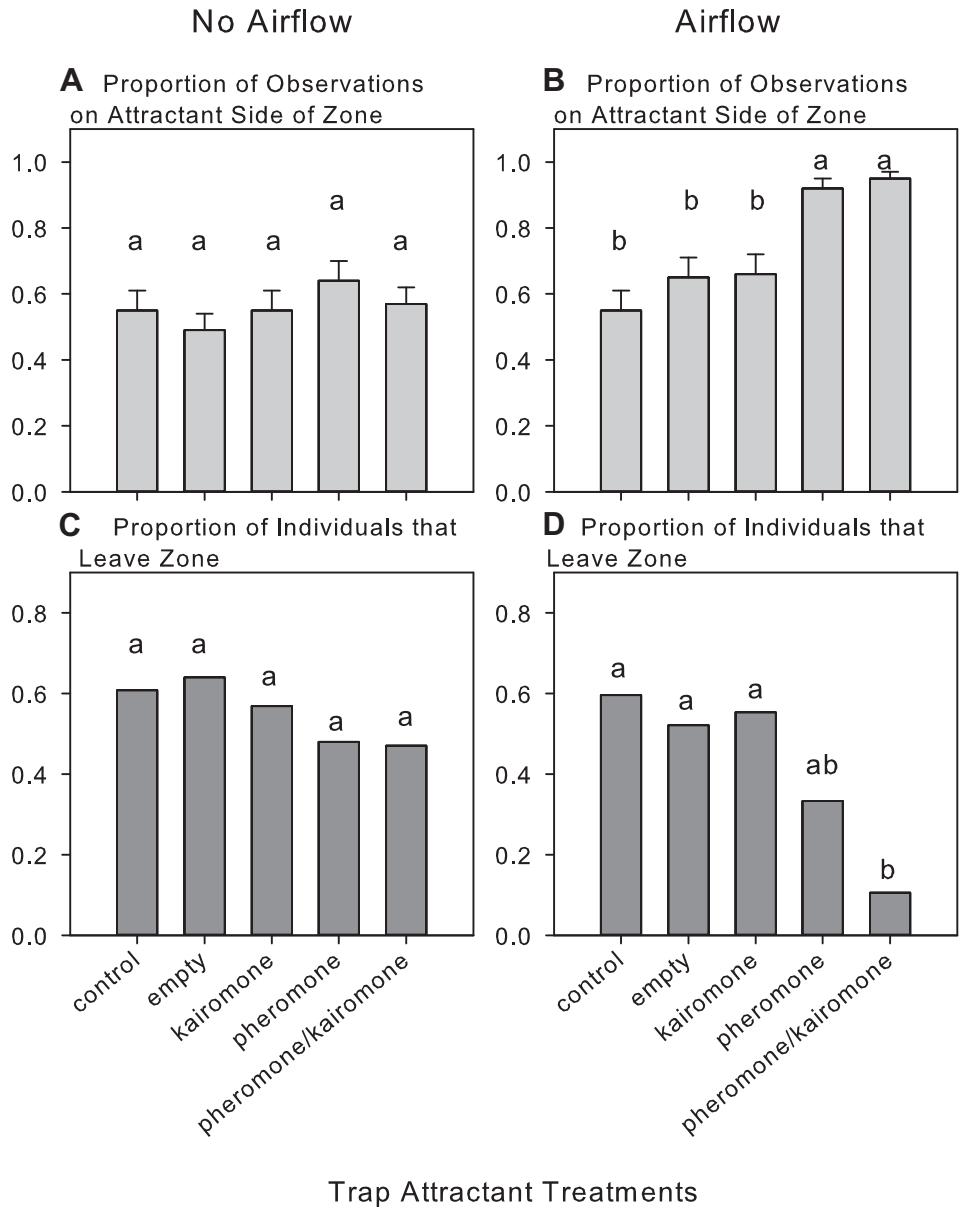

Fig. 4. Proportion of observations of released *Tribolium castaneum* on the side of the observation zone containing the source (no trap control, empty trap, or trap baited with kairomone, pheromone, pheromone/kairomone) without (A) and with (B) airflow and the proportion of released beetles leaving the observation zone for the same treatment combinations (C,D). Letters above bars indicate statistical differences, with bars that share a letter not being significantly different from each other within a graph ($P > 0.05$).

evaluate this assumption, the number of beetles that leave the observation zone, return and interact with the trap was assessed. For a subset of the observations, beetles were also evaluated over a larger area encompassing 300 by 175 cm. In 119 observations of individual beetles, 21 (18%) re-entered the observation zone, but by the end of the full observation period had left again, while 11 individuals (9%) returned and were still in the observation area at the end of the observation period, and only 1 individual (1%) returned and encountered a trap. Thus, leaving the observation zone makes it unlikely that a beetle will encounter a trap, even if observation had not been terminated.

Evaluation of movement pathway metrics provides additional insight into the mechanism behind the beetle's response to trap/attractants and could potentially detect responses missed in the other metrics analyzed above. Analyses of the combined strain and sex dataset were performed using GLM. Average step length (number of cells visited in a time step, which indicates rate of movement) was significantly affected by treatments ($F = 5.53$, d.f. = 9, 474, $P < 0.0001$): step lengths were shorter (i.e., slower movement) with airflow ($P < 0.0001$) and when traps contained pheromone (with and without kairomone) ($P \leq 0.0319$), and interaction between airflow and pheromone was significant ($P = 0.0339$). With airflow, beetles moved more slowly when pheromone and kairomone were present in the traps ($F = 6.45$, d.f. = 4, 231, $P < 0.0001$), but without airflow there was no change in step length among the different attractants ($F = 0.52$, d.f. = 4, 243, $P = 0.7175$) (Table 1). The significant effect of airflow appears to be due primarily on its interaction with attractant type, and not that beetle rate of movement was impacted by airflow alone (e.g., comparing no trap treatments with and without airflow).

The average turn angle, a measure of how linearly beetles moved, was significantly different among the treatments ($F = 4.05$, d.f. = 9, 474, $P < 0.0001$), with attractant type being significant ($P = 0.0006$) and airflow not significant ($P = 0.1572$), with

Table 1
Movement pathway metrics calculated for *Tribolium castaneum* beetles responding to different trap and attractant combinations from Experiment 1.[a]

Airflow Treatments Attractant Type	Step Length (No. cells)	Turn Angle (degrees)	Fractal Dimension
Airflow present			
No Trap	0.6 ± 0.1a	30.7 ± 7.2b	1.285 ± 0.021b
Empty Trap	0. 7 ± 0.1a	37.7 ± 8.6b	1.285 ± 0.030b
Kairomone	0.6 ± 0.0a	34.3 ± 8.1b	1.266 ± 0.024b
Pheromone	0.5 ± 0.05ab	84.0 ± 13.1a	1.396 ± 0.037a
Pheromone/Kairomone	0.3 ± 0.03b	90.0 ± 13.0a	1.351 ± 0.032ab
Airflow absent			
No Trap	0.7 ± 0.1a	44.9 ± 9.8a	1.334 ± 0.038a
Empty Trap	0.8 ± 0.1a	41.9 ± 8.6a	1.312 ± 0.033a
Kairomone	0.8 ± 0.1a	46.3 ± 9.8a	1.293 ± 0.024a
Pheromone	0.8 ± 0.1a	52.3 ± 10.9a	1.313 ± 0.029a
Pheromone/Kairomone	0.7 ± 0.1a	46.8 ± 8.0a	1.344 ± 0.027a

[a] Letters that are different from each other within a column and grouped by airflow treatment are significantly different from each other.

a significant interaction ($P = 0.0063$). With no airflow, average turn angle was not different among the treatments ($F = 0.16$, d.f. = 4, 243, $P = 0.9587$), but with airflow there was a significant difference among trap/attractants ($F = 7.99$, d.f. = 4, 231, $P < 0.0001$) due to traps with pheromone alone or in combination with kairomone having greater turn angles in the paths (Table 1). These results suggest that beetles normally move in a relatively straight path (small turn angles), but when the pheromone was detected, turn angles increased resulting in a less linear path, which is consistent with an area concentrated search. This change in turn angle could also decrease step length since it was measured here as movement between cells and a more convoluted path within a cell would produce a slower rate of movement between cells.

Fractal dimension which is an overall measure of path tortuosity was not significantly affected by the airflow and trap/attractant treatments in the overall model ($F = 1.68$, d.f. = 9, 471, $P = 0.0919$). However, when airflow was present there was a significant difference among trap/attractants ($F = 3.55$, d.f. = 4, 229, $P = 0.0079$): with a trend for a greater fractal dimension, decreased path linearity, when pheromone or pheromone/kairomone was present (Table 1). No difference in fractal dimension was detected among attractants under still air conditions ($F = 0.44$, d.f. = 4, 242, $P = 0.7810$).

3.2. Experiment 2

Release distance did not significantly impact beetle encounters with traps: no airflow and no attractant ($F = 2.49$, d.f. = 4, 75, $P = 0.0501$), no airflow and attractants present ($F = 1.41$, d.f. = 4, 75, $P = 0.2383$), airflow present and no attractants ($F = 1.32$, d.f. = 4, 75, $P = 0.2711$), and airflow and attractants present ($F = 1.08$, d.f. = 4, 75, $P = 0.3705$). Similarly there were no significant differences among release distances in beetles that left the observation zone (no airflow and attractants present ($F = 0.18$, d.f. = 4, 75, $P = 0.9457$), airflow present but no attractants ($F = 0.11$, d.f. = 4, 75, $P = 0.9802$), airflow and attractants present ($F = 0.57$, d.f. = 4, 75; $P = 0.6833$)), except for the no airflow and no attractant combination ($F = 3.24$, d.f. = 4, 75, $P = 0.0165$). This significant difference does not appear to be biologically meaningful, since Ryan–Eiont–Gabriel–Welsch multiple range test indicated that only the 30 and 50 cm distances were different from each other, and they were not different from any other release distances.

Evaluation of the differences in response to traps with and without attractants as a function of release distance can provide insight into the active space around traps, with results showing that with airflow the active space extended to 90 cm, maximum distance tested, but active space was essentially zero when no airflow was present. Beetles were significantly more likely to encounter traps with attractants than those without attractants at all release distances when airflow was present (Fig. 5): 10 ($F = 4.27$, d.f. = 1, 30, $P = 0.0474$), 30 ($F = 10.24$, d.f. = 1, 30, $P = 0.0032$), 50 ($F = 13.22$, d.f. = 1, 30, $P = 0.0010$), 70 ($F = 8.67$, d.f. = 1, 30, $P = 0.0032$), and 90 ($F = 7.77$, d.f. = 1, 30, $P = 0.0091$) cm. Conversely, the proportion leaving the observation zone was reduced with attractants in traps compared to empty traps at all release distances (Fig. 5): 10 ($F = 8.85$, d.f. = 1, 30, $P = 0.0057$), 30 ($F = 7.25$, d.f. = 1, 30, $P = 0.0115$), 50 ($F = 9.01$, d.f. = 1, 30, $P = 0.0054$), 70 ($F = 5.70$, d.f. = 1, 30, $P = 0.0234$), and 90 ($F = 8.85$, d.f. = 1, 30, $P = 0.0057$) cm. However, when airflow was not present there were no differences in beetle encounters with traps with and without attractants (10 ($F = 0.00$, d.f. = 1, 30, $P = 1.0000$), 30 ($F = 0.18$, d.f. = 1, 30, $P = 0.6728$), 50 ($F = 1.04$, d.f. = 1, 30, $P = 0.3151$), 70 ($F = 0.35$, d.f. = 1, 30, $P = 0.5560$), and 90 ($F = 0.35$, d.f. = 1, 30, $P = 0.5560$) cm), nor with beetles leaving of the observation zone (10 ($F = 1.98$, d.f. = 1, 30, $P = 0.1692$), 30 ($F = 0.13$, d.f. = 1, 30, $P = 0.7216$), 50 ($F = 0.53$, d.f. = 1, 30, $P = 0.4725$), 70 ($F = 0.00$, d.f. = 1, 30, $P = 1.0000$), and 90 ($F = 0.00$, d.f. = 1, 30, $P = 1.0000$) cm) (Fig. 5).

Proportion of observations on the side of the observation zone with trap, even with the area on each side of the release point not being the same in this experiment, showed no bias at any distance under still air conditions and a significant bias to the upwind side when trap had attractants at the furthest release distances (Fig. 5). With airflow, GLM analysis of response to traps with and without attractants was significant only at the two furthest release points: 70 cm ($F = 5.11$, d.f. = 1, 30, $P = 0.0311$) and 90 cm ($F = 5.72$, d.f. = 1, 30, $P = 0.0232$). Without airflow, response to traps with and without attractants were not different at any release distance ($P > 0.05$).

There was limited impact of release distance on the movement pathways of the beetles, with airflow being a more significant factor than distance. Mean step length was not significantly different in the overall GLM model ($F = 0.55$, d.f. = 19, 286, $P = 0.5505$). Mean turn angle was significantly different in the overall GLM model ($F = 5.27$, d.f. = 19, 287, $P < 0.0001$), with distance ($P < 0.0001$) and distance by airflow interaction ($P = 0.0026$) being significant. However, distance was only significant because of larger mean turn angle when released close to the trap (10 cm), with all other distances having a similar turn angles. Fractal dimension was also significantly different in overall GLM model ($F = 1.81$, d.f. = 19, 275, $P = 0.0216$), with airflow the only significant factor or interaction ($P = 0.0002$). Movement pathways were slightly less linear with no airflow (1.29 ± 0.01) compared to when airflow was present (1.22 ± 0.01). At the different release distances and airflow treatments, there were no significant differences in beetle movement pathway metrics when exposed to traps with pheromone/kairomone or empty traps ($P > 0.05$), except for step length at 90 cm release distance with airflow ($F = 5.17$, d.f. = 1, 30, $P = 0.0303$).

Taken all together these results suggest that the active space in which beetles respond to attractants in traps under still air conditions is essentially zero (i.e., not detectable even when beetles released as close as 5–10 cm from trap edge), but with air-flow beetles are able to detect and respond to attractant over distances of at least 90 cm. There was little detectable difference in their movement pathways at different distances, suggesting the effect was on the overall direction of the movement and not the rate of movement or tendency to turn.

3.3. Retention of beetles on traps

Previous analyses focused on beetle behavior prior to encountering a trap, but here beetle retention on traps after being encountered is evaluated using data from experiments with Dome

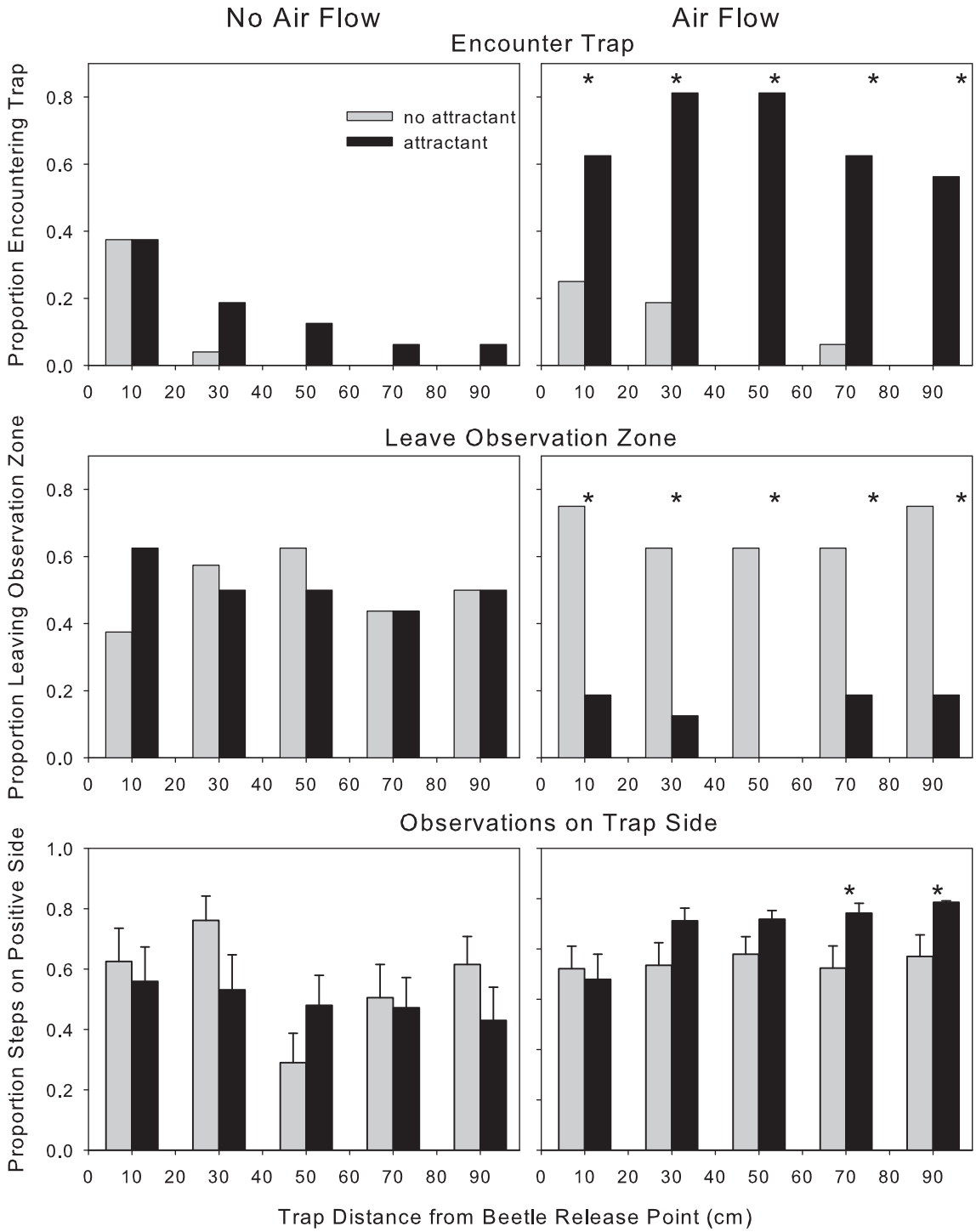

Fig. 5. Proportion of *Tribolium castaneum* released at different distances from the trap that encounter the trap or left the observation zone, and the proportion of observations on the side of the observation zone containing the trap sorted by presence or absence of airflow. The * symbol indicates significant differences between traps with and without pheromone/kairomone attractant at that release distance ($P < 0.05$).

traps with lids. For Dome traps with pheromone/kairomone attractants, 95% of beetles were still on the trap at the end of the observation period when airflow was present ($n = 73$) compared to 64% of beetles remaining ($n = 22$) when airflow was not present, which was significantly greater (Fisher Exact Test, $P = 0.0007$). However, there was no difference between airflow conditions in the proportion of beetles remaining that were captured versus just being present on the trap (Fisher Exact Test, $P = 1.0$). Insufficient numbers of beetles encountered unbaited traps under either airflow or no airflow conditions for further analysis.

4. Discussion

Attraction by insects to pheromones and kairomones is influenced by odor plume characteristics created as a result of wind currents and structures in the landscape. Air movement is one of

the most important abiotic factors impacting the ability of insects to orientate toward a source of attraction (McNeil, 1991). *Tribolium castaneum*'s response to pheromone/kairomone baited traps was strongly influenced by the presence of air moving over the trap toward the beetle. This finding for *T. castaneum* in itself is not surprising, but it does have important implications for use of these attractants in commercial food facilities. Movement of air, both in terms of its velocity and level of turbulence, influences factors such as the distance that the pheromone or kairomone moves from source, the structure of the plume, and the concentration of the attractant at different locations around a source (Bell, 1991; Elkinton and Cardé, 1984; Murlis et al., 1992). Movement of air can also impact the behavior of the responder, in that it can influence the activity levels of individuals, their ability to respond to changes in plume characteristics, and if flight to the source is involved, then higher wind velocity can reduce flight initiation. In the experiments reported here, a relatively low airflow speed was used and evaluation of the controls, no attractants or traps, indicated that walking *T. castaneum* did not respond either positively or negatively to the airflow itself.

Walking insects can rely solely on mechanoreceptor inputs to provide the directional information needed to move upwind toward a chemical attractant source (Bell, 1984). In the absence of directional information, chemical cues can trigger behavioral changes such as area concentrated search that can increase the chance of encountering the source, or directional information can be obtained by sampling at different time points during movement (Bell, 1991). In the experiments reported here, potential attractants were presented with and without directional information due to airflow and results indicate that *T. castaneum* responded strongly to pheromone cues when airflow was present, but did not change their behavior when airflow was absent. This was the case for not only locating the trap, but also for other metrics such as time on trap half of the observation zone, probability of leaving the observation zone, and movement pathway metrics that also indicate detection of attractants. This suggests that beetles are able to use the directional information from air movement to locate sources of attractants, but did not effectively respond to non-directional cues. Under the airflow conditions used in the current experiments, the active space around the trap extended out to the maximum tested distance of 90 cm, but under still air conditions the active space was essentially 0 cm. Although there was a non-significant trend for trap encounters under still air conditions to be greater when pheromone was present, this was likely due to an increased likelihood of entering a trap once it was randomly encountered.

Obeng-Ofori and Coaker (1990b) and Obeng-Ofori (1991) using an olfactometer bioassay also reported that *T. castaneum* responded to pheromone by moving upwind in a directed manner under moving air conditions. They also reported that there was an increase in speed, turning frequency, and turn angle with increasing pheromone concentration with resulting paths that tended to keep the beetle within the plume and to move more linearly upwind. In the current study walking speed decreased with pheromone compared to without pheromone, but results of a no pheromone control were not reported in the earlier studies. Positional differences in beetle behavior relative to the pheromone plume and distance from source could explain some of the difference in response since movement pathways are reported to change with distance from the source (Obeng-Ofori and Coaker, 1990a) and whether beetle was in a discrete or diffused portion of the pheromone plume (Obeng-Ofori, 1991). In addition Obeng-Ofori (1991) reported that *T. castaneum* responded to increasing pheromone concentration under still air conditions as well, with walking speed and turn angles increasing with concentration, but in the current study beetles did not respond under still air conditions. It is possible under the enclosed conditions within an olfactometer beetles responded to the pheromone, but that this might not translate to successful trap location under more realistic conditions.

Even under the best set of conditions, the proportion of beetles encountering traps was relatively low in these experiments (40%) and there was limited influence of strain or sex on the response. This low response in part reflects the challenging conditions under which the beetles were evaluated, although these are arguably more equivalent to those encountered in commercial food facilities. However, most laboratory experiments also tend to show a relatively low level of response by *Tribolium* species to pheromone either with or without food odors. In a series of olfactometer studies using low airflow (0.1–0.3 m/s), the percent positively responding at the shortest exposure times was between 50 and 70% (Obeng-Ofori and Coaker, 1990a, 1990b). Olsson et al. (2006) using a walking bioassay with airflow had percent of individuals responding to pheromone in the 10–20% range in their experiments. Duehl et al. (2011a) found the number of responders to pheromone alone was roughly twice that to the control, but still only approximately 50% of the individuals. This low response may be due to lower motivation to respond to aggregation versus sex pheromones. Low behavioral response may also be a behavioral artifact due to a disturbance response resulting from handling of beetles prior to experiments (Duehl et al., 2011a), with the supposition being that under natural conditions a stronger response could be achieved. Changes in pheromone concentration (Obeng-Ofori and Coaker, 1990b) or blend of stereoisomers (Lu et al., 2011) might also improve the response to the pheromone baited traps. The pattern of pheromone release and the resulting plume due to trap design may also be an important factor that needs further evaluation. Finally, the response to chemical cues could be enhanced by incorporation of visual cues such as light (Duehl et al., 2011c) or dark shapes (Semeao et al., 2011).

Some benefit to combining the kairomone with the pheromone was observed in terms of the proportion of individuals encountering trap; as has been previously reported (Phillips et al., 1993). However, the food oil alone did not appear to have any detectable increase in attraction compared to an empty trap and the attraction to the combined pheromone and kairomone was often similar to the pheromone alone. This limited attraction or arrestment in response to food-based volatiles has been previously observed (Duehl et al., 2011a; Romero et al., 2010; Willis and Roth, 1950), although arrestment at food may be greater when beetles can interact physically with the material (Romero et al., 2010) and after longer periods of starvation (Willis and Roth, 1950). In the current study, the pheromone appears to elicit most of the *T. castaneum* response. Although commercially traps widely use the combination, the benefits for attraction are weakly supported for *T. castaneum*, although the food oil can play other roles including killing insects captured in the trap. A laboratory study looking at pheromone, food, and combination of the two reported a relatively low response to food and pheromone alone and significantly greater response to the combination, although it was at best an additive effect (Phillips et al., 1993). It is also possible that the role of the food volatiles is in the final steps of capture in the trap, since food based oils can lead to greater captures than control mineral oils in pitfall traps (Barak and Burkholder, 1985), but may not be long range attractants. A decrease in the benefits of a combination of pheromone and kairomone under more natural conditions has been reported for *Sitophilus* spp., where synergistic interactions were observed in the laboratory (Trematerra and Girgenti, 1989; Walgenbach et al., 1987), but under field conditions the interactions were at most additive (Hodges et al., 1998; Likhayo and Hodges, 2000).

The role of air movement on *T. castaneum* response to traps has important practical implications because commercial food facilities are complex environments with considerable variation among trap locations in physical and environmental conditions (Semeao et al., 2012) and in wind speed and direction. For example, traps placed near doors, windows, air handling systems, and zones of high activity receive quite a bit of air movement, but traps placed behind pieces of equipment, pallets, or other obstructions may have very limited airflow. These out of the way locations are often where traps for monitoring *T. castaneum* are placed. Traps also tend to be placed along walls or in corners where beetles are more likely to be moving, but these positions can also influence the movement patterns of air and these potential tradeoffs need to be evaluated. Wind speed and direction is also likely to be temporally variable at a location. These differences could create variation in ability of beetles to detect and respond to attractants leading to differential capture in traps that is not related to the density of active insects. Thus the environment in which the traps are placed could have an influence on the strength of response observed in food facilities. Mankin and Hagstrum (1995) found that the stored-product moth *Cadra cautella* (Walker), under windless conditions only responded to a pheromone plume from a distance of approximately 40 cm, while another moth *Plodia interpunctella* (Hübner) exhibited wing fanning, indicating detection of pheromone, at a distance of 4 m (Mankin et al., 1980). However, the reduced active space for walking *T. castaneum* under still air conditions may result in trap encounters being essentially random, but with captures enhanced by the lures.

A low active space around the trap does not necessarily mean that the traps are not effective monitoring tools, since unbaited pitfall traps are among the most frequently used methods to sample epigeal arthropod activity (Greenslade, 1964; Southwood, 1994; Spence and Niemelä, 1994). Pitfall trap designs such as the Dome trap baited with pheromone/kairomone have been widely used for monitoring *Tribolium* species inside food facilities and have been effective at documenting trends in populations (Campbell et al., 2010). However, the potential variation in active space around a trap as a function of airflow pattern variation within a facility, does raise some issues regarding optimal trap placement and how to interpret the results of monitoring programs. For example, two locations with the same level of insect activity could get two very different levels of beetle capture if one had still air and the other was near a source of air movement. While the potential influence of this factor needs further evaluation, results suggest that placing traps near locations with airflow could increase their effectiveness and documenting air movement patterns, both speed and direction, could improve interpretation of monitoring data. Ultimately devices that generate their own airflow might be incorporated into trapping programs.

Overall, the experimental design developed here for evaluating insect response to pitfall type traps used in food facilities provides a good framework for assessing insect response to these devices. It incorporates some of the complexity of monitoring within commercial food facilities, while enabling controlled conditions and replication. An open arena with single beetle releases more accurately depicted the strength of the response to baited traps. Although a range of metrics were measured in the current study, the simple metric of trap encounters provided as reliable an indicator of response as any of the others. This will make performance and analysis of future experiments simpler and similar to other laboratory assays described above while still providing a more realistic test of the strength of the response and providing the flexibility of more detailed analysis if needed. Under these simulated field conditions, results indicate that a pheromone and kairomone combination was the most effective at increasing beetle encounters with a trap, but the highest level of response for any group of beetles and conditions was in the 40–60% range. There is room for improving this level of response as well as a need to evaluate how other biotic and abiotic factors impact the response of this important pest of the food industry.

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