Three-dimensional movement and distribution of *Tribolium castaneum* and *Cryptolestes ferrugineus* in stored wheat at different temperatures and at different times

by

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ABSTRACT

The findings of this study have significant practical implications for the field of biosystems engineering. Understanding the movement and distribution patterns of insects is crucial for developing effective stored grain management protocols. This research investigates the three-dimensional movement and distribution of *Tribolium castaneum* and *Cryptolestes ferrugineus* separately at different temperatures (5, 10, 20, and 30ºC) and for different movement periods (1, 2, 3, and 24 h) in stored wheat with a uniform moisture content of 14.5% (wet basis). The experiments were conducted in a wooden container with internal dimensions of 0.7 × 0.7 × 0.7 m. The wheat was filled into 343 mesh cubes (referred to as cube), each measuring 0.1 × 0.1 × 0.1 m, arranged in seven layers after being loaded into the container. One hundred insects were introduced into the centre cube (also the centre of the container) at the start of the experiment. After the desired movement period, the cubes were removed, wrapped in labelled plastic bags, and sieved, and the insects were recovered and counted. Results indicated that both species exhibited movement speeds > 7.2 m/d in vertical and horizontal directions at higher temperatures (20 and 30ºC). At lower temperatures (5 and 10ºC), their vertical speed was higher than their horizontal speed. *Tribolium castaneum* ceased movement at 5ºC, whereas approximately 13% of *C. ferrugineus* adults continued to move at this temperature. The drift effect and geotaxis influenced the movement and distribution of both species in the vertical direction, while their horizontal movement followed a diffusion pattern. These findings will contribute to developing more effective and targeted pest control strategies in stored grain management.

Keywords: *T. castaneum*, *C. ferrugineus*, three-dimensional study, movement, distribution, temperature, movement period

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1. INTRODUCTION

Canada stands as one of the world's leading producers and exporters of wheat, with its vast agricultural landscape supporting a thriving grain industry (Statistics Canada, 2023). The importance of grain storage systems in preserving the quality and safety of Canada's wheat production cannot be understated. A zero-tolerance policy towards stored grain pests is maintained to recognize the importance of preventing infestations. Insect infestation presents a significant global challenge to grain storage facilities, endangering food security and economic stability. Grain storage environments provide a complex habitat for a wide range of insect species, offering shelter, food resources, and optimal conditions for reproduction. Insects such as weevils, beetles, and moths exhibit diverse movement behaviours which are influenced by factors like grain type, temperature, humidity, and structural features of storage facilities (Jian and Jayas, 2012).

Among the damaging pests, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), commonly referred to as the rusty grain beetle and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), commonly known as the red flour beetle are recognized as the two major grain pests in Canada (Hulasare et al., 2003). These cosmopolitan beetles thrive in stored grain environments, causing extensive damage and contamination. The diet of *T. castaneum* primarily includes flour, broken grains, and other dried foods (Abdullahi et al., 2019). They are known to infest a wide range of commodities, such as spices, beans, and pasta. In contrast, the *C. ferrugineus* beetle primarily feeds on stored grains and other cereals (Hagstrum, 2012). Adults of *T. castaneum* are ten times larger than *C. ferrugineus* and are responsible for causing 15- 20 % more damage to grains (Jayas et al., 1994).

Insect movement and distribution pattern studies involve observing and analyzing how insects navigate, disperse, and establish populations within specific environments such as grain storage facilities. Different species exhibit varied behaviours and preferences for temperature, humidity and grain type, which influence their movement patterns (Anukiruthika et al., 2021). By understanding these patterns, we can identify the key pathways and mechanisms through which pests infiltrate and spread within storage facilities. This knowledge is essential for developing effective pest management and control strategies to prevent infestations and preserve stored grains (Jian, 2019).

Researchers have extensively studied these two species in controlled laboratory settings and field bins to understand their nature and behaviour under various environmental conditions (Bharathi et al., 2023a, Chaubey, 2023). A one-dimensional grain column was developed by Jian et al. (2004) to study the movement of *C. ferrugineus* adults under both linear and dynamic temperature conditions and varying insect densities. The study examined insect behaviour at different time intervals of 1, 3, 6, 12, 24, 72, and 144 h, with insect densities of 2, 12, 24, and 48 adults per kilogram of wheat (A/kg). Additionally, this research was conducted across a wide range of temperatures, moisture gradients, and dockage percentages (Jian et al., 2003, 2005b). Since the initial study provided insights in only horizontal direction, a two-dimensional grain chamber was developed to study horizontal movement in two directions (i.e., X and Y in the same plane) (Jian et al., 2007). This study revealed that *C. ferrugineus* adults follow a diffusion pattern at constant temperature and moisture levels within the 2-D grain chamber. Similarly, the horizontal and vertical movements of *T. castaneum* were also investigated within wheat and corn columns under constant and varying temperature gradients ranging from 20 to 30ºC (Jian et al., 2005b). The first three-dimensional study was conducted by Surtees (1964b), who used cube bags made of netting with openings to facilitate insect movement. The setup consisted of 64 cubes arranged in 4 layers. Insects were introduced at the top surface, and the cubes were removed at the end of the experiment. To address the limitations of this approach, such as the arrangement of bags for each experiment which led to a lack of consistency, and the restriction of movement examination to only the downward direction, Bharathi et al. (2021) modified the setup. Bharathi et al. (2022a) conducted a 24-h three-dimensional study on *C. ferrugineus* adults in wheat at constant temperatures (20 and 30ºC) and moisture contents (12, 14, and 16% wet basis). Additionally, another study by Bharathi et al. (2022b) examined the effects of various insect densities (0.35, 1.77, and 3.53 A/kg), movement periods (6, 24, and 72 h), and temperatures (20, 30 and 35ºC). The results from these studies were consistent with previous 1-D and 2-D research, showing that the insects followed diffusion patterns and exhibited a drift effect in their distribution and movement.

Despite these studies, an extensive three-dimensional laboratory movement study of *T. castaneum* has not yet been reported. The temperature at which *C. ferrugineus* adults cease to move within a three-dimensional setup remains unknown. This study extends the previous research by Bharathi et al. (2022a, b) and aims to understand the movement and distribution of both *T. castaneum* and *C. ferrugineus* in a three-dimensional laboratory setup with 14.5% moisture of the wheat grain at different temperatures (5, 10, 20, and 30ºC) and for different movement periods (1, 2, 3, and 24 h).

2. LITERATURE REVIEW

2.1. Grain storage and infestation

In the era of exceptionally increasing human population growth, the development of strategies to effectively manage cereal production and storage is crucial. According to the United Nations Food and Agriculture Organization (FAO), cereal production in 2023 witnessed a surge of 32 million t (Mt) in harvest. While this upward trend is promising to meet the nutritional needs of the growing population, ensuring that these grains remain viable and safe for consumption throughout the storage process is a formidable challenge. Post-harvest losses pose a major obstacle to storage and processing. It is estimated that around one-third of the harvested grains are lost before reaching the consumers (Kumar and Kalita, 2017). Among the multiple factors contributing to the loss, ranging from 20 to 40%, is substantially caused by insect infestation(Gula, 2023).

Insects cause significant damage to stored grains through various mechanisms. Firstly, they directly consume the grains, reducing the overall quantity available for use. In addition to physical consumption, insects generate filth, such as feces, shed skins, and dead bodies, which contaminate the grain, pose health risks, and can act as nuclei for mould growth. This contamination often produces unpleasant odours and leads to mycotoxin production, further degrading the quality of the grain. Moreover, certain insect species produce webbing that can entangle and bind the grains together, which can plug grain conveying and conveying systems. Consequently, the combined effects of consumption, contamination, and webbing make the grains unsuitable for human consumption (Lal et al., 2017).

Some of the common stored product insects include *Sitophilus oryzae* (L.) (rice weevil), *Sitophilus granarius* (L.) (granary weevil), *Tribolium castaneum* (Herbst) (red flour beetle), *Tribolium confusum* (Jaquelin du Val) (confused flour beetle), *Cryptolestes ferrugineus* (Stephens) (rusty grain beetle), *Oryzaephilus surinamensis* (L.) (saw toothed grain beetle), *Rhyzopertha dominica* (F.) (lesser grain borer), and *Plodia interpunctella* (Hübner) (Indian meal moth). Among these, *C. ferrugineus*, the rusty grain beetle and *T. castaneum*, the red flour beetle are particularly prevalent and are regarded as the most notorious pests in grain storage (Hagstrum, 2016).

2.2. Biology of *Tribolium castaneum* (Herbst) and *Cryptolestes ferrugineus* (Stephens)

2.2.1. *Tribolium castaneum* Identification

The red flour beetle, scientifically known as *Tribolium castaneum* (Herbst), is a member of the Coleoptera order. The primary pest species in genus *Tribolium* consists of two closely related species, *T. castaneum* and *T. confusum*. Both species have similar morphology, biology, and behaviour. However, they can be distinguished by their antennae and thorax characteristics. *Tribolium castaneum* is identified by its antennae ending in a three-segmented club, with the sides of the prothorax homogeneously curved throughout, and with the narrow part of its eyes laterally being 3-4 facets wide. On the other hand, *T. confusum* can be recognized by its antennae ending in a more gradual 4-6 segmented club, prothorax side curve is more pronounced anteriorly, and the narrow part of its eyes laterally are 1-2 facets wide (Baldwin and Fasulo, 2003). *Tribolium castaneum* is believed to have originated from warm and temperate regions of the world and has spread globally due to human activities like agricultural trading and transportation. Its remarkable adaptability to various environments, the tendency to infest stored food products has led to its cosmopolitan and significant notorious pest status in grain storage facilities. *Tribolium castaneum* predominantly infests stored grains, beans, peas, oilseeds, fruits, and starchy materials. They prefer to feed on the germ of damaged wheat kernels, followed by the endosperm. While they target the damaged kernels, the produced harmful secretion affects the product quality and makes them unfit for consumption (Canadian Grain Commission, 2017a).

Life cycle

A. Egg

The egg stage marks the beginning of its life cycle, it is typically laid by the adult females in the food source like flour, grains, and beans. These oval-shaped eggs are usually white or creamy in colour and measure around 0.61 mm in length and 0.3 mm in width. The incubation period ranges from 4 to 7 d (at 29.14 ±2.0ºC and 59.4± 3.0% relative humidity). They often adhere to the surfaces by a sticky substance produced on the coat of eggshell (Devi and Devi, 2015). With an adult female capable of laying 400 to 500 eggs throughout her lifespan and a maximum rate of increase of 70 times per lunar month, the red flour beetle stands out as one of the stored product insects with a high population growth rate (Canadian Grain Commission, 2017a, Sinha and Watter, 1985).

B. Larva

Upon hatching from eggs, the larvae emerge as small brownish-white, worm-like creatures with distinct segmentation along their bodies. The larvae undergo several molts, shedding their exoskeletons to accommodate their increasing size as they feed and grow. These molting stages are known as instars which range from 5-11 phases, influenced by factors such as temperature, relative humidity, and food availability (Abdullahi et al., 2019, Sinha and Watter, 1985). Throughout the larval stage, which can span from 12-13 d, larvae continue to grow reaching a length of approximately 4 to 6 mm. (Devi and Devi, 2015).

C. Pupa

After the larval development phase, they enter the pupal stage, undergoing metamorphosis. Initially, the pupa appears soft, gradually darkening as it matures. Sexual dimorphism in pupal length and duration is evident between males and females. A study conducted at 29.14 ±2.0°C and 59.4± 3.0% RH reported the average length and width of the male pupa as 3.18±0.3 mm and 1.07±0.03 mm respectively, whereas the female pupa length was 4.12±0.01 mm and width 1.15±0.01 mm. Under the conditions previously reported, the pupa duration for males lasted 6-7 d, and for females it lasted 7-9 d (Devi and Devi, 2015). Sex identification of this species is easier in the pupal stage, because as female pupae possess larger genital papillae than male pupae.

D. Adult

Adult red flour beetles typically measure 2.3 to 4.5 mm in length, with a slender, elongated shaped body and fully developed wings. Their exoskeleton, or outer shell is typically reddish-brown in colour and they sometimes live more than 3 yr (Chaubey, 2023, Sinha and Watter, 1985). Male and female adults can be identified by examining their anterior femur, where a sex patch is present in the males (Halstead, 1963). Their optimal developmental temperature ranges from 35 to 37.5°C with a relative humidity of 70%. Under these conditions, their growth period is 19 to 20 d (Hagstrum, 2012, Sinha and Watter, 1985). The life cycle of *T. castaneum* is depicted in Figure 2.1.

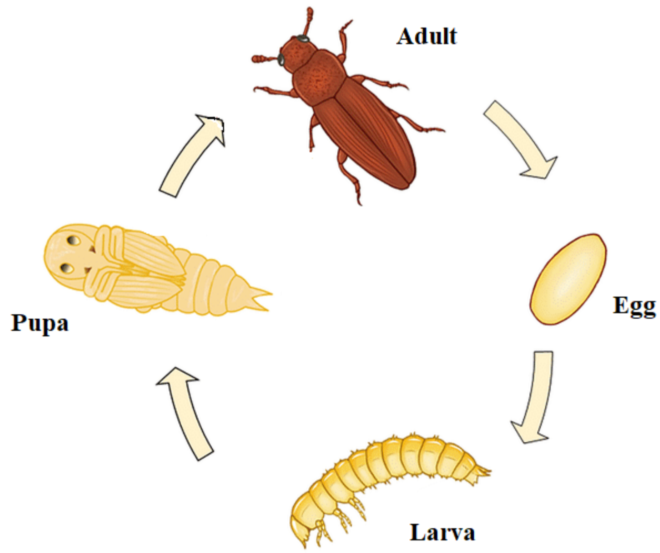

Figure 2.1. Life cycle of the red flour beetle, *Tribolium Castaneum*. Source (Klingler andand Bucher, 2022); reused under licence CC BY 4.0.

2.2.2. *Cryptolestes ferrugineus* Identification

The rusty grain beetle, initially named as *Cucujus ferrugineus* Stephens, has undergone several reclassifications over the years. It was first placed under the genus *Laemophloeus* and subgenus *Cryptolestes* and referred to as *Laemophloeus ferrugineus* (Stephens). Over time, as entomologists studied its distinct characteristics more thoroughly, the beetle was referred to by various names (Rilett, 1949). Today, it is widely recognized and referred to as *Cryptolestes ferrugineus*, placed under its own distinct genus, *Cryptolestes*. The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), is cosmopolitan in nature. Their widespread distribution is due to their remarkable adaptability to various environmental conditions, ranging from warm and humid to cooler regions, and their rapid rate of increase. Its ability to thrive in such diverse climates has contributed to its reputation as one of the most notorious pests in grain storage facilities (Bharathi et al., 2023a). The rusty grain beetle, *Cryptolestes ferrugineus*, primarily feeds on grains such as wheat, barley, rye, and oats, and can occasionally be found in milled products (Canadian Grain Commission, 2017b). The beetles have a high reproductive rate, allowing them to multiply rapidly under favourable conditions. *Cryptolestes ferrugineus* can be distinguished from other *Cryptolestes* species by observing the antennae, which are subequal in length for both males and females, measuring about half the length of the body. Additionally, the presence of an external mandibular tooth in males is a distinctive feature of this species (Bharathi et al., 2023a).

Life cycle

A. Egg

The egg stage is the initial phase in the life cycle. They are small and typically measure about 0.7 to 0.8 mm in length and 0.2 to 0.3 mm in width. They are elongated, slightly curved with a smooth and shiny surface that appears creamy white. A single female can lay up to 200 to 500 eggs over her lifetime. The incubation period generally lasts between 3 to 4 d under optimal conditions of temperature of 32ºC and 75% relative humidity (Bharathi et al., 2023a).

B. Larva

The larval stage follows the hatching of the eggs and is a critical period in the beetle's development. Larvae are slender, elongated and typically white or cream-coloured with two brown projections on the tail end. Upon hatching, they measure about 1 mm in length, but as they grow, they can reach up to 4 mm. The larval stage consists of four to five instars, or growth phases, between which the larvae molt. This stage lasts approximately 2 to 3 wk under optimal conditions. During this period, the larvae burrow into grain kernels and feed on the germ. Their cannibalistic nature also leads them to feed on pupae or eggs (Bharathi et al., 2023a).

C. Pupa

The pupal stage is the transitional phase bridging the larval and adult stages. After completing the four or five instar stages, the larvae enter the pupal stage, undergoing metamorphosis. The pupae are initially creamy white but gradually darken as they mature, signalling the nearing emergence of the adult beetle. The pupae are immobile and remain within the grain kernel or other protected environments, such as cracks and crevices in grain bulk. This stage typically lasts between 3 to 6 d. Optimal conditions for pupation are warm temperatures around 32-35ºC and relative humidity levels of about 75-90% (Bharathi et al., 2023a, Sinha and Watter, 1985).

D. Adult

The adult stage of the rusty grain beetle marks the final phase of its life cycle and is characterized by its reproductive activities and continued feeding. Adult beetles are small measuring about 1.5 to 2.5 mm in length. They have a flattened, elongated body with a reddish-brown colouration. The adult stage demonstrates remarkable cold hardiness, distinguishing *C. ferrugineus* as one of the most tolerant species in this regard. They are capable of development within a temperature range of 20 to 42.5ºC. However, their reproductive rate declines below 23ºC, and they cannot fly when temperatures drop below 21ºC (Bharathi et al., 2023a). Sexual dimorphism in *C. ferrugineus* is evident through several distinct physical and anatomical differences between males and females. Females exhibit a slightly smaller head than their prothorax, possess internal sexual organs that form a distinct Y shape visible through transparency, and feature short tactile styli at the tip of the abdomen. In contrast, males have a head size nearly identical to their prothorax, display a straight line instead of a Y-shaped internal structure, and lack styli (Figure 2.2). Additional differences include females lacking a lateral mandibular tooth and having 5 segments in their hind tarsi, while males have a mandibular tooth and 4 segments in their hind tarsi (Halstead, 1963).

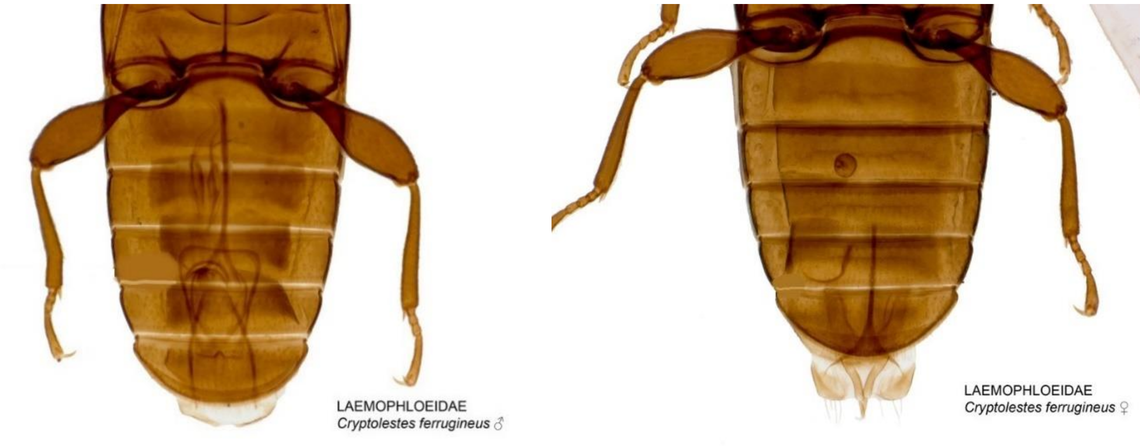

Figure 2.2. Female and Male *Cryptolestes ferrugineus* adults abdomen view under transparent light. Image used from Pest and Disease Image Library (*Scanlon*, 2022) under Creative Commons Attribution-Non Commercial 4.0 International licence (CC BY-NC 4.0).

2.3. Movement Behaviour

2.3.1. Dispersal behaviour

Insects exhibit an active dispersal pattern influenced by a range of internal and external environmental factors. Many experts have studied the distribution patterns of stored product pests throughout time. Research has indicated that a variety of parameters including temperature, moisture content, relative humidity, food source and insect density can significantly affect their movement inside the grain bulk (Anukiruthika et al., 2021). For instance, Surtees (1963) observed that the dispersal pattern of *T. castaneum* was random at temperatures of 25 and 30^{o}C. A noticeable reduction in activity was observed when the temperature was lowered to 15^{o}C with a moisture content of 14%. This indicates that *T. castaneum* activity increases with rising temperatures. In contrast, *C. ferrugineus* exhibited a different response under similar moisture conditions. Despite a reduction in activity at lower temperatures, the dispersion pattern of *C. ferrugineus* remained relatively consistent, showing little variation regardless of temperature changes. This suggests that while *C. ferrugineus* also experiences reduced activity in colder conditions, its overall dispersal behaviour is less influenced by temperature fluctuations compared to *T. castaneum*. Additionally, a study examining spontaneous walking stops (Sm_h) and the critical chilling temperature (CCT), where insects do not move even after shaking, was conducted on four stored pests: *C. ferrugineus*, *T. castaneum*, *S. oryzae*, and *S. zeamais*. The findings revealed that *C. ferrugineus* adults had the lowest Sm_h at 4ºC and CCT at 2ºC. In contrast, *T. castaneum* adults ceased movement between 8ºC and 9ºC, with rapid temperature decreases not significantly affecting their Sm_h (Jian et al., 2015).

According to Surtees (1965), the hydrokinetic variable plays a crucial role in the dispersal of *T. castaneum*, as the adult movement patterns vary according to relative humidity levels. Females typically move more slowly than males because their higher respiratory rates lead to greater water loss. As an adaptive behaviour to conserve water, females reduce their movement. However, in high relative humidity conditions (85%), females travel at speeds comparable to males (Surtees, 1964a). Similarly, Jian et al. (2006) reported that hydrophilic behaviour influenced the dispersal of C. ferrugineus within a 1-D corn grain column. These insects moved faster in dry grain and slower in moist grain as they tended to accumulate in areas with higher moisture content. In drier conditions, *C. ferrugineus* dispersed more extensively as they actively searched for regions with higher moisture levels (Jian et al., 2005a). This behaviour indicates their preference for more humid environments, which drives them to explore further in less favourable dry conditions. Additionally, their vertical dispersion within the grain column was attributed to the drift effect, where movement is influenced by gravity and physical displacement within the grain bulk.

In a 2-D study of *C. ferrugineus* in wheat, Jian et al. (2007) reported that the diffusivity of these insects within the grain column increased with rising temperatures and higher insect densities. This means that the insects spread more widely and rapidly under warmer conditions and in the presence of more insects. Conversely, the diffusivity decreased in conditions of high moisture and over longer periods of movement. In high-moisture environments, the insects tended to congregate in moist areas, reducing their overall spread (Jian et al., 2005a). Also, the initial rapid dispersal likely slowed over extended movement periods as the insects settled into favourable locations within the grain column.

The size of the insects and the type of grain also influence their dispersal patterns. The *C. ferrugineus*, with its smaller body size, moves quickly through the grains. In contrast, *T. castaneum* is approximately twice as large as *C. ferrugineus* and thus takes more time to move through the grain (Bharathi et al., 2023b) . The type of grain can impact how these insects move, with factors such as grain size, shape, and density affecting their ability to disperse within the grain bulk. Jian et al. (2005b) investigated the movement of *T. castaneum* in 1-D vertical and horizontal columns of wheat and corn, both with and without temperature gradients. The study found that in the wheat columns, the adults primarily moved through the side gaps of the box rather than penetrating into the bulk grain. Over 90% of the beetles were recovered from the area where they were initially introduced. In contrast, in the corn columns, less than 30% of the adults were recovered from the introduction area, indicating that they traveled through the larger pore spaces within the corn bulk. This demonstrates that the larger body size of *T. castaneum* restricts its movement in grain bulks with smaller pore sizes such as wheat, while allowing more extensive dispersal in grain types with larger pore spaces, like corn.

2.3.2. Flight activity

Flight activity in insects refers to their ability and tendency to fly, is a key behavioural trait that influencing their dispersal, colonization, and infestation patterns. Numerous studies have been conducted over the years to understand the flight activities of *T. castaneum* and *C. ferrugineus* in both field and laboratory conditions. This activity can be triggered by various environmental factors such as temperature, humidity, light conditions, overcrowding, and the presence of food or mates (Perez-Mendoza et al., 2014, Bharathi et al., 2023a). Flight allows insects to move quickly and cover large distances, enabling them to find and infest new storage facilities. Understanding flight activity helps in predicting how pests spread and identify potential points of infestation and aids in developing pest management strategies to target critical periods when insects are most likely to disperse (Jian, 2019).

In a laboratory study examining the effects of abiotic factors on the flight activity of *T. castaneum*, it was found that temperature and photoperiod were the primary factors influencing flight activity. In contrast, relative humidity and light intensity did not significantly affect flight (Perez-Mendoza et al., 2014). A separate study examined the impact of insect density, sex, age, and food deprivation on the flight activity of *T. castaneum*. Contrary to general expectations, it was found that insect density did not significantly affect flight activity. Additionally, there was no significant difference in flight initiation between male and female beetles, and the presence of food had minimal impact on their flight behaviour (Perez-Mendoza et al., 2011a). Interestingly, *T. castaneum* adults can survive without food for up to 18 d, although their rate of flight initiation increases when food resources are scarce (Perez-Mendoza et al., 2011b). This suggests that limited food availability may stimulate their dispersal efforts. While *T. castaneum* can potentially use flight for dispersal at various life stages, they are most active in their adult stage. However, their flight activity decreases as they age (Perez-Mendoza et al., 2011b) .

The minimum temperature required for flight initiation is 25ºC for *T. castaneum* (Cox et al., 2007) and 20ºC for *C. ferrugineus* (Cox and Dolder, 1995). A field study on the flight activity of *T. castaneum* in subtropical regions of Queensland, Australia, found year-round flight activity, with monthly temperatures ranging from a mean daily minimum of 9ºC to a mean daily maximum of 34.4ºC (Daglish et al., 2017). In contrast, in South Carolina, USA, the lowest temperature when flight activity for *T. castaneum* was observed at 17.2ºC (Throne and Cline, 1994). For *C. ferrugineus*, there was no flight activity during the winter months of June, July, and August in New South Wales, Australia (Holloway et al., 2018). In Kansas and Nebraska, USA, *C. ferrugineus* exhibited high flight activity in September, which then declined through November (Toews et al., 2006).

2.3.3. Aggregation behaviour

Aggregation behaviour in insects refers to the tendency of individuals to group together. This behaviour can be influenced by various factors including environmental conditions, presence of food, mating opportunities, and pheromone secretion (Shorey, 1973). One of the primary mechanisms driving aggregation in beetles is the release of pheromones. These chemical signals can attract species to a particular location, enhancing group formation. The aggregation pheromone produced by male *T. castaneum* is 4,8-Dimethyldecanal (DMD) (Suzuki and Sugawara, 1979) and male *C. ferrugineus* produces ferrulactone I, (E, E)-4, 8-decadien-10-olide, and ferrulactone II, (S)-(Z)-3-dodecen-11-olide (Borden et al., 1979), commonly referred to as *cucujolide* I and II, respectively (Losey et al., 2019). Research has shown that these volatile compounds emitted by the males attract both males and females in both *T. castaneum* and *C. ferrugineus* (Borden et al., 1979; Obeng-Ofori and Coaker, 1990). A trapping experiment conducted by Lindgren et al. (1985), it was revealed that *T. castaneum* exhibited a slight cross-attraction to the pheromone of *C. ferrugineus*. This cross-attraction suggests some level of interspecific pheromone recognition. The presence and quality of food resources are critical determinants of aggregation.

Beetles often aggregate where food is abundant, enhancing their feeding efficiency and reproductive success (Currie et al., 2020). These beetles rely heavily on their olfactory senses to detect environmental cues. An experiment by Obeng-Ofori and Coaker (1990), investigated *T. castaneum's* pheromone attraction range and orientation behaviour and revealed that the beetles utilized chemo-orientation to locate pheromones, demonstrating their ability to navigate towards chemical signals. Traps with higher pheromone concentrations attracted beetles from a wider sampling range, indicating that stronger pheromone signals are more effective in drawing beetles over larger distances. Additionally, the average walking speed of the beetles increased with higher pheromone concentrations, suggesting that the intensity of the pheromone signal enhances their movement efficiency. Moreover, in a 3-D spatial and temporal study involving 1.5 t of wheat grain, notable aggregation patterns were observed for both *T. castaneum* (Jian et al., 2012) and *C. ferrugineus* (Jian et al., 2011). At lower insect densities, these species tended to aggregate more, indicating a high level of clustering. However, as the insect densities increased, the level of aggregation decreased. This reduction in clustering at higher densities is attributed to the repelling effect between individual insects (Jian et al., 2011, 2012). The repulsion likely occurs as competition for resources intensifies, leading the insects to spread out more evenly throughout the grain mass to avoid direct competition and potential conflicts.

2.4. Knowledge gap

Researchers have conducted 1-, 2-, and 3- D studies over time to comprehend the movement patterns and spatial distribution of *T. castaneum* and *C. ferrugineus* within whole grains, both in laboratory and in large grain bulks in full-size bins (Bharathi et al., 2023a ; Jian et al., 2012). Jian et al. (2004) performed a 1-D study on *C. ferrugineus* using a 10 cm by 10 cm by 100 cm grain column to examine their responses to temperature gradients, movement periods, and insect densities. Further research by Jian et al., (2005a) explored the *C. ferrugineus* responses to different moisture gradients. Anukiruthika et al. (2023) expanded on this work by studying the movement of these beetles in grain columns of varying lengths. A 2-D study provided deeper insights into the diffusion pattern behaviour of *C. ferrugineus* in response to varying temperatures, moisture levels, and movement durations (Jian et al., 2007). Jian et al. (2005b) conducted another study examining the movement of *T. castaneum* within wheat and corn columns both vertically and horizontally, with and without temperature gradients.

The first 3-D study on *T. castaneum* and *C. ferrugineus* was conducted by Surtees (1963). In this study, 64 cubes were arranged into 4 layers, filled with grains, and the insects were introduced at the top surface, allowing them to move only downward. Due to the small amount of grains used (25 kg) and the tedious construction of the cubes, repetition and longer distance studies were not feasible. Bharathi et al. (2022a) modified the experimental design by using a wooden container containing 343 mesh cubes arranged in 7 layers, with insects introduced at the centre to allow movement in any direction. A 24-h movement period, 3-D study on *C. ferrugineus* was conducted under constant temperatures (20 and 30ºC) and moisture contents (12.5, 14.5, and 16.5%). They concluded that the distribution of the insects was random in horizontal direction, along with the 'drift' effect in vertical direction, aligning with the results from previous 1-D and

2-D studies. However, they did not determine how *C. ferrugineus* adults behave in different movement periods and low temperatures. Additionally, the movement of *T. castaneum* within the 3-D setup has not yet been reported. Therefore, the primary objective of this study was to investigate the 3-D movement and distribution patterns of *T. castaneum* and *C. ferrugineus* individually. Specifically, the study aimed to observe their behaviour and the distribution variations of adults between the layers, as well as their location preferences inside the wooden container for different movement periods and a range of temperatures.

3. MATERIALS AND METHODS

3.1. Wheat

From Richardson International Ltd., Winnipeg, Manitoba, around 2,000 kg of Canadian Western Red Spring Wheat (Grade No.1, cv. AC Barrie, certified) was obtained and utilized for this study. The wheat underwent cleaning procedures using a 3D shaker (Vibro-Energy Separator, SWECO, Florence, KY) to eliminate any dockage and it was stored in double-layered plastic bags. Subsequently, the wheat was stored in -15^{o}C for a minimum of three weeks to ensure the absence of any insect contaminants (Fields and White, 1997). The moisture content of the received wheat was determined by the oven drying method, where 10 g of wheat sample from each bag was dried at 130^{o}C for 19 h, and the moisture content of the samples was 11±0.5 % (wet basis) (ASABE 2016). The wheat was conditioned to the desired moisture content of 14.5% (wet basis) by adding the necessary amount of distilled water. The mixture was then rotated in the mixer for approximately 35 to 40 min. The conditioned wheat was again stored in double-layered plastic bags at 10^{0}C for 2 to 4 wk. At least four days before to the experiment, the wheat bags were transferred to the environmental chamber (CMP 4030, Controlled Environments Limited, Winnipeg, MB), maintaining the experimental temperature and the moisture content of conditioned wheat was verified. This procedure was repeated for each experiment.

3.2. Experimental setup

The experimental setup was the same as that utilized by Bharathi et al., (2022b). A total of 343 mesh cubes, each measuring 0.1 × 0.1 × 0.1 m were arranged within a wooden container with internal dimensions of 0.7 × 0.7 × 0.7 m (Figs. 3.1 and 3.2). The mesh cubes were constructed using two 91.4 cm metal rods which were bent to create frames and then combined to form a single cube. These mesh cubes were then enclosed by cloth screens with openings of 1.4 × 1.4 mm, which enabled insect movement between cubes while preventing wheat from escaping the cubes (Fig. 3.3). One day before to the experiment, all the cubes were filled with conditioned wheat, a process that took approximately 2-3 h. The mesh cubes were then loaded into the wooden container layer by layer to form aligned rows and columns (Fig. 3.1). The wooden container was then sealed with a lid and double-sided tape was applied along the top edges.

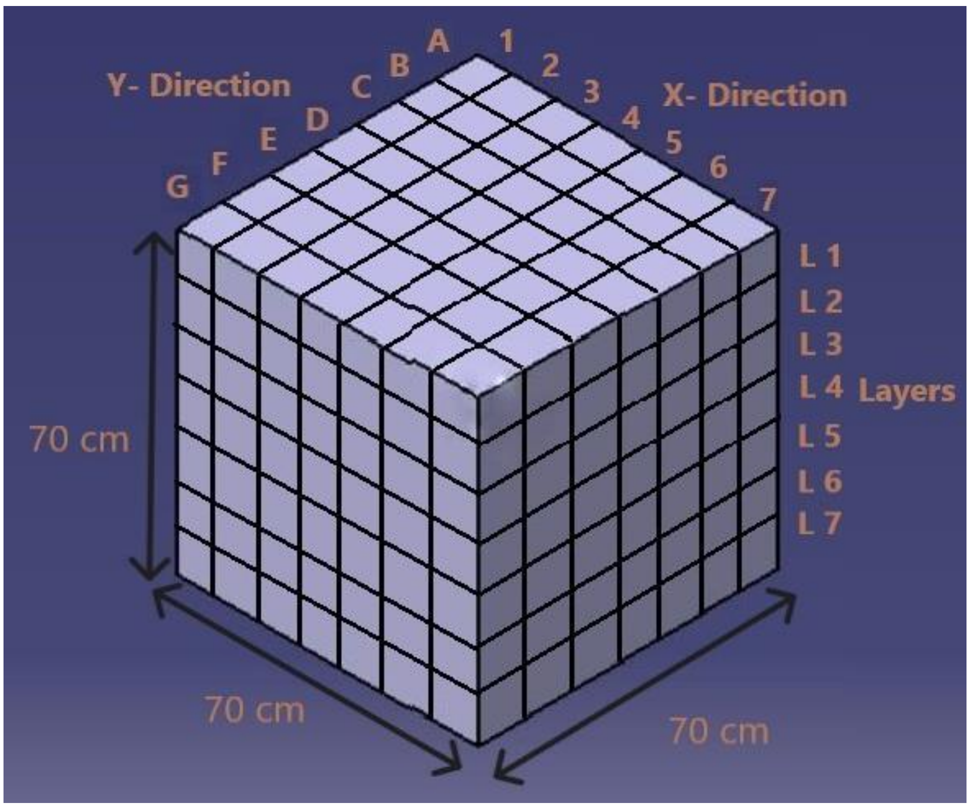

Figure 3.1. Internal dimensions of the wooden container and numeration of each small mesh cubes. Insects were introduced in the L4-D4 cube located at the centre.

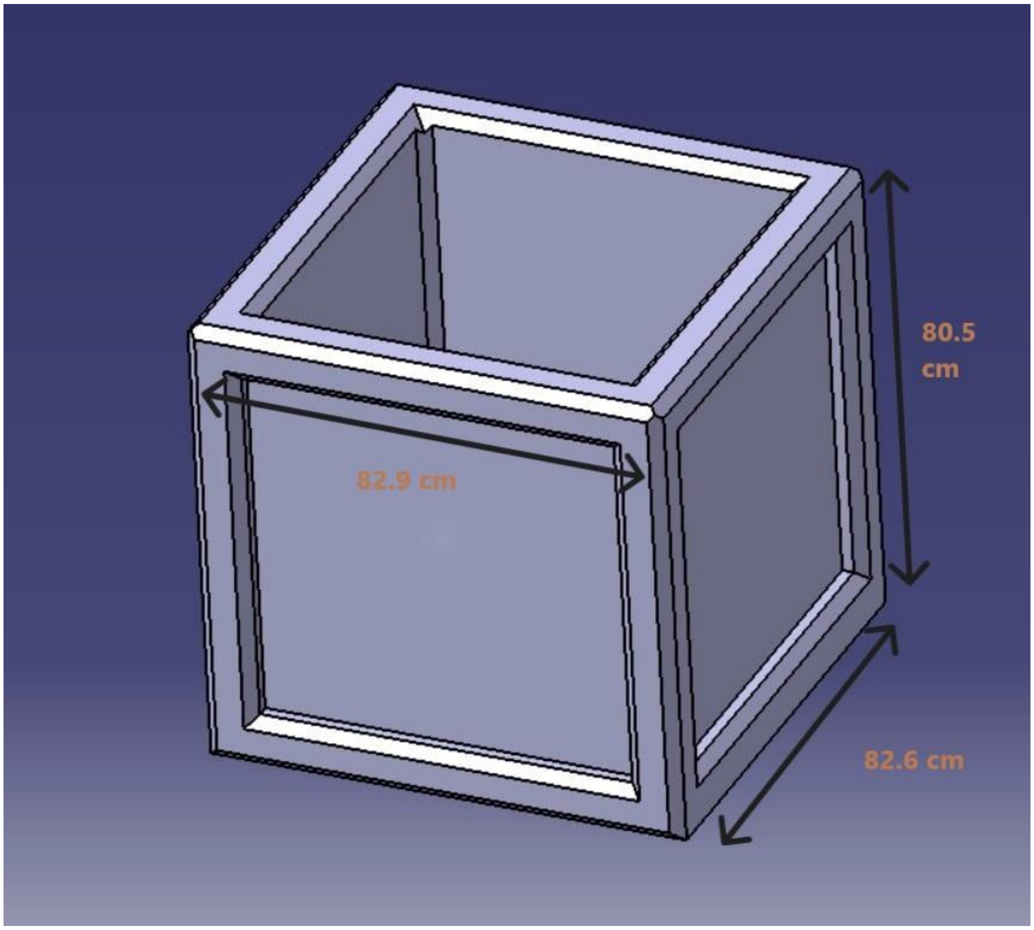

Figure 3.2. The wooden container used for the study (inside dimensions 0.7 × 0.7 × 0.7 m). A lid made with 1.1cm thick plywood was used to cover the container.

Figure 3.3. Constructed mesh cube with dimensions 0.1 × 0.1 × 0.1 m.

3.3. Insects

Both *C. ferrugineus* and *T. castaneum* adults were cultured in a laboratory environment maintained at 30 ± 1ºC and 75 ± 5% relative humidity. For *C. ferrugineus*, a medium consisting of a mixture of whole wheat kernels, cracked wheat, and wheat germ in a mass ratio of 95:5:5 was utilized. Meanwhile, *T. castaneum* was reared on a mixture of wheat flour and brewer's yeast in a mass ratio of 95:5. For all experiments, adults of both insects aged between 1-d to 2-mo were utilized. One day before to each experiment, 100 adults were sieved from the laboratory culture and placed inside a mesh cube for acclimation. This cube contained the same wheat as used for the experiments. Subsequently, the cube was positioned inside a plastic container (33.1×23.5×17.3 cm) for 24-h acclimation within the environmental chamber (Conviron CMP1010, Controlled Environments Ltd., Winnipeg, MB, Canada) set at the desired temperature (10, 20 or 30° C). For the 5ºC experiment with *C. ferrugineus*, a 120-h acclimation period was implemented during which the temperature was gradually reduced at a rate of 5ºC/d, while a 24-h acclimation period was used for all the other temperatures.

3.4. Experimental Procedure

A day before to the experiment, the prepared wheat was filled into the mesh cubes and placed inside the wooden container, except for the cube containing adults, which was placed inside a plastic container. Each mesh cube was labelled for convenience, with the x direction numbered from 1 to 7, the y direction from A to G, and the layers from L1 to L7 (Fig. 3.1). The cube at the top right corner was labelled as L1-A-1, while the central cube containing the adults was labelled as L4-D-4. After 24 h acclimation period, on the day of the experiment, the cube containing the adults was placed at the L4-D-4 location by removing only the centre cubes from the top three layers. The wooden container was then covered with a plywood lid and sealed with double-sided tape. After the desired movement period, the lid was opened, and the cubes were removed. Each cube was placed into a pre-labelled plastic bag according to its position and each bag was tied shut. This process took approximately 30 to 45 min in total and was conducted inside the environmental chamber.

Adults in each labelled cube were separated using a shaker with a No. 14 sieve (1.4 mm mesh openings). Not all introduced adults were successfully recovered, as some adults tended to hide among the wheat kernels. Some adults were stuck on the tapes which was used to seal the lid of the wooden container. A total of 35 *T. castaneum* adults were recovered from the tape for all the tests conducted at 20 and 30ºC while no adults were recovered from the tape at lower temperatures of 10 and 5ºC. Additionally, no adults of *C. ferrugineus* were recovered from the tape under any of the tested conditions.

The mean recovery rate was 93.2% (n=19) for *T. castaneum* and 97.4% (n=18) for *C. ferrugineus*. To account for the missed adults, each replicate's total number of recovered insects was normalized using Eq. 3.1 (Jian et al., 2007).

$$\text{Number of adults in the cube } = \frac{\text{Number of adults recovered from the cube } \times 100}{\text{Total number of adults recovered from all cubes}} \quad (3.1)$$

where 100 represents the number of initially introduced adults.

3.5. Statistical Analysis

A total of 14 experiments, consisting of 7 experiments for *T. castaneum* and 7 experiments for *C. ferrugineus* were conducted. All the experiments except the experiment for *T. castaneum* at 5ºC were replicated three times in 14.5% moisture content wheat at 5, 10, 20 and 30°C for movement periods of 1, 2, 3 and 24 h. Experiment for *T. castaneum* at 5ºC was only conducted once due to the absence of insect movement under this condition. The data used for analyzing and comparing the 24-h movement of *C. ferrugineus* at 30ºC was sourced from Bharathi et al. (2022a).

An ANNOVA followed by a Tukey's test was conducted to assess whether the adults exhibit any preference for specific layers within the setup and to determine if this preference changes with variations in environmental conditions. The test was carried out within different layers for each experimental condition and within each layer across various experiments, all at a 0.05 confidence level. To address the risk of Type I errors associated with multiple comparisons, the Tukey Honestly Significant Difference (HSD) test was employed, ensuring that the confidence level remains at 0.05 during all the comparisons (Greenwood and Banner, 2015; Navarro, 2019).

To examine whether adults exhibit a preference for the boundary or the centre under each experimental condition, each layer was subdivided into three categories: centre, middle and periphery (Fig. 3.4). The adult density in each area was computed (Eq. 3.2), and Tukey test was performed.

$$\text{Adult density} = \frac{\text{Number of adults in the area}}{\text{Total wheat mass in the area}} \tag{3.2}$$

A two-sample location test and Empirical Distribution Function (EDF) statistics were performed using R Studio software (version 4.3.1) to analyze whether adults exhibit similar movement patterns in both horizontal and vertical directions under different temperatures and movement periods (Jian et al., 2002). This analysis involved comparing two temperatures in the same movement period (1 h) and two movement periods at the same temperature (30ºC). The variations in each layer were assessed by the Wilcoxon test, while the cumulative distribution along the vertical direction was analysed using Kolmogorov–Smirnov test and a Mood's median test was conducted to analyse the general movement of the introduced adults. All the analyses were performed separately for both *T. castaneum* and *C. ferrugineus* adults.

To calculate the maximum vertical movement speed of the adults at different temperatures, the distance traveled by the fast moving adult in both the upward and downward directions from the point of introduction within 1 hour at ≥10$^{℃}$ and 24 h at 5$^{℃}$ was calculated. Since the point of introduction is in the middle of the wooden container, the distances travelled to the upward layers 1, 2, and 3 were 30, 20, and 10 cm respectively. In the downward direction, the distances travelled to layers 5, 6, and 7 were 10, 20, and 30 cm respectively. The maximum movement speed in the horizontal direction was the distance from the point of introduction to the location where the furthest-moving adults were recovered, divided by the movement time. The distance from the centre, middle and periphery area were 10, 20, and 30 cm respectively.

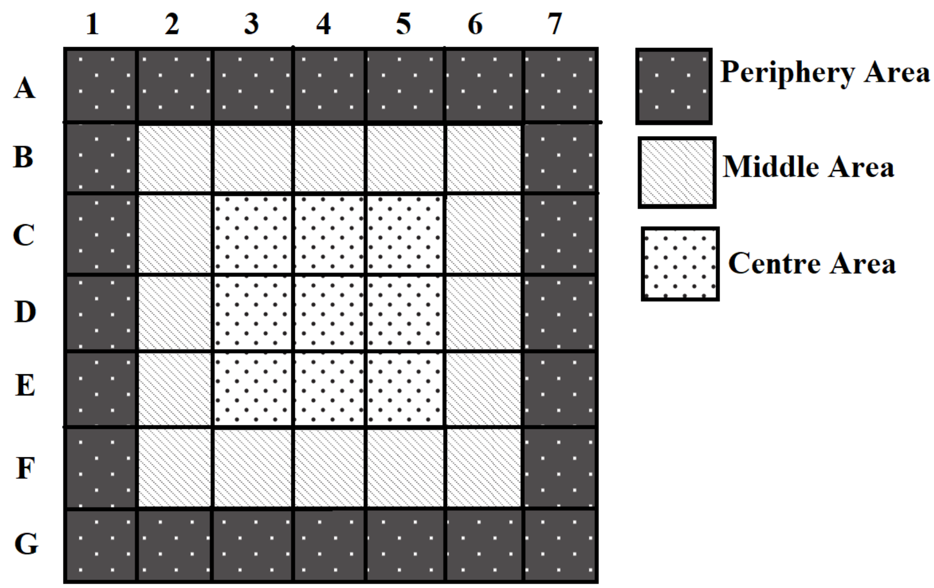

Figure 3.4. Categorization and numeration of mesh cubes inside the wooden container.

4. RESULTS

4.1. Movement and distribution of *Tribolium castaneum* adults

4.1.1. Movement in the vertical direction

At 20 and 30ºC, the adults of *T. castaneum* displayed active movement in both upward and downward directions, and more adults moved down (Figs. 4.1- 4.2). When the temperature was reduced to 10ºC, these adults restricted their movement, and more adults moved up (Fig. 4.2). As the temperature was further decreased to 5ºC, the adults ceased movement (Fig. 4.2). The adults moved at a speed of more than 7.2 m/d at 30 and 20ºC in both upward and downward vertical directions. This speed decreased to 4.8 m/d in the upward direction and 2.4 m/d in the downward direction at 10ºC (Table 4.1).

As the movement duration increased from 1 to 2 h at 30ºC, the upward movement increased from 19% to 22%. However, when further increasing time from 3 to 24 h, more adults moved down. For example, only 8% and 9% adults moved upward in 3 and 24 h, respectively. At 20 and 30^{oC}, the mean fraction of recovered adults in the bottom layer was notably higher compared to the other layers. At 20 and 30ºC in 1 h, 21.1% and 16.9% adults were recovered in the bottom layer, respectively. At 30ºC and with the time increased from 1 to 24 h, the recovered adults in the bottom layer were increased from 16.9% to 42.3% (Table 4.2). The Tukey test also showed the numbers in bottom layer and the insect introduction layer were significantly higher than other layers (Table 4.3). The two-sample location test and EDF statistics revealed that the adults at 20 and 30ºC had similar distribution patterns (Table 4.3). When the temperature was higher than 20ºC, the adults gradually shifted to a downward movement after an initial upward movement.

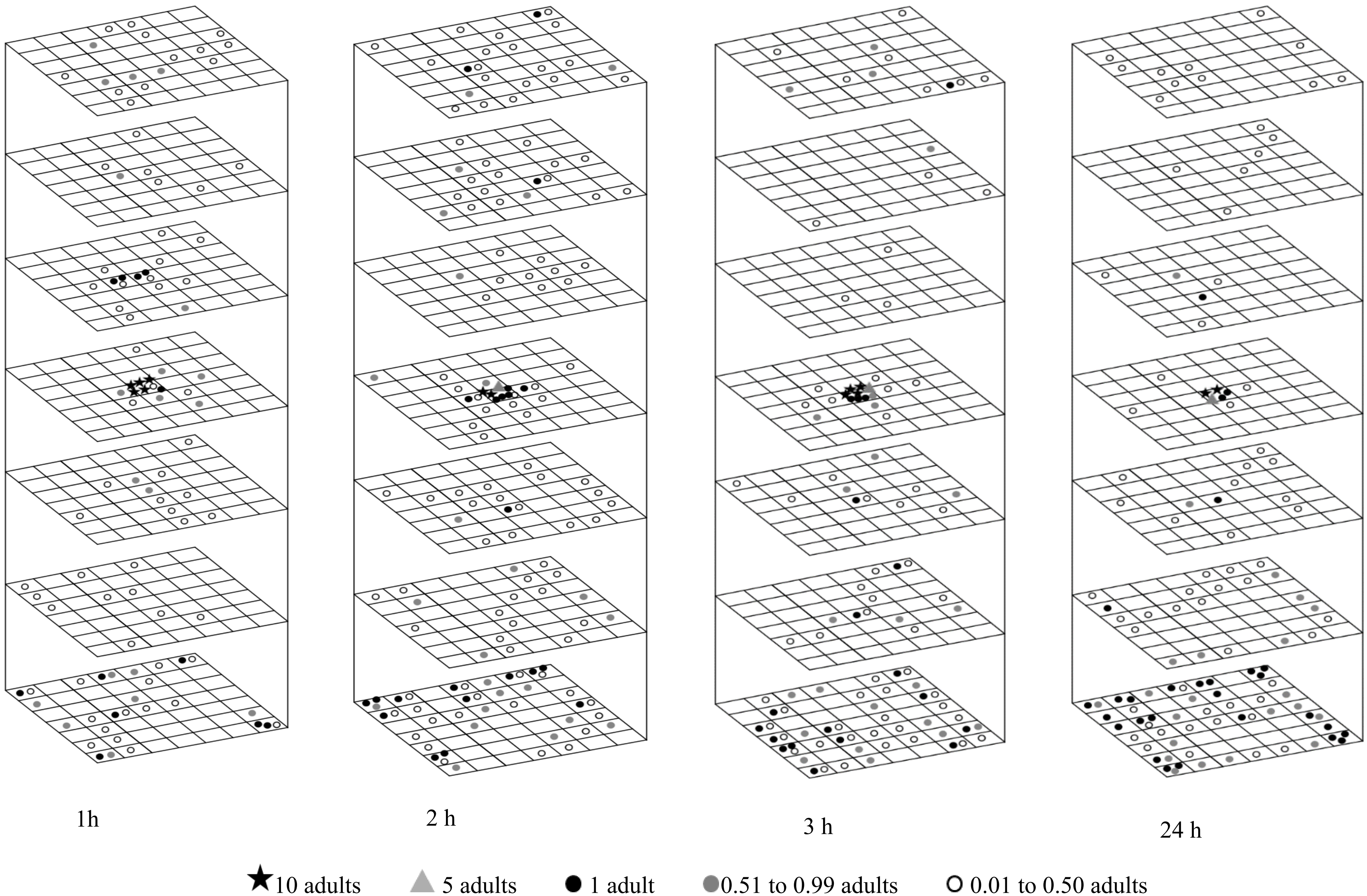

Figure 4.1. Redistribution of 100 *Tribolium castaneum* adults at 30ºC in wheat with 14.5% moisture content in different movement periods.

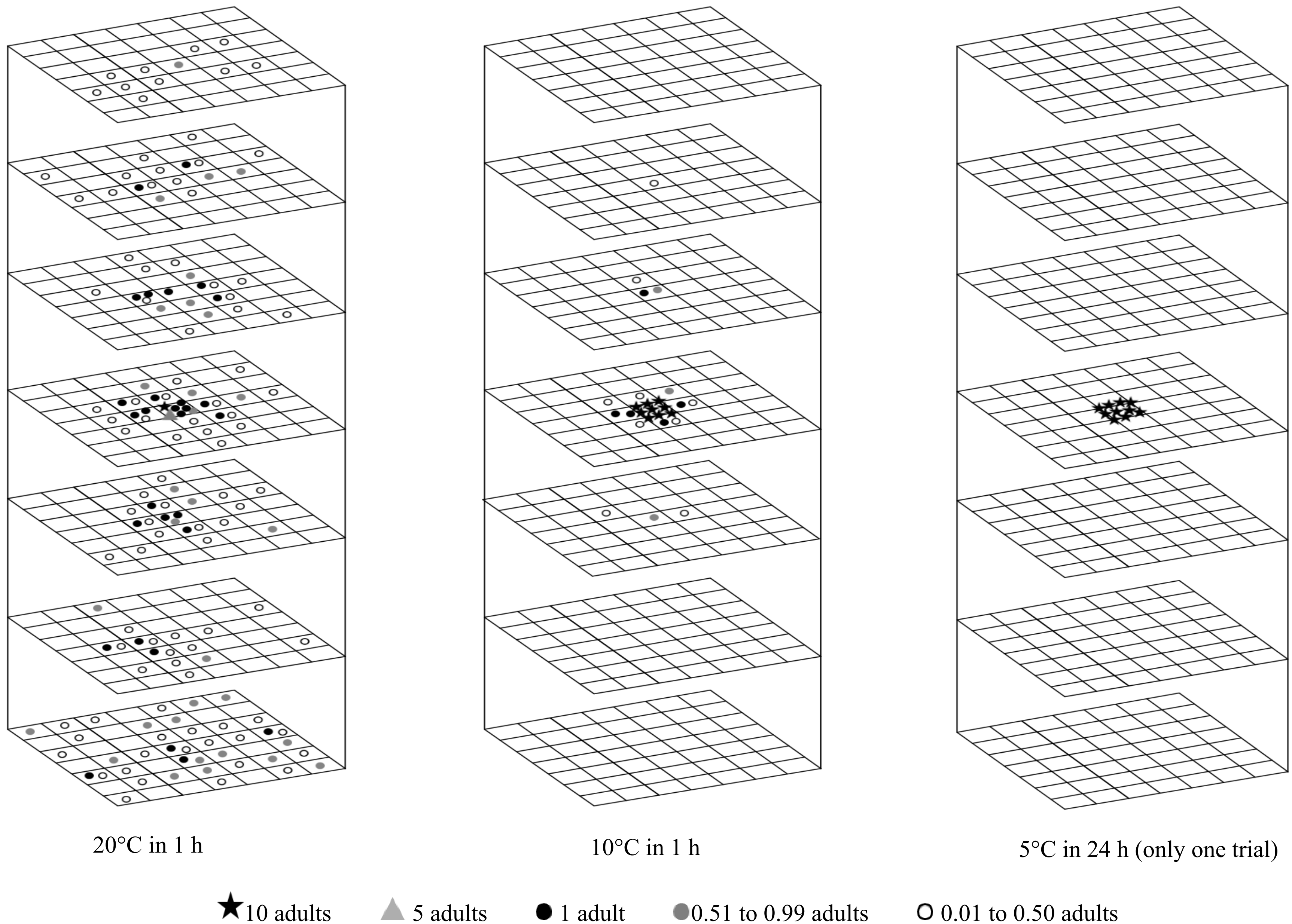

Figure 4.2. Redistribution of 100 *Tribolium castaneum* adults in wheat with 14.5% moisture content at 20, 10 and 5ºC.

Table 4.1. Maximum movement speed of *T. castaneum* in vertical and horizontal direction.

Grain Temperature (^{o}C)	Movement speed (m/d)		
	Vertical direction		Horizontal direction
	Upward direction	Downward direction	
30	>7.2	>7.2	>7.2
20	>7.2	>7.2	>7.2
10	4.8	2.4	2.4

Table 4.2. Recovered adults of *T. castaneum* in different layers at each tested condition

Layers		Tested Conditions [γ]					
		30ºC in 1 h	**30ºC in 2 h**	**30ºC in 3 h**	**30ºC in 24 h**	**20ºC in 1 h**	**10ºC in 1 h**
1		7.9±2.3 $^{\mathbf{b\,A}}$	9.6±6.6 $^{\mathbf{bc\,A}}$	5.1±3.7 $^{\mathbf{b\,A}}$	4.1±1.3 $^{\mathbf{b\,A}}$	4.4±1.0 $^{\mathbf{b\,A}}$	0.0±0.0 $^{\mathbf{c\,B}}$
2		2.9±3.3 $^{\mathbf{b\,A}}$	8.0±4.3 $^{\mathbf{c\,A}}$	1.8±1.7 $^{\mathbf{b\,A}}$	2.2±3.0 $^{\mathbf{b\,A}}$	8.1±6.6 $^{\mathbf{b\,A}}$	0.3±0.6 $^{\mathbf{bc\,A}}$
3		8.3±4.2 $^{\mathbf{b\,AB}}$	4.4±3.2 $^{\mathbf{c\,A}}$	1.1±2.0 $^{\mathbf{b\,A}}$	3.0±0.7 $^{\mathbf{b\,A}}$	12.1±5.2 $^{\mathbf{b\,A}}$	2.0±1.0 $^{\mathbf{b\,B}}$
4		55.8±15.1 $^{\mathbf{a\,B}}$	36.1±4.2 $^{\mathbf{a\,A}}$	53.0±23.5 $^{\mathbf{a\,A}}$	31.6±9.8 $^{\mathbf{a\,A}}$	32.6±11.9 $^{\mathbf{a\,B}}$	96.3±0.6 $^{\mathbf{a\,A}}$
5		4.7±1.6 $^{\mathbf{b\,AB}}$	7.7±1.3 $^{\mathbf{c\,A}}$	5.6±4.8 $^{\mathbf{b\,A}}$	5.6±2.2 $^{\mathbf{b\,A}}$	13.4±7.2 $^{\mathbf{b\,A}}$	1.3±1.1 $^{\mathbf{bc\,B}}$
6		3.6±0.6 $^{\mathbf{b\,AB}}$	8.9±4.2 $^{\mathbf{c\,A}}$	6.8±3.5 $^{\mathbf{b\,A}}$	11.2±5.1 $^{\mathbf{b\,A}}$	8.4±3.5 $^{\mathbf{b\,A}}$	0.0±0.0 $^{\mathbf{c\,B}}$
7		16.9±11.8 $^{\mathbf{b\,AB}}$	25.3±10.8 $^{\mathbf{ab\,A}}$	26.6±18.7 $^{\mathbf{ab\,A}}$	42.3±11.3 $^{\mathbf{a\,A}}$	21.2±6.9 $^{\mathbf{ab\,A}}$	0.0±0.0 $^{\mathbf{c\,B}}$
Tukey test	**F**	18.590	12.920	8.035	20.570	6.047	9080
values	**P**	<0.001	<0.001	0.001	<0.001	0.003	<0.001

[γ] The lowercase letters a, b, and c in a column indicate significantly different mean values among different layers under the same tested condition. The uppercase letters A, B, and C in a row indicate significantly different mean values among the same layers under different tested conditions. Tukey's test at a 0.05 confidence level was used to conduct these comparisons.

Table 4.3. Two-sample location test and EDF statistics result for *T. castaneum* movement and distribution in vertical direction.

Experimental conditions [γ]		**Wilcoxon**		**Kolmogorov-Smirnov**		**Mood median**	
Temperature (^{0}C)	**Time (h)**	**Z**	**p**	**D**	**P**	**Z**	**p**
30	1 vs 2	4.420	<0.001	-0.548	<0.001	1.741	0.082
	2 vs 3	4.145	<0.001	-0.571	<0.001	3.539	<0.001
	3 vs 1	3.207	0.001	-0.405	<0.001	2.915	0.003
	24 vs 1	3.920	<0.001	-0.405	<0.001	1.575	0.115
	24 vs 2	4.858	<0.001	-0.571	<0.001	2.201	0.028
	24 vs 3	3.570	<0.001	-0.429	<0.001	3.210	0.001
30 vs 20	1	4.870	<0.001	-0.524	<0.001	1.139	0.255
30 vs 10		0.663	0.507	-0.262	0.041	4.796	<0.001
20 vs 10		2.251	0.024	-0.405	<0.001	5.723	<0.001

[γ] Comparison between two experiments, with the first column representing the constant experimental condition and the second column representing the variables being compared

4.1.2. Adult movement in the horizontal direction

Their migration in the horizontal direction increased with the increase of both temperature and movement period, and a diffusion pattern in the horizontal direction was observed across nearly all layers (Figs. 4.1- 4.2). At temperatures of 30 and 20ºC, the adults moved with a movement speed of more than 7.2 m/d in the horizontal direction, which was reduced to 2.4m/d at 10ºC (Table 4.1) At the higher temperature of 30ºC and with extended movement periods, the adults in the bottom layer (layer 7) tended to accumulate at the periphery of the container (Fig. 4.1). Although the adults in the introduced layer (layer 4) moved upward and downward over time, a high density of adults remained at the centre location, indicating that many adults did not move and stayed within their introduced location under all tested conditions (Table 4.4). The statistical analysis confirmed these observations (Table 4.4). For instance, at 30ºC, adults consistently remained in the centre location of the introduced layer (layer 4) rather than the middle and periphery location during various movement periods (Table 4.4). Therefore, the adults moved both upward and downward from their introduced location, exhibiting a diffusion horizontal distribution during migration. Their movement was constrained by the boundary of the wooden container, leading them to remain near the periphery in the bottom layer (layer 7).

Table 4.4. Recovered densities of *T. castaneum* adults in different areas in the same layer at the same tested conditions.

Layer	Time (h) †	Centre	Middle	Periphery	Tukey test values	
					F	P
			30ºC µ			
1 γ	1	0.2±0.2 a	0.2±0.1 a	0.1±0.1 a	0.424	0.673
	2	0.3±0.3 a	0.2±0.2 a	0.2±0.1 a	0.377	0.701
	3	0.3±0.2 a	0.0±0.1 a	0.2±0.1 a	1.717	0.257
	24	0.1±0.1 a	0.1±0.1 a	0.1±0.1 a	0.726	0.522
2 γ	1	0.2±0.3 a	0.0±0.1 a	0.1±0.1 a	1.361	0.326
	2	0.4±0.2 a	0.2±0.2 a	0.1±0.1 a	2.299	0.182
	3	0 a	0.0±0.4 a	0.1±0.1 a	1.317	0.336
	24	0.1±0.2 a	0.3±0.1 a	0.1±0.1 a	0.274	0.769
3 γ	1	0.8±0.5 a	0.1±0.1 b	0.1±0.0 ab	6.363	0.033
	2	0.3±0.2 a	0.1±0.1 a	0.3±0.1 a	3.792	0.086
	3	0.1±0.1 a	0.1±0.1 a	0 a	0.503	0.628
	24	0.3±0.0 a	0 b	0.0±0.1 b	213.500	<0.001
4 γ	1	7.2±2.2 a	0.1±0.1 b	0.0±0.1 b	32.870	<0.001
	2	4.5±0.4 a	0.1±0.1 b	0.1±0.1 b	429.500	<0.001
	3	7.0±3.4 a	0.1±0.2 b	0 b	11.720	0.008
	24	4.1±1.5 a	0.0±0.1 b	0.0±0.1 b	23.010	0.002
5 γ	1	0.4±0.3 a	0.0±0.1 a	0.1±0.1 a	3.567	0.095
	2	0.4±0.2 a	0.1±0.1 a	0.2±0.0 a	4.192	0.073
	3	0.3±0.5 a	0.1±0.1 a	0.1±0.2 a	0.694	0.536
	24	0.3±0.2 a	0.1±0.1 a	0.1±0.0 a	2.972	0.127
6 γ	1	0.1±0.2 a	0.0±0.1 a	0.1±0.1 a	1.250	0.352

	2	0.2±0.3 [a]	0.2±0.2 [a]	0.3±0.1 [a]	0.285	0.762
	3	0.4±0.4 [a]	0.1±0.0 [a]	0.1±0.1 [a]	1.247	0.353
	24	0.1±0.1 [a]	0.1±0.1 [a]	0.5±0.3 [a]	5.128	0.051
7 $^{\gamma}$	1	0.3±0.2 [a]	0.1±01 [a]	0.6±0.6 [a]	1.455	0.305
	2	0.3±0.4 [a]	0.4±0.2 [a]	0.9±0.5 [a]	2.441	0.168
	3	0.5±0.4 [a]	0.4±0.3 [a]	0.9±0.6 [a]	0.801	0.492
	24	0.5±0.5 [b]	0.6±0.1 [ab]	1.6±0.3 [a]	7.983	0.020
			20ºC $^{\mu}$			
1 $^{\gamma}$	1	0.3±0.1 [a]	0.1±0.1 [ab]	0.0±0.1 [b]	5.578	0.043
2 $^{\gamma}$	1	0.7±0.7 [a]	0.1±0.1 [a]	0.1±0.1 [a]	2.198	0.192
3 $^{\gamma}$	1	1.1±0.5 [a]	0.2±0.1 [b]	0.1±0.0 [a]	12.170	0.008
4 $^{\gamma}$	1	3.4±1.3 [a]	0.2±0.1 [b]	0.1±0.1 [b]	22.950	0.002
5 $^{\gamma}$	1	1.2±0.8 [a]	0.3±0.1 [a]	0.1±0.1 [a]	4.796	0.057
6 $^{\gamma}$	1	0.5±0.3 [a]	0.2±0.0 [a]	0.1±0.0 [a]	4.377	0.067
7 $^{\gamma}$	1	0.7±0.1 [a]	0.5±0.2 [a]	0.5±0.3 [a]	0.832	0.480

$^{\mu}$ Grain temperature.

† Movement period

$^{\gamma}$ The alphabets a and b in the same row indicate significantly different mean values among same layers under the same tested condition.

Tukey's test at a 0.05 confidence level was used to conduct these comparisons

4.2. Movement and distribution of *Cryptolestes ferrugineus*

4.2.1. Adult movement in the vertical direction

Cryptolestes ferrugineus adults exhibited a clear preference for downward movement over upward movement in the vertical direction. Their movement increased with rising temperatures. At 30ºC, 79% moved downward and at 5ºC only 6% of adults moved downwards (Figs. 4.3 and 4.4). A similar trend was observed with the movement period. As the duration increased from 1 h to 24 h at 30ºC, the adult's downward movement increased from 54% to 79% (Fig. 4.3). At higher temperatures of 20 and 30ºC, their movement speed exceeded 7.2 m/d in both directions. However, as the temperature decreases to 10ºC, the upward movement speed reduces to 2.4 m/d while the downward movement speed remains above 7.2 m/d. At 5ºC, the movement speed in both directions reduces to 0.2 m/d (Table 4.5).

The bottom layer consistently showed the highest mean fraction of recovered insects compared to the other layers under all tested conditions except at 10 and 5ºC (Table 4.6). At 5ºC, the mean fraction of adults recovered in the introduced layer was high (87%), but this recovery gradually decreased to 10% as the temperature increased to 30ºC. At 20 and 30ºC, the two-sample location test and EDF statistics indicated no significant variations, with the adults displaying similar movement patterns. Therefore, adults had the similar movement patten regardless of the temperature (Table 4.7). In contrast, movement at 10ºC was different from movement at both 20 and 30ºC (Table 4.7). This indicates that higher temperatures (higher than 10^{oC}) facilitate greater movement and dispersal in the vertical direction, while lower temperatures restrict movement.

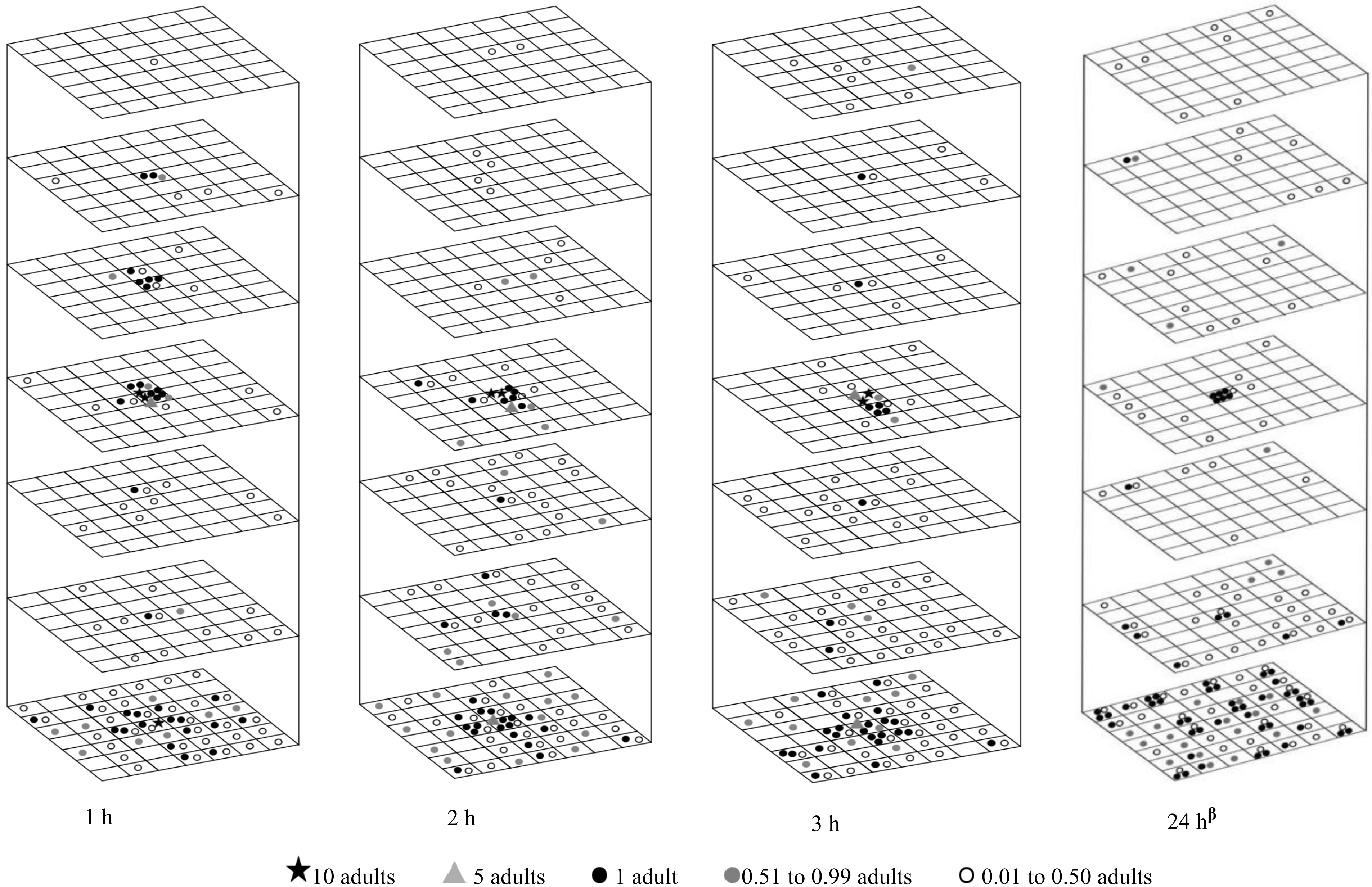

Figure 4.3. Redistribution of 100 *Cryptolestes ferrugineus* adults at 30ºC in wheat with 14.5% moisture content for a movement period of 1 h to 24 h. Note: [β] Image used from Bharathi et al. (2022a).

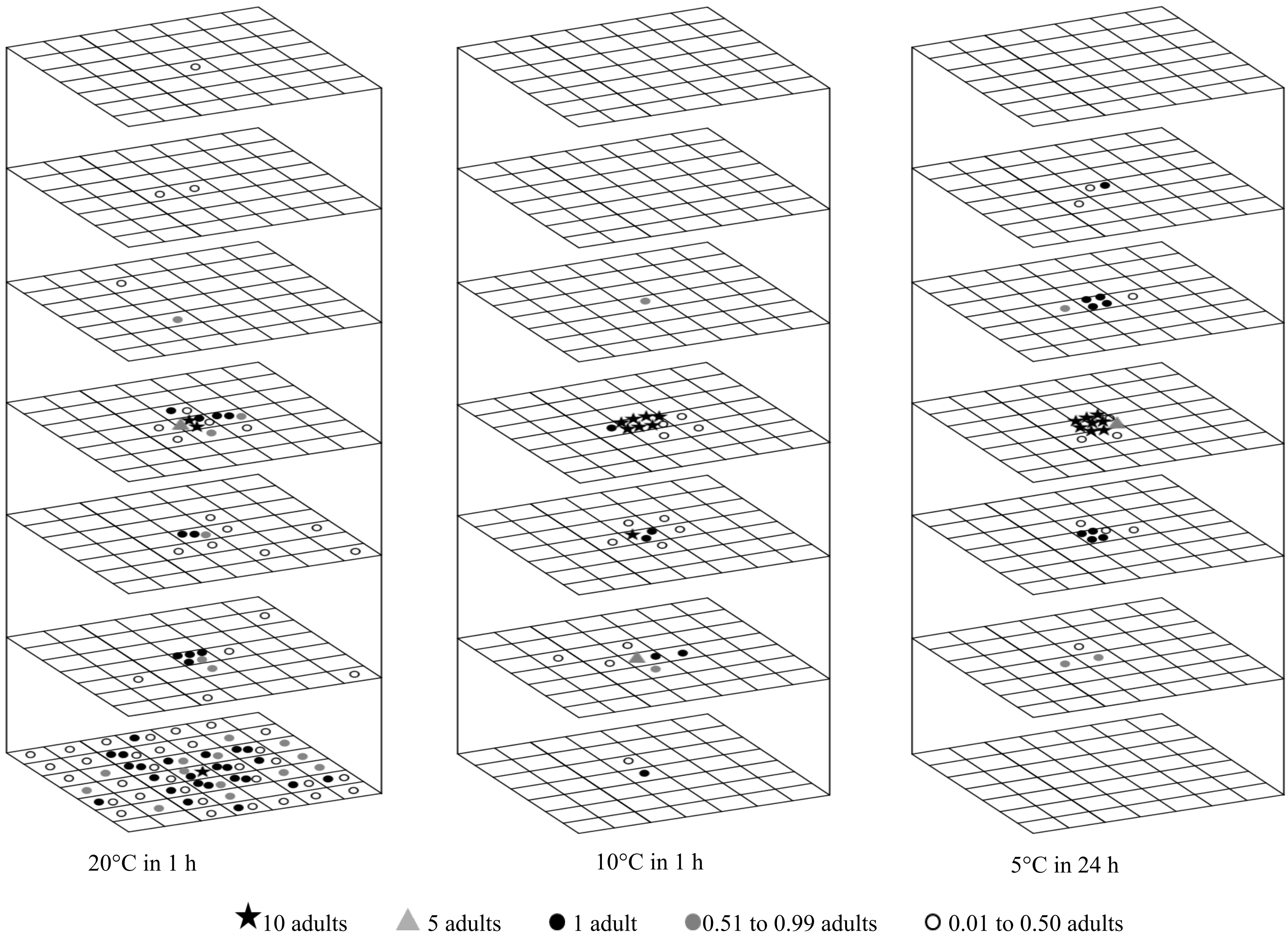

Figure 4.4. Redistribution of 100 *Cryptolestes ferrugineus* adults after1 h movement period in wheat with 14.5% moisture content at 20, 10ºC and after 24 h (with acclimation time of 5 d where the temperature was reduced gradually) at 5ºC

Table 4.5. Maximum movement speed of *C. ferrugineus* in vertical and horizontal direction.

Grain Temperature (^oC)	Movement speed (m/d)		
	Vertical direction		Horizontal direction
	Upward direction	Downward direction	
30	>7.2	>7.2	>7.2
20	>7.2	>7.2	>7.2
10	2.4	>7.2	4.8
5	0.2	0.2	0.1

Table 4.6. Recovered adults of *C. ferrugineus* in different layers for each tested condition.

Layers		Tested conditions$^{\gamma}$						
		30ºC in 1 h	**30ºC in 2 h**	**30ºC in 3 h**	**30ºC in 24 h$^{\beta}$**	**20ºC in 1 h**	**10ºC in 1 h**	**5ºC in 24 h**
1		0.4±0.6 $^{b\ A}$	0.7±1.3 $^{b\ A}$	2.7±0.6 $^{b\ A}$	2.4±1.6 $^{b\ A}$	0.3±0.6 $^{b\ A}$	0 $^{b\ A}$	0 $^{b\ A}$
2		3.8±2.2 $^{b\ A}$	1.4±1.7 $^{b\ A}$	2.0±1.8 $^{b\ A}$	3.8±4.9 $^{b\ A}$	0.7±1.2 $^{b\ AB}$	0 $^{b\ B}$	1.7±2.0 $^{b\ A}$
3		6.9±3.0 $^{b\ A}$	2.9±3.4 $^{b\ A}$	3.1±1.0 $^{b\ A}$	4.8±3.2 $^{b\ A}$	1.0±1.0 $^{b\ B}$	0.7±10.2 $^{b\ B}$	5.0±4.0 $^{b\ A}$
4		35.2±15.4$^{a\ A}$	36.3±12.8$^{a\ A}$	32.7±14.2$^{a\ A}$	10.0±4.2$^{b\ A}$	41.8±17.3$^{a\ A}$	73.7±17.9$^{a\ A}$	86.9±7.5 $^{a\ A}$
5		4.2±2.6 $^{b\ A}$	6.9±3.2 $^{b\ A}$	5.5±4.3 $^{b\ A}$	3.5±2.2 $^{b\ A}$	5.5±3.2 $^{b\ A}$	14.6±8.2 $^{b\ A}$	5.1±3.5 $^{b\ A}$
6		5.9±3.2 $^{b\ A}$	11.8±9.6 $^{b\ A}$	11.6±8.8 $^{b\ A}$	15.1±9.9$^{b\ A}$	6.5±3.6 $^{b\ A}$	9.1±10.6 $^{b\ A}$	1.7±0.6 $^{b\ A}$
7		43.7±19.5$^{a\ A}$	39.9±4.0 $^{a\ B}$	42.3±5.8 $^{a\ B}$	60.4±7.9$^{a\ A}$	44.3±17.4$^{a\ A}$	2.0±1.0 $^{b\ B}$	0 $^{b\ B}$
Tukey test values	**F**	9.874	19.640	16.900	41.700	13.1700	29.950	239.400
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: $^{\beta}$ Data used from Bharathi et al. (2022a)

$^{\gamma}$ The lowercase letters a, b, and c in a column indicate significantly different mean values among different layers under the same tested condition. The uppercase letters A, B, and C in a row indicate significantly different mean values among the same layers under different tested conditions. Tukey's test at a 0.05 confidence level was used to conduct these comparisons.

Table 4.7. Two-sample location test and EDF statistics results for *C. ferrugineus* movement and distribution in vertical direction.

Experimental conditions $^{\gamma}$		**Wilcoxon**		**Kolmogorov-Smirnov**		**Mood median**	
Temperature (^{0}C)	**Time (h)**	**Z**	**p**	**D**	**p**	**Z**	**p**
30	1 vs 2	3.520	<0.001	-0.452	<0.001	4.022	<0.001
	2 vs 3	3.732	<0.001	-0.452	<0.001	3.415	<0.001
	3 vs 1	3.769	<0.001	-0.429	<0.001	2.441	0.0145
	24 vs 1	3.769	<0.001	-0.429	<0.001	2.894	0.004
	24 vs 2	3.657	<0.001	-0.452	<0.001	3.856	<0.001
	24 vs 3	3.957	<0.001	-0.429	<0.001	2.273	0.023
30 vs 20	1	2.782	0.005	-0.405	<0.001	4.359	<0.001
30 vs 10		1.719	0.086	-0.357	0.003	4.749	<0.001
20 vs 10		0.769	0.442	-0.333	0.006	5.505	<0.001
5 vs 30	24	1.857	0.063	-0.333	0.005	4.725	<0.001

$^{\gamma}$ Comparison between two experiments, with the first column representing the constant experimental condition and the second column representing the variables being compared.

4.2.2. Adult movement in the horizontal direction

An increase in temperature and movement period resulted in greater diffusion of *C. ferrugineus* adults (Figs. 4.3 and 4.4). The adults moved horizontally at speeds more than 7.2 m/d at 30ºC and 20ºC, but this speed decreased to 4.8 m/d at 10ºC and further reduced to 0.1 m/d at 5ºC (Table 4.5). At 30ºC, the adults exhibited high diffusion and were spread throughout the wooden container due to the high movement speed (Fig. 4.3). However, when the temperature was low (10 and 5ºC), a high density of adults was found near the centre location, close to their introduction area (Fig. 4.4). For instance, at 30ºC more than 40% adults were recovered at the bottom layer (layer 7) (Fig. 4.3, Table 4.8) and adults almost uniformly distributed in different areas (Fig. 4.3, Table 4.8). Therefore, the adults had no preference for different areas. At 20ºC, higher density of adults was recovered in the centre location in all layers (Fig. 4.4, Table 4.8). These results confirmed that the mobility of *C. ferrugineus* adults was significantly reduced by low temperatures.

Table 4.8. Recovered adult densities of *C. ferrugineus* in different areas in the same layer at the same tested conditions.

Layer	Time (h) †	Centre	Middle	Periphery	Tukey test values	
					F	P
			30ºC $^{\mu}$			
1 $^{\gamma}$	1	0.1±0.1 ^{a}	0 ^{a}	0 ^{a}	1.000	0.422
	2	0.1±0.2 ^{a}	0 ^{a}	0 ^{b}	1.000	0.422
	3	0.2±0.2 ^{a}	0.3±0.0 ^{a}	0.0±0.1 ^{a}	2.385	0.173
	24$^{\beta}$	0 ^{a}	0.1±0.1 ^{a}	0.1±0.1 ^{a}	1.245	0.353
2 $^{\gamma}$	1	0.4±0.1 ^{a}	0.1±0.1 ^{a}	0.0±0.01 ^{b}	21.800	0.002
	2	0.2±0.3 ^{a}	0.0±0.1 ^{a}	0 ^{b}	0.833	0.480
	3	0.2±0.3 ^{a}	0 ^{a}	0.0±0.1 ^{a}	0.840	0.477
	24$^{\beta}$	0.1±0.1 ^{a}	0 ^{a}	0.2±0.2 ^{a}	1.361	0.326
3 $^{\gamma}$	1	0.9±0.5 ^{a}	0.0±0.1^{b}	0 ^{b}	10.290	0.012
	2	0.3±0.4 ^{a}	0.1±0.1 ^{a}	0.0±0.1 ^{a}	0.735	0.518
	3	0.3±0.2 ^{a}	0.0±0.1 ^{a}	0.0±0.1 ^{a}	3.036	0.123
	24$^{\beta}$	0 ^{a}	0.1±0.1 ^{a}	0.2±0.1 ^{a}	3.523	0.097
4 $^{\gamma}$	1	4.5±2.1 ^{a}	0.0±0.1 ^{b}	0.1±0.1 ^{b}	13.370	0.006
	2	4.4±1.8 ^{a}	0.1±0.1 ^{b}	0.1±0.1 ^{b}	16.890	0.003
	3	4.2±1.5 ^{a}	0.1±0.1 ^{b}	0.0±0.1 ^{b}	21.850	0.002
	24$^{\beta}$	0.9±0.7 ^{a}	0.0±0.1 ^{a}	0.1±0.1 ^{a}	4.555	0.063
5 $^{\gamma}$	1	0.4±0.5 ^{a}	0 ^{a}	0.1±0.1^{a}	1.880	0.232
	2	0.3±0.3 ^{a}	0.1±0.1 ^{a}	0.2±0.2 ^{a}	0.611	0.573
	3	0.3±0.2 ^{a}	0.2±0.2 ^{a}	0.1±0.1 ^{a}	1.754	0.251
	24$^{\beta}$	0 ^{a}	0 ^{b}	0.2±0.1 ^{b}	7.618	0.023
6 $^{\gamma}$	1	0.4±0.2 ^{a}	0.1±0.1 ^{a}	0.1±0.1 ^{a}	4.092	0.076
	2	0.6±0.5 ^{a}	0.1±0.1 ^{a}	0.3±0.3 ^{a}	1.299	0.340
	3	0.6±0.7 ^{a}	0.3±0.3 ^{a}	0.2±0.1 ^{a}	0.530	0.614

	24$^{\beta}$	0.4±0.5 ^{a}	0.2±0.2 ^{a}	0.5±0.3 ^{a}	0.467	0.648
7 $^{\gamma}$	1	3.1±1.3 ^{a}	0.6±0.4^{b}	0.7±0.3 ^{ab}	8.938	0.016
	2	2.9±0.3 ^{a}	0.5±0.1 ^{b}	0.6±0.2 ^{b}	88.610	<0.001
	3	3.0±0.7 ^{a}	0.5±0.1 ^{b}	0.7±0.5 ^{b}	27.490	0.001
	24$^{\beta}$	2.0±0.3 ^{a}	0.7±0.5 ^{b}	1.8±0.2 ^{a}	9.853	0.013
			20^{o}C$^{\mu}$			
1 $^{\gamma}$	1	0.1±0.1 ^{a}	0 ^{a}	0 ^{a}	1.000	0.422
2 $^{\gamma}$	1	0.1±0.2^{a}	0 ^{a}	0 ^{a}	1.000	0.422
3 $^{\gamma}$	1	0.1±0.2 ^{a}	0.0±0.1 ^{a}	0 ^{a}	0.742	0.515
4 $^{\gamma}$	1	5.6±2.3 ^{a}	0 ^{b}	0 ^{b}	17.550	0.003
5 $^{\gamma}$	1	0.7±0.4 ^{a}	0.0±0.1 ^{b}	0 ^{b}	7.764	0.022
6 $^{\gamma}$	1	0.8±0.4 ^{a}	0.0±0.01^{b}	0.0±0.1^{b}	9.314	0.015
7 $^{\gamma}$	1	2.8±1.0 ^{a}	1.0±0.6 ^{b}	0.5±0.3 ^{b}	9.470	0.014

Note: $^{\beta}$Data used from Bharathi et al. (2022a)

$^{\mu}$ Grain temperature.

† Movement period

$^{\gamma}$ Different letters in the same row indicate significantly different mean values among same layers under the same tested condition. Tukey's test at a 0.05 confidence level was used to conduct these comparisons

5. DISCUSSIONS

5.1. Effect of temperature, time, and grain bulk dimension on movement and distribution

The 3-D movement and distribution of *T. castaneum* and *C. ferrugineus* adults were influenced by both temperature and movement period. Even though both adults moved in both horizontal and vertical directions, the movement speed of both species was significantly influenced by temperature (Figs. 4.1-4.4; Tables 4.1-4.8). Their vertical and horizontal movements decreased noticeably as the temperature reduced from 30 to 5ºC. They reached the boundary of the wooden container due to the high movement speed at the high temperatures within 1 h. In 1-D study by Jian et al. (2004a), which investigated the movement of *C. ferrugineus* within stored wheat using a 10 × 10 × 100 cm grain column. Both vertical and horizontal directions were analyzed under linear and dynamic temperature gradients ranging from 25 to 35ºC. The study involved four insect densities and movement periods of 12, 24, 72, and 144 h. The results indicated that adults moved at speeds exceeding 10.8 m/d in the vertical direction and up to 6 m/d in the horizontal direction within the grain column. Our 3-D study showed that the adults exhibit movement speeds of more than 7.2 m/d in both vertical and horizontal directions at higher temperatures of 30 and 20ºC. While the vertical movement speed in our study is similar to the 1-D study, the higher horizontal speed observed in our results could be due to the size of the grain container. In the 1-D study, the adults had limited space, whereas inside the 3-D setup they had more space to move horizontally. Therefore, even though adults could have more winding paths in 3-D containers than that in 2-D containers or 1-D columns, their movement in both vertical and horizontal directions would not be influenced by the dimension and size of the grain bulk if they could move in the direction. This observation is important for grain storage management, as the measured insect movement under laboratory conditions can be applied to grain storage bins of any shape and size.

The recovery rate of *C. ferrugineus* adults from the introduced cube at 20 and 30ºC in 1 h ranged from 35% to 42%. The aggregation behaviour observed in the *C. ferrugineus* can be attributed to pheromone production during the acclimation period. This is supported by Bharathi et al. (2022a), who reported recovery rates in the introduced cube of their 24 h 3-D studies in wheat with 12.5 % moisture content at 20 and 30ºC for *C. ferrugineus* were between 12% and 13%. The difference in recovery rates from our results could be due to the higher moisture content and shorter movement periods. This novel finding adds to the existing body of knowledge on pest behaviour. Similarly, in a study by Halliday and Blouin-Demers (2016) investigating the Ideal Free Distribution (IFD) of *T. castaneum* adults, it was found that mixed-sex groups of adults aggregated more than female-only groups due to the secretion of aggregation pheromones. This aggregation behaviour can cause a deviation from their IFD. This finding has practical implications for pest control strategies, as it explains the high recovery rate of *T. castaneum* adults in the introduced cube at temperatures of 30 and 20ºC, which ranged from 32% to 55%. Based on these observations, it can be concluded that within a 3D bulk environment, temperature had a great impact on the movement of insects, while the movement period had a relatively minor effect on their distribution.

5.2. Distribution pattern and movement behaviour

In our 3-D study, we observed that at 30ºC over a 24-h period, *C. ferrugineus* adults exhibited a 20% higher downward movement compared to *T. castaneum*. Jian et al. (2005b) explained the more downward movement of *C. ferrugineus* compared to *T. castaneum* was due to the drift effect caused by their smaller body size relative to *T. castaneum*. Most researchers categorized this downward movement as a geotaxis behaviour (Bharathi et al., 2022b; Jian et al., 2002, 2005a, 2006). This suggests that body size and geotactic responses play significant roles in the vertical movement patterns of these pests under different environmental conditions. In a laboratory study using a modified dispersal apparatus consisting of interconnected containers and tubes to track insect movement, it was found that *T. castaneum* shows a greater tendency to climb up in high-density conditions (Arnold et al., 2017). This behaviour suggests that dispersal may be influenced by interactions such as repulsion from conspecifics of the same sex. *Tribolium castaneum* adults showed more upward movement than *C. ferrugineus* adults. Specifically, in 2 h at 30ºC, 17% more adults of *T. castaneum's* were recovered in the top layers than that of *C. ferrugineus*. This difference becomes even more prominent at the temperature of 20ºC in 1 h, where the upward movement of *T. castaneum* surpasses the rusty grain beetle movement by approximately 23%. In our current study, since we did not differentiate adults by sex, it is likely that high densities of individuals of the same sex could exist, potentially leading to increased repulsion and thereby promoting dispersal among the adults. Similar findings have been reported in the literatures (Bharathi et al., 2023b; Surtees, 1963; Jian et al., 2005b, 2012).

The random horizontal movement behaviour observed at high temperatures, specifically at 20ºC for 1 h and at 30ºC for durations of 1, 2, 3, and 24 h in both species contributes to the diffusion pattern within the 3D setup. These findings are consistent with previous 1-D and 2-D studies, which demonstrated that the randomness in their movement is a key factor in creating diffusion patterns (Jian et al., 2004b, 2007) . This random movement results in an even spread of insects throughout the grain mass, preventing the formation of clusters and ensuring a more uniform distribution. Although insects possess strong olfactory senses, the physical structure of bulk grain poses significant challenges to their communication. Within the dense and compact grain mass, the diffusion of pheromones is restricted, and the concentration of these chemical signals is reduced, making it difficult for insects to detect and respond to them effectively (Obeng-Ofori and Coaker, 1990). This is proven in a laboratory study by Currie et al. (2020) who examined the attractiveness of pheromones in *C. ferrugineus* adults with and without the presence of grain inside an apparatus monitored by an infrared camera. It was found that within the grain, there was no attraction between conspecifics at low insect densities. The researchers attributed this lack of attraction to the absorption of pheromones by the grain and the absence of air currents to disperse the pheromones effectively. Similarly, insects that might use acoustic signals for communication are also compromised in bulk grain environments. As these signals travel through the grain, they tend to weaken and the physical structure of the grain acts as a barrier and reduces the range and clarity (Mankin et al., 2011). It also obstructs their line of sight, resulting in a lack of visual cues. So consequently, the beetles move in a more random pattern as they navigate through the obstructed environment (Jian et al., 2009). This consistency in behaviour across different studies (Bharathi et al., 2022a; Jian et al., 2002, 2007) highlights the insect's tendency for random dispersal in a stable environment.

5.3. Different movement behaviours between different species.

The results of this 3-D study indicate distinct movement behaviours for *T. castaneum* and *C. ferrugineus* adults at 30ºC. The *T. castaneum* adults initially move upwards, then downwards, and finally move horizontally. In contrast, *C. ferrugineus* adults start by moving downwards, then upwards, and followed by horizontal movement. This suggests that each species has unique behavioural responses to temperature, influenced by their physiological and ecological adaptations (Anukiruthika et al., 2021). In a separate study by Jian et al. (2005b), the movement of *T. castaneum* was analyzed in both horizontal and vertical wheat and corn columns with and without temperature gradients. They discovered that over 90% of *T. castaneum* remained in their introduced area for 6 d within the wheat grain column when temperatures were uniform. This high aggregation rate was attributed to the smaller granular size of the wheat bulk, which made it difficult for the beetles to move through the grain. As their large body size relative to the wheat pores created a physical barrier that hindered their ability to disperse into the bulk, resulting in a higher concentration of beetles in the initial area. These findings partially explain the difference between species, and their biological behaviour such as food preference, preyed and prey, and mating behaviour also influence their movement. These physical and biological factors together shaped the movement and distribution patterns among species.

A study on the movement and distribution of *C. ferrugineus* and *T. castaneum* adults was conducted on 300 t of wheat in a 10-m diameter bin from September 2019 to October 2021 in Winnipeg, Canada (Bharathi et al., 2023b). The results showed that *C. ferrugineus* adults, released at the top layer at the start of the experiment, initially moved downwards and then spread horizontally. In contrast, most of the *T. castaneum* adults remained near their introduction point but some were also found throughout the bin. Both adult species were found near the warmer walls of the grain bin. However, in our study, both species exhibited higher densities at the periphery of the bottom layer (layer 7). This might be caused by the physical boundary limitation of the wooden container in our study. In a separate study conducted by Collins and Conyers (2009) on the movement of *O. surinamensis* and *S. granarius* adults inside a bin with a surface area of 9 m^2 and a depth of 4 m filled with 30 t of wheat, it was reported that *O. surinamensis* adults exhibited an upward movement pattern without horizontal dispersion, while *S. granarius* adults showed less upward movement. Another study on the movement and distribution of *S. zeamais* by Zhang et al. (2020) in a bin with a diameter of 1.2 m and a depth of 1.2 m filled with 1 t of wheat reported that the adults demonstrated less upward and more downward movement in the vertical direction. The observed differences in movement behaviour among various insect species can be attributed to factors such as their point of introduction into the grain bulk, the presence of temperature and moisture gradients within the storage environment, and the factors mentioned in the previous paragraph. These findings highlight the species-specific movement and distribution patterns in stored grain environments.

6. CONCLUSIONS

From the three-dimensional study of the movement of *Tribolium castaneum* and *Cryptolestes ferrugineus* adults the following conclusions can be drawn:

1. Both adult species had movement speeds of more than 7.2 m/d in both vertical and horizontal directions at high temperatures of 30 and 20ºC. At lower temperatures of 10 and 5ºC, the movement speed of both species decreased, with vertical movement speed exceeding horizontal movement speed. However, *T. castaneum* ceased all movement at 5ºC.

2. In both species, a large proportion of individuals remained within the same cube they were introduced in. This could be attributed to various factors, including the production of aggregation pheromones, the fact that suitable conditions were found in this cube, or because the large size of their bodies hindered their dispersal.

3. The movement and distribution of both adult species in the vertical direction were influenced by geotaxis and drift effects, while they followed a diffusion pattern in the horizontal direction.

4. Inside the grain bulk, *T. castaneum* initially moved in upward, then downward and finally moved in the horizontal direction. In contrast, *C. ferrugineus* first moved downward, then upward followed by horizontal movement.

5. The results align with earlier one-, two- and three-dimensional studies, confirming the influence of temperature and movement period on the behaviour and distribution of these two species.

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APPENDIX

Table A1. Normalized insect count of *T. castaneum*

Layers	Environmental Conditions						
	30°C in1 h	**30°C in 2 h**	**30°C in 3 h**	**30°C in 24 h**	**20°C in 1 h**	**10°C in 1 h**	**5°C in 24 h**
1	7.9	9.6	5.1	4.1	4.4	0.0	0
2	2.9	8.0	1.8	2.2	8.1	0.3	0
3	8.3	4.4	1.1	3.0	12.1	2.0	0
4	55.8	36.1	53.0	31.6	32.6	96.3	100
5	4.7	7.7	5.6	5.6	13.4	1.3	0
6	3.6	8.9	6.8	11.2	8.4	0.0	0
7	16.9	25.3	26.6	42.3	21.2	0.0	0

Table A2. Normalized insect count of *C. ferrugineus*

Layers	Experiment conditions						
	30°C in 1 h	**30°C in 2 h**	**30°C in 3 h**	**30°C in 24 h$^{\beta}$**	**20°C in 1 h**	**10°C in 1 h**	**5°C in 24 h**
1	0.4	0.7	2.7	2.4	0.3	0.0	0.0
2	3.8	1.4	2.0	3.8	0.7	0.0	1.7
3	6.9	2.9	3.1	4.8	1.0	0.7	5.0
4	35.2	36.3	32.7	10.0	41.7	73.7	86.6
5	4.1	6.9	5.5	3.5	5.4	14.6	5.0
6	5.9	11.8	11.6	15.1	6.5	9.1	1.7
7	43.7	39.9	42.3	60.4	44.3	2.0	0.0

Note: $^{\beta}$ Data used from Bharathi et al. (2022a)

Table A3. Recovered adults of *T. castaneum* in different layers for all the tested conditions.

Layers		**Environmental Conditions**$^{\gamma}$					
		30°C in 1 h	**30°C in 2 h**	**30°C in 3 h**	**30°C in 24 h**	**20°C in 1 h**	**10°C in 1 h**
1		7.92±2.25 $^{\mathrm{b\,A}}$	9.60±6.58 $^{\mathrm{bc\,A}}$	5.12±3.66 $^{\mathrm{b\,A}}$	4.10±1.26 $^{\mathrm{b\,A}}$	4.36±1.02 $^{\mathrm{b\,A}}$	0.00±0.00 $^{\mathrm{c\,B}}$
2		2.88±3.29 $^{\mathrm{b\,A}}$	8.01±4.26 $^{\mathrm{c\,A}}$	1.82±1.72 $^{\mathrm{b\,A}}$	2.23±2.93 $^{\mathrm{b\,A}}$	8.07±6.57 $^{\mathrm{b\,A}}$	0.33±0.58 $^{\mathrm{bc\,A}}$
3		8.29±4.15 $^{\mathrm{b\,AB}}$	4.39±3.22 $^{\mathrm{c\,A}}$	1.14±1.97 $^{\mathrm{b\,A}}$	2.99±0.69 $^{\mathrm{b\,A}}$	12.05±5.18 $^{\mathrm{b\,A}}$	2.01±1.00 $^{\mathrm{b\,B}}$
4		55.75±15.06 $^{\mathrm{a\,B}}$	36.10±4.18 $^{\mathrm{a\,A}}$	53.00±23.48 $^{\mathrm{a\,A}}$	31.56±9.76 $^{\mathrm{a\,A}}$	32.61±11.90 $^{\mathrm{a\,B}}$	96.32±0.59 $^{\mathrm{a\,A}}$
5		4.67±1.63 $^{\mathrm{b\,AB}}$	7.74±1.32 $^{\mathrm{c\,A}}$	5.56±4.82 $^{\mathrm{b\,A}}$	5.57±2.17 $^{\mathrm{b\,A}}$	13.38±7.20 $^{\mathrm{b\,A}}$	1.34±1.12 $^{\mathrm{bc\,B}}$
6		3.60±0.61 $^{\mathrm{b\,AB}}$	8.88±4.19 $^{\mathrm{c\,A}}$	6.80±3.49 $^{\mathrm{b\,A}}$	11.19±5.07 $^{\mathrm{b\,A}}$	8.37±3.50 $^{\mathrm{b\,A}}$	0.00±0.00 $^{\mathrm{c\,B}}$
7		16.90±11.84 $^{\mathrm{b\,AB}}$	25.26±10.76 $^{\mathrm{ab\,A}}$	26.56±18.70 $^{\mathrm{ab\,A}}$	42.34±11.27 $^{\mathrm{a\,A}}$	21.17±6.92 $^{\mathrm{ab\,A}}$	0.00±0.00 $^{\mathrm{c\,B}}$
Tukey test values	**F**	18.59	12.92	8.035	20.57	6.047	9080
	P	6.296e-06	5.304e-05	0.0006795	3.403e-06	0.002676	<2.2e-16

$^{\gamma}$ The lowercase letters a, b, and c in a column indicate significantly different mean values among different layers under the same tested condition. The uppercase letters A, B, and C in a row indicate significantly different mean values among the same layers under different tested conditions. Tukey's test at a 0.05 confidence level was used to conduct these comparisons

Table A4. Recovered adult densities of *T. castaneum* in different areas in the same layer for all the tested conditions

Layer	**Time (h)** †	**Centre**	**Middle**	**Periphery**
		30ºC$^{\mu}$		
1 $^{\gamma}$	1	0.24±0.22 ^{a}	0.24±0.08 ^{a}	0.14±0.11 ^{a}
	2	0.34±0.30 ^{a}	0.20±0.22 ^{a}	0.20±0.10 ^{a}
	3	0.25±0.23 ^{a}	0.03±0.05 ^{a}	0.15±0.08 ^{a}
	24$^{\beta}$	0.10±0.09 ^{a}	0.06±0.05 ^{a}	0.13±0.08 ^{a}
2 $^{\gamma}$	1	0.24±0.3 ^{a}	0.03±0.05 ^{a}	0.04±0.06 ^{a}
	2	0.44±0.24 ^{a}	0.19±0.19 ^{a}	0.10±0.14 ^{a}
	3	0 ^{a}	0.03±0.4 ^{a}	0.07±0.09 ^{a}
	24 $^{\beta}$	0.10±0.17 ^{a}	0.29±0.05 ^{a}	0.06±0.10 ^{a}
3 $^{\gamma}$	1	0.77±0.47 ^{a}	0.05±0.09 ^{b}	0.09±0.03 $^{a\ b}$
	2	0.30±0.15 ^{a}	0.12±0.12 ^{a}	0.34±0.06 ^{a}
	3	0.05±0.09 ^{a}	0.06±0.10 ^{a}	0 ^{a}
	24 $^{\beta}$	0.30±0.01 ^{a}	0 ^{b}	0.04±0.03 ^{b}
4 $^{\gamma}$	1	7.22±2.16 ^{a}	0.11±0.12 ^{b}	0.02±0.03 ^{b}
	2	4.49±0.35 ^{a}	0.08±0.08 ^{b}	0.07±0.06 ^{b}
	3	6.96±3.43 ^{a}	0.13±0.23 ^{b}	0 ^{b}
	24 $^{\beta}$	4.12±1.48 ^{a}	0.03±0.05 ^{b}	0.02±0.03 ^{b}
5 $^{\gamma}$	1	0.39±0.30 ^{a}	0.03±0.05 ^{a}	0.07±0.06 ^{a}
	2	0.40±0.16 ^{a}	0.14±0.13 ^{a}	0.15±0.03 ^{a}
	3	0.33±0.46 ^{a}	0.10±0.09 ^{a}	0.09±0.15 ^{a}
	24 $^{\beta}$	0.30±0.15 ^{a}	0.11±0.13 ^{a}	0.09±0.03 ^{a}
6 $^{\gamma}$	1	0.14±0.15 ^{a}	0.03±0.05 ^{a}	0.10±0.05 ^{a}
	2	0.15±0.26 ^{a}	0.17±0.22 ^{a}	0.28±0.14 ^{a}
	3	0.39±0.43 ^{a}	0.08±0.00 ^{a}	0.14±0.02 ^{a}
	24 $^{\beta}$	0.10±0.09 ^{a}	0.11±0.05 ^{a}	0.45±0.25 ^{a}
7 $^{\gamma}$	1	0.34±0.22 ^{a}	0.14±010 ^{a}	0.63±0.57 ^{a}
	2	0.30±0.39 ^{a}	0.39±0.16 ^{a}	0.90±0.46 ^{a}
	3	0.48±0.36 ^{a}	0.44±0.31 ^{a}	0.86±0.61 ^{a}
	24 $^{\beta}$	0.51±0.54 ^{b}	0.56±0.11 $^{a\ b}$	1.56±0.30 ^{a}

		20°C$^{\mu}$		
1 $^{\gamma}$	1	0.29±0.14 ^{a}	0.14±0.09 $^{a\ b}$	0.02±0.03 ^{b}
2 $^{\gamma}$		0.69±0.69 ^{a}	0.11±0.05 ^{a}	0.07±0.08 ^{a}
3 $^{\gamma}$		1.12±0.48 ^{a}	0.19±0.13 ^{b}	0.05±0.00 ^{b}
4 $^{\gamma}$		3.69±1.26 ^{a}	0.23±0.12 ^{b}	0.05±0.05 ^{b}
5 $^{\gamma}$		1.21±0.83 ^{a}	0.25±0.14 ^{a}	0.05±0.05 ^{a}
6 $^{\gamma}$		0.534±0.32 ^{a}	0.19±0.04 ^{a}	0.09±0.04 ^{a}
7 $^{\gamma}$		0.68±0.09 ^{a}	0.49±0.17 ^{a}	0.48±0.32 ^{a}
		10°C$^{\mu}$		
1 $^{\gamma}$	1	*Nil*	*Nil*	*Nil*
2 $^{\gamma}$		0.04±0.08 ^{a}	0 ^{a}	0 ^{a}
3 $^{\gamma}$		0.27±0.13 ^{a}	0 ^{b}	0 ^{b}
4 $^{\gamma}$		12.89±0.08 ^{a}	0 ^{b}	0 ^{b}
5 $^{\gamma}$		0.18±0.16 ^{a}	0 ^{b}	0 ^{b}
6 $^{\gamma}$		*Nil*	*Nil*	*Nil*
7 $^{\gamma}$		*Nil*	*Nil*	*Nil*

† Grain temperature.

$^{\mu}$ Movement period

$^{\gamma}$ Different letters in the same row indicate significantly different mean values among same layers under the same tested condition. Tukey's test at a 0.05 confidence level was used to conduct these comparisons.

Nil indicates no insect movement in the specified layer

Table A5. Recovered adults of *C. ferrugineus* in different layers for all the tested conditions.

Layers		Experiment conditions$^{\gamma}$						
		30ºC in 1 h	**30ºC in 2 h**	**30ºC in 3 h**	**30ºC in 24 h$^{\beta}$**	**20ºC in 1 h**	**10ºC in 1 h**	**5ºC in 24 h**
1		0.35±0.61 $^{b\ A}$	0.73±1.27 $^{b\ A}$	2.73±0.60 $^{b\ A}$	2.41±1.57 $^{b\ A}$	0.34±0.59 $^{b\ A}$	0 $^{b\ A}$	0 $^{b\ A}$
2		3.84±2.25 $^{b\ A}$	1.44±1.69 $^{b\ A}$	2.04±1.76 $^{b\ A}$	3.78±4.87 $^{b\ A}$	0.68±1.18 $^{b\ AB}$	0 $^{b\ B}$	1.67±2.01 $^{b\ A}$
3		6.89±2.96 $^{b\ A}$	2.89±3.37 $^{b\ A}$	3.07±1.02 $^{b\ A}$	4.84±3.20 $^{b\ A}$	1.02±1.02 $^{b\ B}$	0.69±10.19 $^{b\ B}$	5.01±3.99 $^{b\ A}$
4		35.16±15.40$^{a\ A}$	36.25±12.82$^{a\ A}$	32.73±14.20$^{a\ A}$	10.01±4.20$^{b\ A}$	41.74±17.25$^{a\ A}$	73.68±17.92$^{a\ A}$	86.90±7.53 $^{a\ A}$
5		4.12±2.61 $^{b\ A}$	6.92±3.20 $^{b\ A}$	5.48±4.31 $^{b\ A}$	3.45±2.17 $^{b\ A}$	5.45±3.15 $^{b\ A}$	14.55±8.22 $^{b\ A}$	5.05±3.51 $^{b\ A}$
6		5.94±3.15 $^{b\ A}$	11.83±9.64 $^{b\ A}$	11.63±8.79 $^{b\ A}$	15.14±9.88$^{b\ A}$	6.48±3.64 $^{b\ A}$	9.06±10.55 $^{b\ A}$	1.68±0.59 $^{b\ A}$
7		43.69±19.52$^{a\ A}$	39.94±4.00 $^{a\ B}$	42.31±5.78 $^{a\ B}$	60.37±7.87$^{a\ A}$	44.30±17.34$^{a\ A}$	2.02±0.98 $^{b\ B}$	0 $^{b\ B}$
Tukey test values	**F**	9.874	19.64	16.9	41.7	13.17	29.95	239.4
	P	0.0002329	4.505e-06	1.114e-05	3.842e-08	4.758e-05	3.258e-07	2.76e-13

Note: $^{\beta}$ Data used from Bharathi et al. (2022a)

$^{\gamma}$ The lowercase letters a, b, and c in a column indicate significantly different mean values among different layers under the same tested condition. The uppercase letters A, B, and C in a row indicate significantly different mean values among the same layers under different tested conditions. Tukey's test at a 0.05 confidence level was used to conduct these comparisons

Table A6. Recovered adult densities of *C. ferrugineus* in different areas in the same layer for all the tested conditions.

Layer	Time (h) †	Centre	Middle	Periphery
		30ºC$^{\mu}$		
1 $^{\gamma}$	1	0.05±0.08 ^{a}	0 ^{a}	0 ^{a}
	2	0.10±0.17 ^{a}	0 ^{a}	0 ^{b}
	3	0.23±0.21 ^{a}	0.26±0.04 ^{a}	0.03±0.06 ^{a}
	24$^{\beta}$	0 ^{a}	0.05±0.05 ^{a}	0.09±0.12 ^{a}
2 $^{\gamma}$	1	0.37±0.08 ^{a}	0.05±0.09 ^{b}	0.02±0.03 ^{b}
	2	0.15±0.25 ^{a}	0.03±0.04 ^{a}	0 ^{b}
	3	0.18±0.32 ^{a}	0 ^{a}	0.03±0.03 ^{a}
	24 $^{\beta}$	0.05±0.08 ^{a}	0 ^{a}	0.17±0.21 ^{a}
3 $^{\gamma}$	1	0.87±0.46 ^{a}	0.03±0.05 ^{b}	0 ^{b}
	2	0.26±0.42 ^{a}	0.05±0.05 ^{a}	0.02±0.03 ^{a}
	3	0.27±0.24 ^{a}	0.03±0.04 ^{a}	0.03±0.03 ^{a}
	24 $^{\beta}$	0 ^{a}	0.08±0.08 ^{a}	0.19±0.13 ^{a}
4 $^{\gamma}$	1	4.52±2.12 ^{a}	0.03±0.05 ^{b}	0.05±0.05 ^{b}
	2	4.39±1.80 ^{a}	0.13±0.13 ^{b}	0.09±0.12 ^{b}
	3	4.15±1.51 ^{a}	0.08±0.13 ^{b}	0.03±0.06 ^{b}
	24 $^{\beta}$	0.92±0.68 ^{a}	0.03±0.05 ^{a}	0.14±0.06 ^{a}
5 $^{\gamma}$	1	0.41±0.49 ^{a}	0 ^{a}	0.05±0.05 ^{a}
	2	0.28±0.25 ^{a}	0.10±0.12 ^{a}	0.17±0.22 ^{a}
	3	0.32±0.21 ^{a}	0.15±0.21 ^{a}	0.05±0.09 ^{a}
	24 $^{\beta}$	0 ^{a}	0 ^{b}	0.17±0.11 ^{b}
6 $^{\gamma}$	1	0.37±0.17 ^{a}	0.08±0.14 ^{a}	0.11±0.11 ^{a}
	2	0.56±0.48 ^{a}	0.13±0.05 ^{a}	0.30±0.30 ^{a}
	3	0.55±0.72 ^{a}	0.28±0.27 ^{a}	0.18±0.11 ^{a}
	24 $^{\beta}$	0.41±0.50 ^{a}	0.21±0.20 ^{a}	0.47±0.27 ^{a}
7 $^{\gamma}$	1	3.08±1.32 ^{a}	0.63±0.44 ^{b}	0.66±0.28 $^{a\,b}$
	2	2.86±0.34 ^{a}	0.53±0.12 ^{b}	0.58±0.23 ^{b}
	3	3.02±0.65 ^{a}	0.49±0.12 ^{b}	0.66±0.47 ^{b}
	24 $^{\beta}$	2.00±0.30 ^{a}	0.73±0.52 ^{b}	1.80±0.24 ^{a}

		20ºC^μ		
1 ^γ	1	0.05±0.08 **a**	0 **a**	0 **a**
2 ^γ		0.09±0.15 **a**	0 **a**	0 **a**
3 ^γ		0.09±0.16 **a**	0.03±0.04 **a**	0 **a**
4 ^γ		5.58±2.31 **a**	0 **b**	0 **b**
5 ^γ		0.68±0.41 **a**	0.03±0.04 **b**	0 **b**
6 ^γ		0.77±0.43 **a**	0.03±0.04 **b**	0.02±0.03 **b**
7 ^γ		2.82±0.99 **a**	0.95±0.60 **b**	0.53±0.29 **b**
		10ºC^μ		
1 ^γ	1	*Nil*	*Nil*	*Nil*
2 ^γ		*Nil*	*Nil*	*Nil*
3 ^γ		0.09±0.16 **a**	0 **a**	0 **a**
4 ^γ		9.86±2.4 **a**	0 **b**	0 **b**
5 ^γ		1.95±1.1 **a**	0 **b**	0 **b**
6 ^γ		1.17±1.34 **a**	0.25±0.04 **a**	0 **a**
7 ^γ		0.27±0.13 **a**	0 **b**	0 **b**
		5ºC^A		
1 ^γ	24	*Nil*	*Nil*	*Nil*
2 ^γ		0.22±0.28 **a**	0 **a**	0 **a**
3 ^γ		0.67±0.53 **a**	0 **a**	0 **a**
4 ^γ		11.59±1.00 **a**	0 **b**	0 **b**
5 ^γ		0.68±0.47 **a**	0 **b**	0 **b**
6 ^γ		0.22±0.08 **a**	0 **b**	0 **b**
7 ^γ		*Nil*	*Nil*	*Nil*

Note: ^β Data used from Bharathi et al. (2022a)

† Grain temperature.

^μ Movement period

^γ Different letters in the same row indicate significantly different mean values among same layers under the same tested condition. Tukey's test at a 0.05 confidence level was used to conduct these comparisons.

Nil indicates no insect movement in the specified layer