

Movement Behavior of Red Flour Beetle: Response to Habitat Cues and Patch Boundaries

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Environ. Entomol. 39(3): 919–929 (2010); DOI: 10.1603/EN09324

ABSTRACT Movement behavior determines the success or failure of insects in finding important resources such as food, mates, reproductive sites, and shelter. We examined the response of female red flour beetles (*Tribolium castaneum* Herbst: Coleoptera: Tenebrionidae) to habitat cues by quantifying the number of individuals that located a patch (either with or without flour) in response to the distance released from the patch, air movement over the arena, and food-deprivation status. We also investigated how patch characteristics, such as resource amount and presence of cover, influenced time taken to find a flour patch, the frequency of entering or leaving, and residence time within the patch. Although the proportion of beetles successfully locating the patch decreased as a function of release distance, the probability that beetles reached the patch was ultimately unaffected by whether flour was present or not, suggesting that search behavior in red flour beetles may exhibit a simple distance-decay function. Significantly more beetles reached the patch when they had not been food deprived and air was flowing over the arena, which indicates that walking beetles may orient to airflow, exhibiting anemotaxis. Results of the second experiment showed that, on first encounter, fewer beetles entered patches with a greater amount of flour; but once they had entered, they left them less frequently than patches with less resource. Beetles entered covered patches more quickly than uncovered patches irrespective of resource amount, which indicates that shelter is perhaps more important to red flour beetles than resource levels in determining whether to enter patches.

KEY WORDS movement behavior, air movement, habitat quality, shelter, *Tribolium castaneum*

Knowledge of insect movement behavior is essential for understanding and modeling dispersal and population structure as well as developing biologically-based integrated pest management programs. Insect movement behavior is determined by many factors, including movement ability, perceptual resolution (the smallest distance over which a resource is detected), resource quality and distribution, and internal and external stimuli (Bell 1990, 1991). Typically, resources (e.g., habitat, food, shelter, and mates) required by insects are patchily distributed within the landscape (Wiens 2001). As a result, search behavior may be hierarchical, with different behavioral mechanisms operating at the scale of an entire landscape (e.g., within patches [movement within a relatively homogeneous patch], between-patch, and at the in-

terface between patches and the surrounding matrix [i.e., at the patch edge]). The ability to locate resources is affected not only by the ability of insects to detect resources and move among patches, but also how they interact with patch edges (e.g., move into or out of habitat patches, follow habitat edges) (Hansen and di Castri 1992, Haddad 1999, Ries and Debinski 2001). Characteristics of the patch, the patch boundary (e.g., size, shape, and permeability of patches), and the individual (e.g., movement behavior, perceptual resolution, and physiology) all determine the probability that an insect will cross the boundary and enter the patch (Stamps et al. 1987, Wiens 1992). By studying the movement behavior of an insect, in response to experimental manipulations of resources and other physical factors, we can gain insights concerning how it perceives and searches for resources in its environment. In the current experiment, we focus on how female red flour beetle (*Tribolium castaneum*: Coleoptera: Tenebrionidae Herbst) responds to a single flour patch from a distance and, secondly, how it responds to various patch attributes (i.e., resource amount, presence of cover, boundary characteristics).

The red flour beetle is an important worldwide pest of stored products. Successful dispersal from one patch to another drives the ability of stored-product insect populations to persist, thereby challenging the efficacy of control tactics (Campbell and Arbogast

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2004). Red flour beetle is well adapted to heterogeneous landscapes, readily dispersing among several resource patches over its lifetime (Naylor 1961). Females will distribute eggs among multiple patches, and progeny complete development within small amounts of resource (Campbell and Hagstrum 2002, Campbell and Runnion 2003). The well-developed dispersal ability of red flour beetle has contributed greatly to its pest status, yet information on red flour beetle movement outside of food patches is very limited because early studies focused only on emigration from patches (Korona 1991) or because the experimental design constrained movement to artificial channels between patches (Naylor 1961, Lomnicki and Krawczyk 1980, Ben-Shlomo et al. 1991). Campbell and Hagstrum (2002) investigated how red flour beetle exploited patches of food on a scale larger than previously studied, and Toews et al. (2005) documented the role of food, spatial structure, and trap position on trap catches in simulated warehouses; however, more studies on movement and foraging behavior are needed because the decreased reliance on pesticides for control requires a better understanding of the biology, behavior, and ecology of stored-product insects. The prominence of red flour beetle as an experimental model (Sokoloff 1974) has led to its extensive use as a genetic model for Coleoptera. This species also serves well as a behavioral model for the response of insects to patchy resources because they are adapted to environments that are inherently patchy across a range of scales (Campbell and Runnion 2003, Romero et al. 2009). Development of new management strategies for this and other coleopteran pests is promising if behavioral information is synthesized with ecological and genetic information.

In previous studies, we observed how female red flour beetles responded to the pattern of flour patches on a landscape. The beetles moved more slowly, their pathways were more convoluted, and they remained longer within landscapes where resource (flour patches) was highly or moderately fragmented compared with landscapes with a clumped resource distribution (Romero et al. 2009). Landscape effects on movement patterns were also influenced by the type of activity in which individuals were engaged (i.e., foraging or oviposition). On fragmented landscapes, beetles chose to oviposit in a smaller number of patches than they had previously visited when not engaged in oviposition (Romero 2007). We studied the movement response of females rather than males because mated females respond to flour patches as potential oviposition sites, which is important in terms of population growth and dispersion. Flour conditioning has been shown to affect dispersal and suppress oviposition in red flour beetles (Ghent 1963, Sokoloff 1974). Conditioning results from the following: 1) depletion of nutritive content; 2) accumulation of feces, exuviae, and dead imagoes; and 3) defensive compounds, such as quinones produced by beetles as the colony grows in a limited resource. Females removed from conditioned flour and placed into fresh flour for 24 h experience a release from oviposition

suppression (Romero 2007) and, thus, will actively locate resources (i.e., fresh flour) in which to oviposit (Sonleitner and Guthrie 1991). Females removed from conditioned flour and deprived of flour for 24 h experience a delay in the release from oviposition suppression and do not begin ovipositing until 48 h after removal from the colony (Romero 2007).

A beetle's perception of patch structure and the spatial scale(s) at which it responds to the abundance and distribution of resources may depend upon whether it perceives habitat pattern through direct physical encounter with patches or if it perceives patches from a distance. Red flour beetles appear to have only weak attraction to flour resources (Barrer 1983, Willis and Roth 1950), unless they are already occupied by conspecifics that have conditioned the resource by the chemical compounds they secrete (Prescott 1970, Obeng-Ofori 1991); but the distances over which flour beetles are able to detect resource patches is unknown. The objective of the current study was to investigate the following: (1) how beetles respond to patches from a distance, and (2) how patch characteristics influence both beetle behavior and the probability of entering the patch. The ability of red flour beetles to detect resource patches from a distance is likely to be influenced by many factors, but in the first experiment, we tested two factors that might influence search behavior, as follows: an exogenous factor (directional air movement) and an endogenous factor (food deprivation). We hypothesized that, if beetles respond to cues associated with air movement, then the proportion of beetles locating a flour patch will be greater when air is moving over it. In regard to food deprivation, we hypothesized that a greater proportion of food-deprived beetles would locate the flour patch than those that had not been deprived because beetles would be hungry, or searching for a suitable oviposition site. In our second experiment, we hypothesized that higher quality patches (providing more resource and shelter) would be entered more quickly and have a higher retention rate than lower quality patches (with less flour and no shelter). To separate the shelter effect from the amount of resource, we also included a treatment with a lower amount (and height) of flour that had a cover that provides enhanced shelter. All patch types tested provided adequate resources for development, although red flour beetles lay more eggs and have an increased probability of survival to adulthood in larger amounts of flour (Campbell and Runnion 2003), so there may be fitness benefits to females in selecting patches with a large amount of flour that provide more shelter and resource.

Materials and Methods

Insects Used in the Study. We used female red flour beetles taken from colonies founded with ≈ 40 individuals collected from a flour mill ≈ 18 mo (≈ 14 generations) before experiments were conducted. Each subcolony used in the experiment had been initiated with ≈ 50 adults and maintained in 0.25 liters of wheat/

brewer's yeast mixture (95/5%) in an environmental chamber ($27 \pm 0.03^\circ\text{C}$; $56 \pm 5\%$ RH; 14:10 d/night cycle). We standardized beetle age at the time of the experiment to 3 wk \pm 4 d by removing the initial adults after they had been allowed to mate and oviposit in the subcolony for 4 d. This standardization controlled for potential differences in movement behavior as a result of sex, age, and mating status. Females were determined by immobilizing beetles by chilling and observing under the microscope whether they lacked the setaceous patch that is present on the first femur of males (Good 1936). Individual beetles were used in experiments only once. Voucher specimens were deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research under Lot 195.

Experiment One: Response to Habitat Cues

Treatments and Experimental Arena. As a measure of how beetles respond to habitat cues, we evaluated the relative ability of female red flour beetles to locate a resource patch (i.e., flour) under various conditions. To evaluate whether beetles orient toward a resource patch before physically encountering edges, we measured the number of beetles reaching a predetermined location (referred to in this study as a patch) with flour either present or absent. Our estimation of response to resource assumes that beetles respond behaviorally to perceived stimuli, but it is of course possible that beetles perceive stimuli to which they do not respond. Because the behavioral response to resource stimuli is likely to be influenced by various factors, we experimentally manipulated air movement and feeding status and tested beetle responses at different release distances from the patch. We removed beetles from a subcolony 24 h before use in the experiment, determined sex, and placed a group of 20–24 individuals in each of two 88×12 -mm disposable polystyrene petri dishes containing a piece of folded 70-mm-diameter grade one filter paper. For each release-distance category (2, 4, 8, or 16 cm from the patch), we assigned beetles to a feeding status and air-movement treatment combination: they were either provided ≈ 6.0 g of fresh flour until the start of a trial (fed, F) or kept without flour for 24 h beforehand (unfed, U). We tested half of the beetles in each feeding status group in a wind tunnel (airflow rate of 0.01 m/s, as used in Obeng-Ofori and Coaker 1990) with the air flowing (A) across the patch toward the beetle, and the other half with no airflow (N). We evaluated their movement relative to the flour (a patch 54 cm downwind of the air source) as well as movement relative to the absence of flour (patch without flour: control) (Fig. 1). For the flour patch, ≈ 50 ml of flour was spread evenly over a 4-cm-wide strip (flour patch). Beetles were released along the midline between the sides of the wind tunnel. We designated a line parallel to, and 32 cm downwind from, the patch edge as the extent of the arena. Observations were terminated after beetles crossed this line, regardless of whether or not they had encountered the patch. For the trials with flour in the

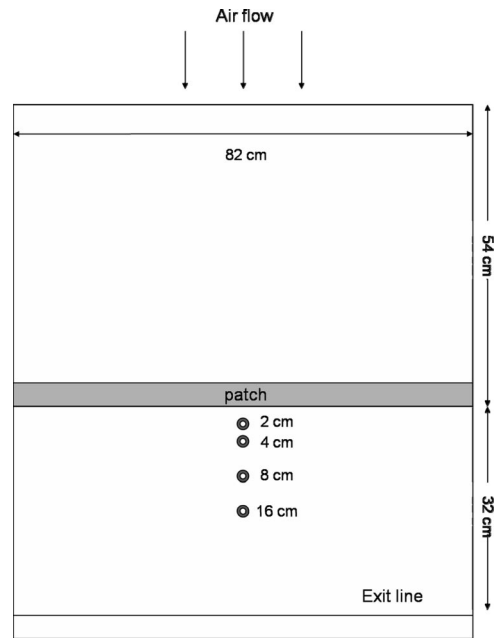


Fig. 1. Diagram of the experimental arena in the wind tunnel. Female red flour beetles (*T. castaneum*) were released at labeled points 2, 4, 8, and 16 cm downwind from the flour patch.

patch and for the control trials (patch without flour), we scored the patch as “found” if they encountered either type of patch.

Experimental Design. We tested the effects of feeding status, air movement, and distance from the patch on beetle movement as whole-plot factors within split plots, in which flour was either present or absent. The four feeding status \times air treatment combinations, designated FA, UA, FN, and UN, in conjunction with the four distance treatments, resulted in 16 treatment combinations that were randomized within each split plot. We tested all whole-plot combinations (16 with flour present + 16 with flour absent) within a block on the same day between the hours of 1200 and 1900. We randomized the order of the whole-plot factors as well as the split-plot factors within each block each day. A single beetle subcolony was used for each experimental block. Blocks were replicated over a 12-wk time period totaling 32 replicates of each resource \times distance \times air \times feeding status combination.

We used two beetle-release methods in experiments. In the first 14 trials, an individual female beetle was held under a 1.5-cm-diameter glass vial for a 90-s acclimation period at the appropriate release point, with the vial removed at the start of the trial. In the second method (18 replicates), we used forceps to place the beetle in a paper release envelope, in which it could acclimate, but was able to leave at will. The envelope consisted of two circular layers of filter paper (3 cm diameter) that were taped around the edges, leaving four evenly spaced 5-mm exit holes. We switched to the second release method because, in the

first group of trials, some beetles climbed up the side of the vial and so couldn't be released in a manner consistent with the others. Because there were no significant differences between release methods for any of the response variables, data from the two methods were pooled for final analyses.

Upon release, the beetle was observed by eye until one of the following occurred: 1) the beetle reached the patch; 2) the beetle exited the arena; 3) the beetle climbed and remained on a side wall of the wind tunnel for >10 s; or 4) the beetle remained in the observation area for 10 min. The outcome of each trial was scored according to the four criteria mentioned above and the result entered into Observer software (Noldus Information Technology 2003a). This software was also used during the experiment to track the beginning and ending time of each trial and, subsequently, to group responses for further analysis. The floor of the wind tunnel was wiped clean with alcohol and lined with a single sheet of clean white paper between split plots to eliminate any remaining flour residue from the previous flour treatment. Trials were conducted under ambient light, temperature, and humidity conditions ($22.0 \pm 10.0^\circ\text{C}$; $21.0 \pm 7.0\%$ RH).

Data Analyses. A generalized linear mixed model for binomial data (Proc GLIMMIX, SAS Institute 2002) was used to examine the relationship between the number of beetles that reached or did not reach the patch (4-cm side strip) dependent on the split-plot factor (presence or absence of flour). Within each resource treatment, the four air movement \times feeding status combinations and their interactions were analyzed for differences after nesting within distance categories. Analysis of variance (ANOVA) (Proc MIXED, SAS Institute 2002) was used to determine the role of distance, feeding status, and airflow on the amount of time beetles spent in the experimental arena. Means were considered significantly different at $\alpha = 0.05$ using Tukey's honestly significant difference.

Experiment Two: Response to Patch Characteristics

Treatments and Experimental Arena. To determine how patch quality influences beetle response to patch boundaries and their decision to enter patches, we manipulated patch characteristics and examined various aspects of beetle movement behavior in an experimental arena (Fig. 2). Using a complete randomized block design, we modified resource amounts in a single, 5×5 -cm patch, either uncovered with 2.0 g of flour, uncovered with 0.6 g of flour, or covered with 0.6 g of flour. To cover the flour patch, a flat piece of 5×5 -cm cardstock was held 3 mm parallel to the floor of the arena by a size 2 insect pin at each corner.

The experimental arena consisted of a square polystyrene petri dish bottom ($22.5 \times 22.5 \times 2.2$ cm) that was covered by white bond paper to facilitate movement. The size of the arena was constrained by the camera resolution and ability of the behavioral software to track a small rapidly moving insect (≈ 4 mm). Red flour beetles were unable to walk up the smooth

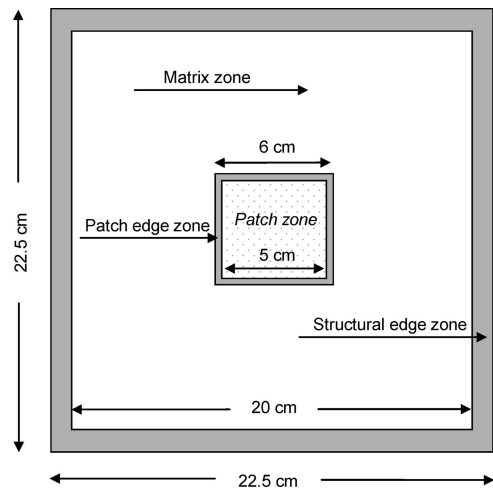


Fig. 2. Experimental arena with various zones used to observe red flour beetle (*T. castaneum*) response to patch treatments described in the text as: high amount of resource, a patch with 2.0 g of flour and a 1-mm high edge; low amount of resource, a patch with 0.6 g of flour and a low edge ≈ 0.25 mm; and cover, a low resource (0.6 g) patch covered by a 5×5 -cm flat cardboard cover supported 3 mm above the flour surface.

sides of the dish, limiting their movement to the floor. Habitat patches were created in the center of each arena by sifting a measured amount of flour over a cardboard template with a 5×5 -cm cutout. We removed the templates before placement of the beetles in the arena.

We removed beetles from a single subcolony immediately before trials, determined sex, and placed females individually into the petri dish arena to acclimate for 2–3 h before the start of the trial. Beetles were free to explore the petri dish arena; however, we kept beetles from contacting the patch during acclimation by placing a glass petri dish cover (88×12 mm) over each patch. Arenas with acclimating beetles were placed in an environmental chamber under 40-watt incandescent lighting set at 28°C until trials were run. We removed an individual petri dish arena from the acclimation chamber and placed it into the experimental chamber with the same environmental conditions as the acclimation chamber. We then uncovered the flour patch, and recorded beetle movement for 10 min with a digital video camera (Sanyo Day/Night Color CCD Camera model VCC-4594 with Pentax 3–8 m 1:1.0 D/N lens). Five blocks were run on sequential days, with four replicates of each treatment per block (20 replicates per treatment total) in a complete randomized block design.

Measures of Beetle Response. We measured the behavioral responses of beetles to patch boundaries in several ways. To determine whether movement changes in proximity to patch boundaries, we calculated pathway metrics for beetles located in one of three zones within the arena, as follows: 1) the patch edge, a 0.5-cm-wide zone surrounding the patch, one

beetle length in width; 2) the matrix, a 7-cm-wide zone between the patch edge zone and the arena-edge zone; and 3) the arena edge, a 1.25-cm-wide zone adjacent to the inside walls of the arena (Fig. 2). The metrics included the following: velocity; total distance traveled; and angular velocity, a measure of pathway complexity (Bell 1991). These metrics could not be calculated for beetles in the patch itself because movement could not be tracked either because of the cover over the patch or because beetles tunneled into the flour.

To determine effects of resource amount and cover on retention time in the patch, we calculated the proportion of time spent in the patch as well as in the other zones. To determine whether patch treatment influenced the time it took beetles to enter the patch, we calculated time from the beginning of each trial until the beetle reached both the patch edge and the patch itself. These measures allowed us to calculate the time from initial contact with the patch edge zone until first entering the patch.

To more directly assess permeability of the patch boundary, we quantified several behaviors. We recorded whether or not the beetle entered the patch on the first encounter with the boundary. We also calculated cumulative frequencies of entering or leaving the patch. Data on the frequency of beetles entering the patch were only collected for the high- and low-resource treatments because it was not possible to observe the beetles in the covered patch.

Data Analyses. Ethovision behavioral tracking software (Noldus Information Technology 2003b) was used to calculate pathway metrics and other measures from digital video recordings. ANOVA (ANOVA on ranks, Proc GLM, Tukey's test at $\alpha = 0.05$) was used to test means of behavioral measures for significant differences among main factors (patch treatments) and main factor \times zone interactions. We transformed proportions using the arcsine-square root method (Zar 1999) before analyses. We used χ^2 test of association (Pearson's exact) to test for significant differences ($P < 0.05$) in the distribution of frequencies of behaviors at patch boundaries (e.g., entering or leaving the patch) among patch treatments.

Results

Response to Habitat Cues. Contrary to expectations, presence of flour was not a significant predictor of beetle success in reaching the patch ($F = 1.37$; $df = 1, 31$; $P = 0.251$). As expected, significantly fewer beetles reached the patch, both with and without flour, as release distance increased to 8 and 16 cm ($F = 21.47$; $df = 3, 845$; $P < 0.0001$) (Fig. 3). Approximately 63% of beetles reached the patch (with and without flour) at the two closest release distances combined (2 and 4 cm) compared with 43% at the 8-cm release distance (with and without flour). At the 16-cm release distance, 45% of beetles reached the patch containing flour, whereas only 27% reached the target area without flour resource (Fig. 3). Beetles spent similar amounts of time in the arena at all release distances

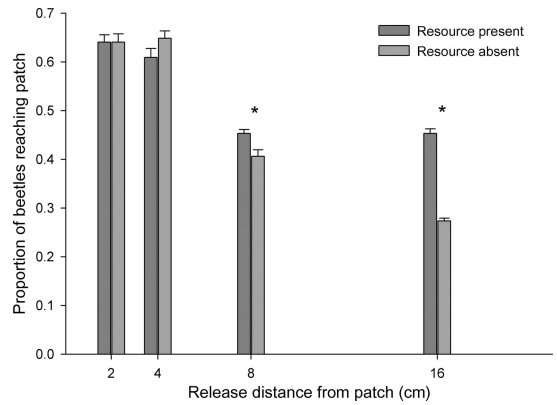


Fig. 3. Proportions of female red flour beetles (*T. castaneum*) reaching a patch (results with and without flour pooled) when released 2, 4, 8, or 16 cm from the patch. Asterisks indicate the two distances (8 and 16 cm) in which results for the combined treatments (with and without flour) were significantly different from the results of the 2- and 4-cm release distances (GLIMMIX procedure, $\alpha = 0.05$).

($F = 1.20$; $df = 3, 983$; $P = 0.3102$), indicating that release distance did not constrain the beetles' ability to reach the patch.

A significantly greater proportion of the individuals that had fed and had air flowing over the arena (FA) reached the patch or target area compared with the other treatments: unfed with no airflow (UN; $t = -2.34$; $df = 3, 845$; $P = 0.019$); fed with no airflow (FN; $t = -2.78$; $df = 3, 845$; $P = 0.006$); unfed with airflow (UA; $t = -2.29$; $df = 3, 845$; $P = 0.0022$) (Fig. 4). Contrary to expectations, $\approx 65\%$ of fed (F) beetles oriented upwind compared with 54% of food-deprived beetles (unfed or U; Fig. 4). Food deprivation had no significant effect on the amount of time that beetles spent in the arena ($F = 0.33$; $df = 1, 845$; $P = 0.563$).

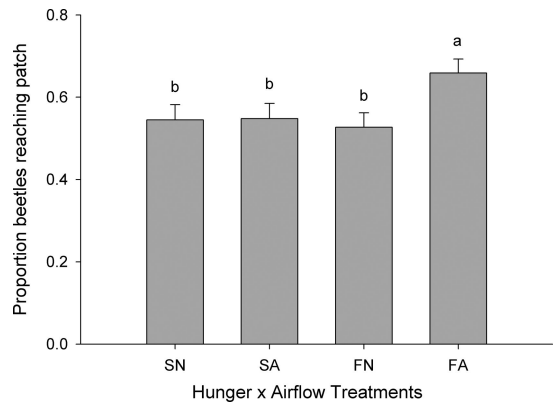


Fig. 4. Proportions of female red flour beetles (*T. castaneum*) reaching a patch (results with and without flour pooled) for the following treatments: food deprived with no air flowing (SN), food deprived with air flowing (SA), fed with no air flowing (FN), and fed with air flowing (FA). Bars with the same letter are not significantly different (GLIMMIX procedure, $\alpha = 0.05$).

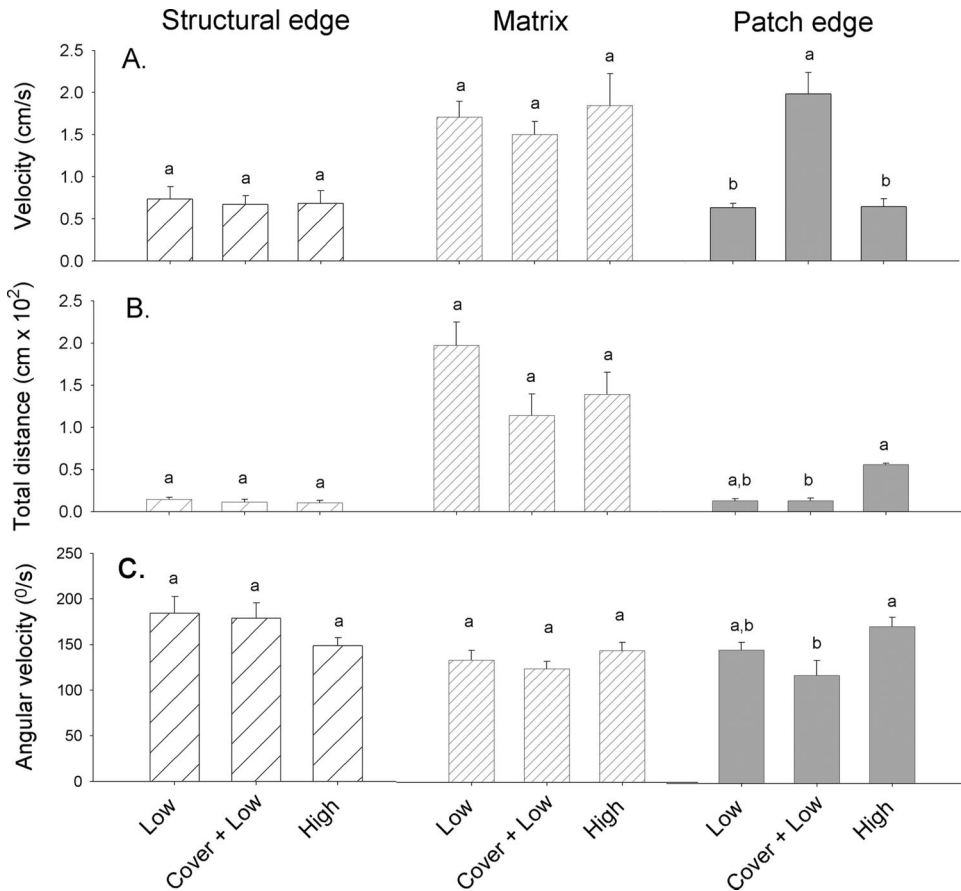


Fig. 5. Mean \pm SEM of (A) velocity, (B) total distance moved, and (C) angular velocity of red flour beetle (*T. castaneum*) pathways in various zones of the experimental arena in response to flour patch treatments consisting of low resource, cover with low resource, and high resource. Bars with the same letter are not significantly different (ANOVA on ranks, GLM procedure, $\alpha = 0.05$) within each plot.

Response to Patch Characteristics

Pathway Metrics. Patch characteristics influenced beetle movement patterns when analyzed for the total arena (three arena zones combined). Beetles moved 28% faster in the arena in the presence of a covered patch compared with both high- and low-resource treatments that lacked patch covers ($F = 5.79$; $df = 2, 149$; $P = 0.0036$). The total distance that beetles moved was significantly less in arenas with covered patches than in the arenas with the low- and high-resource uncovered patches ($F = 5.86$; $df = 2, 149$; $P = 0.0036$). Beetles did not differ in the degree of angular velocity in the arena among all treatments ($F = 1.95$; $df = 2, 149$; $P = 0.1457$).

Dividing the arena into different zones (arena edge, matrix, and patch edge) for analysis, beetle movement pathways differed among the patch-type treatments only in the zone around the patch (i.e., the patch edge), and not in the matrix or the arena-edge zones (Fig. 5). In the covered patch treatment, beetles moved 3 times faster in the patch-edge zone compared with either high ($P < 0.0001$)- or low ($P = 0.0002$)-

resource patches, which did not differ from each other ($F = 6.05$; $df = 4, 149$; $P = 0.0002$) (Fig. 5A). The total distance moved by beetles within the patch-edge zone of the high-resource patch was statistically similar to the distance moved within the patch-edge zone of the low-resource patch ($P = 0.092$), but significantly greater than within the patch-edge zone of the covered patch ($P = 0.011$; $F = 2.83$; $df = 4, 149$; $P = 0.0268$) (Fig. 5B). There were significant differences in the angular velocity of beetles in the patch-edge zone ($F = 4.08$; $df = 2, 149$; $P = 0.024$) (Fig. 5C); in the covered patch treatment, beetles turned 1.5 times less than in the high-resource treatment, indicating a more linear path. Beetles turned at a similar rate at the patch edge of the covered and the low-resource treatment patch treatments ($P = 0.344$), and angular velocity was not significantly different between the high- and low-resource treatments ($P = 0.3505$).

Time Allocation. Beetles allocated time differently to the patch-edge and patch zones depending on patch treatment (Fig. 6). The time it took the beetles to enter the patch-edge zone ($F = 1.46$; $df = 2, 42$; $P =$

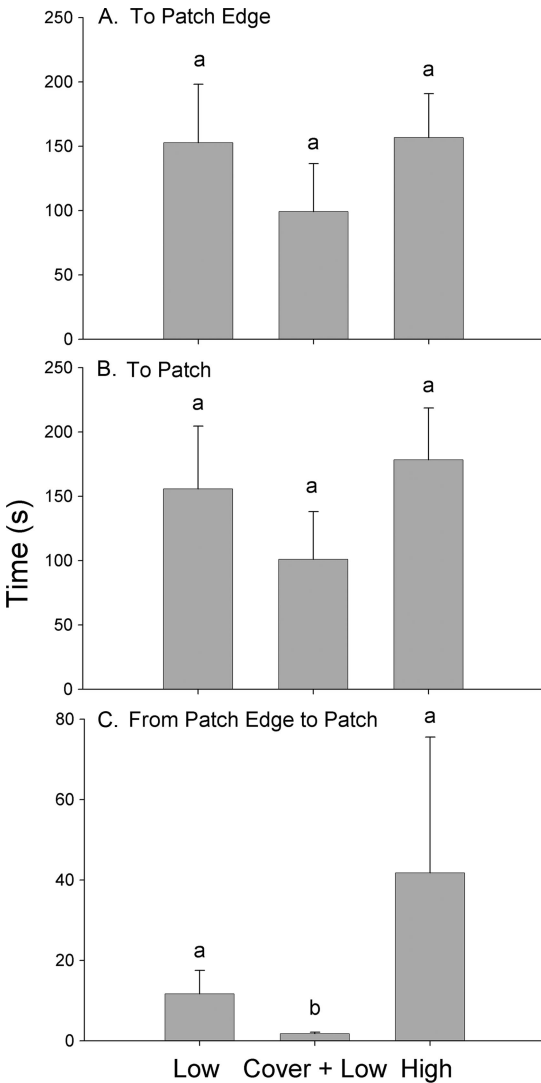


Fig. 6. Mean ± SEM for time (s) from start of experiment for red flour beetle (*T. castaneum*) to enter (A) patch edge zone, (B) patch, and (C) patch after entering the patch edge zone in response to patch treatments: low resource, cover with low resource, and high resource. Bars within a plot with the same letter are not significantly different (ANOVA on ranks, GLM procedure, $\alpha = 0.05$).

0.2437) and the patch itself ($F = 1.69$; $df = 2, 42$; $P = 0.1982$) was similar among patch treatments, although the trend was for time to be shorter in the covered patch treatment. However, once beetles entered the patch-edge zone of covered patches, they entered the patch more quickly than in other patch types ($F = 6.46$; $df = 2, 39$; $P = 0.0038$) (Fig. 6C). More time was spent at the edge of the high-resource patch (13%) than at the edge of the covered patch (1%) ($P = 0.01$). Time spent at the edge of the high-resource patch was similar to that at the edge of the low-resource patch ($P = 0.446$), but was more variable: time spent in the high-patch-edge zone ranged from 1.8 to 209.2 s, and

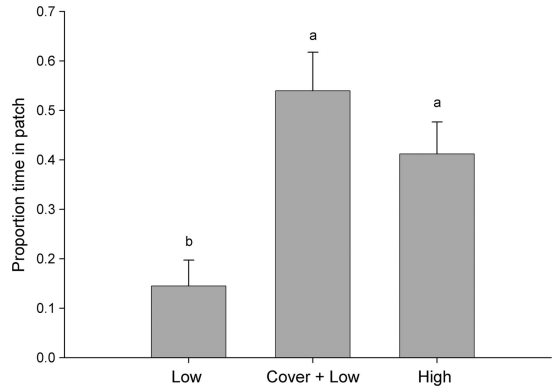


Fig. 7. Mean ± SEM of proportion of time spent in patch by red flour beetle (*T. castaneum*) in response to the three patch treatments of low resource, cover with low resource, and high resource. Bars with the same letter are not significantly different (ANOVA on ranks, GLM procedure, $\alpha = 0.05$).

time spent in the low-patch-edge zone ranged from 1.8 to 42.0 s. After entering a resource patch, beetles spent less time in low-resource patches than in covered (39% less time, $P = 0.0015$) and high-resource (26% less time, $P = 0.024$) patches (Fig. 7).

Patch Permeability. When beetles first encountered the patch edge from within the matrix (Table 1), patch edges with a low amount of resource or a covered patch with a low amount of resource were initially more permeable to beetles than patches with a high-resource amount (Pearson's exact test, $\chi^2 = 17.799$, $df = 2$, $P < 0.0001$) (Table 1). There were more total encounters with the edge of the high-resource patch (3.9 encounters/individual) than with covered (2.45/individual) or low-resource (2.85/individual) patches. When the total encounters with the patch edge were calculated for each treatment, significantly fewer encounters with the high-resource patch edge resulted in the beetle entering the patch, compared with the low-resource and covered patches (Pearson's exact test, $\chi^2 = 16.677$, $df = 2$, $P < 0.0001$). For beetles approaching the patch edge from the interior of the patch, there was a greater probability of exiting a low-resource patch (79%) than exiting a high-re-

Table 1. Probabilities that red flour beetles (*Tribolium castaneum*) enter a patch on the first encounter

Patch treatment	First encounter	Cumulative encounters			
	Entering	Entering		Exiting	
	%	n	%	n	%
Low	50	57	61	38	71
Cover	60	49	84	-	-
High	0	78	47	53	51

Treatments consist of a patch with a low amount of resource, a cover and a low amount of resource, and a high amount of resource. Also shown are probabilities of beetles entering and exiting the patch over cumulative encounters with the patch edge. Percentages are based on total number of encounters during a 10-min observation period ($n = 20$ beetles per treatment).

source patch (50%) (Pearson exact test, $\chi^2 = 16.677$, $df = 2$, $P = <0.0001$).

Discussion

Movement of insects in response to landscape structure (With et al. 1999, Goodwin and Fahrig 2002), including patch boundaries (Collinge and Palmer 2002, Haynes and Cronin 2006), can elucidate mechanisms underlying movement behaviors (With 1994a, b). In our first experiment, red flour beetles did not appear to perceive flour from a distance and move toward it. Our results support the findings of previous studies reporting that red flour beetles are not highly attracted to fresh flour or whole grains, unless they contain aggregation pheromone, are damaged or deteriorating, or are a source of water for desiccated beetles. Willis and Roth (1950) tested attraction by *T. castaneum* to fresh flour and found that attraction of beetles, starved in the absence of water, increased as the water content of the flour increased. Surprisingly, they found that attraction to fresh flour (10–12% water content) decreased with the number of days that beetles were starved in the presence of water. Phillips et al. (1993) reported that *T. castaneum* was attracted to a commercially prepared food product and to several different grain-derived oils, but not attracted to volatiles derived from whole grain. They concluded that *T. castaneum* was attracted to the oils and food product because the beetle prefers damaged or fungus-infected grains that have volatile components similar to these compounds. In addition, red flour beetles were more attracted to a combination of the commercial food product and a synthetic pheromone, 4,8-dimethyldecanal, than to either the food product or pheromone alone. In contrast to the current experiment, all studies mentioned above were conducted in small arenas with beetles and the food or pheromone cues within a relatively small area. Our data seem to suggest that dispersing red flour beetles use a ranging pattern of movement until they are arrested by pheromone and food volatile cues.

When held with food before trial, significantly more individuals moved upwind and encountered the patch (with and without food) than when no airflow was present. These data suggest two things about movement of red flour beetles. First, beetles orient their movement in relationship to air movement in both the presence and absence of flour. Schooley and Wiens (2003) showed that successful orientation by cactus bugs (*Chelinidia vittiger*; Hemiptera: Coreidae) to their host plant depended on patch size, structure of the intervening matrix, and the direction of the prevailing wind; but the bugs also oriented upwind even when outside of a patch network. Obeng-Ofori (1991) found that the behavioral responses of male *T. castaneum* to aggregation pheromone in an olfactometer were similar in the presence of still and moving air, but did not test beetle response to air movement in the absence of the pheromone. Insects predominantly use three orientation mechanisms: chemotaxis, kinesis, and anemotaxis (Shorey 1973), but the latter is con-

sidered the most important for orientation to distant odors (Bell and Kramer 1979, Kennedy 1978). Our data suggest that anemotaxis plays a role in the orientation response of red flour beetle.

Second, our data suggest that female beetles actively move upwind in the presence of airflow if they are physiologically ready to oviposit. The response we observed could be explained by the fact that the fed females, having been removed from conditioned flour and placed into fresh flour for 24 h before the experiment, were sufficiently released from oviposition inhibition and were actively dispersing and/or seeking resources for oviposition. Females that had been removed from conditioned flour, but deprived of fresh flour for 24 h before the experiment, may not have been sufficiently released from the effects of oviposition inhibition and, thus, were not in a dispersive state and/or actively seeking resources for oviposition. An individual's responsiveness to resource cues as well as their general movement behavior may change depending on the activity in which they are engaged. McIntyre and Wiens (1999) found that in landscapes with randomly distributed food patches, food-deprived darkling beetles (*Eleodes extricata* Say, Coleoptera: Tenebrionidae) turned more frequently over a larger distance than fed beetles. In landscapes with clumped or uniformly distributed food resources, food-deprived *E. extricata* moved over a smaller area, a behavior indicative of area-restricted foraging (Bell 1991). Morales and Ellner (2002) reported that the confused flour beetle (*Tribolium confusum*; Coleoptera: Tenebrionidae), in experimental microlandscapes, alternated between exploratory behavior (with a high variance in turning angles) to behavior typical of escape from an unsuitable environment (linear movement), which influenced their rate of spatial spread. Results of research examining patch finding and orientation to resource patches in insects have been mixed and indicate that species may vary widely in their search strategies. *Trirhabda borealis* (Coleoptera: Galerucinae), a beetle specializing on goldenrod, exhibited no significant orientation toward goldenrod when released up to a meter away from the plants (Goodwin and Fahrig 2002). However, McIntyre and Vaughn (1997) reported that darkling beetles (*E. extricate* and *Eleodes hispilabris*; Coleoptera: Tenebrionidae) oriented to food by olfaction, over distances up to 80 cm. Many insects, including red flour beetle, appear to have considerable flexibility in their movement behavior. A single mechanism may be insufficient to explain their movement; integration of multiple mechanisms, in response to the context of exogenous and endogenous cues, is probably necessary for insects to find needed resources and must be considered to accurately explain observed movement responses.

If a species has relatively good dispersal abilities, but limited long-distance perceptual abilities, then information about the quality and location of available resources will be limited as they move about in a landscape, and patches may be found only through direct contact. Our previous research demonstrated

that search tactics of red flour beetles appear to be significantly influenced by their physical interactions with patch edges. In landscapes where resource patches are close together (<5 cm, with a higher amount of patch edge), they engaged in fine-scale foraging behavior (more convoluted pathways with less displacement); however, in landscapes where patches were farther apart (>12 cm, with a lower amount of patch edge), they engaged in a broader scale searching behavior, indicative of a ranging strategy (more linear pathways with greater net displacement) (Romero et al. 2009). To further determine whether beetles are able to evaluate patches before actually entering them, we focused on how movement pathways and permeability of patch boundaries might differ in response to variation in patch quality. In our experiment, changes in beetle movement indicated that beetles responded to boundaries and evaluated patch quality only when <1 cm from the patch. These results further suggest that covered patches were assessed visually as a shelter very rapidly once beetles were close to the patch. Willis and Roth (1950) mentioned that large numbers of red flour beetles aggregated in unbaited corrugated cardboard traps in their laboratory, and Good (1936) observed that adults and larvae will conceal themselves under any suitable object that is near food. The importance of cover in patch selection has been reported for the pine weevil, *Hyplobius abietis* (Coleoptera: Curculionidae), which preferred shelters that had opaque rather than transparent covers (Björklund 2008). Beetles in our study most probably entered patches with covers more quickly than those without because this type of patch simultaneously supplied two primary resources for the insects: food and shelter.

It is clear that cover, supplying beetles with lower light or more thigmotactic stimuli, played a significant role in both permeability and residence time. Long residency times indicate that covered patches were the most preferred of all tested patch types, even though the amount of food resource contained in the patch was less than the high-resource patch, albeit similar to the low-resource patch. A preference for cover and/or a propensity to move in covered areas has been reported for a carabid beetle, *Alba parallelipipedus*, even when covered areas offered more resistance to movement (Charrier et al. 1997). Several studies with other animal taxa have indicated that, initially, individuals may choose patches affording cover, but that emigration out of these patches is higher over time than patches containing higher quality resources (Chan-McLeod and Moy 2007, Hogberg et al. 2002, Tittler et al. 2001).

We took into account that the difference between edge characteristics of the two uncovered resource patches could also affect permeability in ways not related to the amount of resource. The edge of the high-resource patch was ≈ 4 times higher than the edge of the low-resource patch (≈ 1 and ≈ 0.25 mm, respectively). Upon first encounter with a high-resource patch, not one beetle entered, indicating that edge structure (vertically higher) may have initially

created a barrier to movement. However, additional encounters with the high-resource patch ultimately increased apparent permeability (i.e., beetles were ultimately more likely to enter high-resource than low-resource patches). Delaying entry into patches could have fitness consequences for dispersers because they may lose contact with a potentially high-quality patch; but if a disperser maintains contact with the patch edge (visually, tactically, or by olfaction), this cost could be lessened.

Although low-resource patches were initially entered more frequently than high-resource patches, beetles ultimately left these patches more frequently than high-resource patches, resulting in asymmetrical permeability (Wiens 1992). Fewer transitions out of the high-resource and covered patches resulted in longer residence times in both of these patch types. Once inside the high-resource patch, higher viscosity (resistance to movement) of the deeper flour may have retained beetles; or they may have preferred the high-resource patch because it afforded both more resource and protection than the low-resource patch, providing more fitness benefits in terms of food for offspring and protection from predation and desiccation. It is possible that beetles could have marked the high-resource patch with aggregation pheromone during initial contact, which would influence their acceptance during subsequent encounters. Over longer observation periods, the residence time of red flour beetles in high-resource patches could be greater than in covered (but low-resource) patches, although at the end of our observation period (10 min) the two patch types had similar retention.

Insects must efficiently locate and accurately assess resources to maximize their fitness and achieve population growth. In insect pests, behavioral traits associated with dispersal and resource use are usually highly developed and are a major factor in their pest status. By focusing on these traits, through experimental manipulation of various exogenous and endogenous factors, mechanisms that pest insects use to locate and choose resources may be elucidated. Results of our research highlight several aspects of movement behavior of female red flour beetle, but more information is needed, specifically how endogenous and exogenous cues interact to influence orientation to resources. One of the major goals of pest management is to control movement of insects into crops or commodities. Therefore, understanding movement behavior is necessary if effective monitoring, biological control, and integrated pest management systems are to be developed.

Acknowledgments

We thank David Margolies and Matt Grieshop for reviewing earlier drafts of this manuscript. This work was supported in part by United States Department of Agriculture/Cooperative State Research, Education, and Extension Service (Risk Assessment and Mitigation Program) under Agreement 2005-51101-02358. This is Contribution 09-046-J of the Kansas Agricultural Experiment Station, Kansas State University (Manhattan, KS).

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Received 28 October 2009; accepted 7 March 2010.
