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Influence of landscape pattern in flour residue amount and distribution on *Tribolium castaneum* (Herbst) response to traps baited with pheromone and kairomone

James F. Campbell*

USDA, Agricultural Research Service, Center for Grain and Animal Health Research, 1515 College Ave, Manhattan, KS 66502, USA

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ABSTRACT

Tribolium castaneum (Herbst), the red flour beetle, is a major pest of food facilities and is typically monitored using traps that capture walking individuals. In flour mills the accumulation of residues of flour on surfaces has the potential to influence beetle movement and response to trap attractants. Different flour residue landscapes of habitat (flour) within a matrix of no flour with the following characteristics were created: low abundance (10% coverage) and fragmented distribution, high abundance (30% coverage) and fragmented distribution, low abundance and clumped distribution, high abundance and clumped distribution, 0% coverage, and 100% coverage. Response of individual beetles to traps placed on top of these landscapes was evaluated; traps were either baited with aggregation pheromone and kairomone or contained no attractants. Encounters with the two types of traps were not significantly different for any of the specific landscapes, but greater numbers tended to encounter traps with attractants on fragmented landscapes and 100% flour landscapes. Combining landscape types, the proportion of beetles encountering pheromone + kairomone-baited traps (0.61) was not greater than the proportion encountering empty traps (0.50). However, when combining just the fragmented landscapes there was a significantly greater response to traps with attractants (78%) than traps without (50%), but no difference in response on the combined clumped landscapes. Movement pathways, analyzed using video recordings of beetles, showed a general trend for 0% and 100% habitat landscapes to be most different from each other and 10% and 30% habitat landscapes tended to group together, but only for maximum distance traveled in an interval, velocity, and mean turn angle were significant differences observed. Results suggest that fragmented landscapes may have some impact on beetle response to attractants, but a potential mechanism for this needs further evaluation.

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1. Introduction

Tribolium castaneum (Herbst), the red flour beetle, is a major pest of food facilities, such as mills, manufacturing plants, warehouses, and retail stores (Campbell et al., 2010). Traps used to monitor *T. castaneum* inside food facilities are typically pitfall designs that capture walking individuals and are baited with aggregation pheromone and food-based kairomone lures (Burkholder, 1990; Chambers, 1990; Mullen, 1992; Phillips, 1997; Phillips et al., 2000; Campbell, 2012). How *T. castaneum* respond to commercially available traps and attractants may be influenced by the landscape of physical and environmental conditions at the locations where traps are placed inside a food facility. Semeao et al. (2012) found that trap locations within a flour mill with larger captures tended to have greater flour dust accumulation, higher temperatures, and closer proximity to milling equipment. Romero et al. (2009) found that *T. castaneum* movement patterns were influenced by the distribution pattern of flour accumulations. Presence of food in the environment has also been shown to reduce *T. castaneum* captures in traps (Stejskal, 1995). These findings suggest that accumulation of food material such as flour on surfaces might influence how beetles interact with traps placed out to monitor them. If this is true it could influence the effectiveness of pheromone + kairomone-baited traps and impact how captures should be interpreted in different types of environments.

During milling and other manufacturing processes, fine particles of grain-based material can be produced and released into the air and these particles can settle and accumulate on surfaces. In

* Tel.: +1 785 776 2717; fax: +1 785 537 5584.
E-mail address: james.campbell@ars.usda.gov.

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addition, grain-based material can be released onto surfaces during equipment operation, mechanical failures, or cleaning, and result in spillage accumulations on surfaces. The milling of wheat kernels into flour is a process that especially tends to produce a large amount of flour dust and spillage, and accumulations of this material can be an important resource supporting populations of *T. castaneum* within flour mills. Housekeeping to remove these accumulations of food material at regular intervals is an important component of a food facility sanitation program, but at any given time surfaces may have a residue of food material. This food residue can directly impact insect captures since it can coat sticky surfaces or fill pitfall traps and therefore reduce trap efficacy. The development of covers for pitfall traps used in food facilities is a result of this issue. However, food residues may also indirectly impact captures in traps through their influence on insect movement (Romero et al., 2009) and nutritional status (Fedina and Lewis, 2007).

Landscape structure, the amount and distribution of a habitat type, can have an impact on an organism's foraging behavior. The residue of food material on surfaces within a food processing facility, which can be considered as habitat for *T. castaneum*, can have its own spatial structure of presence and absence and depth of accumulations (Semeao et al., 2012). Neutral landscape models have been used to investigate how *T. castaneum* respond to spatial pattern in habitat abundance and distribution, and shown that beetles move more slowly and tortuously as the individual patches of flour habitat change from clumped to fragmented in distribution and as a result beetles tend to remain longer within individual patches of flour and on landscape as a whole (Romero et al., 2009). Thickness of flour residue patches also influenced *T. castaneum* movement, particularly in terms of movement across patch edges and time spent in patches of flour (Romero et al., 2010). Given that flour residue pattern influences beetle movement, as revealed using neutral landscape models, it may also influence the probability of beetles encountering monitoring traps when placed in food facilities.

Walking *T. castaneum* exhibit a behavioral response to food and aggregation pheromone, and traps targeting walking individuals typically use a combination of these two types of attractants. The aggregation pheromone, 4,8-dimethyldecanal, is produced by feeding males and is attractive to both sexes (Suzuki, 1980; Suzuki et al., 1984). The pheromone occurs in four different forms and is released by males at a 4:4:1:1 [(4*R*,8*R*):(4*R*,8*S*):(4*S*,8*R*):(4*S*,8*S*)] ratio (Lu et al., 2011). Multiple studies have evaluated how *T. castaneum*, and *Tribolium confusum* Jacquelin DuVal which shares the same pheromone (Suzuki and Sugawara, 1979), respond to pheromone (Sokoloff, 1974; Ryan and O'Ceallachain, 1976; O'Ceallachain and Ryan, 1977; Levinson and Mori, 1983; Boake and Wade, 1984; Barak and Burkholder, 1985; Obeng-Ofori and Coaker, 1990; Obeng-Ofori, 1991; Lewis and Austad, 1994; Olsson et al., 2006; Verheggen et al., 2007; Duehl et al., 2011; Campbell, 2012). *Tribolium castaneum* and *T. confusum* also respond to food odors (Willis and Roth, 1950; Phillips et al., 1993), but attraction to flour appears to be negligible (Hughes, 1982; Romero et al., 2010). The response to pheromones by *T. castaneum* can be increased by addition of food odors, but the increase in response appears limited (Phillips et al., 1993; Campbell, 2012). However, these studies have only evaluated beetle response under simplified conditions where there was little potential for landscape features and competing attractants to influence the strength of the response.

The food volatile, gustatory, and physical cues associated with a landscape containing flour could impact *Tribolium* spp. response to pheromone and kairomone attractants in traps, but the nature of this impact is difficult to predict. Flour accumulations may enhance captures through the interaction of pheromone and food odors increasing attraction or through inducing a more tortuous search path that retains beetles in the vicinity of the trap. Alternatively, flour accumulation could reduce captures in traps by reducing probability of encounter either by presenting competing attractants or by limiting beetle dispersal distance. Campbell (2012) described an experimental approach for evaluating response to traps that provides a realistic evaluation of the strength of insect response under simulated field conditions. A modification of that approach is used here to assess how flour residue pattern influences beetle response to pheromone + kairomone-baited traps, using neutral landscape models of flour distribution originally developed by Romero et al. (2009). Still air conditions were used in this study, even though attraction to traps is stronger under moving air conditions (Campbell, 2012), so that results could be more directly compared to conditions in Romero et al. (2009) and because this represents conditions typical of trap locations in flour mills.

2. Materials and methods

Tribolium castaneum originally collected from a flour mill within two years of conducting the study, was maintained on wheat flour and brewers yeast (5% by weight) in an incubator set at 25 °C, 65% rh, and 14:10 light:dark cycle. Beetles between two and three weeks after adult emergence were collected for use in experiments. Beetles were transferred individually to 30 ml clear plastic cups (Jet Plastica Industries, Hatfield, PA) that contained flour and cracked wheat sufficient to just cover the bottom of the cup. Beetles were held for approximately 48 h in an incubator under conditions described above prior to start of experiments.

The experimental arena simulated the floor/wall junction along which traps are typically placed when used for monitoring. The arena consisted of a floor made from a 61 cm by 61 cm piece of particle board that had been spray painted white and a wall made from a piece of white laminated board (61 cm long × 20 cm wide × 1.5 cm thick). The smooth laminate coating on the wallboard prevented beetles from climbing. The floor was covered with a piece of white paper cut large enough to extend slightly over the sides of the floor. Then the wall was placed standing on its side perpendicular to the floor, and aligned along north edge of floor. Tape was used to mark off a 50 cm by 45 cm observation zone, with one 50 cm edge being the wall.

Different flour residue landscapes (50 × 50 cm) of habitat (unbleached white flour) and matrix (no flour) developed in Romero et al. (2009) were used in this experiment. Experimental landscapes consisted of two grain sizes (2 × 2 and 10 × 10 cm) within two levels of habitat abundance (10 and 30% coverage of landscape), with four different random maps created for each abundance by grain size combination using RULE software program (Gardner, 1999). Holding the landscape extent constant while varying grain size produced landscapes with different degrees of habitat aggregation (i.e., fragmented and clumped), with specific landscape characteristics described in detail in Romero et al. (2009). Additional landscapes with 0% and 100% flour were also included in experiment. Heavy cardstock templates of each landscape pattern with cutouts for cells with flour were used to create these patterns of flour habitat patches (see Romero et al. (2009) for detailed description). The appropriate cardboard template for a given treatment was aligned next to the wall in the experimental area and centered within observation zone. Flour was applied evenly over the template and paper covering the floor to a depth of approximately 1 mm using a 60 mesh sieve to distribute the flour. The template was then removed to create the pattern of flour and matrix on the floor. Between each experimental replication the paper and flour were removed and replaced with a new piece of paper and a new flour landscape pattern.