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Effects of Insect Density, Movement Period, and Temperature on Three-Dimensional Movement and Distribution of Adult *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae)

Vimala S. K. Bharathi, Digvir S. Jayas,[1] and Fuji Jian

Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, R3T 5V6, Canada, and [1]Corresponding author, e-mail: digvir.jayas@umanitoba.ca

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Abstract

Knowledge on three-dimensional (3D) movement and distribution of *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) in grain bulks assists in the prediction of their distribution inside a bin. The following experiments were conducted to determine the 3D dispersal patterns of adult *C. ferrugineus* in wheat with 14.5% moisture content: 1) at various insect densities (0.35, 1.77 and 3.53 A/kg (adults/kg) at 20°C and in 24 h movement period; 2) in different movement periods (6, 24, and 72 h) at 20°C and 0.35 A/kg insect density; and 3) at different temperatures (20, 30 and 35°C) at 0.35 A/kg density in 24 h movement period. To create the densities of 0.35, 1.77, and 3.53 A/kg, 100, 500, and 1,000 adults were introduced in about 285 kg wheat, respectively. The 285 kg of wheat was kept in 343 mesh cubes, which in turn were packed in a wooden box. The introduced adults were counted at the end of the movement periods. Adult *C. ferrugineus* tended to move downward from the point of introduction, and then diffused throughout the grain bulk. The effects of insect densities, movement periods, and temperatures on the dispersion pattern of insects were similar in 1D columns, 2D chambers, and 3D grain bulk.

Key words: *Cryptolestes ferrugineus*, stored grain, insect density, movement period, three-dimension

Cryptolestes ferrugineus (Stephens), the rusty grain beetle, is a cosmopolitan secondary pest that is found in tropical and temperate climate zones. Adults and larvae mostly feed on germs of the wheat and cause a significant loss to the stored grain. In the absence of germ, they feed on endosperms of the grain (Rilett 1949). Larvae of *C. ferrugineus* were observed to better survive than any other insect species found (adult *C. ferrugineus*, adults and larvae of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), adult *Lathridius minutus* (L.) (Coleoptera: Latridiidae), and larvae of *Trogoderma* sp.) in the samples obtained during transportation and storage of durum wheat in an inland terminal elevator in Canada (Smith and Loschiavo 1978). Flinn and Hagstrum (1998) concluded that even in winter months, some adults of *C. ferrugineus* move to centers of bins and continue to multiply in large unaerated grain bins.

Over the decades, the effects of various factors such as temperature, moisture content (mc) and their gradients, dockage, temperature acclimation, age, insect density, and geotaxis on the movement and distribution of *C. ferrugineus* have been studied (Flinn and Hagstrum 1998, Jian et al. 2002, 2003, 2004a, 2005a, 2005b, 2005c, 2006, 2007). Their movement was also caused due to drift, random and scattered orientations (Jian et al. 2009a). Most of these studies were performed in one-dimensional (1D) columns or two-dimensional (2D) chambers. Surtees (1964a) developed a technique to study the three-dimensional (3D) dispersion behavior of insects. The method involved arranging 64 3-inch-cube bags inside a 12-inch cube box in four layers of 16 each. The 3-inch bags, made of Terylene net, allowed the movement of insects but held the grain. Known number of insects were introduced on the top layer of the 12-inch cube and after the required amount of movement time, the 3-inch cube bags were taken out and the number of insects at each location were determined. Using this technique, Surtees (1964b) analyzed the 3D dispersion of *C. ferrugineus*, *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae), *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae), *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), and *T. castaneum* in 25 kg wheat at 14% mc. However, restricted insect movement in downward direction only, short movement distance, time-consumption, and lack of repetition were identified to be the major drawbacks of this technique. So, Bharathi et al. (2021) modified the method by using metal rod-based mesh cubes, which reduced the time taken to arrange the cubes and ensured the repetition of the experiment with minimal errors. The metal rod-based mesh cubes were used to study the 3D movement and distribution of *C. ferrugineus* at constant temperatures and moisture contents. The study involved introducing the known number of insects at the center of the grain bulk, thus facilitating the study of movement of insects in all the directions. However, these studies did not consider the effects of different insect densities and movement periods in 3D.

Intraspecific insect density plays an important role in development, life span, rate of oviposition, and mortality of *C. ferrugineus* (Smith 1966). Moreover, the increase in insect density influences the distribution and diffusivities of *C. ferrugineus* in both 1D columns and 2D chambers (Jian et al. 2004a, 2007). Watters (1969) reported that the density of 51 to 510 A/kg did not affect the emigration of *C. ferrugineus*; while density higher than this range might affect emigration due to interaction between individuals (Jian and Jayas 2009). In general, density affects the ability of insects to find a mating partner. Hence, it is important to study the influence of insect density on their movement and distribution in 3D grain bulk.

The present work filled this knowledge gap by studying the movement and distribution of adult *C. ferrugineus* in wheat bulks held in a 3D chamber at different insect densities and movement periods. In addition, we tried to understand the behavior of *C. ferrugineus* adults at high temperature (35°C) by comparing with the observations of published work at 20 and 30°C (Bharathi *et al.* 2021).

Materials and Methods

Wheat

About 1,500 kg of Canada Western Red Spring wheat (*Triticum aestivum* L., Grade No.1, AC Barrie), obtained from Cargill Ltd. (Winnipeg, MB), was used in this study. The wheat was cleaned using a 3D shaker (Vibro-Energy Separator, SWECO, Florence, KY) to remove dockage. The wheat was placed at −15°C for at least 3 wk to make sure that the grain does not have any other insects (Fields and White 1997). Moisture content of the wheat was determined using the oven drying method by drying about 10 g samples, in triplicate, at 130°C for 19 h (ASABE 2016). The wheat mc was adjusted to 14.5% (wet basis) by adding desired amount of distilled water and mixing in a rotating drum. The conditioned wheat was stored in double layer plastic bags at 10°C for 2 to 4 wk. The moisture content of the wheat was measured before each experiment to confirm whether it was 14.5 ± 0.1%. At least 4 d before each experiment, the wheat in the double-layered plastic bags was placed inside the environmental chamber (CMP 4030, Controlled Environments Limited, Winnipeg, MB) which was set at the desired temperature.

Experimental Set Up

The experimental set up used in this study was similar to the one described by Bharathi et al. (2021). Briefly, 343 mesh cubes (0.1 x 0.1 x 0.1 m each) filled with 14.5% mc wheat were packed in a wooden box with inner dimensions of 0.725 x 0.725 x 0.784 m. The opening of the mesh covering the cubes was 1.4 x 1.4 mm and the mesh cubes were the same as the ones described by Bharathi et al. (2021). The wooden box was located inside an environmental chamber that was set at desired experimental temperatures (20 or 35°C). The wooden box was covered with a wooden lid during the experimental period. None of the introduced insects moved out of the wooden box during the entire study.

Adult *C. ferrugineus* Preparation

Adults *C. ferrugineus* of age 1 d to 2 mo at the start of each experiment were obtained from cultures reared on 90% whole wheat kernels, 5% cracked wheat, and 5% wheat germ, at 30 ± 1°C and 75 ± 5% relative humidity (RH), in the dark. The desired number of adults were introduced into one mesh cube holding about 0.83 kg of 14.5% mc wheat. The mesh cube was kept inside a plastic box (0.34 x 0.23 x 0.12 m). This plastic box was kept at the experimental condition for insect acclimation. After 24 h acclimation, the mesh cube was introduced at the center of the wooden box.

Experimental Procedure

After the prepared wheat was filled into each mesh cube (about 0.83 kg in each mesh cube), the 343 mesh cubes were labelled based on their locations (in terms of layer and x- and y-directions) inside the wooden box. The layers were numbered 1 to 7 from top to bottom of the wooden box. The x-direction was also numbered 1 to 7, while the y-direction was marked A to G (Fig. 1). Thus, the mesh cube located at the top left corner in Fig. 1 in the top layer was labeled as L1-A-1. The mesh cube with the acclimated adults was located at L4-D-4.

After 343 mesh cubes were packed into the wooden box, the wooden box was kept at the desired experimental conditions for at least 24 h to ensure the wheat reached to the desired experimental condition. After 24 h insect acclimation, the mesh cube with the acclimated adults was introduced at the center of the wooden box (L4-D-4) by moving out the cubes above L4-D-4. After the cube with the acclimated insects was placed, the moved cubes were put back at their previous locations and the wooden box was covered with the lid immediately. After the desired time of insect movement, the mesh cubes were dismembered from the wooden box. Each of the dismembered mesh cube was placed in a plastic bag. This dismembering procedure was completed in less than 45 min.

The adult number inside each mesh cube was counted by sieving (sieve opening of 850 µm). According to Jian et al. (2007, 2015), not all the introduced adults could be sieved out from the wheat and some of them might hide inside grain kernels. The adult recovery rate for all experiments was 96.01 ± 0.44% *(n = 15)*. Thus, the adult number retrieved from each mesh cube was adjusted by using the following equation.

$$Number\ of\ adults\ in\ the\ cube = \frac{A_{cube} \times A_{initial}}{A_{final}} \quad (1)$$

Where; A_{cube} is the number of adults retrieved from the mesh cube, $A_{initial}$ is the total number of adults introduced initially, and A_{final} is the total number of adults retrieved from the experiment.

Data Analyses

Five experiments with different insect densities, movement periods, and temperatures were conducted (Table 1). There were three replications for each experiment. These experiments were designed to compare insect movement: 1) in 24 h at different insect densities (0.35, 1.77, and 3.53 A/kg) at 20°C; 2) in different movement periods (6, 24 and 72 h) at 20°C with 0.35 A/kg density; and 3) at different temperatures (20, 30, and 35°C) for 24 h with 0.35 A/kg density. To obtain the densities of 0.35, 1.77, and 3.53 A/kg, 100, 500, and 1,000 adults were used. The results of the experiments with density 0.35 A/kg at 20 and 30°C in wheat with 14.5% mc for 24 h have been adopted from Bharathi *et al.* (2021). The experiments with 500 and 1,000 introduced adults were normalized to 100 adults, so their movement and distribution could be compared at the same level. All statistical analyses were performed using SAS® OnDemand for Academics (SAS Institute Inc., Cary, NC).

Vertical Distribution

The adults retrieved from each layer were summed up and used to conduct the Tukey's test (α = 0.05) within different layers of the same experiment, or in the same layer under different insect densities,

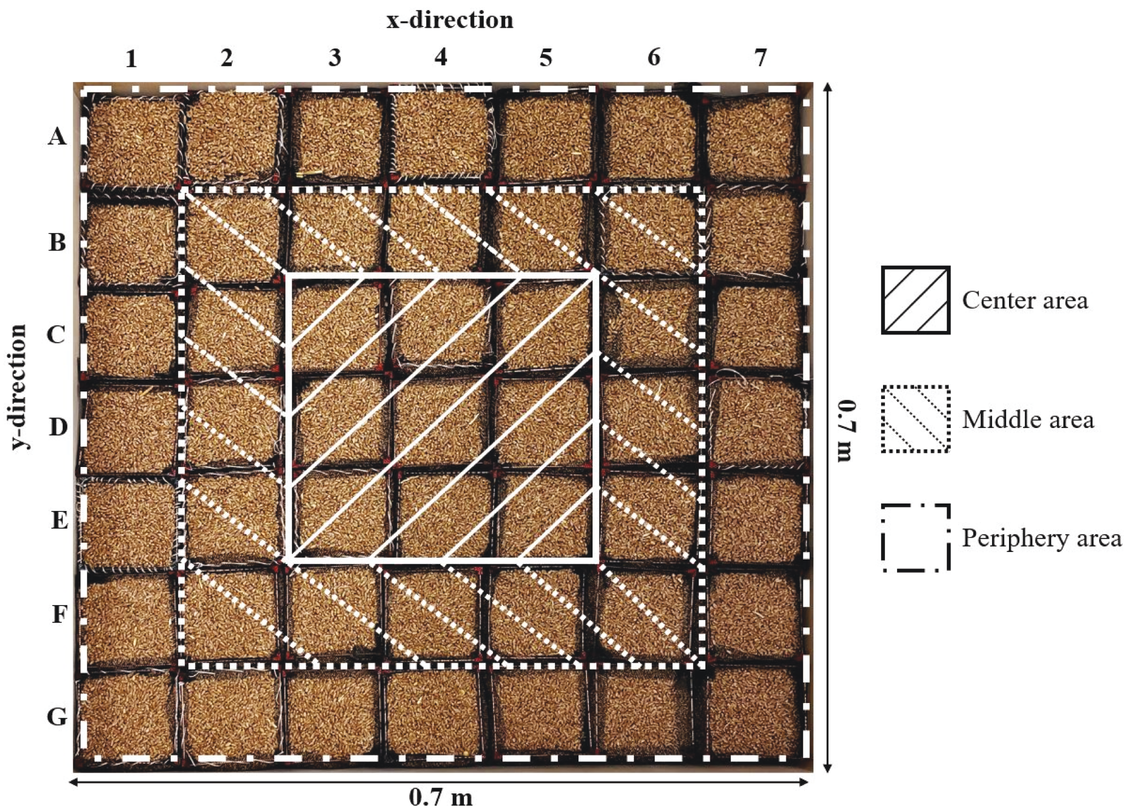

Fig. 1. Numeration and categorization of the mesh cubes filled with wheat, in each layer. The size of each mesh cube was 0.1 x 0.1 x 0.1 m.

Table 1. Experimental conditions conducted using 14.5% moisture content of wheat

Adults introduced	Movement period (h)	Temperature (°C)
100$^{\varepsilon}$, 500, 1,000	24	20
100	6, 24$^{\varepsilon}$, 72	20
100	24	20$^{\varepsilon}$, 30$^{\lambda}$, 35

Data reported by Bharathi *et al.* (2021) as redistribution of 100 adults at 20$^{\varepsilon}$ and 30°C$^{\lambda}$ in wheat with 14.5% mc for 24 h.

temperatures, and movement periods. To determine whether adults preferred moving up or down, the number of insects recovered from the upper half of the box (layers 1, 2, and 3) were multiplied with 30, 20, and 10 (distance traveled by the insects, in cm), respectively. The summation of these products was termed as upward direction. Likewise, the summation of the products obtained by multiplying the number of insects recovered from the bottom half (layers 5, 6, and 7) with the constants 10, 20, and 30, respectively, was termed as downward direction. Upward movement was assigned as positive, while downward was negative. If sum of the upward and downward movements was positive, the net movement was assumed as upward movement, otherwise as downward movement.

The insect movement and distribution of adult *C. ferrugineus* at different insect densities, movement periods, and temperatures were compared by performing the Two-Sample Location Test (Wilcoxon and Median) and Empirical Distribution Function (EDF) (Kolmogorov-Smirnov) statistic (Jian et al. 2005a). The summations of each layer were calculated and two experiments at different densities, movement periods, or temperatures were compared.

Horizontal Distribution

For the purpose of understanding the horizontal distribution, each layer of the wooden box (layers 1 to 7) was classified into three areas, namely, Center, Middle, and Periphery (Fig. 1). The densities of *C. ferrugineus* adults at each area were calculated by dividing the total number of adults retrieved from the area by the total quantity of wheat in the area. Tukey's test (α = 0.05) was conducted for the calculated densities to understand if the adults had any horizontal preference in any of the layers at all tested experimental conditions.

Results

Vertical Distribution

At all tested experimental conditions, summations of upward and downward movement were negative. Thus, adults generally had a downward movement irrespective of insect densities, movement periods, and temperatures.

At 20°C and when 1,000 insects were introduced, about 44% of the insects moved downward and 40% moved upward at the end of 24 h movement period. At the same temperature and movement period, when 500 and 100 insects were introduced, around 64% and 55% insects, respectively, were found at the bottom half of the box; while only 7% and 23%, respectively, moved upwards. Significant number of insects were found in layers 4 and 7 when 100 and 500 insects were introduced (Table 2). Therefore, insects distributed more uniformly in all layers when 1,000 insects were introduced, and lower percentage of insects were found in introduction layer at this high insect density than those when ≤ 500 insects were introduced. These results indicated that high insect densities would force adults to occupy the chamber more evenly.

At 20°C, about 51%, 55%, and 57% of the 100 introduced adults were recovered from the bottom half of the box after 6, 24 and 72 h of movement period, respectively, and about 12%, 23%, and 17% were found at the upper half of the box, respectively. However, about 36% of the insects were recovered from the introduction layer (layer 4) after 6 h, while only 22% and 25% were recovered from the layer 4, after 24 and 72 h, respectively. Similarly, higher percentage of insects were found at the insect introduction cube (L4-D4) after 6 h (25%), as compared to those after 24 (13%) and 72 h (15%). Moreover, adults distributed more uniformly throughout the box after 72 h, while significantly higher number of insects were found at layers 4 and 7, after 6 h and 24 h of movement period (Table 2). Therefore, the introduced adults generally moved in the downward direction at first, then moved up and distributed inside the wheat.

At 20, 30, and 35°C, only 23, 11, and 20% of adults moved up when 100 insects were introduced, respectively, in 24 h of movement period (Table 2), while about 55%, 79%, and 65% of insects were recovered from the bottom half of the wooden box, respectively. However, at 20°C, higher percentage of insects (22%) were recovered from the insect introduction layer (layer 4), as compared to those at 30°C (10%) and 35°C (15%). At all the tested temperatures, higher number of insects were observed in the bottommost layer of the box. These results indicated that higher the temperature, faster the movement of insects.

Even though the recovered adult numbers varied with varying insect densities, movement periods, and temperatures, two-sample location tests, and EDF statistics showed that no significant differences existed in the movement and cumulative distribution of the *C. ferrugineus* adults inside the wooden box, at the tested experimental conditions (Table 3). These results indicated that insects had the similar distribution pattern in vertical direction under various tested conditions.

Horizontal Distribution

At all the seven layers of the performed experiments, the insect densities at the center were higher than or equal to those at middle and periphery areas (Table 4). In layer 4, more insects (higher insect density) were found at the center area, for all the experiments (Figs. 2–6). Tested insect densities, movement periods, and temperatures did not have any effect (Table 4) on the insect densities present at different areas (center, middle, and periphery) for all the seven layers. These results indicated the introduced adults preferred the introduced location and when they move up or down, they exhibit uniform horizontal distribution, at constant environmental conditions.

Discussion

About 51% to 79% of the insects were found to move downward from the point of introduction under the tested experimental conditions, except at insect density of 3.53 A/kg. This result is in

Table 2. Mean insect number of *Cryptolestes ferrugineus* adults recovered at different layers in wheat with 14.5% mc

Experiments^{ε}	Layer 1	Layer 2	Layer 3	Layer 4	Layer 5	Layer 6	Layer 7
100A-24P-20T	$7.8 \pm 1.9^{a,A,K,X}$	$6.3 \pm 1.4^{a,A,K,X}$	$9.2 \pm 1.2^{a,A,K,X}$	$21.7 \pm 0.8^{ab,AB,K,X}$	$6.9 \pm 1.4^{a,A,K,X}$	$8.8 \pm 1.6^{a,A,K,X}$	$39.2 \pm 3.1^{b,A,K,X}$
500A-24P-20T^{λ}	$0.5 \pm 0.2^{a,A}$	$2.1 \pm 0.4^{a,A}$	$4.2 \pm 1.6^{a,A}$	$29.4 \pm 3.7^{b,A}$	$16.6 \pm 3.6^{ab,A}$	$15.3 \pm 5.3^{ab,A}$	$31.8 \pm 1.5^{b,A}$
1,000A-24P-20T^{λ}	$16.2 \pm 8.8^{a,A}$	$10.8 \pm 2.7^{a,A}$	$13.3 \pm 3.0^{a,A}$	$15.3 \pm 2.4^{a,B}$	$7.9 \pm 2.5^{a,A}$	$7.5 \pm 1.9^{a,A}$	$29.0 \pm 6.5^{a,A}$
100A-6P-20T	$2.1 \pm 0.5^{a,K}$	$4.1 \pm 1.9^{a,K}$	$6.2 \pm 1.7^{a,K}$	$36.1 \pm 0.8^{b,K}$	$11.5 \pm 3.0^{a,K}$	$7.6 \pm 1.4^{a,K}$	$32.3 \pm 1.2^{b,K}$
100A-72P-20T	$2.4 \pm 2.0^{a,K}$	$5.6 \pm 2.4^{a,K}$	$9.1 \pm 0.5^{a,K}$	$25.0 \pm 5.8^{a,K}$	$15.4 \pm 2.7^{a,K}$	$17.7 \pm 4.7^{a,K}$	$24.8 \pm 5.9^{a,K}$
100A-24P-30T	$2.4 \pm 0.4^{a,X}$	$3.8 \pm 1.3^{a,X}$	$4.8 \pm 0.9^{a,X}$	$10.0 \pm 1.1^{a,X}$	$3.5 \pm 0.6^{a,X}$	$15.1 \pm 2.7^{a,X}$	$60.4 \pm 2.1^{b,X}$
100A-24P-35T	$8.4 \pm 2.3^{a,X}$	$5.2 \pm 1.3^{a,X}$	$6.6 \pm 2.0^{a,X}$	$14.6 \pm 3.2^{a,X}$	$5.9 \pm 1.2^{a,X}$	$11.5 \pm 1.9^{a,X}$	$47.7 \pm 6.6^{b,X}$

^{ε}Numbers before A, P, and T are the number of introduced adults, movement period (h), and temperature (°C) of wheat, respectively.

^{λ}Insect numbers in the experiments with 500 and 1,000 introduced adults were normalized to 100.

^{a,b}Different lowercase alphabets within the same experimental conditions (in the row) represent the significantly different mean values using Tukey's test at the level (α) 0.05.

^{A,B,K,X}Different uppercase alphabets represent the significantly different mean values in the same layer (in the column) with different insect densities (AB), different movement periods (K), or different temperatures (X) using Tukey's test at the level (α) 0.05.

Table 3. Results of two-sample location tests and EDF statistics for adult *Cryptolestes ferrugineus* movement and distribution in three-dimensional set up

Experiments^{ε}			Wilcoxon		Median		Kolmogorov-Smirnov	
			Z	P > |Z|	Z	P > |Z|	KSa	P > KSa
Insect density	24P, 20T	100A vs 500A^{λ}	0.2556	0.7983	−0.5151	0.6065	0.8018	0.5412
		500A^{λ} vs 1,000A^{λ}	0.0000	1.0000	0.5151	0.6065	0.8018	0.5412
		100A vs 1,000A^{λ}	−0.7667	0.4433	−1.5452	0.1223	0.8018	0.5412
Movement period	100A, 20T	6P vs 24P	−0.6389	0.5229	−0.5151	0.6065	0.8018	0.5412
		24P vs 72P	−0.1278	0.8983	−0.5151	0.6065	0.5345	0.9375
		6P vs 72P	−0.2556	0.7983	−0.5151	0.6065	0.5342	0.9375
Temperature	100A, 24P	20T vs 35T	0.3833	0.7015	0.5151	0.6065	0.5345	0.9375
		30T vs 35T	−0.8944	0.3711	−0.5151	0.6065	1.0690	0.2032

^{ε}Comparison between two experiments. Numbers before A, P, and T are the number of introduced adults, movement period (h), and temperature (°C) of wheat with 14.5% mc, respectively.

^{λ}Insect number in the experiments with 500 and 1,000 introduced adults were normalized to 100.

Table 4. Insect densities of adult *Cryptolestes ferrugineus* at different areas of each layer under different experimental conditions ($n = 3$)

		Area		
Layer	Experimental conditions$^{\varepsilon}$	Center	Middle	Periphery
1	500A-24P-20T$^{\lambda}$	0.1 ± 0.0^a	0^a	0^a
	1,000A-24P-20T$^{\lambda}$	1.2 ± 0.6^a	0.4 ± 0.2^a	0.1 ± 0.1^a
	100A-6P-20T	0.1 ± 0.1^a	0.1 ± 0.0^a	0^a
	100A-72P-20T	0.3 ± 0.2^a	0^a	0^a
	100A-24P-35T	0.3 ± 0.1^a	0.2 ± 0.01^a	0.2 ± 0.1^a
2	500A-24P-20T$^{\lambda}$	0.2 ± 0.1^a	0^b	0^b
	1,000A-24P-20T$^{\lambda}$	0.7 ± 0.1^a	0.3 ± 0.1^{ab}	0^b
	100A-6P-20T	0.2 ± 0.1^a	0.1 ± 0.1^a	0.1 ± 0.0^a
	100A-72P-20T	0.7 ± 0.3^a	0^a	0^a
	100A-24P-35T	0.3 ± 0.2^a	0^a	0.1 ± 0.0^a
3	500A-24P-20T$^{\lambda}$	0.5 ± 0.2^a	0^a	0^a
	1,000A-24P-20T$^{\lambda}$	1.3 ± 0.5^a	0.2 ± 0.1^a	0.1 ± 0.0^a
	100A-6P-20T	0.6 ± 0.2^a	0.1 ± 0.0^a	0.1 ± 0.1^a
	100A-72P-20T	1.2 ± 0.0^a	0^b	0^b
	100A-24P-35T	0.4 ± 0.1^a	0.1 ± 0.0^b	0.1 ± 0.1^{ab}
4	500A-24P-20T$^{\lambda}$	3.9 ± 0.5^a	0.1 ± 0.0^b	0^b
	1,000A-24P-20T$^{\lambda}$	1.8 ± 0.3^a	0.1 ± 0.0^b	0^b
	100A-6P-20T	4.3 ± 0.3^a	0.2 ± 0.1^b	0.1 ± 0.1^b
	100A-72P-20T	3.3 ± 0.7^a	0.1 ± 0.0^b	0^b
	100A-24P-35T	1.2 ± 0.3^a	0.1 ± 0.1^b	0.2 ± 0.0^{ab}
5	500A-24P-20T$^{\lambda}$	1.9 ± 0.5^a	0.1 ± 0.0^b	0^b
	1,000A-24P-20T$^{\lambda}$	0.8 ± 0.3^a	0.1 ± 0.0^a	0^a
	100A-6P-20T	1.1 ± 0.3^a	0.1 ± 0.0^a	0.1 ± 0.0^a
	100A-72P-20T	1.9 ± 0.3^a	0.1 ± 0.0^b	0^b
	100A-24P-35T	0.2 ± 0.1^a	0.1 ± 0.0^a	0.2 ± 0.1^a
6	500A-24P-20T$^{\lambda}$	1.8 ± 0.7^a	0.2 ± 0.0^a	0^a
	1,000A-24P-20T$^{\lambda}$	0.6 ± 0.3^a	0.1 ± 0.0^a	0.1 ± 0.0^a
	100A-6P-20T	0.3 ± 0.0^a	0.2 ± 0.0^a	0.2 ± 0.0^a
	100A-72P-20T	2.0 ± 0.7^a	0.1 ± 0.1^a	0.1 ± 0.0^a
	100A-24P-35T	0.4 ± 0.1^a	0.1 ± 0.0^a	0.4 ± 0.1^a
7	500A-24P-20T$^{\lambda}$	2.9 ± 0.2^a	0.4 ± 0.1^b	0.2 ± 0.1^b
	1,000A-24P-20T$^{\lambda}$	1.6 ± 0.5^a	0.6 ± 0.2^a	0.5 ± 0.2^a
	100A-6P-20T	1.9 ± 0.5^a	0.6 ± 0.2^a	0.5 ± 0.1^a
	100A-72P-20T	1.6 ± 0.7^a	0.6 ± 0.1^a	0.3 ± 0.1^a
	100A-24P-35T	1.4 ± 0.6^a	1.1 ± 0.1^a	1.2 ± 0.2^a

[ε]Numbers before A, P, and T are the number of introduced adults, movement period (h), and temperature (°C) of wheat with 14.5% mc, respectively.

[λ]Insect number in the experiments with 500 and 1,000 introduced adults were normalized to 100.

[a,b]Different alphabets within the same row represent the significantly different mean values using Tukey's test at level (α) 0.05.

accordance with the observations of various other researchers (Loschiavo 1974, 1983, White et al. 1993, Jian et al. 2004b, 2006, 2009a, Bharathi et al. 2021), who found that a considerable portion of *C. ferrugineus* adults prefer to move downwards from the introduction location, owing to their drift effect, smaller body size, and preference. When insects move up or down from the point of introduction, it was observed that they tend to distribute uniformly in horizontal direction at uniform environmental conditions. Our results are in accordance with the findings of Surtees (1964b) who performed three-dimensional dispersion analysis of different insect species and found that *C. ferrugineus, T. castaneum,* and *R. dominica* exhibited homogenous lateral dispersion and heterogenous vertical dispersion.

Jian et al. (2004a) concluded that lower density of insects (2 A/kg) led to aggregation, while higher insect density (>12 A/kg) resulted in uniform dispersion in 1D studies. The effect of insect density between 2 and 12 A/kg on insect dispersion pattern would be in the middle of aggregation and uniform dispersion. Jian et al. (2007) showed that the 2D diffusivities of adult *C. ferrugineus* at densities of 1.67 and 6.67 A/kg in 14.5% mc wheat at 30°C for 3 h of movement, were significantly different. However, the diffusivity did not vary significantly when the insect density was increased from 1.67 to 3.33 A/kg of wheat. The result of the current 3D study is in accordance with these findings. Briefly, the insect densities of 0.35, 1.77, and 3.53 A/kg with initial insect counts of 100, 500, and 1,000 insects, respectively, did not show significant difference in 3D movement and distribution. In addition, more recovery of insects (Table 2) at density of 0.35 and 1.77 A/kg, in layers 4 and 7, is in line with the findings of Jian et al. (2004a), who showed aggregation occurs at lower densities. At 3.53 A/kg, the insects were uniformly distributed throughout the wooden box (Fig. 3). These consistent results in 1, 2, 3D movement might be explained by insect movement behaviors under different conditions. For example, in wheat without temperature and moisture differences, insects at high density tend to find their mating partner more easily, as opposed to lower insect densities, where they need to search and find their mating partner with the help of aggregation pheromones (Loschiavo et al. 1986).

In 6 h movement period at 20°C, significantly higher insects were recovered at insect introduction layer (layer 4), followed by the most bottom layer (layer 7). Conversely in 24 h, more insects were retrieved from layer 7, followed by layer 4. In 72 h, the insects were more uniformly distributed in all layers (Table 2). Thus, these results indicated that insects moved from the introduction location to the bottom most layer and then, moved up and gradually distributed uniformly over time. These results are in harmony with those of 1D column experiments, where Jian et al. (2004a) showed that the distribution of *C. ferrugineus* adults was unstable for the first 24 h and after which, the insects distributed uniformly with time, at any tested insect densities (2, 12, 24, and 48 A/kg). The 2D diffusivity of insects at 72 h was found to be statistically same as that at 24 h (Jian et al. 2007). Similarly, no significant change in the 3D movement and distribution, with respect to different movement periods (6 h, 24 h, and 72 h), was observed in the current study.

The higher number of insects at the most bottom layer at 30°C, as compared to 20°C could be explained by the fact that higher temperature increases the movement speed of the *C. ferrugineus* adults (Loschiavo 1983). The results of the current study are in agreement with the findings of Jian et al. (2007), who reported that the diffusivity of adult *C. ferrugineus* increased with increase in temperature. In general, faster movement of insects leads to increased number of contacts with individual insects in the proximity, which in turn results in "pseudo crowding" of insects (Kunert et al. 2005). Thus, the decrease in number of insects at the most bottom layer at 35°C could be caused by the increased movement speed which might have further enhanced the dispersal of insects (Jian et al. 2004a). In addition, increase in movement at higher temperature explains the higher recovery of insects from the insect introduction layer (layer 4) at 20°C than those at 30°C. Among the insects recovered from layer 4, higher percentage of insects were recovered in the middle and periphery areas, at 35°C (38%), compared to those at 30°C (31%) and 20°C (27%). This confirms the temperature effect on the movement speed and distribution pattern of insects (downward, followed by upward and horizontal movement).

Following interpretations can be made from this study:

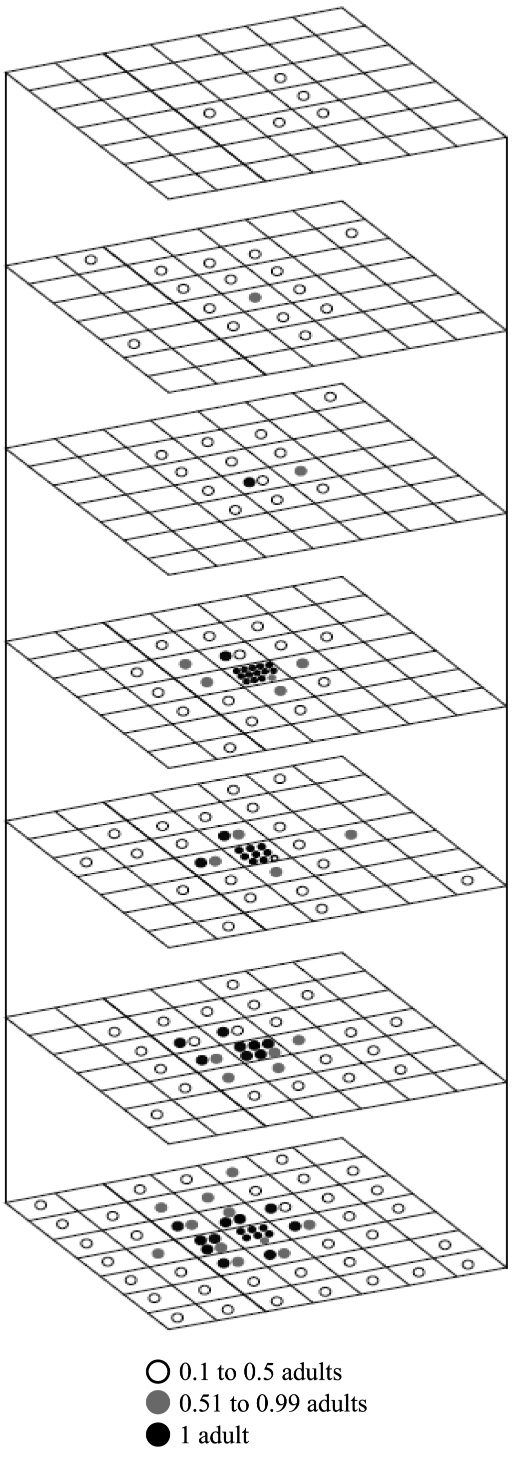

Fig. 2. Redistribution of 500 *Cryptolestes ferrugineus* adults at 20°C in wheat with 14.5% mc for a period of 24 h. The adult numbers have been normalized to 100 adults for the purpose of comparison. Different dots show the mean insect number over three replicates.

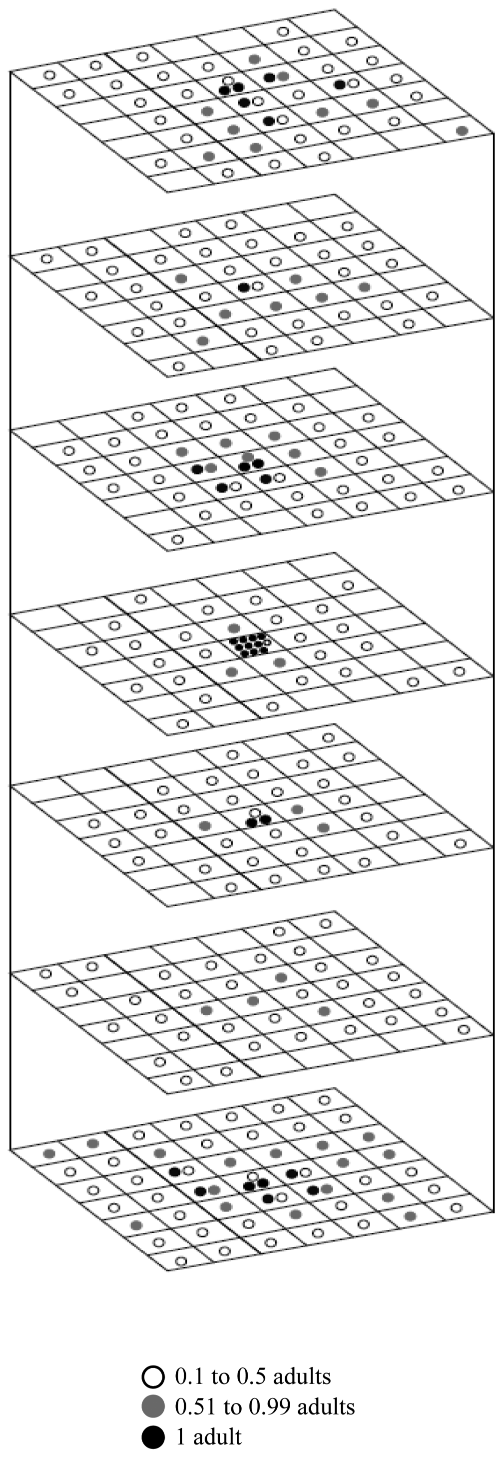

Fig. 3. Redistribution of 1,000 *Cryptolestes ferrugineus* adults at 20°C in wheat with 14.5% mc for a period of 24 h. The adult numbers have been normalized to 100 adults for the purpose of comparison. Different dots show the mean insect number over three replicates.

1. In wheat bulks with uniform temperature and moisture content, *C. ferrugineus* adults moved downward initially, then moved up and horizontally.
2. Insects disperse more at high insect densities than at low densities.
3. Adult *C. ferrugineus* move faster at higher temperature (35°C) than at low temperature (20°C).
4. The effects of insect densities, movement periods, and temperatures observed in 3D grain bulk were similar to those observed in 1D columns and 2D chambers.

In general, grains are stored inside bins after harvest. During storage, seasonal weather changes result in moisture migration and temperature gradients in the stored grain, which in turn affects the movement

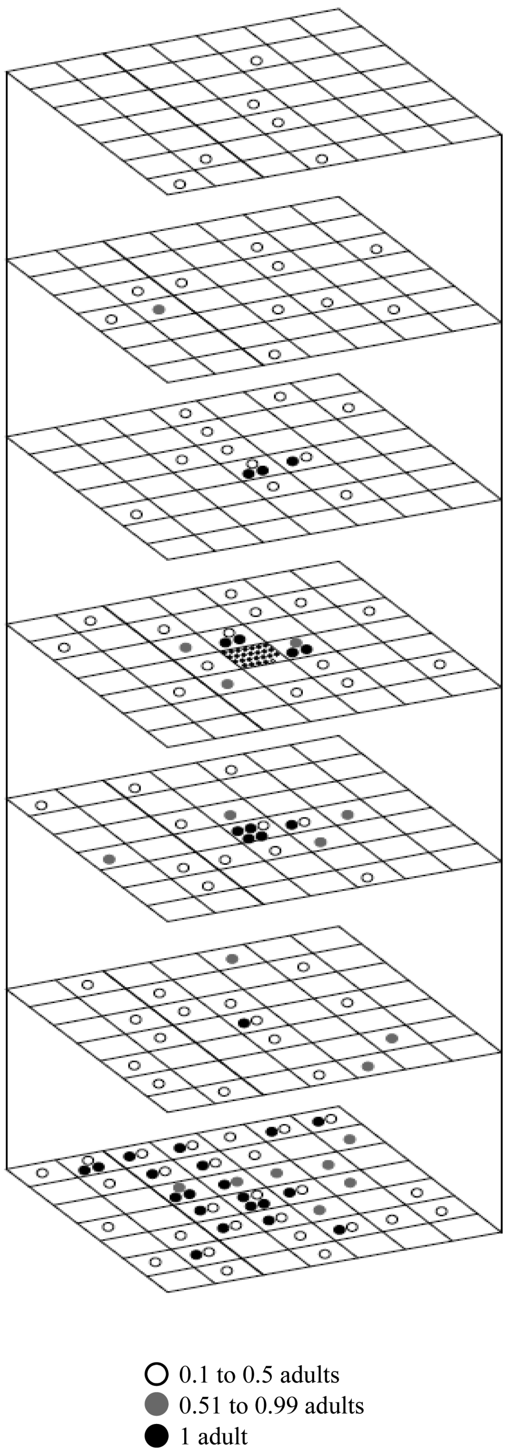

Fig. 4. Redistribution of 100 *Cryptolestes ferrugineus* adults at 20°C in wheat with 14.5% mc for a period of 6 h. Different dots show the mean insect number over three replicates.

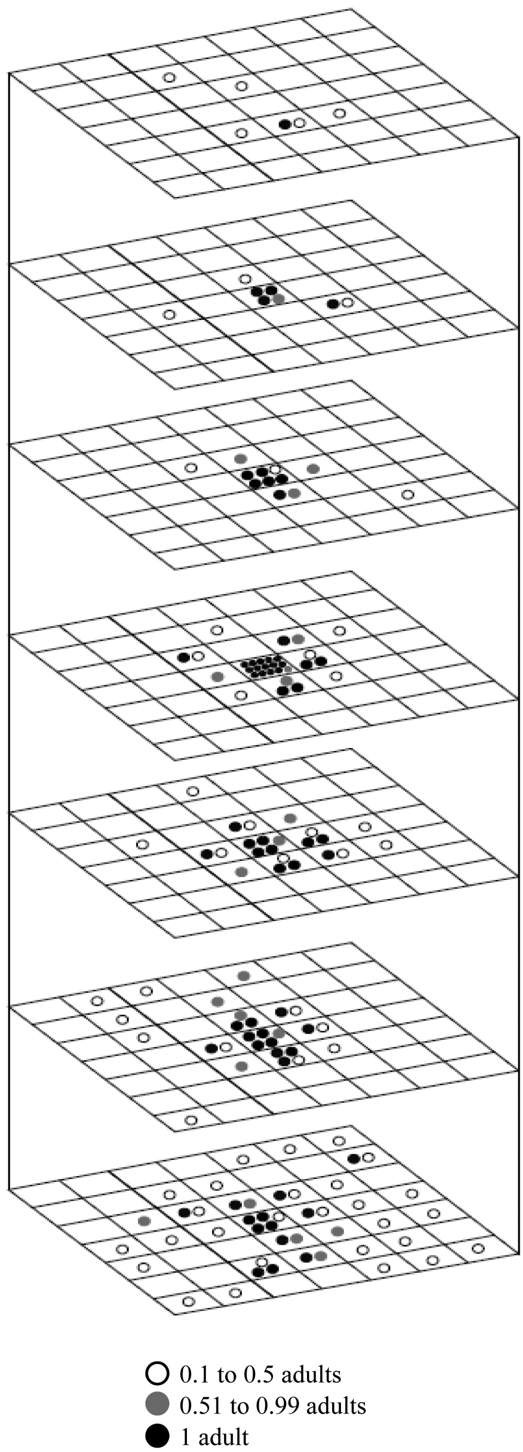

Fig. 5. Redistribution of 100 *Cryptolestes ferrugineus* adults at 20°C in wheat with 14.5% mc for a period of 72 h. Different dots show the mean insect number over three replicates.

of insects (Toews et al. 2003, Jian et al. 2009b). In addition, the density of insects at a particular location affects their dispersion, due to competition. The present study helps in understanding the dispersion pattern of insects at various insect densities, movement periods, and temperatures. However, further research is needed to study the influence of temperature and moisture gradients in 3D chambers to completely understand the behavior of insects in grain bulks.

According to Jian et al. (2005b), insects that are 1-d, 1-, 5-, 10-, and 20-week old and mixed-age adults did not show any difference in their distribution in the absence of temperature gradient. Thus, the age of the adults used in the current study (1 d to 2 mo old) could not have affected the movement pattern of insects. However, to the best of our knowledge, no report exists on the effects of different physiological states of *C. ferrugineus* on their movement and distribution

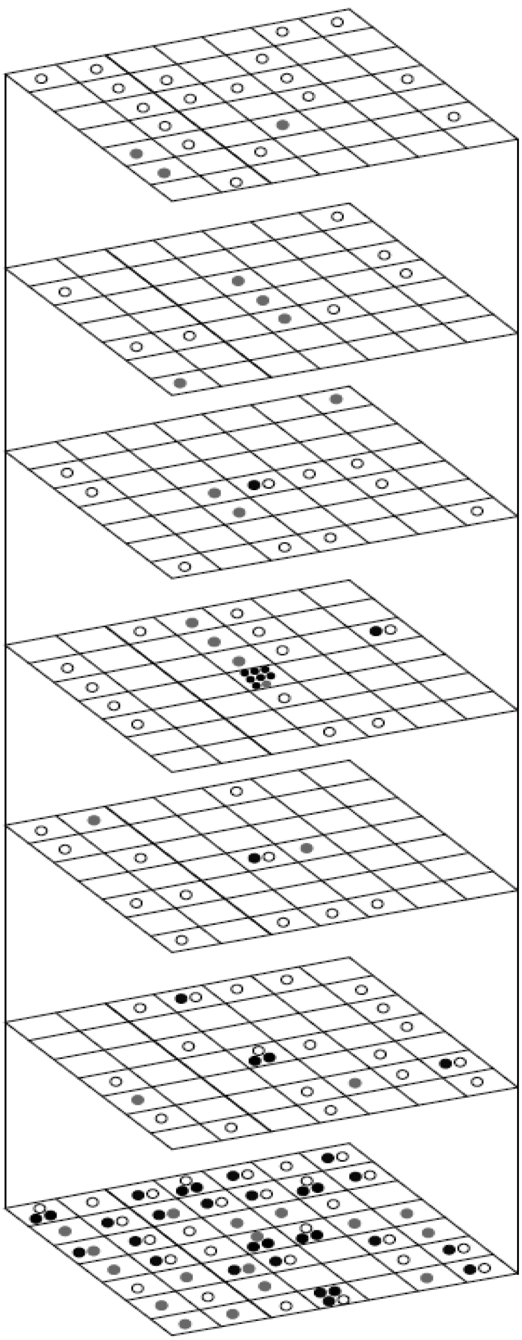

Fig. 6. Redistribution of 100 *Cryptolestes ferrugineus* adults at 35°C in wheat with 14.5% mc for a period of 24 h. Different dots show the mean insect number over three replicates.

in 3D grain bulk. Hence, it would be interesting to consider these factors in the future studies.

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Author Contributions

VB: Data Curation, Formal analysis, Investigation, Methodology, Writing – original draft. DJ: Conceptualization, Methodology, Funding acquisition, Supervision, Writing – review & editing. FJ: Conceptualization, Methodology, Supervision, Writing – review & editing.

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