Population Dynamics of *Tribolium Castaneum* (Red Flour Beetle) Under Optimal and Sub-Optimal Conditions

by

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ABSTRACT

Population dynamics of red flour beetles, *Tribolium castaneum* (Herbst) was determined using three different sizes of grain patch (bulk), specifically, small (0.03 kg wheat), medium (2 kg wheat) and large (14 kg wheat), at different temperature profiles. The temperature profiles tested were 21, 25, 30, 35˚C, T-decrease (30˚C in the first 4 weeks and then decreased 1˚C /week to -10˚C) and T-increase (21˚C in the first two weeks and then increased 1˚C /week to 38˚C). Three male and three female adults were introduced into each grain patch (bulk) at the start of experiments. Numbers of adults in the grain patch (bulk) were counted every 28 days up to 30 weeks. The population dynamics of the *Tribolium castaneum* (insect numbers) were strongly influenced by the temperature profiles, storage time and grain patch (bulk) size. The insect population increased after 4 week of introduction inside all the grain patches. Later, the number of both offspring and adults showed drastic variation with respect to temperature and storage time under different patch sizes. The peak number or density of insects also showed variation with time for different temperatures and patch sizes. The peak live adult density was the highest in the small patch at each temperature profile. The peak live adult density in the small patch was 300 ± 50, 673 ± 118, 689 ± 48, 1100 ± 150, 1150 ± 150 and 1133 ± 94 adults/kg at 21, 25, 30, 35˚C, T-decrease and T-increase, respectively.

Keywords: red flour beetle (*Tribolium castaneum* (Herbst)), population dynamics, patch (bulk) size, temperature

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	analysis of variance
GLM	general linear model
hp	Horsepower
MC	moisture content on wet basis
PVC	polyvinyl chloride
REG	regression
RH	Relative humidity (%)
SAS	Statistics Analysis System
T	temperature in ˚C
wb	wet basis
Td	T-decrease
Ti	T-increase

1. INTRODUCTION

Approximately half billion-tonne of the stored grain is lost each year globally due to insect infestation (Kumar and Kalita 2017). About 20 million tonnes of wheat was stored on-farm in Canada in 2019 (Canadian Grain Commission 2019). To safely store such a large quantity of grain for some time before it reaches the market, the industry uses an on the farm storage system (Muir 1998). Poor storage conditions can cause losses in the stored grain due to the combined action of insects, moulds, rodents and other pests (Lucia and Assennato 2006). About 5% of stored grain is destroyed by insects globally (Tipples 1995).

Over 600 pest insect species have been recorded in stored products throughout the world (Bousquet 1990). Out of these species, only some cause serious damage to grains. Beetles are the major insects responsible for considerable grain loss (Hill 1990). The transportation of grain and other goods around the world with some insects in it across the world, adaptations by insects have facilitated their wide-spread. Successful exploitation of relative dry human food by stored product insects is due to the reservoirs in the wild environment (such as forest and grassland), tolerance to stored environments (temperature and relative humidity), suitable food habits, the high rate of reproduction, high survival rate of pest insects in the absence of food and their morphological adaptations (White 1995).

Red flour beetle (*Tribolium castaneum* (Herbst)) is the second most common beetle in stored wheat found in Canada (Hulasare et al. 2003). The life cycle of *T. castaneum* varies from 164-194 days and passes through several development stages from egg to adult. It is essential to understand insect population dynamics to prevent the loss of stored grain, which helps devise effective pest control practices. Population dynamics is studying the population's size and age composition as a dynamic system taking into consideration driving factors such as birth rate, death rate, immigration and emigration. The understanding of population dynamics helps in the design and development of a robust integrated pest management program (Chidawanyika et al. 2012). Hagstrum et al. (1998) stated that the application of nonchemical control in an integrated pest management program depends upon the understanding of insect ecology. It is essential first to understand the population dynamics to model the insects' ecology (Jian et al. 2018).

Several studies were conducted to explore the impact of temperature on insect population dynamics. The ideal temperature for the development of the beetles ranges from 32-37˚C (Małek et al. 2015; Singh and Prakash 2015). The physical limits of successful development and multiplication are 20-40˚C and 10-95% relative humidity; the optimum ranges are 32-35˚C and 70-75% relative humidity (Sinha and Watters 1985). Temperatures below 20˚C inhibit the development of many insects of stored products (Hagstrum et al. 1998). At suboptimal environmental conditions, the simulation model of population growth enhances the detection and control of *T. castaneum* (Scharf et al. 2015).

Many number of lab-scale studies have been conducted to forecast the influence of environmental temperature and size of the patch on the population dynamics of the *T. castaneum* (White 1987; Hagstrum and Throne 1989; Maier et al. 2006; Rustamani et al. 2014). However, very few studies on stored product insect's population dynamics have been conducted at conditions expected during bulk storage of grain in bins (Jian et al. 2018). Even limited attempts were made to understand the effects of sub-optimal temperature or patch size on the population of *T. castaneum* on a large scale. The current research focuses on exploring the combined impact of suboptimal conditions on the beetles' population dynamics with the following objectives.

The development of *T. castaneum* was studied at conditions similar to what can be expected in storage bins in Canada. Specific objectives were to determine the impact of constant and fluctuating temperatures on the population and mortality of *T. castaneum* (including adult and offspring) in small, medium and large wheat patches for 30 weeks and to characterize the interaction effect of these factors (temperature, patch size and storage time) on insect population dynamics.

2. LITERATURE REVIEW

2.1 Stored grain loss due to insect infestation

Humans harvest grain as an important source for their diets globally. Wheat is one of the major cereal crops, represents 28.7% (734 million tonnes) of global grain production (Shahbandeh 2020). Canada was the seventh-largest producer of wheat (34 million tonnes) in 2019 and most of the harvested grain is stored in farm bins with a capacity of 25 to 250 tonnes in each bin (Muir 1998). Such storage is one of the concerns of farmers and elevator managers due to the potential risk of infestation (Mann 1995).

Stored wheat loss can be categorized as a direct loss (weight) and indirect loss (quality and nutrition) occurring due to biotic (fungi, insect, mites, birds and rodents) and abiotic (temperature and humidity) factors. The unpleasant odours are the first sign of infestation and this smell is mainly produced from biomass (produced by moulds), body parts, frass and spoiled food particles. Insect and mould metabolic activities, such as respiration, produce heat and moisture, induce the development of hotspots and multiplication of microorganisms (Divagar 2018). Severely infested wheat is unsuitable for human consumption and cannot be used for seeding (Neethirajan et al. 2006). Postharvest losses could reach up to 24% of the total food produced globally, mostly due to insect infestation. The loss due to insect infestation varies from 9% in developed nations to more than 20% in developing nations (Paul et al. 2020). Stored grain losses could be limited to 2% in developed countries where the grain is stored and managed optimally; however, losses could reach up to 50% in undeveloped countries due to poor stored grain management (Jayas 2012). About 2.7% of wheat is lost during farm and market storage in the four regions of Ethiopia (Abdullahi and Haile 1991). Along with several qualitative losses (germination loss and loss of nutrition), the weight loss caused by *T. castaneum* infestation could reach up to 2% of stored wheat at 27 ± 2˚C and 70 ± 5% relative humidity and insect density of 40 adults/kg (Padin et al. 2002). To prevent the qualitative and quantitative losses in stored grain, Canadian Grain Commission enforces the policy of no insect pests in commercialized grain (Canada Grain Act 1985).

2.2 Stored grain pest insects

Insects and mites are important parts of the stored-grain ecosystem (Lacey 1988). Around 670 out of approximately 42000 insect pests are responsible for infesting stored grain globally (Rajendran 2000). A summary of the major insects and pests found in grain and their optimal and minimum growth temperatures and RH are given in Table 1. The rate of insect infestation is relatively low in Canada due to a temperate climate with low temperatures and dry conditions. Out of approximately 9000 insect pests found in Canada, about 60 are responsible for infesting the stored grain (Sinha and Watters 1985). Some of the common insect pests of stored grain are *Sitrotroga cerealella* (Olivier), *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), *Sitophilus granarius* (L.), *Trogoderma granarium* Everts, *Tribolium confusum* Jacquelin du Val, *Cryptolestes ferrugineus* (Stephens) and *Tribolium castaneum* (Herbst) (Loschiavo et al. 1984). Among these *T. castaneum* (red flour beetle) and *C. ferrugineus* (rusty grain beetle) are the two main grain insects (Hulasare et al. 2003). Adult of *T. castaneum* is ten times heavier than *C. ferrugineus* adult and causes more damage (15–20%) to stored wheat (White 1995). Due to extended connection with stored grain, *T. castaneum* is labelled as a major insect in cereal processing facilities (Campbell and Runnion 2003). *T. castaneum* belongs to the order Coleoptera, one of the largest orders of insects containing more than 15000 species, of which about 600 are linked to stored products. In a study of on-farm storage bins in Kansas state, Schwitzgebel and Walkden (1944) found that *T. castaneum* enters stored wheat from April through October. Insect infestation of grain occurs from May to November in Canada (Smith 1985).

Table 1. Summary of major species infesting grain in the world

Scientific name	Common name	Minimum growth temperature (˚C)	Minimum growth RH (%)	References
Acarus siro (L.)	Flour mite	7	65	Aspaly et al. (2007); Howe (1965)
Corcyra cephalonica (Staint.)	Rice moth	18	30	Howe (1965)
Cryptolestes ferrugineus (Stephens)	Rusty grain beetle	23	10	Bishop (1959)
Ephestia elutella (Hübner)	Warehouse moth	10	30	Woodroffe (1951)
Oryzaephilus surninamensis (L.)	Sawtoothed grain beetle	21	10	Howe (1965)
Plodia interpunctella (Hübner)	Indian meal moth	18	40	Tzanakakis (1959)
Rhyzopertha dominica (F.)	Lesser grain borer	23	30	Howe (1965)
Sitophilus granarius (L.)	Granary weevil	15	50	Eastham and Mccully (1943)
Sitophilus oryzae (L.)	Rice weevil	17	60	Howe (1965)
Sitrotroga cerealella (Olivier)	Angoumois grain moth	16	30	Howe (1965)
Tribolium castaneum (Herbst)	Red flour beetle	22	10	Sinha and Watters (1985)
Tribolium confusum Jacquelin du Val	Confused flour beetle	21	10	Sinha and Watters (1985)
Trogoderma granarium Everts	Khapra beetle	24	1	Howe (1965)

Tribolium castaneum can be reared easily, has a short life cycle and can develop under a wide range of environmental settings. Therefore, it is a model insect to test thermal ecology and associated factors (Campbell and Runnion 2003; Scharf et al. 2015). Because of its nonlinear demographic dynamics, *T. castaneum* is regarded as the ideal candidate to study population dynamics (Costantino et al. 1997) and is the researchers' conventional choice to study behavioural ecology (Campbell and Hagstrum 2002). Therefore, extensive studies have been published in the literature about the impact of temperature on the population fluctuations of *T. castaneum* in different volumes of stored grain (White 1987; Maier et al. 2006; Rustamani et al. 2014; Atta et al. 2020).

2.3 Life cycle and morphology of *T. castaneum*

One life cycle of *T. castaneum* completes in 164 to 194 days and has four stages as egg, larva (seven instars), pupa and adult. Numbers of biotic and abiotic factors govern the development of each stage. Hagstrum and Subramanyam (2006) found that the average growth time from egg to adult varies from 41.8 days at 25˚C to 21.7 days at 35.5˚C. Development from egg to adult completes in 19 to 20 days at 35-37.5˚C and 70% RH (Mahroof and Hagstrum 2012). Females lay approximately 24 eggs/day (Thirupathi 2010; Devi and Devi 2015).

Eggs of *T. castaneum* are oblong, white and covered with a sticky fluid that allows debris particles to adhere to them. Pupae are white. The cream colour larvae have three pairs of legs. A newly emerged adult is pale brown and within a day the cuticle hardens and turns dark reddish-brown. Devi and Devi (2015) found the average length of 0.59, 6.22, 3.81-4.12 and 3.06-3.70 mm for *T. Castaneum* eggs, larva (7^{th} instar), pupa and adult, respectively. The mean body weight of *T. castaneum* is 200 x 10^{-5} g (Soliman and Hardin 1971). An adult's last few antennae segments are extremely extended and look like a club with a tip. The head margins are nearly continuous in the eyes. *Tribolium castaneum* adults have hardened protective forewings (elytra) and have membranous hind wings, which are folded beneath the elytra. The adult males on their posterior side of the fore femur have a setiferous patch that is absent in the females (Bousquet 1990). The female pupa of *T. castaneum* has larger genital papillae (just anterior to the pointed urogomphi) than those of the male. Both adults and pupae of *T. castaneum* can be sexed; however, sexing pupae instead of adults is easier since there is hardly any movement in pupae compared to adults (Sreeramoju et al. 2016).

2.4 Biology

Tribolium castaneum females' fecundity improved significantly with temperature from 24 to 34˚C at low adult densities (Park and Frank 1948; White 1987). The oviposition increased with temperature (Soliman 1987). The highest egg-laying (20 eggs per day) was observed at low insect density (at 34˚C) (Park and Frank 1948). Zakladnoi and Ratanova (1987) reported 22˚C as the minimum temperature for reproduction in *T. castaneum*. A higher temperature during adult development significantly influenced the reproduction in *T. castaneum* (Scharf et al. 2015). Young larvae of *T. castaneum* were generally extra heat tolerant compared to other stages (Mahroof et al. 2003). However, almost all mortality occurred in young larvae and pupae at 20˚C and only up to 13% mortality was seen between 22 to 35˚C (Howe 1956).

The female *T. castaneum* adult's survival changed significantly with only a 4˚C increase in the environmental temperature from 30 to 34˚C (Grazer and Martin 2012). One reason for reduced survivorship at increased temperature could be the loss of water from the body of the adult male and female beetles (Hadley 1994). At a lower temperature below the development threshold (20˚C), the insect's mortality depends upon the exposure time, temperature and species (Fields 1992). The mortality threshold for the *T. castaneum* was 20˚C at 30% RH (Singh and Prakash 2015). In general, the lower the temperature (below 20˚C), the greater the mortality (Fields and White 1997).

Behaviour of stored grain insects is influenced by the interaction of several physical, chemical and biotic factors including temperature, RH, food availability and moisture content of the grain (Cox and Collins 2002). Once the favourable condition is met, females of *T. castaneum* search for a mating partner and mate multiple times (Sbilordo and Martin 2014) and warmth helps in their mating activity (Grazer and Martin 2012). Based on the patch's suitability (physiological state and resource quality), the female determines the number of eggs to be laid (Charnov and Skinner 1985).

Covered patches were preferred over uncovered patches for the movement by *T. castaneum* due to their shelter seeking behaviour (Romero et al. 2010). Insects reared at an elevated temperature (34˚C) and shifted to a lower temperature (26˚C) did not mate at all (Scharf et al. 2015). As the *T. castaneum* adults preferred to move to the edge of the patch, the density increased near the edge of the patch instead of the center; however, the egg dispersal pattern indicates that females spent less time inside a patch (Campbell and Hagstrum 2002). Movement behaviour inside the patch varies with temperature and MC among *T. castaneum* adults, as both male and female adults moved twice as fast in the grain at 18% MC when compared to grain at 14% MC (Surtees 1964). Full-grown larvae are very active and move to the surface to seek shelter for pupation; however, they prefer living concealed in the food away from light (Mahroof and Hagstrum 2012).

2.5 Population dynamics

Population dynamics refers to the study of birth rate, death rate, immigration and emigration, affecting its size and age composition. Researchers report the surrounding climate during the investigation of insects' population dynamics, which substantially impacts the critical attributes of population dynamics. The key attributes of population dynamics include survival (mortality), mating, oviposition and fecundity. These key attributes are mostly governed by temperature, space (patch size), food, RH and moisture content (MC) of the grain. This section discusses the impact of these environmental factors on the population dynamics of *T. castaneum*.

2.5.1 Patch size (volume) and quality

A habitat patch is a discrete area having a fixed contour, spatial and configuration used by a species to grow population or find other resources (Atkins et al. 2019). The grain bin or flour resting inside milling equipment in a flour mill could be an example of a food patch. Entry and emigration inside the food patch by *T. castaneum* depend upon insect density, thermal quality and food abundance of the patch (Ziegler 1977; Halliday et al. 2015). Soon after insects enter a fresh patch of stored grain under optimal temperatures and RH, their growth would be maximum with low mortality due to high quality and quantity of resources such as food and low competition (Smith et al. 1966). The patch's size and quality play a significant role in their search for resources and mating partners. A larger patch has apparent benefits such as freedom to move and locate the resources when exhausted at one place (Jian et al. 2018). There exists a strong correlation between the oviposition in *T. castaneum* and patch size (Campbell and Runnion 2003). Exhausting resources impact the insects' capability to grow and survive, leading to a phenomenon called negative density dependence. The negative density dependence is strong in grain patches maintained at higher temperatures and the oviposition decreases sharply as the patch size decreases (Halliday et al. 2015).

Dispersal is a survival and colonization mechanism; adults of *T. castaneum* show that higher dispersal behaviour develops faster and more eggs are laid than the adults that exhibit low dispersal at high population density (Zirkle et al. 1988). Inside large patches (grain/flour) *T. castaneum* adults are highly mobile (Surtees 1964; Hagstrum and Leach 1973); the insects also quickly disperse from patches of flour for stages up to the adult stage (Ziegler 1977). *T. castaneum* follows a non-uniform dispersal depending on the moisture and temperature gradients inside the food patch (Zirkle et al. 1988). During temperature variation, the adults moved to warmers areas; however, due to tight void spaces in the grain patch, their movement is limited (Jian et al. 2005). Dispersal in *T. castaneum* depends on the deterioration of habitat and increases with population density due to conditioning of the patch (by the accumulation of frass, depleting nutrients and release of toxic defensive secretions)

(Ziegler 1977; Ogden 1969). The adults of *T. castaneum* move to a colder and less dense (low competition) habitat soon after the insect density increases at warmer patches of grain (Halliday et al. 2015).

In low insect density, a larger patch increases the difficulty in discovering the other mating sex (Jian et al. 2018). An insect density ≥ 1pair/250 g of grain was a weakening factor for fertility (White 1987). Sonleitner and Guthrie (1991) suggested that due to high insect density, the build-up of waste materials (frass and ethylquinone repellent) act as a signal to stop oviposition and disperse to avoid egg mortality (Flinn and Campbell 2012; Scharf et al. 2015). The high population density in *T. castaneum* suppresses oviposition behaviour due to sub-lethal effects on the development of depleted diets (Arthur et al. 2019). In the event of decreased favourable niches due to feeding, patch size could be a major contributor to the population dynamics. The female of *T. castaneum* tended to lay more eggs with an increase in the size of the flour patch increased (Campbell and Runnion 2003). In small and medium patches, *T. castaneum* beetles were forced to live within finite space and regulated oviposition rate (Halliday et al. 2015).

There exists a significant correlation between insect population and grain moisture and MC ≥ 10.8% (wb) is favourable for population growth (Dars et al. 2001). The maximum population (243 insects in a 14 d) was reported from August to October for *T. castaneum* in wheat having MC 11.4% (wb) under laboratory conditions (Dars et al. 2001). Depending on grain moisture and temperature gradients, *T. castaneum* exhibits a non-uniform dispersal behaviour (a survival and colonization mechanism) at high population densities (Cox and Collins 2002).

2.5.2 Temperature

The average temperature in unventilated grain silos (up to 5.6 m diameter) in the fall and winter declines at approximately 1˚C/week in southern Manitoba (Fields and White

1997). Temperature variation influences the stored grain (Jian et al. 2018), creating a need to test the climatic variability's net impact on population dynamics (Estay 2011). Hulasare et al. (2003) reported a positive correlation between the increasing temperature and the adult population of *T. castaneum*. Population growth rate varies with the species of the insects; however, the increase in this rate could be 20-60 times per month at 25–35˚C (Jian et al. 2016). The fluctuating temperature will affect the number of offspring and could influence population dynamics as the number of offspring significantly increased with increased (from 26 to 34˚C) grain temperatures during adult development in *T. castaneum* (Scharf et al. 2015).

A high temperature (30 to 35˚C) is preferred by *T. castaneum* to develop and thrive. The ability to withstand a suboptimal temperature and survival depends on the stage of development of the insect. But, when the temperature of the environment deviates from the optimum range, the population dynamics of insects are affected. A temperature ranges of 30 to 35˚C at 70% RH is optimum for *T. castaneum* (Singh and Prakash 2015). *Tribolium castaneum* rapidly expands in population when the temperature is equal to or slightly exceeds 27˚C in rice flour (Arthur et al. 2019). The temperature during adult development strongly influences the reproduction in *T. castaneum* (Scharf et al. 2015). The respiratory and metabolic rates of insects increase with temperature (Mbata and Phillips 2001). Low grain temperatures (18˚C) reduce activity and minimize population growth in *T. castaneum* (Maier et al. 2006).

Beetles choose their favoured temperature instead of food in the low-temperature patch; however, were forced to use suboptimum temperatures to obtain sufficient food to maximize fitness as competition increases (Halliday et al. 2015). The effect of temperature fluctuations on population dynamics depends on whether mean temperatures in question are below or above the thermal optimum of *T. castaneum* (Malek et al. 2015). The acclimation differences among various stages of *T. castaneum* might affect successive susceptibility at elevated temperatures when exposed to gradually increasing temperatures (Mahroof et al. 2003).

Soon after the insects enter the stored grain; they disperse randomly with no preference and gather at favourable locations (Jian et al. 2009). The negative density dependence increases with temperature. During adult development, temperature plays a key role in affecting the offspring count in *T. castaneum* (Scharf et al. 2015). The proportion of eggs that emerge as adults decreases with increasing temperature and insect density (Halliday et al. 2015). Fewer offspring were observed for *T. castaneum* under aeration at 18˚C (Maier et al. 2006). The longevity of *T. castaneum* adults decreased at low temperatures (White 1987).

In *T. castaneum* the oviposition rate doubled (from 0.052 to 1.04 per week) when the temperature increased from 24 to 29˚C, however, the oviposition rate increased slightly from 29 to 34˚C (Park and Frank 1948; Howe 1962). Grazer and Martin (2012) reported a higher reproduction and dispersal in *T. castaneum* at warmer temperatures (30˚C-34˚C) than at other temperatures outside this range. The fertility of young adults increases from 0 at 20˚C to 5 offspring/d per female at 35˚C and at 50% RH (White 1987).

Crowding and mean density of *T. castaneum* adults are strongly correlated with the temperature between 20 to 30˚C in stored wheat (Jian 2019). The survivorship of the *T. castaneum* adults reduced from 250 to 0 days with an increase in the temperature from 25 to 42.5˚C or descend to 7.5˚C at 70% RH (White 1987).

The temperature change significantly influences adult count in *T. castaneum and* the average adult count increased in four weeks from 66 to 289 when temperature increased from 15 at 25˚C (Hulasare et al. 2005). The rate of offspring production (offspring/week) of *T.*

castaneum increased up to four weeks and then declined quickly after four weeks at 30˚C and 34˚C at 70% RH (Grazer and Martin 2012).

2.5.3 Relative Humidity

The optimum relative humidity for the development of *T. castaneum* is 70% (at optimum growth temperatures). The impact of RH and temperature were identical on the oviposition in *T. castaneum* (Howe 1962); however, the insect mortality remains unaffected by relative humidity (Mahroof et al. 2003). The minimum RH at which the larvae of *T. castaneum* survive is 40% in wheat and the mortality of larvae increased significantly at the lower RH with no survival at 35% RH (Howe 1956; White 1987). The development periods of pupae remained unaffected by RH; however, adult longevity declined significantly with a decrease in RH (White 1987). *Tribolium confusum* and *T. castaneum* exhibit an identical relationship with RH (Howe 1960). No mortality was observed in *T. confusum* adults up to 50 days at 30% RH in a temperature range of 25-35˚C (White 1987).

2.5.4 Cannibalism

Many species of stored-product insects eat the inactive stages under crowded conditions like eggs or pupae of their own or other species (Hagstrum et al. 1998). Excessive cannibalism of eggs by larvae plays a key role in limiting the density of immatures in *T. castaneum* (Ziegler 1977). Cannibalization plays a determinant role in the *T. castaneum* population's size by affecting survival rate, development, fecundity and reduced competition.

There are possibilities of larval cannibalism under high insect density (White 1995). There was no cannibalization noticed at low egg density (20 eggs/kg) (White 1987). High egg cannibalism and reduction in fecundity significantly influenced the larval number (Sonleitner 1961). Flinn and Campbell (2012) found that larvae would have a greater rate of cannibalism. Male adults cannibalize less due to a different level of nutrition requirement than females. Larva cannibalizes up to 12 eggs compared to seven eggs by adult *T. castaneum* female. The egg cannibalism by adult *T. castaneum* was more in pre-infested flour compared to the fresh flour (Sonleitner 1961). At a larval (*T. castaneum*) density of 2 larvae/g of wheat flour, up to 60% of eggs were cannibalized (Flinn and Campbell 2012).

2.6 Knowledge Gap

Grains can be safely stored for a prolonged period by appropriate monitoring and management of interactions among biotic and abiotic factors (Jayas 2012). Understanding insect population dynamics and its interactions with the physical variables of the atmosphere surrounding the grain would help develop appropriate monitoring techniques. To devise an effective control strategy and curb the growing insect population, the understanding of insect population growth and decline trends are vital (Hagstrum et al. 1998; Jian et al. 2018). The population of *T. castaneum* is determined by several density-dependent factors (mating, oviposition, productivity, survival and mortality) (Park et al. 1965; Kawamoto et al. 1989). Researchers studied the effect of temperature and RH (Howe 1956; White 1987; Fields 1992; White 1995; Fields and White 1997; Hulasare et al. 2005; Maier et al. 2006; Singh and Prakash 2015), grain moisture, patch size (Campbell and Hagstrum 2002; Campbell and Runnion 2003; Halliday et al. 2015) on the population of *T. castaneum* in the stored-grain. However, the researchers so far tested the effects of several factors on population dynamics of the *T. castaneum* in the smaller laboratory-scale setups. In grain bins, insects are present in a much larger patch (250 tonnes) than the laboratory size grain patches (up to 1 kg) (Jian et al. 2018). There is a lack of published literature about the influence of the size of the grain patch (bulk size) and fluctuating temperatures on the population dynamics of *T. castaneum.*

3. MATERIALS AND METHODS

3.1 Wheat

Hard red spring wheat (variety: "AC Barrie”, CWRS Grade-1) was obtained from a local elevator (Cargill Ltd.) in Manitoba. The initial germination of the wheat was determined by following a method reported by White (1987) and was found to be ≥ 95%. About 2000 kg of wheat was cleaned using a three-dimensional shaker (Vibro-Energy® Separators, SWEKCO, Florence, KY, USA) and a 10-mesh sieve (2 mm openings). The wheat moisture content was then measured by following the ASABE standard (ASABE 2016a) and the initial moisture content was 13.5% (wet basis). After cleaning, the moisture content of the wheat was adjusted to 14.5% by adding 11.7 mL of distilled water per kg of wheat (Jian et al. 2018). To even the moisture distribution among kernels, wheat with added distilled water was tumbled for half an hour in a tumbler type batch mixture having a batch capacity of 75 kg. The conditioned wheat was kept in double-layered plastic bags (sealed by rubber elastic bands) at room temperature for two weeks. Before use, wheat was disinfected by freezing at -15˚C for three weeks (Fields and White 1997) and then warmed to the desired experimental temperatures.

3.2 Rearing and sexing of insects

Insects were reared in a culture of whole wheat kernels (the same wheat as mentioned before), wheat germ and cracked wheat (16:1:1 by mass) at 30˚C and 70 ± 5% RH. A procedure reported by Jian et al. (2018) was followed to produce known aged adults. Adults were initially separated from the culture by sieving and selected by aspiration. About 100 adults were introduced in a jar with about 2 kg feed and incubated at 30˚C and 70% RH to allow mating and egg-laying for five days. The jar containing the insects and feed was covered with a screen lid covered with one layer of filter paper to allow air exchange. After five days of egg-laying, the adults were sieved out and the diet with the eggs was incubated at the rearing condition. About 21 days later, around 2000 pupae were separated and sexed. The sex of the pupae was determined using the method reported by Halstead (1963) by checking pupal genital papillae under a microscope. Female and male pupae were separated and kept in glass vials containing 30 g wheat flour at 16% MC (wet basis). These vials were incubated for three weeks at 30˚C and 70% RH. The emerged adults after incubation were introduced into different sizes of grain containers for testing.

3.3 Grain containers

The experimental setup included small (0.03 kg), medium (2 kg) and large (14 kg) sizes of wheat bulks (patches). Grain containers used to hold these grain bulks were used by Jian et al. (2018). For the small, medium and larger patches, 50 mL glass vials (inner diameter 28 mm and 80 mm long), PVC columns with 2.6 L capacity (diameter 150 mm and length 150 mm) and PVC columns with 18 L capacity (diameter 150 mm and length 1020 mm) were used, respectively. The columns were filled with wheat, leaving a 150 mm gap from the top rim of the large column and 50 mm of the medium column. Each of the small vials was filled with 30 g of wheat, leaving 20 mm from the vial's top rim. All the vials and columns were cleaned and disinfected using soap and hot water before wheat was loaded.

3.4 Insect growth conditions

The temperature and RH settings were similar to the experiments conducted by Jian et al. (2018). Four growth cabinets (CMP6050, CONVIRON, Winnipeg, Manitoba, Canada) were set at 21˚C, 25˚C, 35˚C and for temperature increase (T-increase) and two walk-in environment chambers (C1010, CONVIRON, Winnipeg, Manitoba, Canada) were set at 30˚C and for temperature decrease (T-decrease). For T-decrease, grain was kept at 30˚C for the first four weeks and reduced by 1˚C/week until 10˚C, then reduced by 5˚C per week until it reached −10˚C and maintained at −10˚C for a week. For T-increase, the starting temperature was 21˚C and kept for two weeks, then gradually raised by 1˚C/week until 38˚C and maintained at 38˚C for five weeks. The RH of the growth cabinets and environmental chambers for all experiments was set at equilibrium RH corresponding to the incubation temperatures to maintain the grain moisture (14.5% wb). The RH was calculated using the modified Henderson equation as being in an equilibrium of 14.5% moisture wheat and using constants from ASABE standard (ASABE 2016b).

3.5 Experimental procedure

The experiments were conducted over a year due to the practicality of sieving, counting and number of incubators. In the first six months, experiments at 25˚C, 30˚C, 35˚C were conducted and the remainder of experiments were conducted in the next six months. Once the grain temperature reached the desired temperature, three pairs of 10-day-old male and female (three virgin males and three virgin females) were introduced in each vial or column. The initial insect density inside the large, medium and small patches was 0.43, 3 and 200 adults/kg of wheat, respectively. Each column was covered with a layer of 1.6 mm mesh nylon cloth and a vial with a screened lid to allow air exchange. Sieving and counting of the adults were carried out at 4, 8, 12, 16, 20, 30 weeks for T-decrease and at 4, 8, 12, 16, 20, 24 weeks for all other treatments. Wheat samples were sieved with a #10 sieve (2 mm opening) on a shaker (AS400, Retsch GmbH, Haan, North Rhine-Westphalia, Germany) for 3 min. Adult insects were counted using a laboratory aspirator (1/6 hp) (5KH33DN16JX, Marathon Electric, Mississauga, Ontario, Canada). After counting, the adults were discarded, the shifted diet (including larvae, pupae, eggs and dockage) was mixed with the wheat sieved which was re-incubated at 30˚C and 70% RH for four weeks. The purpose of this re-incubation was to count the offspring (emerged adults) by following the above procedure. Three replicates were conducted for each temperature and patch size.

3.6 Data analyses

Adult numbers at the first counting time were presented in this thesis. The number of emerged adults counted after 4-week re-incubation was referred to as offspring at the first counting time. The adults (live and dead) and offspring (emerged adults after re-incubation) were combined and termed as 'all insect' during the data analysis. Data (insect numbers) at each storage period (N) were transformed using Log_{10}^{N} to normalize the data and equalize the variation of analysis. The effect of temperature, the mass of wheat bulk (patch size) and storage time on insect population was analyzed by conducting three-way factorial analyses (General Linear Model, mixed procedure SAS 2019). The Pearson product correlation and polyserial correlation were used to test correlation among the attributes of the *T. castaneum* population (number of adults and offspring, the density of adult and offspring, the mortality of adult and offspring and all insects). All tests were evaluated at a 95% confidence level. The moisture content of wheat was 14.5 ± 0.2% in all treatments throughout the study, hence not presented in the results.

4. RESULTS

4.1 Effect of patch size on the population

Size of the grain patch (grain volume) significantly influenced (Table 2) all insect number (Fig. 1), live adult number (Fig. 2), offspring number (Fig. 3), adult and offspring density (Figs. 4 and 5) and adult mortality (Fig. 6). All insect number was different among the three-grain patches (Fig. 1) and increased with the size of the grain patch.

Table 2. Effect of storage period (Weeks), patch size (Patch) and grain temperature (Temperature) on all insect number, offspring number, adult mortality and insect density (Three-way ANOVA, P<0.050) of *T. castaneum*

Parameters	DF	F Value	P
All insect number (N=126)			
Weeks	6	8.54	<0.0001
Temperature (˚C)	5	24.48	<0.0001
Patch	2	51.7	<0.0001
Weeks*Temperature	30	1.98	0.0122
Temperature*Patch	10	10.84	<0.0001
Weeks*Patch	12	4.39	<0.0001
Adult mortality (N=126)			
Weeks	6	6.83	<0.0001
Temperature (˚C)	5	9.53	<0.0001
Patch	2	10.17	0.0002
Weeks*Temperature	30	2.39	0.0021
Temperature*Patch	10	3.51	0.0011
Weeks*Patch	12	3.07	0.002
Offspring number (N=126)			
Weeks	6	4.64	0.0006
Temperature (˚C)	5	9.22	<0.0001
Patch	2	43.36	<0.0001
Weeks*Temperature	30	1.4	0.1334
Temperature*Patch	10	5	<0.0001
Weeks*Patch	12	3.01	0.0024
Offspring density (N=126)			
Weeks	6	4.22	0.0013
Temperature (˚C)	5	10.51	<0.0001
Patch	2	39.45	<0.0001
Weeks*Temperature	30	1.42	0.1235
Temperature*Patch	10	3.65	0.0008
Weeks*Patch	12	2.3	0.0173
Adult density (N=126)			
Weeks	6	6.39	<0.0001
Temperature	5	5.97	0.0002
Patch	2	40.31	<0.0001
Weeks*Temperature	30	1.21	0.262
Temperature*Patch	10	2.84	0.0059
Weeks*Patch	12	1.87	0.0575

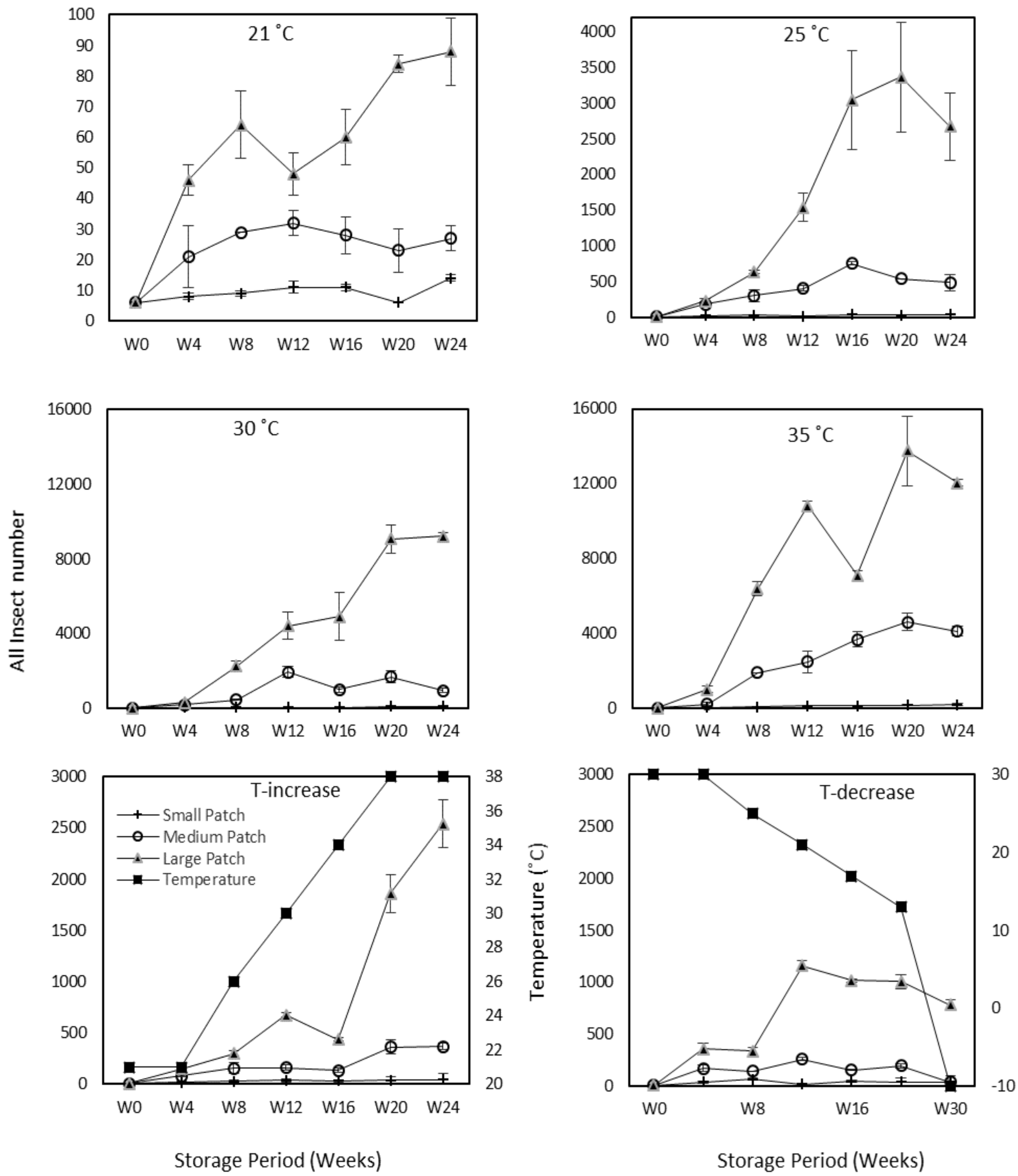

Fig. 1. All insect (adults and offspring) number of *T. castaneum* at different temperatures, grain patches and storage periods.

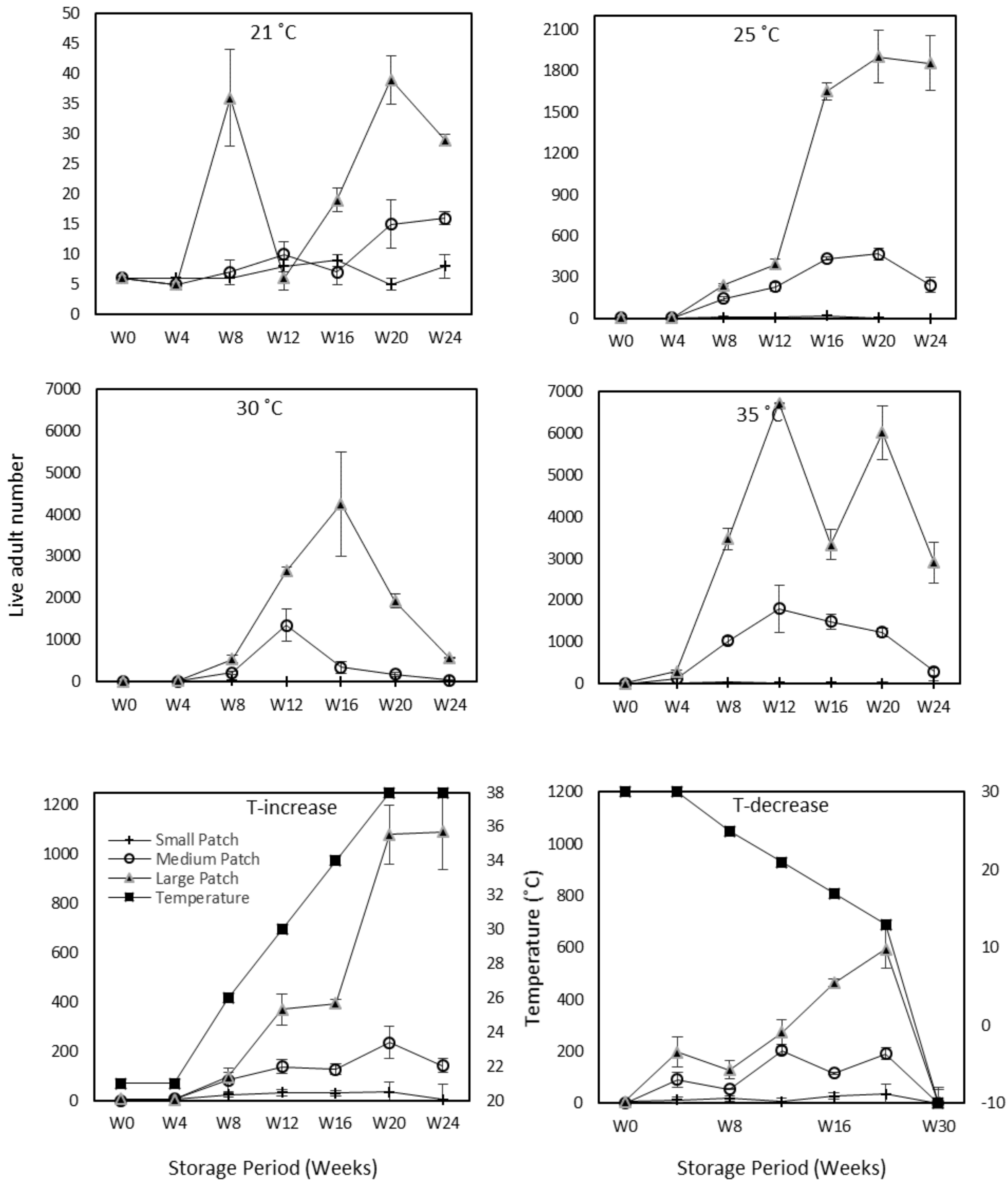

Fig. 2. Live adult number of *T. castaneum* at different temperatures, grain patches and storage periods.

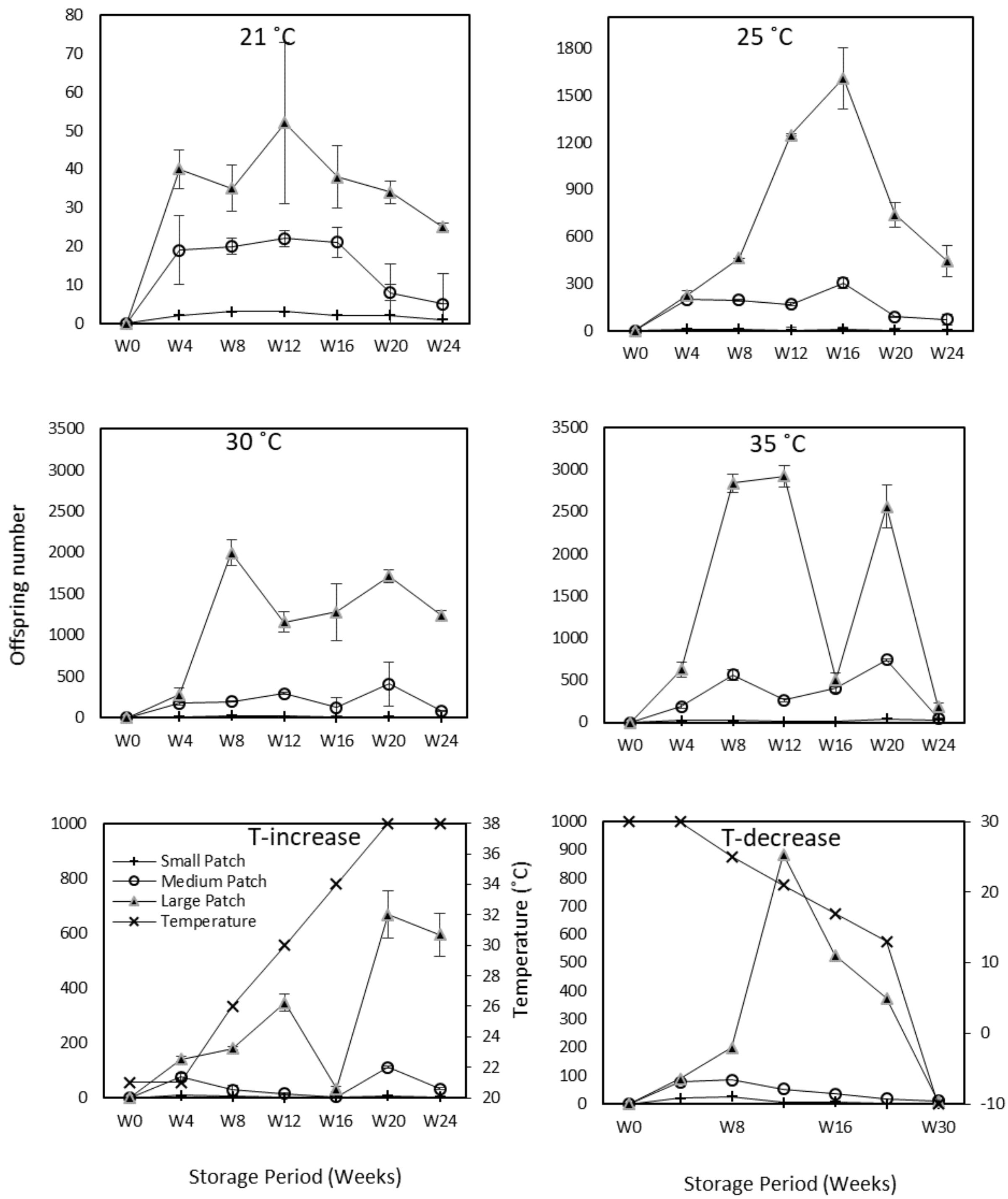

Fig. 3. Offspring number of *T. castaneum* at different temperatures, grain patches and storage periods.

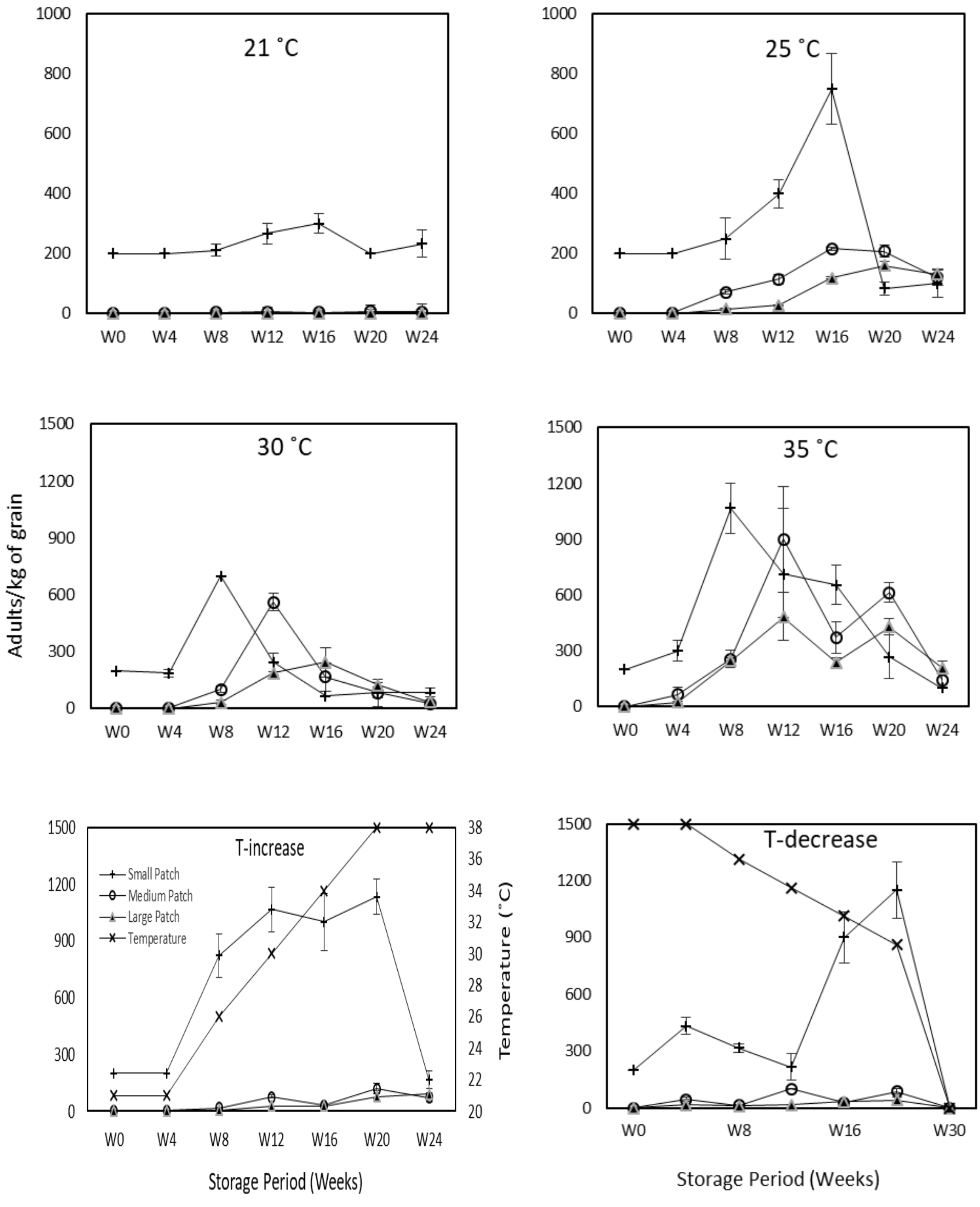

Fig. 4. Live adult density (adult/kg) of *T. castaneum* at different temperatures, grain patches and storage periods.

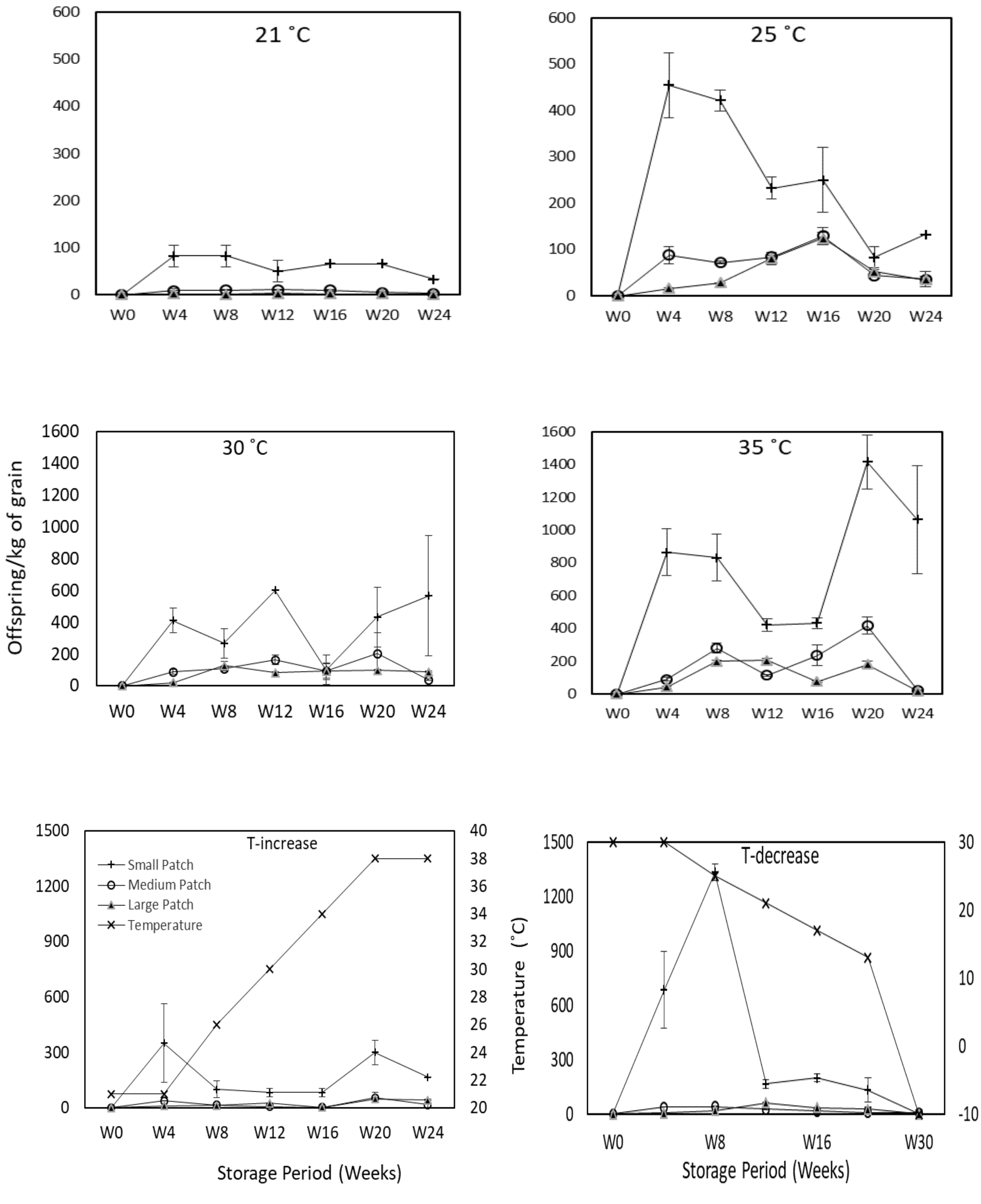

Fig. 5. Offspring density (Offspring kg^{-1}) of *T. castaneum* at different temperatures, grain patches and storage periods.

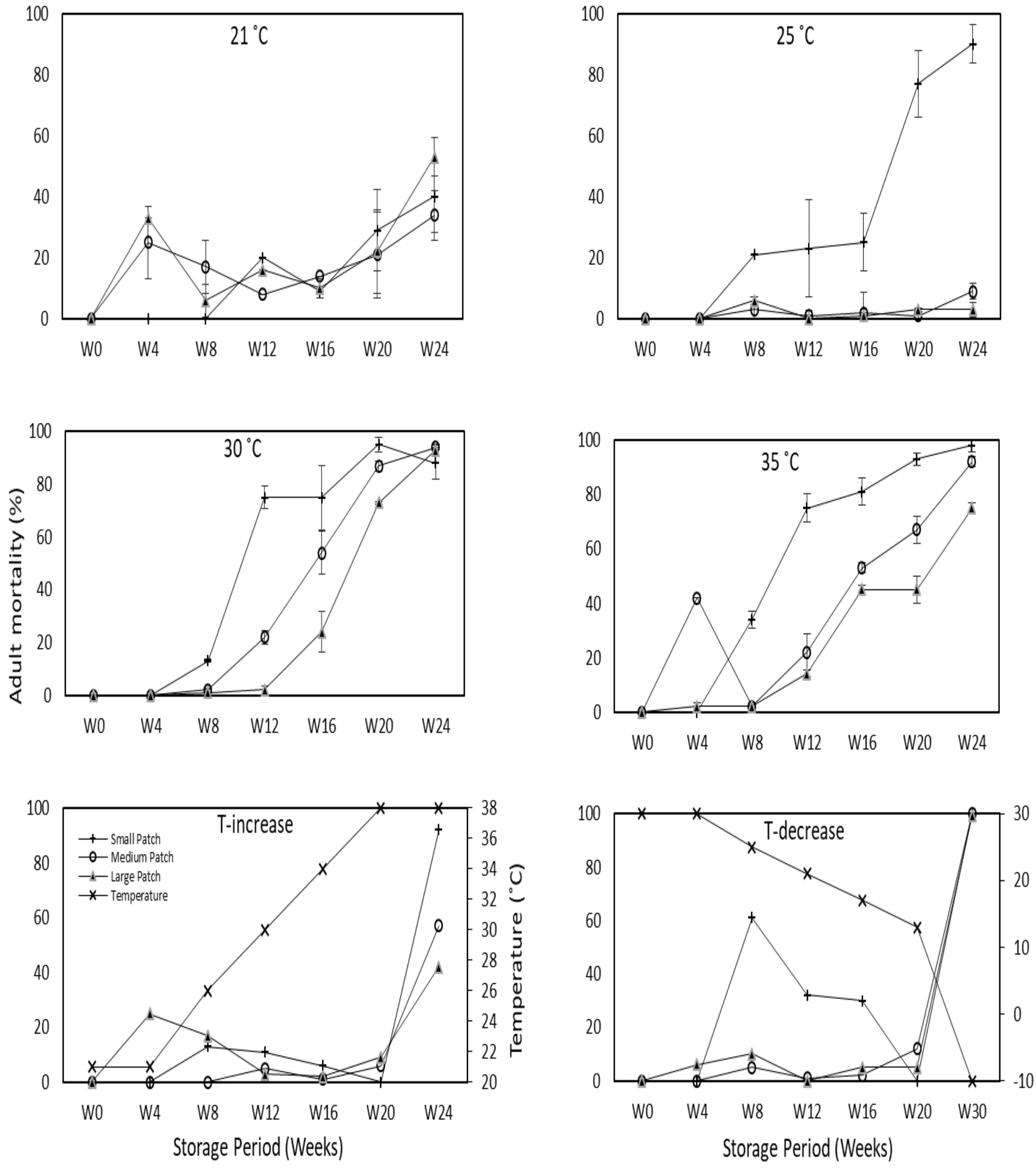

Fig. 6. Adult mortality (%) of *T. castaneum* at different temperatures, grain patches and storage periods.

All insect number had a strong correlation with an adult number, offspring number, adult density, offspring density and adult mortality in all the three grain patches (Table 3). Adult density and adult mortality inside the small patch had a lower correlation with all insect number. The adult number was strongly correlated with the offspring number, adult density and offspring density (Table 3) in three patches. Offspring number had a strong correlation with adult density and offspring density inside the small and large patch (Table 3).

Table 3. Pearson correlation coefficients among all insect number, Adult number, Offspring number, Adult density, Offspring density and Adult mortality in different patches (N = 42)

	Small Patch	**Medium Patch**	**Large Patch**
All insect number v/s Adult number	0.81	0.68	0.85
All insect number v/s Offspring number	0.94	0.87	0.72
All insect number v/s Adult density	0.07	0.73	0.85
All insect number v/s Offspring density	0.68	0.74	0.75
All insect number v/s Adult mortality	0.07	0.76	0.80
Adult number v/s Offspring number	0.76	0.60	0.76
Adult number v/s Adult density	-0.06	0.51	0.99
Adult number v/s Offspring density	0.63	0.72	0.81
Adult number v/s Adult mortality	-0.11	0.45	0.44
Offspring number v/s Adult density	-0.15	0.37	0.76
Offspring number v/s Offspring density	0.55	0.46	0.98
Offspring number v/s Adult mortality	-0.16	0.38	0.26
Adult density v/s Offspring density	0.01	0.66	0.81
Adult density v/s Adult mortality	0.97	0.95	0.44
Offspring density v/s Adult mortality	0.05	0.72	0.28

Adult density had a strong correlation with offspring density and adult mortality in medium patches; however, had a strong correlation with offspring density in the large patch and adult mortality in the small patch (Table 3). Offspring density was strongly correlated with adult mortality inside the medium patches (Table 3).

Peak density of live adults was the highest inside the small patch compared to the medium and large grain patches (Fig. 4) and was influenced by the size of the grain patch. The highest value of peak live adult density in the small patch was 1150 ± 150 adults/kg.

4.2 Effect of constant and fluctuating temperatures on insect density

Grain temperature significantly influenced (Table 2) all insect number (Fig. 1), live adult number (Fig. 2), offspring number (Fig. 3), adult and offspring density (Figs. 4 and 5) and adult mortality (Fig. 6). All insect number was different among at each temperature treatment (Fig. 1) and increased with the increase in temperature.

There exists a strong correlation between all insect count, adult number, adult mortality at 21, 25, 30, 35˚C and Ti; however, the all insect count has a strong negative correlation with adult density at 21˚C and had a strong correlation with the adult number at Td (Table 4). The adult number was strongly correlated with the offspring number at 21˚C, 35˚C and Ti; however, was strongly correlated with adult mortality at all the temperature treatments (Table 4). Offspring number had a strong correlation with adult mortality at 21˚C, 25˚C and Ti (Table 4). The offspring density was strongly correlated with adult density at 21˚C, 25˚C and Ti (Table 4).

Table 4. Pearson correlation coefficients among all insect number, Adult number, Offspring number, Adult density, Offspring density and Adult mortality at different Temperatures (N = 21)

	21	25	30	35	Ti	Td
All insect number v/s Adult number	0.87	0.82	0.78	0.66	0.88	0.90
All insect number v/s Offspring number	0.59	0.77	0.90	0.75	0.82	0.33
All insect number v/s Adult density	-0.56	-0.11	-0.03	0.03	-0.23	-0.30
All insect number v/s Offspring density	-0.49	-0.21	-0.23	-0.33	-0.15	-0.20
All insect number v/s Adult mortality	0.81	0.97	0.61	0.92	0.97	0.78
Adult number v/s Offspring number	0.66	0.34	0.47	0.55	0.50	0.31
Adult number v/s Adult density	-0.44	-0.20	0.07	0.11	-0.20	-0.24
Adult number v/s Offspring density	-0.39	-0.14	-0.24	-0.09	-0.12	-0.12
Adult number v/s Adult mortality	0.73	0.68	0.91	0.56	0.92	0.81
Offspring number v/s Adult density	-0.21	0.00	-0.12	-0.06	-0.13	-0.14
Offspring number v/s Offspring density	-0.23	-0.17	-0.17	-0.29	-0.07	-0.10
Offspring number v/s Adult mortality	0.54	0.84	0.28	0.46	0.71	-0.12
Adult density v/s Offspring density	0.81	0.51	0.24	0.25	0.50	0.31
Adult density v/s Adult mortality	-0.34	-0.05	0.22	0.11	-0.23	-0.24
Offspring density v/s Adult mortality	-0.34	-0.20	-0.20	-0.28	-0.17	-0.20

*Ti = 21˚C in the first two weeks and then increased 1˚C /week to 38˚C
*Td = 30˚C in the first 4 weeks and then decreased 1˚C /week to -10˚C

The adults and offspring density fluctuated as the grain temperature changed during T-increase and T-decrease experiments (Fig. 4). The mean density of live adults at 35˚C was higher than 30˚C and 25˚C which was further higher than the mean density of live adults at 21˚C in the small patches (Fig. 4). The peak density of live adults was influenced by the grain temperature and increased with the increase in temperature inside each grain patch (Fig. 4). The peak live adult density in the small patches were: 300 ± 50, 673 ± 118, 689 ± 48, 1100 ±

150, 1150 ± 150 and 1133 ± 94 adults/kg at 21, 25, 30 and 35˚C and T-decrease and T-increase, respectively. The peak density of live adults inside the medium patch of grain at 21˚C was different than the peak density at 25˚C (Fig. 5). The peak density of live adults was different inside the large grain patches between 21 and 25˚C, 30 and 35˚C (Fig. 5). There was a significant difference in the offspring density between 21 and 30˚C, 21 and 35˚C, 25 and 35˚C irrespective of the size of the grain patch. The offspring density at 35˚C was higher than the offspring densities at 21, 25, 30, T-increase and T-decrease in the small patches.

Insect (live adult and offspring) densities at fluctuating temperatures (T-increase and T-decrease) followed a similar trend as seen at the constant temperatures resulting in a peak density (Fig. 4 and 5); however, there was no significant difference between the live adult and offspring densities at the two fluctuating temperature experiments.

4.3 Effects of interactions among temperature, storage period and patch size

The interactions of grain temperature, patch size and storage period had a significant influence (Table 2) on the population parameters (all insect population, offspring count, adult and offspring morality, adult and offspring density) of *T. castaneum*, except for the interaction between storage period and temperature for offspring count, adult and offspring densities.

The coefficient of determination inside the large patch was higher than 0.69 at each temperature experiment (Table 5). The values of coefficient of determination associated with the large patches were higher than that with the medium as well as small patches each temperature except 35˚C (Table 5). The value of coefficient of determination for medium patches was higher than small patches at ≤25˚C; however, the values were higher in the small patches at temperatures ≥30˚C (Table 5).

Table 5. Coefficient of determination (r^2) between generations and previous generations inside different patches of grain at different grain temperatures (N=7)

Grain Patch	Insects	Grain Temperature (˚C)					
		21	25	30	35	Ti *	Td*
Small Patch	All insects	0.50	0.50	0.96	1.00	0.96	0.17
	Live adults	0.15	0.01	0.13	0.07	0.11	0.03
Medium Patch	All insects	0.52	0.74	0.47	0.97	0.96	0.03
	Live adults	0.84	0.67	0.01	0.17	0.75	0.04
Large Patch	All insects	0.90	0.91	1.00	0.84	0.98	0.69
	Live adults	0.38	0.87	0.19	0.41	0.87	0.12

*Ti = 21˚C in the first two weeks and then increased 1˚C /week to 38˚C
*Td = 30˚C in the first 4 weeks and then decreased 1˚C /week to -10˚C

The size of the grain patch influenced the density of live adults instead of the storage period at 21˚C and fluctuating temperatures (Ti and Td) (Fig. 4); however, the storage period was the major influencing factor on the density of live adults at 35˚C (Fig. 4). The offspring density was mostly influenced by grain patch size instead of the storage period at all the constant temperatures. The peak density of live adults inside the small patch of grain kept at constant and fluctuating temperatures were different among 21-25˚C, 30-35˚C, 35-T-increase except among 25-30˚C and T-increase and T-decrease (Fig. 5). The live adult density was higher in small patches compared to medium and large patches of wheat at 21˚C, 25˚C, T-increase and T-decrease (Fig. 4). Similarly, the live adult density was higher inside the medium patches compared to large patches at 21˚C.

The peak live adult density inside small, medium and large grain patches was 1133 ± 94, 118 ± 31 and 77 ± 8 adults/kg, respectively, achieved in 20 weeks under T-increase conditions (Fig. 4). The peak density at 30 and 35˚C was achieved in 8 weeks but achieved in 16 weeks at 21 and 25˚C inside the small patches. The peak density of live adults inside the medium patch of wheat was different between 21 and 25˚C (Fig. 5). The peak density of live adults was different inside the large grain patches at 21 and 25˚C or 30 and 35˚C (Fig. 5).

5. DISCUSSION

5.1 Effect of patch size

The larger the patch, the higher the adult and offspring number. Adults of *T. castaneum* are highly mobile in the large patches (Surtees 1964; Hagstrum and Leach 1973) and relocate to a fresh resource when the quality of the grain at the current location declines (Jian et al. 2018). In the fresh grain, the grain has its highest quality, there is low competition (Smith et al. 1966); insects disperse quickly and develop faster (Hagstrum and Leach 1973) and females likely to lay more eggs (Campbell and Runnion 2003). Therefore, higher growth of the insect population was observed at the beginning of the experiments and a higher number in the large patch. Female reduce the oviposition rate in a small patch (Halliday et al. 2015).

Peak density of live adults was influenced by the size of the grain patch and was highest inside the small patch compared to the medium and large grain patches. The reason could be high initial insect density, fresh grain, low competition (Longstaff 1994; Jian et al. 2018) and the reason mentioned in the previous paragraph. Likely sources of egg disappearance include a reduction in oviposition, cannibalism of eggs by adults and larvae. Such effects might result in a reduction in insect density and quantities in the later weeks of the experiment.

The peak densities of adults and offspring were calculated based on the assumption of uniform distribution of the insects inside three patches. However, peak density might be higher than calculated at certain locations of the column due to the clumped distribution of *T. castaneum*. Inter-granular space is a barrier for the movement *T. castaneum* adults into a deep wheat bulk (Jian et al. 2005; Jian et al. 2012). *Tribolium castaneum* prefer the grain surface and the top layer of one tone wheat bulk (Jian et al. 2012). Adult aggregation caused by volatile compounds produced by adult *T. castaneum* could be another reason (Jian et al.

2012). These results are in contrast to the findings of *C. ferrugineus* by Jian et al. (2018), in which the rusty grain beetles were uniformly distributed throughout the column.

5.2 Variation of insect density at constant and fluctuating temperatures

Density of adults and offspring increased significantly with an increase in temperature inside each grain patch. Higher temperatures result in high mating and oviposition and thus high insect density (Maier et al. 2006). Extended exposure at elevated temperature enhances the metabolic activity, rate of development, mating and mortality. At higher temperatures, the grain is also softer and easier to chew, the softer grain could help the insect have more food and grow its population and density (Throne 1991; Jian et al. 2018). There is low negative density dependence at low temperatures (Scharf et al. 2015). The crowding and scarcity of food resulted in negative density dependence and a drop from the peak (Halliday et al. 2015).

The trends of Live adult density at T-increase were identical to T-decrease in small patch except in week 12. A gradual increase in the population of stored wheat insects is seen until grain temperature starts to reduce in the autumn (Hagstrum 1987). While during the T-decrease (from high to low temperatures) the increased rate of oviposition and short development periods at initial high temperatures could have lead to high insect density. Thus insect densities at the two fluctuating temperatures were identical following a similar trend.

5.3 Effects of interactions among temperature, patch size and storage time

All insect population, offspring count, adult mortality, adult and offspring density were influenced by the interactions among grain temperature, patch size and storage time. Interactions between time (months) and the temperature had a significant effect on the adult population of *T. castaneum* (Arthur et al. 2019). All insect count increased with time and temperatures. The storage time was the major influencing factor on the density of live adults at 35˚C. The peak adult density was the highest inside the small patch of grain stored at 35˚C.

Stored grain insect behaviour is influenced by the interaction of several physical, chemical and biotic factors including temperature, RH, food availability and moisture content of the grain (Cox and Collins 2002).

After 4 weeks, the live adult density inside the small patches increased as the temperature increased. The peak adult density was quickly reached in the small patches at higher temperatures. The reason could be higher initial density inside the small patches (Jian et al. 2018). Under both Ti and Td conditions, the density of insect (adult and offspring) altered due to the fluctuation in the temperature of grain (Figs. 4 and 5). Under both fluctuating temperature conditions, the population of insect reached the peak density determined at 35°C. Thus, the interaction among the storage period and temperature influenced the insect population dynamics.

At high temperature, the number of insect inside small and large patches were majorly dependent on the number of previous insect compared to medium patches. A high initial density of insect would produce a higher number of insect in the subsequent generations. All insect number was correlated with their previous insect number. At the same location, a strong correlation exists between previous and current insect density of rusty grain beetles (Jian et al. 2012). Higher insect density would result in more insects in the succeeding generations (Jian et al. 2012). Due to negligible effects of size limitation, crowding and waste buildup and lack of time to reach the peak, density of insect inside a grain bin would be majorly influenced by grain moisture, temperature and previous insect number (Jian et al. 2018).

5.4 Comparison of the population dynamics between *T. castaneum* and *C. ferrugineus*

This study was conducted at the same grain conditions that were studied for the population dynamics of *C. ferruginous*. In general, the number of *C. ferrugineus* under same conditions were higher than that of *T. castaneum*. The insect number between the two insect species followed the similar trend for each temperature and patch size except the large patches at 21˚C where all the insect number gradually decreased after 60 days for *C. ferrugineus* compared to *T. castaneum*. This could be due to the fact that at 25˚C the insect number for *C. ferrugineus* continuously increased compared to *T. castaneum*. The adult and offspring count among the two species fluctuated identically with temperature and patch size with higher number of adults in *C. ferrugineus* compared to *T. castaneum.*

The peak density of the *C. ferrugineus* at different temperatures and path sizes is the same, while the *T. castaneum* is different. Difference between the peak densities inside small and medium patches at 35˚C in *C. ferrugineus* was 20 times of same difference in *T. castaneum*. The adult mortality trends were identical except mortality of *T. castaneum* rise, fall and then rise where as increased continuously in *C. ferrugineus* with storage period at T-decrease in small patches. This could be due to the small body size and the ability of *C. ferrugineus* to move even at lower temperatures and thrive compared to *T. castaneum* (Jian et al. 2015).

6. CONCLUSIONS

Patch size had a strong influence on the population parameters of *T. castaneum* and the population size of all insects increased with an increase in patch size. The peak adult density increased at constant temperatures from 21 to 35˚C, however, was not significantly different when compared between fluctuating temperature conditions. The interaction among the factors had a significant effect on the total insect population and the peak adult density was the highest inside the small patch of grain stored at 35˚C. Peak density was achieved quickly in the small patch of grain at higher temperatures (≥30˚C). Insect numbers inside all patches were dependent on the previous insect number.

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APPENDIX

Table A1. Insect count (Adult and offspring) and insect mortality (Adult and offspring) in the three grain patches at 21˚C

Storage Period (Weeks)	Patch*	Adult Count	Offspring Count	Adult Mortality	Offspring Mortality
0	1	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	2	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	3	6 ± 0	0 ± 0	0 ± 0	0 ± 0
4	1	6 ± 0	2 ± 1	0 ± 0	0 ± 0
4	2	5 ± 1	19 ± 9	1 ± 1	0 ± 0
4	3	5 ± 1	40 ± 5	0 ± 1	0 ± 0
8	1	6 ± 1	3 ± 1	0 ± 0	0 ± 0
8	2	7 ± 2	20 ± 2	1 ± 1	2 ± 0
8	3	36 ± 8	35 ± 6	1 ± 1	5 ± 2
12	1	8 ± 1	3 ± 1	0 ± 1	0 ± 0
12	2	10 ± 2	22 ± 2	0 ± 0	3 ± 2
12	3	6 ± 2	52 ± 21	0 ± 0	8 ± 3
16	1	9 ± 1	2 ± 0	0 ± 0	1 ± 0
16	2	7 ± 2	21 ± 4	0 ± 0	3 ± 0
16	3	20 ± 3	38 ± 8	2 ± 0	3 ± 1
20	1	5 ± 1	2 ± 0	3 ± 1	1 ± 1
20	2	16 ± 5	8 ± 2	5 ± 4	2 ± 2
20	3	39 ± 5	34 ± 3	7 ± 1	10 ± 0
24	1	8 ± 2	1 ± 0	5 ± 1	1 ± 0
24	2	16 ± 1	5 ± 0	7 ± 3	2 ± 0
24	3	29 ± 1	25 ± 1	23 ± 5	12 ± 2

*Patch 1, 2 and 3 refer to small, medium and large patches.

Table A2. Insect count (Adult and offspring) and insect mortality (Adult and offspring) in the three grain patches at 25˚C

Storage Period (Weeks)	Patch*	Adult Count	Offspring Count	Adult Mortality	Offspring Mortality
0	1	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	2	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	3	6 ± 0	0 ± 0	0 ± 0	0 ± 0
4	1	5 ± 1	11 ± 2	0 ± 1	0 ± 0
4	2	6 ± 0	198 ± 11	0 ± 0	3 ± 0
4	3	6 ± 1	224 ± 31	0 ± 0	6 ± 6
8	1	10 ± 5	10 ± 0	9 ± 5	0 ± 0
8	2	146 ± 15	196 ± 7	9 ± 7	3 ± 3
8	3	242 ± 7	464 ± 0	13 ± 4	20 ± 2
12	1	9 ± 4	4 ± 0	4 ± 0	0 ± 0
12	2	231 ± 35	168 ± 9	1 ± 0	5 ± 1
12	3	396 ± 38	1250 ± 4	3 ± 1	54 ± 9
16	1	21 ± 4	11 ± 2	9 ± 2	0 ± 0
16	2	435 ± 11	303 ± 36	12 ± 6	6 ± 5
16	3	1655 ± 62	1613 ± 195	27 ± 10	67 ± 8
20	1	4 ± 3	2 ± 0	19 ± 3	0 ± 0
20	2	471 ± 43	89 ± 4	8 ± 7	1 ± 0
20	3	1906 ± 194	741 ± 80	73 ± 16	38 ± 23
24	1	2 ± 2	4 ± 0	25 ± 2	0 ± 0
24	2	243 ± 55	72 ± 34	31 ± 4	1 ± 1
24	3	1858 ± 201	445 ± 101	82 ± 21	16 ± 5

*Patch 1, 2 and 3 refer to small, medium and large patches.

Table A3. Insect count (Adult and offspring) and insect mortality (Adult and offspring) in the three grain patches at 30˚C

Storage Period (Weeks)	Patch*	Adult Count	Offspring Count	Adult Mortality	Offspring Mortality
0	1	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	2	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	3	6 ± 0	0 ± 0	0 ± 0	0 ± 0
4	1	6 ± 1	12 ± 2	0 ± 0	4 ± 2
4	2	9 ± 3	176 ± 26	0 ± 0	4 ± 2
4	3	13 ± 5	277 ± 80	0 ± 0	8 ± 3
8	1	13 ± 1	24 ± 18	5 ± 5	3 ± 5
8	2	205 ± 35	197 ± 10	6 ± 1	8 ± 3
8	3	540 ± 88	1999 ± 160	9 ± 2	93 ± 3
12	1	8 ± 2	18 ± 0	27 ± 8	0 ± 0
12	2	1346 ± 389	292 ± 15	236 ± 157	56 ± 7
12	3	2641 ± 115	1156 ± 123	49 ± 37	303 ± 140
16	1	5 ± 4	5 ± 0	21 ± 18	1 ± 2
16	2	335 ± 152	125 ± 122	514 ± 115	41 ± 9
16	3	4259 ± 1253	1280 ± 345	1350 ± 645	274 ± 47
20	1	2 ± 3	13 ± 5	52 ± 17	0 ± 0
20	2	167 ± 52	407 ± 267	1213 ± 407	42 ± 45
20	3	1922 ± 169	1715 ± 74	5018 ± 859	200 ± 83
24	1	5 ± 3	5 ± 5	42 ± 22	0 ± 0
24	2	37 ± 12	82 ± 11	820 ± 331	20 ± 5
24	3	553 ± 13	1245 ± 53	6206 ± 2714	142 ± 31

*Patch 1, 2 and 3 refer to small, medium and large patches.

Table A4. Insect count (Adult and offspring) and insect mortality (Adult and offspring) in the three grain patches at 35˚C

Storage Period (Weeks)	Patch*	Adult Count	Offspring Count	Adult Mortality	Offspring Mortality
0	1	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	2	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	3	6 ± 0	0 ± 0	0 ± 0	0 ± 0
4	1	9 ± 2	26 ± 4	0 ± 0	2 ± 2
4	2	130 ± 1	187 ± 25	2 ± 2	1 ± 1
4	3	290 ± 30	627 ± 88	9 ± 4	8 ± 6
8	1	32 ± 4	25 ± 4	10 ± 8	9 ± 5
8	2	1026 ± 96	563 ± 62	24 ± 14	21 ± 9
8	3	3470 ± 257	2836 ± 113	49 ± 38	43 ± 37
12	1	25 ± 11	13 ± 1	67 ± 22	5 ± 1
12	2	1801 ± 570	265 ± 16	344 ± 97	19 ± 9
12	3	6728 ± 16	2920 ± 132	779 ± 679	46 ± 35
16	1	20 ± 3	13 ± 1	89 ± 16	4 ± 2
16	2	1486 ± 173	405 ± 51	1699 ± 234	58 ± 22
16	3	3331 ± 359	506 ± 81	2847 ± 477	229 ± 179
20	1	8 ± 4	42 ± 4	115 ± 19	28 ± 18
20	2	1227 ± 107	741 ± 16	2558 ± 418	542 ± 106
20	3	6020 ± 647	2558 ± 254	5157 ± 1195	1177 ± 216
24	1	0 ± 0	32 ± 9	138 ± 65	44 ± 24
24	2	291 ± 97	50 ± 4	3750 ± 191	59 ± 22
24	3	2910 ± 490	181 ± 53	8902 ± 1250	174 ± 72

*Patch 1, 2 and 3 refer to small, medium and large patches.

Table A5. Insect count (Adult and offspring) and insect mortality (Adult and offspring) in the three grain patches at T-increase

Storage Period (Weeks)	Patch*	Adult Count	Offspring Count	Adult Mortality	Offspring Mortality
0	1	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	2	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	3	6 ± 0	0 ± 0	0 ± 0	0 ± 0
4	1	6 ± 0	10 ± 6	0 ± 0	0 ± 0
4	2	6 ± 0	76 ± 13	0 ± 0	0 ± 0
4	3	5 ± 1	141 ± 11	1 ± 1	3 ± 2
8	1	25 ± 4	6 ± 2	1 ± 2	0 ± 0
8	2	83 ± 5	29 ± 17	0 ± 0	1 ± 1
8	3	97 ± 19	179 ± 6	20 ± 6	6 ± 2
12	1	32 ± 4	2 ± 0	3 ± 1	0 ± 0
12	2	139 ± 26	15 ± 4	7 ± 0	0 ± 0
12	3	369 ± 66	346 ± 31	13 ± 7	7 ± 1
16	1	30 ± 5	2 ± 0	0 ± 1	0 ± 0
16	2	128 ± 21	4 ± 1	1 ± 0	1 ± 0
16	3	394 ± 19	32 ± 10	10 ± 4	5 ± 3
20	1	34 ± 3	8 ± 2	0 ± 0	0 ± 0
20	2	237 ± 64	111 ± 4	16 ± 2	5 ± 4
20	3	1079 ± 121	667 ± 86	112 ± 7	49 ± 10
28	1	5 ± 1	4 ± 1	37 ± 2	0 ± 0
28	2	142 ± 19	32 ± 4	195 ± 16	1 ± 1
28	3	1090 ± 156	594 ± 79	950 ± 180	27 ± 9

*Patch 1, 2 and 3 refer to small, medium and large patches.

Table A6. Insect count (Adult and offspring) and insect mortality (Adult and offspring) in the three grain patches at T-decrease

Storage Period (Weeks)	Patch*	Adult Count	Offspring Count	Adult Mortality	Offspring Mortality
0	1	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	2	6 ± 0	0 ± 0	0 ± 0	0 ± 0
0	3	6 ± 0	0 ± 0	0 ± 0	0 ± 0
4	1	13 ± 1	20 ± 0	0 ± 0	12 ± 6
4	2	92 ± 26	78 ± 3	0 ± 0	1 ± 2
4	3	198 ± 76	88 ± 8	12 ± 20	2 ± 0
8	1	18 ± 11	25 ± 0	12 ± 4	0 ± 0
8	2	55 ± 10	84 ± 9	3 ± 2	1 ± 0
8	3	129 ± 6	198 ± 29	22 ± 24	8 ± 3
12	1	7 ± 2	5 ± 2	2 ± 1	0 ± 0
12	2	204 ± 3	53 ± 12	3 ± 3	9 ± 5
12	3	274 ± 49	883 ± 66	2 ± 1	19 ± 3
16	1	27 ± 4	6 ± 2	12 ± 3	0 ± 0
16	2	116 ± 5	35 ± 8	3 ± 1	5 ± 2
16	3	464 ± 21	525 ± 37	28 ± 13	36 ± 8
20	1	37 ± 5	2 ± 2	0 ± 0	0 ± 0
20	2	191 ± 29	19 ± 7	27 ± 15	3 ± 4
20	3	595 ± 23	373 ± 61	36 ± 14	37 ± 10
30	1	0 ± 0	0 ± 0	39 ± 2	0 ± 0
30	2	0 ± 0	11 ± 3	27 ± 4	1 ± 0
30	3	0 ± 0	9 ± 7	764 ± 68	22 ± 9

*Patch 1, 2 and 3 refer to small, medium and large patches.