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Response of *Tribolium castaneum* and *Tribolium confusum* adults to vertical black shapes and its potential to improve trap capture

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ABSTRACT

Tribolium castaneum and *Tribolium confusum* can be monitored in food processing facilities using traps baited with pheromones and kairomones, but beetle response to traps might be enhanced by adding visual cues. Against a white background, *T. castaneum* adults were more likely to visit black pillars than white pillars when presented with a choice (e.g., 73% of beetles visited black and 17% visiting white pillar), and visits to black pillars increased with pillar height. When tested against a black background, beetles did not show a significant preference for either color pillar regardless of height. When comparing beetle's captures in pheromone/kairomone baited traps placed in front of a white or black panel in a white arena under high, low, or dark light conditions, more beetles were captured in traps in front of black panels under both high and low light conditions, but not under dark conditions. A similar pattern of capture under low light and dark conditions was also found for the closely related species *T. confusum*. In a larger scale choice test, the same pattern of greater *T. castaneum* captures in traps in front of black panels than white panels was obtained, whether traps were placed in corners or along walls. Our results suggest that captures in monitoring traps could be increased by adding dark vertical shapes behind trap locations or placing traps near dark structures.

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1. Introduction

Integrated pest management of stored-product insects in food processing facilities relies on monitoring data to guide management decisions and to evaluate the effectiveness of the program (Barak et al., 1990; Burkholder, 1990). Often this monitoring data is generated using pheromone and/or kairomone baited traps that rely on insect olfaction to facilitate captures, but typically don't exploit other sensory modalities such as vision (Chambers, 1990). However, in other systems color preferences by economically important insects have been demonstrated (Southwood, 1978; Prokopy and Owens, 1983), and visual cues, typically attractive colors and trap shapes, have been exploited to monitor and manage insect pests (e.g., Hoback et al., 1999; Strom and Goyer, 2001; Athanassiou et al., 2004). Although response to color and shape by stored-product insects is relatively poorly understood, traps that combine olfactory cues such as pheromones and kairomones with visual cues may offer the potential to increase captures of stored-product insects.

Exploitation of visual signals in insect monitoring programs fall into three general categories: lights that attract insects, colored objects that are attractive because of their specific reflectance, and shapes or silhouettes that stand out against a contrasting background. Pinero et al. (2006) found that female melon flies (*Bactrocera cucurbitae* (Coquillett)) were primarily attracted to objects of hemispherical shape associated with either yellow, white, or orange pigments. Mainali and Lim (2010) found that western flower thrips (*Frankliniella occidentalis* (Pergande)) were more attracted to flat yellow sticky traps on a black background and suggested that the black background may help the insect to perceive reflectance with minimal interception from other sources of reflectance. There is considerable variation among insect species in their color preference, but attractive colors typically resemble suitable habitats for mating, oviposition, or feeding (e.g., Hoback et al., 1999; Cilek, 2003; Döring et al., 2004; López-Guillén et al., 2009). For example, many herbivorous insects respond to the color yellow or white, which corresponds to the peak color reflectance of plants (Prokopy and Owens, 1983). Insect species that attack trees such as bark beetles respond to vertical trunk silhouettes for landing and short range orientation, and traps that provide appropriate visual silhouettes are usually more efficient (Lanier, 1983; Finch and Collier, 2000).

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Tribolium castaneum (Herbst), red flour beetle, and *Tribolium confusum* Jacquelin du Val, confused flour beetle, (Coleoptera: Tenebrionidae) are major pests of food processing facilities, especially wheat and rice mills, and walking beetles can be monitored using traps based on a pitfall design (e.g., Mullen, 1992) and baited with pheromone and kairomone attractants. These traps have been used successfully to monitor pest populations of *T. castaneum* (e.g., Campbell et al., 2010), but the level of attraction by flour beetles to these baited traps is widely, although anecdotally, reported as relatively weak. Reza and Parween (2006) showed that *T. castaneum* larvae and adults differed in their tendency to aggregate on different colors when presented with a choice between different color surfaces. Of the colors tested in this study, adults of *T. castaneum* exhibited a tendency to aggregate only on black surfaces. Here we evaluate if adult *T. castaneum* are actually attracted to the color black and if the height of the black shape impacts the level of attraction. We also evaluate if these black shapes when combined with a commercially available pheromone/kairomone baited trap can increase the effectiveness of these traps at capturing beetles. Finally, we confirmed if *T. confusum* exhibited a similar increase in trap captures with black shapes.

2. Materials and methods

2.1. Experimental conditions

Tribolium castaneum and *T. confusum* adults used in the experiments were obtained from laboratory colonies maintained in 0.94 l glass jars containing wheat flour fortified with 5% (by weight) brewer's yeast (ICN Biomedicals, Aurora, OH). Both colonies were established within 10 months before experiments were conducted by mixing ~100 adults collected from food facilities in Kansas, USA. The colonies were maintained at 28.0 ± 0.5 °C, 60.0 ± 5.0% r.h. and 14:10 light:dark cycle (mean ± SE). Only adults less than two months old were used in experiments and collected the same day as experiments were conducted. Beetles were held for at least 30 min in Petri dishes without food inside the experimental chambers prior to the start of the experiment. Individual beetles were only used once in an experiment.

Experiment 1 was conducted inside a chamber (4.7 m long by 4.7 m wide by 2.4 m tall) located in a room without controlled temperature and r.h. and where lighting resulted primarily from natural sunlight. To reduce environmental variability all replicates were performed between 1300 h and 1600 h. The mean ± SE environmental conditions in chamber during experiments were 21.3 ± 0.4 °C air temperature, 22.7 ± 0.8% r.h., and 380.5 ± 2.3 lux light intensity. All subsequent experiments were conducted in an environmental chamber (6.1 m long by 4.9 m wide by 2.4 m tall) held at 25.0 ± 0.0 °C, 66.5 ± 0.0% r.h. and 24 h light (214.9 ± 0.3 lux). Temperature and r.h. were measured using either a data logger (HOBO® H8 family, Onset Computer Corp., Pocasset, MA) or handheld meter (Kestrel® 3000, Nielsen-Kellerman, Boothwyn, PA), and light intensity was monitored using light meter (Model EA33, Extech Instruments Corporation, Waltham, MA).

2.2. Experiment 1: are *T. castaneum* adults attracted to black shapes?

Beetle response when presented with a choice between black or white pillars of different heights, when the background color was either black or white, was evaluated. This enables us to determine if beetle response was due to the dark shape or just due to contrast with the background. An experimental arena was constructed inside chamber described above from four foam core boards (1 × 1 m squares) taped together to form a cube, with the top open and the floor of the chamber forming the floor of the box. Depending on the treatment conditions, the inside of the box was covered with either white or black paper (Art Kraft Duo-Finish paper, C2F Inc., Beaverton, OR). We created three different types of cuboids (pillars) out of corrugated cardboard with dimensions of 14 × 14 cm and height of either: 2 cm (short), 14 cm (medium), or 33 cm (tall). The heights of the pillars were selected to give a range of heights, with the tallest pillar height predicted to be perceived by the beetle as an extremely tall shape given the size of the beetle and the release distance used in the first experiment. The pillars were covered with the same type of paper used inside the box, either black or white depending on the treatment conditions.

At the center of the arena floor, a square (50 × 50 cm) was marked off with a pencil and this was the observation zone for recording beetle behavior. In each replicate, we placed one black pillar and one white pillar of the same height at opposite sides of the square marked on the floor (adjacent to the line but outside the observation zone). Individual *T. castaneum* adults were placed inside 1.5 mL centrifuge tubes, acclimated under conditions in arena prior to release, and then released by placing an opened and inverted tube at the center of the observation zone and removing the tube after beetle inside was observed to upright and walking. After release, the time it took for either of the following events to occur was recorded: reach the edge of the observation zone, reach the black pillar, or reach the white pillar. Observations were terminated after either of these three events occurred or if 300 s had elapsed. Replications consisted of individual beetles exposed to pairs of pillars of the same height against either a black or a white background. Within, first the white and then the black arena color, the order of the pillar height pairs was completely randomized, and within each height the orientation of the pillars was alternated (North–South and East–West orientations). A total of 25 replicates of each treatment were performed against the black background, and a total of 30 replicates of each treatment, except for one treatment that had 31 replicates, were performed against the white background.

Data was analyzed in SAS version 9.1.2 (SAS Institute, Cary NC) using contingency table analysis (PROC FREQ). For each color background, the contingency tables had 3 rows (pillar height) and three columns (beetles reach edge, reach black pillar, reach white pillar). From a total of 166 beetles tested, only 3 beetles remained in the observation zone for the 300 s and they were excluded from analysis. Chi-square test was used for testing the null hypothesis that the size of the pillars did not affect the events and to evaluate differences in the number of beetles reaching black and white pillars.

2.3. Experiment 2: how is the movement behavior of *T. castaneum* impacted by black pillars of different heights?

Movement pathways of individual beetles when exposed to pillars of different colors and heights were evaluated in a no-choice experiment. Individual beetles were observed in a wood arena (120 cm long by 120 cm wide and 23.5 cm tall) painted white (Zinsser Bulls Eye 1–2–3 primer, Rustoleum, Vernon Hills, IL) and held in chamber and under environmental conditions described above. The wood arena was covered by a box made of white paper (Art Kraft Duo-Finish paper) (120 cm long by 120 cm wide by 130 cm tall) with an observation hole cut in the center of the top through which a video camera could be inserted. This paper box enabled a video camera to be placed at the necessary height above the floor of the arena to record beetles and also provided a monochromatic white environment in which to observe response to black shape. The observation zone inside the wood arena in which activity of individual beetles was recorded was 50 × 50 cm.