Journal of Stored Products Research 54 (2013) 17–22

Contents lists available at SciVerse ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Movement of *Tribolium castaneum* within a flour mill

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ARTICLE INFO

Article history:
Accepted 15 March 2013

Keywords:
Red flour beetle
Flour mill
Mark-recapture
Dispersal
Heat treatment

ABSTRACT

The colonization of food processing plants by stored-product pests and their distribution within a facility depend, in part, on their dispersal ability. In this case study, we relied on self-mark recapture to evaluate the ability of *Tribolium castaneum*, the red flour beetle, to move among floors within a flour mill and the effects of a heat treatment on insect activity. Marking stations with pheromone and fluorescent powder were placed on each of five floors in the mill, and two techniques were used to recover marked individuals (trapping and direct collection of individuals from the floor). Considering both recovery techniques, *T. castaneum* was able to move among floors, but the majority of individuals remained on the same floor where they were marked (86%). Most individuals captured on a different floor were captured on a floor below the one they were marked (70%) and adjacent to it (87%). There was a spike in the number of beetles captured during heat treatment, but not an increase in movement of marked beetles between floors. These results suggest that the rate of heating was sufficient to prevent beetles time to move to cooler floors to escape heat. *T. castaneum* movement among floors needs to be taken into account when identifying sources of infestation and targeting pest management.

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1. Introduction

Stored-product insects associated with food processing facilities inhabit an environment made up of resource patches that are isolated and discontinuous, both within structures and within the broader urban and/or rural landscape (Campbell, 2005). The ability to move through this environment and to find and colonize these resource patches contributes to their reproductive success in food facilities and their economic impact as a pest. As a result of this environment and behavior interaction, populations of stored-product insects in many food processing facilities are spatially and temporally patchy in distribution. Understanding these patterns of distribution and movement can improve the implementation and interpretation of monitoring programs and the targeting of pest management. A number of studies have evaluated the spatial distribution of insect captures in traps (Arbogast et al., 2000; Strother and Steelman, 2001; Campbell et al., 2002; Trematerra and Sciarretta, 2004; Trematerra et al., 2007), but those evaluating patterns of movement are much more limited.

Stored-product pests are readily trapped outside of grain storage and processing structures and often far away from anthropogenic structures (Sinclair and Haddrell, 1985; Throne and Cline, 1989; Fields et al., 1993; Dowdy and McGaughey, 1994; Doud and Phillips, 2000). This suggests that they are highly mobile in outside environments and capable of long-distance flight, but since dispersal was not directly measured these captures may also indicate feral populations in close proximity to the traps. Mark recapture techniques have been used to measure dispersal of stored-product insects: lesser grain borer, *Rhyzopertha dominica* (F.), was shown to be a strong flyer and capable of dispersing considerable distances (Campbell et al., 2006; Mahroof et al., 2010); warehouse beetle, *Trogoderma variabile* Ballion, and Indianmeal moth, *Plodia interpunctella* (Hübner) were found to be highly mobile outside of a food processing facility (Campbell and Mullen, 2004); and Indianmeal moth was demonstrated to move into a flour mill (Campbell and Arbogast, 2004). Evaluation of movement patterns inside facilities is typically more difficult to perform, in part because of the limited numbers available to self-mark and restrictions on releasing insects. Campbell et al. (2002) used self-mark recapture to measure *T. variabile* mobility within a processing plant, and documented beetles moving across multiple floors and up to 216 m within a warehouse.

Tribolium castaneum (Herbst) is a major pest of stored grain, cereal products, and other stored commodities. This species is often found in environments where grain and grain-based products are processed and stored (Sinclair and Haddrell, 1985;

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0022-474X/$ – see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.jspr.2013.03.004

Arbogast et al., 2000; Campbell and Arbogast, 2004). Flour mills are favored habitats because the milling process produces large amounts of flour and other byproducts that can accumulate inside equipment, on surfaces, and inside cracks and voids in the building structure. Favorable temperature conditions for development and movement are also maintained inside mills throughout the year (Campbell et al., 2010). *T. castaneum* do appear to have patchy distributions within mills (Semeao et al., 2012a), but since this species appears to disperse primarily by walking, their dispersal ability is not well understood. Flight can be initiated throughout the adult stage, but conditions such as low food or lack of conspecifics can trigger greater flight initiation (Perez-Mendoza et al., 2011a,b). While *T. castaneum* is not often observed flying inside flour mills, regular patterns of flight activity have been reported in food facilities (Ho and Boon, 1995). Ecological and molecular techniques have also been used to indicate that beetles do leave storage locations and disperse into surrounding areas (Ridley et al., 2011). Outside wheat flour mills *T. castaneum* abundance tends to be reduced, but beetles are regularly captured at low levels in outside traps targeting both walking and flying individuals (Campbell and Arbogast, 2004; Semeao et al., 2013). Semeao et al. (2012a) reported that the spatial distribution pattern of *T. castaneum* captures across floors of a flour mill changed over time, which suggests either shifts in the distribution of infestation or an ability to move among floors. Campbell et al. (2002) attempted to use mark recapture to measure *T. castaneum* dispersal, but was not successful in recovering marked beetles, presumably because densities were low.

Understanding the level of dispersal of *T. castaneum* within flour mills, particularly in terms of movement between floors, would assist in determining the spatial extent of populations, the potential for invasion and colonization, and interpreting patterns in pheromone trap capture. Here we present the results of an experiment using self-mark recapture to evaluate the ability of *T. castaneum* to move among floors within a flour mill. We took advantage of a situation where the population was large and appeared primarily confined to the inside of the mill since this would facilitate the recapture of marked beetles. In response to the large number of beetles, a heat treatment was applied during the course of monitoring. This provided an additional opportunity to measure if the increased insect activity associated with the heating up of the building increases beetle movement among floors, potentially enabling them to avoid lethal temperatures.

2. Materials and methods

2.1. Study site

This study was conducted at the pilot-scale Hall Ross flour mill at Kansas State University. The mill is a concrete structure composed of five floors (364 m^2 each floor). The mill is a tightly sealed structure and at the time of the study had an infestation of *T. castaneum* inside the mill. Given the tightness of the structure and the findings of Semeao et al. (2012a) that very few adults were captured in traps placed outside the mill this infestation is considered to represent a relatively closed population within the mill. Monitoring and a self-mark recapture study were conducted within the mill between July 28 and October 10, 2008. On each floor, four self-marking stations and five recapture traps were placed at locations chosen based on proximity to pieces of milling equipment or potential to be encountered by individuals walking across the floor. Five recapture traps were also placed along walls outside the mill. Temperature and relative humidity on each floor of the mill were monitored throughout the study using HOBO data loggers (Onset Computers, Pocasset MA). Average temperature and relative humidity during the monitoring period were 27.03 ± 0.02 °C and 37.27 ± 0.04%, respectively.

2.2. Self-marking stations

Self-marking stations were constructed using traps described in Semeao et al. (2013), which were a modified corrugated cardboard trap design (Likhayo and Hodges, 2000). Briefly, these stations consisted of two layers of corrugated plastic held between two metal plates arranged to form a 9 × 9 cm square and containing a 3 × 3 cm open space in the center. Approximately 2 g of fluorescent powder (Day-Glo Color, Cleveland, OH) and one *Tribolium* spp. pheromone lure (Trécé, Adair OK) was placed in the open center of each marking station. Marking stations on the same floor were filled with the same color fluorescent powder, but each floor had a unique color [1st floor: green (Signal Green pigment, A-18-N); 2nd floor: yellow (Saturn Yellow pigment, A-17-N); 3rd floor: orange (Fire Orange pigment, AX-14-N); 4th floor: blue (Horizon Blue pigment, A-19) and 5th floor: magenta (Corona Magenta pigment, A-21)]. On August 29, 2008 all marking stations were removed from the mill but the recapture traps were left in place until October 10, 2008.

2.3. Recapture traps

Recapture traps were Dome™ pitfall traps (Trécé, Adair, OK, USA) containing a pheromone lure for *Tribolium* spp. attached to the underside of the lid. The interior floor of the trap bottom, instead of containing a food based oil as is usually added, was coated with Tangle-Trap sticky coating (Tangle Foot Co., Grand Rapids, MI). The sticky coating was used to restrain the captured insects to avoid cross contamination of marking powder and to avoid the marking powder migrating into the kairomone oil. While the kairomone oil does increase insect response to the trap, the pheromone contributes to the majority of the insect response (Campbell, 2012). Traps were changed every week, except as noted below. The traps were taken to the laboratory where beetles were inspected under long wave (365 nm) ultraviolet light (Black Ray Lamp Model UVL-21, UVP, Upland, CA) for the presence of fluorescent powder. Observations were made under a dissecting microscope (Wild M3Z, Heerbrugg, Switzerland) at 40× magnification to detect small amounts of the fluorescent powder.

2.4. Heat treatment

A heat treatment was applied to the flour mill beginning at ~4 pm on August 13 and ending at ~1 am on August 17, 2008. Maximum temperatures achieved at data logger locations were 48 °C, 47 °C, 47 °C, 50 °C, 47 °C for 1st through 5th floors, respectively, and were reached during the evening of August 16 and held for 2, 3, 6, 4, 2 h, respectively (Fig. 1). All traps in the mill were replaced with new traps immediately before the beginning of the heat treatment and these traps were replaced immediately after the heat treatment finished.

2.5. Collection of dead beetles

Before and especially after the heat treatment, dead beetles were observed on the floors of the mill. Samples of these beetles were collected before and after the heat treatment, and presence or absence of fluorescent marking powder was determined as described above. Before the heat treatment, because of lower numbers, beetles were collected off the floor wherever they were observed. After the heat treatment, beetles were much more