Laboratory and field studies and mathematical modelling of the three-dimensional distribution of adult *Cryptolestes ferrugineus* in wheat bulk

by

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ABSTRACT

Cryptolestes ferrugineus (Stephens), commonly referred to as rusty grain beetle, is a cosmopolitan pest and can survive in wide environmental conditions, including temperate and tropical areas with humid and dry environments. Understanding its movement and distribution in bulk grain would help to develop an effective stored grain management protocol. The one- and two-dimensional movement of the insects have been well established under laboratory conditions. The current work aimed to address the knowledge gap in the three-dimensional movement of rusty grain beetle under laboratory and field conditions.

To understand the three-dimensional movement and distribution of *C. ferrugineus*, an experimental setup, consisting of 343 cubes arranged inside a wooden box, was designed. Using the designed setup, the experiments were performed in the laboratory at various uniform temperatures (20, 30, and 35°C), moisture contents (12.5, 14.5, and 16.5%), insect densities (0.35, 1.77, and 3.53 adults (A) /kg), and movement periods (6, 24, and 72 h). The results of the study revealed that (i) at uniform environmental conditions, *C. ferrugineus* adults moved downward initially, then moved up and horizontally; (ii) the movement of adults was faster at higher temperature (35°C) than at low temperature (20°C); (iii) insects tend to disperse more at higher densities (3.53 A/kg); (iv) the effects of temperatures, moisture contents, insect densities, and movement periods observed in three-dimensional grain bulk were similar to those reported in one-dimensional grain columns and two-dimensional grain chambers.

To perform a field experiment, about 300 t of wheat were filled inside an un-aerated, 10 m diameter corrugated steel bin in Winnipeg, Canada. About 75000 *C. ferrugineus* adults (insect density of 0.25 A/kg) were introduced at the centre surface of the bin in September 2019 and about the same number of *Tribolium castaneum* (Herbst) adults were introduced at the same location during September 2020. The bin was equipped with temperature sensors and insect sensors (Insectors®) and the hourly temperature and insect counts were monitored from September 2019 to October 2021. In addition, about 20-g grain samples were collected at each of the 36 locations (at various depths and radial locations) from September 2019 to August 2021 for every month. The movement and distribution of *C. ferrugineus* observed during the field study were similar to the laboratory study under similar environmental conditions. In addition, the analytical solutions to calculate the three-dimensional diffusivity of insect movement were proposed, and the diffusivity was calculated at various homogeneous environmental conditions.

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The success and final outcome of this project required a lot of guidance and assistance, and I am extremely fortunate to have got this all along the completion of the study.

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Chapter 1. Introduction

1.1 Scope of the research

The food grain storage system, often called as a man-made ecosystem, is an integral part of the process between grain production and consumption (Jayas et al., 1995). Canada is the second main exporter of small grains (wheat, barley, oats, rye, and triticale) globally, with 23.8 million tonnes exported in 2021 (FAOSTAT, 2023). Moreover, wheat is one of the crops with the highest economic value in Canada and is considered one of the major export earners among all other agricultural products (Statistics Canada, 2023). During the export of grains, zero-tolerance towards stored grain insects is considered in decision making (Phillips and Throne, 2010).

More than 600 species of beetles, 70 species of moths, and 355 species of mites are known to cause various losses to stored grain products, including quantitative, qualitative, nutritional, seed viability, and commercial losses as well as the loss in the form of damage to storage structures (Bharathi et al., 2017; Kumar, 2017). In addition, the consumption of grains by insects can result in the formation of fines, dusts, and broken kernels, which in turn can affect airflow through the grains (Mason and McDonough, 2012). These losses can be prevented by proper management practices and protocols including proper storage structures, sanitation, monitoring, and use of pesticides when necessary. Understanding the insect movement and distribution is a prime requisite to developing a proper stored grain management protocol.

Cryptolestes ferrugineus (Stephens) (Coleoptera: Laemophloeidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), commonly known as the rusty grain beetle and the red flour beetle, respectively, are the most important grain pest species in Western Canada (Hulasare et al., 2003; Madrid et al., 1990). *Cryptolestes ferrugineus* is cosmopolitan and ubiquitous. It wanders inside the stored grain bins in quest of suitable locations for their survival, growth, and multiplication (White, 1995). Factors including relative humidity (r.h.) (Surtees, 1963), gas composition (White et al., 1993), temperature (Jian et al., 2007), moisture content of the grain (Jian et al., 2005a) and their gradients (Jian et al., 2003, 2004b, 2005a), presence/ absence of light (Park and Lee, 2017), density of the insects (Jian et al., 2004a), level of dockage (Jian et al., 2005a), insect species (Jian et al., 2012), presence of other arthropods (Flinn and Hagstrum, 1995) and age of the insects (Jian et al., 2005b) have been found to influence the movement and distribution of insects, inside a grain bulk. One- and two-dimensional movement of *C. ferrugineus* has been well studied (Jian, 2003; Jian et al., 2007, 2008). However, knowledge on three-dimensional movement of *C. ferrugineus* is virtually nonexistent.

1.2 Objectives of the study

Based on an extensive literature research (Chapter 2) and considering the economic importance of *C. ferrugineus*, the main objective of the current study was to understand the three-dimensional distribution of *C. ferrugineus*. To achieve this main objective, the following sub-objectives were investigated:

1. Study the three-dimensional movement and distribution of *C. ferrugineus* adults in stored wheat under constant temperatures, moisture contents, insect densities, and movement periods.
2. Conduct a full-size bin experiment that involves:
    a. the monitoring of temperature and relative humidity for a period of 26 months;
    b. evaluation of the quality parameters such as moisture content and germination of the grains stored in the bin;
    c. introduction of *C. ferrugineus* during the first year and *T. castaneum* the following year, inside a full-size bin and monitoring the insect numbers and their movement over time.
3. Calculation of the three-dimensional diffusivity of *C. ferrugineus.*

1.3 Hypothesis of the study

The hypothesis of the study is that the three-dimensional movement and distribution of *C. ferrugineus* in grain can be determined under laboratory conditions for each individual environmental factors such as temperature, moisture content, insect density, and movement period, and these characterized movements associated with each factor can be mathematically modeled to provide a comprehensive understanding of insect behaviour within a grain bin.

1.4 Overview of the study

The detailed plan of work of the current study is clearly illustrated in Figure 1.1

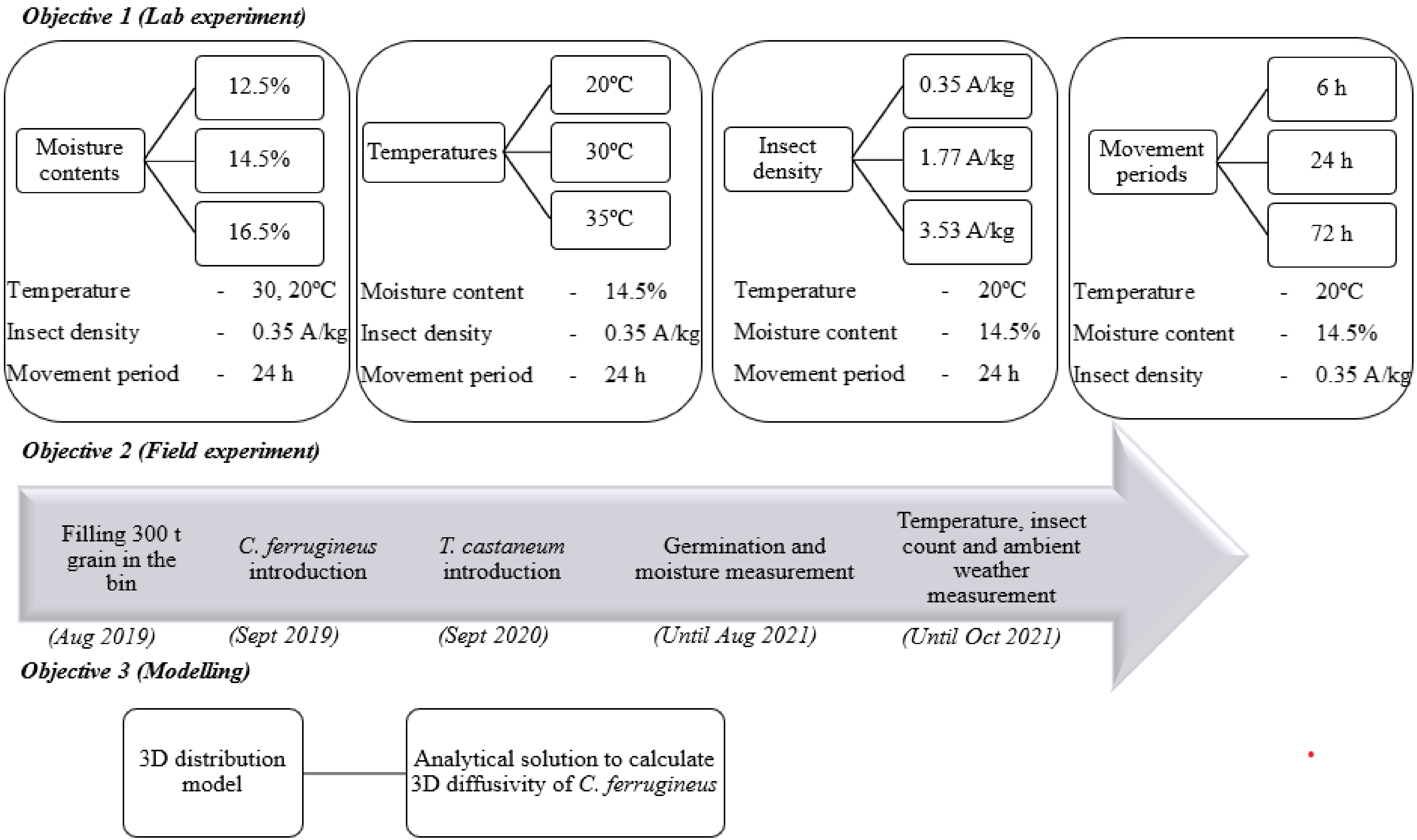

Figure 1.1. Overview of the study.

1.5 Outline of the thesis

The contents of this thesis have been structured and presented as different chapters as follows:

Chapter 2 reviews literatures related to the biology, distribution, and ecology of *C. ferrugineus*. Extensive review on the models developed to describe *C. ferrugineus* movement and population dynamics has also been made.

Chapter 3 focuses mainly on the objective 1, which is the laboratory experiments performed to analyze the three-dimensional movement and distribution of *C. ferrugineus*. This chapter is the compilation of the following two published articles.

a. **Bharathi, V. S. K.,** Jayas, D. S., Jian, F., Morrison, J., 2021. Three-dimensional movement and distribution of *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) adults in stored wheat under constant temperatures and moisture contents. *Environmental Entomology*, 51 (1), 11-21. https://doi.org/10.1093/ee/nvab109.
b. **Bharathi, V. S. K.,** Jayas, D. S., Jian, F., 2022. Effects of insect density, movement period, and temperature on the three-dimensional movement and distribution of adult *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae). *Journal of Insect Science*, 22 (3), 1-9. https://doi.org/10.1093/jisesa/ieac020.

Chapter 4 discusses the experimental plan as well as the results and discussion of the experiments performed from September 2019 to October 2021 in a 10-m diameter corrugated steel bin filled with 300 t of wheat. This chapter focuses on the objective 2 of the thesis and is a compilation of the following three published articles.

a. **Bharathi, V. S. K.,** Jian, F., Jayas, D. S., 2023. Study on 300 t of wheat stored in corrugated steel bin for two years in Canada. Part I – Temperature and moisture profiles of the grain. *Journal of Stored Products Research*, 100, 102057. https://doi.org/10.1016/j.jspr.2022.102057.
b. **Bharathi, V. S. K.,** Jayas, D. S., Jian, F., 2023. Study on 300 t of wheat stored in corrugated steel bin for two years in Canada. Part II – Movement and distribution of *Cryptolestes ferrugineus* (Stephens) and *Tribolium castaneum* (Herbst). *Journal of Stored Products Research*, 100, 102062. https://doi.org/10.1016/j.jspr.2022.102062.

c. **Bharathi, V. S. K.,** Jian, F., Jayas, D. S., 2022. Effects of weather on temperatures of the grain bin components and headspace of a 10-m diameter corrugated steel bin. *Canadian Biosystems Engineering/Le génie des biosystèmes au Canada*, 64, 3.1-3.11. https://doi.org/10.7451/CBE.2022.64.3.1.

Chapter 5 focuses on the objective 3 and consists of the analytical solution to the simplified transport equation and the algorithm and results of the three-dimensional diffusivity of *C. ferrugineus*, at various environmental conditions considered in chapter 3 (Objective 1).

Chapter 6 explains the summary and the key findings of the research in brief. Moreover, the recommendations for future research have also been provided.

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Chapter 2. Review of Literature

2.1 Biology and ecology of rusty grain beetle (*Cryptolestes ferrugineus* (Stephens))

2.1.1 Introduction

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), is a pest of considerable economic importance around the world. To understand the geographical distribution of *C. ferrugineus*, the countries in which this insect has been reported are mapped in Figure 2.1. and listed in Table 2.1. The country names are listed based on the political borders known today. The current official names of the countries are presented in Table 2.1, even if the literatures cited the former names. For instance, the current official name 'Myanmar' is adopted, even if the literatures published before the 1990s refer to the country as 'Burma'. The United Kingdom represents the countries including England, Wales, Scotland, and Northern Ireland. *Cryptolestes ferrugineus* is a cosmopolitan stored grain pest. Due to the world trade and its ability to develop under wide environmental conditions, it has established in temperate and tropical areas ranging from humid to dry as well as cool to warm climates.

Cryptolestes ferrugineus is found mostly in the following stored products: wheat, maize, barley, sorghum, oats, flour, groundnuts, beans, cassava, rice, sunflower seeds, palm kernels, cacao beans, and cotton seeds. Even though *C. ferrugineus* can develop on botanicals such as *Pimpinella anisum* (Anise), *Hibiscus sabdariffa* (roselle), *Coriandrum sativum* (Coriander), *Matricaria chamomilla* (Chamomile), *Glossostemon bruguieri* (Mogat) and *Origanum majorana* (Marjoram), it thrives in stored grain (Abdelghany et al., 2010). They are found in farms, maltings, mills, warehouses, storage bins and other storage structures (Howe and Lefkovitch, 1957). *Cryptolestes ferrugineus* can rapidly multiply and damage the grain, at suitable conditions, and leave the hollow grain as leftovers. In countries like Canada with cold winters, the establishment of stored grain pests were limited. However, *C. ferrugineus* was identified as one of the major grain pests in Canada since early 1940s. The species has been found in several Roman archaeological excavations such as England and Israel (Kislev and Simchoni, 2007). Since it is widely distributed in the world, various researchers have extensively explored its ecology, behaviour, and control techniques.

A review of literature was conducted using the databases Web of Science and Scopus with the search terms such as "Rusty grain beetle", "*Cryptolestes ferrugineus*" and "*Laemophloeus* *ferrugineus*". On January 1, 2023, a total of 380 and 412 papers (in English and other languages) were retrieved from Web of Science and Scopus databases, respectively. The papers from both sources were merged and the duplicates were removed. A total of 483 distinct research publications from 1949 to 2023 were compiled. The additional literature that is not included in Web of Science and Scopus has been retrieved from Google Scholar and discussed in the current chapter. To categorize the country of investigation, the affiliation of the authors was taken into consideration. For an article with authors from multiple countries, the country of the first author was assumed. From Figure 2.2, it can be observed that most of the literature on *C. ferrugineus* was published from Canada (171), followed by the USA (80) and the UK (47). The surge in the number of publications on *C. ferrugineus* over the years (Figure 2.3) highlights the economic importance of this insect.

A comprehensive publication on the biology, ecology, and detection of *C. ferrugineus* is not yet available in the literature. In 1949, Rilett summarized the biology of *C. ferrugineus* in detail. In 2009, Jian and Jayas provided a detailed review focusing mainly on the movement of the insect. However, several aspects of its behaviour have been explored by various researchers around the world. Hence, the current review aims to summarize the relevant literature on the biology and ecology of *C. ferrugineus,* to provide a detailed comprehension of one of the most important pests of the world.

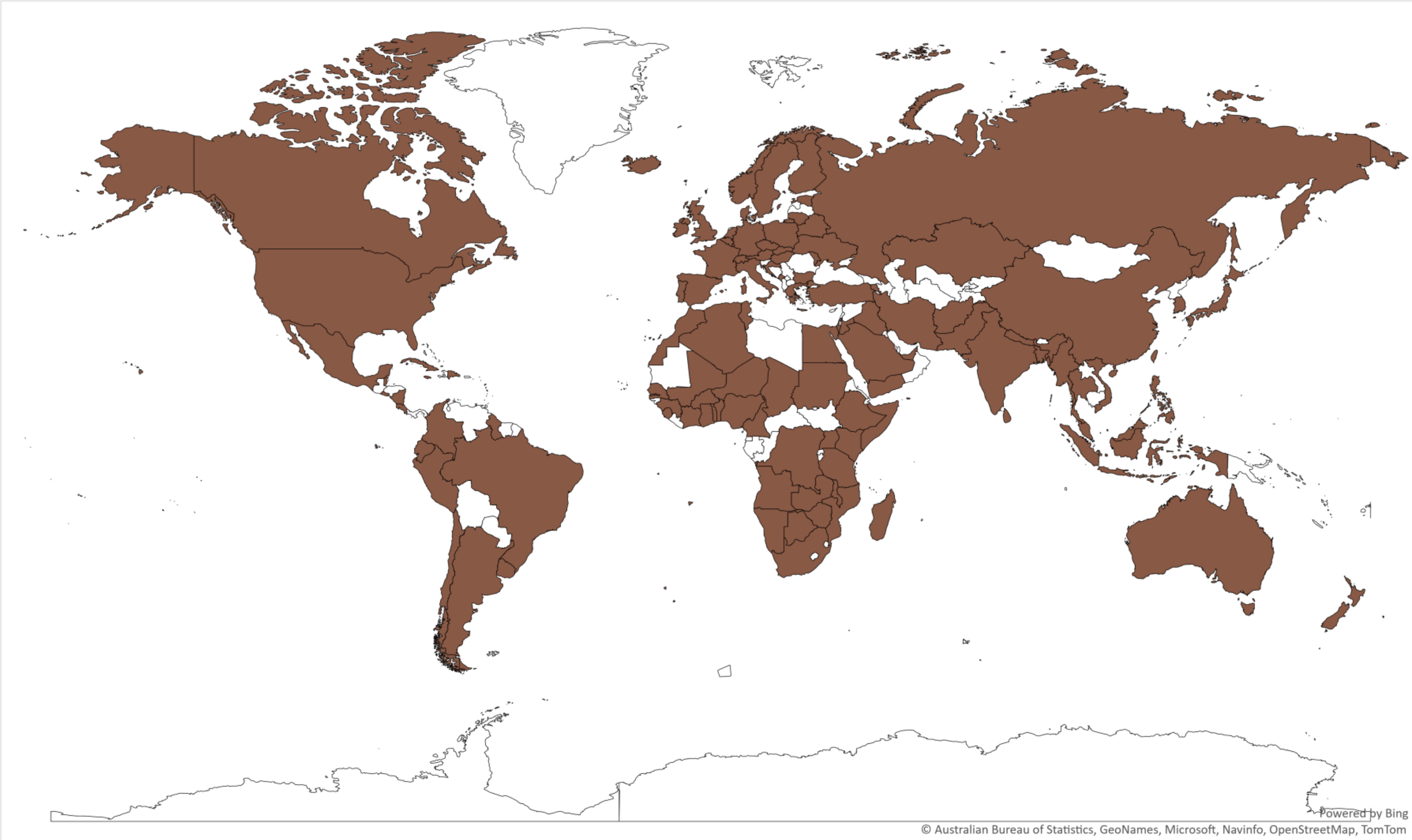

Figure 2.1. Map showing the occurrence of *Cryptolestes ferrugineus* as of January 1, 2023.

(The map is based on the information available from the literature listed in Table 2.1; however, due to its ability to survive at wide environmental conditions, C. ferrugineus *could possibly be present in other countries as well).*

Table 2.1. List of countries where *Cryptolestes ferrugineus* have been identified. (*Cryptolestes ferrugineus* is probably established in countries other than listed here, however, we could not find sources to confirm their presence in other countries).

Countries	References
Afghanistan	Lefkovitch (1962), Hagstrum et al. (2013)
Algeria	Howe and Lefkovitch (1957), Hagstrum et al. (2013)
Angola	Paim et al. (2018)
Argentina	Howe and Lefkovitch (1957), Hagstrum et al. (2013)
Armenia	Hagstrum et al. (2013)
Australia	Howe and Lefkovitch (1957), Sinclair and Bengston (1980), Barrer (1983)
Austria	Berger and Hetfleis (1985), Hagstrum et al. (2013)
Bangladesh	Haines (1974), Mills (1983), Hagstrum et al. (2013)
Belarus	Tsinkevich (2005)
Belgium	Casteels et al. (1996), Moermans et al. (1998), Hagstrum et al. (2013)
Belize	Howe and Lefkovitch (1957)
Benin	Hell et al. (2003), Bakoye et al. (2017)
Botswana	Allotey et al. (2017)
Brazil	Howe and Lefkovitch (1957), Trematerra et al. (2004)
Bulgaria	Tsvetkov et al. (1983)
Burkina Faso	Waongo et al. (2015), Baoua et al. (2016)
Cabo Verde	Hernandez et al. (2005)
Cameroon	Parasian et al. (2018)
Canada	Howe and Lefkovitch (1957), Liscombe and Watters (1962), (Loschiavo 1975), Smith and Barker (1987), Hagstrum et al. (2013)
Chad	Trematerra et al. (2003)
Chile	Hagstrum et al. (2013)
China	Howe and Lefkovitch (1957), Wang et al. (2006)
Colombia	Caiza (2016), Agrosavia (2022)
Congo	Hagstrum et al. (2013)
Costa Rica	Directorate of Plant Protection, Quarantine and Storage (2019)

Cote d'Ivoire	Tah et al. (2011), Parasian et al. (2018)
Croatia	Kalinovic and Ivezic (1994)
Cuba	Lorenzo (1997)
Czech Republic	Hubert et al. (2002), Stejskal et al. (2003), Hagstrum et al. (2013)
Denmark	Hallas (1986), Hagstrum et al. (2013)
Dominican Republic	Parasian et al. (2018)
Ecuador	Haines (1974), Hagstrum et al. (2013)
Egypt	Abdelghany et al. (2010)
El Salvador	Ochoa et al. (2014)
Estonia	GBIF (2023)
Ethiopia	Haines (1974), Hagstrum et al. (2013), CAB (2014)
Finland	Hagstrum et al. (2013), GBIF (2023)
France	Leblanc et al. (2014)
Gambia	Howe and Lefkovitch (1957)
Germany	Bahr (1980), Hagstrum et al. (2013)
Ghana	Hagstrum et al. (2013), Baoua et al. (2016), Danso et al. (2018), Manu et al. (2019)
Greece	Buchelos and Athanassiou (1999), Guerra (1992), Hagstrum et al. (2013)
Guinea	Directorate of Plant Protection, Quarantine and Storage (2020)
Guyana	Howe and Lefkovitch (1957), Hagstrum et al. (2013)
Hungary	EPPO (1999)
Iceland	Olafsson (2008) recited from Forbes and Milek (2014)
India	Mahla (2001), Hagstrum et al. (2013)
Indonesia	Erdiansyah et al. (2018)
Iraq	Al-Bekr (1972) recited from Al-Salihi and Al-Azawi (1985)
Iran	Hagstrum et al. (2013), Thomas and Ghahari (2016)
Ireland	Alexander and Anderson (2012)
Israel	Hagstrum et al. (2013)
Italy	Trematerra and Gentile (2003)
Jamaica	Howe and Lefkovitch (1957)
Japan	Sinha and Utida (1967), Sonda (1970), Hagstrum et al. (2013)

Jordan	Antary and Thalji (2017)
Kazakhstan	Sarsenbayeva et al. (2018)
Kenya	Howe and Lefkovitch (1957), Giles (1969), Haines (1974), Hagstrum et al. (2013)
Lithuania	Ostrauskas and Taluntyte (2004), Hagstrum et al. (2013)
Luxembourg	GBIF (2023)
Madagascar	Hagstrum et al. (2013)
Malawi	Howe and Lefkovitch (1957), Hagstrum et al. (2013)
Malaysia	Howe and Lefkovitch (1957), Hagstrum et al. (2013)
Mali	Haines (1974), Hagstrum et al. (2013)
Malta	Halstead and Mifsud (2003)
Mexico	Corral et al. (1992), Córdova Ballona et al. (2011), Hagstrum et al. (2013)
Montenegro	GBIF (2023)
Morocco	Howe and Lefkovitch (1957), Hagstrum et al. (2013)
Mozambique	Covele et al. (2020)
Myanmar	Howe and Lefkovitch (1957)
Namibia	Stejskal et al. (2006)
Nepal	Gurung (2002)
Netherlands	Pals and Hakbijl (1992), Hagstrum et al. (2013)
New Zealand	Howe and Lefkovitch (1957), Chapman et al. (2016)
Nicaragua	Haines (1974), Giles and Leon (1974), Hagstrum et al. (2013)
Niger	Bakoye et al. (2017)
Nigeria	Howe and Lefkovitch (1957), Lale and Yusuf (2000), Ukeh and Udo (2008)
Norway	Hagstrum et al. (2013), GBIF (2023)
Pakistan	Hagstrum et al. (2013), Wakil et al. (2014)
Peru	Howe and Lefkovitch (1957), Haines (1974), Hagstrum et al. (2013)
Philippines	Haines (1974), Hagstrum et al. (2013)
Poland	Klejdysz and Nawrot (2010), Hagstrum et al. (2013)
Portugal	Howe and Lefkovitch (1957), Hagstrum et al. (2013), GBIF (2023)
Republic of Korea	Kim et al. (1988)
Republic of Moldova	Irina et al. (2019)

Russia	Howe and Lefkovitch (1957), Andreev (1991), Hagstrum et al. (2013)
Saudi Arabia	Haines (1974), Hagstrum et al. (2013), CAB (2014)
Senegal	Faye et al. (2022)
Sierra Leone	Haines (1974), Hagstrum et al. (2013)
Singapore	Howe and Lefkovitch (1957), Hagstrum et al. (2013), CAB (2014)
Slovakia	Hagstrum et al. (2013)
Somalia	Lavigne (1991), Hagstrum et al. (2013)
South Africa	Howe and Lefkovitch (1957), Hagstrum et al. (2013)
Spain	Pascual-Villalobos et al. (2006), Hagstrum et al. (2013), Castañé et al. (2020)
Sri Lanka	Howe and Lefkovitch (1957), Ganesalingam (1976), Hagstrum et al. (2013)
Sudan	Howe and Lefkovitch (1957), Haines (1974), Hagstrum et al. (2013), CAB (2014)
Sweden	Mathlein (1971), Hagstrum et al. (2013)
Switzerland	Hagstrum et al. (2013), GBIF (2023)
Tanzania	Howe and Lefkovitch (1957), Hagstrum et al. (2013)
Thailand	Howe and Lefkovitch (1957)
Timor-Leste	Handayani et al. (2019)
Togo	Baoua et al. (2016)
Tunisia	Howe and Lefkovitch (1957), Hagstrum et al. (2013)
Turkey	Er et al. (2016), Özgen et al. (2018), Toğantimur and Özder (2019)
Uganda	Hagstrum et al. (2013)
Ukraine	(Department of Agricultural Extension 2015)(Ostrauskas and Taluntyte 2004)
United Arab Emirates	Hagstrum et al. (2013)
United Kingdom	Prickett et al. (1990), Hunter et al. (1973)
United States of America	Arbogast and Mullen (1988), Hagstrum (1989), Throne and Cline (1994) Sedlacek et al. (1998)
Uruguay	Howe and Lefkovitch (1957), Hagstrum et al. (2013)
Vietnam	Štusák et al. (1986), Hagstrum et al. (2013)

Yemen	Haines (1974), Hagstrum et al. (2013)
Zambia	Howe and Lefkovitch (1957)
Zimbabwe	Howe and Lefkovitch (1957), Giga and Katerere (1986), Hagstrum et al. (2013)

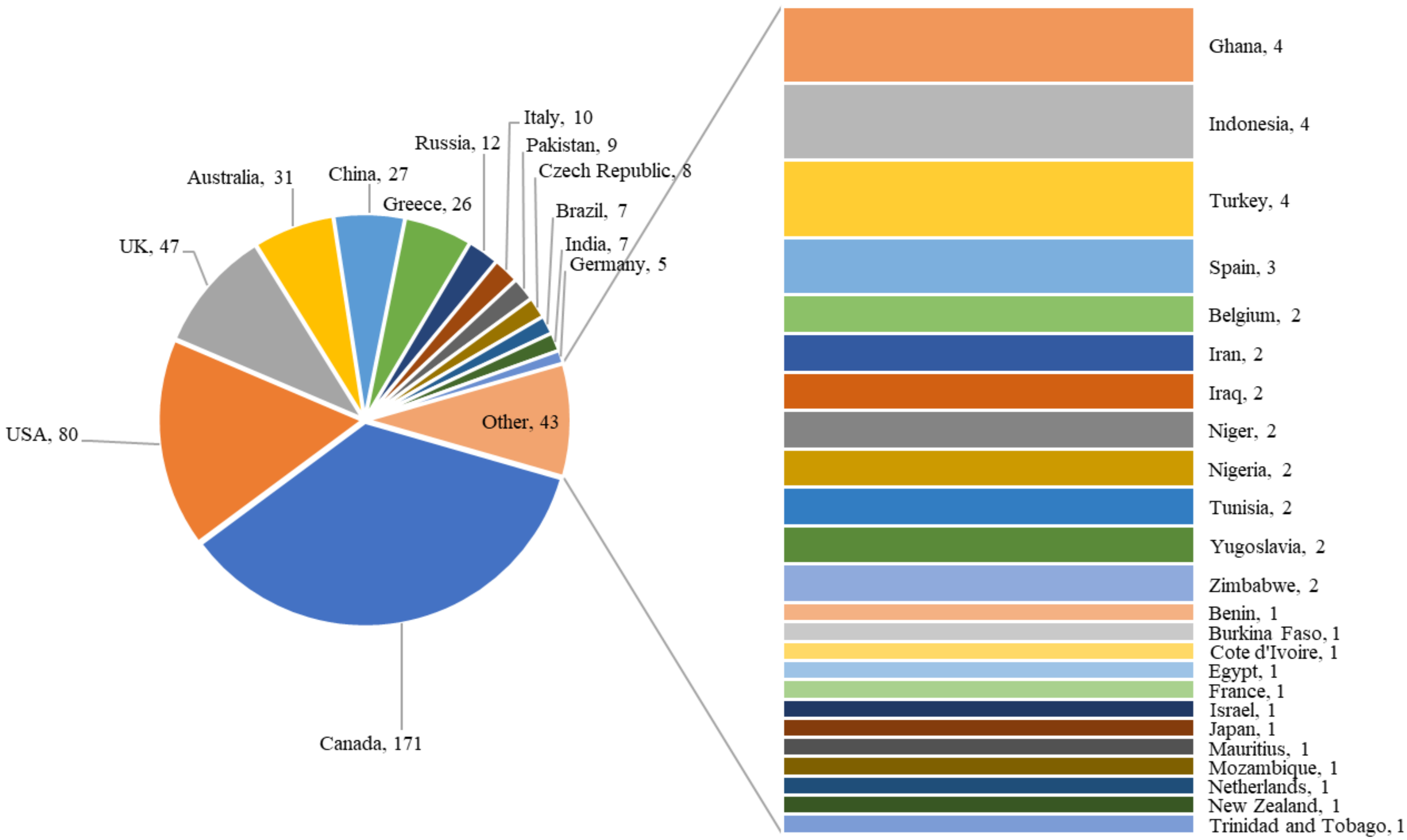

Figure 2.2. The number of publications on *Cryptolestes ferrugineus* from different countries compiled using the data retrieved from 483 publications from Web of Science and Scopus from 1949 to 2023 (as of January 1, 2023).

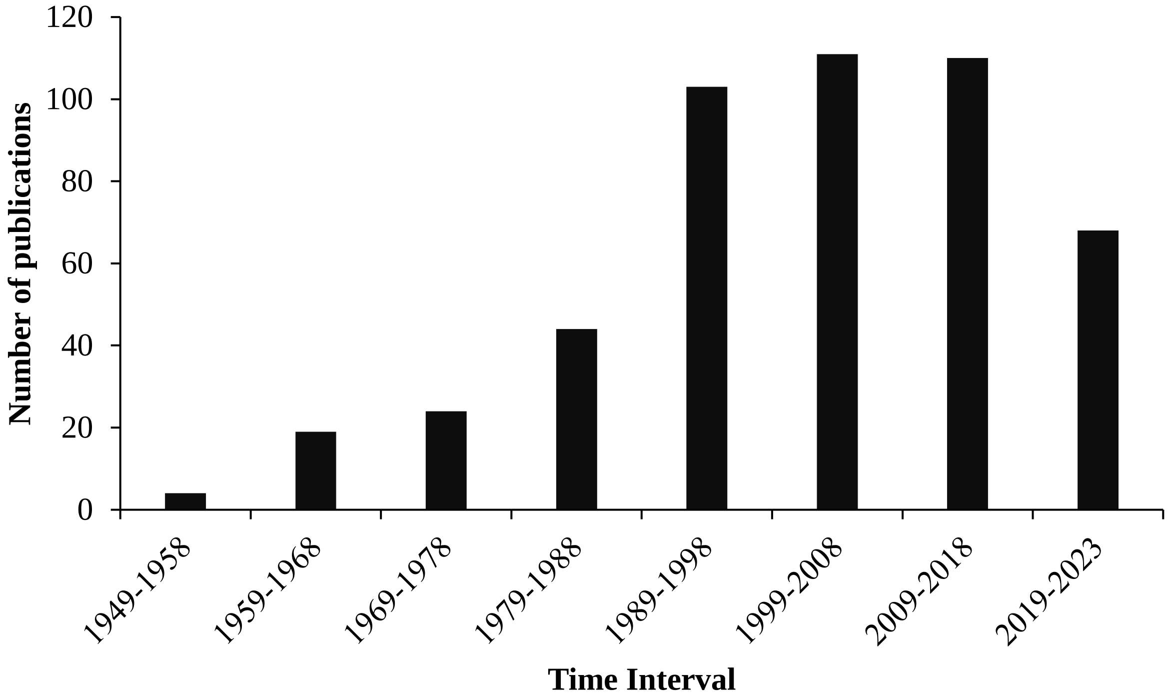

Figure 2.3. The number of publications on *Cryptolestes ferrugineus* at different time intervals compiled using the data retrieved from 483 publications from Web of Science and Scopus from 1949 to 2023 (as of January 1, 2023).

Table 2.2. Identification of different species of *Cryptolestes* (Arbogast, 1991; Halstead, 1993)

Part	*Cryptolestes ferrugineus*	*Cryptolestes pusillus*	*Cryptolestes turicicus*
Antennae	Subequal in male and female; half as long as the body	Longer in males than females; two-third length as their body	Longer in males than females; equal to or longer than their body
External mandibular tooth	Present in male	Absent in male	Absent in male
Head	Transverse ridge near dorsal posterior margin is absent	Transverse ridge near dorsal posterior margin is present	Transverse ridge near dorsal posterior margin is present
Pronotum	Narrowed posteriorly, especially in males	Transverse, slightly narrowed posteriorly in males	Nearly quadrate
Number of rows of setae between first and second and between second and third elytral striae	Four	Four	Three

2.1.2 Identification and other names

The rusty grain beetle, also known as the rust-red grain beetle, was initially described by James Francis Stephens in 1831 under the name *Cucujus ferrugineus*. *Cryptolestes* was listed as a subgenus under the genus *Laemophloeus* and the insect was referred to as *Laemophloeus ferrugineus* (Stephens) by Leng; whereas Casey claimed that *Cryptolestes* could be referred to as an individual genus due to its distinct nature and this was agreed by other researchers such as Sheppard (Rilett, 1949). The insect was also referred to using other names such as *Cucujus monilicornis* (Stephens, 1831), *Laemophloeus concolor* (Smith, 1851), *Laemophloeus obsoletus* (Smith, 1851), *Laemophloeus carinulatus* (Wollaston, 1877), *Laemophloeus emgei* (Reitter, 1887), *Laemophloeus alluaudi* (Grouvelle, 1906) (Halstead, 1993). In mid-20th century, the insect was often referred as *Laemophloeus ferrugineus* (Stephens) in the literatures. However, currently, *Cryptolestes ferrugineus* is widely used.

There are about 50 species globally present in the genus *Cryptolestes* (Ganglbauer, 1899), but only nine are considered as pests of stored products: *C. capensis* (Waltl, 1834), *C. cornutus* (Thomas and Zimmerman, 1989), *C. divaricatus* (Grouvelle, 1898), *C. ferrugineus*, *C. klapperichi* (Lefkovitch, 1962), *C. pusillus* (Schönherr, 1817), *C. pusilloides* (Steel and Howe, 1952), *C. turcicus* (Grouvelle, 1876), *C. ugandae* (Steel and Howe, 1952). Differentiation of *C. ferrugineus* from other *Cryptolestes* spp. could be performed by identifying the morphological differences in those species as listed in Table 2.2 (Arbogast, 1991; Halstead, 1993). Moreover, several researchers (Wang et al., 2014; Varadínová et al., 2015; Chen et al., 2020) proposed identification of different species of *Cryptolestes* (*C. ferrugineus, C. pusillus, C. turcicus, C. pusilloides* and *C. capensis*) based on mitochondrial cytochrome c oxidase subunit I (COI) barcode region. Vendl et al. (2019) studied the tarsal and inter-claw adhesive structure of *C. ferrugineus* using a scanning electron microscope and reported the following observations: 1) The length-to-width ratio of tarsi is about 9.5; 2) the first tarsomere is short and small (almost the same shape as the next tarsomere); 3) the last tarsomere is the longest among other tarsomeres; 4) the ventral side of the tarsomeres and the pre-tarsi do not have any adhesive structures; 5) a pair of apical setae on the unguitractor is present; 6) the lateral margin of the terminal tarsomere contains two pairs of setae; whereas, the medial part of the ventral side of the margin is trapezoidal. The absence of adhesive structures in the tarsomeres is responsible for their inability of this species to climb inclined and smooth surfaces.

2.1.3 Biology and development

2.1.3.1 *Life stages*

a. Egg

The female *Cryptolestes ferrugineus* deposits eggs in small gaps in the grain kernels (under the outer layer of the seed coat), between the grain kernels, small crevices, or fractures in any structures, or in debris with the help of their substitutional ovipositor. Those caudal segments are generally present retracted in the abdomen. During oviposition, those segments protrude out to facilitate the placing of the egg at a suitable location. A terminal segment called ‘styli’ aids in the suitable orientation of the egg. Each female could lay about 200 to 500 eggs (Canadian Grain Commisision, 2021). The eggs appear to be white, moderately translucent with length and width in the range of 0.68 to 0.81 mm and 0.20 to 0.30 mm, respectively. The eggshell after emergence has distinct iridescence. Under suitable environmental conditions (at 32ºC and 75% relative humidity), the eggs hatch in three to four days (Rilett, 1949). Eggs do not hatch below 15ºC (Kawamoto et al., 1990).

b. Larva

Once the egg is ready to hatch, the larva breaks the membrane around itself (termed as ‘chorion’) through a series of movements. The larva continuously produces those movements until its head emerges from the egg. Then, the larva crawls out of the eggshell with the help of its legs and a series of to-and-fro movements. Then, the larva starts its exploration of food. The larva mainly feeds on the germ portion of the wheat, but also feeds on endosperm during germ scarcity. The amount of food consumed depends on the environmental condition. At suitable conditions, the larva stays inside a kernel of wheat and forms a burrow through the consumption of wheat germ. It ejects the fecal material and molted exuviae through the opening created by the female adult during oviposition or created by the larvae to enter the wheat germ (Rilett, 1949). The size of larva ranges from 1 to 4 mm (Canadian Grain Commisision, 2021).

Larvae of *C. ferrugineus* are four instars, which implies that they moult four times and becomes a pupa after the last moulting. The first, second, third and fourth instar larval stages last about three to four, two to five, two to five, and five to eight days, respectively, at suitable conditions. The first instar larva is white in color; whereas, the fourth instar larva becomes light tan in color. The fourth instar larvae have a head, thorax, and abdomen. At the end of the abdomen, caudal hooks are present, which aid in backward movement of the larvae. The mouth parts of larvae and adults are similar. On evaluating the bioenergetics of *C. ferrugineus*, Campbell and Sinha (1978) reported that the immature stages assimilated about 66% to 79% of the food consumed. They also reported that during development, the proportion of assimilated energy converted into tissue growth/ biomass was from 3% (early first-instar larvae) to 23% (older larvae).

Since *C. ferrugineus* is cannibalistic in nature, larvae feed on pre-pupae or pupae. Before entering the next stage of development, the fourth instar larva enters the burrow of the wheat and seals the burrow using debris and excrement through silken threads. Sometimes, they also pupate in other locations such as crevices or the space between the grain kernels. Two papillae, which are slightly and distinctly noticeable in the third and fourth instars, respectively, were reported to be responsible for the silk thread formation (Rilett, 1949). Compared to other *Cryptolestes* species such as *C. turcicus*, which can produce tough silk, strong enough to produce cocoon, *C. ferrugineus* forms fragile silk, which can only hold debris, bran and excrement in place (Lefkovitch and Milnes, 1963).

c. Pupa

The pupal stage lasts about three to six days at 32ºC and 75% relative humidity. Initially, the pupa is white and over time, it turns into a light tan colour, with a triangular shape to some extent. The eyes of the pupae look dark brown in colour (Rilett, 1949).

d. Adult

The adult emerged from pupa is light tan in colour, which turns into a rusty brown color in one or two days (Figure 2.4). Immediately after emergence, the membranous pair of wings are stretched for a short duration, after which they fold beneath the elytra. The length of the adult is in the range of 1.70 to 2.34 mm and antennal length ranges from 0.70 to 1.14 (Reid, 1942). A day or two after emergence, the adults start mating, the oviposition begins, and the cycle continues. White and Bell

(1993) reported that the isolated virgin adults have greater life span than the mated adults. The mean life span of adults ranges from 12 to 32 wk depending on the density and sex ratio (White and Bell, 1993). *Cryptolestes ferrugineus* can develop at temperature range from 20 to 42.5ºC (White et al., 1995). *Cryptolestes ferrugineus* is one of the most cold-tolerant species with the adult being the most cold-hardy stage (Smith, 1970). At temperature below 23ºC, the rate of reproduction decreases; at temperatures below 21ºC, the insects cannot fly (Canadian Grain Commisision, 2021). On the other hand, Cox and Dolder (1995) reported that the minimum temperature for *C. ferrugineus* flight was 20ºC, whereas in the laboratory cultured strains for a period of over 20 years, one insect was reported to flew at 17.5ºC. The female: male sex ratio of *C. ferrugineus* adults were reported to be 1:0.64 (Rilett, 1949) and 1:0.69 (Hulasare et al., 2005) on wheat and 1.1:0.8 on dates (Al-Salihi and Al-Azawi, 1985). The longevity of female *C. ferrugineus* is longer than that of males (Bishop, 1959).

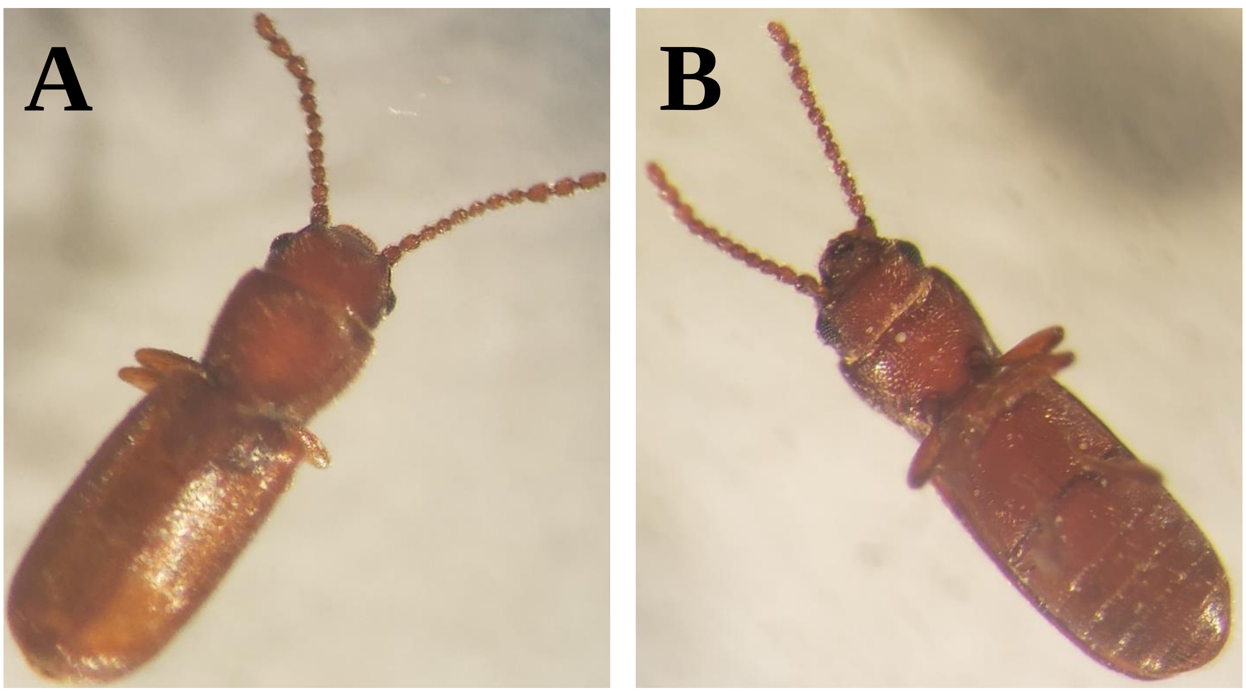

Figure 2.4. Dorsal view (A) and Ventral view (B) of *Cryptolestes ferrugineus* adult.

2.1.3.2 *Sexual dimorphism*

Male and female *C. ferrugineus* can be differentiated by observing their genitalia (Figure 2.5). The tarsi of female *C. ferrugineus* are all five-segmented (with tarsal formula 5-5-5); whereas those of males are four- and five-segmented (with tarsal formula 5-5-4). On the other hand, in females, the styli are present on the ninth abdominal segment, whereas, it is absent in males. The male *C. ferrugineus* have a larger head and wider thorax than the female (Rilett, 1949). The sex difference could also be observed in the mandibles. Precisely, the male mandible has a toothlike projection on the lateral ventral side near the base; while the female does not have the projection (Rilett, 1949; Boukouvala et al., 2022). Chambers et al. (1990) reported that the sexual differences of *C. ferrugineus* could be also identified based on the electroantennogram responses of the adults and showed greater electroantennogram amplitude in females than males.

2.1.4 Effects of various environmental parameters on the biology of *Cryptolestes ferrugineus*

2.1.4.1 *Temperature*

Temperature is one of the main factors that influences the population dynamics of *C. ferrugineus* (Jian et al., 2018). An extensive review on the application of temperature to control stored product insects is available (Fields, 1992). At -10ºC, the LT_{50} (lethal time for 50% of a population) values for egg, young larva, old larva, pupa and adult were reported to be 8, 4, 16, 11 and 91 h, respectively (Ganesan et al., 2021). Ashby (1961) reported that the rate of respiration and development of *C. ferrugineus* proportionally increased with the rise in temperature, at the range of 21 to 33ºC. The development rate of *C. ferrugineus* eggs is linearly related to temperature (T) (Egg development rate, D = 0.0169T - 0.258) (Kawamoto et al., 1990).

Temperature change is one of the well-known methods to control *C. ferrugineus* population. The presence of ice-nucleating active bacteria was reported to influence the cold tolerance of *C. ferrugineus.* Fields (1993) reported that the application of *Pseudomonas syringae*, an ice-nucleating active bacteria in the form of freeze-dried powder on grain increased the supercooling point of *C. ferrugineus* and thus, increased its mortality at -10ºC. An ice-nucleating fungus, *Fusarium avenaceum* was also explored for their ability to increase the supercooling point of *C. ferrugineus*. However, *P. syringae* is more effective than *F. avenaceum*, since *P. syringae* has about 1000 times more ice nuclei per gram than *F. avenaceum* (Fields et al., 1995). Lazzari et al. (2006) adopted artificial chilling to control the population of stored grain species such as *Oryzaephilus surinamensis* (L.), *C. ferrugineus*, *Rhyzopertha dominica* (Fabricius), and *Sitophilus* spp. After 28 days, when the average temperature of the grain bulk dropped to 15ºC, about 77%

reduction in insect population was observed. However, Bareil et al. (2018) reported that 0ºC for 3 months was not sufficient to completely eradicate *C. ferrugineus*.

Acclimation

Acclimation is one of the important parameters which determines the cold tolerance levels of insects. When acclimated to low (15 to 5ºC) temperatures for some time, most stored product insects were found to increase their cold tolerance by 2 to 10 times (Fields, 1992). The acclimated *C. ferrugineus* were reported to be more cold hardy than the non-acclimated ones (Fields, 1993). Precisely, *C. ferrugineus* acclimated at 15, 10 and 5ºC consecutively for two weeks at each temperature had LT_{50} and LT_{90} (lethal time for 90% population) of 24 and 42 days, respectively, at -10ºC; whereas, unacclimated *C. ferrugineus* had LT_{50} and LT_{90} of 1.4 and 2.7 days, respectively, at the same temperature (Fields et al., 1998). Smith (1970) reported that the LT_{50} values of acclimated adults increased by 9 and 56 times, respectively, at -6 and -12ºC, compared to the unacclimated adults. In addition, the supercooling points of *C. ferrugineus* adults were -16.5, -20 and -21ºC for unacclimated, acclimated at 15ºC, and acclimated at 15ºC followed by acclimation at 4ºC, respectively. In cold acclimated *C. ferrugineus*, trehalose, amino acids including proline, asparagine, valine, lysine, leucine, isoleucine, alanine, phenyl alanine glutamic acid and aspartic acid as well as phosphoethanolamine (a phospholipid precursor) were higher than unacclimated *C. ferrugineus* (Fields et al., 1998). Furthermore, the acclimation increased the mean fresh weights of *C. ferrugineus* (Evans, 1981). The acclimation temperature was found to affect the behaviour of *C. ferrugineus* than the exposure time (Jian et al., 2005c). Burks and Hagstrum (1999) examined the rapid cold hardening ability of five different species (*C. ferrugineus, O. surinamensis, R. dominica, S. oryzae*, and *T. castaneum*) and reported that *C. ferrugineus* is capable of rapid cold hardening than other tested species.

2.1.4.2 *Moisture content*

The damper grains facilitate convenient feeding of grains than the dry grains for *C. ferrugineus*. The development of *C. ferrugineus* is limited when the moisture content of the grain or relative humidity is below 12% or 40%, respectively (Canadian Grain Commisision, 2021). Throne (1990) studied the progeny of *C. ferrugineus* at different moisture contents (11.3, 12.4, and 14.8%) and reported that the number of offspring produced on damaged grain increased linearly with moisture content. Similarly, Throne and Culik (1989) reported that the corn maintained at 75% relative

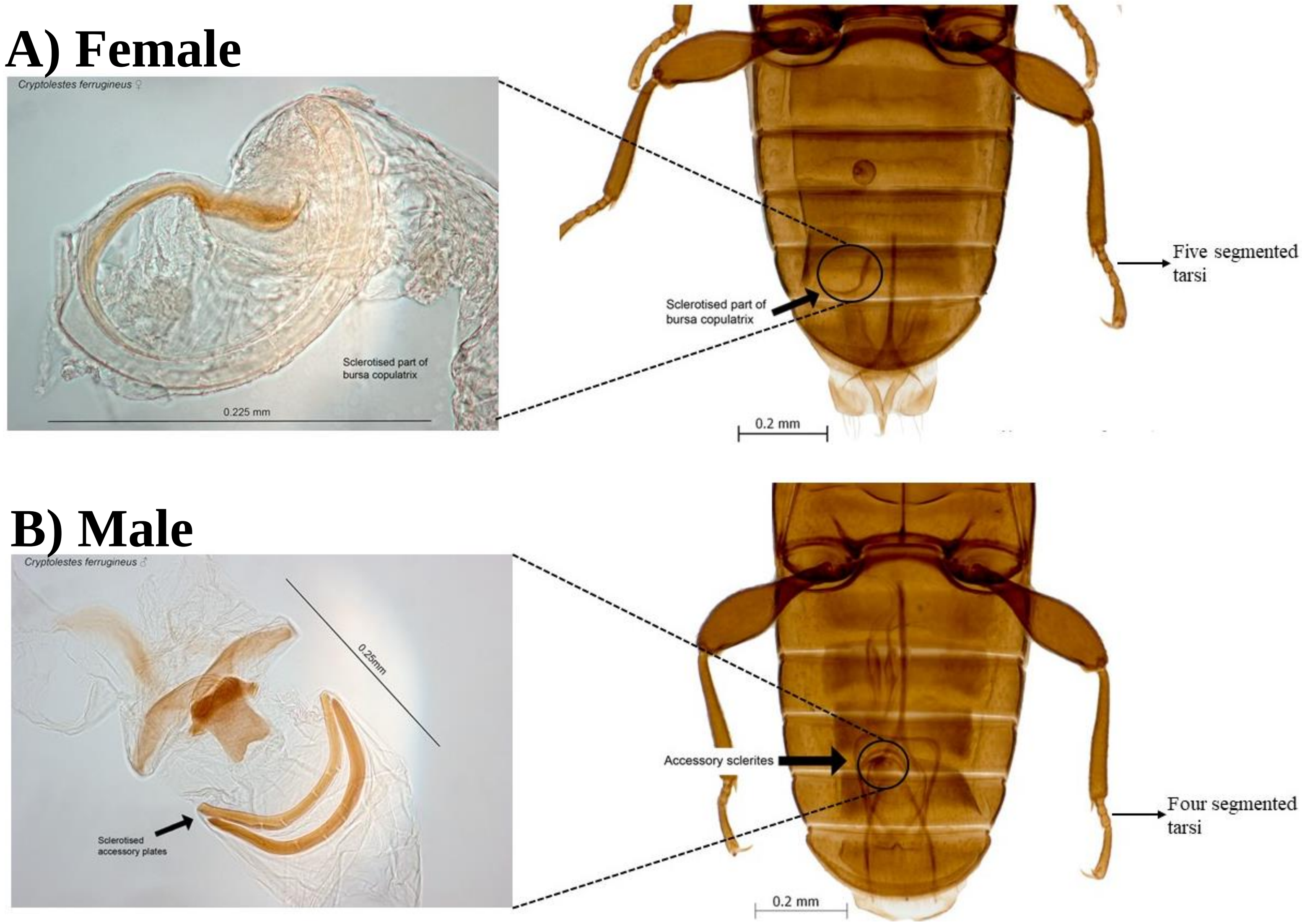

Figure 2.5. Female (A) and male (B) abdomen and genitalia of *Cryptolestes ferrugineus*. Image source: Pia Scanlon, adapted from Padil.gov.au under Creative Commons Attribution-Non Commercial 4.0 International license (Szito, 2012)

humidity showed higher progeny production and lower development time for *C. ferrugineus*, when compared with those at 43% relative humidity. Bishop (1959) reported that the egg production and longevity of *C. ferrugineus* increased with increase in relative humidity. However, Kawamoto et al. (1990) studied the mortality and development of *C. ferrugineus* egg at different relative humidities (50, 60,70, 80, and 90%) and reported that relative humidity does not affect the mortality and development of eggs. With increase in temperature, the effect of humidity on *C. ferrugineus* rate of oviposition was reported to be more pronounced (Smith, 1965).

2.1.4.3 *Diet*

The type and quality of food greatly influences the survival, growth, and multiplication of *C. ferrugineus.* However, certain studies found contradictory results regarding the suitability of diets for *C. ferrugineus*. Some of them are highlighted in this section. Larvae of *C. ferrugineus* have better survival and faster development in a wheat kernel with germ than those without a germ or on bran or white flour (Rilett, 1949). Moreover, the oviposition rate on whole-wheat flour was greater than wheat kernel at all tested densities (4, 16, and 64 pairs), except 1 pair per vial (Smith, 1966a). Tuff and Telford (1964) reported that *C. ferrugineus* were not able to invade sound kernels, whereas they could infest seeds with damaged grain coats. Similarly, Throne and Culik (1989) reported higher progeny production and decreased development duration on cracked corn compared to undamaged kernels. However, the level of cracking on the corn did not significantly affect the survival of the immature stages of *C. ferrugineus* (Throne, 1992). Shufran et al. (2013) performed a laboratory experiment on the host suitability of pecan and wheat for various stored-product insects and reported that *C. ferrugineus* were observed to produce more immatures on unsorted pecan, cracked pecan, and nutmeats than on in-shell pecan; however, only fewer adults were observed on different types of pecans than wheat. This implies that pecans lack certain dietary requirements for *C. ferrugineus*. White and Loschiavo (1988) reported that the slower developmental time and higher larval mortality of *C. ferrugineus* on oats compared to wheat was due to the nutritional insufficiency and unpalatability of oats. Even though *C. ferrugineus* can survive on hemp seed and its dockage, it does not flourish (Hamilton et al., 2021). Also, *C. ferrugineus* prefers wheat kernels as compared to canola and rapeseed (Loschiavo and Lamb, 1985). Durum Kyle, Coulter, and Medora are suitable wheat varieties for the oviposition and development of *C. ferrugineus* (White and Loschiavo, 1988). Jagadeesan et al. (2013) evaluated the suitability of nineteen grain-based diets on the number of live adult progeny developed and concluded that diet containing (a) barley flour, (b) rolled oats and cracked sorghum, (c) wheat flour and barley flour, and (d) cracked sorghum alone resulted in higher progeny production of laboratory strains, whereas diet containing (a) rolled oats and cracked sorghum, (b) wheat flour and barley flour and (c) barley flour alone were suitable for field collected strains. They also reported that diets containing cracked sorghum were better than those containing cracked maize or wheat. This could be because, the laboratory strain used was cultured in diet containing rolled oats, cracked sorghum and yeast for 5 generations prior to the experiment; besides, the insects were collected from stored sorghum. They hypothesized that the literatures published on the successful culturing of *C. ferrugineus* on corn (Throne and Culik, 1989; Throne, 1992) could have been collected from stored maize. The diet also influences the cold tolerance of *C. ferrugineus.* For instance, the LT_{50} of *C. ferrugineus* adults at -10ºC in grain, flour and Brewer's yeast, and flour alone were 104, 79, and 42 h, respectively; and, the supercooling points were -20.6, -22.9, and -19.4ºC, respectively (Ganesan et al., 2021). From an experiment performed to reveal the life history of *C. ferrugineus*, Al-Salihi and Al-Azawi (1985) revealed the following: number of eggs per female (558), egg incubation period (3.2 days), egg hatching (96.8%), larval period (70.3 days), pre-pupal period (3.6 days) and pupal period (6.1 days), adult life span (6 months and 6 days) at 30ºC and 70% relative humidity.

2.1.4.4 *Insect density*

Crowding plays a significant role in the population dynamics of *C. ferrugineus* since crowding can facilitate high egg damage, high larval mortality due to cannibalism and adult mortality due to fighting (Jian et al., 2018a). Smith (1966a) performed an experiment to evaluate the effect of *C. ferrugineus* crowding on its rate of oviposition, development and mortality and reported the following observations: 1) At 30ºC and 70% relative humidity, the number of eggs produced per female per day were 6.4 and 1.5 when 1 and 64 pairs of adults, respectively, were present in the vial containing 0.5 g flour; whereas, in 1 g wheat kernel, the number of eggs produced per female per day was 5.6 and 0.75 in the presence of 1 and 64 pairs of adults, respectively. 2) Development time (egg to adult) on 0.5 g flour were 24 and 87.1 d with the initial larval count of 1 and 32, respectively, per vial. 3) Mortality of the insects increased with density. White and Bell (1993) reported that the amount of energy outflow and the physical injury during copulation affects the survival of insects at different densities and at different sex ratios. These results reveal that insect density affects the oviposition, development, and mortality of *C. ferrugineus.* A study on the population dynamics of *C. ferrugineus* revealed that the population dynamics of *C. ferrugineus* are influenced by patch size and temperature (Jian et al., 2018a, b). Moreover, they also reported that the total insect number and kernel infestation percentage were positively correlated.

2.1.5 Ecology and behaviour

2.1.5.1 *Refuge-seeking behaviour*

Refuge-seeking behaviour is the ability of the stored grain pests to hide in the structural cracks and crevices of the storage structure, which contain grain residues. The refuge provides food and shelter to the insects, in addition to protecting them from insecticide treatments. The hidden insects emerge out and reinfest nearby grain when the conditions are favourable. Even in the absence of food, insects were reported to be attracted towards the refuge, possibly for the physical contact around the body of *C. ferrugineus*. This could also be the reason for their occurrence near the container boundary during laboratory experiments (Cox and Parish, 1991).

On analyzing the samples obtained from structural cracks and surfaces from 34 empty storage structures in the Prairie provinces of Canada (Manitoba, Saskatchewan, and Alberta), *C. ferrugineus* were obtained from 36% of the sampled structures (Liscombe and Watters, 1962). The effects of different temperatures, refuge contents, food availability, and different strains have been evaluated on the refuge-seeking behaviour of *C. ferrugineus* (Cox et al., 1989; Cox and Parish, 1991). Cox et al. (1989) observed the refuge-seeking behaviour of different strains of *C. ferrugineus* at different temperatures (15, 20, 25, and 30°C) and reported that about 45% and 20-30% of the insects were found to remain inside the refuge at the end of 2 wk for *C. ferrugineus* strains that were reared in the laboratory for over 25 yr and those obtained from grain stores and mills in the UK, respectively. Moreover, they also observed that the refuge-seeking behaviour of different strains of *C. ferrugineus* varied with varying temperatures. The refuge-seeking behaviour of *C. ferrugineus* females was greater than males, and that of adults 0-3 wk old was greater than

10-12 wk and 16-18 wk old adults (Cox et al., 1990). This was because the refuge would have attracted females for oviposition and attracted younger adults since oviposition is greater in younger adults than older ones (Smith, 1965).

2.1.5.2 *Flight activity*

The flight activity of insects determines their ability to infest the stored grains in different bins. The level of infestation inside a grain bin varies with the number of insects immigrating into the bin. Understanding the flight activity pattern of insects under different environmental conditions could help in planning the frequency of management practices such as aeration and fumigation. The flight activity of *C. ferrugineus* depends on external factors such as air temperature, wind direction, wind speed and daylength (Nansen et al., 2004; Holloway et al., 2018). During a flight activity study of *C. ferrugineus* in southern New South Wales, Australia, Holloway et al. (2018) observed no flight activity during the winter months (June, July and August). *Cryptolestes ferrugineus* captured on glue boards installed in and around the warehouses of Kansas and Nebraska, USA were reported to peak in early September and decline through early November (Toews et al., 2006). Hagstrum (2001) studied the immigration of insects in 34 bins with varying capacities (36 to 238 t) containing hard red winter wheat on 12 farms, from July to December 1998, in Kansas, USA and observed the immigration of *C. ferrugineus* in all the 34 bins. The drop in immigrated insect count was reported when the ambient temperature dropped. Thus, *C. ferrugineus* shows seasonal variation in flight activity and immigration. This is because the minimum temperature for flight initiation of *C. ferrugineus* is 20ºC (Cox and Dolder, 1995). Hagstrum (1989) observed the distribution of *C. ferrugineus* in three farms in Kansas, ISA and reported that most of the *C. ferrugineus* infestation occurs after the grain was loaded into the bin. In addition, the number of insect counts decreased from the top layers. This implies that *C. ferrugineus* adults fly and reach the top of the bin and then distribute to other parts of the grain inside the bin.

2.1.5.3 *Mating behaviour*

Male and female adults of *C. ferrugineus* start mating within one or two days after they emerge. When a male identifies a potential female, the male adult turns and follows the female. Boukouvala et al. (2022) performed an experiment to evaluate the lateralization of males during courtship and mating, and reported that most (41%) *C. ferrugineus* males showed left-biased approach (turning 180º to their left) towards females; whereas, 34%, 14% and 11% approached females in right-side, back side, and front side, respectively. Moreover, they also revealed that the left-biased males showed a shorter duration of mate recognition and chasing as well as lower copulation attempt duration, with high successful mating attempts compared to right-biased males. The male follows the female by nudging the tip of female's abdomen with the male's head. Once the female stops, the male strokes the female elytra with its antenna. The male continues its efforts to succeed by crawling on the back of the female and turning. Only the flickering of female's antenna was reported during the process. Once the male and female are coupled, the first copulation was observed to last for 105 min, followed by separation for 20 min. Then, the second and third copulations were observed for 35 and 95 min, respectively. During coition, the male and female *C. ferrugineus* are firmly attached since the aedeagus is inserted into the genital tract of the female deeply (Rilett, 1949).

2.1.5.4 *Chemical ecology*

Pheromones

Pheromones are chemical substances produced by insects with the intention to affect the behaviour of other individuals of the same species. *Cryptolestes ferrugineus* males produce pheromones namely (E, E)-4,8-dimethyl-4,8-decadien-10-olide (ferrulactone I) and (3Z,11S)-3-dodecen-11-olide (ferrulactone II) (Borden et al., 1979). Researchers have shown the possibility of isolating the pheromones from *C. ferrugineus* (Wong et al., 1983). The naturally produced ratio of ferrulactone I to ferrulactone II by *C. ferrugineus* was reported to be 1.6:1.0 (Lindgren et al., 1985). Borden et al. (1979) reported that *C. ferrugineus* adults of mixed sex and age responded to the odour of mixed-sex adults, frass, pentane extracts of frass and Porapak Q-captured volatiles from adults or frass. The pheromones are produced in the alimentary canal and/ or the Malpighian tubules. Moreover, they also observed responses from both the sexes to the volatiles from males. Similarly, Currie et al. (2020) reported that in the absence of air currents and food for feeding, male and female *C. ferrugineus* were attracted toward a single male in an apparatus of 10 cm length; when the grain was present, a single male was not enough to attract *C. ferrugineus*. However, a significant number of females were found attracted to 50 males.

Several researchers have shown different ways to synthesize the aggregation pheromones (Oehlschlager et al., 1983). For instance, Sakai and Mori (1986) synthesized the aggregation pheromones ferrulactone I and II with the help of (2E, 6E)-farnesol and ethyl (S)-3-hydroxybutanoate, respectively. On the other hand, Cheskis et al. (1993) and Czeskis et al. (1993) synthesized ferrulactone II using 2-carboxyethyltriphenylphosphonium bromide; whereas, Keinan et al. (1991) synthesized ferrulactone II using *Thermoanaerobium brockii* alcohol dehydrogenase (TBADH)-generated bifunctional chiron. According to Oehlschlager et al. (1987), the aggregation pheromones produced by *C. ferrugineus* (ferrulactone I and II) can act alone as well synergistically. Moreover, *C. ferrugineus* species is not cross-attracted to the pheromones produced by other species such as *Oryzaephilus Mercator*, *O. surinamensis*, *C. turcicus*, and *C. pusillus* (Schonherr) (Oehlschlager et al., 1987). Chambers et al. (1990) analyzed the electroantennogram (EAG) responses of the males and females of *C. ferrugineus* and reported that females produced EAG with higher amplitude. Thus, the greater response of females to the pheromones produced by males implies the importance of pheromones in mate identification and courtship. While determining the flight activity of *C. ferrugineus* in farms in south-eastern Australia, Holloway et al. (2018) reported that more females were trapped in traps with pheromone (female: male ratio of 3:1); whereas in passive trapping, the female: male ratio of the *C. ferrugineus* caught were 1:1. Similarly, during a seasonal flight activity study in the grain storage sites in South Carolina, USA, Throne and Cline (1994) observed more *C. ferrugineus* females at all the tested sites. These results further confirm the higher attraction of females towards the pheromone.

2.1.5.5 *Heat production*

Cryptolestes ferrugineus multiplication is associated with grain heating; however, at very low density (less than 5 adults/ kg), they cannot initiate heating (Smith, 1978). Smith (1983) performed an experiment and concluded that the increase in moisture content of wheat facilitated the heating of grain as well as a rapid increase in *C. ferrugineus* population. Thus, high multiplication of *C. ferrugineus* is a consequence of grain heating and not the cause.

On evaluating the effects of temperature, moisture content, level of wheat breakage, insect density, age and the stage of *C. ferrugineus* on the heat production, Cofie-Agblor et al. (1996a, b) reported the following conclusions: 1) Exponential increase in heat production by adults with increase in temperature from 15 to 35ºC; 2) Increase in heat production with increase in moisture constant; however, the rate of increase from 15 to 18% was lower than that from 12 to 15%; 3) Increase in heat production with increase in level of wheat breakage; however, the rate of increase from 10 to 20% breakage was lower than that from 0 to 10%; 4) Heat production of adults and larvae were in the range of 0.72 to 21.47 µW/ insect and 0.37 to 17.53 µW/ larvae, respectively, at the tested conditions (temperatures: 15, 20, 25, 30 and 35ºC; moisture content: 12, 15 and 18% wb; 4 week old adults and second, third and fourth instar larvae); 5) maximum rate of heat production was observed in adults of the age of 4 weeks old; 6) Heat production of adults varied with insect density.

2.1.5.6 *Movement and distribution inside the grain*

The movement of insects inside the grain can be random (non-directional) or biased (non-random or directional). Under uniform environmental conditions, insects tend to wander inside the grain randomly and reach biologically suitable locations. On the other hand, individual insects move in a non-random direction in search of food, refuge, mating partner or to escape predators due to non-uniform environmental conditions in stored grain structures (Jian et al., 2009a). The biased movement is also influenced by pheromones, host stimuli, the presence of other organisms such as parasitoids and predators, and other physical stimuli such as temperature, moisture content, gas concentration, dockage, foreign materials, light, and radiation (Jian, 2003). More detailed information on the factors influencing the movement and distribution of *C. ferrugineus* is available elsewhere (Jian and Jayas, 2009). The current section focuses mainly on the movement and distribution of *C. ferrugineus* because of physical stimuli.

The one-, two- and three-dimensional movement of *C. ferrugineus* have been extensively studied at various environmental conditions in the laboratory (Jian et al., 2002, 2004a, b, 2005a; Bharathi et al., 2021a, 2022). *Cryptolestes ferrugineus* adults prefer warmer grain than their surrounding cooler grain, in the absence of other factors and the adults could detect the temperature gradient in less than 1 h (Flinn and Hagstrum, 1998; Jian et al., 2004b). Moreover, the movement pattern of male and female adults did not vary significantly in the presence of temperature gradients (Jian et al., 2004a), whereas 1 day old adults were observed to be slower in response to temperature gradients than older adults (Jian et al., 2005c). On comparing the temperatures of spontaneous walking stops (SM), CCT (nil movement after shaken) and minimum movement (TM) of stored product insects including *C. ferrugineus, T. castaneum, S. oryzae* and *S. zeamais,* (Jian et al., 2015) reported that *C. ferrugineus* showed the lowest SM (4ºC), CCT (2ºC) and TM (6.3 to 8.9ºC) temperatures. Moreover, the rate of temperature decrease also influences the TM temperature of *C. ferrugineus* (Jian et al., 2015). The two-dimensional diffusivity of *C. ferrugineus* was reported to increase with an increase in temperature and insect number as well as a decrease in moisture content and movement period (Jian et al., 2007). White et al. (1993a) reported that *C. ferrugineus* adults prefer to move towards elevated carbon dioxide levels; whereas, prolonged exposure to higher levels of carbon dioxide is lethal to the insects. Adult *C. ferrugineus* prefer damp and mould infected grain rather than dry grain since damp grain is soft and easy to oviposit into. Moreover, *C. ferrugineus* also feeds on the mould itself (Surtees, 1965). At insect density higher than two adults per kilogram of wheat, *C. ferrugineus* adults tend to disperse inside the grain; whereas, at lower insect density, they tend to aggregate (Jian et al., 2004b). Similarly, while determining the spatial and temporal distributions of *C. ferrugineus* adults inside a grain bin containing 1.5 t of wheat, Jian et al. (2011) reported that the level of aggregation decreased with increase in insect density. This is because at lower density, aggregation would increase their possibility of meeting mating partners.

Due to its smaller size, the movement speed of *C. ferrugineus* is faster than larger stored grain insects such as *Rhyzopertha dominica* (Flinn and Hagstrum, 2011). The movement of adults in the vertical direction (speed greater than 10.8 m/d) was faster compared to horizontal direction (maximum speed of 6 m/d) at 14.5% moisture wheat (Jian et al., 2004b). Jian et al. (2006) reported that the speed of *C. ferrugineus* movement varies with the moisture content of the wheat due to its hydrophilic behaviour. *Cryptolestes ferrugineus* adults showed faster downward movement in dry corn (13.5% moisture content) than in corn with 15.5% and 17.5% moisture content. Despite their preference to move downward, more *C. ferrugineus* adults were reported to be present at the top or middle areas of a grain bulk when the moisture content of the grain at those areas were comparatively higher than the bottom areas (Loschiavo, 1983).

Initially after introduction adult *C. ferrugineus* prefer to move downward, due to their small size, drift effect and preference, at uniform environmental conditions (Jian et al., 2009a; Bharathi et al., 2022, 2023). Then, *C. ferrugineus* adults move gradually upward in the presence of biologically suitable conditions at the upper portion of the grain and distribute inside the grain mass depending on the grain condition (Jian et al., 2004b; Bharathi et al., 2023). Bharathi et al. (2023) reported that the activity of *C. ferrugineus* inside a 300 t wheat grain bin is reduced near the boundary when the temperature dropped during Winter (especially when temperature dropped below 2.5ºC and resumed activity when temperature increased above 4.5ºC).

In the presence of multiple factors, one factor typically becomes predominant. For instance, in the presence of temperature gradients and moisture differences, grain temperature was found to be the predominant factor in the presence of lower moisture differences (14.5% and 16.5%); whereas, the grain moisture content was the predominant factor in the presence of higher moisture differences (12.5% and 16.5%) (Jian et al., 2005a). In a horizontal column experiment in the presence of carbon dioxide and moisture gradients, carbon dioxide level was observed to be the predominant factor over the grain moisture content (Parde et al., 2004). Bharathi et al. (2023) observed the movement and distribution of *C. ferrugineus* inside a grain bin filled with 300 t of wheat for 26 months in Winnipeg, Canada. They reported that *C. ferrugineus* inside the grain bin followed a movement and distribution pattern similar to those reported in the laboratory experiments under similar environmental conditions.

Briefly, temperature gradients and moisture differences are the predominant factors that influence the movement and distribution of *C. ferrugineus*; whereas, the presence of mould, type of food, dockage, intergranular grain spaces and ventilation are trivial factors that influence the movement and distribution of *C. ferrugineus* adults (Jian and Jayas, 2009). Based on limited research, carbon dioxide gradient also seems to be a significant factor (White et al., 1993a) but more research is required on the movement of insects under carbon dioxide gradients. Thus, the behaviour of the *C. ferrugineus* is the result of exploration for a location that is biologically suitable and physically comfortable for their survival, growth, and multiplication.

2.1.6 Interaction with other organisms

2.1.6.1 *Interspecific interaction*

Tribolium castaneum: Hulasare et al. (2003) reared *C. ferrugineus* and *T. castaneum* alone and in combination at three different insect densities (250, 500, and 1000 adults/ kg of wheat, referred to as low, medium, and high densities, respectively) at two different moisture contents (12% and 15%) of wheat. For the combination case, the insect densities of each species were 250, 500 and 1000 adults/ kg for low, medium, and high densities, respectively. They observed significantly lower *C. ferrugineus* adult population, when reared alone, as compared to that of the combination, at all the tested densities, at 12% moisture content. They hypothesized that, the smaller-sized first instar larvae of *C. ferrugineus* could have had difficulty in penetrating the wheat germ in dry grain; whereas, in combination, *T. castaneum* could have damaged the grain and made easier for *C. ferrugineus* larvae to enter and feed. On the other hand, in damp grain (15% moisture content), the larvae would have penetrated easily, and the adults would have fed well and as a result, in 15% moisture grain, *C. ferrugineus* reared alone had a higher adult population than those in combination. Suresh et al. (2001) observed the cannibalistic behaviour of both the species and concluded that *T. castaneum* adults (weigh about 2 mg) are more effective cannibals than *C. ferrugineus* (weigh about 0.2 mg) due to their larger size. Nevertheless, the immature stage of *C. ferrugineus* stay hidden under the seedcoat and it is difficult for *T. castaneum* to discover them and prey on them; whereas, *C. ferrugineus* preys effectively on the exposed stages of *T. castaneum* such as eggs, larvae and pupae (Suresh et al., 2001). Overall, the presence of *C. ferrugineus* restricts the survival of *T. castaneum* in wheat and wheat feed. However, these interaction might differ in the presence of ample food (Lefkovitch, 1968). A similar finding was reported by Nansen et al. (2009), who analyzed 129 grain silos in Kansas from 1999 to 2001 and reported that the presence of *C. ferrugineus* reduces the population of *T. castaneum*, while the presence of *T. castaneum* did not affect *C. ferrugineus*.

Cryptolestes turcicus: Lefkovitch and Milnes (1963) studied the interaction of *C. turcicus* and *C. ferrugineus* and reported that the *C. turcicus* adult survival in the presence of *C. ferrugineus* depends on the initial number of *C. turcicus*; while, the survival of *C. ferrugineus* depends majorly on the environment. In the presence of *C. turcicus* larvae at higher density, *C. ferrugineus* delay the induction of pupation and in some cases, the delay in pupation could lead to loss in its pupation ability. Moreover, the metamorphosing stage of *C. ferrugineus* is susceptible to cannibalism since their cocoon contains little silk and are fragile; whereas, *C. turcicus* forms a tough silk cocoon and are protected inside them (Lefkovitch and Milnes, 1963).

Cryptolestes pusillus: The lactones produced by *C. ferrugineus* were reported to attract *C. pusillus* (Chambers et al., 1990). When an equal number of *C. ferrugineus* and *C. pusillus* were introduced on wheat and maize at different temperatures, White et al. (1995) reported that *C. ferrugineus* multiplied better on warmer temperatures (30 and 35ºC on wheat and 35ºC on maize); whereas, *C. pusillus* multiplied better on colder temperature (20ºC).

Sitophilus oryzae (L.): The presence of *C. ferrugineus* in wheat inhibited the growth of *S. oryzae* (Lefkovitch, 1968).

Lasioderma serricorne (F.): The presence of *C. ferrugineus* restricted the survival of *L. serricorne* in limited wheat feed. On the contrary, the presence of *L. serricorne* in wheat encouraged the growth of *C. ferrugineus* since *L. serricorne* is a primary pest and *C. ferrugineus* is a secondary pest and so the damage to wheat kernels by *L. serricorne* could have led to easy access to food for *C. ferrugineus* (Lefkovitch, 1968).

Rhyzoperthe dominica (F.): The presence of *C. ferrugineus* decreases *R. dominica* infestation; while, the presence of *R. dominica* does not affect *C. ferrugineus* population (Nansen et al., 2009).

2.1.6.2 *Wasps*

Several wasps have been identified as parasitoids for *C. ferrugineus* and are used as biological control agents. *Cephalonomia waterstoni* (Gahan) has been identified as one of the best biological control agents against *C. ferrugineus*. They are advantageous because they effectively follow the kairomonal trail inside the grain (Howard and Flinn, 1990), have ability to feed on all the instars, have generation time half that of the host and are extremely host-specific. Adult females *Cephalonomia waterstoni* paralyze, feed and oviposite on their hosts. *Cryptolestes ferrugineus* larvae paralyzed by *C. waterstoni* cannot advance into the next developmental stage and as a result, those larvae are available as oviposition sites for a minimum of 2 weeks (Flinn, 1991). Flinn and Hagstrum (1995) developed a model to predict the phenology of *C. waterstoni* and *C. ferrugineus* in relation to the grain temperature and reported that the effect of the parasitoid on the host is the highest when released during the first production of fourth instar larvae. The combined application of an insecticide and a parasitic wasp could result in effective control of *C. ferrugineus*. For instance, Flinn et al. (2006) reported that the combined application of transgenic avidin maize powder and the parasitoid wasp, *Theocolax elegans* (Westwood) drastically reduced the population of *C. ferrugineus*, compared to *T. elegans* alone, in maize.

2.1.6.3 *Mites*

Mites such as *Acarophenax lacunatus* (Cross & Krantz) (Oliveira et al., 2007) and *Cheyletus eruditus* (Schrank) (Barker, 1991) have been identified as biological control agents of *C. ferrugineus*. *Acarophenax lacunatus* was observed to parasitize the eggs and reduce the larval population of *C. ferrugineus*. Thus, these parasitic mites were able to successfully reduce the instantaneous rate of *C. ferrugineus* increase (Oliveira et al., 2003).

2.1.6.4 *Fungi*

Cryptolestes ferrugineus feeds on certain fungal species as supplementary or alternative food source. Nevertheless, in the absence of grain, *C. ferrugineus* feeds mainly on fungi. Sinha (1965) reported that *C. ferrugineus* completed its development on 10 species of fungi (*Absidia orchidis* (Vuill.) Hagem, *Alternaria tenuis* sensu Wiltshire, *Curvularia tetramera* (McKinney) Boedijn, *Fusarium moniliforme* Sheld, *Helminthosporium sativum* P., K & B., *Mucor sphaerosporus* Hagem, *Nigrospora sphaerica* (Sacc.) Mason, *Penicillium cyclopium* Westl., *Stemphylium botryosum* Wallr. and *Trichothecium roseum* Lk.,) among 23 species tested. The shortest and longest developmental periods were about 22 and 34 days, respectively, on *T. roseum* and *F. moniliforme*. Similarly, Loschiavo and Sinha (1966) studied the oviposition, feeding, and aggregation of *C. ferrugineus* in the presence of different species of seed-borne fungi and revealed that *N. sphaerica*, *M. sphaerosporus*, *Hormodendrum cladosporiodes* (Fres.) Sacc. and *C. tetramera* were the most suitable fungi for oviposition and feeding. The differences in responses of *C. ferrugineus* were observed for different species from the same genus. For instance, *P. terrestre* was not suitable for oviposition and feeding; whereas, *P. cyclopium* and *P. funiculosum* were moderately suitable. On the other hand, *C. ferrugineus* were observed to feed moderately on *Aspergillus flavus*; and did not lay eggs, but were observed to lay few eggs and feed slightly on *A. fumigatus*. Aggregation of *C. ferrugineus* was observed on the grain kernels containing mycelia and spores of *N. sphaerica* (Loschiavo and Sinha, 1966).

Entomopathogenic fungi such as *Beauveria varroae*, *B. bassiana* and *Purpureocillium lilacinum* were found to be associated with *C. ferrugineus* in wheat and maize samples in Central and South Anatolia in Turkey (Er et al., 2016). Similarly, Wakil et al. (2014) explored the naturally occurring entomopathogenic fungi infecting stored grain insects in Punjab, Pakistan and reported the fungal species such as *Alternaria alternata*, *A. solani*, *Aspergillus flavus*, *A. fumigatus*, *A. parasiticus*, *A. niger*, *Bipolaris oryzae*, *C. lunata*, *Fusarium oxysporium*, *Helminthosporium oryzae*, *P. capsulatum*, *P. chrysogenum*, *Phomopsis* sp. and *Rhizopus stolonifers.* Among the insect species tested, *C. ferrugineus* (0.1% occurrence) was less affected by entomopathogenic fungi than *Tribolium castaneum* (Herbst) (0.3% occurence) and *Sitophilus oryzae* (L.) (0.2% occurence). However, *C. ferrugineus* eggs are resistant to *B. bassiana* infection (Lord, 2009). In 1988, Dr. Isabelle I. Tavares identified an undescribed fungal parasite species in the genus *Dimeromyces* (Ascomycetes: Laboulbeniales) on the last visible abdominal segment near the ovipositor base of *C. ferrugineus* (Throne, 1989). Lord et al. (2010) reported that *Nosema oryzaephili* microsporidia at 10^6 spores/g of diet resulted in about 99% infection on *C. ferrugineus* larvae after three weeks of exposure.

2.1.6.5 *Bacteria*

Ünal and Koçak (2019) reported the association of endosymbionts such as *Wolbachia*, *Rickettsia* and *Spiroplasma* with *C. ferrugineus*.

2.1.6.6 *Protists*

Mattesia oryzaephili and *M. dispora* were reported to be one of the pathogens that infect *C. ferrugineus*. Lord (2003) reported that at the dose rate of 10^5 oocysts/g of diet, the mortality and infection rate of fourth instar of *C. ferrugineus* were higher with *M. oryzaepili* than *M. dispora*. Thus, the presence of these two *Mattesia* species could lead to a decline *C. ferrugineus*.

2.1.7 Models developed

Over the years, several attempts have been made to develop mathematical models, focusing primarily on the population dynamics and movement behaviour of *C. ferrugineus*. Campbell and Sinha (1990) developed a computer simulation model for *C. ferrugineus* population dynamics and its bioenergetics, which was reported to provide the predicted number of insect life stages and bioenergetic variables similar to those of the observed values during *C. ferrugineus* population growth phase. Woods et al. (1997) used a population dynamics model to simulate the potential number of *C. ferrugineus* generations in various regions in Canada based on yearly harvest and weather data from 1952 to 1990 and reported that the harvest temperature and the date could facilitate prediction of regions with potentially higher *C. ferrugineus* population. Mani et al. (2001a) developed a *C. ferrugineus* - induced hot spot model by combining a) a heat transfer model (three-dimensional, finite element), b) population dynamics model, c) heat production model, and d) *C. ferrugineus* movement model, and predicted the development of hot spot. Mani et al. (2001b) compared the population dynamics of *C. ferrugineus* predicted by the hot spot model (with feedback from the insect model to the temperature model) and a spatial model (without feedback) based on the conditions in Winnipeg, Canada and Topeka, Kansas, and concluded that the predictions of hot spot model were better compared to realistic scenario than the spatial model, since the hot spot model considers the effects of insect movement and heat production as well as the variable heating around the bin wall. Flinn et al. (1992) developed a spatial model by coupling population dynamics model and two-dimensional bin temperature model and validated the model using the field data from a bin situated in Kansas, USA. Similarly, Jian et al. (2004c) coupled the insect distribution model and the temperature model and predicted the distribution of insects inside a bin using the 1990 weather data of Winnipeg, Canada. The model predicted that a higher percentage of adults remain at the centre of the bin when high-temperature gradients and low temperature in the boundary exist. Jian et al. (2007a) developed a time-varying distributed-delay model to predict the survival rate of *C. ferrugineus* adults at constant or transient temperatures at varying relative humidities. The model also considered the adult acclimation at different temperatures and moisture fluctuations inside the stored grain bin. They compared the predicted results with the experimental data from two granaries for 4 months and observed no significant difference. Jian et al. (2008) reported that the population redistribution of *C. ferrugineus* adults could be modeled and solved by the diffusion equation and finite difference method, respectively. Jian et al. (2007b) calculated the two-dimensional diffusivity and predicted the adult number at different locations inside wheat. They concluded that *C. ferrugineus* distribution follows a diffusion pattern, under constant environmental conditions in stored wheat. Jian (2021) developed a basic model structure to correlate the biological age of *C. ferrugineus* with its oviposition, ageing and mortality and concluded that the predicted data were consistent with the measured data in the literature.

2.1.8 Concluding remarks from the literature review

A large number of studies performed by researchers around the world on the ecology and behaviour of *C. ferrugineus* demonstrates the global importance of this species. Mobility is one of the crucial behaviours of *C. ferrugineus*, that increases their likelihood of finding partners and suitable locations inside the stored grain, to facilitate growth and multiplication. Understanding the movement and distribution of *C. ferrugineus* inside the stored grain ecosystem is crucial to reduce the stored grain losses that occur due to *C. ferrugineus* infestation. Even though, the movement and distribution pattern of *C. ferrugineus* in 1D and 2D has been studied extensively, a knowledge gap exists on the movement and distribution of *C. ferrugineus* in 3D. Thus, the current work aimed to understand the 3D movement and distribution of *C. ferrugineus* in the laboratory as well as commercial scale grain bin. In addition, the diffusivity of *C. ferrugineus* in 3D has been calculated using the experimental data collected from the laboratory studies. Moreover, the proposed experimental method and the analytical solution could be extended for other stored product insects.

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Chapter 3. Three-dimensional movement and distribution of *C. ferrugineus*

3.1 Three-dimensional movement and distribution of *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) adults in stored wheat under constant temperatures and moisture contents

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3.1.1 Abstract

Understanding the movement and distribution of insects inside a grain bin would help to develop an effective stored grain management protocol. The three-dimensional movement and distribution of adult *Cryptolestes ferrugineus* (Stephens) at 20 and 30°C were determined in a 0.7 x 0.7 x 0.7 m^3 (internal dimensions) wooden box filled with wheat of uniform moisture contents (12.5 ± 0.1%, 14.5 ± 0.1% and 16.5 ± 0.1% wet basis). The wheat at a constant moisture content was placed into 343 mesh cubes (0.1 ×0.1 ×0.1 m) and placed inside the wooden box. The center mesh cube in the box had one hundred adult insects introduced at the beginning of the experiment. After 24 h, the 343 mesh cubes were removed from the wooden box in less than 45 min. Finally, the contents of each mesh cube were sieved, and the insects counted. Each experiment was replicated three times. A maximum of 17% of insects stayed in the initial cube (center of the wooden box). About 50 to 88% of the introduced adults moved downward from the introduction location across all the studied temperatures and moisture contents. This study showed that *C. ferrugineus* movements over 24 h in three dimensions follow a diffusion pattern in the horizontal direction and move downward due to the “drift” effect and geotaxis in the vertical direction.

Keywords: Stored grain, Movement and distribution, Uniform temperature, Uniform moisture content

3.1.2 Introduction

Mobility is one of the salient features for most living organisms. In general, stored-grain insects, being an integral part of a stored-grain ecosystem, move inside stored grain bins in search of biologically and physically suitable locations for their growth and multiplication (White, 1995). Various abiotic factors (grain temperature and moisture content) and biotic factors (density of the insects, presence of other species of insects, mold, fungi, predators and/or parasitoids) associated with the stored grain ecosystem influence their movement and distribution (Loschiavo, 1983; Flinn and Hagstrum, 1995; Jian, 2003). Hence, a complete understanding of their movement and distribution is essential in developing a proper management protocol.

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), is the most prevailing insect, infesting stored grains worldwide (Howe and Lefkovitch, 1957; Sinha and Watters, 1985; Prakash et al., 2003; Canadian Grain Commission, 2019). Though the adults of *C. ferrugineus* can survive relatively dry conditions (Surtees, 1963), they prefer warmer and moist areas over cooler and drier regions inside grain bulks (Flinn and Hagstrum, 1998; Jian et al., 2005a). In the past five decades, several researchers have demonstrated that the movement and distribution of *C. ferrugineus* adults inside stored grain bulks are affected by factors including but not limited to temperature (Jian et al., 2007), moisture content (Loschiavo, 1983; Jian et al., 2007) and their gradients (Flinn and Hagstrum, 1998; Jian et al., 2002, 2003, 2005a). Yet, how the adults respond to the change in moisture content and temperature of the grain in three dimensions remains an enigma.

Precisely, Jian et al. (2004a) developed a one-dimensional (1D) grain column and studied the movement of *C. ferrugineus* adults at various environmental conditions (linear and dynamic temperature gradients and different percentages of grain dockage) at specific insect densities (2, 12, 24 and 48 adult per kilogram (kg) of wheat) for a variety of time periods (12, 24, 72 and 144 h). Moreover, the developed grain column extends the study of insect movement to a wide range of temperature and moisture gradients (Jian, 2003; Jian et al., 2004a, 2005a, 2005b). However, this design provides insight into insect movement only in one dimension at a given time. Hence, a two-dimensional (2D) grain chamber was developed, which facilitates bi-directional temperature gradients instead of the unidirectional gradients of the 1D column (Jian et al., 2004b). The 2D chamber design allowed the 2D movement of *C. ferrugineus* adults to be studied. The findings of that study agreed with the observations of the 1D column. A slight modification enabled a study to determine the diffusivity of *C. ferrugineus* inside the wheat during constant environmental conditions (Jian et al., 2007). The first 2D chamber considers the horizontal and vertical (gravity) directions, while the latter only considers horizontal directions. The study results revealed that *C. ferrugineus* follows a diffusion pattern at constant temperatures and moisture contents in 2D.

To study the three-dimensional (3D) movement of insects, Surtees (1964a) proposed a method to analyze the 3D dispersion pattern that involved arranging 64 3-in (7.62 cm) cube bags (made of mesh having 10 openings per inch) to hold the tested grain. After placing the 64 bags (16 cubes each in 4 layers) inside a transparent box, insects were introduced at the top surface of the cube and the cube bags were dismantled after the desired amount of time. However, this method limits the complete understanding of the insect behaviour as the insects were introduced only at the top surface, restricting their movement to a downward direction only. Also, the grain used was only about 25 kg, and hence, longer distance movement behaviours could not be revealed. In addition, the construction of cube bags was time-consuming and causing a lack of repetition because it was difficult to form the bags into cubes manually and make the same cube repeatedly. Hence, to address this drawback, Bharathi et al. (2021) developed a design that used metal rod cubes covered with mesh screens and demonstrated that the developed mesh cubes did not influence *C. ferrugineus* movements inside the grain bulk.

While extensive studies of 1D (Jian, 2003; Jian et al., 2004a, 2005a, 2005b) and 2D (Jian et al., 2004b, 2007) movement of *C. ferrugineus* are available, a comprehensive study of the 3D movement of *C. ferrugineus* in a large grain bulk is needed to understand the movement of insects in a real-time scenario. Therefore, the present study aimed to investigate the movement pattern of *C. ferrugineus* adults at uniform environmental conditions (different constant temperatures and moisture contents) in a 3D setup.

3.1.3 Materials and methods

3.1.3.1 *Wheat*

The Canada Western Red Spring Wheat (Grade No.1, cv. AC Barrie, certified), used in this study, was received from Cargill Ltd., Winnipeg, Manitoba. A three-dimensional shaker (Vibro-Energy®

Separators, SWEKCO, Florence, KY, USA) was used to clean the approximate 1500 kg of wheat to remove dockage and fine materials. The moisture content of the wheat was adjusted by adding the desired amount of distilled water in a rotating drum to obtain the desired moisture contents (12.5 ± 0.1%, 14.5 ± 0.1% and 16.5 ± 0.1%, wet basis). This work used a standard oven-drying method to determine the moisture content of the wheat by drying about 10 g of samples, in triplicate, at 130°C for 19 h (ASABE, 2016). The conditioned grain was placed in double-layered plastic bags, secured and maintained at 10°C, for at least 2 wk, and the moisture content was measured to verify that the desired moisture content was reached. Before each experiment, the wheat samples were kept at -15°C for at least 3 wk to ensure the wheat was free of any arthropods (Fields and White, 1997). The moisture content of the wheat samples was determined before each insect movement test.

3.1.3.2 *Experimental setup*

The mesh cubes (size 0.1 ×0.1 ×0.1 m^3) used in this study were described by Bharathi et al. (2021). Briefly, the edges of a cube were made of 12 metal rods of 1.6 mm diameter. These 12 rods were fastened and connected using eight polylactic acid corners, fabricated using a 3D printer (Cartesio W, MaukCC, Maastricht, Netherlands). The metal rod cube was enclosed with a clothing screen with 1.4 × 1.4 mm^2 openings. This mesh facilitated the free movement of insect adults but not wheat (Bharathi et al. 2021). A total of 343 mesh cubes were made, filled with wheat (≈ 285 kg total) of the desired moisture content and located inside a wooden box with 1.1 cm sheet and external framing along the four walls using 3.7 cm by 8.2 cm boards (Figure 3.1). The mesh cube construction arranged the 343 mesh cubes into a large cube of 0.7 × 0.7 × 0.7 m^3 (Figure 3.2) inside the slightly larger interior of the wooden box (Figure 3.2). It took about 2 to 3 h to fill the cubes and complete the arrangement. A lid covered the wooden box during the entire insect movement period. Double-sided tape sealed the top edges of the wooden box to prevent insects from escaping. No insects were found on the surface of the tape during the entire study.

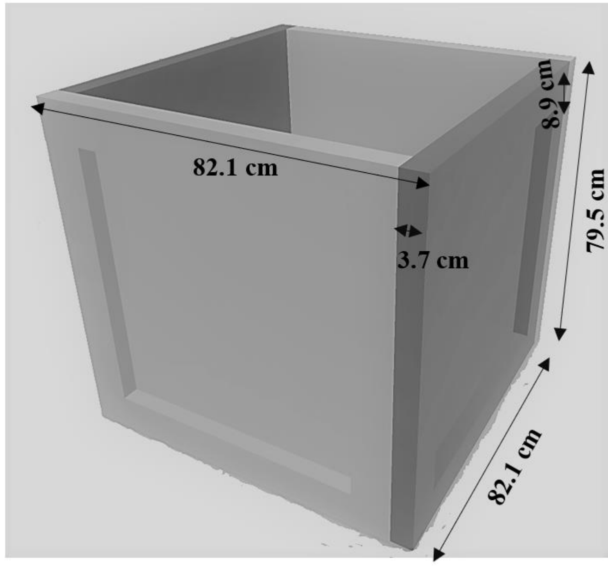


Figure 3.1. The wooden box used for testing insect movement. It has an interior of 72.5 x 72.5 x 78.4 cm to enable placement of cubes. The lid, bottom and interiors are 1.1 cm thick plywood. The four side panels are framed on the exterior with 8.2 cm x 3.7 cm wood boards. The lid fits exactly into the top and the bottom matches the outer dimensions of the exterior framing.

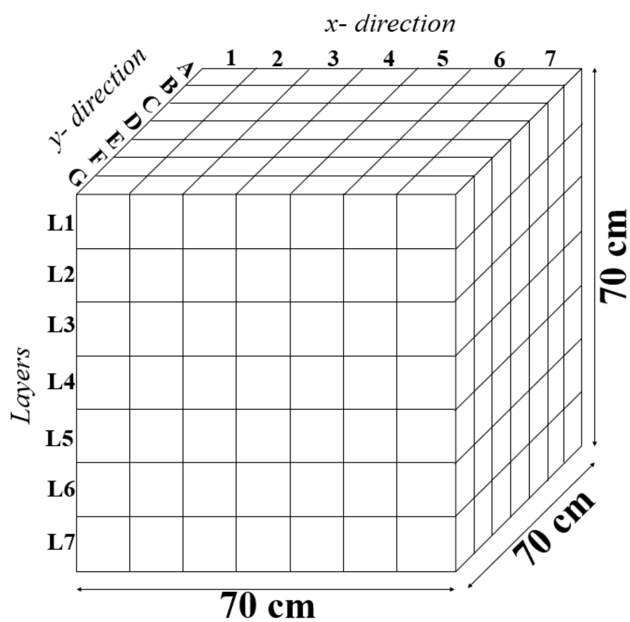


Figure 3.2. Numeration of mesh cubes in the assembled 3D wheat. In the graph, each 10×10×10 cm3 small cube is a mesh cube. Insects were introduced at the center of cube, designated as L4-D-4 in the schematic.

3.1.3.3 *Insects*

Cryptolestes ferrugineus were reared at 30 ± 1°C and 75 ± 5% relative humidity (r.h.) on whole wheat kernels, cracked wheat, and wheat germ (90:5:5 on weight ratio), in the dark. About 100 adults of mixed sex, with age 1 d to 2 mo old at the start of the experiment, were sieved out of the culture jar. The sieved adults were held inside one of the prepared mesh cubes (size 0.1 ×0.1 ×0.1 m^3) containing about 0.83 kg wheat (the same wheat and moisture content as that of the tested wheat), which was then kept inside a closed plastic box (0.34 x 0.23 x 0.12 m^3) with a lid. This box, containing the wheat in a mesh cube, was placed in the environmental chamber (CMP 4030, Controlled Environments Limited, Winnipeg, Manitoba) for 24 h, at the experimental temperature and r.h., for acclimation purposes. Throughout the experiment, the insects were kept in the dark.

3.1.3.4 *Experimental procedure*

A total of six experiments were conducted with the combinations of three moisture contents (12.5, 14.5 and 16.5%) and two temperatures (20 and 30°C). Each experiment was replicated three times, and the insect movement period for each experiment was 24 h.

At least four days before the test, the prepared wheat (sealed inside double plastic bags) was warmed inside an environmental chamber set at the desired experimental temperature. After this warming, the mesh cubes filled with the warmed wheat (except for the mesh cube with the acclimated adults) were placed inside the wooden box. For convenience, the mesh cubes were numerated and marked based on their locations (Figure 3.2). For instance, “L1-A-1” represented the cube A-1 in layer 1, and the insect introduction cube (the mesh cube with the acclimated adults) was L4-D-4 (Figure 3.2). After 24 h acclimation, the cube containing the acclimated insects and wheat was carefully placed inside the wooden box at D-4 in layer 4 (i.e., the “insect introduction cube”). After this introduction, the D-4 cubes in layers 3, 2, and 1 were carefully placed on top of the introduction cube. Twenty-four hours later, the lid was removed, and each mesh cube was drawn out of the wooden box and placed inside a plastic bag with pre-marked locations, and then the bag was secured with a knot. The total dismantling time was about 30 to 45 min for each experiment. This entire operation was conducted inside the environmental chamber at the desired temperature.

The adults inside each cube were sieved out from the wheat using sieve no. 20 (opening 850 µm). The mean recovery rate of the introduced adults for all the experiments conducted was 93.72 ± 0.71% *(n = 18)* because some adults might hide inside wheat kernels and could not be sieved out (Jian et al., 2007, 2015). Therefore, the number of adults recovered from each cube was adjusted using the total adults recovered and the number of initially introduced adults in each replication, using the following formula (Jian et al., 2007):

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

Where 100 is the number of initially introduced adults.

3.1.3.5 *Statistical analysis*

To determine whether the adults prefer to move to selected layers of the setup and to determine if this preference varied with the change in environmental conditions, the adult numbers in each cube were normalized using Equation 3.1 and the sum of adults at each layer were calculated. The summations in each layer were used to conduct ANOVA, followed by the Tukey's test within different layers under each experimental condition and within each layer of different experiments.

To determine whether the insects prefer to move towards downward or upward direction, the number of insects in layers 1, 2 and 3 were multiplied with 30, 20 and 10, respectively and the obtained values were added, and these were considered as upward direction values. Similarly, the number of insects in layers 5, 6 and 7 were multiplied with 10, 20 and 30, respectively and the summation of the obtained values were considered as downward direction values. The upward and downward direction values were analyzed using Tukey's test for all the experimental conditions.

Each layer was categorized into three areas (Center, Middle, and Periphery areas, Figure 3.3) to determine whether the adults prefer the boundary or the center at the given experimental condition. The adult density in each area was calculated using Equation 3.2 and then used to conduct the Tukey's test for all seven layers.

$$\textbf{Adult density} = \frac{\textbf{Number of adults in the area}}{\textbf{Total wheat mass in the area}} \quad (3.2)$$

To answer the question: whether the adults have a similar movement and vertical distribution under different temperatures and grain moisture contents, two-sample location test and Empirical Distribution Function (EDF) statistic were conducted (Jian et al., 2002) using SAS (SAS® University Edition, SAS Institute, North Carolina, USA). The summations in each layer, obtained after normalizing each cube using Equation 3.1, were used for the two-sample location test and EDF statistics. The Wilcoxon test gives information on the variation in each layer, the median test provides the general movement direction of the introduced adults, and Kolmogorov-Smirnov test indicates the variation in the cumulative distribution in the wooden box in the vertical direction. The comparison was between two different moisture contents at the same temperature or two different temperatures at the same moisture content.

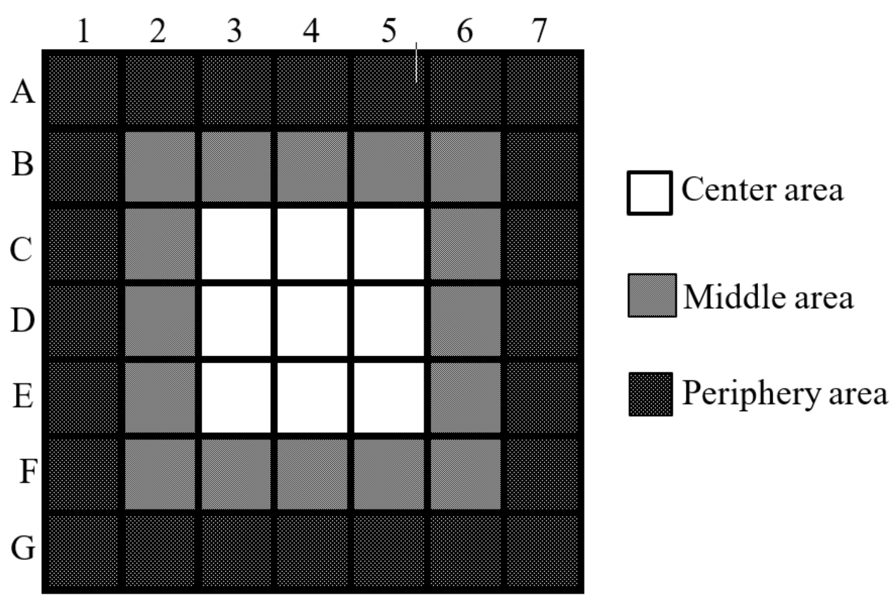


Figure 3.3. Numeration and categorization of the mesh cubes in each layer.

To understand the effects of temperatures, moisture contents and their interaction on the movement and distribution of insects, the number of insects in layers 1, 2, 3, 4, 5, 6 and 7 was multiplied with 30, 20, 10, 0, -10, -20 and -30, respectively. These numbers were the distance moved by the insects from the point of introduction in centimeters. The upward movement was considered positive, while the downward movement was considered negative. The obtained product values from each layer were summed to obtain a single value for each replicate. Then, Factorial test was conducted using the SAS software.

3.1.4 Results

3.1.4.1 *Adult movement direction*

a. Vertical distribution of adults in 3D setup

At 16.5% moisture content and 30°C, 88.1 ± 3.6% *C. ferrugineus* adults moved downwards from the point of introduction; on the other hand, at the same moisture content, only 50.9 ± 7.1% of adults moved downwards, at 20°C. Similarly, at 14.5% moisture content, 55 ± 4.4% and 79 ± 1.0% adults were found at the bottom half of the box, at 20 and 30°C, respectively. Contrarily, at 20°C, about 23.3 ± 3.2% and 25.5 ± 12.5% of insects had moved upward from the point of introduction, at 14.5 and 16.5% moisture contents, respectively. At 30°C and 14.5 and 16.5% moisture content, only 11 ± 2.7% and 5.2 ± 1.7% of insects had moved upward, respectively. At high moisture content (16.5%), more downward movements of insects were observed at higher temperature (30°C) than their corresponding lower temperature (20°C) (Figure 3.4). At all the tested experimental conditions (except at cooler (20°C) and moister (16.5%) condition), significantly higher insects were observed at the bottom-most layer (Layer 7), as compared to all the other layers (Table 3.1). Also, more than 50% of the *C. ferrugineus* adults had moved down after 24 h of introduction for all the tested experimental conditions. By comparison, less than 30% of the insects had moved into the top three layers of the box.

At 20°C, a decrease in the trend of insects moving downwards was observed with an increase in moisture content. While, at 30°C, significantly increasing trend of insects moving downwards was observed with an increase in moisture content (Figure 3.4). Briefly, at 20°C, with 12.5, 14.5 and 16.5% moisture content, the mean fraction of recovered insects from the bottom half of the box was about 64%, 55% and 50%, respectively. While at 30°C, with 12.5, 14.5, and 16.5% moisture content, the mean fraction of recovered *C. ferrugineus* adults found in the bottom half of the box was 58%, 79%, and 88%, respectively. Contrarily, at 20°C temperature and 12.5, 14.5 and 16.5% moisture contents, the top half of the box yielded about 23%, 23% and 25% of the recovered insects, respectively. At 30°C, as moisture content increased from 12.5% to 16.5%, a decreasing mean fraction of insects, 30%, 11% and 5% respectively, had moved upward.

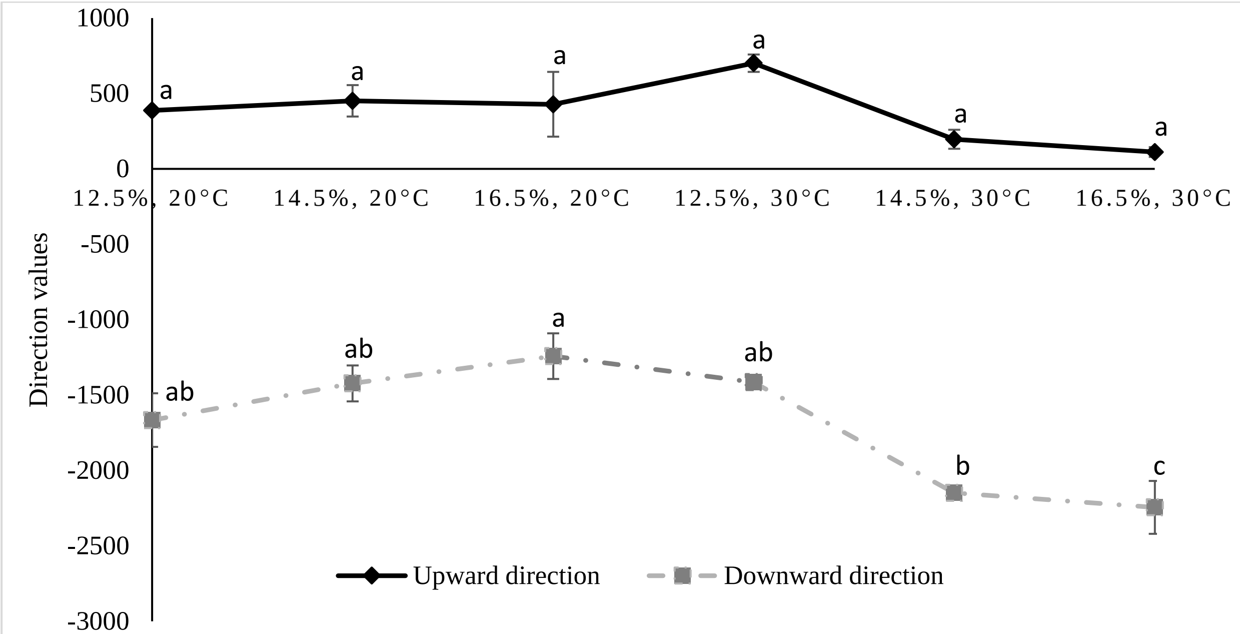


Figure 3.4. Movement of *Cryptolestes ferrugineus* adults in upward and downward directions from the point of introduction (center) at different environmental conditions (n = 3).

The first and the second numbers along the X axis are the moisture content (%) and temperature (°C), respectively.

[a, b, c] Different alphabets within the same line represent the significantly different mean values using Tukey's test at the level (α) 0.05. The direction value along the Y axis is the sum of the products of the insect number and the movement distance (cm).

Table 3.1. Mean insect number of adult *Cryptolestes ferrugineus* at each layer under different experimental conditions

Experimental conditions		Layers	Mean insect number at each layer
Temperature$^{\alpha}$ (°C)	Moisture content$^{\beta}$ (% wb)		
20	12.5	1	5.57 ± 0.57$^{a, A}$
		2	4.91 ± 0.61$^{a, A}$
		3	12.24 ± 0.34$^{a, A}$
		4	13.33 ± 3.10$^{a, A}$
		5	7.38 ± 1.28$^{a, A}$
		6	10.51 ± 1.05$^{a, A}$
		7	46.05 ± 4.13$^{b, A}$
	14.5	1	7.76 ± 1.87$^{a, AB}$
		2	6.34 ± 1.45$^{a, A}$
		3	9.17 ± 1.22$^{a, A}$
		4	21.74 ± 0.78$^{ab, A}$
		5	6.95 ± 1.45$^{a, A}$
		6	8.81 ± 1.64$^{a, A}$
		7	39.23 ± 3.06$^{b, A}$
	16.5	1	2.11 ± 0.50$^{a, A}$
		2	13.14 ± 4.40$^{ab, A}$
		3	10.24 ± 2.49$^{ab, A}$
		4	23.61 ± 3.32$^{ab, A}$
		5	10.11 ± 1.55$^{ab, A}$
		6	8.31 ± 1.67$^{ab, A}$
		7	32.49 ± 2.16$^{b, A}$
30	12.5	1	16.39 ± 1.24$^{a, B}$
		2	7.47 ± 0.48$^{ab, A}$
		3	5.98 ± 0.71$^{b, A}$

		4	$12.30 \pm 0.57^{ab, A}$
		5	$9.73 \pm 1.27^{ab, A}$
		6	$12.65 \pm 0.91^{ab, A}$
		7	$35.49 \pm 1.12^{c, A}$
	14.5	1	$2.41 \pm 0.43^{a, A}$
		2	$3.78 \pm 1.33^{a, A}$
		3	$4.84 \pm 0.87^{a, A}$
		4	$10.01 \pm 1.14^{a, A}$
		5	$3.45 \pm 0.59^{a, A}$
		6	$15.14 \pm 2.69^{a, A}$
		7	$60.37 \pm 2.14^{b, A}$
	16.5	1	$2.10 \pm 0.28^{a, A}$
		2	$1.75 \pm 0.32^{a, A}$
		3	$1.39 \pm 0.43^{a, A}$
		4	$6.64 \pm 1.35^{a, A}$
		5	$9.78 \pm 1.72^{a, A}$
		6	$20.33 \pm 0.78^{a, A}$
		7	$58.01 \pm 4.39^{b, A}$

$^{\alpha}$ Nominal grain temperature (°C).

$^{\beta}$ Nominal grain moisture content (%).

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

^{A,B} Different uppercase alphabets represent the significantly different mean values within layers of different experimental conditions, using Tukey's test at the level (α) 0.05.

The maximum observed difference in the mean number of insects aggregated at the insect introduction location was 16.5% moisture content at 20°C (17%) and 30°C (2%). This difference decreased with a decrease in moisture content. For the 14.5% moisture content cases, about 13% of insects were recovered at 20°C, while 6% of insects were recovered at 30°C. On the other hand, at 12.5% moisture content, about 9% and 4% of insects were recovered at 20 and 30°C, respectively. The mean insect number at the insect introduction layer was found to be in the range of 6% to 24%, over the tested conditions (Table 3.1).

Table 3.2. Insect density of adult *Cryptolestes ferrugineus* at different areas of each layer under different experimental conditions.

Layer	Experimental conditions		Area		
	Temperature [A] (°C)	Moisture content [B] (% wb)	Center	Middle	Periphery
1	20	12.5	0.28 ± 0.11[a]	0.18 ± 0.04[a]	0.05 ± 0.03[a]
		14.5	0.64 ± 0.40[a]	0.08 ± 0.04[a]	0.10 ± 0.08[a]
		16.5	0.05 ± 0.04[a]	0.03 ± 0.02[a]	0.07 ± 0.04[a]
	30	12.5	0.50 ± 0.25[a]	0.34 ± 0.04[a]	0.41 ± 0.06[a]
		14.5	0[a]	0.05 ± 0.02[a]	0.09 ± 0.05[a]
		16.5	0[a]	0.03 ± 0.02[ab]	0.09 ± 0.01[b]
2	20	12.5	0.52 ± 0.10[a]	0.08 ± 0.04[b]	0[b]
		14.5	0.45 ± 0.26[a]	0.08 ± 0.04[a]	0.09 ± 0.04[a]
		16.5	0.53 ± 0.38[a]	0.19 ± 0.10[a]	0.34 ± 0.18[a]
	30	12.5	0.20 ± 0.16[a]	0.11 ± 0.06[a]	0.23 ± 0.05[a]
		14.5	0.05 ± 0.04[a]	0[a]	0.17 ± 0.10[a]
		16.5	0[a]	0[a]	0.09 ± 0.03[b]
3	20	12.5	1.08 ± 0.14[a]	0.16 ± 0.07[b]	0.11 ± 0.05[b]
		14.5	0.84 ± 0.32[a]	0.11 ± 0.02[a]	0.08 ± 0.04[a]
		16.5	0.48 ± 0.34[a]	0.16 ± 0.07[a]	0.23 ± 0.08[a]
	30	12.5	0.15 ± 0.00[a]	0.03 ± 0.02[a]	0.23 ± 0.07[a]
		14.5	0[a]	0.08 ± 0.04[a]	0.19 ± 0.06[a]
		16.5	0.05 ± 0.04[a]	0.03 ± 0.02[a]	0.04 ± 0.03[a]
4	20	12.5	1.60 ± 0.68[a]	0.03 ± 0.02[a]	0.05 ± 0.03[a]
		14.5	2.13 ± 0.13[a]	0.11 ± 0.09[b]	0.22 ±0.07[b]
		16.5	2.56 ± 0.82[a]	0.08 ± 0.04[a]	0.18 ± 0.01[a]

	30	12.5	0.70 ± 0.10^{a}	0.08 ± 0.04^{b}	0.30 ± 0.04^{b}
		14.5	0.93 ± 0.32^{a}	0.03 ± 0.02^{a}	0.14 ± 0.03^{a}
		16.5	0.42 ± 0.13^{a}	0.03 ± 0.02^{a}	0.16 ± 0.08^{a}
5	20	12.5	0.90 ± 0.35^{a}	0.05 ± 0.04^{a}	0^{a}
		14.5	0.59 ± 0.42^{a}	0.05 ± 0.02^{a}	0.09 ± 0.04^{a}
		16.5	0.89 ± 0.50^{a}	0.03 ± 0.02^{a}	0.16 ± 0.05^{a}
	30	12.5	0.35 ± 0.15^{a}	0.09 ± 0.04^{a}	0.30 ± 0.14^{a}
		14.5	0^{a}	0^{a}	0.17 ± 0.05^{b}
		16.5	0.61 ± 0.27^{a}	0.11 ± 0.06^{a}	0.19 ± 0.04^{a}
6	20	12.5	1.08 ± 0.25^{a}	0.13 ± 0.04^{b}	0.04 ± 0.01^{b}
		14.5	0.63 ± 0.46^{a}	0.05 ± 0.04^{a}	0.17 ± 0.10^{a}
		16.5	0.46 ± 0.23^{a}	0.05 ± 0.04^{a}	0.21 ± 0.04^{a}
	30	12.5	0.45 ± 0.19^{a}	0.11 ± 0.05^{a}	0.40 ± 0.02^{a}
		14.5	0.42 ± 0.24^{a}	0.21 ± 0.09^{a}	0.47 ± 0.13^{a}
		16.5	0.52 ± 0.21^{a}	0.37 ± 0.27^{a}	0.59 ± 0.18^{a}
7	20	12.5	2.20 ± 0.75^{a}	0.93 ± 0.02^{a}	0.88 ± 0.08^{a}
		14.5	1.78 ± 0.24^{a}	0.61 ± 0.02^{b}	0.91 ± 0.29^{a}
		16.5	1.26 ± 0.67^{a}	0.45 ± 0.05^{a}	0.87 ± 0.13^{a}
	30	12.5	0.60 ± 0.12^{a}	1.02 ± 0.37^{a}	0.89 ± 0.16^{a}
		14.5	2 ± 0.14^{a}	0.73 ± 0.25^{b}	1.81 ± 0.11^{a}
		16.5	0.94 ± 0.13^{a}	1.19 ± 0.15^{a}	1.78 ± 0.47^{a}

[A] Nominal grain temperature (°C).

[B] Nominal grain moisture content (%).

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

b. Horizontal distribution of adults in 3D setup

The distribution of *C. ferrugineus* adults is shown in Figure 3.6 - Figure **3.10** and horizontally, there is neither directional nor location bias, except at the insect introduction layer. More insects stayed in the introduction cube than in the middle or periphery cubes within the introduction layer (layer 4). However, statistically, the center area did not show a significant difference in insect density compared to the middle and the periphery, except at 14.5% moisture content at 20°C and 16.5% moisture content at 30°C. This could be attributed to the higher standard error observed in the center area of layer 4, at almost all the tested experimental conditions, except at 14.5% moisture content at 20°C and 16.5% moisture content at 30°C (Table 3.2).

3.1.4.2 *Insect distribution in 3D setup at uniform temperatures and moisture contents*

Figure 3.6Figure 3.10 show the distribution of *C. ferrugineus* adults at uniform temperatures (20 and 30°C) and moisture contents (12.5, 14.5 and 16.5%). Adult *C. ferrugineus* introduced at the center of the 3D wheat bulk did not show a significant difference in movement and distribution, with various uniform temperatures and moisture contents during the 24 h study (Table 3.3).

The results of two-way ANOVA (Table 3.4) showed that (1) temperature significantly affected the movement and distribution of *C. ferrugineus* adults; (2) moisture content did not have significant effect on the insect movement and distribution; (3) the interaction between temperature and moisture content has significant effect on the insect movement and distribution.

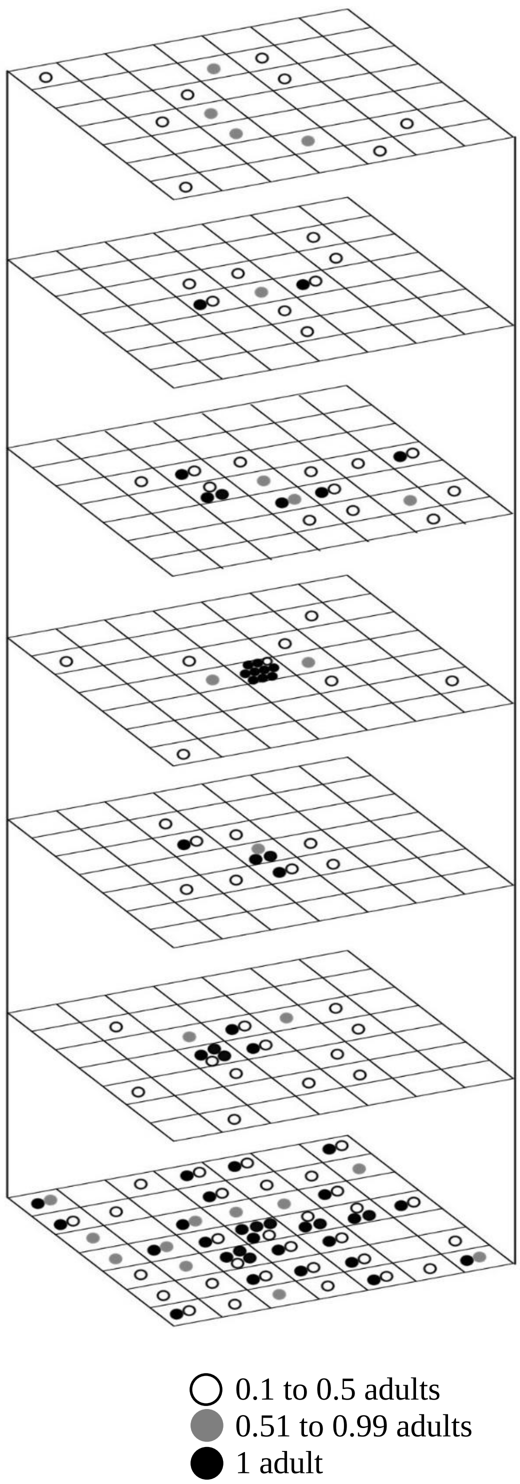


Figure 3.5. Redistribution of *Cryptolestes ferrugineus* adults at 20°C in wheat with 12.5% mc. Different dots show the mean insect number over three replicates.

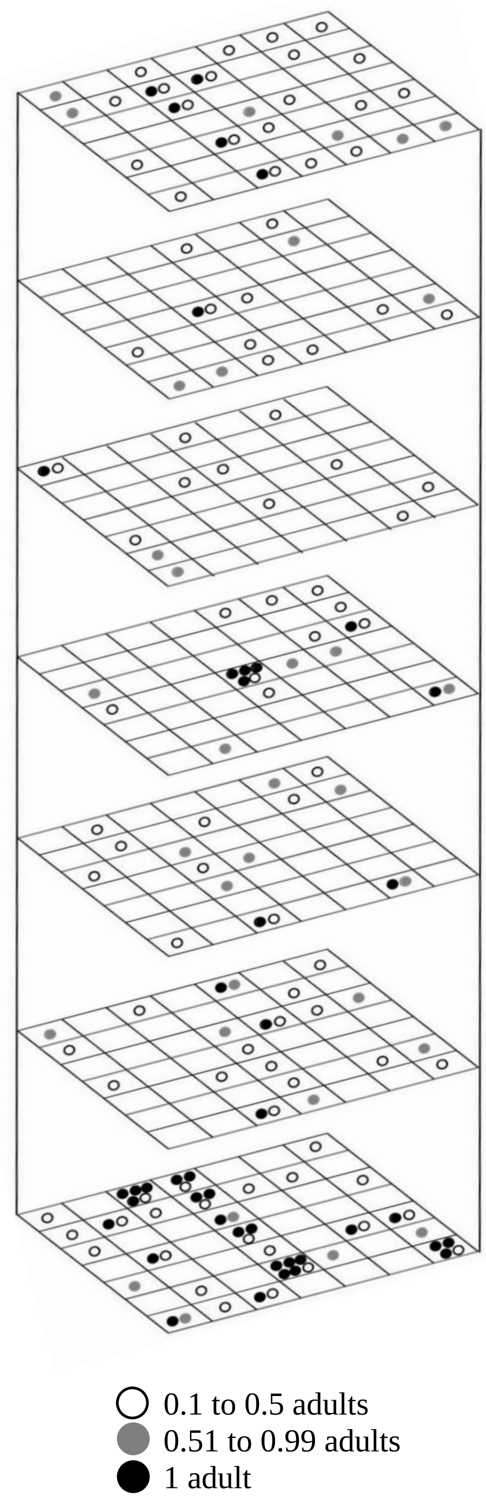


Figure 3.6. Redistribution of *Cryptolestes ferrugineus* adults at 30°C in wheat with 12.5% mc. Different dots show the mean insect number over three replicates.

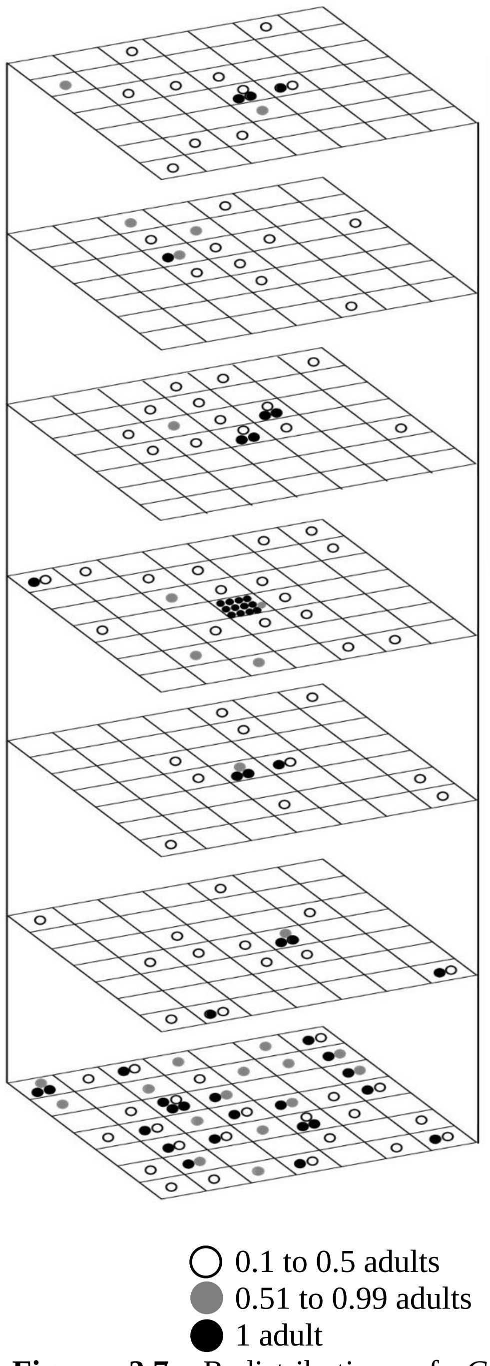


Figure 3.7. Redistribution of *Cryptolestes ferrugineus* adults at 20°C in wheat with 14.5% mc. Different dots show the mean insect number over three replicates.

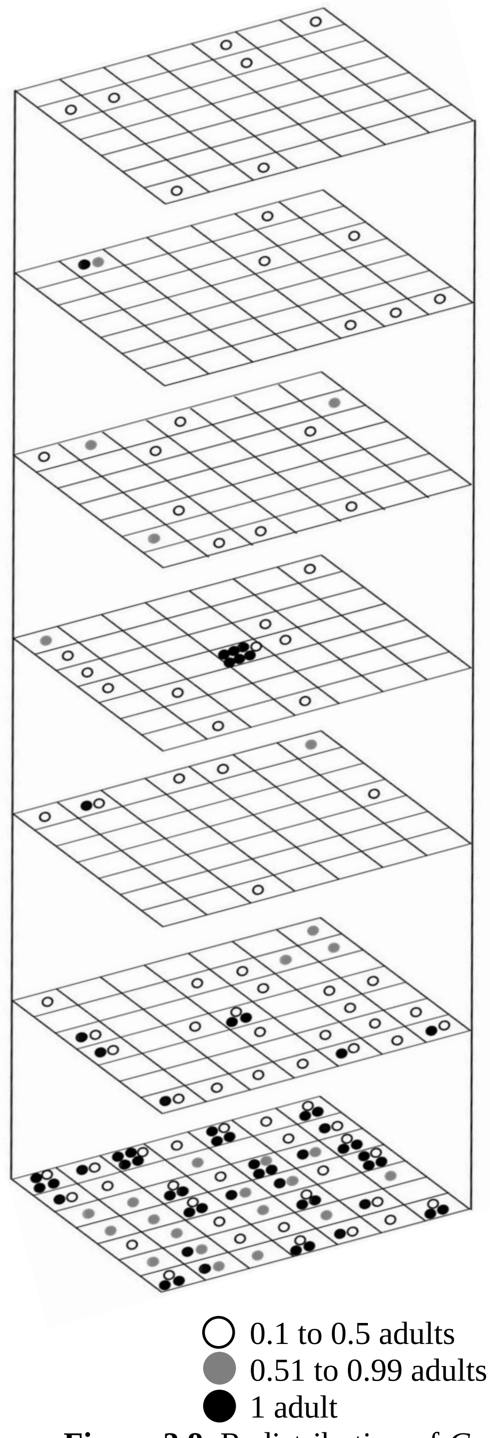
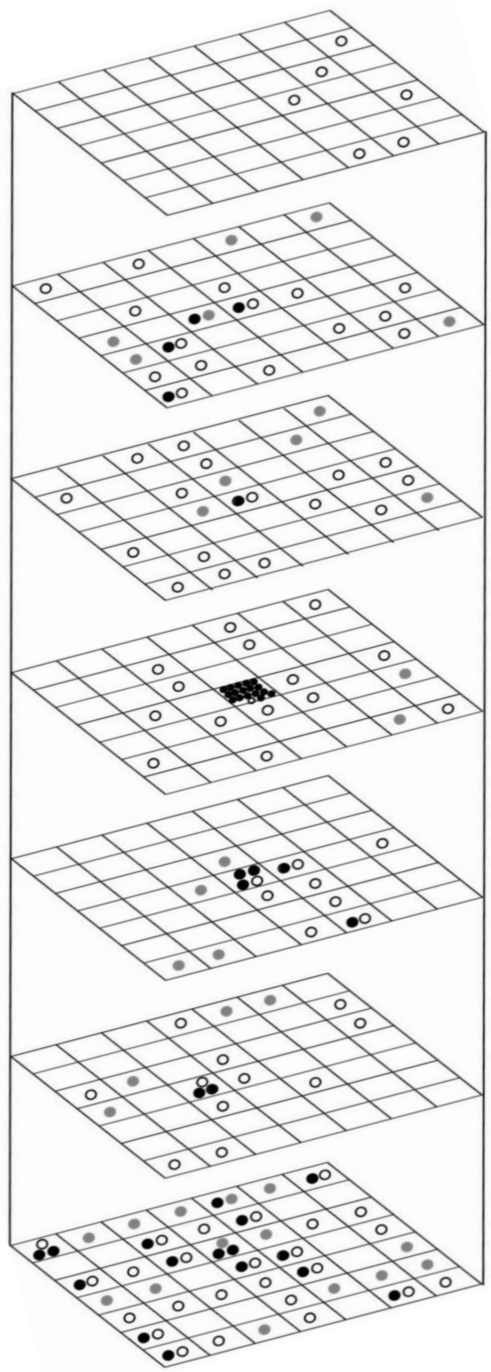
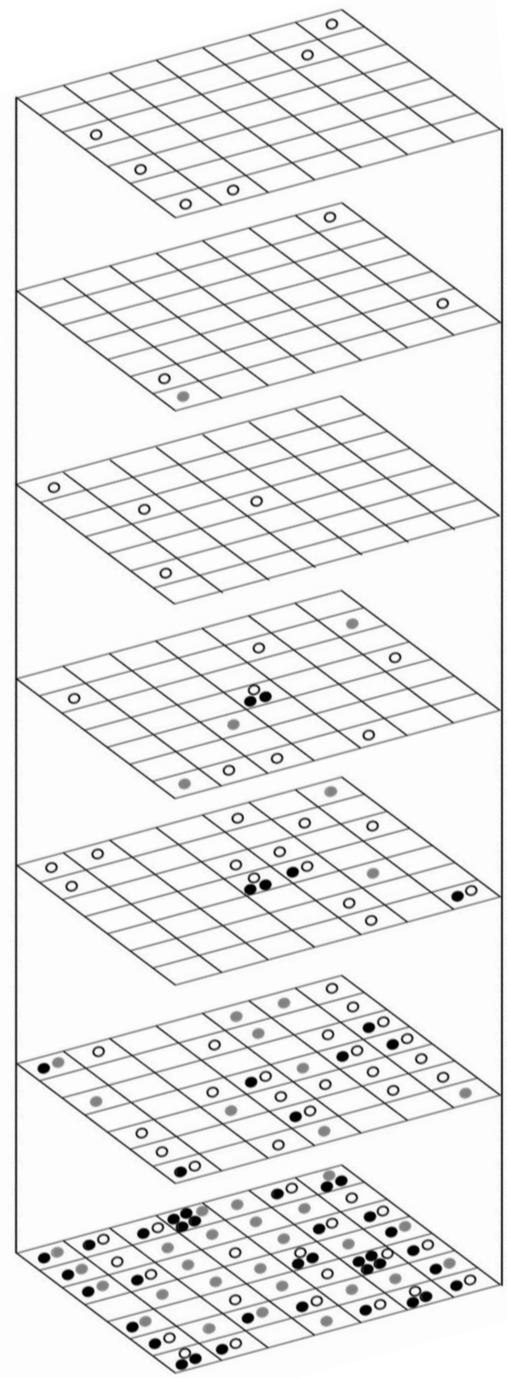


Figure 3.8. Redistribution of *Cryptolestes ferrugineus* adults at 30°C in wheat with 14.5% mc. Different dots show the mean insect number over three replicates.



○ 0.1 to 0.5 adults
● 0.51 to 0.99 adults
● 1 adult

Figure 3.9. Redistribution of *Cryptolestes ferrugineus* adults at 20°C in wheat with 16.5% mc. Different dots show the mean insect number over three replicates.



○ 0.1 to 0.5 adults
● 0.51 to 0.99 adults
● 1 adult

Figure 3.10. Redistribution of *Cryptolestes ferrugineus* adults at 30°C in wheat with 16.5% mc. Different dots show the mean insect number over three replicates.

Table 3.3. Results of two-sample location tests and EDF statistics for *Cryptolestes ferrugineus* adults' movement and distribution in three-dimensional setup.

Experiments [a]		Wilcoxon		Median		Kolmogorov-Smirnov	
		Z	P > |Z|	Z	P > |Z|	KSa	P > KSa
12.5[b]	20[c] vs 30[c]	-0.6389	0.2615	-0.5151	0.6065	0.5345	0.9375
14.5[b]	20[c] vs 30[c]	0.8944	0.3711	0.5151	0.6065	1.0690	0.2032
16.5[b]	20[c] vs 30[c]	1.1500	0.2502	1.5452	0.1223	0.8018	0.5412
20[c]	12.5[b] vs 14.5[b]	0.000	1.0000	0.5151	0.6065	0.5345	0.9375
	12.5[b] vs 16.5[b]	-0.1278	0.8983	0.5151	0.6065	0.5345	0.9375
	14.5[b] vs 16.5[b]	-0.6389	0.5229	-1.5452	0.1223	0.8018	0.5412
30[c]	12.5[b] vs 14.5[b]	1.1500	0.2502	0.5151	0.6065	1.0690	0.2032
	12.5[b] vs 16.5[b]	0.8944	0.3711	0.5151	0.3032	0.8018	0.5412
	14.5[b] vs 16.5[b]	0.5111	0.6093	-0.5151	0.6065	0.8018	0.5412

[a] Comparison between two experiments. First column represents the constant experimental conditions, and the second column represents the comparison.

[b] Nominal grain moisture content (% wb).

[c] Nominal grain temperature (°C).

Table 3.4. The effects of uniform temperatures, moisture contents and their interaction on the movement and distribution of *Cryptolestes ferrugineus* adults

Source [a]	F [b]	P [c]
Treatments [d]	5.82	0.0059**
Temperature	8.09	0.0148*
Moisture content	2.39	0.1340
Interaction	8.11	0.0059**

[a] Degrees of freedom of the treatment, temperature, moisture content and the interaction between temperature and moisture content were 5, 1, 2, and 2, respectively.

[b] F value of the factorial test.

[c] Probability of the F-Value at $P<0.05$* and $P<0.01$**, respectively.

[d] Effects of all factors on insect movement.

3.1.5 Discussion

In our 24 h movement study, more than 50% of the insects had moved down from the point of introduction. A similar result was observed in an insect movement study inside a 1D wheat column, where more than 45% of the introduced *C. ferrugineus* were observed at the bottom section (White et al., 1993). Similarly, Jian et al. (2004b) found that more than 40% of *C. ferrugineus* adults were found in the bottom layer of the 2D grain chamber at uniform temperature after 24 h of introduction. According to Loschiavo (1983), *C. ferrugineus* adults prefer the bottom sections of grain at uniform moisture contents and temperatures. This behaviour of *C. ferrugineus* is the result of the "drift" effect caused by insects falling between the grain kernels during the movement (Jian et al., 2006). Besides, considering the smaller size of *C. ferrugineus*, it has been estimated that they could slip at least five times in a minute during their movement on the surface of the grain. In addition, these tiny insects fall into the pore spaces in the wheat grain bulk (Jian et al., 2009a). Moreover, Jian et al. (2004b) showed that *C. ferrugineus* adults exhibit positive geotaxis behaviour at uniform environmental condition. These aspects explain why a significant portion (50% to 88%) of the insects moved towards the bottom of the wooden box, in the tested experimental conditions. Our results also indicated that many insects tend to move towards the bottom of the grain bulk, especially when the temperature (30°C) and moisture content (16.5%) were high. Jian et al. (2005b) found the adults preferred to move down in the first 24 h and moved up after 24 h in vertical columns with 0%, 5% and 10% uniform dockage. Their study also indicated that the high percentage of dockage influences the movement of insects in one-dimension. Since our study was conducted with clean grain in 24 h, this re-moving up behaviour was not observed.

In a study involving the movement of *C. ferrugineus* adults in 1D grain column, in both horizontal and vertical directions, Jian et al. (2003) showed that the speed of the insect movement increased with increasing temperature. This response to temperature could be why lower temperatures had an increased recovery of insects at the introduction location than their corresponding higher temperature. Additionally, from the results of our study, the influence of temperature on insect movement is seen to increase with increasing moisture content.

The current study trials recovered a range of 2% to 17% of insects from the insect introduction location, while at other cubes, the insects obtained were in the range of 0% to 5%. In general, higher insect densities could incite uniform dispersion due to a reduced need for movement to identify mating partners. On the contrary, at low insect density, the insects might need to travel to a comparatively long distance to find their mating partner, making it difficult to reproduce and multiply. In such cases, insects are attracted towards aggregation pheromones (Loschiavo et al., 1986). In our study, the aggregation of insects at the center location might be due to the aggregation pheromones produced by the insects during the 24 h acclimation. Jian et al. (2004a) showed that at insect densities higher than two adults/kg of wheat, *C. ferrugineus* adults showed uniform distribution and at lower insect densities, insects tended to aggregate. In this study, the introduced insect density (≈ 0.35 adults/kg of wheat) inside the grain bulk was lower than two adults/kg of wheat, and this could also account for the aggregation of insects inside the grain bulk.

During the study of the movement of *C. ferrugineus* adults in 1D grain columns filled with wheat, with and without temperature gradients, Jian et al. (2003) showed that aggregation pheromones have a minor influence on insect movement and distribution in the presence of temperature gradient and geotaxis. The current study also confirms that a lower portion of insects (less than 17% of the introduced adults) tend to aggregate at the insect introduction location in the absence of temperature gradients. Therefore, the smaller amount of aggregation is likely due to the influence of aggregation pheromones, and most insects moved downwards due to the drift and geotaxis effect, as explained earlier.

In our study, about 5% to 30% insects were recovered from top layer. A 3D dispersion pattern study of *Sitophilus granarius* (L.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (Fabricius) and *C. ferrugineus* adults in a uniform wheat bulk conducted by Surtees (1964b), showed that more insects (40%) were recovered in the top quarter of the bulk (the insect introduction layer) and only 23% were recovered from the bottom of the bulk, at 25°C and 14% moisture content. The study also showed the clumping behaviour of *C. ferrugineus* at the insect introduction location as well as the homogeneous lateral dispersion characteristics of insects. The insects were introduced at the upper surface of the top layer and hence, the insect introduction layer and the top layer are the same, in that case; while our study clearly differentiated the top layer as well as the insect introduction layer. In addition, Surtees (1964b) performed each replicate for about two weeks, while ours was a 24 h movement study. These reasons could be attributed for the accumulation of more insects at the top of the grain bulk during the 3D dispersion study by Surtees (1964b).

In general, the insects cannot use their visual perception to look a long distance inside the grain due to the hindrance of the stored-grain (Jian et al., 2009a). This lack of long-distance perception explains their random distribution as they move away from the introduction location. From the results of our study, it is observable that the adult *C. ferrugineus* (1) did not show any significant difference in insect density in the horizontal direction, except at few layers, at the tested experimental conditions (Table 3.2); (2) did not prefer any direction in each layer (Figure 3.6Figure 3.10); (3) did not show any significant difference in movement and distribution at uniform moisture contents and temperatures (Table 3.3). Overall, the movement of *C. ferrugineus* inside a grain bulk was found to be random in the horizontal direction.

According to Surtees (1964c), the insects, including *S. granarius*, *O. surinamensis* and *T. castaneum* individual adults, did not show any directional bias in uniform environmental conditions. The lack of directional bias implies that these adults showed random movement, also is known as “diffusion,” and was demonstrated for *C. ferrugineus* in 2D by Jian et al. (2007). The current study also confirms the diffusion pattern of *C. ferrugineus* in the horizontal direction, and the vertical movement of *C. ferrugineus* is influenced by the "drift" effect and geotaxis. Moreover, Jian et al. (2006) showed that *C. ferrugineus* adults exhibit similar distribution, irrespective of the size of the grain column. In other words, the size of the grain column does not have a significant effect on the insect distribution. Hence, the 3D distribution observed in this study can be assumed for the actual bin filled with wheat with similar insect densities at constant environmental conditions.

From the results of this study, the following conclusions can be drawn.

1. *Cryptolestes ferrugineus* adults tend to follow a diffusion pattern in the horizontal direction at a uniform temperature and moisture content.
2. A portion of *C. ferrugineus* adults accumulate around the introduced cube, at constant environmental conditions.
3. More *C. ferrugineus* adults moved downward over upward in uniform environmental conditions.
4. A movement pattern similar to 1D grain columns and 2D grain chambers was observed in a 3D grain bulk.

The current work provides insight into the 3D movement of insect pests (*C. ferrugineus*) at constant environmental conditions for a period of 24 h. However, the grains stored inside a bin undergo various temperature and moisture changes due to naturally occurring air movement, as a result of change in ambient weather conditions (Jian et al., 2009b). These temperature and moisture gradients could affect the movement and distribution of insects. In addition, the activity of insects varies at different time periods in a day and also in different days, due to the change in ambient temperatures throughout the day (Toews et al., 2003). Thus, the current approach needs to be expanded to study the effects of various temperature and moisture gradients for different time periods with different adult densities in the grain.

3.1.6 Author contributions

VB: Data Curation, Formal analysis, Investigation, Methodology, Writing – Original draft. FJ: Methodology, Supervision, Writing – review & editing. DJ: Methodology, Funding acquisition, Supervision, Writing – review & editing. JM – 3D printing of corners, Writing – review & editing.

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3.2 Effects of insect density, movement period and temperature on three-dimensional movement and distribution of adult *Cryptolestes ferrugineus*

(This section has been published in a peer-reviewed scientific journal)

Bharathi, V. S. K., Jayas, D. S., Jian, F., 2022. Effects of insect density, movement period, and temperature on the three-dimensional movement and distribution of adult *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae). *Journal of Insect Science,* 22 (3), 1-9. https://doi.org/10.1093/jisesa/ieac020.

3.2.1 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 (mc)*:* 1) at various insect densities (0.35, 1.77 and 3.53 A/kg (adults/kg) at 20ºC and in 24 h movement period; 2) in different movement periods (6, 24, and 72 h) at 20ºC and 0.35 A/kg insect density; and 3) at different temperatures (20, 30 and 35ºC) 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 1000 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.

Keywords: *Cryptolestes ferrugineus,* Stored grain, Insect density, Movement period, Three-dimension

3.2.2 Introduction

Cryptolestes ferrugineus (Stephens), the rusty grain beetle, is a cosmopolitan secondary pest of grain that is found in tropical and temperate climate zones. Adults and larvae mostly feed on germs of the grain 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 of adults of *C. ferrugineus* move to centres of bins and continue to multiply in large unaerated grain bins.

Over the decades, the effects of various factors such as temperatures, moisture contents (mc) and their gradients, dockage, temperature acclimation, age, insect densities, 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 behaviour 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 was determined. Using this technique, Surtees (1964b) analysed 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, 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, 1966b). Moreover, the increase in insect density influences the distribution and diffusivities of *C. ferrugineus* in both 1D columns and 2D chambers (Jian et al., 2004b; Jian et al., 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 behaviour of *C. ferrugineus* adults at high temperature (35ºC) by comparing with the observations of published work at 20 and 30ºC (Bharathi et al., 2021).

3.2.3 Materials and methods

3.2.3.1 *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 -15ºC for at least 3 wk to make sure that the grain does not have any other insects (Fields and White, 1997). Moisture content (mc) of the wheat was determined using the oven drying method by drying about 10 g samples, in triplicate, at 130ºC 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 10ºC 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.

3.2.3.2 *Experimental setup*

The experimental setup 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.

3.2.3.3 *Adult* Cryptolestes 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 ± 1ºC 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.

3.2.3.4 *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 (e.g., layer and direction of x and y) 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 (Figure 3.11). Thus, the mesh cube located at the top left corner in Figure 3.11 in the top layer was labelled as L1-A-1. The mesh cube with the acclimated adults was located at L4-D-4.

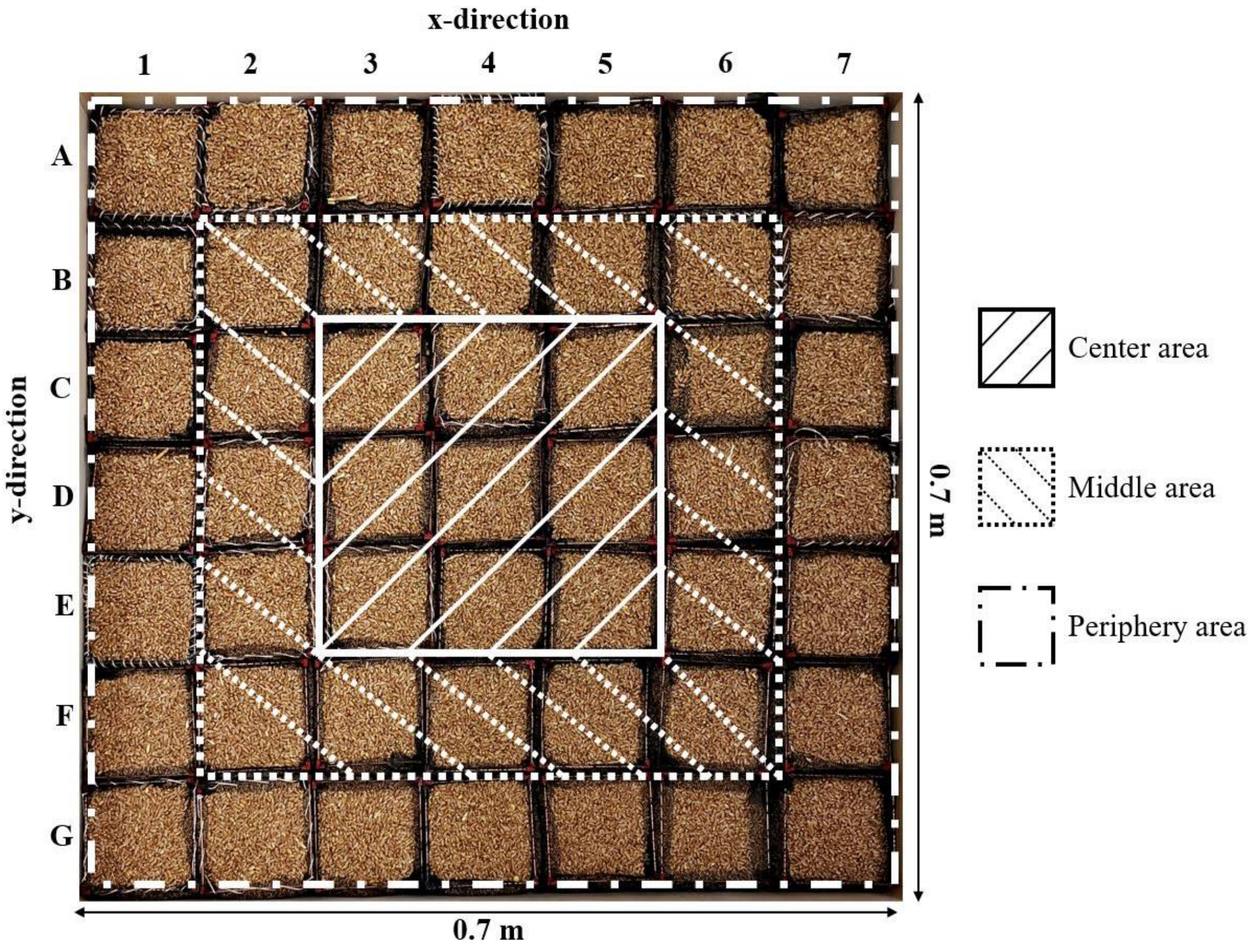


Figure 3.11. Numeration and categorization of the mesh cubes filled with wheat, in each layer. The size of each mesh cube was 0.1 x 0.1 x 0.1 m.

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.

$$\textbf{Number of adults in the cube} = \frac{\mathbf{A_{cube}} \times \mathbf{A_{initial}}}{\mathbf{A_{final}}}$$

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.

3.2.3.5 *Data analyses*

Five experiments with different insect densities, movement periods, and temperatures were conducted (Table 3.5). 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 20ºC; 2) in different movement periods (6, 24 and 72 h) at 20ºC 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 1000 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 1000 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).

Table 3.5. Experimental conditions conducted using 14.5% moisture content of wheat.

Adults introduced	Movement period (h)	Temperature (ºC)
100^{ε}, 500, 1000	24	20
100	6, 24^{ε}, 72	20
100	24	20^{ε}, 30^{λ}, 35

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

Vertical distribution

The adults retrieved from each layer were summed up and used to conduct ANOVA, followed by Tukey's test (α = 0.05) within different layers of the same experiment, or in the same layer under different insect densities, temperatures and movement periods. To determine whether the adults moved up or down, the number of insects recovered from the upper half of the box (layers 1, 2 and 3) were multiplied by 30, 20 and 10 (distance travelled 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 (Figure 3.11). 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.

3.2.4 Results

3.2.4.1 *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 20ºC and when 1000 insects were introduced, about 44% of the insects moved downward and 40% 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 3.6). Therefore, insects distributed more uniformly in all layers when 1000 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 20ºC, 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) (25%) after 6 h, 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 3.6). Therefore, the introduced adults generally moved in the downward direction at first, then moved up and distributed inside the wheat.

Table 3.6. 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 ± 1.9 [a, A, K, X]	6.3 ± 1.4 [a, A, K, X]	9.2 ± 1.2 [a, A, K, X]	21.7 ± 0.8 [ab, AB, K, X]	6.9 ± 1.4 [a, A, K, X]	8.8 ± 1.6 [a, A, K, X]	39.2 ± 3.1 [b, A, K, X]
500A-24P-20T[λ]	0.5 ± 0.2[a, A]	2.1 ± 0.4[a, A]	4.2 ± 1.6[a, A]	29.4 ± 3.7[b, A]	16.6 ± 3.6[ab, A]	15.3 ± 5.3[ab, A]	31.8 ± 1.5[b, A]
1000A-24P-20T[λ]	16.2 ± 8.8[a, A]	10.8 ± 2.7[a, A]	13.3 ± 3.0[a, A]	15.3 ± 2.4[a, B]	7.9 ± 2.5[a, A]	7.5 ± 1.9[a, A]	29.0 ± 6.5[a, A]
100A-6P-20T	2.1 ± 0.5[a, K]	4.1 ± 1.9[a, K]	6.2 ± 1.7[a, K]	36.1 ± 0.8[b, K]	11.5 ± 3.0[a, K]	7.6 ± 1.4[a, K]	32.3 ± 1.2[b, K]
100A-72P-20T	2.4 ± 2.0[a, K]	5.6 ± 2.4[a, K]	9.1 ± 0.5[a, K]	25.0 ± 5.8[a, K]	15.4 ± 2.7[a, K]	17.7 ± 4.7[a, K]	24.8 ± 5.9[a, K]
100A-24P-30T	2.4 ± 0.4[a, X]	3.8 ± 1.3[a, X]	4.8 ± 0.9[a, X]	10.0 ± 1.1[a, X]	3.5 ± 0.6[a, X]	15.1 ± 2.7[a, X]	60.4 ± 2.1[b, X]
100A-24P-35T	8.4 ± 2.3[a, X]	5.2 ± 1.3[a, X]	6.6 ± 2.0[a, X]	14.6 ± 3.2[a, X]	5.9 ± 1.2[a, X]	11.5 ± 1.9[a, X]	47.7 ± 6.6[b, X]

[ε] Numbers before A, P, and T are the number of introduced adults, movement period (h), and temperature (ºC) of wheat, respectively.

[λ] Insect numbers in the experiments with 500 and 1000 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 3.7. Results of two-sample location tests and EDF statistics for adult *Cryptolestes ferrugineus* movement and distribution in three-dimensional setup.

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[β] vs 1000A[λ]	0.0000	1.0000	0.5151	0.6065	0.8018	0.5412
		100A vs 1000A	-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 (ºC) of wheat with 14.5% mc, respectively.

[λ] Insect number in the experiments with 500 and 1000 introduced adults were normalized to 100.

Table 3.8. Insect densities of adult *Cryptolestes ferrugineus* at different areas of each layer under different experimental conditions (n = 3).

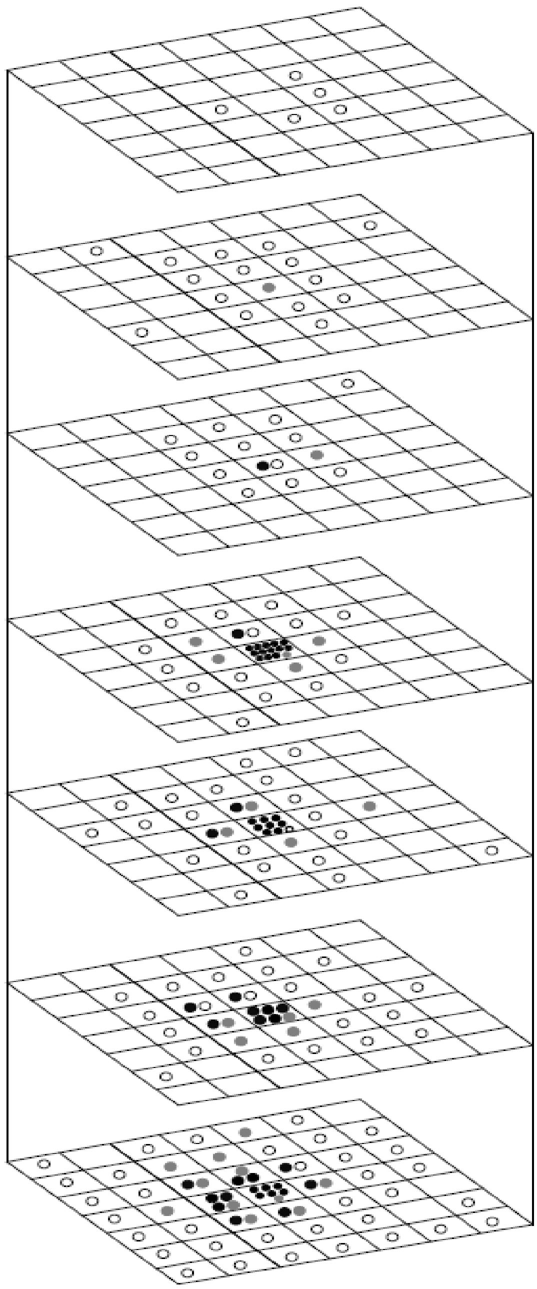
Layer	Experimental conditions[ε]	Area		
		Center	Middle	Periphery
1	500A - 24P - 20T[λ]	0.1 ± 0.0[a]	0[a]	0[a]
	1000A - 24P - 20T[λ]	1.2 ± 0.6[a]	0.4 ± 0.2[a]	0.1 ± 0.1[a]
	100A - 6P - 20T	0.1 ± 0.1[a]	0.1 ± 0.0[a]	0[a]
	100A - 72P - 20T	0.3 ± 0.2[a]	0[a]	0[a]
	100A - 24P - 35T	0.3 ± 0.1[a]	0.2 ± 0.01[a]	0.2 ± 0.1[a]
2	500A - 24P - 20T[λ]	0.2 ± 0.1[a]	0[b]	0[b]
	1000A - 24P - 20T[λ]	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[λ]	0.5 ± 0.2[a]	0[a]	0[a]
	1000A - 24P - 20T[λ]	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[λ]	3.9 ± 0.5[a]	0.1 ± 0.0[b]	0[b]
	1000A - 24P - 20T[λ]	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[λ]	1.9 ± 0.5[a]	0.1 ± 0.0[b]	0[b]
	1000A - 24P - 20T[λ]	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[λ]	1.8 ± 0.7[a]	0.2 ± 0.0[a]	0[a]
	1000A - 24P - 20T[λ]	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}
	500A - 24P - 20T[λ]	2.9 ± 0.2^{a}	0.4 ± 0.1^{b}	0.2 ± 0.1^{b}
	1000A - 24P - 20T[λ]	1.6 ± 0.5^{a}	0.6 ± 0.2^{a}	0.5 ± 0.2^{a}
7	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% moisture content, respectively.

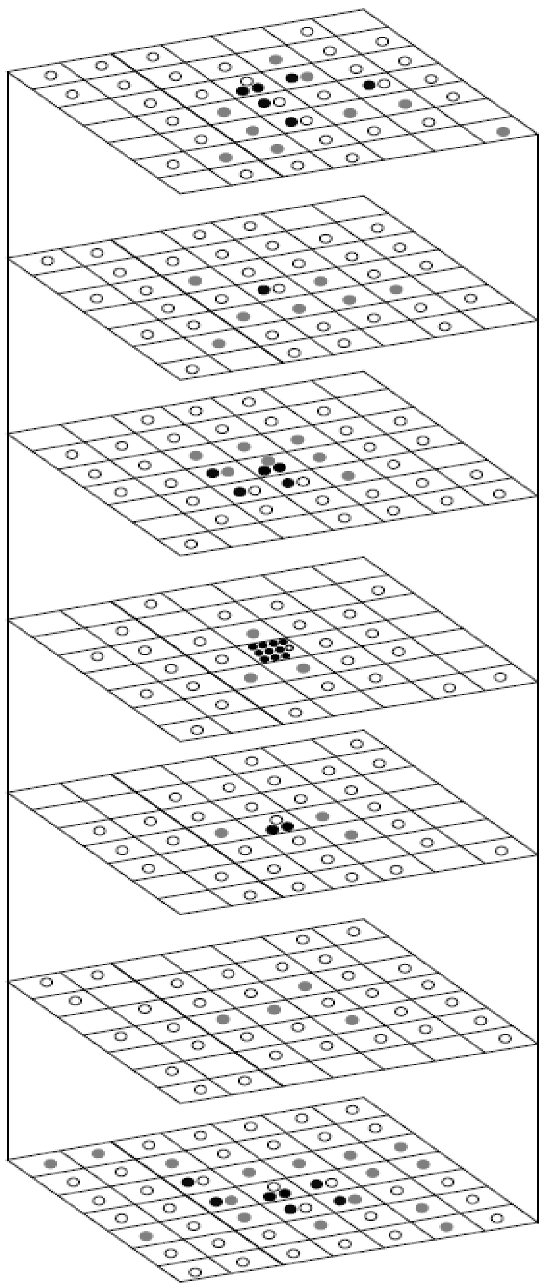
[λ] Insect number in the experiments with 500 and 1000 introduced adults were normalized to 100.

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



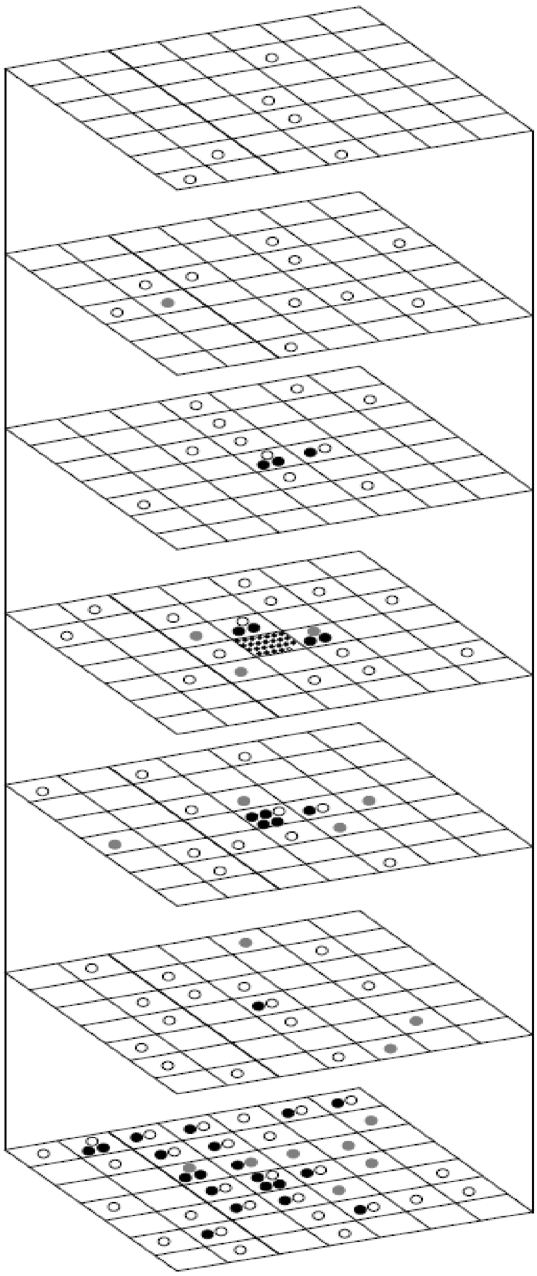
○ 0.1 to 0.5 adults
● 0.51 to 0.99 adults
● 1 adult

Figure 3.12. 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



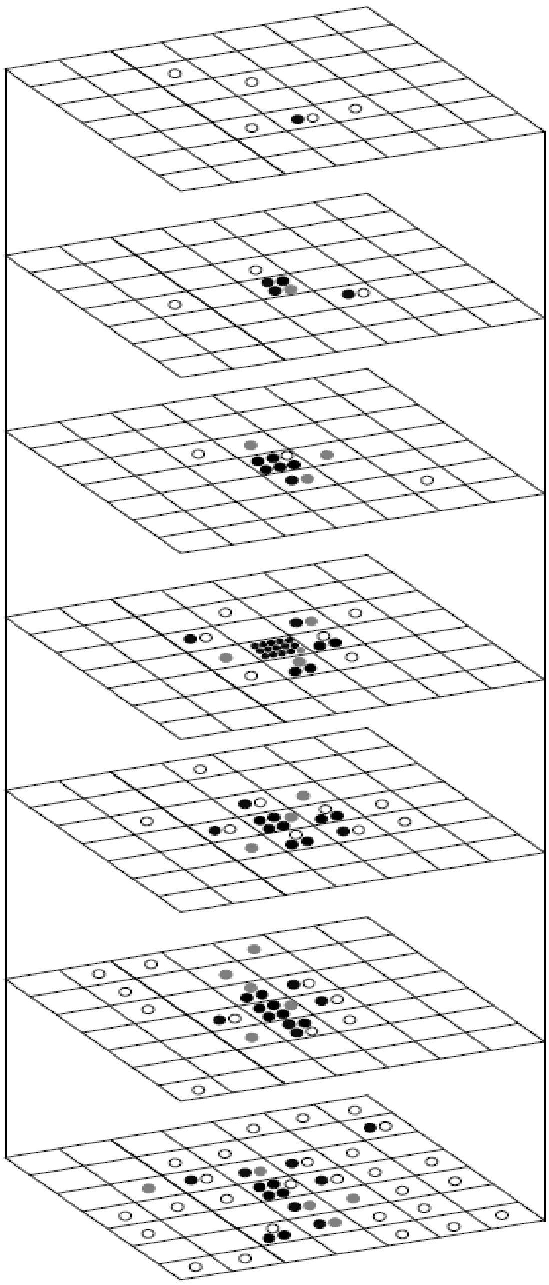
○ 0.1 to 0.5 adults
● 0.51 to 0.99 adults
● 1 adult

Figure 3.13. Redistribution of 1000 *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.



○ 0.1 to 0.5 adults
● 0.51 to 0.99 adults (grey)
● 1 adult

Figure 3.15. 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.



○ 0.1 to 0.5 adults
● 0.51 to 0.99 adults (grey)
● 1 adult

Figure 3.14. 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.

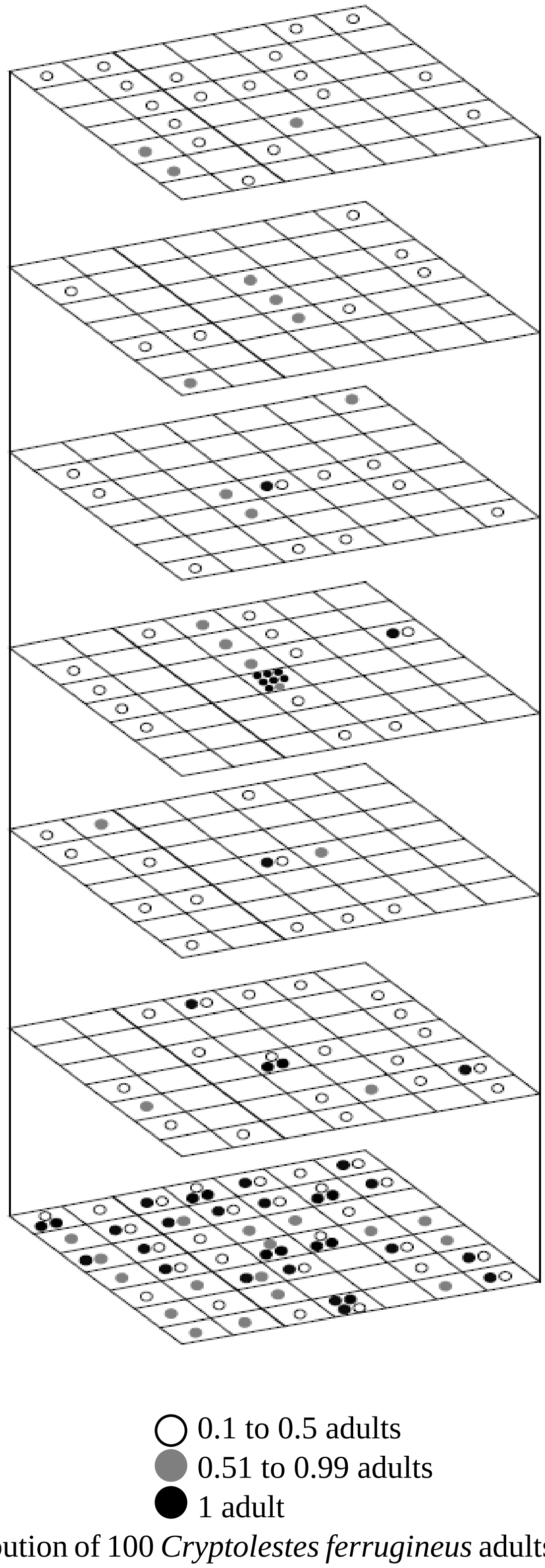


Figure 3.16. Redistribution of 100 *Cryptolestes ferrugineus* adults at 35°C in wheat with 14.5% mc for a period of 24 h. Different dots show the mean insect number over three replicates.

At 20, 30 and 35ºC, only 23, 11, and 20% of adults moved up when 100 insects were introduced, respectively, in 24 h of movement period (Table 3.6), while about 55%, 79% and 65% of insects were recovered from the bottom half of the wooden box, respectively. However, at 20ºC, higher percentage of insects (22%) were recovered from the insect introduction layer (layer 4), as compared to those at 30ºC (10%) and 35ºC (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 3.7). These results indicated that insects had the similar distribution pattern in vertical direction under various tested conditions.

3.2.4.2 *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 3.8). In layer 4, more insects (higher insect density) were found at the center area, for all the experiments (Figure 3.12 - Figure 3.16). Tested insect densities, movement periods and temperatures did not have any effect (Table 3.8) 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.

3.2.5 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 accordance with the observations of various other researchers (Loschiavo, 1974a, 1983; White et al., 1993b; Jian et al., 2004d, 2006, 2009a; Bharathi et al., 2021), who found that a considerable portion of *C. ferrugineus* adults moved 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 30ºC 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 1000 insects, respectively, did not show significant difference in 3D movement and distribution. In addition, more recovery of insects (Table 3.6) 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 (Figure 3.13). These consistent results in 1D, 2D and 3D movement might be explained by insect movement behaviours 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 20ºC, 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 3.6). 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 30ºC, as compared to 20ºC 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 35ºC could be caused by the increased movement speed which might have further enhanced the dispersal of insects (Jian et al. 2004b). In addition, increase in movement at higher temperature explains the higher recovery of insects from the insect introduction layer (layer 4) at 20ºC than those at 30ºC. Among the insects recovered from layer 4, higher percentage of insects were recovered in the middle and periphery areas, at 35ºC (38%), compared to those at 30ºC (31%) and 20ºC (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:

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 (35ºC) than at low temperature (20ºC).
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 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 behaviour 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) should 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 in 3D grain bulk. Hence, it would be interesting to consider these factors in the future studies.

3.2.6 Author contributions

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

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Chapter 4. Study on 300 t of wheat stored in corrugated steel bin for two years

4.1 Temperature and moisture profiles of the grain

(This section has been published in a peer-reviewed scientific journal)

Bharathi, V. S. K., Jian, F., Jayas, D. S., 2023. Study on 300 t of wheat stored in corrugated steel bin for two years in Canada. Part I – Temperature and moisture profiles of the grain. *Journal of Stored Products Research,* 100, 102057. https://doi.org/10.1016/j.jspr.2022.102057.

4.1.1 Abstract

Grain temperatures, moisture contents and germination inside an un-aerated, 10 m diameter corrugated steel bin filled with 300 t of wheat with an initial moisture content of 12.5 ± 0.1% (w.b.), were monitored from September 2019 in Winnipeg, Canada. The hourly temperatures were recorded until October 2021, while moisture content and germination were monitored every month until August 2021. During the 26-month period, the temperatures inside the bin were above 7.8°C, at least at one of the measured locations. The temperatures and the moisture contents of the grain varied with changes in ambient weather conditions. The grain moisture changes and temperature fluctuations near the surface of the bulk and temperature fluctuation near the walls were higher than those inside the grain bulk. The temperature gradient-induced convective currents and the hot spot development triggered the moisture migration inside the bin. The presence of high moisture grains (as a result of snow leakage) inside the grain induced a hot spot development, which in turn influenced the grain temperature and moisture content. Germinability of the grain near the surface and inside the grain bulk dropped faster than those near the walls, in the presence of insect infestation. The drop in ambient temperature could either cease the hot spot or lower the grain temperature.

Keywords: Grain bin; temperature gradients; moisture migration; insect activity; wheat storage

4.1.2 Introduction

Stored grain is a man-made ecosystem, where the interactions of biotic and abiotic factors such as temperature, moisture content, insects, mites, rodents, birds, and microorganisms occur. Temperature and moisture content of the grain play a significant role in influencing the properties of the grain stored in a bin (Jayas and White, 2003). Temperature change inside the stored grain occurs due to the interaction of internal sources (such as grain respiration, insect and microbial activity), and external conditions (ambient weather such as wind velocity, solar radiation and ambient air temperature) (Converse et al., 1973). Structural parameters of the bin such as size, shape, type of material, type of foundation, and number and size of openings also influence the temperature of the stored grain (Jian and Jayas, 2022).

Temperature gradients induce convection currents inside stored grain bulks, which in turn results in moisture migration in the grain bulk (Thorpe, 1982). The degree of moisture migrated inside the bin depends on the initial moisture content and temperature of grain mass, in addition to ambient conditions and the structural parameters of the bin (Al-Amri and Abbouda, 2000; Jian and Jayas, 2022). However, significant moisture changes inside a bin as a result of moisture migration take long time (weeks to months) owing to the low convection currents (about 6 to 15 m/day) (Gough et al., 1990; Jian and Jayas, 2022).

Interactions among biotic and abiotic factors involve many complex processes (Jian and Jayas, 2012). Therefore, it is difficult to characterise each interaction between two individual factors. One approach to solve these complex processes is to develop mathematical models (Jayas, 1995) and many mathematical models have been developed over the past few decades (Alagusundaram et al., 1990a, 1990b; Montross et al., 2002a; Jian et al., 2005; Quemada-Villagómez et al., 2020; Wang et al., 2020). To validate the developed models, detailed experiments involving monitoring maximum possible parameters are required. Jian et al. (2009) monitored the temperature fluctuations and moisture migrations in wheat stored inside a metal silo for a period 15 months and reported that temperature fluctuations and moisture migrations at the surface of the grain and temperature fluctuation near the wall were higher than those inside the grain mass because wheat acts as an insulating material owing to their low thermal conductivity (0.181 to 0.202 W/(m.K)) and diffusivity (8 to 9.3 x 10^{-8} m^2/s). However, the capacity of the grain used for the study (20 t of wheat) was small compared to the commercially available grain storage bins (Limay-Rios et al., 2017). Moreover, Jian et al. (2009) used wheat of moisture content 13.6% (wet basis), without insect infestation. Respiration of insects can produce heat and water (Sinha and Wallace, 1966), and hence influence the grain temperature and moisture content. In addition, the presence of plenum influences the temperature and moisture distribution inside the bin differently as compared to those in contact with soil foundation (Jian and Jayas, 2022). No published study that considers these complex interactions (temperature, moisture content, insect infestation, and presence of plenum) in a large (300 t or more) stored grain ecosystem is available.

As a result of climate change, especially damper winters and hotter summers, the grains stored in improperly aerated bins could undergo rapid deterioration because of the increase in moisture content and temperature (Moses et al., 2015). To tackle this, it is advised to reduce the height of silos as short as possible in warmer climate areas (Kumar, 2021). The change in the abiotic factors (such as moisture content and temperature) could affect the biotic factors and their interaction in the stored grain ecosystem (Jayas, 1995). Understanding the effect of ambient weather on the temperatures of the stored grain can help tackle the problems associated with climate change on the stored grain.

Due to interaction of various biotic and abiotic factors and the lower thermal diffusivity of grains than the bin wall, localized warm temperature zones occur inside the grain bulk, which are often referred to as 'hot spots'. The warmer temperature or higher moisture content or both in hot spot locations often result in degradation of the grains. The hot spot is usually initiated by the multiplication of microorganisms (Sinha and Wallace, 1965). The increase in moisture content of the grain due to condensation, snow or rainwater leakage or moisture migration through convection currents, favors the growth of microorganisms in the grain, which in turn leads to fungi-induced hot spot (Sinha and Wallace, 1965). Unexpected leakage of snow through the loading hole in the current study changed the course of the study. Thus, the current study reported the process of a hot spot (from initiation, development to the death of the hot spot) and its influence on the quality of the grain. In the current study, the wheat was also highly infested by the introduced insects (rusty grain beetles and red flour grain beetles). No such data has been reported in a large grain bin (capacity 300 t or more) under such complex condition.

The aim of the current study was to: 1) monitor temperatures, moisture contents, and germination at various locations inside an un-aerated, 10-m diameter corrugated steel bin containing 300 t of wheat, for a 26-month storage period; 2) characterize the effect of bin configurations (such as plenum) and hot spot development on these measured parameters; and 3) evaluate the effect of weather change on grain temperature.

4.1.3 Materials and methods

4.1.3.1 *Grain bin and wheat*

The bin used for this study was a flat-bottom cylindrical-wall corrugated steel bin with 10 m diameter and 8 m height (3 m high conical roof and 5 m high cylindrical part), located in the south end of Winnipeg, Manitoba, Canada. The bin was mounted on a 0.5 m high concrete foundation. A 0.35 m deep plenum chamber and a perforated floor were installed above the foundation. Inside the plenum, metal frames supported the perforated floor, so air inside the plenum could freely move. A layer of poly tarp with the hole size less than 1 mm was placed on the floor before the loading of the wheat to prevent the escape of insects. The tarp did not hinder air exchange between the plenum and the grain inside the bin. The bin consisted of a conical roof with 0.9 m diameter opening at the centre to facilitate grain loading and four manholes along the four directions (north, south, east, and west). Four ladders were installed on the outside of the bin to access the manholes to facilitate sampling. In addition, the bin was also equipped with a ladder inside the bin on the north side. The bin also consisted of a pulley, winch, and harness system, to ensure safety of personnel entering the bin. Besides, the bin was equipped with a chute and two auger conveyors to facilitate unloading of grain. One sweeping auger was located on the perforated floor and the another unloading auger was present along the north-west direction under the floor in the plenum. During the grain storage period, the sweeping auger was placed at the location as same as that of the unloading auger. The bin was neither airtight nor artificially aerated throughout the experimental period. Therefore, air exchange between the inside of the bin (the headspace and plenum) and the outside environment was possible. A total of about 300 t of wheat (AAC Goldrush, No. 2 Canada Western Red Winter Wheat with the initial moisture content of 12.5 ± 0.1%, w.b., harvested from southern Manitoba, Canada) was filled inside the bin during August 7 - 15, 2019, in five batches of 60 t each. Further details of loading of the grain into the bin was reported by Salarikia et al. (2021). After completion of the grain loading, the grain surface was manually levelled, and the grain depth was about 4.1 m. The wheat was unloaded from the bin in November 2021. Therefore, data collected from September 1, 2019, to October 31, 2021, were analyzed and are reported in this article.

4.1.3.2 *Insect introduction*

In order to study the effect of insects on the quality of the stored wheat, about 75000 adults of *Cryptolestes ferrugineus* (Stephens) were introduced on the centre surface of the wheat bulk on 6th and 10th of September 2019 (37500 adults each time). The overall adult density was 0.25 adults/kg (A/kg) of wheat after the second introduction. On 9th and 13th of September 2020, about 75000 adults of *Tribolium castaneum* (Herbst) were introduced at the same location as that of *C. ferrugineus* introduction in two batches (37500 adults each time). Insects heavily infested the wheat during the study period. The results of the insect movement and distribution inside the bin were presented in section 4.2 (Bharathi et al., 2022a). However, the effect of insect multiplication on the temperature, moisture content and germination of the wheat are discussed in the current section.

4.1.3.3 *Temperature measurement*

Nine cables (OPI Systems Inc., Calgary, AB) containing temperature (resolution: ±0.1ºC) and relative humidity (resolution: ±5%) sensors were installed at 0.85 m from the centre of the bin along the north direction (referred to as centre (C)), 2.25 m from the centre of the bin (referred to as HR and numbered from 1 to 6, in order, from north and in clockwise direction (Figure 4.1b), and 0.6 m away from the walls (referred to as east and west) (Figure 4.1). The cable locations were chosen to measure the temperature at the critical locations (such as center and half radius) and along different directions. Since the temperatures at the walls were measured using the thermocouples (Bharathi et al., 2022b), the cables near the walls were limited. Each cable had three sensors (referred to as S and numbered as 1, 2 and 3 from top to bottom) inside the grain. The depth of S1, S2 and S3 in C cable from the surface of the grain were 1.1, 2.3 and 3.5 m, respectively; while the depth of those at HR (HR1, HR2, HR3, HR4, HR5 and HR6), east and west locations were 0.7, 1.9 and 3.1 m from the grain surface, respectively. The details of the sensors located in the headspace and those on the floor, roof, and sidewalls have been reported elsewhere (Bharathi et al., 2022b). The temperature data of S2 in cable C and HR5 were missing throughout the study period due to sensor malfunctioning. The hourly temperatures and relative humidity at 25 locations inside the grain were recorded from September 1, 2019 to October 31, 2021. The data from the west cable were missing from December 21, 2019 to March 28, 2020 and from January 24 to March 16, 2021 and the data from east cable were missing for a period of 67 d in winter at various intervals. The missing data period at all the other locations was less than 20 d.

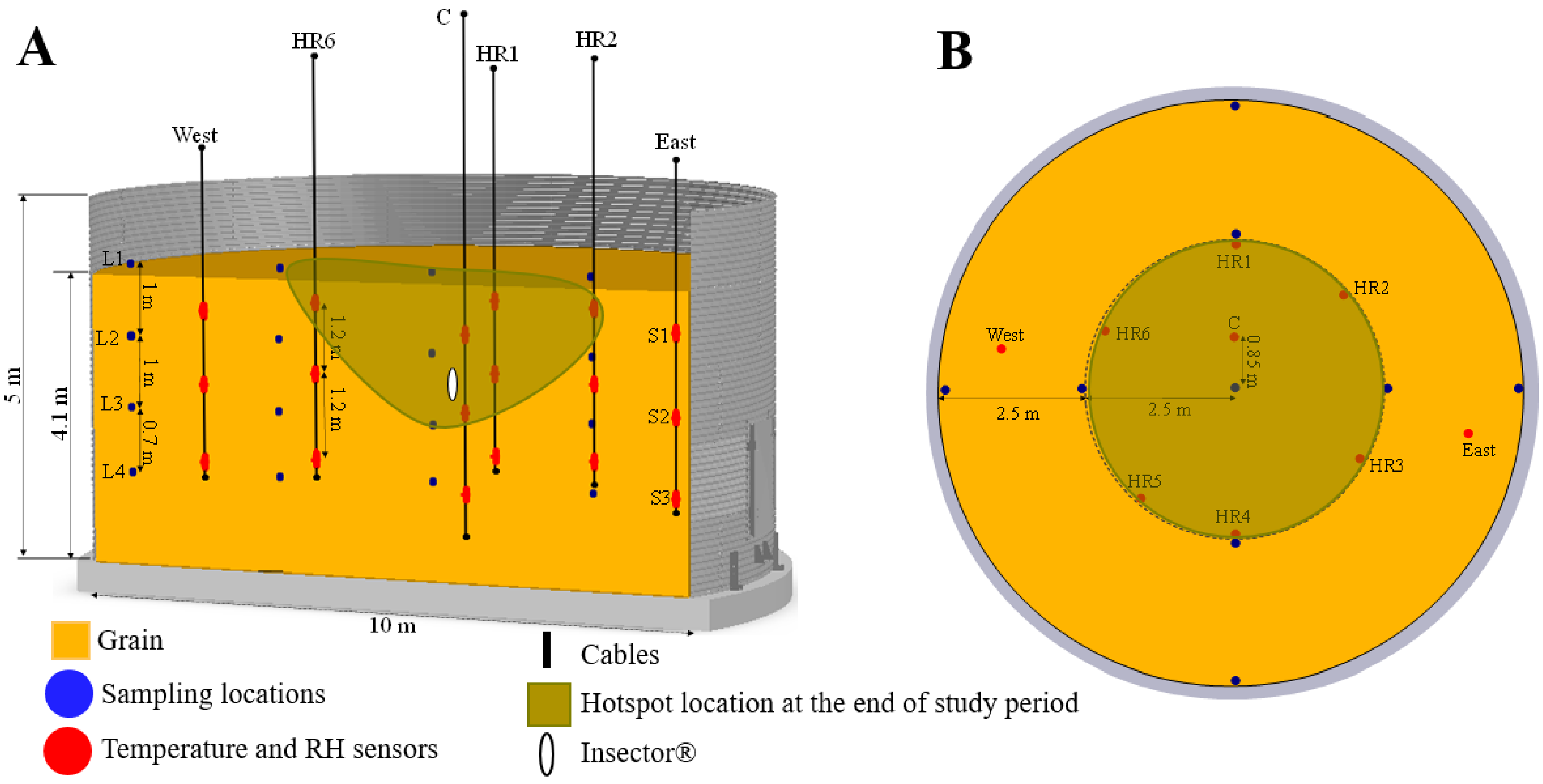


Figure 4.1. Locations of cables and grain samples collected (Cross-sectional view (A) and Top view (B)) from a 10 m diameter corrugated steel bin containing 300 t of wheat. In the graph, ‘C’ is the cable located at 0.85 m from the centre of the bin along the north direction, and ‘HR’ represents the cables located at half radius and numbered from 1 to 6, in order, from north in clockwise direction. ‘West’ and ‘East’ are the cable located at 0.6 m away from the walls in the west and east direction, respectively. ‘L’ is the location of the layers of grain sampled and numbered from 1 to 4, in order, from top to bottom of the grain. ‘S’ is the sensor located on the cables and numbered from 1 to 3, in order, from the surface to the bottom of the grain. The sensors measured hourly temperature and relative humidity.

Ambient weather data were collected at 10 m from the bin in the south-east direction from September 1, 2019, to October 6, 2021, for every 30 min, using a weather station. The weather station included a temperature and relative humidity probe (CS215, Campbell Scientific, Edmonton), a barometric pressure sensor (CS106, Campbell Scientific, Edmonton), a pyranometer (CS300, Campbell Scientific, Edmonton), a tipping bucket rain gage (TE525M, Campbell Scientific, Edmonton) and a wind monitor (05103, Campbell Scientific, Edmonton) in order to measure air temperature (°C) and relative humidity (%), barometric pressure (kPa), average solar radiation (W/m^2), precipitation (mm), wind speed (m/s) and wind direction (degrees clockwise with reference to the north), respectively. The details of the weather data have been reported elsewhere (Bharathi et al., 2022b). In this paper, the period from November 1 to March 31 was referred to as Cold Temperature Period and April 1 to October 31 was referred to as Warm Temperature Period. Also, the periods from November 1, 2019, to October 31, 2020, and November 1, 2020, to October 31, 2021, were referred to as Years I and II, respectively.

4.1.3.4 *Moisture content and germination measurement*

From September 2019 to August 2021 and for every month (between the dates 21 to 25 of each month), wheat samples were collected at the centre and at 0.15 m and 2.5 m away from the wall along the north, south, east, and west directions (total of nine locations, Figure 4.1). At each location, about 20 g of sample was collected at each of four grain depths: 0 (grain surface, referred to as L1), 1 (referred to as L2), 2 (referred to as L3), and 2.7 m (referred to as L4) (Figure 4.1). A bullet probe sampler was used to collect the sample at each location, and grain at the surface (L1) was collected at first, followed by those at 1 m (L2), 2 m (L3) and 2.7 m (L4). Moisture contents of the grain samples were determined using the standard oven-drying method which involves drying 10 g of samples at 130°C for 19 h (ASABE, 2016a). Germination of the grain was assessed by placing 25 randomly selected wheat kernels in a Petri dish with moistened filter paper and held at room temperature for 7 d (Wallace and Sinha, 1962).

4.1.3.5 *Statistical analysis*

Even though the experimental design was to sample the same location at different sampling times, the sampled grain in different sampling times might not have been exactly taken from the same location due to the operational difficulties in probing. In order to understand the effect of the sampling location variation, three replicates were collected from each location in the sampling months of May and July 2021. Standard errors of moisture content and germination observed during May were in the range of 0.0 to 0.3% and 0.0 to 9.7%, respectively. Standard errors of moisture content and germination in July were 0.0 to 0.4% and 0.0 to 10.7%, respectively. Even though these standard errors might be acceptable for the big scale of study during the tested period, there were outliers in the germination data. Therefore, the germination of a particular month was compared with the germinations in the previous three months. The germination of the month that was less by 15 percent point than that of the later three months and the germination that was more by 15 percent point than that of the previous three and later three months were considered as outliers. These outliers were excluded in the data analyses. Moreover, it was difficult to insert the sampling probe at the center location from January 2021 due to cake formation of the grain in the hot spot. The germination at the center after January 2021 was considered as missing data.

Due to the scale of this study and workload of the sampling, only one bin was used. Therefore, there was no replicate of the collected data except the measured moisture contents and germinations in May and July 2021.

To compare the temperature and moisture content data measured at various locations inside a 10 m diameter bin, paired t-tests ($\alpha = 0.05$) were performed. The moisture content data measured inside the bin at different times were compared using student t-test ($\alpha = 0.05$). SAS® OnDemand for Academics (SAS Institute Inc., Cary, NC) was used to perform the statistical analyses. To handle the missing data during paired t-tests, elimination of the pairs corresponding to the missing data was adopted. For instance, while comparing the hourly temperatures of the grain near the east and west walls, if the data near the west wall was missing for a particular time, the corresponding grain temperature recorded at that particular time near the east wall was eliminated.

4.1.4 Results

4.1.4.1 *Hot spot inside the bin*

During second week of October 2019, a heavy snowstorm occurred in Winnipeg. As a result, snow could have blown into the bin through the openings around the loading hole. Since the headspace and grain temperature during that period was higher than 0°C (Bharathi et al., 2022b), the snow would have melted and led to the moisture increase at the top center (about 1 m diameter and increased to 14.2% on the surface of the grain bulk). Condensation was not observed during this period. Thus, the melted snow might have resulted in mould growth and multiplication, and a hot spot was probably initiated at the location (could not prove at that time because there was no temperature sensor at the location). Later in February 2020, a massive snow leakage occurred through the openings around the loading hole into the bin and a cone shaped snow pile (about 0.75 m diameter at the cone base) was formed at the center surface of the grain, because the headspace was at sub-zero temperature at that time (Bharathi et al., 2022b). The snow was removed as soon as it was noticed. However, some of snow melted and drastically increased the moisture content (to 22.4% during February 2020) of the surface grain at the snow pile location. The sudden drop in germination at the center location from January 2020 confirms the fungal growth at that location. Since the headspace temperatures were lower during winter, the hot spot did not develop completely until July 2020. The temperature at 1.1 m below the surface (S1) at centre increased from July 15, 2020 and reached the highest temperature 35.4°C on August 31, 2020. Meanwhile, inside the hot spot, the insects *(C. ferrugineus*) multiplied (Bharathi et al., 2022a). As the hotpot developed and was enlarged, the temperature at the S1 HR location of the bin increased and reached the temperature as similar to that of the S1 centre location during the last week of August 2020 (Figure 4.2). The highest temperature recorded at S1 was 37.2°C at HR2 location during September 3 - 5, 2020, which was the highest grain temperature recorded during the period of study. During September 2020, the S1 highest temperatures at centre and HR2 were 1 and 2.8°C higher than the maximum ambient temperature recorded in 2020 (34.4°C on July 3, 2020), respectively. From the first week of September 2020, the temperature at HR (HR 1 to 6) and centre locations started to drop; however, the rate of temperature drop at the center location was lower than those at the HR locations (HR 1 to 6). These drops in temperatures implied that the hot spot gradually ceased to exist. The grain temperature slowly reduced and reached as low as 4.5 ± 0.3°C (average) on March 10, 2021, at the S1 HR locations and 7.8°C on April 20, 2021, at the centre. These were the minimum grain temperatures reached during Cold Temperature Period 2021 on S1 locations. This temperature drop was caused by the decreased headspace and ambient temperature. Different drop rates at different locations in the same layer might be caused by different grain depths (because S1 sensor at the centre location was at 1.1 m below from the surface, while those at HR locations were at 0.7 m below the grain surface) as well as different distances from the sidewall. Therefore, the end of hot spot development period was considered until the end of October 2020. However, the gradual drop in germination of the grain at the centre (L3 and L4) and half radius locations (L2) from November 2020 implied that the higher concentration of mould in the hot spot could have affected the grains in the boundary of the hot spot over time. Unfortunately, the temperature of the grain at the S2 centre location was not recorded throughout the experimental period. However, the temperature measured by the Insector® (Figure 4.1) at the centre location at 1.7 m from the grain surface (Bharathi et al., 2022a) from mid-January to the end of October 2021 (Figure 4.2 (S2)) confirms the presence of hot spot near S2. Thus, at the end of this study, the hot spot extended to 2 m at the centre location (vertically) below the grain surface and about 5 m horizontally with an ellipsoid shape (larger near the top surface) (Figure 4.1).

The increase in moisture contents on surface (L1) and 1 m (L2) at centre location (Figure 4.3A) during November 2019 confirms the leakage of snow through the loading hole. The surface grain moisture content changed from 22.4% (February 2020) to 16.7% during March 2020 and maintained 17% in April 2020. When the temperature of grain increased, the moisture content of the surface grain at centre started to drop slowly and reached to the minimum 11.9% in August 2020. The moisture drop might be caused by the transfer of water to the headspace due to the rise in headspace temperature during Summer. The drop in moisture content at 1 m (L2) at centre location from December 2019 also confirmed the hot spot initiated at the centre location.

As a result of the developed hot spot, atypical pattern of moisture and temperature change was recorded inside the wheat bin. Therefore, in order to understand the temperature and moisture profile of the bin under normal storage conditions, the temperature data from October 15, 2019, to October 31, 2020, at centre and HR locations of S1 and the moisture content data during the same period at the surface (L1) and 1 m (L2) below the surface at centre were considered as 'hot spot period' and excluded from the following results, unless otherwise mentioned.

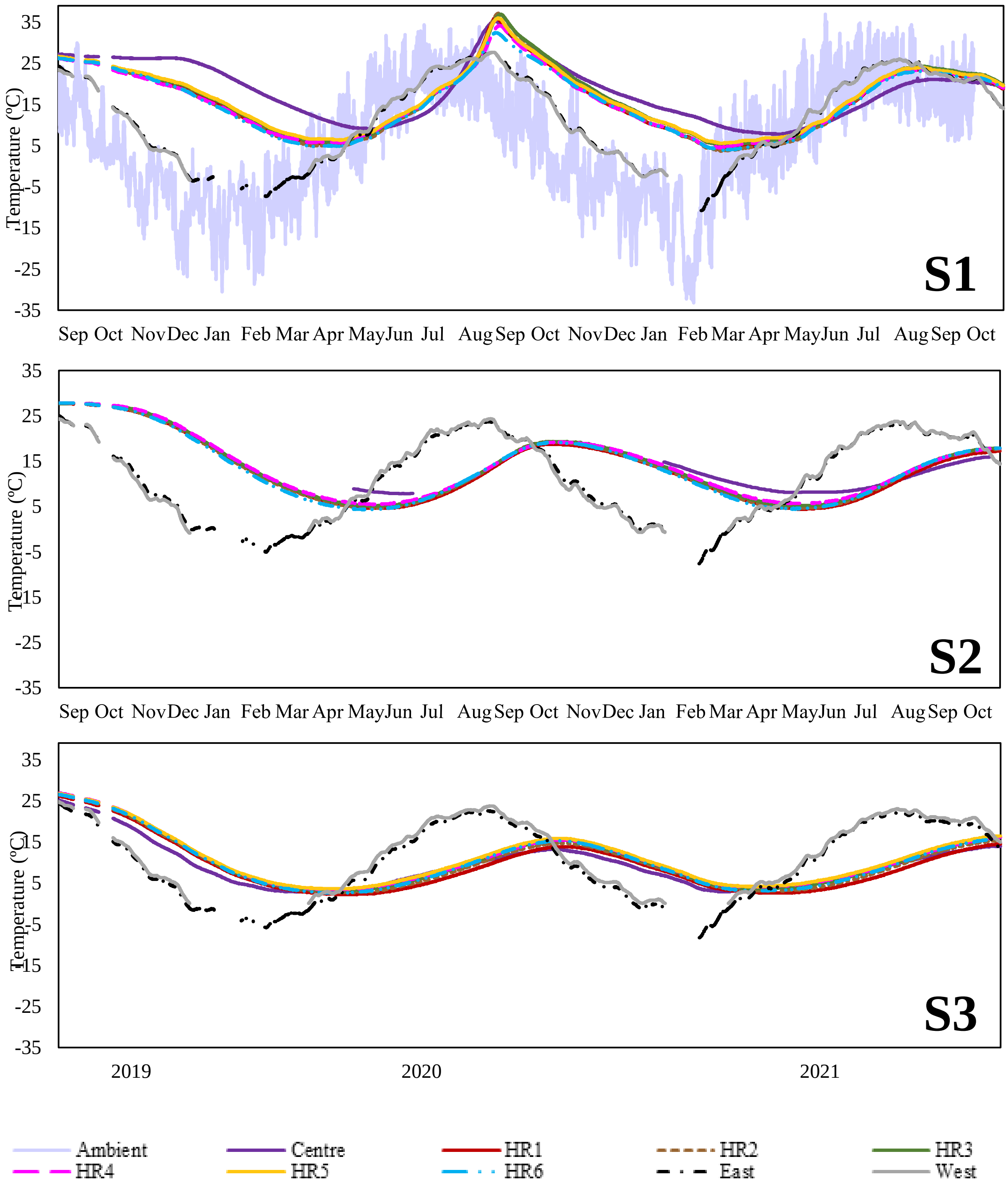


Figure 4.2. Grain temperatures at different locations and grain depth (0.7 (S1), 1.9 (S2), and 3.1 m (S3) below the surface of the grain). Refer Figure 4.1 for the cable locations. The sensors along the center cable were at 1.1 m (S1), 1.7 m (S2) and 3.5 m (S3) below the grain surface.

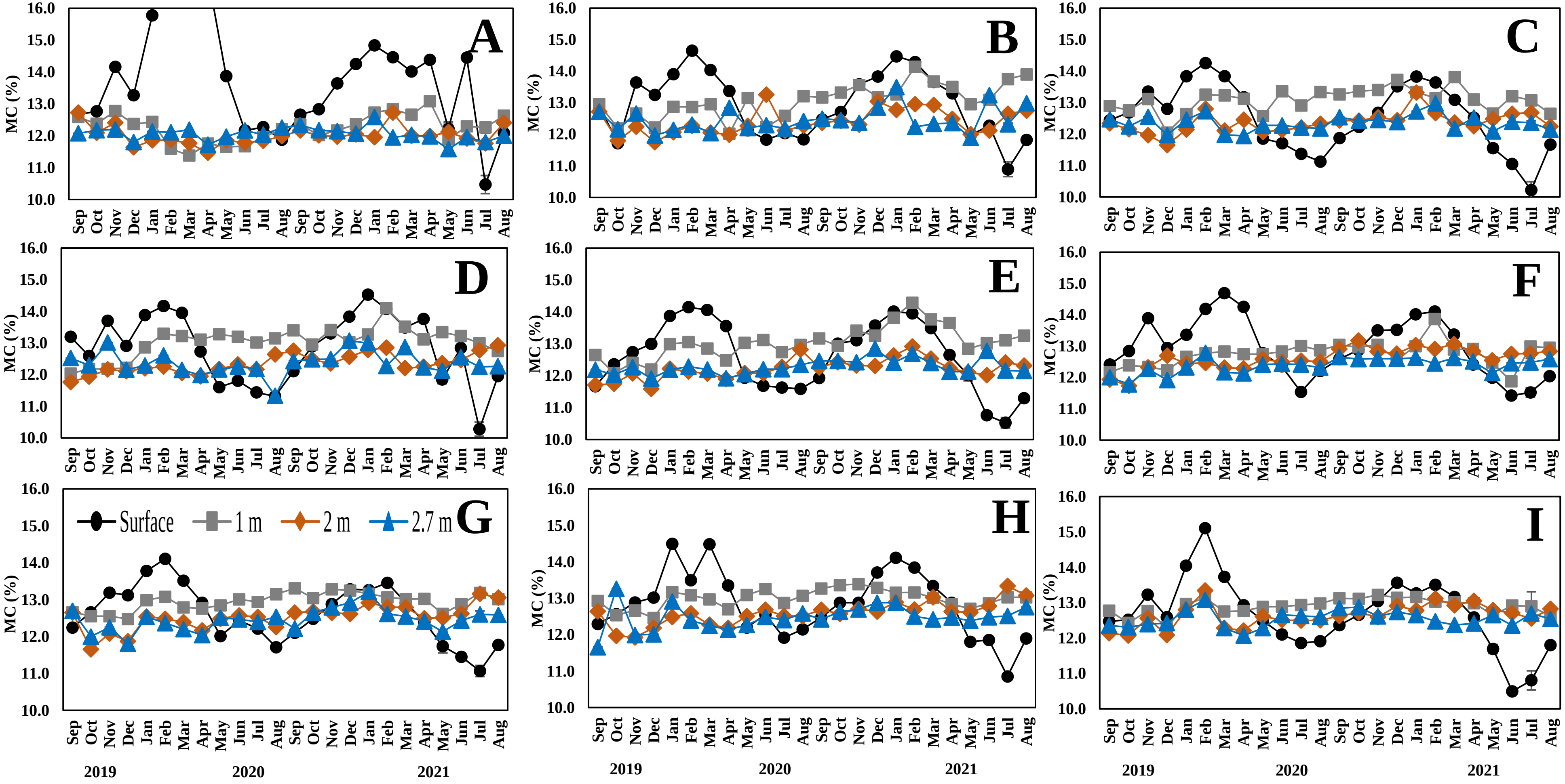


Figure 4.3. Grain moisture contents at different locations and grain depths. A represents the centre of the bin. B, C, D, and E represent the locations at 2.5 m from the north, south, east, and west sides of the wall, respectively. F, G, H, and I represent the locations at 0.15 m from the north, south, east and west sides of the wall, respectively. Surface, 1m, 2m and 2.7 m refer to L1, L2, L3 and L4, respectively.

4.1.4.2 *Grain temperature*

a. Maximum and minimum grain temperatures

The temperature inside the grain changed with change in ambient temperature (Figure 4.2). The grain near the bin wall and the surface followed the ambient temperature and exhibited a substantial change in temperature as compared to those inside the grain bulk. The rate of change of grain temperature varied with varying locations inside the grain. Lower temperature fluctuations were observed at HR locations of S2 (Standard deviation ± 6.5°C) and centre and HR locations of S3 (Standard deviation ± 5.6°C), as compared to those near the walls (Standard deviation ± 8.6°C), during the 26-month study period. During the Warm Temperature Period, the highest grain temperature was at the west wall, followed by those near east wall, HR, and centre (Figure 4.4). The highest grain temperature recorded at centre, HR, east and west wall of S3 were lower than their corresponding locations of S2 and S1. These results indicated that the grain near the boundary of the bin experienced a highest temperature change than those inside the grain bulk. The temperatures of the grain at centre and HR of S1 (near the surface) took about 2 mo to reach their highest temperatures, after the ambient reached its highest temperature. The grains at centre and HR of S2 and S3 reached their highest temperatures during the month of October in 2020 and 2021 (about 3 and 4 mo after the ambient reached its highest temperatures, respectively). During 2021, the grains at centre and HR of S1 reached their highest temperatures on August 20, 2021.

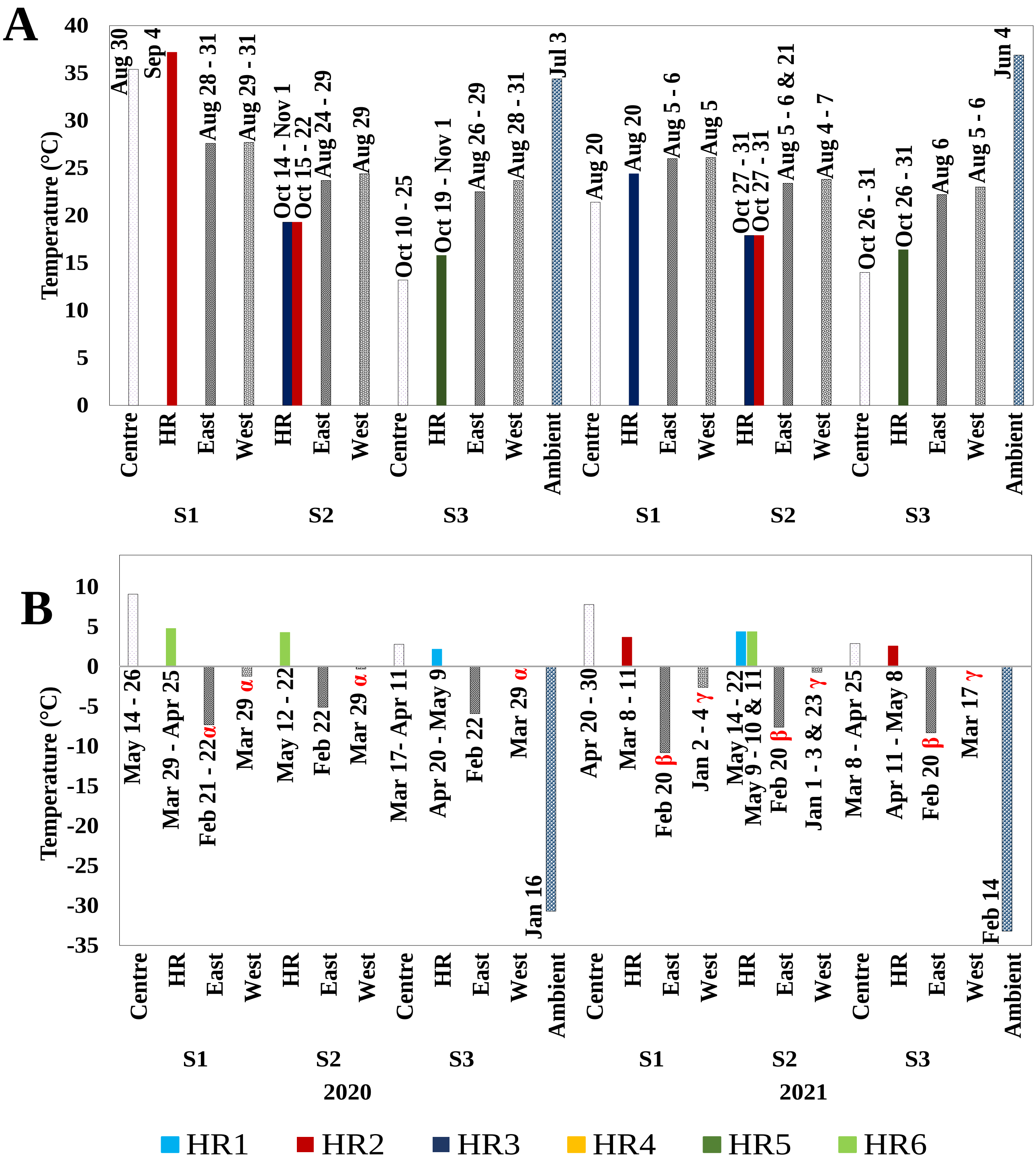


Figure 4.4. Maximum (A) and minimum (B) grain temperatures recorded at various locations with different depths from the surface of the grain, along with their date of occurrence. In the graph, α, β and γ (red bold letters in Figure 4.4 b) imply that the data from January 1 to March 28, February 3 to 19, and January 23 to March 16 were missing at the specified location in that particular year. The lowest temperature could have occurred anywhere in the missing time period. Refer Figure 4.1 for the cable locations.

During Cold Temperature Period, the grains near the east wall were the coldest, followed by those near west wall, HR, and centre (Figure 4.4). The S3 minimum temperatures at HR were lower than those of S2 during 2020 and 2021. The S1, S2 and S3 at HR reached their lowest temperatures during March – April, May, and April – May, respectively. Since the data at different locations near the wall were missing during Cold Temperature Period, the exact timeline of the lowest temperatures cannot be compared. Among the lowest temperatures recorded at various locations, S1 of centre location was observed to be the highest during 2020 and 2021.

During the 26-month study period, the lowest ambient temperatures recorded was -33.2°C on February 14, 2021. The recorded lowest grain temperature was -10.8°C at 0.6 m from the wall in the east side of the bin at S1 on February 20, 2021. Thus, the wheat grain near the walls did not reach the lowest ambient temperature. The temperatures inside the grain at HR and centre were higher than 2.2°C, at all layers and the temperature was higher than 7.8°C, at least at one of the measured locations, at a given time, during the 26-month study period.

The minimum grain temperatures near the walls in 2021 were lower than those in 2020, despite higher ambient temperature in 2021 Cold Temperature Period (-6.6 ± 0.2°C) than 2020 (-8.4 ± 0.1°C). Similarly, the maximum grain temperature near the walls at all layers (S1, S2 and S3) were higher in 2020 than those in 2021, despite higher ambient temperature in 2021 Warm Temperature Period (16.6 ± 0.1°C) than those in 2020 (13.5 ± 0.1°C). This implies that the maximum and minimum grain temperatures reached inside the grain depends not only on the ambient temperature but also on the previous year grain temperatures. Precisely, the higher temperature difference associated in warming up the grain during Warm Temperature and higher temperature difference associated in cooling the grain during Cold Temperature Period might influence the maximum and minimum grain temperatures, respectively.

b. Mean grain temperatures at different locations

The mean grain temperatures increased with increase in distance from the bottom of the bin during the 26-month experimental period. The S1 mean temperatures (including hot spot period) at centre, HR1, HR2, HR3, HR4, HR5, HR6, east and west locations were higher by 9.4, 7.0, 6.3, 6.5, 5.5, 6.1, 5.6, 1.5 and 0.6 than those recorded at S3, respectively. As a result of warming effect of headspace and the hot spot development at the top center (Figure 4.1), the mean temperature of the grain near the surface were higher and near-ambient conditions in the plenum did not affect the grain temperature in a similar way.

Temperatures at south side (HR4) were higher than those at north (HR1) at S2 (Paired t-test; $P < 0.0001$; $t = -356.46$; $N = 18469$) and S3 (Paired t-test; $P < 0.0001$; $t = -411.69$; $N = 18469$) by 0.7 and 1.4°C, respectively. These results indicated that the grain in the south side of the bin was hotter than that in the north side of the bin. At S1, the hourly temperatures of HR1 and HR4 recorded during the study period were significantly different (Paired t-test; $P < 0.0001$; $t = 14.44$; $N = 18469$); however, the mean temperatures of HR1 and HR4 (North and South, respectively) during the study period were same (15.6 ± 0.1°C). The atypical pattern at HR1 and HR4 locations of S1 could have been caused by the developed hot spot and the influence of headspace. At S3, the grain near the west wall was hotter (Paired t-test; $P < 0.0001$; $t = -379.62$; $N = 14762$) than those near the east wall throughout the experimental time. While at S2, the grain near the west wall was hotter in Warm Temperature Period, by 0.3°C, in 2020 (Paired t-test; $P < 0.0001$; $t = -32.51$; $N = 5135$) and 2021 (Paired t-test; $P < 0.0001$; $t = -81.81$; $N = 5134$); those near the east wall was hotter in Cold Temperature Period of 2020 (Paired t-test; $P < 0.0001$; $t = 71.06$; $N = 1253$) and 2021 (Paired t-test; $P < 0.0001$; $t = 54.16$; $N = 2308$) by 0.9 and 0.6°C, respectively. Similarly, at S1, the grain near the east wall was hotter in Cold Temperature Period of 2020 (Paired t-test; $P < 0.0001$; $t = 44.32$; $N = 1253$) by 0.3°C, whereas during Cold Temperature Period of 2021, the hourly temperatures of the grain near the east and west wall were statistically different (Paired t-test; $P < 0.0001$; $t = 15.19$; $N = 2308$). However, the difference was just 0.1°C. Considering the resolution of the temperature sensor (±0.1°C), the difference is considered negligible. During the Warm Temperature Period, the mean temperatures near the east and west walls were equal in 2020 (16.9 ± 0.1°C) and 2021 (17.6 ± 0.1°C), whereas the hourly temperatures were significantly different in 2020 (Paired t-test; $P < 0.0001$; $t = -7.96$; $N = 5135$) and 2021 (Paired t-test; $P < 0.0001$; $t = -9.75$; $N = 5135$). These results indicated that more solar radiation on the west side of the wall during Warm Temperature Period and more solar radiation on the east side of the wall during Cold Temperature Period significantly contributed to grain temperature differences in the bin. The atypical pattern at S1 could have been due to the influence of headspace temperature on the surface grain.

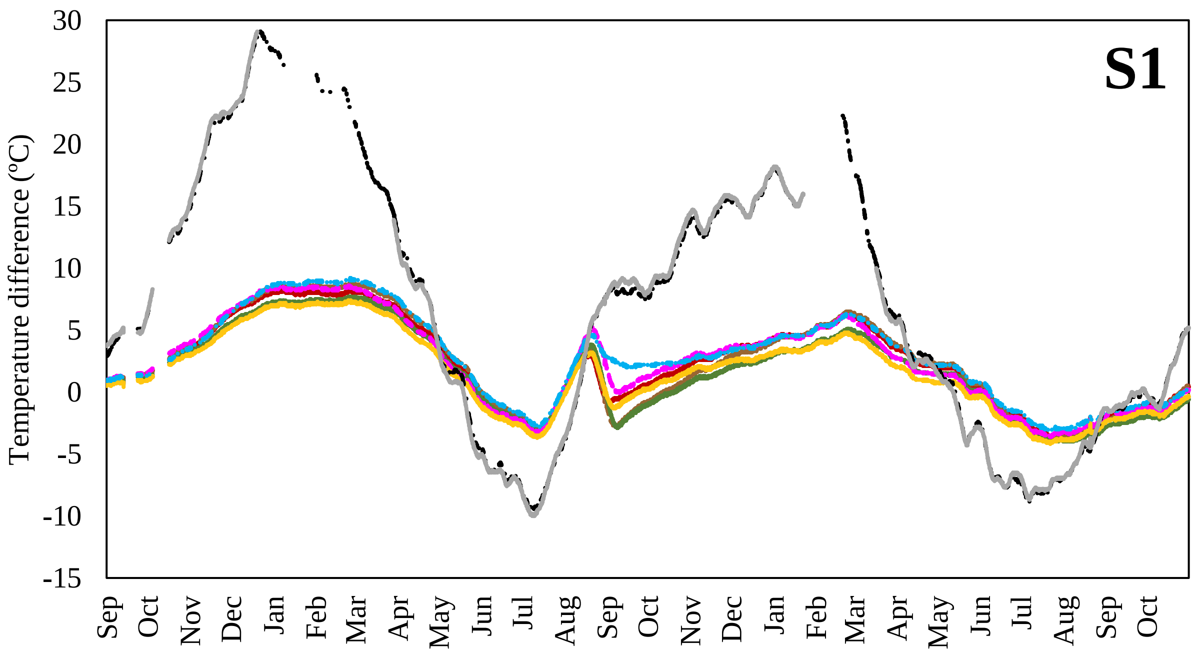
During Warm Temperature Period, the temperature of grain near the wall increased, followed by those at centre and HR locations, at S3. Similarly, in Cold Temperature Period the temperature near the wall dropped first, followed by those at the centre and HR locations at S3. This was because the sensor at HR locations of S3 were about 1 m from the floor and around 2.5 m from the walls, whereas the sensor at centre was 0.6 m away from the floor. The shorter distance between the plenum and the centre location could have influenced the temperature pattern of centre than those at HR locations. One month delay at HR locations (April to May) to reach their minimum temperature during 2020 and 2021, as compared to those at centre (March to April) of S3 also confirms that the change in ambient temperature had higher impact on grains near the centre than those at HR of S3. Moreover, the sensor near the east and west walls were 0.6 m away from the wall. This implies that the effect of solar radiation and ambient temperature on the temperature of the grains near the sidewalls were greater than those near the floor.

Even though the bin was filled with the warmer grain (26.8 ± 0.1°C) initially, and there was a hot spot developed in the top center (Figure 4.1), the temperature of the grain inside the bin dropped over time. The average S1 and S2 grain temperatures at centre and HR locations were higher in year I (November 1, 2019, to October 31, 2020) than their corresponding locations in year II (November 1, 2020, to October 31, 2021). The S3 grain temperatures at centre and HR, in year I, were higher than or equal to those in year II. Contrarily, the temperatures near the walls were higher in year II than those in year I by an average of 0.8°C, at all three layers (S1, S2 and S3). The average increase in ambient temperature by 1.8°C in year II could have influenced the grain temperatures near the wall (Bharathi et al., 2022b). Thus, the temperature of the grain at the centre location of a bin will gradually decrease over storage period.

4. Grain temperature differences/gradients inside the bin

The temperature differences between centre and other locations at S1 and S3 are illustrated in Figure 4.5. The temperature difference between centre and other locations at S2 could not be calculated because of the missing centre temperature data. The S3 temperature difference between centre and HR1 (north) were higher, during Warm Temperature Period and lower during Cold Temperature Period, compared to temperature difference between centre and other HR locations, since the temperature at HR1 were lower than other HR locations. The temperature difference patterns at S1 were different as compared to those at S3, because of the influence of headspace temperature and the hot spot development. The highest temperature difference (29.1°C) at S1 (including the hot spot period) was observed between the centre and walls during December 20, 2019, when the temperature near the walls started to drop due to the decrease of the ambient temperature, while the centre remained warm. Drastic decrease in S1 temperature differences and slight increase in S3 temperature differences were observed in year II than year I. The decrease in S1 temperature differences could be explained by the higher centre temperature as a result of hot spot at S1 in year I and the drop in temperatures in year II. A slight drop in temperatures at half radius and wall locations at S3 could be attributed to the slight increase in S3 temperature differences in year II.

The gradual increase in temperature difference observed at S1 location (Figure 4.5 (S1)) from September 2019, could be attributed to the higher rate of temperature drop at half radius locations than center location due to the influence of headspace, since the sensor at half radius locations were at 0.7 m below the surface, whereas the sensor at center location was at 1.1 m below the surface. As a result, the influence of headspace was higher at half radius locations than those at center location. In addition, the shorter distance of the half radius locations from the sidewall than those at center could have also influenced the temperature drop rate. The increase in temperature difference from mid-July to August 2020 could be attributed to the increase in temperature at the center location (S1) as a result of hot spot development, when the temperature of the grain warmed up in Warm Temperature Period of 2020. Therefore, the increase in grain temperature must have resulted in the growth and multiplication of microorganisms. The sudden drop in temperature difference during last week of August 2020 was because of temperature increase at the half radius location due to enlargement of the hot spot. Later, during first week of September, the temperature difference gradually started to increase because of the influence of headspace temperature at the half radius locations. The atypical pattern in temperature differences at S1 (Figure 4.5 (S1)) were due to the influence of headspace and the hot spot development. On the other hand, Figure 4.5 (S3) followed a typical sinusoidal pattern of temperature difference.



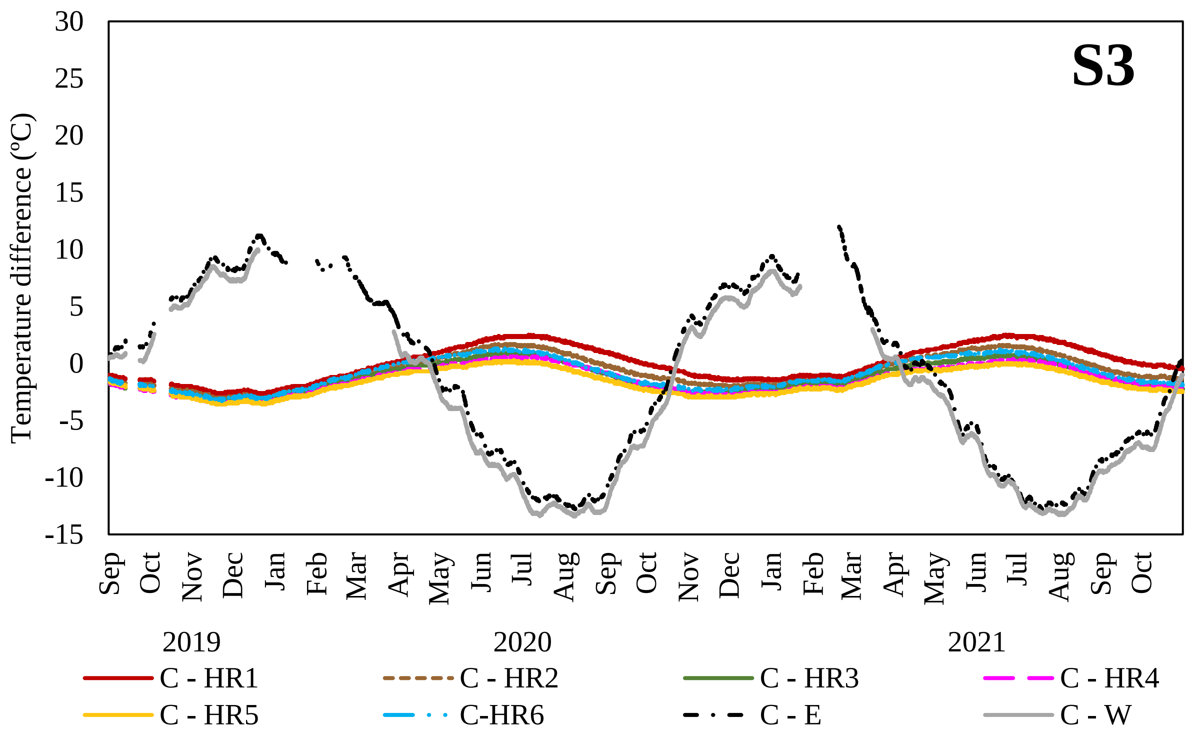


Figure 4.5. Temperature differences between center and other locations at 0.7 m (S1) and 3.1 m (S3) from the surface of the grain. The sensors in the center cable were at 1.1 m (S1) and 3.5 m (S3) from the surface. Refer to Figure 4.1 for the cable locations. Differences > 0 implies that the temperature at center is higher than those at other locations.

4.1.4.3 *Grain moisture content*

The average moisture contents of the grain measured at 36 different locations at the start (August 2019) and the end (August 2021) of the experiment were the same (12.5 ± 0.1%) (Student t-test; $P > 0.7084$; $t = 0.38$; $N = 36$). However, moisture fluctuations occurred during the study period (Figure 4.3). Surface grain had the largest fluctuation of moisture content. The average absolute change in moisture content at all the tested surface grain locations was 0.4 ± 0.0% per month with the maximum of 6.6% and minimum of 0.0%. The maximum moisture change was observed at the centre location due to snow melting. The maximum (15.1%) and minimum (10.2%) moisture contents (excluding the effect of hot spot) were recorded on the surface near the west side of the wall and south half radius location during February 2020 and July 2021, respectively. The average moisture contents of the grain on the surface at south and west sides of the wall were significantly (paired t-test; $P > 0.0021$; $t = 3.47$; $N = 24$) lower than those at north side at both wall and half radius locations. This was because north side was colder than other locations because of the reduced solar radiation. On comparing the grain moisture contents at various locations on the surface with those at 2.7 m below the surface

(L4), surface moisture contents were higher during Cold Temperature Period (due to rewetting) and lower or equal during Warm Temperature Period (due to drying), than those at 2.7 m below the surface (L4). The surface grain at all the tested locations gained moisture during Cold Temperature Period and lost moisture during Warm Temperature Period. An average difference of 1.7% was observed between the moisture contents of the surface grain during Cold Temperature Period (November to April) and those during Warm Temperature Period (May to October). This moisture change pattern was similar to the result reported by Jian et al. (2009). The average moisture content differences between Cold and Warm Temperature Periods inside the grain bulk (L2, L3 and L4) were insignificant. The average moisture contents of the grain sampled at various locations at L1, L2, L3 and L4 were 13.7 ± 0.1, 13.0 ± 0.0, 12.4 ± 0.0 and 12.4 ± 0.0%, respectively, during Cold Temperature Period; and 12.0 ± 0.1, 12.8 ± 0.0, 12.4 ± 0.0 and 12.3 ± 0.0%, during Warm Temperature Period. The moisture content of the grain at 1 m depth from the surface (L2) were higher than or equal to those at 2 m (L3) and 2.7 m (L4). The maximum moisture changes of 1.6% occurred at a particular location inside the grain bulk (L2, L3 and L4), which is in accordance with the reports of Jian and Jayas (2022), according to which the maximum moisture content increase of 1.5% can occur at a location inside a stored bin because of the convection currents.

4.1.4.4 *Grain germination*

The initial germination of the wheat was 98.4 ± 0.4%. The germination of the grains near the surface and 1 m below the surface dropped below 85%, at least at one location, within 4 months (December 2019). The germination of the grain at the top centre dropped drastically below 60% from January 2020. The hot spot initiated as a result of snow leakage near the top centre could have led to mould growth and higher insect activity, which in turn could have resulted in germination drop. The germination was above 70% at all sampled locations, except the centre, during the first year. Even though the moisture content at half radius locations was not influenced by the developed hot spot, the quality of the grain at 1 m depth (L2) at the half radius location and at 2 m (L3) and 2.7 m (L4) at centre dropped gradually below 60% from December 2020, as a result of extended hot spot development at these locations. The germination near the walls was above 60% throughout the study. Insects at walls had less activities than that at other locations because of the lower temperature near the walls during Cold Temperature Period (Bharathi et al., 2022a). Moreover, the hot spot developed did not influence the grain near the walls.

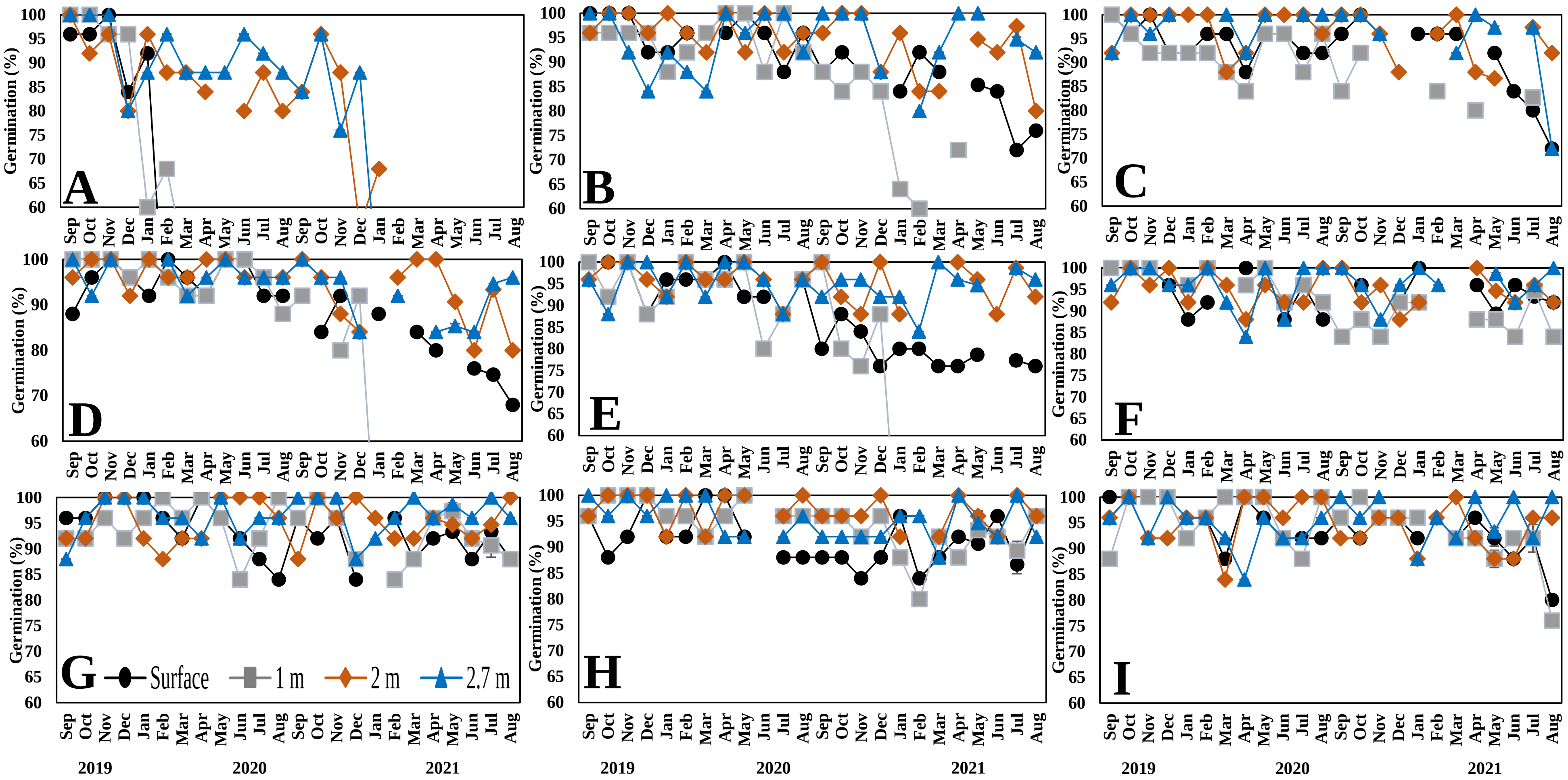


Figure 4.6. Grain germination at different locations and grain depths. A represents the centre of the bin. B, C, D, and E represent the locations at 2.5 m from the north, south, east, and west sides of the wall, respectively. F, G, H, and I represent the locations at 0.15 m from the north, south, east, and west sides of the wall, respectively.

4.1.5 Discussion

4.1.5.1 *Hot Spot development*

In the current study, the initiation of hot spot was identified from the following observations 1) the temperature recorded at S1 centre location (1.1 m depth) was hotter than the temperature recorded at half radius locations at S2 (1.9 m depth) from the first week of November 2019, even though the ambient and headspace temperatures were gradually decreasing. 2) Unlike all other locations, the temperature at S1 centre location stayed almost constant (26.3°C) from the first week of November to mid-December 2019; while the temperature at all other locations dropped because of drop in ambient temperature. This implies that even though the drastic increase in temperature of the grain was not observed because of hot spot development, the grain near the top centre remained warmer irrespective of the change in headspace temperature. 3) The sensor at the centre cable was located at 0.85 m away from the exact centre of the bin along the North direction. According to Sinha and Wallace (1965), during the hot spot development stage, the temperature at the centre of the hot spot can reach 65°C and the grain that is 45 cm away from the hot spot centre can still be at 10°C. Thus, the temperature at other locations near the top centre could have possibly been warmer than the measured temperature. 4) Mouldy/ dark-colored kernels were observed from the samples collected at the top centre location for the moisture content and germination analyses. 5) Germination of the grains at 1 m below the surface (L2) at the centre location dropped to 60% during January 2020. However, the increase in moisture content was not observed at the hot spot location during this period. This could be because of the following reasons: (i) low initial grain moisture content (12.5%), (ii) sampling was performed at exact centre of the bin and at 2.5 m distance from the centre. However, increase in moisture content could have occurred at any other location in between the centre and 2.5 m distance. (iii) the hot spot was located near the top surface of the grain bulk and hence, the moisture produced in the hot spot location would have moved into the headspace and not into the grain, (iv) as a result of the shallow grain depth (4.1 m) and the resistance provided by the presence of perforated floor with the tarp, the air moved vertically downward near the walls during Cold Temperature Period could have moved horizontally at the bottom and reached the bottom centre of the grain bulk and then, moved vertically upward at the centre (v) the convection currents produced as a result of high temperature in the hot spot would be high (Jayas, 1995). As a result of the convection currents, the moisture inside the grain could have moved up into the headspace (Bejan, 1978; Smith and Sokhansanj, 1990). Moreover, during unloading of the grain in November 2021, we observed a chunk of dark colored grain of size more than 1.5 m high and 1 m diameter (approximate). The temperature data and the visible grey-colored grains observed at the centre and half radius locations confirm that: 1) the mould formation and temperature increase had affected the grain for about 5 m diameter and 2 m depth; 2) the moisture increase mainly occurred in the area in contact with snow water; and 3) the development of hot spot did not significantly increase the moisture content of the dry grain. Moreover, the flowability of the grain at the core of the hot spot (about 1 m diameter) were nil (because of the caking); whereas the grain at the boundary of the hot spot were free-flowing even if the germination has dropped below 60%.

The high temperature and moisture content in and around the hot spot attracted insects and facilitated the multiplication of *C. ferrugineus*. Sinha and Wallace (1965) observed drop in temperature of the hot spot as a result of inactivation of fungi at higher hot spot temperature (e.g., higher than 40°C) and by drying of the grain due to convection currents induced by the hot spot. In our study, the hot spot gradually disappeared when ambient temperature decreased. The low headspace temperature during winter might have cooled-down the hot spot present at the top centre. The higher headspace relative humidity did not influence the moisture content of the grain inside the hot spot since the air would equilibrate with the surface grain first. Another reason for the ceased hot spot might be that the grain at the boundary of the hot spot did not increase its moisture content, so microorganism could not be extensively developed.

4.1.5.2 *Grain temperature*

In the present work, larger temperature fluctuations were observed near the boundaries of the bin than those inside the grain bulk. Several researchers (Jayas et al., 1994; Jian et al., 2005; Lo et al., 1975) also concluded that the rate of change in grain temperatures decreased with increase in distance from the wall. In the current study, wheat at 0.6 m away from the walls reached highest yearly temperatures during last and first week of August 2020 and 2021, respectively, at all three layers (S1, S2 and S3). This was because the highest ambient temperature recorded in 2021 (first week of June) was a month earlier than that recorded in 2020 (first week of July). The thermal diffusivity and conductivity of wheat are lower as compared to those of the silo wall (galvanized steel). Precisely, the thermal diffusivity of wheat and galvanized steel are 1.09×10^{-7} and 1.2 to 1.8×10^{-5} m^2/s, respectively; while, the thermal conductivity of wheat and galvanized steel are 0.181 to 0.202 W/(mK) and 40 to 60 W/(mK), respectively (Chang, 1986; Jayas et al., 1994; Jian and Jayas, 2022). The lower thermal diffusivity of wheat explains the longer time taken to reach the ambient temperature, while the lower thermal conductivity of wheat could be attributed to the lower highest grain temperature reached near the walls (27.7°C), than that of the ambient (36.9°C). A time lag of two months were reported between the highest temperature reached near the wall and those at centre inside a 6.6 m diameter corrugated steel bin containing wheat (Chang et al., 1993). According to Jayas et al. (1994), the time lag between the ambient and the centre temperature increases with increase in bin diameter. The current study confirmed those conclusions.

In a study involving monitoring of temperatures at various locations inside two 6.6 m diameter bins filled with wheat, Chang et al. (1993) reported that the temperatures of the grain at 0.3 m from the south wall of the bin were higher by 3.5°C than those from the north wall as a result of solar radiation. The result of the current study also confirms that the grain along the south side of the bin were hotter than those along the north side. In the current study, diurnal variation in temperature was not observed near the walls (measured at 0.6 m away from the sidewalls), since the grains that are within 0.15 m from the wall are only affected by the diurnal temperature (Converse et al., 1973; Jayas, 1995). Moreover, Chang et al. (1993) also reported that the average temperature of grain at 0.3 m from the bin floor were lower than those at 0.3 from the surface, which is in line with the findings of this study where the mean temperatures of S1 (grains near the surface), were higher than those of S3 (near the plenum). Our results also confirm that the temperatures of the grain near the plenum change slowly as compared to those at the walls and the top surface (Muir and Jayas, 2000).

This study demonstrated an atypical pattern of temperature distribution in a farm bin due to the hot spot development. Even though nine temperature cables were installed in the bin, the development of the hot spot was not clearly identified at the initial state of the hot spot development. The temperature difference because of hot spot was observed from the measured grain temperature only during summer months (from mid-July) and the hot spot development was observed for three months. The hot spot ceased by itself even though it was located at the top center of the grain bulk and the size of the hot spot reached nearly 5 m diameter. Therefore, monitoring grain quality by using only temperature cables was not enough. Verified mathematical model should be used to detect the atypical pattern of temperature distribution at the early state of the hot spot development. Other monitoring methods such as sampling and CO_2 monitoring should be also used.

4.1.5.3 *Grain moisture content*

Wallace and Sinha (1962) examined the temperature, moisture, germination and fungal relation of wheat samples collected from various granaries in Manitoba and Saskatchewan during fall and winter months of 1957 to 1960 and reported that the grain at the surface were the dampest in all the tested bins. Lo et al. (1975) predicted that the moisture content of the surface grain inside a grain bin increases during fall and winter and decreases during spring and summer. The results of the current study also show that the moisture contents at the surface of the grain bulk were higher during Cold Temperature Period and lower during Warm Temperature Period. The surface moisture content increased by an average of 3.3 ± 0.4 % from February (winter) to July (summer) during 2020 and 2021, while the average moisture content change at all other locations during the same period was 0.2%. Chang et al. (1994) reported decrease of 2 to 2.5 percentage points on moisture content of wheat near the top surface during summer and less than 0.2 percentage points moisture content change in all other layers, inside a 6.6 m diameter corrugated steel bin with aeration. The results of the current study are in line with the observations of moisture fluctuation study conducted inside a metal silo filled with 20 t of wheat for a period of 15 months (Jian et al., 2009). In the current study, the change in moisture content on the surface of the grain bulk could be explained by two phenomena: 1) the influence of headspace environment on the surface grain. Briefly, as a result of warmer headspace temperature during Warm temperature Period, the surface grain lost moisture (drying) and because of lower headspace temperature and high RH, the surface grain gained moisture (rewetting), during Cold Temperature Period. 2) During Cold Temperature Period, as a result of temperature gradient, the warmer air inside the grain bulk absorbs moisture and moves upward. When warmer air encounters the colder grain near the surface, it releases the moisture to the grain. This phenomenon explains the increase in surface moisture, especially at centre location, during Cold Temperature Period. Obaldo et al. (1991) found that the moisture content of the grain on the top surface of corn was predominantly influenced as a result of change in ambient air conditions. On comparing the moisture migration, temperature fluctuation and natural convection flow of corn and wheat, both the grains exhibited similar pattern (Khankari et al., 1995a). However, due to the lower permeability of wheat (between 1.15×10^{-8} and 7.29×10^{-9} m^2) than corn (between 1.30×10^{-8} and 3.03×10^{-8} m^2), the moisture accumulated at the top of the wheat were lower than those in corn (Khankari et al., 1995a; Montross and McNeill, 2005).

The moisture migration as a result of temperature gradient leads to increase in moisture of the grain at few centimetres below the surface and the centre surface, during winter and fall; whereas during spring and summer, the high moisture grains are found at few centimetres below the surface of the grain or near the bottom of the bin, depending on the temperature gradients and distribution patterns (Jian and Jayas, 2022). The moisture migration inside a stored grain bin occurs as a result of change in ambient conditions and the rate of change depends on the size of bin (Khankari et al., 1995a). According to researchers including Stewart (1975), Jayas (1995), and Khankari et al. (1995b), the moisture accumulates near the bottom during summer because of the convection air flow induced by temperature gradient inside the bin. Hellevang (1987) reported increase in moisture content by up to 0.4% and 0.2% in the grain at 0.6 – 1.8 m deep and those at the bottom centre, respectively; whereas, the surface grain decreased about 2.6% moisture content, in 16 farm bins in North Dakota from April to August. Muir et al. (1980) reported the decrease in moisture content by 1% in the grain at the peak of the cone and increase in moisture content by about 1.5% at 0.3 m below the peak, in five bins containing 25-t wheat and one bin containing 50-t wheat. In the current study, the moisture migration pattern observed was different from those reported by previous researchers. The moisture migration pattern in the current study showed that the moisture migrated from the bottom to the top. During Cold Temperature Period, the cold air could have moved downward along the walls. Because of the sub-zero grain temperatures near the wall, the air does not have a large capacity to hold water. So, grain at the walls would not change moisture. However, when the air reaches the warmer grain near the bottom centre of the grain bulk, it could have absorbed moisture from L3 (2 m) and L4 (2.7 m) and lost moisture to the grain at L2 (1 m) and L1 (surface grain). This explains the moister grain at 1 m depth during Cold Temperature Period. During Warm Temperature Period, the air near the walls moved upward, while the air at the center moved down as a result of warmer temperature near the walls (Jian and Jayas 2022). These explained the moisture increase at the surface and top center of the grain bulk.

4.1.5.4 *Grain germination*

Even though the initial moisture content of the grain used in the current study (12.5%) was less than the safe moisture content of wheat (14.5%) for storage up to a year (Jayas and White, 2003), the leakage of snow into the bin resulted in the heavy spoilage in the bin. This obviously changed our experimental plan. The germination at the top centre dropped from January 2020. It could be mainly because of three reasons: a) fungal and insect multiplication, b) temperature and moisture increase, and c) grain spoilage at the high temperature and moisture content. Wallace and Sinha (1962) and Sinha and Wallace (1966) also observed negative correlation between germination of the grain and the multiplication of storage fungi. The reason for delay in germination drop near the wall could be attributed to the lower temperature as well as the moisture content at the walls in most of the storage time. This low temperature and safe storage moisture content also limited the insect activity (Bharathi et al., 2022a) and fungal multiplication. According to Sinha and Wallace (1966), the outbreak of insects (*Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *C. ferrugineus*) leads to the development of hot spot in stored grain. However, in the current study, the outbreak of insects did not lead to the hot spot development. Temperature and moisture content were the main factors influencing the wheat quality in our study, and multiplication of fungi extremely changed the grain temperature and moisture content. From this study, it can be inferred that compared with fungi, the multiplication of insect might not directly change grain moisture content and temperature.

This study demonstrated the complexity of the grain temperature, moisture content, and germination change in a large farm bin. To understand the complexity of a stored grain ecosystem, comprehensive mathematical model should be developed and verified by using measured field data. The data collected in this study could be used to validate models in an un-aerated bin with a plenum as boundary condition since most of the previously developed models used soil as the boundary condition (Alagusundaram, 1989; Jian et al., 2005). Also, most aerated bins have plenum and comprehensive model must combine mathematical models that predict temperatures and moisture contents for both aerated and non-aerated periods.

4.1.6 Conclusion

From the results of the study, the following conclusions can be drawn:

1. Temperature and moisture on the surface of the grain fluctuated more as a result of change in ambient conditions and the convection currents developed as a result of temperature gradients.
5. The temperature inside the tested 10 m diameter bin was above 7.8°C, at least at one location, at a given time, during the 26-month experimental period. Therefore, both rusty grain beetle and red flour beetle can survive the winter in this bin.
6. The hot spot was mainly developed as a result of fungi multiplication due to increased moisture from moisture migration or snow and not because of the high insect activity.

7. Germinability of the grain near the walls dropped later than those near the surface and inside the grain bulk, in the presence of insect infestation.
8. The grain at the boundary of the hot spot were free flowing even if the germination has dropped below 60%, however, the grain at the core of the hot spot were sticky and non-flowing.
9. The moisture content at 1 m deep was higher than those at 2 and 2.7 m.
10. Drop in ambient temperature could either cease the hot spot or lower the grain temperature.
11. Even though high moisture content could be responsible for the formation of hot spot, the hot spot could spread to wide areas with low moisture content as well, in the presence of insects and microorganisms.

4.1.7 Author contributions

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

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4.2 Movement and distribution of *Cryptolestes ferrugineus* (Stephens) and *Tribolium castaneum* (Herbst)

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4.2.1 Abstract

Movement and distribution of *Cryptolestes ferrugineus* and *Tribolium castaneum* inside a 10 m diameter corrugated steel bin containing 300 t of wheat (initial moisture content of 12.5 ± 0.1%), were monitored by using Insectors® (electronic traps/ e-traps) installed at 65 locations, from September 6, 2019, to October 31, 2021, in Winnipeg, Canada. The data from only 38 locations were available throughout the experimental period due to the failure of some Insectors®. During the first and second week of September 2019, *C. ferrugineus* adults (combined insect density of 0.25 A/kg) were introduced on the centre surface of the grain in two equal batches. During the second week of September 2020, the same number of *T. castaneum* adults (insect density of 0.25 A/kg) were introduced at the same location in two batches. The temperature inside the bin was higher than 7.8°C, at least at one location throughout 26 months. At the end of the study, both *C. ferrugineus* and *T. castaneum* survived inside the bin. No activity of *C. ferrugineus* was recorded near the sidewalls at 0.1 m depth from the grain surface, from February to April 2020, when the temperature dropped below 2.5°C. The activity of *C. ferrugineus* was recorded when the temperature increased above 4.5°C. For *T. castaneum*, the insect activity was not recorded near the sidewalls at 0.1 m depth when the temperature dropped below 22°C in mid-October 2020 and the activity was recorded when the temperature increased above 22°C in mid-July 2021. Eventhough, adult *T. castaneum* were mainly captured at top grain layer (about 1 m depth from the grain surface), they could be captured at any place of the bin. The insect activity near the sidewalls reduced drastically because of the temperature drop. The insect counts as well as the insect activity increased with increase in temperature. Therefore, highest insect counts were recorded inside and around the hot spot for both insects.

Keywords: Grain bin; Insect activity; Temperature gradients; Moisture migration; Wheat storage

4.2.2 Introduction

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), and red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) are cosmopolitan pests that are often found in western Canadian grain bins (Madrid et al., 1990). The *C. ferrugineus* feeds mainly on stored wheat, barley, and other cereals (Rilett, 1949), while *T. castaneum* feeds on stored flour, broken wheat, and other milled products (Cotton, 1956). On undamaged grain, *T. castaneum* exhibits reduced fecundity, low survival, and delayed development rates (Li and Arbogast, 1991). Both are facultatively predaceous and scavengers (Sinha, 1965, 1966) and have about 60- to 70-fold increase rates per month (Fields and White, 1997). *Cryptolestes ferrugineus* is cold-tolerant species (Fields, 1992), with adult being the most cold-tolerant stage (Smith, 1970). On the other hand, *T. castaneum* is cold-susceptible species (Fields, 1992). Eggs and larvae are the most cold-tolerant stages of *T. castaneum* (Arthur et al., 2015). When the temperature was reduced from 25 to 10°C, *C. ferrugineus* adults were found to survive at the end of 40 weeks, while *T. castaneum* adults did not survive at 10°C, at the end of 40 weeks. The 50% acclimated adults of *C. ferrugineus* were found to survive -10°C for a maximum of 40 d (Fields, 1992). The survival rate of the insects varies with the degree of acclimation. *Cryptolestes ferrugineus* tolerates relative humidity as low as 10%, while *T. castaneum* tolerates relative humidity as low as 1% (Howe, 1965).

The presence of *C. ferrugineus* usually can result in the decrease of *T. castaneum* population (Lefkovitch, 1968). This could be attributed to the exposure of different life stages of *T. castaneum* to cannibalism, while the larval and pupal stages of *C. ferrugineus* are protected under the seed coat of grain kernels. Owing to the larger size of *T. castaneum* adults, they exhibit more cannibalistic behaviour than *C. ferrugineus* (Suresh et al., 2001).

Spatial patterns in stored grain bulks are one of the important properties that define the ecological characteristics of insects. Knowledge of three-dimensional spatial and temporal distributions of insects aids in developing effective integrated pest management protocols. Few reports on the spatial distribution of *C. ferrugineus* and *T. castaneum* in stored grain bulks have been published (Jian et al., 2011, 2012a). However, those research were performed in small sized storage units (<50 t grain) for a shorter period of time (< 3 mo). Smith (1978) has introduced *C. ferrugineus* larvae inside two metal granaries containing 27.3 tonnes of wheat and studied the changes in insect density for a period of 4 years. At the end of the study, it was concluded that the biological activity of the low-density *C. ferrugineus* (< 5 insects/kg) did not initiate heating inside the granaries. However, the size of the granaries used were very small for insect movement and distribution because (1) larger the size of the bin, higher the time lag between the ambient and centre grain temperature (Jayas et al., 1994) and (2) grain could be warm enough for the development and multiplication of insects during winter months in large bins. On surveying 83 unique wheat storage bins, during 2011-2013, at various locations across Southwestern Ontario, Limay-Rios et al. (2017) reported that the median capacity of the most of the bins was 300 tonnes. To understand the distribution of insects in a stored bin, it is important to conduct a study in a larger grain bin for a longer period.

To detect the infestation of insects and to understand their distribution pattern, several manual and mechanical devices have been developed in the past few decades. The use of grain probes and traps are most commonly adopted (Lippert and Hagstrum, 1987; Loschiavo et al., 1986; Toews and Phillips, 2002). One of the commercially available electronic versions of pit-fall trap, patented as the Insector® (OPI Systems Inc., Calgary, AB, Canada) has been used to monitor activities and densities of *C. ferrugineus* inside stored wheat (Jian et al., 2012b). When the insect moves inside the bin and enters one of the many openings on the body of the Insector®, they fall into the receptacle section, which contains two pairs of infrared beams. Then, a microcontroller chip analyses the signals and provides information on the size and shape of the insects based on the amount of light blocked from the beam. The chip also records the temperature and time of occurrence and sends all the information to the computer. Based on the size of different insects recorded on the computer system, the Insector® system categorizes the captured insects into different groups of insects. The insects with the similar size and shape (e.g., red flour beetle and confused floor beetle) are categorized into the same group. On the other hand, insects such as rusty grain beetle and red flour beetle (like the present study) are categorized into different groups due to their different sizes and shapes (Shuman, 2003). Jian and Larson (2006) reported a strong correlation, with correlation coefficient of 0.97, between the daily count of Insectors® and the insect density around the Insector® in 25.4 cm range.

Temperature and moisture content of the grain are the major factors that affect the movement and distribution of insects as well as their development and fertility. Insects prefer to move towards the warm temperature locations than their surrounding cold temperature locations, for their survival, growth, and reproduction, owing to the reason that insects are cold-blooded, and their body temperature changes as the ambient temperature changes. The initial growth rate and peak density of *C. ferrugineus* depends mainly on the temperature (Kawamoto et al., 1989).

The response of adult *C. ferrugineus* and *T. castaneum* to various temperatures, moisture contents and their gradients have been well established (Flinn and Hagstrum, 1998; Jian et al., 2003, 2004, 2005a). Adults of *C. ferrugineus* detected the temperature difference in less than an hour (Jian et al., 2004). To understand the distribution of insects with respect to changes in temperature and moisture content, the temperature and moisture profiles of the wheat stored in the 10 m diameter corrugated steel bin had been reported in part I of this article (Bharathi et al., 2023).

The objectives of this study were to (i) determine if the most cold-tolerant (*C. ferrugineus*) and the least cold-tolerant (*T. castaneum*) species found in the western Canadian stored grain ecosystem can survive the winters inside a 10-m diameter grain bin; (ii) to investigate the three-dimensional distribution of *C. ferrugineus* and *T. castaneum* inside a grain bin containing 300 tonnes of wheat for a period of 26 months with known initial insect density.

4.2.3 Materials and methods

4.2.3.1 *Grain bin and wheat*

Information on the grain bin and the wheat used for the study has been reported in Part I of this article (Bharathi et al., 2023). However, for easy reading, the information is summarized here. The 10 m diameter flat-bottom cylindrical corrugated steel bin consisted of a cylindrical part of height 5 m and a conical roof of height 3 m with 0.9 m opening at the centre of the roof (for the purpose of loading) and four manholes. Centre opening, and all manholes had lids and all lids of the openings were closed during the entire period of this study (except during the short sampling periods). A 0.35 m deep plenum and a perforated floor were installed above a 0.5 m high concrete foundation. To prevent insects from running out of the bin through the perforated floor, one layer of poly tarp (with less than 1 mm holes size) was laid on the floor before wheat loading. A chute and two auger conveyors were attached near the floor for the purpose of unloading. No. 2 Canada Western Red Winter Wheat (AAC Goldrush) was used and the moisture content of the wheat at the beginning of the experiment was 12.5 ± 0.1% (w.b.). A total of about 300 t of wheat was loaded into the bin on five alternate days (from August 7 to 15, 2019), with about 60 t loaded on each day. The total grain depth was 4.1 m. Nine cables consisting of temperature and relative humidity sensors were installed inside the bin to measure the temperature and relative humidity inside the grain and the headspace. A total of 38 thermocouples were installed for measuring bin sidewalls, floor, and roof temperatures (Bharathi et al., 2022a).

After loading wheat into the bin, the grain was levelled manually every time and 13 Insectors® (OPI Systems Inc., Calgary, AB) in that layer were installed (Figure 4.7). A total of 65 Insectors® (hereafter, referred to as electronic traps or e-traps) were installed in the bin in five different layers (layers 1, 2, 3, 4 and 5) at a depth of 0.1, 0.9, 1.7, 2.6 and 3.4 m, respectively, from the grain surface. There were two holes at the bottom of each e-trap to facilitate the escape of the captured insects (referred to escaping holes). The e-traps were monitored from August 16 to September 5, 2019, and no insects were detected during this period. This implied that the grain was free of any insect infestation before the introduction of insects.

4.2.3.2 *Insects*

Prior to the experiment, *Cryptolestes ferrugineus* and *T. castaneum* were reared in whole wheat kernels, cracked wheat and wheat germ (90:5:5 w/w), and wheat flour and brewer's yeast (95:5 w/w), respectively, at 30 ± 1°C and 75 ± 5% relative humidity, in the dark. At the start of the experiment, the adults were less than 4 mo old. The masses of 1000 adults (manually counted) of *C. ferrugineus* (in triplicates), separated from the media, were determined using a balance with a resolution of 0.0001 g. Then, about 75000 insects were segregated, based on the mass. The separated 75000 adults were equally divided into four buckets containing about 25 kg of wheat (same as the wheat filled inside the bin) each and the buckets were kept inside the bin for about 10 d for the purpose of acclimation, before introduction. The same procedure was repeated for *T. castaneum*.

About 37,500 *C. ferrugineus* adults were introduced at the centre surface of the wheat bulk on September 6, 2019. Another 37,500 *C. ferrugineus* adults were introduced at the same location on September 10, 2019. These introduced insects made an insect density of 0.25 A/kg of wheat. In September 2020, the same amount (about 75000) of *T. castaneum* adults were introduced at the same location in two batches (on September 9 and 13). The data collected by the e-traps were recorded for a period of 26 months from September 6, 2019, to October 31, 2021. Unfortunately, among 65 e-traps installed, the data from 17 e-traps (Table 4.1), were missing throughout the experimental period. Among the remaining 48 e-traps, 10 e- traps (Table 4.1) stopped recording the data on or before January 5, 2020; only the data from 38 e-traps were recorded until the end of the experimental period. The missing data could be due to any of the following reasons: (a) e-trap malfunction (b) the escaping holes at the bottom of each e-trap were blocked due to dust and/ or dead insect accumulation, so the sensors were blocked by the dust and dead insects.

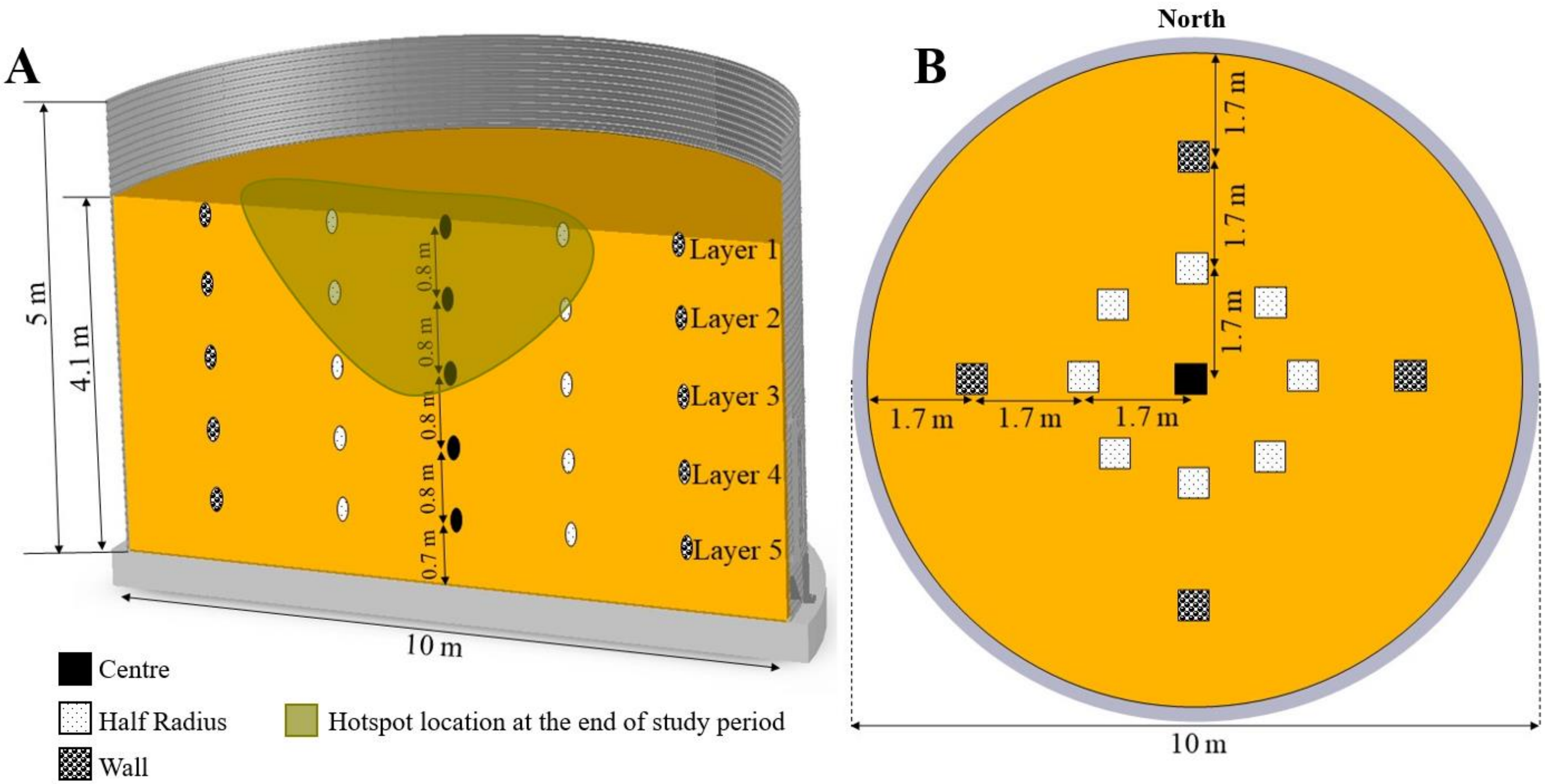

Figure 4.7. Locations of the Insectors® (e-traps) in the 10 m diameter corrugated steel bin filled with 300 t of wheat. There were 13 e-traps in each layer (top view, B), and the e-traps in layer 1 (cross section view, A) were installed at 0.1 m from the surface.

Table 4.1. Insect count data missing periods at different e-trap locations inside a 10 m diameter corrugated steel bin filled with 300 t of wheat.

Layer	Location	Missing period
1	Centre	January 5, 2020 - October 31, 2021
	North half radius	September 6, 2019 - October 31, 2021
	South half radius	November 8, 2019 - October 31, 2021
	East half radius	December 19, 2019 - October 31, 2021
	West half radius	September 6, 2019 - October 31, 2021
	South-east half radius	September 6, 2019 - October 31, 2021
	South-west half radius	September 6, 2019 - October 31, 2021
	South wall	September 6, 2019 - October 31, 2021
	West wall	November 28, 2019 - October 31, 2021
2	Centre	September 6, 2019 - October 31, 2021
	North half radius	September 6, 2019 - October 31, 2021
	South half radius	September 6, 2019 - October 31, 2021
	North wall	September 6, 2019 - October 31, 2021
3	North half radius	September 6, 2019 - October 31, 2021
4	Centre	December 17, 2019 - October 31, 2021
	North half radius	December 17, 2019 - October 31, 2021
	South half radius	September 27, 2019 - October 31, 2021
	West half radius	September 6, 2019 - October 31, 2021
	North-west half radius	September 6, 2019 - October 31, 2021
	South-west half radius	October 5, 2019 - October 31, 2021
	North wall	December 17, 2019 - October 31, 2021
5	Centre	November 16, 2019 - October 31, 2021
	East half radius	September 6, 2019 - October 31, 2021
	West half radius	September 6, 2019 - October 31, 2021
	North-east half radius	September 6, 2019 - October 31, 2021
	North wall	September 6, 2019 - October 31, 2021
	East wall	September 6, 2019 - October 31, 2021

For the case of blockage of escaping holes, a smaller number of insects were recorded occasionally because the blocked sensors could be unblocked due to grain movement during sampling and/ or insects. These data were not used during data analysis.

4.2.3.3 *Experimental procedure*

To understand the horizontal insect distribution, the e-traps were segregated based on their location as centre, half radius and wall at each layer (Figure 4.7) and the average insect counts of the functioning e-traps at half radius and wall locations at each layer were calculated. To understand the vertical distribution of insects, the average insect counts of the functioning e-traps at different layers were calculated. The captured and released insects must move up for at least 0.5 m and enter the e-trap again to be recounted. Thus, considering the size of the bin, the possibility of repeated counting of the same insect around the e-trap is minimum (Jian and Larson, 2006).

To measure the moisture content and the germination of the grain, about 20 g samples were collected at different locations of the bin (Bharathi et al., 2023). In addition, about 10 kg of grain each was collected at nine different locations to analyse the engineering properties of the grain during Summers of 2020 and 2021 (Unpublished). Two Lindgren multiple funnel traps were installed inside the headspace of the bin during the experimental period (Unpublished). No other insect species were observed in these samples and Lindgren multiple funnel traps. Thus, it was assumed that the insects recorded in the e-trap were either *C. ferrugineus* or *T. castaneum*.

Cryptolestes ferrugineus takes about 26 to 38 days to hatch from an egg and to develop into a larva and pupa and finally emerge into an imago, at 26.7°C (Rilett, 1949). The initial temperature of the wheat was 26.8 ± 0.1°C. To compare the distribution of the introduced *C. ferrugineus* adults, a 26-day data (September 6 to October 1, 2019) from the day of introduction were analysed (referred to as Initial Distribution Period). The insect data from October 2 to 31, 2019 was considered as ‘Offspring Period’. The period from November 1 to March 31 and April 1 to October 31 were referred to as Cold and Warm Temperature Periods, respectively.

Due to snow leakage through the gaps between the cover and the loading hole during the second week of October 2019, mould could have multiplied and probably led to hot spot initiation. The temperature and moisture content change as the result of hot spot development were presented Section 4.1 (Bharathi et al., 2023). The developed hot spot influenced the distribution of insects inside the bin. Unfortunately, the influence of the hot spot on insect movement could not be tracked since the e-trap at centre location in layer 1 stopped working from January 5, 2020, and the e-trap at centre location in layer 2 did not work throughout the experimental period. However, the e-traps at the half radius locations in the first and second layers recorded the insect activities. To understand the effect of developed hot spot on the distribution of insects, the following categorization has been made: Initiation period (November 1, 2019, to March 31, 2020), Warm-up period (April 1 to July 15, 2020), Peak temperature period (July 16 to October 31, 2020), and Cool-down period (November 1, 2020, to January 15, 2021). In this study, the immigration and emigration of both insect species into and out of the bin were considered nil.

4.2.4 Results

4.2.4.1 *Cryptolestes ferrugineus distribution*

a. Initial Distribution Period

During this period (September 6 to October 1, 2019), all the recorded insect activities were the activities of the introduced adults. The introduced adults stayed at the centre location and moved vertically downwards towards the bottom of the bin. On the day of introduction (September 6, 2019) about 363, 4, 227 and 10 insects were recorded by the e-traps located at the centre locations of layers 1, 3, 4 and 5 (malfunction occurred at the e-trap at centre location of layer 2), respectively; either one or no insects were recorded at all other locations, except at Layer 2 – East wall (which showed 9 insect count) (Figure 4.8). These results show that immediately after introduction, the insects moved downward from the point of introduction towards the bottom of the bin.

In about 10 days after introduction, the insect counts at layer 1 centre location reduced and the counts at the centre location of other layers increased. This indicated that insects gradually moved down. During the initial distribution period, 78, 17, 94 and 2 Adults/ day (referred to as A/d) were recorded at the centre location of the layers 1, 3, 4 and 5, respectively. The average insect count recorded at the centre location of all the layers were higher than those at half radius locations and those near the walls. The average insect count recorded at half radius locations of layers 1, 2, 3 and 4 were 7.8, 5.2, 2.3 and 1.1 times, higher than those recorded near the walls respectively. Contrarily, the insect count near the walls were higher by 1.5 times than those at half radius locations, at layer 5. This trend implied that as the insects moved downward from the point of introduction, they slowly started distributing horizontally.

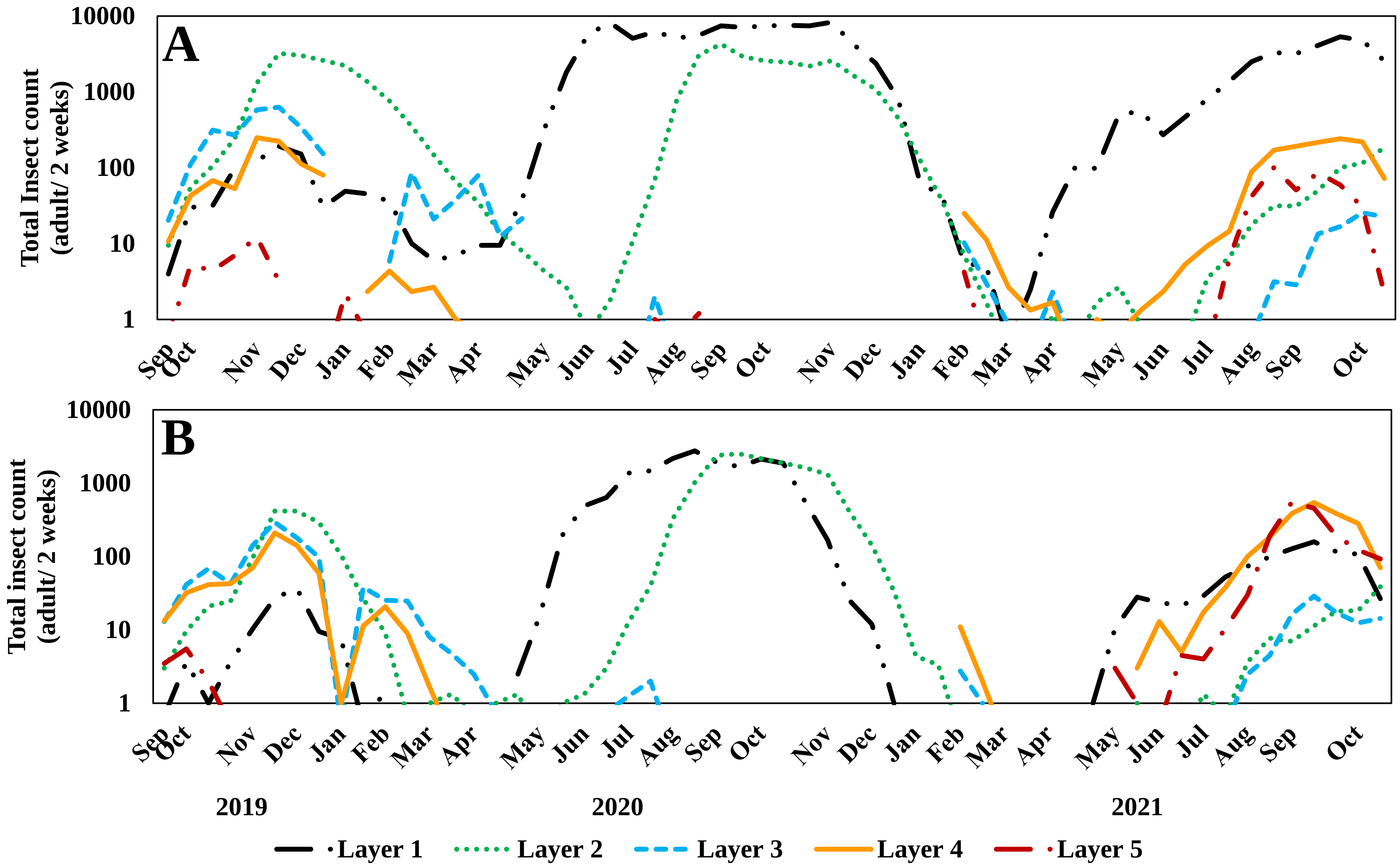

Figure 4.8. Total count of *Cryptolestes ferrugineus* (per e-trap) at half radius (A) and wall (B) locations, every 2 wk, at different layers from September 6, 2019, to October 31, 2021.

b. Offspring Period

During offspring period (October 2 to 31, 2019), the highest number of insects were recorded at the centre locations of all the layers, followed by those at half radius and wall locations. Among the average insect counts recorded at centre location, the e-trap at layer 4 recorded the highest number of adults of 188 A/d, followed by those at layer 1 (117 A/d), layer 3 (38 A/d) and layer 5 (9 A/d). The e-traps at the half radius locations of layer 3 showed the highest average insect count (20 A/d per e-trap), as compared to the count at the half radius locations of all other layers. The e-traps at half radius locations of layers 1, 2, 4 and 5 showed average insect count of 4, 12, 7 and less than 1 A/d per e-trap, respectively. The insect count near the walls were 2, 4 and 3 A/d per e-trap, at layers 2, 3 and 4, respectively; those at layers 1 and 5 showed an average count of less than 1 A/d per e-trap, during the offspring period. These results revealed that during the offspring period, *C. ferrugineus* adults did not prefer to move near the walls of the bin, especially those at layers 1 and 5. This was because the average temperature of the grain near the walls (14.9°C) were lower than those near the half radius and centre locations (24.1°C). The average insect count recorded during the offspring period per day at all the recorded locations was 2.4 times higher than those recorded during the initial distribution period. This increased insect counts might be due to the increase of the total insect number because of insect multiplication.

c. Hot Spot Period

During the initiation period (November 1, 2019, to March 31, 2020), even though the hot spot initiated, the temperature near the walls and the surface were mainly influenced by the ambient temperature. As a result, the highest number of insects were recorded at the centre location of all the layers, followed by those at half radius locations and those near the walls. Among all the half radius locations considered, the highest average insect count was recorded at layer 4 (99 A/d per e-trap), followed by those at layer 3 (13 A/d per e-trap). During the initiation period, the temperature of the grain at centre, half radius, and near the boundary of the bin was 14.7 ± 0.1, 13.6 ± 0.0 and 3.6 ± 0.0°C, respectively (Bharathi et al., 2023). No insect activity was recorded near the walls at layer 1, from February to April 2020, when the temperature dropped below 2.5°C. The insect activity was recorded when the temperature increased above 4.5°C. Moreover, the insect activity near the walls at all other layers reduced drastically because of drop in temperature. Therefore, insect activity was significantly influenced by the grain temperature. Even though the moisture content of the surface grain were higher, the insects did not move to the surface because of the lower temperature during the initiation period (Bharathi et al., 2023).

During November 2019, a sudden increase in insect count at layer 1 and layer 2 (centre and half radius locations) occurred. Precisely, the average insect counts, during November 2019, at centre location of layer 1 drastically increased to 673 A/d and those at half radius location of layer 2 increased to 167 A/d per e-trap. This further confirms the hot spot initiation near top surface of the grain.

From April 1, 2020, to January 15, 2021, the higher insect activity was recorded at layer 1 with an average insect count per day of 249 A/d per e-trap, followed by layer 2 with 72 A/d per e-trap. The layers 3, 4 and 5 had less than 1 A/d. The higher insect activity was recorded at half radius locations, as compared to those recorded near the walls, at layers 1 and 2. This implies that the grain near the surface, especially those near the centre and half radius locations had higher number of insects and high insect activity compared to all other locations, inside the bin. During this period, the temperature of the grain at 0.7 m (16.4 ± 0.2°C) from the surface were hotter than those at 1.9 (12.1 ± 0.2°C) and 3.1 m (9.8 ± 0.1°C); the temperature of the grain at half radius locations (18.3 ± 0.2°C) was hotter than those near the walls (16.9 ± 0.4°C), at 0.7 m from the surface (Bharathi et al., 2023). The insect counts were associated with the grain temperatures which could be due to the higher number of insects and high activity of insects at the warmer locations.

During the warm-up period (April 1 to July 15, 2020), the surface grain temperature warmed up because of the increase in ambient temperature. The higher number of insects recorded at layer 1 (127 A/d per e-trap) showed that the warmer grain had attracted the insects towards the layer 1. Moreover, as the temperature warmed up, the activity of insects slowly increased from June 2020. During the warm-up period, an average of less than 1 A/d per e-trap was recorded at layers 2, 3, 4 and 5.

During peak temperature period (July 16 to October 31, 2020), when the temperature of the grain at layer 2 also increased because of the hot spot development, the insect count increased drastically from less than 1 A/d per e-trap (during April 1 to July 15, 2020) to 152 A/d per e-trap (during July 16 to October 31, 2020) in layer 2. In layer 1, the insect count increased to 411 A/d per e-trap. The average temperature of the grain at 0.7 m from the surface during peak temperature period (25.5 ± 0.2°C) was hotter than the recorded temperature during the warm-up period (10.0 ± 0.2°C). The insect count recorded at layers 3, 4 and 5 remained less than 1

A/d per e-trap. The increase in insect counts at layers 1 and 2 showed the higher insect number and higher activity during peak temperature period.

During the cool-down period (November 1, 2020, to January 15, 2021), the insect counts, and the insect activity started to decrease. In layers 1 and 2, the insect count dropped to 130 and 56 A/d per e-trap, respectively. In layers 3, 4 and 5, no insects were recorded. The temperatures of the grain at 0.7 m, 1.9 m and 3.1 m from the surface were 12.2 ± 0.3, 13.5 ± 0.3, 10.5 ± 0.3°C, respectively. These results indicated the lower activity of the insects at lower grain temperatures.

d. Cold Temperature Period 2021

During cold temperature period 2021 (January 16 to March 31, 2021), as the temperature of the grain dropped, the insect counts also dropped drastically and the insects distributed throughout the bin. The average insect counts at the half radius locations were reduced to 1 A/d per e-trap at layers 1 and 4; while, at layers 2, 3 and 5, the insect counts at the half radius locations were less than 1 A/d per e-trap. The average insect count near the walls were less than 1 A/d per e-trap at all the layers and the total insect count at the half radius locations were higher than those near the walls. The average insect counts recorded, during the months of February and March 2020, was 3 A/d per e-trap whereas, those recorded in 2021 was less than 1 A/d per e-trap. This was caused by the higher grain temperatures around the hot spot, which in turn had increased the insect activity.

e. Warm Temperature Period 2021

During warm temperature period 2021 (April 1 to October 31, 2021), when the temperature of the surface grain increased, the average insect count reached 98 A/d per e-trap in layer 1. The average temperatures of the grain at 0.7 m from the surface were higher by 4.8 and 6.3°C, than those at 1.9 m and 3.1 m, respectively. During warm temperature period 2021, the higher number of adults were recorded near the walls than those at half radius locations at layers 3, 4 and 5. This was because the temperatures of the grain near the wall at 1.9 and 3.1 m from the surface were hotter than those at half radius locations by 6.1 and 7.1°C, respectively. Even though the temperature of the grain at half radius locations at 0.7 m depth from the surface were colder than those near the walls by 1.3°C and the moisture contents of the surface grain at half radius locations and near the walls were equal, higher number of adults were recorded in the half radius locations than those near the walls at the layers 1 and 2. This might indicate a higher insect population and insect activity at the half radius than that at the walls.

4.2.4.2 *Tribolium castaneum distribution*

a. Hot Spot period

Before *T. castaneum* adults were introduced, the hot spot had already developed and reached to the peak temperature period. As a result of higher temperature of the grain near the hot spot, higher counts of adults were recorded at layer 2 half radius locations, in September and October 2020. On the day of introduction (September 9, 2020), *T. castaneum* adults were recorded at the half radius locations of layers 1 and 2 only. On day 4, one adult was recorded near the west wall at layer 2, which is the earliest occurrence of *T. castaneum* adult near the wall. No *T. castaneum* adults were recorded at layers 3, 4 and 5 during the months of September and October 2020. This implied that the introduced *T. castaneum* adults did not move towards the bottom of the bin and distributed horizontally initially (Figure 4.9). This result was consistent with the reports in literature (Surtees, 1964d; Hagstrum, 2000) which state that the red flour beetles mainly stay at top of grain bulks.

During the cool-down period (November 1, 2020, to January 15, 2021), only 2 adults were recorded at layer 1. This result implied the decrease of activity of *T. castaneum* adults due to the decrease of grain temperature in layer 1. The average insect count at layer 2 was 1 A/d per e-trap and no adults were recorded at layers 3, 4 and 5. These data implied that *T. castaneum* adults stayed at the hot-spot location (1 m grain depth) during cool-down period and did not move down.

b. Cold Temperature Period 2021

As temperatures of grain at the surface and in the hot spot location dropped, *T. castaneum* adults started to move downwards, during cold temperature period 2021 (January 16 to March 31, 2021). An average of less than 1 A/d per e-trap was recorded at layers 2, 3, 4 and 5; while no adults were recorded at layer 1. The temperature of the grain at 0.7 m from the surface was 6.5 ± 0.1°C. According to Fields and White (1997), *T. castaneum* adults cannot survive temperature below 10°C. Since the temperature of the surface grain could have dropped below 6.5°C, during Cold Temperature Period 2021, the insects must have moved away from layer 1. At layer 1, near the walls, the *T. castaneum* activity was not recorded from mid-October 2020 to mid-July 2021. This was because the temperature dropped below 22°C from October 15, 2020, and the temperature increased above 22 °C from July 14, 2021. Therefore, even though red flour beetles prefer to multiply and stay at the top of grain bulks, they moved down during winter after the temperature at the top of the grain is reduced.

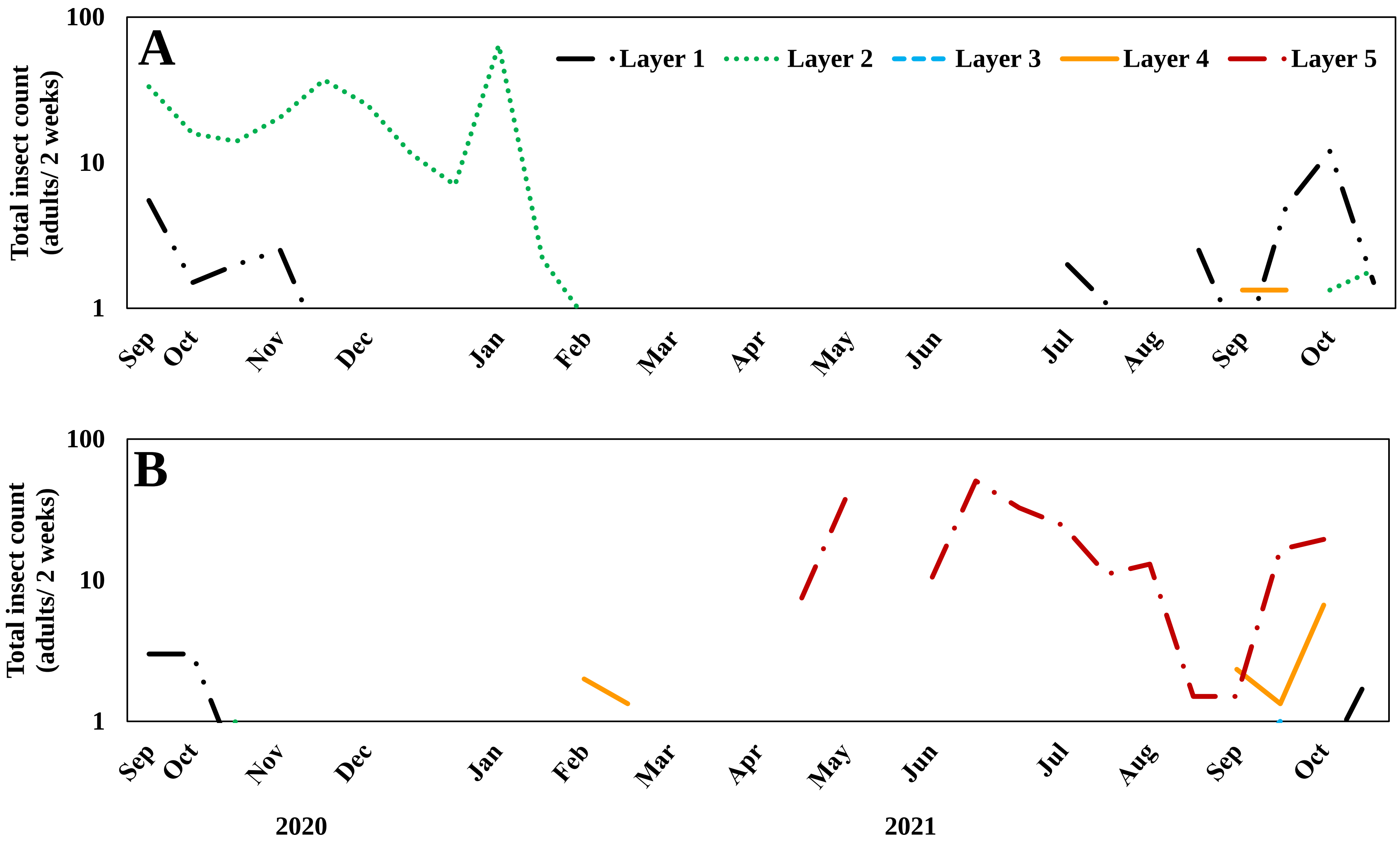

Figure 4.9. Total count of *Tribolium castaneum* (per e-trap) at half radius (A) and wall (B) locations at different layers, every 2 wk, from September 9, 2020, to October 31, 2021.

c. Warm Temperature Period 2021

During warm temperature period 2021 (April 1 to October 31, 2021), at the layers 1 and 2, higher counts were recorded at half radius locations than those near the wall; whereas at layers 3, 4 and 5, higher number of adults were recorded near the wall. These patterns were similar to those observed for *C. ferrugineus* during warm temperature period 2021. These insect counts followed the temperature distribution pattern; higher the temperature, higher the insect counts and activity. Overall, an average of less than 1 A/d per e-trap was recorded inside the grain during the warm temperature period 2021. These results imply that *T. castaneum* adults survived the winter inside the grain bin (the temperature of the grain was greater than 7.8°C, at least at one location, during the winter).

4.2.5 Discussion

On the day of introduction, *C. ferrugineus* adults were observed on centre location of the bottom most layer. This implied that *C. ferrugineus* adults moved downward more than 4 m in less than 24 h. These results are in accordance with the observations of Jian et al. (2012b), who studied the distribution of *C. ferrugineus* activity inside a hopper bottom bin containing 48 t of wheat. Jian et al. (2004) reported that the maximum speed of *C. ferrugineus* through wheat was 6 m/ d and more than 10.8 m/ d in horizontal and vertical direction, respectively. The observations of the current study during the initial distribution period of *C. ferrugineus* are in line with the findings of various researchers (Loschiavo, 1974, 1983; White et al., 1993; Jian et al., 2006, 2009; Bharathi et al., 2021, 2022b), who reported that, in the absence of any gradients, a substantial portion of *C. ferrugineus* adults tend to move downward from the point of introduction because of their smaller size, drift effect and preference. After downward movement, horizontal distribution of adults was observed in the current study, which is in line with the observations of the lab-scale study, conducted by Bharathi et al. (2022b), on the movement and distribution of *C. ferrugineus* inside a 285 kg wheat bulk, with varying insect densities, movement periods, and temperatures. They concluded that *C. ferrugineus* adults showed downward initial movement, followed by upward and horizontal movements, in the absence of temperature and moisture gradients.

The one- and two-dimensional studies conducted by Jian et al. (2003, 2004, 2005a), demonstrated that *C. ferrugineus* adults move towards the warmer location in the presence of temperature gradients and towards the bottom at uniform conditions. The movement of *C. ferrugineus* to warmer locations has been well-established even at the difference of 1°C (Flinn and Hagstrum, 1998). The activity of *C. ferrugineus* was higher at higher temperatures (Bharathi et al., 2022b). The current study confirmed these results in a larger bin. For example, higher number of *C. ferrugineus* and *T. castaneum* were recorded near the south wall of layer 5 during Warm Temperature Period 2021 because grain was warmer at that location. The temperature at the south side was hotter than that at north side due to higher solar radiation at the south of the bin (Bharathi et al., 2022a). Moreover, the activities of insects at the south side were higher than those at north. Smith (1978) also found the residual population of *C. ferrugineus* near the floor close to the south wall of the granary.

Several researchers have shown that *C. ferrugineus* prefer moister grains in the absence of temperature gradients (Jian et al., 2005a; Loschiavo, 1983). Jian et al. (2005a) observed higher responses of *C. ferrugineus* adults when the moisture differences were higher. They reported that higher percentage (95%) of *C. ferrugineus* adults moved towards high moisture grains (16.5%) when the other section of the grain was 12.5% whereas lower percent (65%) of insects moved to 16.5% grains when the other section was 14.5%. They also revealed that in the presence of temperature gradients, when the moisture difference was higher (in the case with 12.5% and 16.5%), the moisture difference was the predominant factor that influenced *C. ferrugineus* movement and distribution; when the moisture difference was lower (in the case with 14.5% and 16.5%), the temperature gradient was the predominant factor. Therefore, even though temperature and moisture content are the two main factors influencing the movement of insects, the major factor at one environmental condition could become a minor factor at other conditions (Jian et al., 2005a). In the current study, the maximum moisture difference observed inside the grain bulk (excluding the surface grain) at any time during September 2019 to August 2021 was 2.3 percentage point (Bharathi et al., 2023). The higher moisture changes (moisture difference between the centre surface location and those inside the grain bulk was 10.8 percentage point) occurred at the surface grain. However, when the moisture content of the surface grain was higher, the temperature was lower. Hence, in the current study, temperature was the main factor that influenced the movement and distribution of the insects. Smith (1978) also concluded that the moisture content did not determine the distribution of *C. ferrugineus* inside the grain bulk in the presence of temperature gradients.

According to Jian et al. (2005a), lower percentage of dockage (5%) does not influence *C. ferrugineus* movement, whereas dockage percentage as high as 10% influences the insect movement. In the current study, the dockage was 0.4 to 0.8% and the grain in the top layer had the maximum percentage of dockage (0.804 ± 0.084%) (Salarikia et al., 2021). Moreover, in the presence of temperature gradients, the effect of dockage on the *C. ferrugineus* movement and distribution is insignificant (Jian et al., 2005a). The current study on insect counts at different locations confirmed these conclusions.

Both *C. ferrugineus* and *T. castaneum* showed the preference to warmer temperatures. However, after adults were introduced into the bin, they showed different movement patterns: lots of *C. ferrugineus* moved down, while *T. castaneum* stayed in the top layer. This might be caused by their different body sizes (Jian et al., 2005b) and the distribution of the temperature gradients. The temperature at the top two layers were warmer during *T. castaneum* introduction period. *Tribolium castaneum* adults (have a larger body size than *C. ferrugineus*) should overcome the smaller pore space in the wheat bulk to move down. This explains why no insects were recorded at the bottom three layers during the months of September and October 2020. During wintertime, when the grain at the boundary became colder, both insects had a higher counts and higher activity inside the wheat bulk and at the hot spot location. Therefore, *T. castaneum* adults could be able to move through wheat bulk if they must do, even though their movement speed is lower than *C. ferrugineus.*

Fields and White (1997) reported that *T. castaneum* did not survive at 10°C in the ground wheat when declined from 25 to 10°C in 36 wk and kept at 10°C for 4 wk (temperatures simulated as that of 12 m-diameter grain bulk); while *T. castaneum* did not survive at 11°C in the ground wheat when declined from 25 to 11°C in 20 wk and kept at 11°C for 4 wk (temperatures simulated as that of 6 m-diameter grain bulk). In addition, the researchers also reported that in the granaries infested with *T. castaneum*, the insects were dead by early February, in two winters. In the current study, *T. castaneum* adults survived even when the temperature of the grain at the recorded locations dropped below 10°C from March 6 to May 15, 2021, and the temperature inside the grain declined from 25 to 10°C in 19 weeks. These differences might be mainly caused by the grain temperature difference in these studies. Fields and White (1997) used 40 to 100 tonnes of wheat, whereas 300 tonnes of wheat was used in the current study. The temperature of the 300 tonnes of wheat was higher than 7.8°C, at least at one location in any time of our study (Bharathi et al., 2023). Therefore, insects of *T. castaneum* could survive at these warmer locations.

A total of 21633 *C. ferrugineus* and 58 *T. castaneum* adults were recorded during October 2021 at 38 active e-traps. This implied that some of *C. ferrugineus* and *T. castaneum* adults survived at the end of the study period. However, the survival rate of the insects could not be estimated because the capture rate of the introduced insects was not known. Also, the introduced insects would have multiplied. Since the insect counts at other 27 locations were not recorded due to the malfunction of the e-traps, the survival of the insects in the bin could be higher than the counted insects by the e-traps. *Cryptolestes ferrugineus* is more cold-tolerant species than *T. castaneum*. This might be the reason of the higher number of *C. ferrugineus* at the end of the study.

Electronic traps are one of the efficient tools to detect insect infestation (Jian and Larson, 2006; Jian et al., 2012b). However, once the Insectors® traps are buried deep inside grain bulks, it is almost impossible to replace them after they start malfunctioning. Moreover, the presence of grain dust and accumulation of dead insects could cover the Insectors® sensors, which could result in the malfunction of Insectors® traps. To overcome these challenges, further improvements need to be done to the Insector® design.

4.2.6 Conclusion

From the results of the current study, the following conclusions were drawn:

1. Both *C. ferrugineus* and *T. castaneum* survived winters inside the bin containing 300 tonnes of wheat stored in a 10 m diameter bin in western Canada.
2. The movement and distribution pattern of both insects in the 300 tonnes of wheat bin were similar to those observed in the laboratory studies at similar environmental conditions.
3. The average count of rusty grain beetle recorded during the offspring period per day at all the recorded locations was 2.4 times higher than those recorded during the initial distribution period.
4. The insect counts as well as the insect activity increased with increase in temperature. Therefore, highest insect counts were recorded inside and around the hot spot for both insects.

5. Immediately after introduction, rusty grain beetles moved vertically downward and then distributed horizontally.
6. The red flour beetles were mainly captured at top grain layer (about 1 m depth from the grain surface). However, they could be captured at any place of the bin.
7. For rusty grain beetle, no insect activity was recorded near the walls at layer 1, from February to April 2020, when the temperature dropped below 2.5°C. The insect activity was recorded when the temperature increased above 4.5°C.
8. For red flour beetle, the insect activity was not recorded near the walls at 0.1 m depth from mid-October 2020 to mid-July 2021, when the temperature was recorded below 22°C during this period.
9. The insect activity near the walls at all other layers reduced drastically because of temperature drop during winter.

4.2.7 Author contributions

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

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4.3 Effects of weather on temperatures of the floor, roof, sidewalls, and headspace of a corrugated steel bin filled with 12.5% moisture content wheat, stored for 26 months in Winnipeg, Canada

(This section has been published in a peer-reviewed scientific journal)

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4.3.1 Abstract

The mean global temperatures are increasing as a result of climate change. To understand how the change in ambient weather influences the temperature of the stored grain, the temperature fluctuation patterns of the floor, roof, sidewalls, and headspace were monitored from mid-August 2019 to the end of October 2021 in Winnipeg, Canada. The bin was filled with 300 t of wheat at an initial average moisture content of 12.5 ± 0.1% (wet basis). The thermocouples were installed at 17, 9, and 12 locations on the floor, roof (outside), and sidewalls (outside) of the bin, respectively. Sixteen temperature and relative humidity sensors were installed at different locations with varying distances from the surface of the grain in the headspace. The ambient weather (air temperature (°C), relative humidity (%), barometric pressure (kPa), average solar radiation (W/m^2), precipitation (mm), wind speed (m/s), and wind direction (degrees with reference to the north)) was also measured near the bin during the study period.

The temperatures of the roof, sidewalls, and headspace were influenced by the ambient temperature and solar radiation. In Year II (November 2020 – October 2021), the floor, roof, sidewalls, and headspace temperatures were higher by 2.1 ± 0.1°C, 3.9 ± 0.1°C, 3.5 ± 0.2°C, and 1.9 ± 0.1°C than that in Year I (November 2019 - October 2020), respectively. The ambient temperature increased by 1.8°C in year II, compared to year I. These results can be used in the prediction of temperatures in grain bins caused by weather changes.

Keywords: Grain bin, headspace temperature, roof temperature, floor temperature, climate change.

4.3.2 Introduction

Change in the weather pattern is one of the frightening effects of climate change (Moses et al., 2015). The Intergovernmental Panel on Climate Change has envisioned a global mean temperature rise of 1.1 to 5.4°C by 2100 (Bale et al., 2002). With the increase in global temperature, the grain stored inside a bin is exposed to warmer temperatures. Interaction between the physical, chemical, and biological factors inside the stored grain ecosystem impacts the quality of the stored grain.

Temperature is one of the most important factors that affect the quality of the grain stored in a bin. The temperature of the grain inside a bin can be affected by various physical and biotic factors (Jian and Jayas, 2022). Grain temperatures inside the bin are also influenced by the heat flux from the sidewalls, headspace, and the floor of the bin (Jian et al., 2005). In addition, the temperature of the grain near the floor in contact with a plenum is differently affected, as compared to those with the soil foundation (Jian and Jayas, 2022). The warmer and humid condition deteriorates the quality of the grain stored in the bin (Jayas et al., 1995). An increase in temperature increases the grain respiration rate, enhances the growth and multiplication of other biotic factors present in the stored grain ecosystem such as insects and microorganisms and decreases the germination ability of the grain over time (Nithya et al., 2011).

Moses et al. (2015) has reported that detailed multidisciplinary research is required to understand the effects of climate change on stored grain ecosystem. Understanding the effects of ambient conditions on the temperatures of the components of bins filled with grain is crucial to maintaining the quality of stored grain as temperatures of these components affect grain temperatures. Free-standing, corrugated galvanized steel, cylindrical bin is a common storage structure used in the Canadian Prairies (Alagusundaram et al., 1990). Limay-Rios et al. (2017) surveyed 83 unique storage bins across Southwestern Ontario, Canada, during 2011-2013 and reported that about 98% of the bins were flat bottom, corrugated galvanized steel with conical roof and the median capacity of the bins were 300 metric tonnes. However, the detailed report on the temperatures of the components of the commercial-sized bin influenced by different ambient temperatures in different years is not available Moreover, reports on the floor temperatures in the presence of a plenum could help develop models using air as the boundary condition rather than the soil.

The aim of the present work was to determine how the weather change influences the temperature fluctuations and gradients at various locations on the floor, roof, sidewalls, and in the headspace of a 10 m diameter bin (flat bottom, corrugated galvanized steel with conical roof) filled with 300 t of wheat, for a period of 26 months. The temperature and moisture profile of the wheat stored in the bin has been reported elsewhere (Bharathi et al., 2023).

4.3.3 Materials and methods

4.3.3.1 *Grain bin*

A flat-bottom, cylindrical, corrugated steel bin with 10 m diameter, 5 m high cylindrical part, and 3 m high conical roof (from the center of the cone to the cone base), located in the south end of Winnipeg, Manitoba, Canada, was used for this study. At the center of the conical roof, there was a 0.9 m opening (vent), which was used for grain loading. The concrete foundation of the bin was 0.5 m high from the ground. A plenum of 0.35 m depth and a perforated floor were present at the bottom of the bin. A poly tarp with 1 mm opening was placed above the perforated floor before the loading of the wheat. A chute (under the perforated floor) and an auger (above the floor) were installed along the north-west direction for grain unloading. Four ladders and four manholes were installed on the north, south, east, and west sides of the bin. About three hundred tonnes of No.2 Canada Western Red Winter Wheat (AAC Goldrush) with an initial average moisture content of 12.5 ± 0.1% (w.b.) (the moisture conte4nt at different locations varied from 11.4% to 13.7%) was loaded into the bin from August 7, 2019, to August 15, 2019, in five batches. The average daily ambient temperature varied from 17.8 to 20.4°C during the loading period (August 7 to 15, 2019). After each loading, the grain surface was leveled manually. The depth of the grain was approximately 4.1 m. Throughout the experimental period, condensation was not observed on the inside surface of the roof or sidewalls.

4.3.3.2 *Weather data collection*

A weather station including a temperature and relative humidity probe (CS215, Campbell Scientific, Edmonton), a barometric pressure sensor (CS106, Campbell Scientific, Edmonton), a pyranometer (CS300, Campbell Scientific, Edmonton), a tipping bucket rain gage (TE525M, Campbell Scientific, Edmonton) and a wind monitor (05103, Campbell Scientific, Edmonton) was installed at 10 m from the bin in the south-east direction. The weather data including air temperature (°C) (resolution: ±0.01°C), relative humidity (%) (resolution: ±0.03%), barometric pressure (kPa) (accuracy: ±1.5 hPa), average solar radiation (W/m^2) (sensitivity: ±5 mV/Wm^{-2}), precipitation (mm) (resolution: ±1 tip), wind speed (m/s) (resolution: ±(0.0980 m/s) / (scan rate in seconds), and wind direction (degrees clockwise with reference to the north) (accuracy: ±3°C), were measured at 30 min interval from September 1, 2019, to October 6, 2021. The accuracy and resolution of the weather data were based on manufacturer's manual. The daily averages of the collected temperature were compared with those recorded at the Forks, Winnipeg, Canada, by Environment Canada (Environment Canada, 2022).

4.3.3.3 *Temperature measurements*

Nine vertical cables (OPI Systems Inc., Calgary, AB) containing different numbers of sensors were installed, in the headspace of the bin from the ceiling to the floor, for measuring the relative humidity (resolution: ±5%), and temperature (resolution: ±0.1°C) (Figure 4.10). The cable at the center location was 0.85 m from the center of the bin towards North and had 3 sensors at the distance of 0.2, 1.4 and 2.6 m from the surface of the grain to ceiling, and those at 2.25 m away from the bin center (HR1, HR2, HR3, HR4, HR5 and HR6) had sensors at 0.6 and 1.8 m from the surface of the grain in the headspace. The cables that were at 0.6 m from the wall on east and west directions consisted of a sensor each at 0.6 m from the grain surface in the headspace.

A total of 17 thermocouples (precision: ±0.5°C) were installed on the floor, which included eight thermocouples at 0.15 m from the sidewalls along the eight directions (north, south, east, west, north-east, north-west, south-east and south-west), and eight at 2.5 m from the center along all the eight directions, and one was at the center of the floor. A total of nine thermocouples were installed on the outside surface of the roof in such a way that one was installed at 0.6 m from the center of the bin along the north direction, four along each of four directions (north, south, east and west) at 0.15 m from the sidewalls and remaining four at 1.5 m from the sidewalls along the four directions. A total of 12 thermocouples were installed on the outside surface of the sidewalls along four directions (north, south, east, and west) with three thermocouples on each side, at the distance of 0.3, 2 and 4 m from the bottom of the bin. Two thermocouples were installed near the floor on the inside and outside surfaces of the south-east side of the wall, to estimate the temperature difference between inside and outside surface of the sidewall. The sensor on the cable near the west side of the wall in headspace and the thermocouple near the south side of the wall on the floor malfunctioned throughout the experimental period.

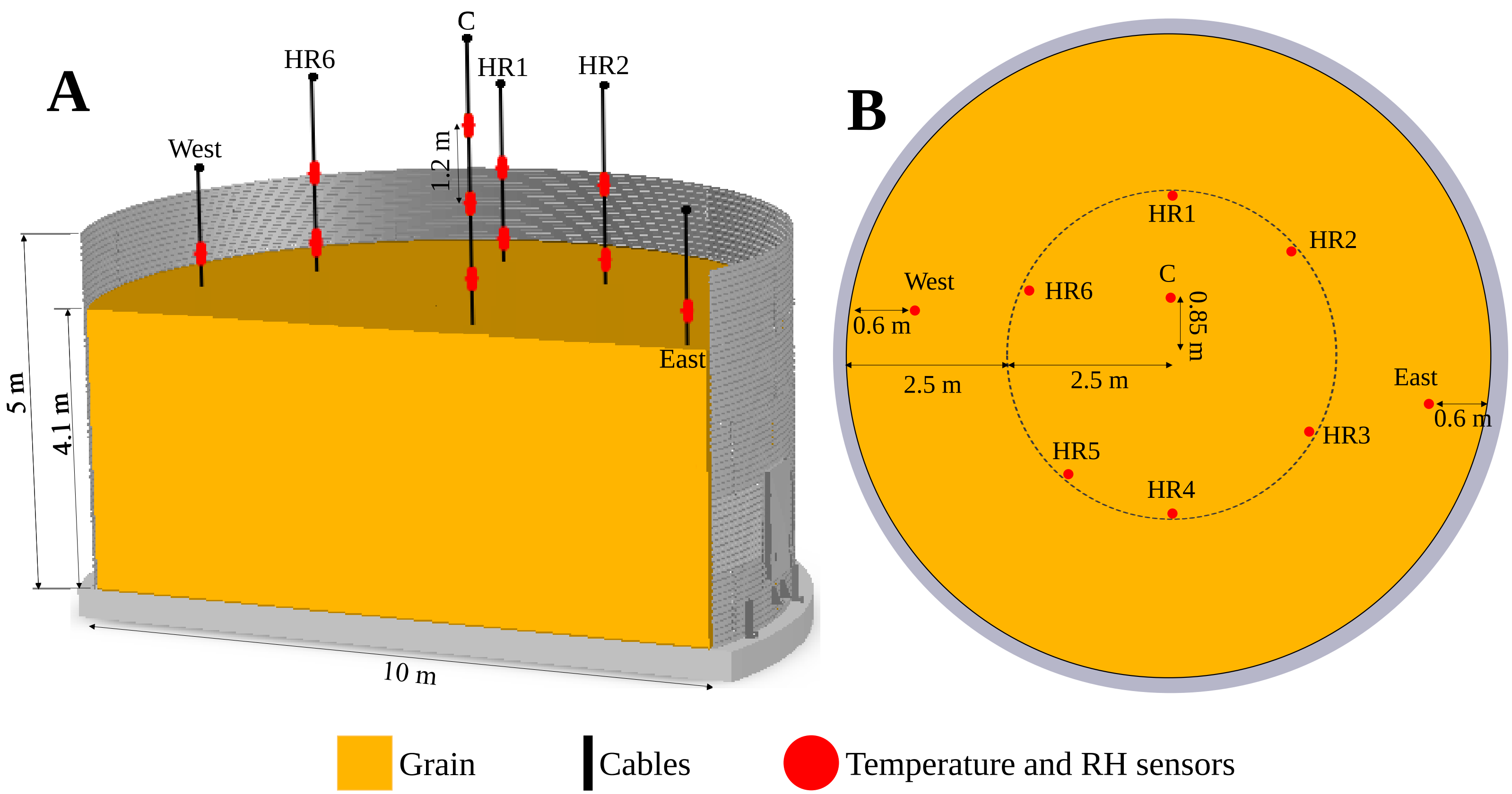

Figure 4.10. Cross-sectional view (A) and top view (B) of the cable locations inside a 10 m diameter corrugated steel bin. The sensors at the cables measured temperatures and relative humidities every hour from August 18, 2019, to October 31, 2021. C represents the cable located at 0.85 m from the centre of the bin along the north direction.

The precision of the thermocouples was tested in the lab using ice water and boiling water. The resolution of the cables inside the headspace have been provided by the company OPI™ Systems Inc., Calgary, AB. In addition, on comparing the temperatures recorded by the thermocouples and the sensors in the cables were observed to be similar after installation (before loading the grain).

Hourly temperature and relative humidity data from OPI cable sensors and hourly temperature from the thermocouples were recorded from August 18, 2019, to October 31, 2021 (26 months). The data from OPI cable sensors were missing for a total of 22 days and those from thermocouples were missing for a total of 62 days, at all locations during the period of study. The data from November 1 to March 31 and those from April 1 to October 31 were referred to as Cold and Warm Temperature Periods, respectively. Also, November 1, 2019, to October 31, 2020, was considered as year I and November 1, 2020, to October 31, 2021, was considered as year II.

4.3.3.4 *Statistical Analysis*

To compare the temperature data recorded at different locations, paired t-test ($\alpha = 0.05$) was performed using SAS® OnDemand for Academics (SAS Institute Inc., Cary, NC).

4.3.4 Results

4.3.4.1 *Weather data*

During the 26-month study, the daily average temperatures recorded near the bin were the same ($P = 0.4487$, $t = -0.76$, $N = 755$) (with negligible temperature difference of 0.03 ± 0.03°C) as those recorded at the Forks, Winnipeg. The maximum and minimum temperatures recorded near the bin during year I were 34.4 and -30.7°C, respectively, while those recorded during year II were 36.9 and -33.2°C, respectively. The average hourly ambient temperature recorded during year II was higher by 1.8°C than those recorded during year I. The higher average solar radiation received in year II (160.1 ± 1.8 W/m^2), as compared to year I (148.3 ± 1.7 W/m^2) could have contributed to the increase in ambient temperature in year II. Even though the maximum precipitation recorded in year II (20.9 mm) was higher than that in year I (8.1 mm), the total precipitation recorded in year II (219.4 mm) was lower than that in year I (233.2 mm). The average ambient relative humidity dropped from 71.9 ± 0.1% in year I to 68.4 ± 0.2% in year II.

4.3.4.2 *Headspace temperatures and relative humidity*

The headspace temperatures inside the bin were influenced by the ambient temperature and solar radiation. The temperatures measured at 16 locations in the headspace were hotter than the ambient temperature by 3.7 ± 0.2°C, during the 26-month study period (Figure 4.11). In the Warm Temperature Period, the headspace temperatures increased or remained the same with increase in height (Table 4.2). The headspace temperatures were the lowest at HR1 (2.25 m away from the center of the bin towards north) during the Warm Temperature Period. The higher average temperatures at HR4 as compared to those at HR1, during the Cold and Warm Temperature Periods, confirmed the higher solar radiation on the south side, as compared to that at the north side. Similarly, Alagusundaram et al. (1990), who predicted the temperature distribution in grain storage bins using a three-dimensional, finite element, heat transfer model, predicted that the south side of the bin was warmer by about 5 to 15°C than the north side. In the current study, the average temperature differences between HR1 and HR4 were 0.6°C (at both 0.6 m and 1.8 m from the surface), during Warm Temperature Period and 1.2°C (at 0.6 m from the surface) and 0.7°C (at 1.8 m from the surface), during Cold Temperature Period. Montross et al. (2002a) also observed the temperature gradients in headspace as a result of solar radiation. They reported that the headspace temperature recorded at 0.4 m from the surface was 6°C lower than those recorded at 1.2 m from the surface during periods of higher solar radiation. In the current study, the average difference between the headspace temperature at 0.6 m and 1.8 m at different locations during the day were the maximum of 1.9 and 0.8°C, during Warm and Cold Temperature Periods, respectively.

The average headspace temperature during year II was hotter than year I and the temperature differences observed between years I and II were 1.6 ± 0.1 and 2.1± 0.0°C, during the Cold and Warm Temperature Periods, respectively. This implies that the increase in ambient temperature increased the temperatures in headspace. The maximum headspace temperatures were recorded at 2.6 m (at centre location) and 1.8 m (at HR4 location) from the surface of the grain, and the minimum headspace temperatures were recorded at 0.6 m (at HR1) and 1.8 m (at HR3) from the grain surface during years I and II, respectively. The hottest headspace temperature recorded in year II (59.6°C), was higher than year I (58.6°C) and the coldest headspace temperature recorded in year II (-32.3°C) was lower than year I (-25.7°C). These data also confirm the influence of the ambient temperature on the temperatures in the headspace inside the bin.

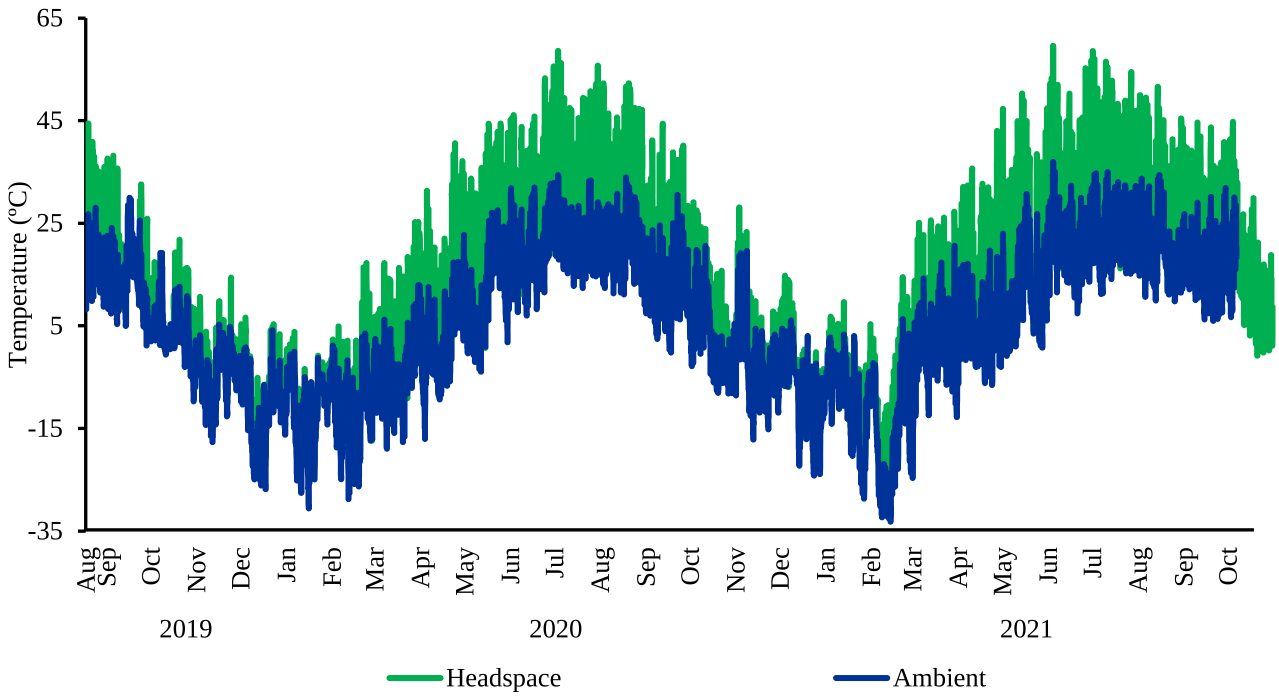

Figure 4.11. Headspace temperature inside the bin filled with 300 t of wheat from August 18, 2019, to October 31, 2021. Only the temperatures recorded at the sensor at the distance of 2.6 m from the grain surface on the centre cable are presented.

The headspace temperature near the surface of the grain could have been impacted by several factors such as (1) ambient temperature, (2) solar radiation, (3) when the headspace air cools down, it moves towards the bottom of the grain through the intergranular airspace, as a result, the air inside the grain moves into the headspace, (4) when the headspace air heats up due to solar radiation in the day and expands, it moves out of the bin, then the air present in the intergranular space moves into the headspace. The combination of these effects could affect temperature of the headspace near the surface of the grain. Moreover, the temperature difference observed between the grain surface and the headspace could have led to the transfer of heat between the grain surface and the headspace. Thus, the headspace temperature near the surface of the grain could have been influenced by grain temperature, in addition to the ambient temperature and solar radiation.

The average headspace relative humidity during years I and II were 68.8 ± 0.2% and 63.4 ± 0.3%, respectively. During year II, the headspace relative humidity decreased as a result of increase in temperature and decrease in ambient relative humidity. The average headspace relative humidity in the years I and II, during the Warm Temperature Period, were 61.2 ± 0.3% and 56.0 ± 0.3%, respectively; and during the Cold Temperature Period were 79.4 ± 0.2% and 73.8 ± 0.2%, respectively. The headspace relative humidity was higher during the Cold Temperature Periods than the Warm Temperature Periods, due to the change in the headspace temperature. Lawrence and Maier (2011) and Lawrence et al. (2012) also reported variations in the temperature and relative humidity in the headspace of a silo as a result of change in solar radiation, wind speed and ambient air infiltration.

4.3.4.3 *Floor temperatures*

Temperatures on the floor, especially near the wall, inside the bin were mainly influenced by the ambient temperature. The temperatures near the wall followed the trend of ambient temperature (Figure 4.12). Temperatures at the center and locations that were 2.5 m away from the center along various directions exhibited temperature lags when compared with those near the sidewalls and the ambient temperature.

The maximum and minimum temperatures recorded during the 26-month experimental period, were 20.0 and -1.0°C at the center of the floor, respectively; were 20.6 and -2.6°C, respectively at 2.5 m from the center; and were 38.4 and -12.0°C, respectively at 0.15 m from the sidewalls. The average absolute temperature gradient between the locations near the wall and their corresponding locations at 2.5 m away from the center was 1.6 ± 0.0°C/m and was 0.4 ± 0.0°C/m between center and 2.5 m away from the center. Among the average temperature differences observed at various locations recorded at 2.5 m from the center, the highest (-2 ± 0.0°C) was observed between north and south direction locations. These results implied that the effect of ambient temperature on the temperature of floor decreased with increase in distance from the sidewalls. Lo et al. (1975) also have predicted that as the distance from the wall increases, the effect of seasonal temperature and moisture change of the wheat stored in a bin decreases.

Among the temperatures recorded at the seven locations near the wall on the floor, the temperatures at the south-east, south-west and east locations were the highest during the Warm and Cold Temperature Periods. Similarly, the temperatures at south, south-east, south-west and east were the highest, during the Warm and Cold Temperature Periods, at 2.5 m from the center of the floor. The floor temperatures along west and north-west direction were lower than those along the east direction. This could be attributed to the presence of the auger from center to outside of the bin in north-west direction. Temperatures along the north direction were the lowest during the Cold Temperature Period, since north side of the bin received lower solar radiation, compared to other locations.

Table 4.2. Mean, maximum (Max) and minimum (Min) temperatures recorded at different locations in the headspace of a 10 m diameter bin filled with 300 t of wheat, for a period of 26 months.

Location[†]	Distance from grain surface (m)	Cold temperature periods						Warm temperature periods					
		Nov 2019 to Mar 2020			Nov 2020 to Mar 2021			Apr to Oct 2020			Apr to Oct 2021		
		Mean (°C)	Max (°C)	Min (°C)	Mean (°C)	Max (°C)	Min (°C)	Mean (°C)	Max (°C)	Min (°C)	Mean (°C)	Max (°C)	Min (°C)
Centre	0.2	-4.9 ± 0.1[a]	17.0	-20.5	-4.8 ± 0.2[a]	21.4	-31.2	18.5 ± 0.2[a]	50.4	-10.8	20.0 ± 0.1[a]	51.8	-5.0
	1.4	-6.4 ± 0.1[b]	22.9	-25.4	-4.8 ± 0.2[b]	28.1	-31.4	18.5 ± 0.2[a]	55.9	-13.8	20.7 ± 0.2[b]	56.8	-5.7
	2.6	-5.8 ± 0.1[c]	25.3	-24.8	-4.1 ± 0.2[c]	25.5	-32.2	19.2 ± 0.2[b]	58.6	-13.4	21.2 ± 0.2[c]	59.6	-5.4
HR1	0.6	-6.7 ± 0.1[a]	20.4	-25.7	-5.2 ± 0.2[a]	23.3	-32.1	17.9 ± 0.2[a]	52.7	-13.9	19.9 ± 0.2[a]	53.4	-5.7
	1.8	-6.4 ± 0.1[b]	23.6	-25.4	-4.8 ± 0.2[b]	26.3	-32.1	18.7 ± 0.2[b]	56.8	-13.8	20.8 ± 0.2[b]	57.9	-5.7
HR2	0.6	-6.3 ± 0.1[a]	20.4	-25.2	-4.8 ± 0.2[a]	23.6	-31.8	18.5 ± 0.2[a]	52.9	-13.4	20.6 ± 0.2[a]	53.4	-5.3

	1.8	-6.2 ± 0.1[b]	22.9	-25.1	-4.6 ± 0.2[b]	25.9	-31.7	18.7 ± 0.2[b]	56.1	-13.5	20.8 ± 0.2[b]	57.4	-5.6
HR3	0.6	-6.4 ± 0.1[a]	20.4	-24.9	-4.9 ± 0.2[a]	23.3	-32.0	18.1 ± 0.2[a]	52.4	-13.4	20.1 ± 0.2[a]	53.0	-5.5
	1.8	-6.2 ± 0.1[b]	24.3	-25.2	-4.6 ± 0.2[b]	26.1	-32.3	18.9 ± 0.2[b]	56.4	-13.6	21.0 ± 0.2[b]	58.3	-5.6
HR4	0.6	-5.5 ± 0.1[a]	21.6	-23.9	-3.9 ± 0.2[a]	24.8	-30.7	18.5 ± 0.2[a]	52.8	-12.4	20.6 ± 0.2[a]	53.1	-4.5
	1.8	-5.8 ± 0.1[b]	25.8	-25.0	-4.0 ± 0.2[b]	28.1	-31.8	19.3 ± 0.2[b]	58.5	-13.2	21.5 ± 0.2[b]	59.6	-5.2
HR5	0.6	-5.9 ± 0.1[a]	22.1	-24.8	-4.4 ± 0.2[a]	25.4	-31.7	18.5 ± 0.2[a]	53.9	-13.1	20.5 ± 0.2[a]	54.3	-5.1
	1.8	-5.6 ± 0.1[b]	25.3	-24.5	-4.0 ± 0.2[b]	28.2	-31.3	19.1 ± 0.2[b]	58.4	-13.0	21.2 ± 0.2[b]	58.8	-5.2
HR6	0.6	-6.0 ± 0.1[a]	21.4	-24.8	-4.5 ± 0.2[a]	24.4	-31.4	18.4 ± 0.2[a]	53.6	-13.2	20.4 ± 0.2[a]	54.3	-5.2
	1.8	-6.3 ± 0.1[b]	24.3	-25.3	-4.7 ± 0.2[b]	26.9	-32.2	18.6 ± 0.2[b]	57.5	-13.8	20.7 ± 0.2[b]	58.4	-5.7

[†] Centre represents the cable located at 0.85 m from the centre of the bin along the North Direction; HR1, HR2, HR3, HR4, HR5 and HR6 represent the cables located at 2.25 m from the centre of the bin (refer to Figure 4.10); data from the sensor at the east and west location near the wall are missing because of sensor malfunction.

[a, b, c] Different lowercase alphabets represent the significantly different mean values recorded using the different sensors at varying distance from the grain surface at the cables located inside the bin, using paired t-test ($\alpha = 0.05$).

The average temperature differences observed between year I and year II were 1.6 ± 0.0, 1.7 ± 0.0 and 2.6 ± 0.1°C at the center and 2.5 and 0.15 m from the sidewall, respectively. Thus, the increase in ambient temperature in year II, affected the floor temperature near the wall, followed by those at 2.5 m away from the center, and center.

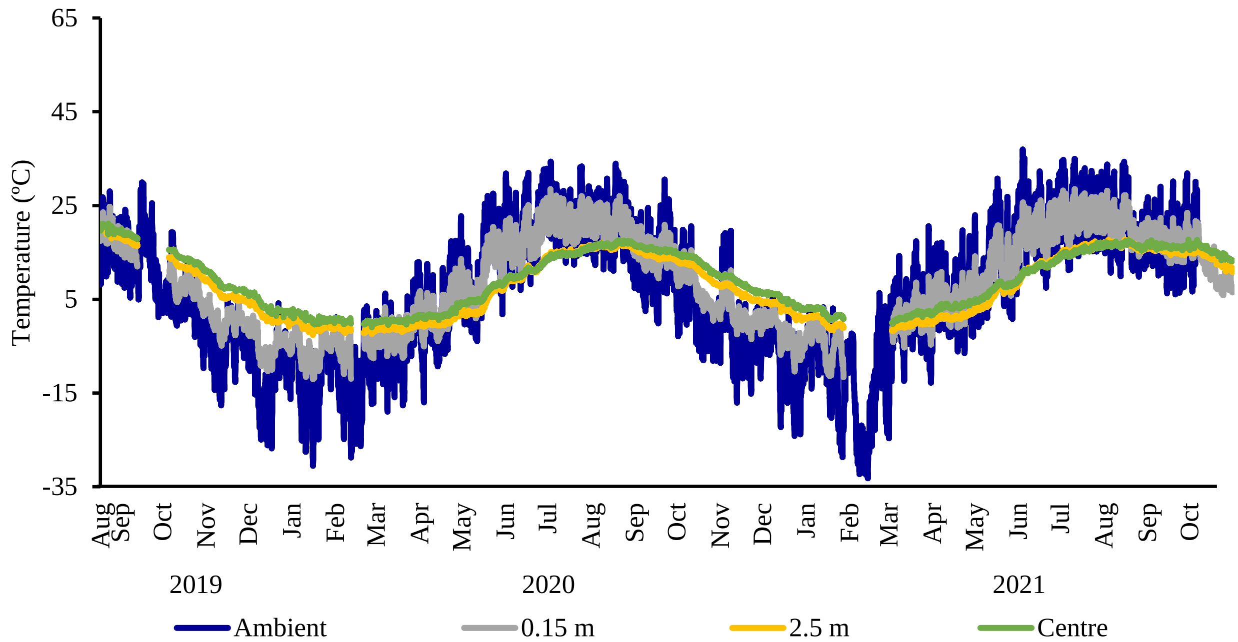

Figure 4.12. Floor temperatures at various locations inside the bin filled with 300 t of wheat from August 18, 2019, to October 31, 2021. Only the temperatures along the north direction (0.15 m and 2.5 m from the sidewall) are presented.

4.3.4.4 *Roof temperatures*

The average temperatures on the outside surface of the roof were 2.5 ± 0.4°C higher than those of the ambient air temperatures during the 26-month experimental period (Figure 4.13). The temperatures on the roof were mainly affected by the ambient temperature and solar radiation. This could be confirmed from Figure 4.14, where the roof temperatures at all the locations were either equal to or higher than the ambient temperature during the Cold and Warm Temperature Periods.

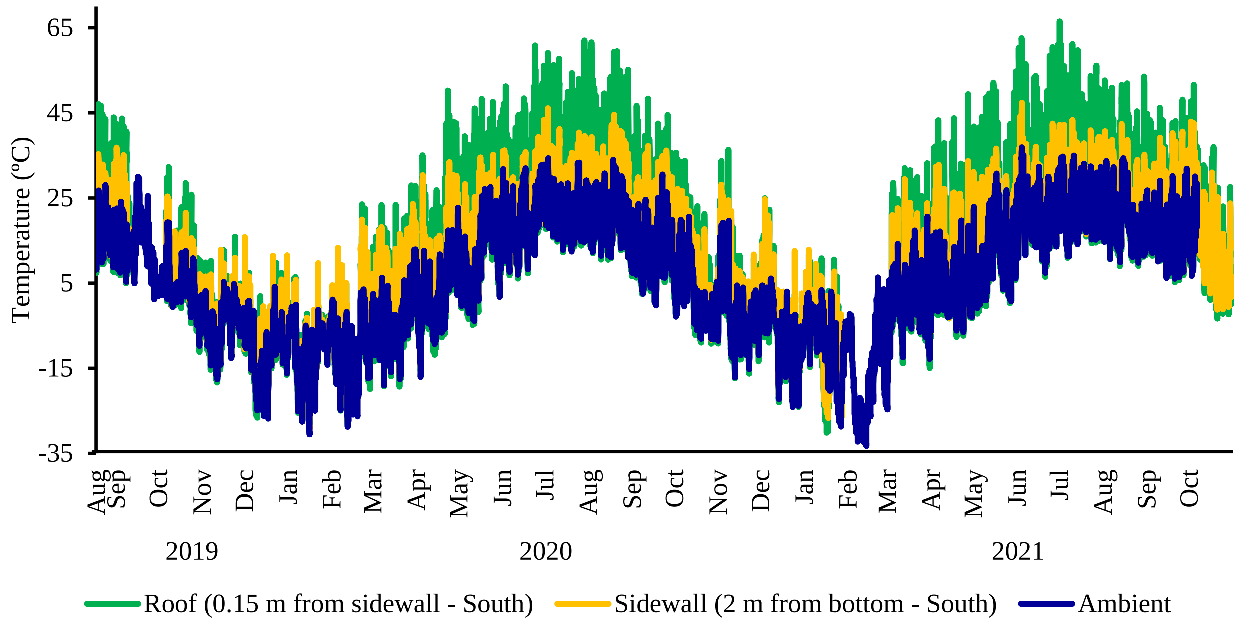

Figure 4.13. Temperatures on the roof and sidewall of a 10 m diameter corrugated steel bin filled with 300 t of wheat from August 18, 2019, to October 31, 2021. Only the temperatures along the south direction are presented.

Temperatures along the south and west directions on roof at 0.15 m from the sidewall, were hotter than those along north and east directions during the 26-month experimental period. This was because south and west directions received more solar radiation than the other directions. Similar to the floor, temperatures on the roof were also higher during year II than year I, due to the higher ambient temperature in year II. The average increase in temperature at various measured locations on the outside surface of the roof during year II, was 3.9 ± 0.1°C, compared to year I. The maximum and minimum temperatures recorded on the outside surface of the roof during year I were 71.7 and -30.6°C, respectively; and those recorded during year II were 71.8 and -33.2°C, respectively. The minimum temperatures reached on the roof were the same as the ambient temperatures, while the maximum temperatures were hotter than the ambient because of the solar radiation. The higher temperature recorded on the south side of the roof, during the day (Figure 4.14a) was mainly because of the higher solar radiation on the south side. Similarly, higher roof temperatures, during the day, especially during Warm Temperature Period (Figure 4.14b) could also be attributed to the incidence of solar radiation on the outside surface of the roof. Lawrence and Maier (2011) reported that the headspace air and wall temperature increased during the day as a result of increased intensity of solar radiation.

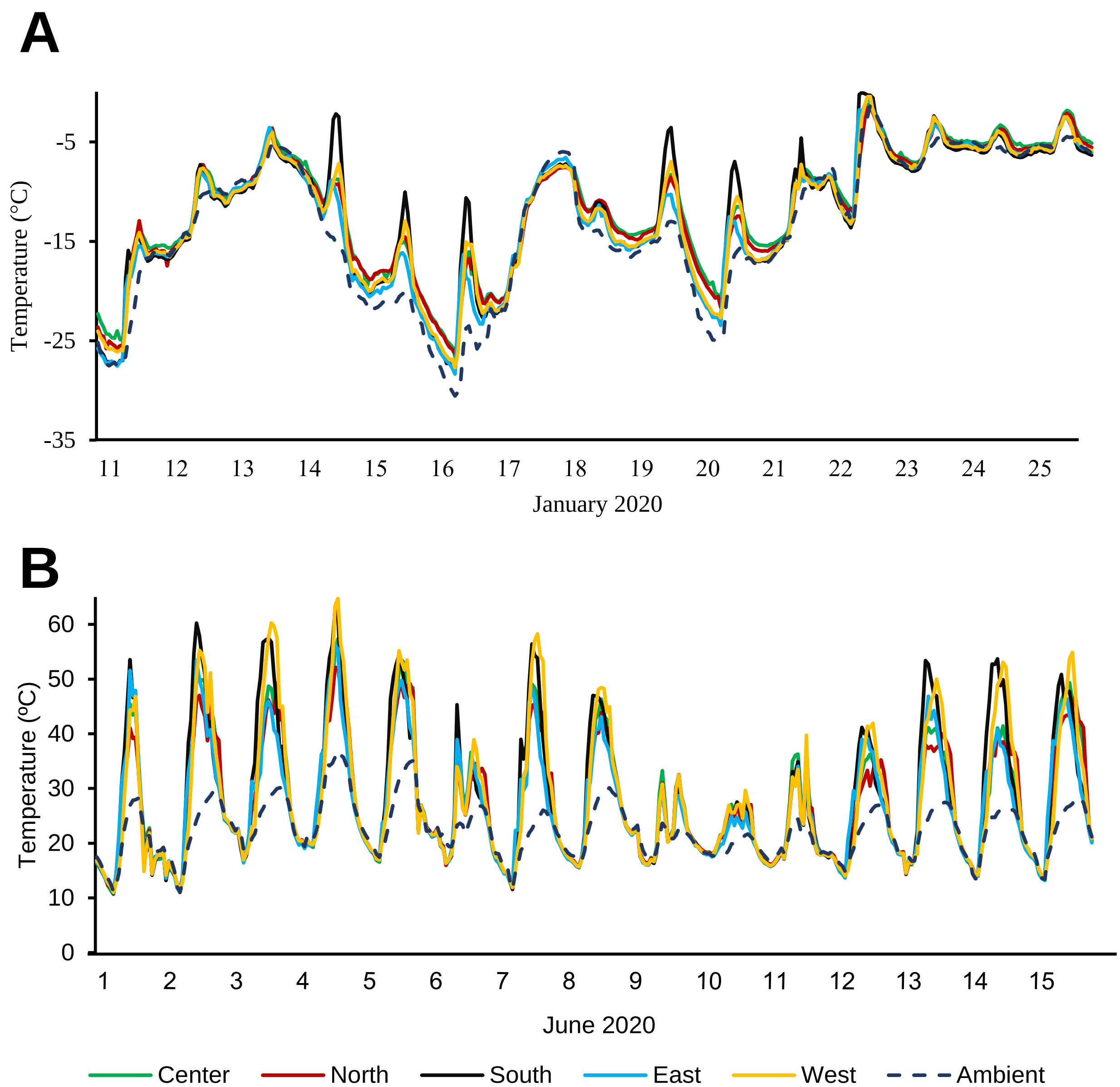

Figure 4.14. Roof temperatures at the center and at 0.1 5 m from the sidewalls along north, south, east, and west directions of a 10 m diameter corrugated steel bin during the Cold Temperature Period (A) and the Warm Temperature Period (B) in 2020. Data for only 15 days are presented.

4.3.4.5 *Sidewall temperatures*

Like the roof, the sidewalls were hotter than the ambient air (Figure 4.13) by 2.5 ± 0.3°C from mid-August 2019 to the end of October 2021. Among the temperatures measured at 12 locations on the outside surface of the sidewalls of the bin, the average temperature at 0.3 m from the bottom of the bin on south side of the wall was the highest (11.4 ± 0.1°C), during the 26 months. The north side of the wall recorded the lowest average temperatures, at all the three locations (0.3 m, 2 m, and 4 m). The locations at the south side of the bin wall were hotter than their corresponding locations at the north side of the wall, during the experimental period. At 0.3 m from the bottom of the bin, east and west sides of the wall recorded equal average temperature (Table 4.3). At 2 m from the bottom of the bin, the temperature at the east side of the wall was hotter than or equal to those at the west side of the wall; while, at 4 m, the temperature at the west side of the wall was hotter. The change in wind direction could have possibly impacted the temperature on the outside surface of the sidewalls.

In general, the temperatures on east and west sides of the wall, increased during forenoon and afternoon, respectively, because of the incidence of solar radiation. The temperatures at 0.3 m from the bottom were hotter than or equal to those at 2 m from the bottom on the wall in all the four directions. The temperatures at 4 m from the bottom were hotter than or equal to those at 2 m, on the wall in all the directions, except east. This was because the temperatures near the bottom of the bin were affected by foundation temperatures and those near the top were affected by the headspace temperatures (Jian et al., 2005).

Like the floor and roof, temperatures on the sidewalls were hotter during year II (average temperature of 10.7 ± 0.5°C) than those during year I (average temperature of 7.3 ± 0.4°C). The maximum and minimum temperatures observed on the outside surface of sidewalls of the bin, during year I, were 59.5 and -30.6°C, respectively; and those recorded during year II were 64.4 and -33.2°C, respectively. These temperatures were lower than those observed on the roof. The temperature on roof were colder during winter and hotter during summer, as compared to those recorded on the sidewalls. The colder surface of roof during winter could be because of the accumulation of snow on the roof; while the hotter surface of roof during summer could be attributed to the higher solar radiation on the surface of the roof, as compared to the sidewalls.

Table 4.3. Mean, maximum (Max) and minimum (Min) temperatures recorded at the outside surface of the sidewall at different distances from the bottom of a 10 m diameter bin filled with 300 t of wheat.

Year ⇩	Distance from the bottom (m) ⇨	North			South			East			West		
		0.3	2	4	0.3	2	4	0.3	2	4	0.3	2	4
Year I[†]	Mean (°C)	6.1 ± 0.1$^{a, A}$	5.8 ± 0.2$^{b, A}$	6.0 ± 0.2$^{c, A}$	9.8 ± 0.2$^{a, B}$	6.9 ± 0.2$^{b, B}$	8.2 ± 0.2$^{c, B}$	8.8 ± 0.2$^{a, C}$	8.0 ± 0.2$^{b, C}$	6.9 ± 0.2$^{c, C}$	8.8 ± 0.2$^{a, C}$	7.5 ± 0.2$^{b, D}$	7.6 ± 0.2$^{c, D}$
	Max (°C)	42.6	44.3	42.2	55.2	46.1	50.8	51.7	51.0	47.7	57.9	56.6	59.5
	Min (°C)	-25.2	-29.2	-29.6	-23.5	-29.2	-29.0	-23.4	-27.3	-29.0	-22.0	-28.6	-29.2
Year II[‡]	Mean (°C)	9.4 ± 0.1$^{a, A}$	9.4 ± 0.1$^{b, A}$	9.7 ± 0.2$^{c, A}$	13.2 ± 0.2$^{a, B}$	10.7 ± 0.2$^{b, B}$	11.8 ± 0.2$^{c, B}$	12.2 ± 0.2$^{a, C}$	11.6 ± 0.2$^{b, C}$	10.6 ± 0.2$^{c, C}$	12.1 ± 0.2$^{a, C}$	11.1 ± 0.2$^{b, D}$	11.3 ± 0.2$^{c, D}$
	Max (°C)	42.2	45.0	43.3	55.8	47.4	64.4	51.8	50.6	46.7	55.1	52.9	55.8
	Min (°C)	-22.2	-26.4	-27.3	-22.4	-26.7	-27.1	-20.4	-25.1	-26.9	-21.0	-25.7	-26.8

[†] Year I represents the period from November 1, 2019 to October 31, 2020; [‡] Year II represents the period from November 1, 2020 to October 31, 2021.

[a, b, c] Different lowercase alphabets represent the significantly different mean values within the different locations at the same side of the wall, using paired t-test (α = 0.05).

[A, B, C, D] Different uppercase alphabets represent the significantly different mean values within the same locations at different sides of the wall, using paired t-test (α = 0.05).

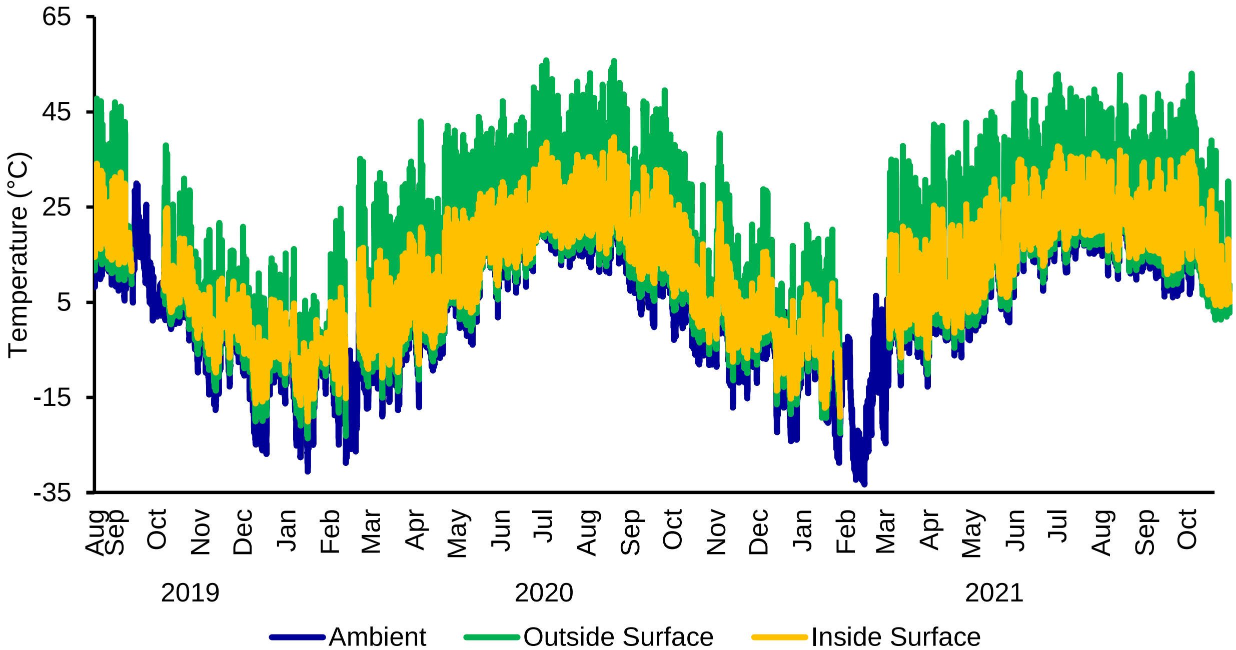

Figure 4.15. Temperatures on the outside and inside surface of the south-east side of the wall near the floor of a 10 m diameter corrugated steel bin filled with 300 t of wheat from August 18, 2019, to October 31, 2021.

On comparing the temperatures recorded on the outside and inside surface of the south-east side of the wall near the floor (Figure. 4.15), the outside surface was hotter by 1.3°C than the inside surface, during both years in the Warm Temperature Period, and the inside surface was hotter to the outside surface, by 0.5 and 0.2°C, during year I and II, respectively, in the Cold Temperature Period. The temperature recorded on the thermocouple located at the inside surface of the wall near floor was hotter by 2.3 and 4.6°C, respectively, as compared to the ambient temperature, during the Warm and Cold Temperature Periods. This could be attributed to the presence of grain inside the bin and the effect of solar radiation on the metal surface. These data can be used to validate the mathematical model which involves prediction of the inside surface of the sidewall using the temperatures measured on the outside surface, ambient conditions and the properties of the wall material.

4.3.5 Discussion

The current study found that the main factor influencing the temperatures on the floor, roof, sidewalls and in the headspace of the bin was the solar radiation, besides ambient temperature. Montross et al. (2002a) reported that the predicted temperatures of the roof, wall and average corn temperature were significantly affected by the absorption of the solar radiation. Montross et al. (2002b) reported the headspace temperature as high as 45°C, inside a 2.74 m diameter corrugated steel bin, in the presence of higher solar radiation; whereas, the headspace temperature was reported to drop to 35°C, during intermittent solar radiation period. The average final predicted corn temperature was found to be about 1.4 to 2.0°C warmer, when the solar radiation and wind speed were included. The grains near the periphery (the wall and the headspace) of the bin was found to be significantly influenced by the solar radiation. Moreover, the heat transfer between the grain surface and headspace air is also influenced by the solar radiation (Montross et al., 2002b). The headspace air near the roof is higher than those near the walls as a result of solar radiation (Lawrence and Maier, 2011). These results are in accordance with the findings of the current study.

By including solar radiation as one of the influencing factors, the temperatures of the stored grain can be predicted more accurately. During prediction of moisture movement in a non-aerated grain mass, Montross and Maier (2001) observed the reduction in average corn temperature and moisture accumulation when the solar radiation was neglected, and the impermeable boundary conditions were assumed. Alagusundaram et al. (1990) also included the weather data (solar radiation, wind velocity and ambient air temperature) in the prediction of temperature distribution in stored grain bins. . The results of the current study could be used to validate mathematical model which involves the prediction of the temperature of the bin components using the ambient conditions, which in turn could be used to predict the temperature of the grain stored inside the bin using the initial temperature and moisture of the grain.

The results of the current study can be used to understand the influence of climate change on the temperature profile of the bin and hence, on the temperature and quality of the stored grain. Warmer temperatures favor the growth and multiplication of insects, mites, and mould. Jian et al. (2018) found that the first two factors influencing the *Cryptolestes ferrugineus* (Stephens) population were the temperature and initial insect numbers. *Cryptolestes ferrugineus* at 25°C can reach its peak density in less than 80 days, while it needs 140 days at 21°C. Tripathi et al. (2021) found a similar trend for the *Tribolium castaneum* (Herbst). Sravanthi et al. (2013) observed visible mould in red lentils stored at 40°C by the end of 3 weeks, whereas at 30°C only after 16 weeks. However, they observed no visible mould throughout the period of storage (16 weeks), at 10 and 20°C. So, the possibility of outbreak of the insects, mites, and mould inside the grain cannot be overlooked and the appropriate measures needs to be taken to prevent/ reduce their outbreak. Considering the effect of increasing global mean temperature on the stored grain and the pest and mould multiplication, further modifications on grain storage structure are required. For example, the temperature of the stored grain can be reduced by adopting appropriate management practices such as aeration and turning. Moreover, with change in global temperature patterns, modifications to meet the safe storage conditions and development of appropriate stored grain management protocols which consider the effect of ambient temperature change are required.

The change in grain quality depends not only on the ambient weather, but also on various other factors such as presence or absence of other biotic factors in the grain bulk such as insects and microorganisms, initial grain temperature and moisture content, accumulation of moisture at a particular location due to leakage of snow/ water into the bin, condensation or moisture migration due to convection currents. Considering the complexity of the interaction of these factors, it might be misleading to compare the quality of the wheat grain stored inside the bin based on ambient condition only. Hence, the change in grain quality, moisture content and temperature has been reported in detail elsewhere (Bharathi et al. 2023).

4.3.6 Conclusion

During the study period, the ambient temperature was higher by 1.8°C in year II, than year I. As a result, the average temperatures in year II were higher by 1.9 ± 0.1°C in the headspace, 2.1 ± 0.1°C on the floor, 3.9 ± 0.1°C on the outside surface of the roof and 3.5 ± 0.2°C on the outside surface of the sidewalls, than those in year I. The temperature distribution varied with varying locations on the floor, roof, sidewalls and in the headspace. On the floor, the effect of ambient weather decreased with increase in distance from the sidewalls. The south side of the bin received more solar radiation than north side. The roof was colder in Cold Temperature Period and warmer in Warm Temperature Period, than sidewalls. This study showed that the floor, roof, sidewall, and headspace temperatures of the bin were differently influenced by the solar radiation. During the 26-month period, the temperatures on the floor, roof, sidewalls and in the headspace of the bin were warmer by 2.5 ± 0.3°C, 2.5 ± 0.4°C, 2.5 ± 0.3°C and 3.7 ± 0.2°C than the ambient air temperature, respectively. . In addition to the ambient temperature, the temperature of the bin at various locations also depends on the incidence of solar radiation.

4.3.7 Author contributions

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

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Chapter 5. Three-dimensional diffusivity of insects

5.1.1 Introduction

Diffusion has been adopted to model movement of insects inside the stored grain ecosystem (Banks et al., 1985). Jian et al. (2007) calculated the 2D diffusivity (or rate of diffusion) of *C. ferrugineus* at varying combinations of temperatures (20, 25, 30, and 35°C), moisture contents (12.5, 14.5, and 16.5%), number of adults (125, 250, and 500), and time periods (3, 6, 12, 24, and 72 h). Prediction of insect movement and distribution in a dynamic environment, such as the actual bin, requires consideration of various factors, as mentioned in the previous chapters. Jian et al. (2008) developed a transport equations-based model to predict the distribution of *C. ferrugineus* adults and used the numerical (finite difference) and analytical method to estimate the coefficients related with the transport equations. They compared the data predicted from the model with those obtained from the experiments conducted using 1D wheat columns and 2D wheat chambers. But there are no published literatures available on the prediction of 3D distribution of *C. ferrugineus*.

Table 5.1. Experimental conditions considered for the calculation of 3D diffusivity of *Cryptolestes ferrugineus*

Experiments$^{\alpha}$	Temperature (°C)	Moisture contents (%)	Adults introduced	Movement period (h)
Temperatures	20, 30	12.5	100	24
	20, 30, 35	14.5	100	24
	20, 30	16.5	100	24
Moisture contents	20	12.5, 14.5, 16.5	100	24
	30	12.5, 14.5, 16.5	100	24
Insect densities	20	14.5	100, 500$^{\beta}$, 1000$^{\beta}$	24
Movement periods	20	14.5	100	6, 24, 72

$^{\alpha}$Data reported in objective 1 (Bharathi et al. 2021, 2022) based on distribution of *C. ferrugineus* inside a 3D grain bulk

$^{\beta}$The insect numbers in the experiments with 500 and 1000 adults were normalized to 100.

In this chapter, the analytical solution to solve the transport equations was proposed to predict the 3D diffusivity of *C. ferrugineus*, under homogenous environment at different temperatures (20, 30, and 35°C), moisture contents (12.5, 14.5, and 16.5%), movement periods (6, 24, and 72 h), and adult numbers (100, 500 and 1000) (Table 5.1). Since the diffusivity values in horizontal, vertical (downward) and vertical (upward) are different for *C. ferrugineus*, the grain bulk used to perform the experiments (explained in objective 1) were divided into three sections based on the layers. Considering the numbering of the layers from 1 to 7, where 1 is the topmost layer and 7 is bottom most layer, the layers from 1 to 3 were considered as section 1 (with upward and horizontal movement), layer 4 was considered as section 2 (with only horizontal movement), layers from 5 to 7 were considered as section 3 (with downward and horizontal movement).

5.1.1.1 *Simplified transport equation*

$$\frac{\partial u}{\partial t} = D_x \frac{\partial^2 u}{\partial x^2} + D_y \frac{\partial^2 u}{\partial y^2} + D_z \frac{\partial^2 u}{\partial z^2} - B_x \frac{\partial u}{\partial x} - B_y \frac{\partial u}{\partial y} - B_z \frac{\partial u}{\partial z} + \alpha$$

where,

u = u (x,y,z,t), the spatial-temporal population density of the insect (adults/m^3 at any time t);

x, y, z = coordinate of a position (m)

D_x, D_y, D_z = diffusivity in x, y, and z directions, respectively (m^2/s)

B_x, B_y, B_z = bias movement velocity in the x, y, and z directions, respectively (m/s)

α = net change in insect count

For a 3D grain bulk with the volume L x W x H, the boundary conditions are:

$$\frac{\partial u}{\partial x} = 0 \; at \; x = \frac{L}{2} \; and \; x = -\frac{L}{2}$$

$$\frac{\partial u}{\partial y} = 0 \; at \; y = \frac{W}{2} \; and \; y = -\frac{W}{2}$$

$$\frac{\partial u}{\partial z} = 0 \; at \; z = \frac{H}{2} \; and \; z = -\frac{H}{2}$$

Initial condition, $u = N_0 \delta(x)\delta(y)\delta(z) at\; t = 0$, which implies an instantaneous source; where, N_0 is the number of insects introduced and $\delta(x)\delta(y)\delta(z)$ = 1 at x = 0, y = 0, z = 0 when t = 0.

5.1.2 Analytical solution

According to Carslaw and Jaeger (1959), the Green's function for 3D are the product of three 1D solutions. Moreover, we assume that the movement of insects in 1D does not affect their movement in other two dimensions. Thus, the analytical solution for homogenous 3D can be modified from one- and two-dimensional solutions reported by Jian et al. (2008):

$$\boldsymbol{u(x,y,z,t)} = \frac{\boldsymbol{N_0}}{\boldsymbol{ABC}}\left\{\mathbf{1}+\mathbf{2}\sum_{\boldsymbol{n=1}}^{\infty}\boldsymbol{e}^{\left[-\boldsymbol{D_H}\left(\frac{\boldsymbol{n\pi}}{\boldsymbol{A}}\right)^2\boldsymbol{t}\right]} * \boldsymbol{cos}\left[\frac{\boldsymbol{n\pi}}{\boldsymbol{A}}\left(\boldsymbol{x}+\frac{\boldsymbol{A}}{\mathbf{2}}\right)\right]\boldsymbol{cos}\left(\frac{\boldsymbol{n\pi}}{\mathbf{2}}\right)\right\}$$

$$* \left\{\mathbf{1}+\mathbf{2}\sum_{\boldsymbol{n=1}}^{\infty}\boldsymbol{e}^{\left[-\boldsymbol{D_H}\left(\frac{\boldsymbol{n\pi}}{\boldsymbol{B}}\right)^2\boldsymbol{t}\right]} * \boldsymbol{cos}\left[\frac{\boldsymbol{n\pi}}{\boldsymbol{B}}\left(\boldsymbol{y}+\frac{\boldsymbol{B}}{\mathbf{2}}\right)\right]\boldsymbol{cos}\left(\frac{\boldsymbol{n\pi}}{\mathbf{2}}\right)\right\}$$

$$* \left\{\mathbf{1}+\mathbf{2}\sum_{\boldsymbol{n=1}}^{\infty}\boldsymbol{e}^{\left[-\boldsymbol{D_V}\left(\frac{\boldsymbol{n\pi}}{\boldsymbol{C}}\right)^2\boldsymbol{t}\right]} * \boldsymbol{cos}\left[\frac{\boldsymbol{n\pi}}{\boldsymbol{C}}\left(\boldsymbol{z}+\frac{\boldsymbol{C}}{\mathbf{2}}\right)\right]\boldsymbol{cos}\left(\frac{\boldsymbol{n\pi}}{\mathbf{2}}\right)\right\}$$

where,

x, y and z are the coordinates at the centre of the section (m)

u(x,y,z,t) is the insect number in any section at time t (h)

N_0 is the initial insect number at the center of the 3D box

A, B and C are the dimensions of the 3D box

n is set from 1 to 20 for the purpose of calculation

D_H and D_V are diffusion rates (m^2/h) in horizontal and vertical directions

5.1.3 Calculation of 3D diffusivity using analytical method

To calculate the 3D diffusivity at different environmental conditions, the experimental data reported in objective 1, were used. Insect numbers in each element inside a 3D grain bulk at a particular environmental condition were calculated using the following equations:

1. To calculate the insect numbers in the layers 1 to 3, the following equation was used,

$$\begin{aligned} u(x,y,z,t) = \frac{N_0}{ABC}&\left\{1+2\sum_{n=1}^{\infty} e^{\left[-D_H\left(\frac{n\pi}{A}\right)^2 t\right]} * \cos\left[\frac{n\pi}{A}\left(x+\frac{A}{2}\right)\right]\cos\left(\frac{n\pi}{2}\right)\right\} \\ &* \left\{1+2\sum_{n=1}^{\infty} e^{\left[-D_H\left(\frac{n\pi}{B}\right)^2 t\right]} * \cos\left[\frac{n\pi}{B}\left(y+\frac{B}{2}\right)\right]\cos\left(\frac{n\pi}{2}\right)\right\} \\ &* \left\{1+2\sum_{n=1}^{\infty} e^{\left[-D_{V1}\left(\frac{n\pi}{C}\right)^2 t\right]} * \cos\left[\frac{n\pi}{C}\left(z+\frac{C}{2}\right)\right]\cos\left(\frac{n\pi}{2}\right)\right\} \end{aligned} \tag{5.1}$$

2. To calculate the insect numbers in the layer 4, the following equation was used,

$$\begin{aligned} u(x,y,t) = \frac{N_0}{AB}&\left\{1+2\sum_{n=1}^{\infty} e^{\left[-D_H\left(\frac{n\pi}{A}\right)^2 t\right]} * \cos\left[\frac{n\pi}{A}\left(x+\frac{A}{2}\right)\right]\cos\left(\frac{n\pi}{2}\right)\right\} \\ &* \left\{1+2\sum_{n=1}^{\infty} e^{\left[-D_H\left(\frac{n\pi}{B}\right)^2 t\right]} * \cos\left[\frac{n\pi}{B}\left(y+\frac{B}{2}\right)\right]\cos\left(\frac{n\pi}{2}\right)\right\} \end{aligned} \tag{5.2}$$

3. To calculate the insect numbers in the layers 5 to 7, the following equation was used,

$$\begin{aligned} u(x,y,z,t) = \frac{N_0}{ABC}&\left\{1+2\sum_{n=1}^{\infty} e^{\left[-D_H\left(\frac{n\pi}{A}\right)^2 t\right]} * \cos\left[\frac{n\pi}{A}\left(x+\frac{A}{2}\right)\right]\cos\left(\frac{n\pi}{2}\right)\right\} \\ &* \left\{1+2\sum_{n=1}^{\infty} e^{\left[-D_H\left(\frac{n\pi}{B}\right)^2 t\right]} * \cos\left[\frac{n\pi}{B}\left(y+\frac{B}{2}\right)\right]\cos\left(\frac{n\pi}{2}\right)\right\} \\ &* \left\{1+2\sum_{n=1}^{\infty} e^{\left[-D_{V2}\left(\frac{n\pi}{C}\right)^2 t\right]} * \cos\left[\frac{n\pi}{C}\left(z+\frac{C}{2}\right)\right]\cos\left(\frac{n\pi}{2}\right)\right\} \end{aligned} \tag{5.3}$$

where,

x, y, and z are the coordinates at the centre of the section (m)

u (x,y,z,t) is the insect number in the section at time t (h)

A, B, and C are the total distances available for the insects to move in x, y, and z directions, respectively

N_0 is the initial insect number at the center of the 3D box

n is set from 1 to 20 for the purpose of calculation

D_H is diffusion rate (m^2/h) in the horizontal direction

D_{V1} and D_{V2} are diffusion rates (m^2/h) in the vertical direction for upward and downward movements, respectively.

The algorithm to calculate the 3D diffusivity at any given environmental condition, using the analytical solution, is provided in Figure 5.1. Python version 3.11 was used to perform the calculations. During estimation, the initial diffusivity was assumed as 0 to 0.01 m^2/h with increment of 10^{-5} m^2/h. To increase the accuracy and optimize the computing time, the diffusivity range of resulted values with smaller increment were considered, and the calculation was repeated for the same range with the increase in increment. For instance, if the resulted range of diffusivity for an experiment with different trials was from 0 to 1 x 10^{-5}, with the increment 10^{-5}, the calculations were repeated with the estimated diffusivity from 0 to 1.6 x 10^{-5} with increment of 10^{-6}. The increments were used from 10^{-5} to 10^{-7} at the end of the calculation. The $D_{H,}$ D_{V1} and D_{V2} values were considered in all the possible combinations within the range at a given increment. For instance, if the range for $D_{H,}$ D_{V1} and D_{V2} values were 0 to 1 x 10^{-5}, with the increment 10^{-5}, the insect numbers were calculated for $D_{H,}$ D_{V1} and D_{V2} values of (0,0,0), (0, 0, 1 x 10^{-5}), (0, 1 x 10^{-5}, 0), (0, 1 x 10^{-5}, 1 x 10^{-5}), (1 x 10^{-5},0,0), (1 x 10^{-5}, 0, 1 x 10^{-5}), (1 x 10^{-5}, 1 x 10^{-5}, 0), and (1 x 10^{-5}, 1 x 10^{-5}, 1 x 10^{-5}).

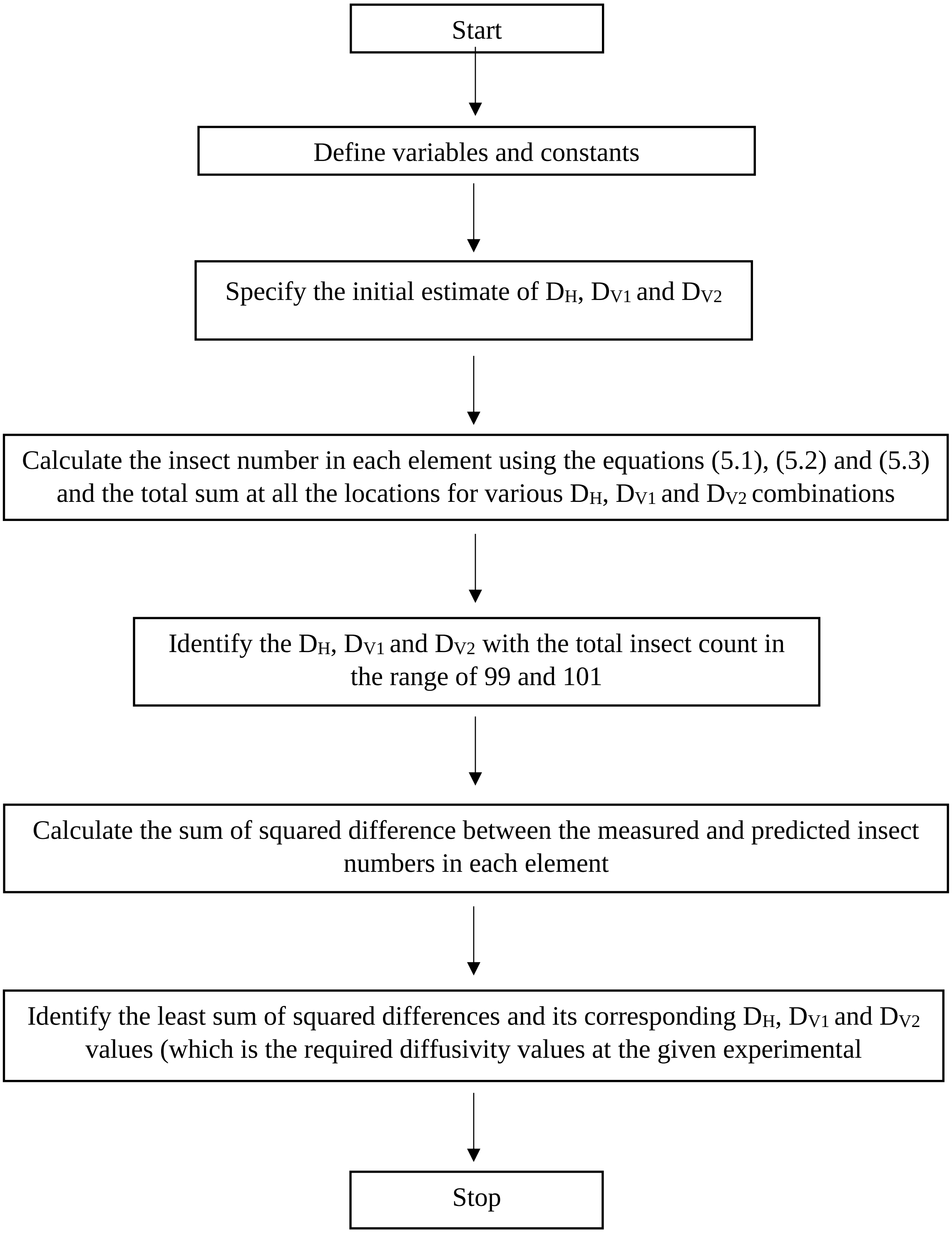

Figure 5.1. Flow chart of the procedure to calculate three-dimensional (3D) diffusivity

Table 5.2. Calculated mean 3D diffusivities (horizontal and vertical) at different environmental conditions

Environmental conditions[ε]			D_H x 10^{-5} m^2/ h	D_{V1} x 10^{-5} m^2/ h	D_{V2} x 10^{-5} m^2/ h	Least squared difference
12.5M-100A-24P	Temperature (°C)	20	6.7 ± 0.2[a, A]	11.8 ± 2.5[a, A]	25.1 ± 12.5[a, A]	356.2 ± 25.7
		30	6.6 ± 0.0[a, A]	14.9 ± 0.0[a, A]	30.7 ± 12.6[a, A]	431.2 ± 48.4
14.5M-100A-24P		20	6.7 ± 0.0[a, A]	13.0 ± 1.4[a, A]	8.3 ± 3.6[a, A]	315.3 ± 12.5
		30	6.6 ± 0.0[a, A]	13.3 ± 1.4[a, A]	32.7 ± 5.5[a, B]	412.7 ± 40.9
		35	6.6 ± 0.1[a, A]	12.7 ± 1.9[a, A]	19.7 ± 8.4[a, A]	307.9 ± 43.0
16.5M-100A-24P		20	7.4 ± 0.3[a, A]	7.0 ± 2.5[a, A]	17.3 ± 11.0[a, A]	411.6 ± 27.2
		30	7.0 ± 0.1[a, A]	7.3 ± 0.3[a, A]	13.7 ± 1.0[a, A]	493.0 ± 30.3
20T-100A-24P	Moisture contents (%)	12.5	6.7 ± 0.2[a, A]	11.8 ± 2.5[a, A]	25.1 ± 12.5[a, A]	356.2 ± 25.7
		14.5	6.7 ± 0.0[a, A]	13.0 ± 1.4[a, A]	8.3 ± 3.6[a, A]	315.3 ± 12.5
		16.5	7.4 ± 0.3[a, A]	7.0 ± 2.5[a, A]	17.3 ± 11.0[a, A]	411.6 ± 27.2
30T-100A-24P		12.5	6.6 ± 0.0[a, A]	14.9 ± 0.0[a, A]	30.7 ± 12.6[a, A]	431.2 ± 48.4
		14.5	6.6 ± 0.0[a, A]	13.3 ± 1.4[a, A]	32.7 ± 5.5[a, B]	412.7 ± 40.9
		16.5	7.0 ± 0.1[a, A]	7.3 ± 0.3[b, A]	13.7 ± 1.0[a, B]	493.0 ± 30.3
20T-14.5M-24P	Initial adult numbers	100	6.7 ± 0.0[a, A]	13.0 ± 1.4[a, A]	8.3 ± 3.6[a, A]	315.3 ± 12.5
		500	8.3 ± 0.3[b, A]	2.3 ± 0.3[b, A]	27.0 ± 18.9[a, A]	399.2 ± 41.6
		1000	6.7 ± 0.1[a, A]	14.0 ± 0.8[a, A]	21.0 ± 5.7[a, A]	177.0 ± 19.4
20T-14.5M-100A	Movement periods (h)	6	33.3 ± 1.2[a, A]	16.0 ± 3.1[a, B]	29.7 ± 0.7[a, A]	431.2 ± 49.9
		24	6.7 ± 0.0[b, A]	13.0 ± 1.4[a, A]	8.3 ± 3.6[b, A]	315.3 ± 12.5
		72	2.3 ± 0.1[c, AB]	0.007 ± 0.0[b, B]	3.7 ± 0.8[b, A]	351.2 ± 27.6

[ε]Numbers before M, T, A and P are the moisture contents (%), temperature (°C), number of adults introduced, and movement periods (h) of the experiment, respectively.

[a,b,c]Different lowercase alphabets within the different experimental conditions (in the column) represent the significantly different mean diffusivity values in the same direction at different experiments using Tukey's test at the level (α) 0.05.

[A,B]Different uppercase alphabets within the diffusivities (D_H, D_{V1}, D_{V2}) (in the row) of same experimental condition represent the significantly different mean diffusivity values at different directions within the same experimental conditions using Tukey's test at the level (α) 0.05.

5.1.3.1 *Data analyses*

To determine the horizontal and vertical diffusivities of *C. ferrugineus* at different temperatures, moisture contents, insect densities, and movement periods, the data reported by (Bharathi et al., 2021a, 2022) dealing with distribution of *C. ferrugineus* were used. A total of 11 experimental results with three trials at each experimental condition were used (Table 5.1). The horizontal and vertical diffusivities of *C. ferrugineus* at different environmental conditions were compared using Tukey's test, performed using SAS® OnDemand for Academics (SAS Institute Inc., Cary, NC).

The insect numbers in each element at one set of environmental conditions were predicted using the average diffusivity at each condition using the equations (5.1), (5.2) and (5.3). The sum of the predicted numbers at different environmental conditions were calculated to confirm if the law of conservation is maintained with 1% error.

5.1.3.2 *Results and discussion*

All the predicted insect numbers calculated by using the average diffusivities were within the range of 99 to 101 for all the experimental conditions, except the experiment conducted at 14.5% moisture content, 20°C with 1000 number of adults (which was normalized to 100 for the purpose of calculation) for a period of 24 h, for which the total predicted number of insects inside the grain bulk were 101.9. This could be solved by further increasing the accuracy of the diffusivities. However, considering the complexity involved in computing time and the availability of computing resources, this was not conducted in this study.

The 3D diffusivities calculated for different experimental conditions are reported in Table 5.2. The diffusivities in shorter movement period (6 h) were higher than those in longer movement period (72 h). This implies that the insects dispersed faster immediately after introduction due to crowding at the introduced location, after which, they dispersed slower. Moreover, the calculated diffusivity at 3D grain bulk were lower than the 2D diffusivity reported by (Jian et al., 2007).

Even though the proposed method could be used to calculate the 3D diffusivity at different environmental conditions, the calculated diffusivities in 24 and 72 h movement period might not be correct because the insects reached the bottom layer within 6 h of movement period, and the insects were forced to wander inside the grain bulk, irrespective of the different rate of diffusion at different environmental conditions. Hence, the experiments must be performed at shorter movement period or larger grain bulk to calculate the reliable diffusivities of insect at various environmental conditions. Moreover, the determined analytical solution must be compared with the numerical solution in the future.

5.1.4 References

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Chapter 6. Summary and Conclusions

6.1 Key findings of the research

Laboratory experiments

1. The designed experimental setup could be used to study the three-dimensional movement and distribution of the insects.
2. *Cryptolestes ferrugineus* adults showed downward movement initially, followed by upward and horizontal movement, inside a grain bulk with uniform temperatures and moisture contents.
3. A portion of the introduced adults remain around the introduced location at constant environmental conditions.
4. Adult *C. ferrugineus* disperse more at high insect densities than at low densities.
5. Insects' movement increases with increase in temperature, i.e., moving slower at low temperatures and faster at high temperatures studied.
6. The movement pattern in three-dimensional grain bulk were similar to those reported in one-dimensional grain columns and two-dimensional chambers.

Field experiments

7. Temperature and moisture on the surface of the grain fluctuated more than those inside the grain bulk due to change in ambient conditions.
8. The temperature inside the tested 10 m diameter bin was higher than 7.8°C, at least at one location, at any time, during the 26-month study period. Thus, both *C. ferrugineus* and *T. castaneum* survived the winter in the bin.
9. The hot spot was mainly developed because of fungi multiplication due to high moisture grains and not because of the insect activity. However, the hot spot could spread to wide areas with low moisture content in the presence of insects and microorganisms.
10. Germinability of the grain near the walls dropped later than those near the surface and inside the grain bulk, in the presence of insect infestation.

11. The grain at the boundary of the hot spot were free flowing even if the germination has dropped below 60%; however, the grain at the core of the hot spot were sticky and non-flowing.
12. Drop in ambient temperature could cease the hot spot by lowering the grain temperature.
13. The movement and distribution patterns of the insects inside a 300 tonnes wheat bin were similar to those observed in the laboratory at similar environmental conditions.
14. The insect count and insect activity increased with increase in temperature. Thus, the highest insect counts were recorded in and around the hot spot for both insects.
15. *Tribolium castaneum* adults were captured mainly on the top grain layer (about 1 m depth from the grain surface). However, they could be captured anywhere in the bin in small numbers.
16. The insect activity near the walls at all the layers reduced drastically because of drop in temperature during the winter.

Modeling

17. The diffusivities in shorter movement periods are higher than those in longer movement periods.
18. The 3D diffusivities of *C. ferrugineus* were lower than the 2D diffusivities reported.

6.2 Contributions to knowledge

1. *Design and applicability of experimental setup:* The designed experimental setup for studying the 3D movement and distribution of insects in grain bulk demonstrates its effectiveness and suitability for such investigations. This contributes to the development of methodologies for studying insect behaviour in realistic grain storage environments.
2. *Movement patterns of* C. ferrugineus*:* The laboratory experiments reveal the movement patterns of *C. ferrugineus* in grain bulk with uniform temperatures and moisture contents. The observation of initial downward movement, followed by upward and horizontal movement provides valuable insights into the behavioural dynamics of the species in relation to environmental conditions.

3. *Influence of insect density on dispersal*: The laboratory experiments indicate that *C. ferrugineus* adults disperse more at higher insect densities compared to lower insect densities. This knowledge contributes to our understanding of population dynamics and the potential for infestation spread based on insect densities in grain storage.
4. *Consistency of movement patterns in different grain environments:* The finding that the movement patterns observed in the three-dimensional grain bulk were similar to those reported in 1D columns and 2D chambers, suggest the consistent behaviour of *C. ferrugineus* across different grain storage environments. This knowledge helps us understand the behaviour of insects with minimal time and resource investment.
5. *Overwintering survival of* C. ferrugineus *and* T. castaneum*:* The finding that both *C. ferrugineus* and *T. castaneum* survived the winter in the grain suggests their ability to withstand low temperatures and highlights the importance of pest management strategies throughout the year, including the winter months.
6. *Field validation of laboratory findings:* The similarity between the movement and distribution patterns of insects observed in the field study and the laboratory findings under similar environmental conditions enhances the credibility and applicability of the laboratory results. The field validation contributes to the reliability of laboratory-based studies on insect behaviour in grain storage.

Overall, the knowledge about insect movement and distribution in 3D is essential to detect and manage the insects inside the grain bins. This information can be useful during sampling as well as to help place the traps in appropriate locations inside the grain bin. In addition, the insect movement and distribution information and the data obtained from traps at particular locations at the given environmental conditions can help understand the population ecology of insects inside the bin.

6.3 Recommendations for future research

1. A laboratory experiment on the effects of different temperature and moisture gradients on the 3D movement and distribution of *C. ferrugineus* could be conducted.
2. A study on 3D movement and distribution of *T. castaneum*, second most common beetle found in Western Canadian stored grain ecosystem, could be performed using a similar experimental setup.
3. A similar laboratory experiment which involves understanding the interaction effect of *C. ferrugineus* and *T. castaneum* on their 3D movement and distribution could be performed.
4. A similar laboratory experiment with shorter movement period or larger grain bulk could be performed to calculate the 3D diffusivities of *C. ferrugineus* at different temperatures, moisture contents, and insect densities.
5. The 3D diffusivity of *C. ferrugineus* could be calculated using the numerical method and the diffusivities calculated using the numerical and analytical methods could be compared.
6. The 3D diffusivity of *T. castaneum* could be calculated using numerical and analytical methods.