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Design of an experimental setup to evaluate movement of adult insects in stored wheat

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Abstract

Stored grain losses, especially due to insect infestations, is one of the critical issues in preserving stored grains. In general, insects move inside stored grain bulks in search of suitable conditions for survival and multiplication. A complete understanding of the movement and distribution of insects inside stored grain bulks is of prime importance for developing proper management protocols. To analyze the movement of *Cryptolestes ferrugineus* (Stephens) and *Tribolium castaneum* (Herbst) inside wheat bulk, 0.1 m sized cubes were made using metal rods of 1.6 mm diameter. Polylactic acid corners fabricated using a 3D printer were used to fasten the metal rods. These cubes were covered by a cloth screen with an opening of 1.4×1.4 mm^2. To evaluate the effects of the cube and screen on the movement of *C. ferrugineus*, a test was conducted by arranging 9 cubes to form a cuboid of size 0.3 m x 0.3 m x 0.1 m. The cuboid was filled with wheat at 16.5% moisture content and 100 adults were released at the center of the cuboid. The movement was stopped after 24 h. The same experiment was repeated without mesh cubes. Similarly, the effects of the cube and screen on the movement of *T. castaneum* was evaluated using the same experimental set up. The mesh cubes had no significant effect on the movement of *C. ferrugineus* and *T. castaneum.*

Keywords: Movement, Distribution, *Cryptolestes ferrugineus, Tribolium castaneum ,* Wheat, Three-dimensional, Stored grain ecosystem, Insects, Management protocols, Mesh cubes

Introduction

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) are the major economical granivorous pests associated with the Western Canadian stored grain ecosystem (Hulasare et al., 2003; Madrid et al., 1990). These insects move inside stored grain bins for various physical and biological reasons. One- and two-dimensional movements of insects inside grain columns and square boxes having grains with different temperatures and moisture contents, have been well established (Jian et al., 2003, 2004a, b, 2005a, b, c, 2007).

Surtees (1964) proposed a method to analyse the three-dimensional dispersion pattern of various insect species including *Sitophilus granarius* (L.), *Oryzaephilus surinamensis* (L.), *T. castaneum*, *C. ferrugineus,* and *Rhyzopertha dominica* (F.). The method used 3-inch cube bags made of 10 mesh net. The bags filled with wheat were arranged inside a Perspex cube and the insects were introduced at the top. The movement of insects, after a particular time, was studied by taking out the bags and placing them in prenumbered jars and counting the insects at each location, after sieving them. The major drawback of this method was the time associated in arranging the cubes (2 to 3 h to construct 4 layers of 16 cubes per layer), because of the considerable care required for proper arrangement. To address this drawback, our study used metal rods forming a cube and covered with a cloth screen. These metal rods maintained the rigidity of the bags even after filled with grains, thus reducing the time taken to construct the three-dimensional grain bulk. The objective of the present study was to determine whether the designed mesh cubes could be used to study the movement of *C. ferrugineus* and *T. castaneum* in three dimensions by comparing them to the insect movement in grain bulk without the use of mesh cubes.

Materials and methods

Wheat

Canada Western Red Spring Wheat (Grade no.1, cv. 'AC Barrie' certified) of moisture content 16.5 ± 0.1% was used in this study. A standard oven drying method was used to determine the moisture content of the wheat by drying 10 g samples at 130°C for 19 h in triplicates (ASABE, 2016).

Insects

The insect cultures of *C. ferrugineus* were reared in a media prepared with whole wheat kernels, cracked wheat, and wheat germ (90:5:5 w/w); whereas the cultures of *T. castaneum* were reared on wheat flour and brewer's yeast (95:5 w/w). Both the cultures were kept at 30 ± 1°C and 70 ± 5% RH in the dark. Mixed-sex adults with the age 1 d to 2 mo old, at the start of each experiment, were used for the study.

Experimental setup

Nine mesh cubes of 0.1 m each side, were prepared by fastening mild steel metal rods of 1.6 mm diameter using polylactic acid corners (Fig. 1). The polylactic acid corners were printed using a 3D printer (Cartesio W, Mauk CC, Maastricht, Netherlands). A cloth screen with an opening of 1.4 x 1.4 mm^2 was used to enclose the metal cube on all the sides, except the top so as to facilitate filling and emptying of the cube. The mesh size of the screen was chosen specifically to facilitate the movement of insects through it, while holding the wheat inside. The prepared mesh cubes were arranged in the form of a 3 x 3 cube, inside a wooden box with the inner dimension of 0.3 x 0.3 x 0.13 m^3 (Fig. 2). The wooden box was filled with wheat to the depth of 0.1 m. The top of the wooden box was covered with a cardboard sheet to prevent the influence of light, and then fastened with double-sided tape to prevent insects from escaping.

The location of the mesh cubes inside the wooden box were prenumbered as shown in Fig. 2. The same wooden box without mesh cubes was used as the control.

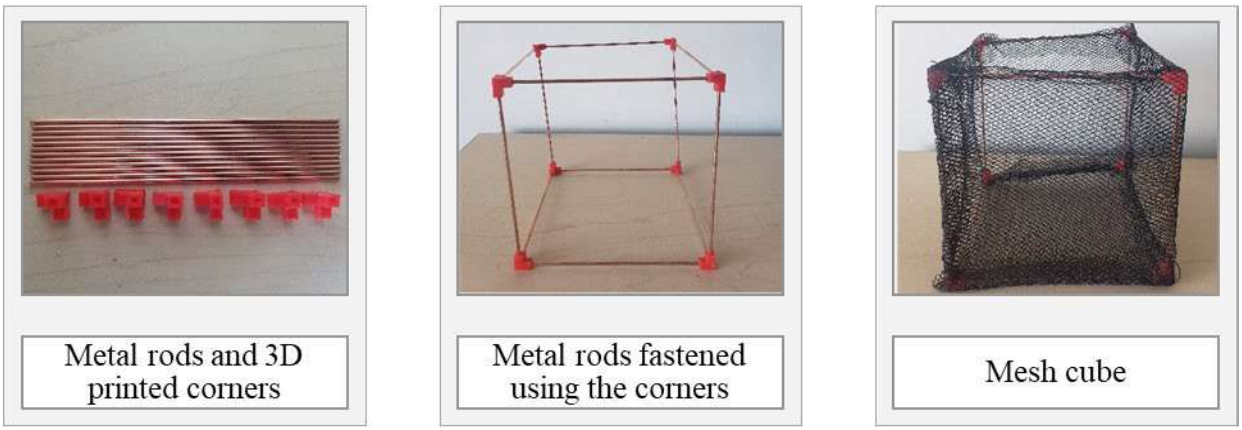

Fig. 1. Step by step preparation process of mesh cubes.

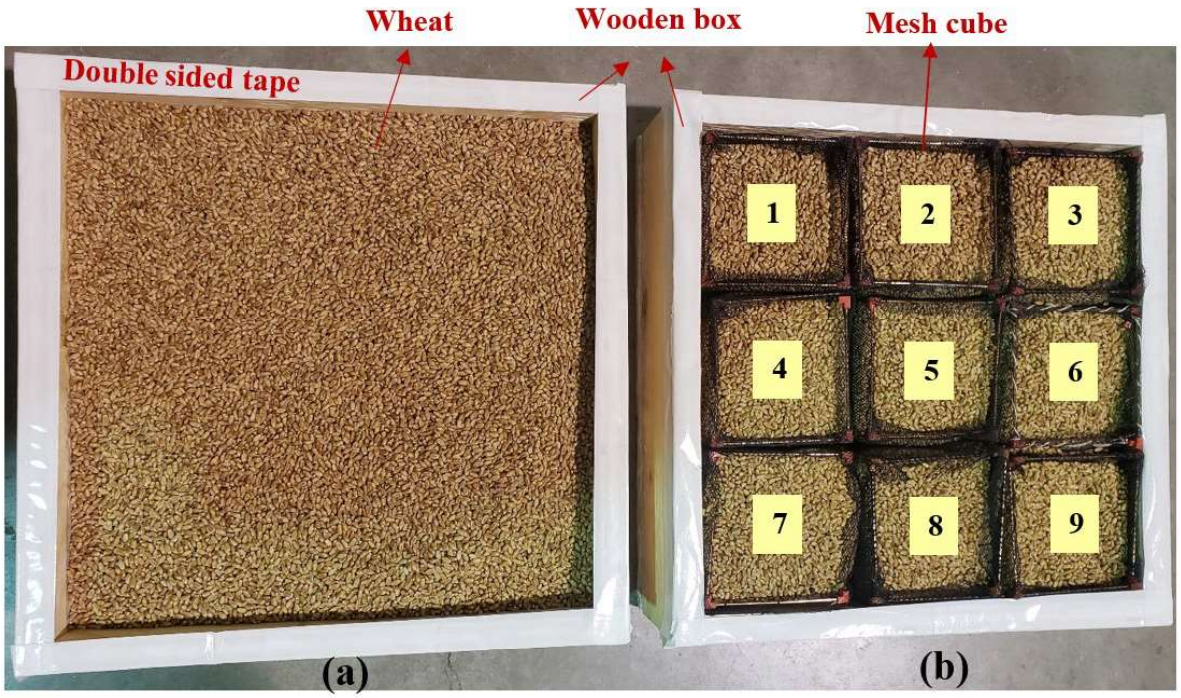

Fig. 2. Experimental set up: Wooden box without (a) and with (b) mesh cubes. The numbers are the marked locations of the mesh cube

Experimental procedure

To perform the experiments, 200 *C. ferrugineus* adults were sieved from the culture and placed in two different glass vials, each containing 100 insects. The insects in the vials were released at location 5 of the wooden box (center of the box) pre-filled with wheat, and with or without mesh cubes. After 24 h, the mesh cubes were carefully taken out of the wooden box and placed in the plastic bags that had prenumbered locations and secured tightly with a knot to prevent the insects from escaping. For the wooden box without mesh cubes, the wheat was segregated into 9 locations, similar to the ones with mesh cube, using the cardboard sheets. The cardboard sheets restricted the further movement of insects. The wheat in each section was vacuumed out along with the insects and then placed inside the plastic bags and secured tightly. A similar procedure was used to evaluate the effect of cubes and mesh screen on the movement of *T. castaneum.* Each experiment was repeated 3 times. The adults were sieved out from the wheat and the number of adults present at each location were counted. In addition, the wheat samples used for each experiment were kept at -15°C for 3 wk in order to eliminate any insect infestation before starting the experiment. The mean recovery rates were 92.7 ± 1.08% (n = 6) and 94.3 ± 0.94 % (n = 6) for *C. ferrugineus* and *T. castaneum*, respectively.

Statistical analysis

For the purpose of data analysis, the number of adults recovered from each box was adjusted to the initial number of adults introduced (i.e., 100) as follows (Jian et al., 2007).

$$Number\ of\ adults\ in\ each\ location = \frac{Number\ of\ adults\ recovered\ from\ each\ location \times 100}{Total\ number\ of\ adults\ recovered\ from\ that\ particular\ replication}$$

To determine if the developed mesh cube affected the movement and distribution of insects, the Two Sample Location Test and the Empirical Distribution Function (EDF) statistical tests (Jian et al., 2002) were conducted (SAS Institute, North Carolina, USA). These tests provided information on the difference in location, median location and the distribution of insects between the experiments with mesh cubes, and those without mesh cubes. The Wilcoxon, Median, and Kolmogorov-Smirnov options were used to perform the analysis.

Results and discussion

No significant differences in movement and distribution of *C. ferrugineus* and *T. castaneum* inside the wooden boxes (with or without mesh cubes) were observed (Table 1). These results indicated that the developed mesh cubes did not have significant effect on the movement of both *C. ferrugineus* and *T. castaneum* and can be used to study the three-dimensional movement of insects by locating the exact position of the insects after a required amount of time, with minimal external disturbances. Moreover, the rapid construction and dismantling of cubes not only reduce the time needed, but also improve the efficiency of the study.

Table 1. Results of the two sample location tests and EDF statistics to compare the movement of *Cryptolestes ferrugineus* and *Tribolium castaneum* in a box with and without the metal cubes covered with mesh screen.

Experiments		Wilcoxon		Median		Kolmogorov-Smirnov	
		Z	P > |Z|	Z	P > |Z|	KSa	P > KSa
With mesh cube vs without mesh cube	*Cryptolestes ferrugineus*	-0.2649	0.7911	-0.4581	0.6469	0.4714	0.9794
	Tribolium castaneum	-0.1766	0.8598	-0.4581	0.6469	0.4714	0.9794

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