

**Effects of Developmental Stage, Cold Acclimation and Diet on the Cold Tolerance of three
Species of *Cryptolestes* (Coleoptera: Laemophloeidae)**

by

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Abstract

Flat grain beetles (Coleoptera: Laemophloeidae) are common stored-product insect pests in Canada, infesting cereals in grain bins and equipment and end products in flour mills. This thesis reports a comparative study on the cold tolerance of three species of *Cryptolestes*: *Cryptolestes ferrugineus*, *Cryptolestes turcicus* and *Cryptolestes pusillus*.

The cold tolerance of different life stages (egg, young larva, old larva, pupa and adult) of the three species reared on flour mixed with brewer's yeast was measured by holding insects at -10°C for different durations. Probit analysis was performed to estimate the lethal time to 50 and 95% mortality. This was done with non-acclimated individuals (only held at 30°C) or cold-acclimated individuals (held at 18, 10 and 5°C , for 1 week/temperature). In general, the adults were the most cold-hardy stage for each of the species. Acclimated insects were anywhere from 1 (no cold-acclimation) to 14-fold more cold-tolerant than the corresponding unacclimated stage and species. *Cryptolestes ferrugineus* was most cold-tolerant species (requiring 58 d to reach 95% mortality, acclimated adult), *C. turcicus* was the next most cold-tolerant (39 d to reach 95% mortality) and *C. pusillus* was the least cold-tolerant (11 d to reach 95% mortality).

The cold tolerance of adults reared on three diets was measured both for acclimated and non-acclimated insects. In general, the adults reared on grain diet (whole wheat kernels, cracked wheat kernels and wheat germ (90:5:5 proportions) were the most cold-tolerant, adults reared on white-wheat flour and brewer's diet (95:5 proportions) had the next highest cold tolerance followed by the adults reared on 100% white-wheat flour. Supercooling point (SCP) of insects ranged from -20.6 to -26.7°C . In general, acclimated insects had slightly lower SCP than non-acclimated insects.

Possible management strategies using cold temperatures, a comparison to previous studies on *Cryptolestes* and cold tolerance of other species are discussed.

Chapter 1: Literature Review

Stored-products

Humans are responsible for dispersing plant and insect species to many parts of the world where they were not originated (Gippet et al., 2019). Insects often infest the food stored on the ships, and these pests then went on to infest local storages upon arrival at the ports of entry. Despite the nuisance the insects cause during transportation, trade and commerce is unavoidable as an important source of income and livelihood (Robinet et al., 2009; Ward et al., 2006). The importance of managing pests and other damaging organisms lies in the fact that it is a loss of time, money, resources and efforts that went into producing the products in storage. This is far worse than that damage caused in the field, as it does not involve harvesting, processing and storage costs. Stored-product insects cause major losses of revenue, raw material and replacement costs in the U.S. it is estimated at \$9.6 billion in monetary losses in a 5 year period in 2014 (Hartzer, 2016).

Historically, food storage began as agriculture began, to save seeds to plant the next season and to provide food security (Levinson and Levinson, 1989). It is an important component of the supply chain that can potentially equilibrate fluctuations from the harvest season to the rest of the year. Storing food is a behavior evident in many other animals like small vertebrates (squirrel and mice) and insects (ants and bees). They forage food in the summer or when food is available and store it to ensure availability when food is not available. This behavior is very common among animals and insects living in temperate regions where the winters are harsh and foraging is hard or impossible (de Saulieu and Testart, 2015; Pollok et al., 2000). When civilization started flourishing and humans started to cultivate crops, insects, rodents and many other higher animals competed for food (Dunkel, 1985; Kuijt and Finlayson, 2009; Levinson and Levinson, 1989). As much as there is a competition for food in the field, insects, humans and other animals compete for the same source of food when in storage. Humans store plant produce in confined environments; vertebrate and invertebrate organisms compete with humans for this food (Bourne, 1977). Stored-product insects are specialized and well adapted for survival as they can sense the volatile compounds of the stored-plant products, search, live and multiply in dry environment (Adler, 2008).

Storage structures form an ecosystem with micro-climate consisting of its own microflora and fauna, mostly invertebrates like insects and mites and vertebrates such as rodents and birds. Stored-product insects have adapted to living in the artificial storage structures where food products are stored for a very short period of time like fresh vegetables and fruits waiting to be taken to the market, or for longer periods of time like grains that are stored over months and years. The food products stored generally include food with low moisture content; cereals, cereal products, pulses, nuts, dry fruits, oilseeds and spices; and foods with higher moisture content; fresh fruits and vegetables including tubers and roots; meat and meat products. Fresh-food products are stored only for a small amount of time until they are moved from the point of production to the point of consumption before their quality is compromised with time. Being inside a confined environment provides shelter from abiotic and biotic factors outside making it easier for the insects to multiply at higher rates in stored-products. *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) can multiply by 60 to 70-fold (Hulasare et al., 2003) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) and *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae) can multiply by 15 to 30-fold per month if left unchecked (Bell, 2013).

Safe storage is important to minimize losses during storage to meet the marketing requirements in terms of both quality and quantity. The commodities that are stored for a long time include dry grains like wheat, rice, maize, millets and grains for seeding purposes. Plant products like spices, tobacco, mushrooms, dried plant materials, bird seeds, pet foods and tubers like potato and turmeric; animal products like fish meal, and dried meat are also stored for future use. Storing food products helps reduce waste when there is surplus production or reduced demand. Sometimes certain products are kept in storage to improve their quality characteristics and increase the demand. An example is curing of tobacco, where the tobacco leaves are kept in storage at natural temperature and humidity range so as to aid in slow fermentation/ ageing of the leaves and to fulfil manufacturing requirement. *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) does not prefer fresh tobacco in the field, but cured tobacco in storage (Ashworth, 1993).

Mechanical harvesting can damage the grains and makes them more vulnerable to attack by stored-product pests. Approximately 50% of wheat threshed mechanically is damaged, while

there is only 1% damage when hand-threshed (Tuff and Telford (1964). This physical damage results in the grains becoming favourable for feeding and development and increased rate of reproduction. This is true for most of the insects including the major pests like *Cryptolestes* spp. (Fraenkel and Blewett, 1943; LeCato and McCray, 1973; Sinha, 1975; Tuff and Telford, 1964).

The type of storage depends on the expected duration of storage, demand, the type and handling of the products. Long-term storage of plant produce is usually done using bags in the tropics in warehouses (FAO, 1985). Grains do not need as much maintenance as required by fresh produce. The warehouses should be properly maintained following all the procedures required to keep the structure free from insect and rodent pests. The floor and walls should be sealed of any cracks and crevices which might act as refuge for insects. Aeration is an important factor to be considered as highly humid structure with no air circulation will only aid in growth of fungi, mites and secondary pests and ruin the quality of the produce (FAO, 1985). Aeration helps to reduce moisture content and prevent moisture content gradients in the grain bulk. The bins used for storing grains have a duct at the bottom for aerating the grains inside (Pabis et al., 1998). Aerating and turning the grains in hopper-bottomed and flat-bottomed bins can help minimize problems arising due to trapped moisture. It is important to aerate the grains stored although they are comparatively dry as problems like moisture migration and condensation may occur (Lu et al., 2010).

The storage conditions to be maintained should be within a strict range of moisture content and temperature. Moisture content of 14-18% in cereal grains is favourable for insect growth and survival. Keeping the grain moisture content between 8-12% helps reduce insect growth. Temperature range of 25-35°C is optimal for growth and multiplication of most stored-grain insects; oviposition and fecundity of most stored-product insects increases with increases in temperature within the range of 25 to 35°C (Sinha and Watters, 1985). Mold activity is further increased by insect infestation as the feeding activity exposes the endosperm by damaging seeds. This forming a microhabitat within the infested grains which makes it even harder to control to spread of mould. Hence, the grain temperatures should be monitored regularly (Evans, 1980; Sinha and Watters, 1985). Temperatures below 20°C prevent most insects from completing development. Temperatures below 2°C do not allow the development of mites and most insects under humid conditions (FAO, 1985; Fields, 1992). The developmental period and longevity of

insects also depends on the temperature. When the temperatures are below the optimum for development, the developmental period increases and so does the longevity due to slower rate of metabolism and growth.

Stored-product Insects

Stored-product pests can cause 5-10% damage in temperate conditions and 20-30% damage in the tropical regions (Nakakita, 1998; Rees, 2004). Pests in storage environments can be insect or non-insect pests (mites, fungi, rodents like mice and squirrels and birds). Stored-product insects are mostly Coleoptera and Lepidoptera. Among the approximately 150 insects that attack stored-products, only about 15 are common, causing most of the damage, and they are *Cryptolestes* spp., *Ephesita* spp., *L. serricorne*, *Oryzaephilus* spp., *P. interpunctella*, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), *Sitophilus* spp., *Sitotroga cerealella* (Oliver) (Lepidoptera: Gelechiidae), *Stegobium paniceum* (L.) (Coleoptera: Anobiidae) and *Tribolium* spp. Some of these insects were found in ancient Egyptian tombs; *Tribolium* spp., *S. granarius*, *L. serricorne*, *S. paniceum*, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *R. dominica* demonstrating that these insects have been pests since the dawn of agriculture (Abdelgalil et al., 1987; Chaddick and Filce Leek, 1972; Solomon, 1965).

The diversity of stored-product insects in storage structures depends on the type of commodity stored and preferences of the insects. Although many stored-product insects have a feeding preference for the germ, which is rich in protein and oil, they can be classified as generalist and specialist feeders i.e. monophagous or polyphagous (Hengeveld, 1979). Generalists are insects that can feed on a wide variety of stored food such as the drugstore beetle, *S. paniceum* which can feed on herbal and medicinal plant products, grain and grain products, spices, dried fruits, seeds, dried fish, pet food, coffee beans, chocolate; upholstery, books and manuscripts. It is a very serious pest in museums attacking the plant and animal specimens on display. Specialists are insects that can only feed on a narrow range of stored food like *Sitophilus granarius* and *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) which are seed feeding insects (Ignatowicz et al., 2011; Panagiotakopulu, 2003).

These insects can be primary or secondary feeders depending on the type of mouth parts, age/ life stage and food product. Primary feeders are insects that feed on stored-products causing

direct damage i.e. they can pierce through the seed coat and damage unbroken commodities. Primary feeders are also known as internal feeders as they feed on endosperm and germ and develop inside the grains and complete their life cycle. Some examples include *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), *S. granarius*, *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae), *R. dominica* and *S. cerealella*. Secondary feeders can only feed on products that are already damaged either by the primary feeders or dockage and handling as their mouth parts are not strong enough to cause direct damage to intact grains or seeds. Most grains are damaged by handling and transport before they are stored making it easier for the insects to feed and get established. They are also known as external feeders as they feed and develop on cracked or broken kernels and grain dust and flour and enter the seeds only through damages inflicted by the internal feeders. Some examples include, *C. ferrugineus*, *C. turcicus*, *C. pusillus*, *O. surinamensis*, *P. interpunctella*, mites and psocids (Hartzer, 2016; Sinha, 1971).

Canada has zero tolerance for live insects in food as per the regulations of the Canadian Grain Act (Anonymous, 2018). In the USA, the Food and Drug Administration (Anonymous (2009), has set standards for the amount of insect materials that can be present in a commodity before marking it as contaminated. In wheat and rye, the presence of two live insects in 1000 g makes it to be graded as infested. In corn, barley, oats, soybeans and sorghum different criteria are used depending on the insects infesting them. The grains are considered infested if 1000 g contain either one live weevil, one live weevil and five or more other live insects or no live weevil and ten other live insects which can cause damage to the grains.

The microhabitat in the stored grain ecosystem can be easily manipulated to suit the needs of optimum storage. Maintaining moisture content in seeds below 14 to 10% moisture content (depending upon the seed) helps to prevent mold growth, as fungi require enough water for spore germination. Dry grains will not permit fungal growth and eliminate previous infections (Sinha and Sinha, 1992; Sinha et al., 1992; Wallace et al., 1981; Wicklow, 1994). However, storage fungi are well adapted to dry conditions unlike field fungi and the mycotoxins produced by the storage fungi like *Fusarium* Link (Hypocreales: Nectriaceae), *Aspergillus* Micheli (Eurotiales: Trichocomaceae) and *Penicillium* Link (Eurotiales: Trichocomaceae) are

toxic to animals including humans. Their presence can cause caking and germ damage compromising the grain quality (FAO, 1985).

The stored-product environment provides a safe structure for the insects to survive in as they are not affected by the biotic and abiotic factors from the open field directly. Insects which prefer hiding in the cracks and crevices of buildings and inside or in between grains are said to have refuge-seeking behavior. They might seek refuge for a certain period of time when they are vulnerable and emerge later when suitable conditions return (Coombs and Freeman, 1955; Liscombe and Watters, 1962). This behavior can differ between different insect species and strains of the same species as demonstrated by Pinniger (1975) in *T. castaneum*. This behavior helps the insects to escape insecticidal treatments in the stored-grain structures and ensures their survival. *Cryptolestes ferrugineus* demonstrates refuge-seeking behavior which varies among different strains and the difference increases with decrease in temperature. It is also found that *C. ferrugineus* prefers to remain in the refuge grains most of their adult life. This provides a great advantage for their survival (Pinniger and Chambers, 1986).

Important Stored-product Insects

Rhyzopertha dominica is a cosmopolitan pest of many cereals and leguminous crops in many parts of the world (Hagstrum, 1994; Hagstrum and Flinn, 1994). The adult and larva can infest whole grains. Zettler (1991) reported losses of over \$500 million per year of the post-harvest products in the U.S. Red flour beetle, *T. castaneum* is a cosmopolitan pest found all over the world and is a major pest attacking groundnuts. They cause a damage of 4.5% weight loss in a year, 27% loss of germination in 6 months (Applebaum, 1969; Howe, 1965a; Parkin, 1956). *Tribolium* spp. are reported to be one of the most common cosmopolitan pests of great importance in the stored-product environment and processing plants (Campbell and Arbogast, 2004; Campbell et al., 2004; Sokoloff, 1972).

Cryptolestes ferrugineus, *O. surinamensis* (cold-hardy), *R. dominica* (moderately cold-hardy), *S. oryzae* (moderately cold-hardy) and *T. castaneum* (cold-susceptible) these are the most common stored grain insects in Canada. The most common insects found in stored grain environment in Canada are *C. ferrugineus* and *T. castaneum* (Sinha and Watters, 1985), *Cryptolestes* will be covered in detail below. *Tribolium* spp. can also attack animal feeds in

storage and are found to cause damage up to 10-20% and loss of a billion tonnes annually (Baker, 1988; IFIF; Mason and McDonough, 2012). *Tribolium castaneum* is a very important pest of plant based animal feeds in storage facilities, the most common being corn meal, wheat meal, by-products of soybean and sunflower (Jagadeesan et al., 2013; Mahroof and Hagstrum, 2012; Rees, 2004). *Oryzaephilus surinamensis* is a major pest of stored grains (Armstrong and Howe, 1963). It is a secondary pest as it can only feed on damaged grains, but can become a primary feeder when the grains are damaged by mechanical harvesting (Fraenkel and Blewett, 1943; Howe, 1973).

Dermestids are pests in houses, museums, feed mills and pet food manufacturers and warehouses. They are used for cleaning bones in a short amount of time as a well-established colony can clean a deer skull in 2-3 days (Hefti et al., 1980). There about 500-700 species worldwide, some examples are *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and *Dermestes lardarius* L. (Coleoptera: Dermestidae). *Trogoderma granarium* is a serious pest attacking wheat and also found in cereals, millets, leguminous crops and meat (Hinton, 1945) and is predominant in tropics and sub-tropics of Asia and Africa. The larva feeds on stored-products causing loss of quality and quantity of the produce and compromises the health of consumers by contaminating with exuvia and hairs (Burgess, 2008; Jood et al., 1993; Jood et al., 1996). It is a quarantined pest in Canada and many other countries and if identified the whole shipment is destroyed and sent back to the exporter (Anonymous, 2019). *Dermestes lardarius* feeds on products of animal origin and wood. It is a serious pest in poultry, feeding on the wooden structures and growing in the excreta piled up near the poultry house. This pest exhibits difference in development, longevity and rate of oviposition depending on the temperature and humidity (Coombs (1978).

There are a few moths that can be a problem in grain. *Sitotroga cerealella* is a major pest in grains most importantly in maize. Although it is a primary pest and the larva develops inside the kernels, they cannot reach below 20 cm of grain in a bulk, hence it causes limited damage to the grain mass (Shahjahan, 1974, 1975). *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) is a widespread stored-product moth attacking wheat, maize, cacao, dried onions, garlic and dried fruit and nuts. The moth is found to locate the food source using the odor of the grains in storage (Bell, 1983). *Plodia interpunctella* is a very destructive moth and primary pest in Canadian

warehouses and grain elevators feeding on stored cereals, peanuts and dried fruits in storage. Food becomes contaminated with webbing and frass. It is cold-hardy and can survive winters in granaries. *Pyralis farinalis* (L.) (Lepidoptera: Pyralidae) is a moderately cold-hardy secondary moth pest infesting damaged grains, potatoes, wheat bran and dry fruits in the Canadian granaries, flour mills and silos (Sinha and Watters, 1985).

Control of Stored-product Insects

Stored-product environments like grain bins, processing machinery and flour mills provide refuge for a number of pests which infest the grains. Canadian grains are marketed for their high quality, and no live insects are tolerated for domestic or international markets as specified by The Canadian Grain Act, 1985 (Anonymous, 2018). Canada was the 6th largest producer of wheat in the world with an estimated yield of 33 million t in 2019 (FAO, 2020). Although many different insect control methods have been proposed, finding the right combination is important and it depends on the geographical distribution, location, climate, species and the extent of damage. Some of the methods used for the control of stored-product pests involves the use of chemical insecticides by contact and fumigation, physical control by modifying the temperature, air and humidity in storage structures, irradiation and use of bio-control agents like parasites of stored-product pests (Campbell, 2004; Hagstrum and Subramanyam, 1995; Hansen, 2007). Conditioning of grains after harvest like grain chilling and aeration can reduce damage from insects and molds (Maier and Rulon, 1998).

Early records suggest that humans have always been in a battle with the pests that were competing with them for food, especially in storage. Evidence can be found in Assyrian (8th-7th century B.C.), the Old Testament records (10th-6th century B.C.) and in the book of Exodus on the various infestations that were faced like lice, body mites, flies and mosquitoes and desert locusts (Abivardi, 2001). Book of the Dead suggests that the practice of mummification was started by the early Egyptians to save the dead bodies from insect infestation as they believed in resurrection (Budge and Romer, 2008). There is evidence that people used dry earth to protect from insect and mite infestations around 1640 B.C (David, 1992). The Egyptians also left records of fumigation in the 26-20th century B.C. It was a religious ritual to burn resins and medicinal herbs on an altar using incense burners. The use of sulphur as a fumigant was mentioned in Homer's odyssey and practiced in Roman culture (Levinson and Levinson, 1990).

Integrated Pest Management

Integrated pest management (IPM) is the concept of integrating control measures from different disciplines of pest management. It includes mechanical, cultural, chemical and biological control, sanitation and microclimate management. IPM is aimed at controlling the insect populations from reaching levels that can cause economic damage; it considers the economic threshold levels of pests in a given area which makes the approach unique. It focusses on information and balanced use of alternatives to chemicals to satisfy food, work and environment safety requirements (Phillips and Zhao, 2002).

The strategies include training the facilitators, keeping the storage space clean before and after harvest, monitoring, trapping and identifying the insect and non-insect pests in and around the facility, testing for resistance to insecticides, formulating control strategies involving-chemicals, temperature control, modified atmosphere, bio-control agents and pheromone application (Mori et al., 2006). Different IPM strategies are available for different groups of insect pests, mold and microbial control.

Chemical Control

Chemicals are used for treating the storage structures and grains. Sanitation is an important criterion for managing pests in storage as grain residues in and around storage structures are an important source of infestation and cracks and cervices in the structures house insect pests to allow chemicals to work effectively. Some of the chemicals used for treating buildings and bins are chlorpyrifos-methyl, malathion, deltamethrin and Cyfluthrin; pirimiphos-methyl can be directly applied on stored commodities (Arthur and Subramanyam, 2012).

The use of chemicals to control stored-product insects has its own benefits such as cost effectiveness, easy application and wide-spread availability. As much as they play a useful role in saving the stored-products at the producing and marketing ends, consumers are concerned about pesticide residues in their food; there is development of resistance and resurgence of stored-product insects to chemicals applied continuously to control them, especially malathion (Assie et al., 2007; Bughio and Wilkins, 2004). Hence, more studies are focused on the development of physical control measures (Banks and Fields, 1995) that leave no residues in food commodities and have few side effects when consumed by humans and livestock (Zettler

and Keever, 1994). When used for controlling insects of museum specimens, chemicals help control and prevent further infestation but it also results in contamination and damage of the specimens (Pinniger and Child, 2002).

Fumigation is a common and effective chemical control method. Fumigants are toxic volatile gases which can kill insect pests hidden inside the seeds and in cracks of the buildings as they can reach places that cannot be reached by sprays or aerosols. In stored-product ecosystems they are used in granaries, primary and terminal elevators, flour mills and warehouses to kill insects and mites. Fumigation is used in enclosed structures which can be sealed for the fumigants to work effectively and can be dangerous when proper procedures of application are not followed. Fumigants leave no chemical residues and can achieve high mortality rates (Kells et al., 2001).

The most commonly used fumigant in Canada and around the world is phosphine (hydrogen phosphide) (Chaudhry, 1997; Pimentel et al., 2008). It can spread evenly and penetrate through structures and grains on reaction with water in the atmosphere. It has garlic-like odor and is applied as pellets or plates, mainly as aluminum phosphide and less frequently as magnesium phosphide. The metal phosphide reacts with the moisture in the air to produce phosphine. There are also gaseous formulations of phosphine that generate the gas quicker and more accurately. Presence of minimum electric equipment in grain bins, silos and buildings are ideal for treatment with phosphine due to its corrosive nature (Phillips et al., 2012). Sulfuryl fluoride is a fumigant used to treat storage structures and also food. It has been used most recently as a replacement for methyl bromide in food-processing plants (Fields and White, 2002). Ethyl formate is a naturally occurring ester which is used against sucking pests in field (Johnson et al., 2010; Winks and Ryan, 1990), and is now being used in stored-products. Although fumigants may provide better control, they are toxic to beneficial insects such as predators and parasitoids and can pose a greater risk to applicators and people working around fumigated structures. Hence, several countries are banning the use of certain chemical fumigants which are known to cause harm. Canada and the U.S. banned the use of liquid fumigants as they were reported to leave residues in food (Fields and Muir, 1995). Methyl bromide is almost entirely banned because it is an ozone depleting substance (Fields and White, 2002).

Issues with Chemical Control

There are several reasons grain managers would like to reduce or even eliminate the use of insecticides to control stored-product insects. 1. Insecticides are costly. 2. Training is required for the application, difficulty in obtaining licenses. 3. Consumers are concerned about insecticide residues in grain (Fishwick, 1988) even with registered insecticides. 4. Insecticide resistance is a growing problem, with resistance to malathion and phosphine found in some populations in Canada (Cato et al., 2017; Sinha and Watters, 1985). 5. Some insecticides can cause grain quality loss. Diatomaceous earth reduces test weight. 6. Insecticides can cause resurgence of insect populations due to the elimination of predators and parasites (Banks and Fields, 1995; Santos, 2006). 7. Apart from accumulation of chemical residues in food grains, the use of synthetic insecticides have also led to environmental pollution (Leidy et al., 1993; Wright et al., 1993). Therefore, there is interest in finding alternatives to insecticides to control stored-product insects.

Rhyzopertha dominica and *C. ferrugineus* have become resistant to the fumigant phosphine making it hard to control. *Cryptolestes ferrugineus* with higher level of resistance to phosphine is a predominant pest among stored-product pests (Nayak et al., 2013). Experiments to determine the extent of resistance of *C. ferrugineus* to phosphine established that the insect died only when treated with high concentrations of phosphine at 200 ppm and resistant strains died at 550 ppm after 45 days (Cao et al., 2010).

Contact insecticides are usually applied by spraying liquid or dust formulations directly on the surface of storage structures or grains. Their mode of action is generally by absorption of the formulation through the exoskeleton or ingestion by the insects. The nervous system is compromised as the chemicals bond with the neuro receptors in the place of neurotransmitters (acetylcholinesterase) (Rowlands, 1971b). The use of some chemical insecticides is known to kill fish and livestock and can be a risk to the environment. This is a potential threat to not-target organisms like birds and mammals and contamination of water sources. Examples include organochlorine compounds like DDT and lindane which are banned for use on stored-products; organophosphorus compounds like azamethiphos, which are highly effective but extremely toxic to mammals working as a stomach poison, chlorpyrifos, diazinon, dichlorvos, fenitrothion and malathion which was widely used in the 1960s but now removed from the market due to higher level of resistance in a lot of stored-product pests; carbamates like bendiocarb and carbaryl

which acts by inhibiting cholinesterase but has less mammalian toxicity (Arthur and Subramanyam, 2012). Pyrethrins, derived from chrysanthemum flowers are used in combination with PBO, piperonyl butoxide as an effective way to control pests although not widely used. Common pyrethroids are bioallethrin, cypermethrin, deltamethrin, fenvalerate and pyrethrum (Kljajic and Peric, 2006; Rowlands, 1971a).

Temperature

The many stored-product insects can live for more than a year under favourable conditions of temperature and humidity (Birch, 1953; Park et al., 1961). Even before the discovery of insecticides, physical control methods were practiced by the people of ancient Egypt, who used subterranean temperatures for grain storage (Levinson and Levinson, 1989). This is because of the existence of ambient conditions for storage such as, temperatures as low as of 12°C, low oxygen levels, cool and dry environment below the ground level of 4 m. These conditions help in maintaining grain quality and prevent insect infestation (Wang, 1998).

Modern granaries are designed to maintain the grain dry and cool, as the Egyptians did thousands of years ago. There are several ways Canadian farmers cool their grain to prevent insect infestation. The most common method is to use ambient air aeration, using fans to push outside air through the grain. In the middle of the winter, slowly moving grain can break up hot spots and cool the grain slightly. Finally, there are grain refrigeration units that will dry and cool the air passing through the grain. Once cooled, grain will maintain its low temperature long after the ambient air temperature has increased (Mason and Strait, 1998).

The use of extreme temperatures to regulate stored-product pests has gained importance as a means of physical control due to its efficiency in insect control. Cold temperatures will slow down the development and movement of insects inside storage structures (Subramanyam and Hagstrum, 1996). The insects tolerate temperature extremes and this tolerance varies with life stage, the duration of exposure, seasonal acclimation (sudden or gradual reduction in temperatures) and other adaptations (diapause) (Fields, 1992). Reducing the temperatures to less than 13-20°C (depends upon species) stops development and when insects freeze from -10 to -20°C instant death occurs; increasing the temperatures to 40 to 60°C makes the environment lethal to survive (Banks and Fields, 1995).

Temperature plays an important role in the development of immature life stages even when favourable diet is not available. For example, *P. interpunctella* showed increase in population at a temperature range of 25 to 30°C regardless of the diet, whether optimal wheat diet or sub-optimal raisin diet (Fontenot et al. (2012). The temperature range of 15-20°C is the minimum threshold for the growth of stored-product insects and maximum infestation is found to occur between 25-35°C, the most favourable temperature range and the populations start to die out above 35°C in most cases (Cotton, 1963; Surtees, 1963, 1965).

Heat treatment is a very old technique used since ancient times for killing stored-product insects by sun drying. Heat treatment in modern flour mills began as early as 1901 in the U.S. The effectiveness depends on the life stage exposed as immature stages are more susceptible to drying and desiccation. Temperatures as high as 50°C can kill all the life stages of stored-product insects in 24 h; reproduction, metabolism and survival are hindered between 40-50°C; 28-32°C is the most favourable range for optimum reproduction and development and temperatures above 38°C or 13-20°C do not allow development (Fields, 1992). Apart from killing the insect pests by exposing the infested produce to high temperatures, heat treatment is also used in sanitising storage structures and processing equipment. Treating empty storage structures is much more effective due to faster and even heat transfer into spaces not reachable by chemical sprays. However, there might be a relapse in insect populations with the incoming grains for storage like in fumigation (Menon et al., 2001; Menon et al., 2000; Subramanyam et al., 2005).

Heat stress, just like cold stress triggers stress-response biochemicals to protect the insects from desiccation and osmotic stress which leads to death by dehydration. Polyols are sugars which help avoid aggregation of proteins at high temperatures were also reported to maintain the thermal stability of the enzymes by Kim and Lee (1993). According to Dowdy (1999), heat treatment of *T. castaneum* at 50°C for over 30 minutes was very effective when used in combination with diatomaceous earth. Some stored-product insects are heat tolerant such as *L. serricorne*, *Cryptolestes pusillus* (Schönherr) (Coleoptera: Laemophloeidae), *R. dominica*, *S. oryzae* and *T. castaneum* as they can survive up to 44°C (Kirkpatrick and Tilton, 1972). Dermott and Evans (1978) and Evans (1981) mentions that insects can acclimate to heat stress and that internal feeders are hard to kill by heat. Both the cold and heat temperature limits required to kill the insect pests of museum specimens were reviewed by Strang (1992): *S. granarius* and *T.*

granarium can be killed at –18 and 54°C, *S. paniceum* can be eliminated at –18 and 49°C and *L. serricorne* can be eliminated at –12 and 49°C respectively.

Exposing the insects to sub-zero temperatures will result in reduced rate of metabolism, movement and growth and prolonged exposure leads to death (Fields, 1992). Lethal cold temperatures differ between species. This is due to the evolutionary adaptations acquired by insects living in different climatic zones of the world over thousands of years. Understanding the critical lethal cold temperature of insects in different ecological regions helps controlling their populations using physical control measures. Insects in the temperate, arctic and polar regions undergo acclimation as the fall starts and the temperature reduces gradually in winter (Ring, 1981). Acclimation is said to increase the cold tolerance by as much as eight to ten-fold in many cold-tolerant insects (Abdelghany and Fields, 2017).

Some stored-product insects that were tested for cold tolerance are *C. ferrugineus*, *O. surinamensis* (cold-hardy), *R. dominica* (moderately cold-hardy) and *T. castaneum* (cold-susceptible) (Howe, 1965b; Solomon and Adamson, 1955). Research on the relation of chill coma temperatures (mild cold temperatures at which the chill-susceptible insects are in a reversible paralytic stage) to oxygen consumption in acclimated stored grain insects shows that the relationship varies from species to species; *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and *Sitophilus* spp. had lower oxygen consumption (inverse acclimation) (Bell et al., 1977; Evans, 1977), *Ptinus tectus* Boieldieu (Coleoptera: Ptinidae) had a higher oxygen consumption and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) was not affected by acclimation (Edwards, 1958).

Controlling the insect populations using cold temperatures has many significant advantages over chemical control. This includes no chemical residues in food, highly safe for pesticide applicators, control of insecticide-resistant strains and no resurgence of insect populations (Fields, 1992). Cold temperature treatments have some disadvantages and many not always work even in colder regions. In Canada, aeration is necessary for reducing the temperature inside the bins by aerating cold air inside the storage bins. This is only possible with refrigeration equipment and in winter and more time is required to eliminate most of the insect populations (Fields, 2001).

Ice nucleating bacteria like *Pseudomonas syringae* Van Hall (Pseudomonadales: Pseudomonadaceae) were found to decrease the cold tolerance of insects which are cold-hardy, especially freeze-intolerant, under similar cold temperatures (Fields, 1990). They increase the supercooling point (the temperature at which the insect haemolymph freezes) and act as a synergist reducing the cold hardiness of stored-product insects (Strong-Gunderson et al., 1990). *Cryptolestes ferrugineus* showed increased mortality when treated with ice nucleating bacteria as freezing occurred at -9°C rather than at -17°C (Fields et al., 1990). The ice nucleating bacteria are naturally found in the insect gut and can make them vulnerable to cold temperatures even after acclimation. Hence, they can be a very effective bio-control agents when applied topically or when ingested (Lee et al., 1992; Strong-Gunderson et al., 1990).

Desiccation

Most stored-product insects are found to survive better at a range of 60-85% relative humidity (r.h.). Relative humidity affects the rate of development leading to lower survival and in some cases the insect's ability to compete for food. A study by Dawson (1967) found that the difference in r.h. can affect the developmental rate of *T. castaneum* and *T. confusum* as their performance was much better at 70% r.h. than at 40% r.h. Adult *Tribolium* spp. can be cannibalistic and feed on egg, larva and pupa when there is scarcity of food and increased population. Relative humidity changes their cannibalistic behaviour as one strain performed great at 70% r.h., while the other did well at 40% r.h. Low levels of humidity combined with modified atmosphere at 60% carbon dioxide and 9% r.h. results in 100% mortality of *Tribolium* spp. (Jay et al., 1971).

Inert dusts used in grain storage act through desiccation (Ebeling, 1971). Inert dusts are chemically inactive dry powders from different sources of origin used as diluents and carriers. They are used to control arthropod pests of field crops and stored-products and mites. Some inert dusts used are clays, sand, paddy husk, wood ash, minerals like dolomite, magnesite, lime, limestone and common salt, but most are diatomaceous earth (DE). Diatomaceous earth is a powdered form of fossilized skeletal remains of diatoms from fresh or saltwater. Diatoms are made up of opaline silica, shells of which are found in combination with trace amount of aluminum, lime and sodium. DE is food-safe and can kill insects by acting as a desiccant absorbing the cuticular waxes and causing abrasions on the exoskeleton and insects die from

desiccation. DE is very effective in storage environment due to dry storage conditions and no access to water. It can be used by itself or in combination with pyrethrins (Subramanyam and Roesli, 2000).

Some disadvantages of applying DE to grains include its tendency to change the physical properties in terms of bulk density, which in turn reduces the value of the produce. Its effectiveness decreases with increasing moisture content of the grains or relative humidity. (Subramanyam and Roesli, 2000) DE causes increased friction leading to wearing out of machinery. A study by Pinto et al. (2008) found that *C. ferrugineus* was very susceptible to DE as 100% mortality was achieved at the fourth day of exposure. Fields and Korunic (2000) found that adult *C. ferrugineus* population was reduced by 95% and larva by 80% when treated with DE at 0.15 kg/ t of grain. High susceptibility of *C. ferrugineus* to DE is also may be due to its high mobility between the grains treated, as the more DE an insect picks up there is high chance of interaction and death by desiccation (Fields et al., 2001).

Irradiation

A better alternative to using chemical insecticides on stored-food products is using gamma and infra-red radiation. Different life stages of the insect population have different tolerances to the dose of radiation. In *P. interpunctella* 0.25 kGy of gamma radiation affected the pupation and oviposition; 0.5 kGy terminated egg hatch. Pre-adults were the most tolerant stage which were not affected even by 1.0 kGy gamma radiation (Aye et al., 2008).

Irradiation combined with temperature control or fumigation shows promising control of stored-product pests. A study by Kirkpatrick et al. (1973) showed that combining different kinds of radiation (gamma, infra-red and microwaves) increased the effectiveness than using just one kind of radiation in control of *R. dominica*. Similar results was obtained in the study by Cogburn et al. (1971) using infra-red and gamma radiation to control *S. cerealella*. According to Cogburn and Speirs (1972) and Cogburn and Gillenwater (1972), using gamma radiation along with fumigation decreased pest multiplication and progeny production of malathion resistant populations of *T. castaneum*. When irradiated before malathion treatment there was better control of the susceptible strain of *T. castaneum*. Resistant strains showed increased mortality after 2-3 weeks of exposure to 10 krad than when using radiation alone (Adem and Watters,

1985). Microwaves heat the product and the insects and achieved 100% mortality of immature life stages of *T. castaneum* in rye (Vadivambal et al., 2010). Adults of *T. castaneum* and *C. ferrugineus* were killed by microwaves at 500 W and adult of *S. granarius* were killed at 400 W after 28 second exposure (Vadivambal et al., 2008).

Modified Atmospheres

Insect control using modified and controlled atmosphere are a much safer option that can be applied in closed environment compared to the use of chemicals. The principle is to manipulate the gases in the closed atmosphere to levels that are toxic to pests of any kind. The air composition is maintained at lethal levels in a controlled atmosphere and it can be done only during treatment. The method can be applied for stored grains, oil seeds, processed and packaged commodities to maintain high quality for marketing and export (Navarro et al., 2012).

Modified atmosphere or hermetic/ sealed/ airtight storage takes advantage of reduced oxygen levels in a sealed structure due to continuous respiration of living aerobic organisms producing increased levels of carbon dioxide. Hermetic storage was practiced by humans for thousands of years by storing grains under soil in tightly sealed mud pots. Oxygen levels reduce from 21% to 1% and carbon dioxide levels increase from 0.035% to 20% which kills any living insect species and stop fungal growth (Adler et al., 2000; Navarro et al., 2012).

Controlled atmosphere keeps the gas composition constant after adding the gases required to control the pests. Carbon dioxide and nitrogen replaces oxygen cutting off the oxygen required for the aerobic organisms to live. This can be done under low pressure conditions and pumping carbon dioxide at high pressure conditions. Oxygen levels under 2% results in immediate death of insects (Navarro et al., 1978). Adult insects are highly susceptible as they are the most active life stage and require higher amount of oxygen due to increased respiration rate. Increased carbon dioxide levels above 10% results in water loss by permanently opening the spiracles and results in death (Nicolas and Sillans, 1989; White et al., 1995b).

However, for carbon dioxide to have a similar efficacy compared to fumigants and nitrogen requires a long time to be effective and this can result in increased energy consumption for maintaining controlled conditions (Navarro et al., 2012). Stored-product insects like *T. castaneum* can become resistant to low oxygen concentrations or lethal carbon dioxide

concentration with repeated exposures (Donahaye, 1990). According to Rameshbabu et al. (1991), egg which is the most resistant life stage to fumigation can be controlled by combining other physical control measures with modified atmosphere and increased carbon dioxide concentration of 90% and reduced oxygen concentration of 4-0% at unfavourable temperature conditions of 10°C increased the egg mortality of *C. ferrugineus* by 20-22%.

Biological Control

The application of predators and parasitoids for controlling stored-product pests has advantages in terms of consumer health and easy removal from the treated grains by milling. The predators and parasitoids used in insect control can be other arthropods (predators and parasitoids), bacteria, virus or fungi (entomopathogens). They can be generalists feeding on unrelated species or specialists feeding on a few species. Predators and parasitoids can be classified as egg, larval, pupal or adult feeders and egg, larva or pupa parasitoids, respectively based on the life stages they attack (Flinn, 2012).

Grain feeding moths can develop resistance when exposed to *Bacillus thuringiensis* Berliner (Bt) (Bacillales: Bacillaceae), (commercially called Dipel) for a few generations (McGaughey and Beeman, 1988). Spinosad, derived from fermentation of a soil actinomycete, *Saccharopolyspora spinosa* Mertz and Yao (Actinomycetales: Pseudonocardiaceae) is very effective against *R. dominica* when applied at 0.1 ppm (Hertlein et al., 2011). It is less effective on adults of *T. castaneum* and *O. surinamensis* even at 20 ppm (Hertlein et al., 2011).

Examples of predators in stored-product pest control are; *Xylocoris flavipes* (Reuter) (Hemiptera: Lyctocoridae), *Teretriosoma nigrescens* Lewis (Coleoptera: Histeridae), *Lyctocoris campestris* (F.) (Hemiptera: Lyctocoridae), and *Calloides noblis* (Harris) (Coleoptera: Cerambycidae). Some well known parasitoids are *Habrobracon hebetor* Say (Hymenoptera: Braconidae), *Lariophagus distinguendus* (Foerster) (Hymenoptera: Pteromalidae), *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae), *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae), *Cephalomia waterstoni* Gahan (Hymenoptera: Bethyilidae), *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae), *Pteromalus cerealellae* (Ashmead) (Hymenoptera: Pteromalidae) and *Venturia canescens* (Gravenhorst) (Hymenoptera:

Ichneumonidae). When treated with *Metarhizium anisopliae* (Metschnikoff) (Hypocreales: Clavicipitacea), an entomopathogenic fungus, the adult of *R. dominica* showed high mortality (Batta, 2005).

Pheromones

Pheromones are promising agents of insect control as they help reduce the use of pesticides. This has become popular especially in stored-product insect control (of Coleopteran and Lepidopteran pests) as the confined environment provides a positive effect when used for monitoring, mass trapping and mating disruption (Trematerra, 2002). Pheromones are used to detect and monitor insect pests by incorporating them in physical traps like funnel traps and sticky traps in warehouses and flour mills (Pinniger and Chambers, 1986).

Pheromones are produced by either males or females for attracting or warning conspecifics. Aggregation pheromones are produced by one sex, usually the male, and used to attract both sexes mostly towards the source of food. *Tribolium castaneum* and *T. confusum* have a common aggregation pheromone which is used in commercial lures. The aggregation pheromone produced by the males of *R. dominica* attracts both male and females. Sex pheromones are produced by one sex, usually the female, and used to attract the opposite sex for mating (Phillips et al., 2000). This can be within the same species (intraspecific) or in between different species (interspecific) (Pinniger and Chambers, 1986). Interspecific sexual attraction can be found between the males of *P. farinalis*, and *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) as both the species have one or more similar components in the sex pheromone produced.

Synthetic pheromones are used in traps for monitoring and pest control purposes. *Tribolium* beetles produce 4,8-dimethyldecanal (DMD), a volatile pheromone with aggregation and sexual attractant properties (Ryan and O'Ceallachain, 1976). Synthetic DMD is used as a lure in traps for monitoring *Tribolium* populations in stored-product facilities (Campbell et al., 2002). The pheromone of *C. ferrugineus* has been synthesized, and tests in the field showed that more insects were caught in flight traps when the pheromone was combined with the fungal volatile, 1-octen-3-ol (Stevens et al., 2019).

Insect Growth Regulators (IGRs)

The use of insect growth hormones that govern the growth and metabolism of insects to the disadvantage of insect pests is a good substitute for chemical pesticides. This includes juvenile hormone which helps in moulting and metamorphosis, ecdysone which interferes with cuticle formation and chitin synthesis inhibitors can be harmful to all the immature stages of insects. By moderating the amount of these IGRs at specific life stages they can inhibit the basic metabolic activities of the insects (Büchi, 1994).

Juvenile hormone (JH) analogues, when used as liquid and aerosol sprays interferes with the metamorphosis leading to malformation, cutting the life cycle short and resulting in death, ecdysone manipulates cuticle formation resulting in death due to desiccation and chitin synthesis inhibitors (CSIs) inhibits the synthesis of chitin resulting in preventing immature stages from moulting into the next stage. Commercial formulations are diflubenzuron, hydroprene and methoprene. Insect Growth Regulators are used in stored-product pest control against *Tribolium* spp., *S. granarius*, *O. surinamensis*, *R. dominica* and *P. interpunctella* (Edwards et al., 1988; Nakakita et al., 1994). Methoprene (Dicaon II) applied at 1 to 5 ppm. In stored-products they are used against external feeders; *R. dominica*, *O. surinamensis* but it is not effective against internal stages and psocids (Arthur, 2003; Athanassiou et al., 2010).

Insect Cold Tolerance

Poikilothermic organisms (including insects), organisms that do not maintain a constant internal temperature, often have the ability to adapt to cold temperature extremes by overwintering (Block, 1982). Insects have the capacity to adapt to changing environmental conditions and survive until the favorable conditions return. The changes include environmental triggers like extreme heat and cold and different life stages may have different capacity to overwinter in these changing conditions. In order to overwinter, cold hardiness and diapause are the two mechanisms used for winter survival. Insects can either be freeze-tolerant or freeze-intolerant. Freeze-tolerant insects survive the formation of ice crystals while the freeze-intolerant insects cannot. Hence, freeze-intolerant insects resort to the use of cryo-protective agents in order to lower their supercooling point (SCP), and survive in a supercooled state until the favorable conditions return (Block, 1991; Duman et al., 1991). Cryoprotection is an important tool for preserving specimens with intact structural components. Research at cellular organelles

level provides an understanding of cryoprotection which a few invertebrates are capable of. RNA sequences of three strains of *Chymomyza costata* (Zetterstedt) (Diptera: Drosophilidae) were used to understand survival on cryoprotection. mRNA transcripts of warm acclimated strain during early or late diapause were compared to cold-acclimated strain undergoing diapause. Cold-acclimated strain had higher capacity to survive cryoprotection and was extremely freeze tolerant as the transcription of genes increased the capacity of proteins to fold and refold and process so that the larva is able to repair damaged proteins, thus avoiding apoptosis of cellular organelles (Des Marteaux, 2019).

Cold hardiness changes with seasonal changes and increases with gradual decrease in temperature over the fall which gives enough time for the insects to get acclimated before the arrival of freezing conditions. Selecting a suitable and safe place to hibernate and survive the winter's freezing conditions, called hibernaculum, may provide some degree of protection from the harsh environment (Baust, 1982). An example is *Aulacophora nigripennis* (Motschulsky) (Coleoptera: Chrysomelidae) which is a freeze-intolerant and overwinters under the bark, cracks and crevices of trees which protects it from low air temperatures in winter (Watanabe and Tanaka, 1998). However, the synchrony of cold hardening mechanisms can be affected when non-diapausing life stages are exposed to extreme cold. Finding the susceptible life stage can help in developing better management strategies to eliminate insect pests as all stored-product insects are freeze-intolerant.

Supercooling point (SCP), lower lethal temperatures and lethal times are some measures of cold hardiness in insects (Sinclair, 2001; Zachariassen, 1985). Freezing point and supercooling points are different mechanisms associated with cooling of an aqueous solution or water. A clear explanation is provided by Zachariassen (1985) who states in his paper that, "When a sample of water or aqueous solution is cooled, it will normally not freeze when the melting point (MP) is reached but will remain unfrozen even when cooled far below this temperature. A system that remains unfrozen at temperatures below its MP is said to be 'supercooled' and the temperature at which spontaneous freezing occurs in a supercooled system is termed the supercooling point of the system. The melting point of pure water is 0°C but the SCP may be as low as -40°C. The difference between SCP and MP of a system is termed the supercooling capacity (SCC) of the system."

Overwintering in insects is a combination of effects of different triggers or cues from the environment. These triggers can be biochemical, physiological, behavioral or ecological (Marshall and Sinclair, 2015). SCP is the temperature at which the insect body fluid freezes with decreasing temperature (Kronic, 1971). Acclimation to cold starts once the insects clear its gut contents to eliminate ice nucleators present in food. In case of overwintering in adult stage, the gut contents are naturally removed when the larva transitions into pupal stage and hence the cold hardiness is higher; cold hardiness decreases as the pupa develops into adult due to the collection of digestive waste in the gut. This was confirmed by the differences in cold hardiness between fed and unfed *Megachile rotundata* (F.) (Hymenoptera: Megachilidae) (Kronic, 1971) and in stored-product insects and mites (Andreadis and Athanassiou, 2017). Certain biochemical compounds like polyols and solutes of low molecular weight such as glycerol are produced by the insects when acclimating to occupy the gut and eliminate any ice nucleators. Salt (1957) proposed that antifreeze agents like glycerol have a significant role in the cold hardiness of overwintering insects.

The haemolymph in the insect body freezes at sub-zero temperatures (temperatures below 0°C). Those insects that survive in temperate and polar regions of the world are adapted to conditions which would kill many other insects in seconds. They are also evolved to perform regular bodily functions like metabolism, moulting and reproduction in a short warm phase and survive until the return of favorable conditions (Block, 1990). Freeze-intolerant insects avoid freeze damage by supercooling their haemolymph and removing any Ice Nucleating Agents (INAs). This in contrast to freeze-tolerant insects that tolerate freezing by producing INAs extracellularly to avoid intracellular freezing. Both freeze-tolerant and intolerant insects can become cold tolerant by the production of low molecular weight solutes and polyols like myo-inositol (Watanabe and Tanaka, 1997), glycerol, sorbitol, mannitol and trehalose (Zachariassen, 1985). Many studies found that the production of these low molecular weight solutes and glycerol in cold-hardy insects are decreased at a faster rate when the temperature is more than 20°C and vice-versa at low temperatures. Hence the insects start to accumulate polyols when the fall starts and lose them as the spring begins (Ring, 1982). Polyols were found to accumulate at a much faster rate as a response to cold-hardening compared to the response as diapause inducing agent (Lee et al., 1987). Heat shock proteins (HSPs) are found to upregulated in Coleoptera,

Lepidoptera, Diptera and Hymenoptera. HSP are proteins that are produced in response to diapause and plays a vital role in increasing the cold tolerance of insects (Rinehart et al., 2007).

Diapause and cold hardiness are important mechanisms to survive low temperatures that can be potentially used to control the insects as they are the most critical in determining the success of the insect survival. The control strategies focus on disrupting the energy utilization and trade-offs before the initiation of diapause, inducing pre-mature diapause and delaying termination of diapause and interfering with the mechanisms that ensures increased cold hardiness. A physical control strategy is the manipulation of day length which is a cue for induction of diapause and cold tolerance mechanisms. This is achieved by exposing the insect to either short light periods below its threshold/ critical photoperiod or longer light periods beyond the required day light (Saunders, 2002). This was proved by averting diapause in Lepidopterans like *Adoxophyes reticulana* (Hubner) (Lepidoptera: Tortricidae) (Ankersmit, 1968), *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (Neven, 2013) and *Ostrinia nubilalis* (Hubner) (Lepidoptera: Crambidae) (Hayes et al., 1974). In *E. cautella* and *P. interpunctella* the critical photoperiods were found to be 14 h and 13 h and induction of diapause can be manipulated by adjusting the photoperiod (Bell, 1976).

Some studies found that cold tolerance can be made possible in tropical insects which are otherwise susceptible to chilling injury below 5-6°C. This can be accomplished by adding stress-induced free amino acids like proline, which is produced in a lot of plants when subjected to drought and temperature related stresses (Verbruggen and Hermans, 2008). This is made possible in tropical fruit fly, *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae) by feeding the larva with a diet augmented with proline, which acts as a cryoprotectant and rendered the larva freeze tolerant. The treated larva survived freezing at -5°C even when half of their body water froze and upon thawing resumed development, pupated, emerged into healthy adults and reproduced. The proline concentration increased by 7.5-fold in the proline incorporated diet compared to that of the standard diet both acclimated at 15°C (Košťál et al., 2012). Studying the effect of stress-induced amino acids might help understand cold tolerance and the extent to which cold tolerance could be manipulated in stored-product insects.

Influence of Acclimation on Cold Tolerance

Physiological and metabolic changes in response to acclimation are responsible cold hardiness in plants and insects (Denlinger and Lee, 2010). These changes include increased amounts of polyhydric alcohols or polyols and sugars and decreased amount of starch or glycogen. Research found that increased “bound” water within the cellular structures which acts as polarized shells of water that do not freeze. This helps to prevent the denaturation of proteins at low temperatures (Storey et al., 1981). Cold acclimation was found to have an influence on non-diapausing insects but very slight or no effect on the diapausing insects. Khodayari et al. (2012) found that *Tetranychus urticae* Koch (Acari: Tetranychidae) diapausing females had lethal temperature LT_{50} of -20°C (50% mortality of the population) and non-diapausing females had an LT_{50} of -13°C . When acclimated at 5°C for 10 days the LT_{50} of diapausing and non-diapausing females were -22°C and -17°C respectively. Hence acclimation has a slightly positive effect on this chill-tolerant mite species.

Many studies found that the changes in the concentration of various substances in the insect body fluid changes the concentration of the essential substances required for survival like water. Water content was found to have a significant effect on cold-hardy insects as they are adapting to the decreasing temperatures of the environment. During the process water content usually decreases as it is replaced by cryoprotectants hence resulting in dehydration which can be as lethal as freezing (Salt, 1961; Somme, 1964). For example, Ring (1982) found that acclimation to cold temperatures in *Pytho deplanatus* Kirby (Coleoptera: Pythidae) resulted in retaining only 30% of total body water content. This reduced amount of water content is highly lethal for many other insects, however the presence of higher concentrations of cryoprotectants protects the insect from death by dehydration.

According to Somme (1964) and Ring (1982) when the solute concentration of cryoprotectants increases, the water content decreases and hence they have an inverse relationship. As the solute concentration increases the SCP decreases thus helping the insects avoid freezing effectively. This is not the case with freeze-tolerant insects as they are not significantly subjected to water loss as they acclimate. Dehydration in freeze-tolerant insects do not aid in cryoprotection as it does in freeze-intolerant insects. For example, *Phtho americanus* Kirby (Coleoptera: Pythidae) beetles which are freeze-tolerant were able to retain a water content

of 68% when acclimated proving that they can maintain water balance in the haemolymph even under freezing conditions (Ring, 1982).

Differences in the survival of life stages of *C. ferrugineus* when acclimated was studied by Smith (1970) where he found that adult and last instar larva had the maximum survival rate compared to pupa, young larva and egg. In a study by Fields et al. (1990), acclimation increased the lethal time, LT_{50} of *C. ferrugineus* increased from 5 d to 40 d from November to February in field in grain bins. The paper also demonstrates that different life stages have different survival rates at -10°C ; adult was more cold-hardy than egg, fourth and second instar larva.

Many stored-product pests are able to acclimate to cold temperatures. This is measured by the survival at sub-zero temperatures. When temperature goes below $13\text{--}20^{\circ}\text{C}$, development stops depending upon the species, the metabolic rate decreases, movement is slowed, biochemical changes occur and death will eventually occur. Rapid reduction in the grain temperature, rather than gradual reduction can help in eradicating the stored-product insect populations. This is especially important in insects with cold tolerance and needs to be considered while planning the management strategies. Studies suggests that reducing the temperature down to -20 to -30°C can help in eradicating the pest populations (Evans, 1980, 1987; Sømme, 1968).

Effect of Diet on Stored-grain Insect Biology

Some insects can survive toxicity due to consumption of excess compounds present in food like carbohydrates, proteins and fat which might lead to death. *Tribolium castaneum* can successfully convert excess free fatty acids in groundnuts, *Arachis hypogaea* L. (Fabales: Fabaceae) very efficiently and hence avoid the toxic effects like retarded development and mortality (Applebaum, 1969). The interaction between biochemical compounds such as secondary metabolites present in the food and enzymes in the insect gut also affects the development and suitability of that food to the insects. Knowledge on this aspect is important when raising the insects in laboratories for the purpose of performing experiments and preserving live specimens for future research. Baker (1988) found that rice weevil, *S. oryzae* develops best on barley and least on corn. The paper also proves that among the four strains of *Sitophilus* tested the strain Tanzania had the least development and survival on wheat. This was

due to the absence of amylase in the insect gut. Also, cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) showed higher mortality when fed with a diet containing amylase inhibitors. This can prove useful to manage various pests by genetic manipulation of amylase producing genes in plants (Gatehouse et al., 1987). Interspecific crossing of *Phaseolus vulgaris* (L.) (Fabales: Fabaceae) with *Phaseolus lunatus* (L.) (Fabales: Fabaceae) and *Phaseolus acutifolius* (A. Grey) (Fabales: Fabaceae) containing LLP, lectin-like proteins which shows resistance to certain bruchids, *A. obtectus* and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae) helps transfer the resistant genes to *P. vulgaris* (Dobie et al., 1990).

Some compounds present in certain plants, especially seeds of leguminous plants can be toxic to mammals when in a raw state. But cooking them completely changes this toxic property and makes them edible. For example, in lentils and French bean there is a heat-labile growth inhibitor that converts into digestible form of carbohydrate when boiled. A study on the suitability of lentils and French bean to *T. granarium* by Bhattacharya and Pant (1969b), shows that these pulses are not favorable when fed raw. *Phaseolus vulgaris* was used to test its suitability on *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae), *C. maculatus* and *R. dominica* and all these studies proves that it was not a suitable diet to these insects due to the presence of growth inhibitors and pentosans (Bhattacharya and Pant, 1969b).

The successful development of *P. farinalis*, depends on the presence of fungi, yeast and bacteria in flours of wheat and corn, hay and even on an artificial diet (Arbogast and Van Byrd, 1981). Addition of yeast was found to promote feeding and development of stored-product insects especially in secondary pests like *E. cautella* which showed promising development on all 39 varieties of soybean tested, in the presence of yeast and a flour diet. This was not the case in the absence of yeast as the insect failed to develop on 13 of the 39 varieties.

Pulses were found to be deficient in nutrients required by the insects to develop and may also contain non-degradable carbohydrates which cannot be digested (Bhattacharya and Pant, 1969a). The form in which the grains are available has a predominant role in the development and establishment of the stored-product pests. According to LeCato (1976), increased weight, higher progeny production and faster development were observed in both *E. cautella* and *P. interpunctella* when reared on cracked and ground foods. Whole cereals and pulses were not

favorable because of the intact seed coat making it hard or impossible for the insect larva to feed on (Locatelli and Limonta, 1998).

Moisture content of the diet is also an important factor that determines the efficiency of feeding especially by the larva. The moisture content of the diet is in turn influenced by the relative humidity of the atmosphere. The hygroscopic nature of various dried food materials, especially cereal products helps in maintaining an equilibrium with the atmosphere (Pixton, 1967). The importance of moisture in wheat germ for the growth of *P. interpunctella* larva was studied by Silhacek and Murphy (2008) using glycerol as a humectant to increase water retention by wheat germ. As a result, larval growth increased by 10 mg at 28% moisture content, as 20% glycerol increased water retention by 50% at any atmospheric humidity.

Stored-product pests may prefer selective parts of the produce for feeding due to the high nutritional content of those parts compared to others. For example, germ part of the seeds is rich in proteins compared to that of husk, bran and cotyledons. *Tribolium castaneum* prefers the germ portion of groundnut, *Cryptolestes* spp. prefers germ portion of wheat. At the same time, they cannot feed on whole unbroken grains as they are secondary pests and prefer broken grains or halves. Stored-product moths prefer the germ and bran of the grains and hence the diet they feed on should contain either of parts of the grain. This is made available more freely when the grains are damaged and/ or processed into flour and by-products. Some insects like *T. confusum* tend to feed selectively on preferred parts of the grain. Germ, the most nutritious compared to bran and endosperm has higher protein content of 25% and total sugars of 20%; higher digestibility resulting in better growth. Bran has a protein content of approximately 10 to 12% and total sugars of 6% with very low digestible carbohydrates. Endosperm mostly consists of more than 70% starch and 8 to 13% proteins making it the least nutritious part of the grain. Due to these differences in nutritional quality of different parts of the grains the insects tend to feed selectively on germ and endosperm around the germ in cracked and damaged grains. However, this is not possible when the grain is processed into flour by grinding. Hence, *T. confusum* shows reduced growth and weight gain (Bhattacharya and Waldbauer, 1969, 1972; Waldbauer and Bhattacharya, 1973).

The quality of the diet is an important factor that affects the pheromone production in *Tribolium* spp. Ming and Lewis (2010) found that the pheromone produced by males of *Tribolium* spp., DMD increased by three-fold when fed a highly nutritious diet. The study also found that diet quality only influenced the rate of production of DMD and had no effect on the attractant ability of the pheromones to the females. Nutritional quality of the diet affects the oviposition preferences as adult females tend to lay eggs on a nutritious substrate. This was established by Xue et al. (2010) testing *T. castaneum* adult females on different diets at, where the females laid eggs only when on flour. Starchy diets with least nutritional quality were least preferred and did not support egg laying. The same study found that the larva reared on starchy diets did not develop beyond second instars, while flour and brewer's yeast diet led to healthy individuals with higher survival at constant conditions of 28°C and 65% r.h.

The health and survival of the succeeding generations depends not only on the abiotic factors like temperature and humidity but also on the initial population density. When the quantity of the food available is the same which is the case in stored-product environments, higher the number of individuals depending on the limited food available higher is the competition resulting in reduced feeding, weight gain and survival of immature stages. Cannibalism is also a factor to be considered as the competition for food and space leads to the larvae killing and feeding on each other to ensure survival. This is true for a lot of stored-product insect pests such as *T. castaneum* in which increased population densities lead to reduced body weight of the resulting generation; highest weight of 1.7 mg in control and 1.6 mg in wheat bran diet at lowest densities (4 adults/g) and lowest weight of 1.0 mg in the least favored poultry diet at highest densities (12 adults/g) (Assie et al., 2007; Đukić et al., 2016).

There have been no studies on the effect of diet on cold tolerance and acclimation in literature. Understanding the effect diet could have on the insect cold tolerance can help formulate new strategies for managing the stored-product insects.

Biology of *Cryptolestes*

The flat grain beetles (Laemophloeidae) are important pests in stored-products and they cause significant economic damages to food like grains, peanuts and cocoa in storage (Bishop, 1960; Halstead, 1993; Thomas, 1984; Toon et al., 2016). The three species of flat grain beetles:

Cryptolestes ferrugineus (Stephens), *Cryptolestes turcicus* (Grouvelle) and *Cryptolestes pusillus* (Schonherr) are commonly found in stored-products, and there are a few other less common species; *Cryptolestes capensis* (Waltl), *Cryptolestes klapperichii* (Horn), *Cryptolestes pusilloides* (Steel and Howe) and *Cryptolestes ugandae* Steel and Howe (Sinha and Watters, 1985).

Origin: Palearctic

Systematic position:

Kingdom: Animalia

Sub-kingdom: Bilateria

Infrakingdom: Protostomia

Superphylum: Ecdysozoa

Phylum: Arthropoda

Sub-phylum: Hexapoda

Class: Insecta

Sub-class: Pterygota

Infraclass: Neoptera

Superorder: Holometabola

Order: Coleoptera

Sub-order: Polyphaga

Infraorder: Cucujiformia

Superfamily: Cucujoidea

Family: Laemophloeidae

Genus: *Cryptolestes*

Species: *capensis*, *ferrugineus*, *klapperichii*, *pusilloides*, *pusillus*, *turcicus*, *ugandae*

Flat grain beetles/ flat bark beetles/ bran bugs belong to the family Laemophloeidae consists of approximately 450 species of beetles. Those that are found outdoors live under loose tree barks, hence the name flat bark beetles. They are mostly scavengers feeding on dead insect remains and plant debris but are also predators of insects.

The adults are small, 2-3 mm long, flat, elongate, pale reddish brown in color; filiform antenna which is longer in males. The head and thorax are conspicuous and not proportional in size (Table 1.1 and 1.2). Adult females start mating as soon as they emerge from the pupa and can lay 200-500 eggs. The females lay loose eggs on seeds. Adults cannot climb glass or porcelain surfaces. They undergo complete metamorphosis; there is an egg, four larval instars, pupa and adult stages. Eggs are elongate, 0.68-0.81 mm long and 0.20-0.30 mm wide. Larva is campodeiform type and are thin 3 mm upon maturing, translucent, pale white or yellow with a dark head capsule; 2 urogomphi, a dark tail-like projections that are present at the last body segment. Pupa is covered in a thin cocoon inside the grains. Temperature range of 25-32°C, relative humidity of 60-70% and minimum grain moisture content of 12% is favorable for development. Adults are long lived and can survive for four months to more than a year when conditions are favorable. Hence, they can produce more than one generation/year (Hill, 1990; Sinha and Watters, 1985) (Table 1.1, 1.2 and 1.3).

They are distributed worldwide and are most common in warmer tropics although there are a few exceptions. They are an introduced pest in Canada. They are polyphagous pests feeding on whole grains, wheat, maize, sorghum, cassava, groundnuts, flours and dried fruits, nuts and oilcake and molds. They are primary pests or grain feeders. The larva cannot penetrate intact grains. They are capable of feeding and establishing on even the slightly damaged grains. They prefer feeding on the germ portion of grains, hence destroys the grain quality and capacity to germinate. Adult males produce macrolide aggregation pheromone, (z)-3-dodecen-11-olide 4,8-dimethyl-(E,E)-4,8-decadienolide (Pinniger and Chambers, 1986) that attracts both males and females. It was found that methoprene, a juvenile hormone (JH) analogue enhanced the production of aggregation pheromone in *C. ferrugineus* and three other beetles (Pierce et al.,

1986). Sometimes aggregation pheromones are found to have a synergistic relationship. When present together the aggregation pheromones produced by the cucujid beetles *C. ferrugineus*, *C. turcicus* and *C. pusillus* and *Oryzaephilus mercator* (F.) (Coleoptera: Silvanidae) and *O. surinamensis* have an increased attraction of individuals of both the genus (Oehlschlager et al., 1988) (Table 1.4).

Currie (1967) did a comparative study on the relationship between temperature and r.h. (relative humidity) and development, mortality and oviposition of *C. ferrugineus* and *C. pusillus* where he found that the rapid development and rate of increase in the population of *C. pusillus* occurred at 35°C and 90% r.h.; highest oviposition rate and lowest mortality was observed at 32.5°C. *Cryptolestes pusillus* (Currie, 1967) and *C. turcicus* (Lefkovitch, 1962) require the same developmental time at 25°C and below and 90% r.h on finely sieved and unsieved wheat feed. *Cryptolestes pusillus* and *C. ferrugineus* takes approximately the same time to develop around 25°C on flour and wheat germ diet. Lower humidities were found to affect the formation of pupal case in many species of *Cryptolestes*. *Cryptolestes ferrugineus* can develop very well at 40% r.h. unlike *C. pusillus* and *C. turcicus* which can barely survive 50% r.h (Lefkovitch, 1965). According to Currie (1967) egg laying has a pattern where there is maximum oviposition during certain parts of the female's lifetime. In a 12-week period into the female's life cycle, *C. ferrugineus*, *C. turcicus* and *C. pusillus* peaked at oviposition during the 11th, 5-7th and 8-12th weeks, where the maximum number of eggs laid were 350, 100 and 300 eggs per female respectively; the minimum threshold temperature required for egg hatch is 20°C for *C. ferrugineus*, 15°C for *C. turcicus* and 17.5°C for *C. pusillus*.

Reasons to Study *Cryptolestes* Cold Tolerance

Although there have been several studies on the cold tolerance of *C. ferrugineus* not much data is available on the cold tolerance of *C. turcicus* and *C. pusillus* (Table 1.5), which are important pests in Canadian flour mills and grain bins (Sinha and Watters, 1985). Some studies found the egg, larva, pupa and adult stages of *C. ferrugineus* to be moderately cold-tolerant (Evans, 1983; Mathlein, 1961) to highly cold-tolerant (David et al., 1977). *Cryptolestes ferrugineus* adult survived low temperatures throughout the winter in grain bins. The adult survived at -10°C for 24 days (LT₅₀) (Fields et al., 1998) and 40 days (LT₅₀) (Fields, 1990) when acclimated at various temperature regimes; 60% survival was observed at 28 days exposure after

acclimation and cold stress at -9°C (Kawamoto et al., 1990); 56% adult survived 7 days exposure after cold stress at 0°C with no acclimation and 95% adult survived 7 days exposure to 0°C with acclimation (Kahn, 1990); adult had an LT_{50} of 0.6 days when not acclimated and 28 days when acclimated and exposed to at -12°C ; larva and pupa had LT_{50} of 7 days at -5°C (Barker, 1990); egg had LT_{50} of 0.5 days -12°C when not acclimated (Smith, 1970). A study by Burks and Hagstrum (1999) found that *C. ferrugineus* adult had the most tolerance to rapid cold hardening compared to four other species of stored-product beetles. The LT_{50} recorded at -14°C was 157 min for non-acclimated conditions and 1370 min for acclimated conditions. Ice-nucleating bacteria, *P. syringae* was found to increase the mortality of *C. ferrugineus*. When exposed to low temperature at -10°C , 1000 ppm of the bacteria induced freezing at -9°C in 24 hours, compared to -17°C in insects not treated with the bacteria (Fields et al., 1990).

The egg, larva, pupa and adult stages of *C. pusillus* are cold-susceptible (David et al., 1977). *Cryptolestes pusillus* adult, pupa, old larva, younger larva and egg had survival of 26, 61, 83, 98 and 67% (respectively) at 2°C after acclimating at 25°C (Williams, 1954a) (Table 1.5). In another study comparing the low temperature survival of *C. ferrugineus* and *C. pusillus*, *C. ferrugineus* survived temperatures reduced from 25°C to 0°C for up to 40 weeks and stopped egg laying at 16°C ; *C. pusillus* survived less than 24 weeks at a much higher temperature of 11°C and stopped egg laying at 14°C (Fields and White, 1997). There has been very little work on the cold tolerance of *C. turcicus*. The larva and adult stages were considered to be moderately cold-tolerant (Mansbridge, 1936).

This thesis focusses on effects of developmental stage, cold acclimation and diet on the cold tolerance of three species of *Cryptolestes*. A comparative study on these three important species of *Cryptolestes* could help understand and develop control measures using cold temperatures in the grain bins. Also, there is a disagreement in the literature as to what stage is the most cold-tolerant stage for *C. ferrugineus*. Smith (1970), found that non-acclimated *C. ferrugineus*, adult and old larva were more cold-hardy than pupa, young larva and egg. In the study by Fields (1992) adult was found to be the most cold-hardy life stage. However, in a study with large grain bulks of 300 kg, immature stages were shown to be more cold-tolerant than adult (Abdelghany and Fields (2017). Fields (1990) found that adult *C. ferrugineus* were more cold-hardy and required 75 days to reach 95% mortality.

Objectives

The main objective is to determine the survival of three species of *Cryptolestes* (*C. ferrugineus*, *C. turcicus* and *C. pusillus*) at low temperatures. I have divided that general objective into three sub-objectives:

1. To determine which life stage (egg, young larva, old larva, pupa or adult) of *C. ferrugineus*, *C. turcicus* and *C. pusillus* is the most cold-hardy under acclimated and non-acclimated conditions.
2. To determine if diet affects the cold tolerance of *C. ferrugineus*, *C. turcicus* and *C. pusillus* adults using whole wheat-wheat germ-cracked wheat grain (90:5:5), wheat flour-brewer's yeast (95:5) and straight wheat flour.
3. To determine the SCP of egg, young larvae, old larvae, pupae or adults of *C. ferrugineus*, *C. turcicus* and *C. pusillus* under acclimated and non-acclimated conditions; adults of *C. ferrugineus*, *C. turcicus* and *C. pusillus* on different diets under acclimated and non-acclimated conditions.

Table 1.1. Morphological differences between *Cryptolestes* species (Arbogast, 1991; Bousquet, 1990).

Attribute	<i>C. ferrugineus</i>	<i>C. turcicus</i>	<i>C. pusillus</i>
Antennae	Male and female similar	Male much longer than female	Male much longer than female
Male mandible	External tooth	No tooth	No tooth
Head	Dorsal carina not prolonged transversally near dorsal posterior margin	Dorsal carina prolonged transversally ridge near dorsal posterior margin	Dorsal carina prolonged transversally ridge near dorsal posterior margin
Pronotum	Narrowed posteriorly (especially males)	Nearly quadrate	Transverse, slightly narrowed posteriorly in males
Setae in between rows on elytra	Four rows of setae elytral striae	Three rows between second elytral interval	Four rows between second elytral interval
Sclerotization of abdominal tergites	Dark bands, sclerotization	-	Uniformly colored, no sclerotization

Table 1.2. Morphological differences between *Cryptolestes* species (Arbogast, 1991; Bousquet, 1990).





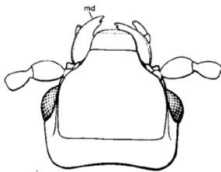
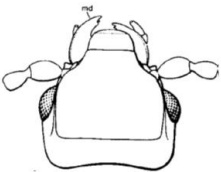

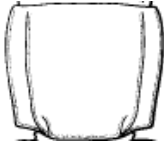

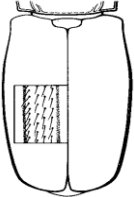
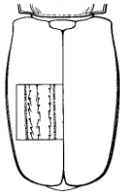
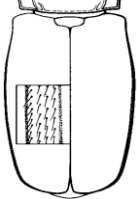


Attribute	<i>C. ferrugineus</i>	<i>C. turcicus</i>	<i>C. pusillus</i>
Antennae			
Head and male mandible			
Pronotum			
Setae in between rows on elytra			
Sclerotization of abdominal tergites			

Table 1.3. Morphological and biological differences between the three *Cryptolestes* spp. (Adapted in part from Sinha and Watters (1985) and Currie (1967)).

Attribute	<i>C. ferrugineus</i>	<i>C. turcicus</i>	<i>C. pusillus</i>
Preferred food	Whole wheat grains	Wheat flour, sometimes grains	Wheat flour
Size (mm)	1.6-4	1.5-2.3	1.5-2
Body length (mm)	2	2	1.75
Dry body mass (mg)	0.28	0.28	0.21
Developmental temperatures (°C)	20-40	18-38	18-38
Developmental humidity (% r.h.)	40-95	45-100	45-100
Developmental period (d)	21	30	22-43
Adult longevity (d)	180-270	180-270	80-600
Fecundity (Eggs/female)	350-400	300-500	150-240
Maximum oviposition rate:			
Temperature (°C)	35	32.5, 27.5	32.5
Humidity (% r.h.)	90	90	90
Rate of increase (fold/4 weeks)	60	50	10
Rate of increase:			
Temperature (°C)	35	28	35
Humidity (% r.h.)	90	90	90
Time to double in number (days)	5	11	7
Cold hardiness	Cold-hardy	Fairly cold-hardy	Not cold-hardy
Climate plasticity index, I _p	570	475	100
Prothorax	Narrow at end	Square	Square

Table 1.4. Comparative biological response characteristics of three species of *Cryptolestes* spp. in literature.

Species	Life stage	Treatment duration (weeks)	Treatment temperatures (°C)	r.h. (%)	Experiment	Mortality (%)	Population (Mean)	Reference
Diet								
<i>C. ferrugineus</i>	Adult	6 weeks	30	70	Diet-Barley + wheat germ (4.5% w/w) + torula yeast (0.5% w/w)		608	Jagadeesan et al. (2013)
<i>C. ferrugineus</i>	Adult	6 weeks	30	70	Barley flour wheat germ (4.5% w/w) + torula yeast (0.5% w/w)	0.26		
<i>C. pusillus</i>	Adult	6 weeks	30	70	Rolled oats + cracked sorghum, wheat flour + Barley flour, Barley flour	2.54	876.1	Jagadeesan et al. (2013)
<i>C. ferrugineus</i>	Adult	11 weeks	30	70	Wheat		1150	White et al. (1995a)
			35	70	Wheat	97		
<i>C. pusillus</i>			20	70	Wheat	6		
<i>C. pusillus</i>	Adult		30	75	Oats cv. Paul		No. of progeny 10.3 ± 1.20	Throne et al. (2003)
			30	75	Oats cv. Milton		0.00 ± 0.00	

Pheromones						
Species	Life stage	Pheromone combinations/ response	Pheromones	No. of beetles collected in Mixture traps	Control	Reference
<i>C. pusillus</i>	Starved Adult	Stimulus-I-81% response Stimulus-II-46% response Stimulus I + II + beetle volatiles- 92% response	(Z)-3- dodecadienolide (I), (Z)-5- tetradecen-13- olide (II) Synergistic to other cucujolides			Millar et al. (1984b)
<i>C. turcicus</i>	Starved Adult	Stimulus I + II-response (Mean+ SE) = 8.5 ± 1.5 Frass volatiles-31.5 gh amount of stimulus = 10.5 ± 0.6 experimental stimulus	(Z,Z)-5,8- tetradecadien-13- olide (I), (Z)-5- tetradecen-13- olide (II) Synergistic enantiomers			Millar et al. (1984a)
<i>C. ferrugineus</i>	Adult in bin	Mixture I: 9:1 of Ferrulactone I & Ferrulactone II Mixture II: 3:4 of Ferrulactone I & Ferrulactone II	Ferrulactone I: 4,8-dimethyl- E,E-4,8- decadienolide & Ferrulactone II: racemic (Z)—3- dodecen-11-olide	2300	1308	Loschiavo et al. (1986)

Insecticide resistance						
Species	Life stage	Treatment duration	Treatment temperatures (°C)	Phosphine treatment (mg/L)	% mortality	Reference
<i>C. ferrugineus</i>	Adult	48 h		2	100	Nayak et al. (2013)
		30 d		0.5	100	Nayak et al. (2010)
		24 d		1	100	Nayak et al. (2010)
<i>C. pusillus</i>	Adult	4 mon	30	1		Tay et al. (2016) Pascual-Villalobos (2006)

Table 1.5. Cold tolerance in literature adapted from Fields (1992).

Species	Life stage	Treatment		Results							References		
		Accl. (°C)	Duration (d)	Non-accl. /cold stress (°C)	Duration (d)	% survival (Duration of exposure, d)						LT ₅₀ (d)	
<i>C. ferrugineus</i>	Adult	5, 10, 15	7 wk	-10	1-63						24	Fields et al. (1998)	
				-10	1-63						1.4		
		20 to 15	Oct.-Nov	-10		50 (5)	5 (10)				40	Fields (1990)	
		20 to 15	Oct.-Nov	-10		50 (6)	5 (11)						
		20 to 15	Oct.-Nov	-10		50 (5)	5 (11)						
		20 to -2	Oct./Feb	-10		50 (40)	5 (73)						
		20 to -2	Oct./Feb	-10		50 (30)	5 (76)						
20 to -2	Oct./Feb	-10		50 (24)	5 (38)								
<i>C. ferrugineus</i>	Adult			15		100 (7)	100 (35)	100 (70)	99 (175)	97 (224)		Kawamoto et al. (1990)	
				10		100 (7)	99 (35)	98 (70)	97 (105)	95 (140)	91 (175)		87 (224)
		30, 15	21	0		100 (7)	100 (14)	100 (21)	98 (28)	94 (42)	83 (49)		56 (77)
		30, 15	21	-9		100 (7)	94 (14)	71 (21)	60 (28)	34 (42)	13 (49)		
<i>C. ferrugineus</i>	Adult	30		15		100 (7)	99 (14)	99 (21)	99 (28)	99 (35)	99 (42)	Kahn (1990)	

		30		10		67 (7)	28 (14)	13 (21)	10 (28)	6 (35)	4 (42)	
		30		5		84 (7)	59 (14)	28 (21)	16 (28)	7 (35)	5 (42)	
		30		0		56 (7)	20 (14)	16 (21)	8 (28)	1 (35)	1 (42)	
		30, 20, 7	7	0		95 (7)	94 (14)	91 (21)	50 (28)	38 (35)	31 (42)	28 (49)
		30 to 5	42	0		94 (7)	91 (14)	86 (21)	85 (28)	84 (35)	79 (42)	44 (49)
		30, 15	7, 7	0		96 (7)	95 (14)	94 (21)	53 (28)	44 (35)		
		30, 15	7, 14	0		90 (7)	90 (14)	86 (21)	74 (28)	0 (35)		
		30, 15	7, 21	0		94 (7)	94 (14)	92 (21)	54 (28)	0 (35)		
<i>C. ferrugineus</i>	Larva, pupa	30		15		50 (18)	1 (245)					
				10		50 (12)	1 (335)					
				5		50 (12)	1 (43)					(Barker, 1990)
				0		50 (10)	1 (30)					
				-5		5 (7)	0 (14)					
<i>C. ferrugineus</i>	Adult	32		9		50 (7)	1 (33)					
				9		50 (11)	1 (24)					(Evans, 1983)
		32 to 13.5	28	13.5		50 (265)	1 (> 364)					
				13.5		50 (144)	1 (294)					

	32 to 9	35	9	50 (75)	1 (162)														
			9	50 (52)	1 (106)														
<i>C. ferrugineus</i>	Adult	30	2	50 (15.3)															
			-6	50 (3.5)															
			-12	50 (0.6)															
		30	-6	52 (3)	21 (5)	3 (9)													
		30, 15	1	-6	98 (24)	24 (50)													
		30, 15	3	-6	100 (3)	25 (5)													
		30, 15	7	-6	99 (3)	83 (5)	46 (9)	35 (14)											
		30, 15	14	-6	98 (3)	98 (5)	98 (9)	91 (14)	79 (21)	56 (28)	13 (35)							Smith (1970)	
		30, 15	21	-6	100 (3)														
		30	-12	0 (3)	0 (5)	0 (9)													
		30, 15	17	-12		33 (5)	31 (9)	19 (14)											
		30, 15	14	-12		80 (5)	41 (9)	41 (14)											
		30, 15	20	-12				76 (14)	77 (22)	51 (28)									
		30, 15	28	-12				75 (14)	74 (22)	61 (28)									
	Pupa	30	2	50 (8.8)															
			-6	50 (1.4)															

				-12	50 (0.3)				
	Larva (10- 17d)	30		2	50 (8.6)				
				-6	50 (2.7)				
				-12	50 (0.8)				
	Larva (1-2d)	30		2	50 (5)				
				-6	50 (1.4)				
				-12	50 (0.1)				
	Egg	30		2	50 (5.3)				
				-6	50 (0.6)				
				-12	50 (0.5)				
<i>C. ferrugineus</i>	Adult	Room	6-7	0	3 (45)	0 (60)			
				-2	27 (25)				Mathlein (1961)
				-4	26 (20)				
				-5	1 (28)				
<i>C. pusillus</i>	Adult	25		2	62 (1)	26 (2)	26 (2.5)	15 (3)	7 (4)
	Pupa	25		2			61 (2.5)		Williams (1954a)
	Larva 4 th I	25		2			83 (2.5)		

Larva 3 rd I	25	2			80 (2.5)			
Larva 2 nd I	25	2			93 (2.5)			
Larva 1 st I	25	2			98 (2.5)			
Egg	25	2	95 (1)	80 (2)	67 (2.5)	39 (3)	13 (4)	

Chapter 2: Materials and Methods

Effects of Developmental Stage, Species and Acclimation on Cold Tolerance

Insects

The adult beetles of *C. ferrugineus*, *C. turcicus* and *C. pusillus* were obtained from the cultures maintained by Agriculture and Agri-Food Canada, Morden Research and Development Centre, Manitoba at the Canadian Wheat Board Centre for Grain Storage Research, University of Manitoba, Winnipeg. *Cryptolestes ferrugineus* had been collected from St. Agathe, at a family farm (Sorin) in Southern Manitoba on 28 October 2013, *C. pusillus* had been collected at an elevator in Montreal from maize imported from India on 6 March 2013 and *C. turcicus* origin is unknown and the lab strain culture was started on 1 January 1990. New cultures for obtaining various developmental stages of the beetles were started by sifting out the adults from stock cultures using an insect shaker (Eberbach Corp., Model 6000 Mid-range reciprocal shaker, St. Belleville, Michigan, USA) and allowing them to oviposit on diet. Approximately 500 adults, approximately 2-weeks old, were introduced onto the medium in glass jars. The flour medium was used to facilitate the easy extraction of the immature developmental stages by sifting (Smith, 1970). Adults were held for 1-3 days, then sifted off of diet to obtain a cohort of eggs 1-3 d old. Insects were held at $30 \pm 1^{\circ}\text{C}$ and $65 \pm 10\%$ r.h. in controlled environmental chambers (Convion, Model No. E8, Winnipeg, Canada) unless stated otherwise.

The glassware used for rearing the various developmental stages were glass jars of various capacities: 4 L and 2.5 L jars. The lids of these jars were prepared for use by sealing with No. 1 Whatman filter paper over the perforated plate of the lid to provide gas exchange, prevent the insect from escaping and to inhibit any mites from contaminating the cultures.

Unbleached white wheat flour and brewer's yeast mixture (95:5) was used as a culture medium. The diet was changed every 3-4 weeks to avoid the growth of mould that can affect insect cultures. The diet stored at 5°C in a walk-in controlled environmental chamber (Convion, Model C1010, Winnipeg) was poured into 4 L glass jars at 2 kg per jar. It was kept at room temperature for at least 24 h before introducing the insects on to diet.

Obtaining Different Developmental Stages

Eggs were obtained by introducing 100 unsexed 0-14 day old adults from the stock cultures onto a fresh white flour (without brewer's yeast) pre-sieved through a sieve with 180 μm openings (#80 mesh) and allowing them to oviposit for 24 h. Eggs were extracted by sifting the medium through a sieve with 250 μm openings (#60 mesh) (Smith, 1970). A pre-test was done to test the hatchability of the eggs obtained by sieving. Approximately 100 eggs were transferred to a Petri dish and held for 5 days to observe the number of eggs hatched. Emergence from eggs was 85-90%.

The eggs collected were counted and carefully transferred on to 20 g of wheat flour and brewer's yeast (95:5) at 50 eggs/vial. Glass vials (37 mL) had perforated lids. The vials were held for different durations to allow insects to develop to young larva, old larva or pupa. After eggs were placed in vials, *C. ferrugineus* and *C. turcicus* were held 3-5 days to obtain young larva, 13-16 days to obtain old larva and 21-24 days to obtain pupa by sifting flour using sieves of 250 μm openings (#60 mesh), 300 μm openings (#50 mesh) and 500 μm (#35 mesh) size, respectively. *Cryptolestes pusillus* takes longer to develop, therefore, 5-6 days were used for young larva, 18-20 for old larva and 25-28 days to obtain pupa by sifting flour using sieves of 250 μm openings (#60 mesh), 300 μm openings (#50 mesh) and 500 μm (#35 mesh) size, respectively.

The adults were obtained by sieving the adults from the stock cultures by sifting them through a sieve of size 600 μm (# 30 mesh). They were introduced at 600-800 adults per jar (4 L) onto 1.5 kg medium of wheat flour and brewer's yeast (95:5). The adult *C. ferrugineus* and *C. turcicus* emerged from the pupa after approximately 28-30 days and those of *C. pusillus* emerged from the pupa after approximately 35-40 days after starting the cultures. They were then sifted out through a sieve of size 600 μm (# 30 mesh) and the adults that turned completely dark (at least 24 h) in colour were used for the experiments.

Non-acclimated and Acclimated Insects

All the developmental stages of *C. ferrugineus*, *C. turcicus* and *C. pusillus* were tested to determine the effect of acclimation on cold tolerance. All the developmental stages were exposed

to a stepped reduction in temperatures over three weeks. The temperatures used for acclimation were below 20°C as it is the lower threshold for the development of *Cryptolestes* (Smith, 1965). All the life stages of the insects were held successively at 18, 10 and 5°C for 1 week/temperature. This range was chosen to simulate cooling of grain in farm bins in Western Canada. For non-acclimation experiments, vials with various the developmental stages were taken from 30°C directly before being placed at -10°C for various durations (Fields and White, 1997; Smith, 1970). The treatment durations were adjusted to better estimate the LT₅₀ and LT₉₅ values as some life stages did not survive long enough to attain LT₅₀ or LT₉₅ (Appendix 1).

Mortality at Low Temperature

To test survival at low temperatures insects were exposed to $-10 \pm 1^\circ\text{C}$ in a chest freezer (ScienTemp Corp., 34-25A, Adrian, Michigan, USA). Vials were placed in polystyrene foam boxes to reduce variation in temperature. There were 50 insects/vial, 3 vials/duration and 5-6 durations for each treatment. There were 3 different experiments: 1, 2 and 3 started at different times. Experiment 1 was started between August and September 2018, experiment 2 was started between March and June 2019 and experiment 3 was started between July and August 2019. The durations at -10°C were adjusted to better estimate the full range of mortality. The final set of treatment durations for non-acclimated conditions were from 0 to 168 hours and acclimated conditions from 0 to 840 hours (Appendices 1 and 2).

After the insects were removed from -10°C, immature stages were held at $30 \pm 1^\circ\text{C}$ and 60-70% r.h. until they emerged as adults. Emergence to adult stage was the criteria to have survived cold exposure. Adult mortality was assessed within 24 h of taking them out -10°C.

Effects of Diet on Adult Cold Tolerance

The adults were obtained by rearing the adults of *C. ferrugineus*, *C. turcicus* and *C. pusillus* from the stock cultures on all three media under investigation, grain diet consisting of whole wheat kernels, cracked wheat kernels and wheat germ (90:5:5, proportions); wheat flour and brewer's yeast (95:5, proportions) and straight wheat flour. *Cryptolestes ferrugineus* is found mostly on wheat grains, *C. turcicus* is found to survive on both wheat grains and flour and *C. pusillus* is mostly found on wheat flour in flour mills (Sinha and Watters, 1985). I used wheat

flour and brewer's yeast for testing the cold tolerance of the different stages as it allow for easy extraction of the different life stages. All the three media were tested for the effect of acclimation, exposure to low temperatures and SCP with adults (Appendix 2).

Adults of the each species was reared on three different diets. One hundred adults of *C. ferrugineus*, *C. turcicus* and *C. pusillus* were counted and weighed (for measuring any difference in weight caused by different diets with different nutritional status) on a balance (Mettler Toledo, Model ME204TE/00, analytical balance, Langacher, Switzerland) with 3 different sets of 100 adults.

Supercooling Points

Supercooling point (SCP) is one measure of cold hardiness. It is the point at temperatures below freezing at which there is a sudden, but a brief rise in insect body temperature due to the latent heat of crystallization or freezing. The temperature at which SCP or freezing occurs is the lethal temperature for *Cryptolestes* as they are freeze intolerant (Carrillo and Cannon, 2005; Zachariassen, 1985).

The SCPs were determined by keeping the developmental stages, egg, young larva, old larva, pupa and adult of *C. ferrugineus*, *C. turcicus* and *C. pusillus* in contact with the thermocouple wires (Smith, 1970) in a freezer at -44°C (ScienTemp, Adrian, Michigan, USA). The thermocouple unit consists of 20 thermocouples connected to HOBO data loggers (Onset UX120-014M, HOBO 4-channel thermocouple logger, Bourne, Massachusetts, USA) which were set to read the temperature every second (Figs. 2.1 and 2.2). There were 16 to 69 individuals for each treatment. They were done in batches of no more than 20 and done all on the same day or on different days depending upon availability of the different stages. Due to lack of insects, it was necessary to do these experiments with two cohorts of insects.

The developmental stages were tested for measuring SCP as soon as they were taken out of 30°C with minimum exposure/ adaptation to room temperature. Petroleum gel (Vaseline) was used to stick certain immature stages such as egg, young larva and pupa to the thermocouple wires as it helped maintain contact (Figure 2.2). The thermocouple unit was connected to data loggers and put inside the deep freezer at -44°C for 1.5 h. The rate of temperature decline from 0

to -10°C was approximately $0.6^{\circ}\text{C}/\text{min}$. As the temperature reduced inside the unit from room temperature to -38°C , the insect body temperature kept reducing until it reached the SCP, when the latent heat of crystallization was released as the haemolymph freezes (Zachariassen, 1985).

Statistical Analysis

Probit Analysis

The mortality analysis was done using probit analysis (Polo Plus 2.0, LeOra Software LLC, Parma, Missouri, USA). Mortality data were transformed using the probit scale, time was transformed using the log scale and the lethal times to 50 and 95% mortality (LT_{50} and LT_{95}) were estimated and the 95% confidence intervals noted. Probit analysis transforms the usual sigmoidal curve (mortality vs duration) to a straight line and estimates the slope and intercept with standard errors and runs a chi-square goodness of fit test between the estimated straight line and the data points. High chi-square value indicates a poor fit of the data to the model. In general chi-square value of 3.884 or higher is considered statistically significant. Values much higher like more than 60 is considered a result of an error or the data not agreeing with the assumptions. Although some chi-square values are higher in my results the higher number of replications and repetition of the experiments three times is considered an asset. This is because increased number of replications strengthens the assumptions when the same conclusion is obtained even with different values achieved. In addition a statistic called the heterogeneity factor (h) is estimated and h greater than 1 indicates a poor fit of the data to the model (Robertson et al., 2017). Finally, poor fit of data to the model is indicated by large spread of the confidence intervals or that the LT_{50} and LT_{95} can not be estimated or the confidence intervals can not be estimated. Sometimes this occurred because the durations were too long and all insects died by the first duration, or the opposite, at the longest durations there was not enough mortality to describe the curve. Durations were adjusted from experiments 1, 2 and 3 to better estimate mortality (Appendices 1 and 2).

There were 50 insects/vial, 5 replicate vials/treatment with 5-6 durations/treatment (including controls). The controls (time = 0 h) were replicated 3 times and maintained at $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and 60-70% r.h. for immature developmental stages to develop into adults before counting for mortality. When counting mortality data, the total number of dead was counted as the sum of

moribund and dead, where moribund is near dead or incapacitated individuals depending on the developmental stages.

Graphic Estimation

For some data sets, the LT_{50} and LT_{95} could not be estimated using probit analysis. I used graphic estimation to approximate the LT_{50} and LT_{95} of the developmental stages of species which were not obtained from probit analysis. The mortality was plotted against duration (Sigma Plot versions 13.0 and 14.0, Systat Software, San Jose, California, USA) to create graphs that could be interpolated to get the durations needed to attain 50 and 95% mortality. Linear regression (Sigma Plot versions 13.0 and 14.0, Systat Software) was used to extrapolate the lines to estimate the durations at 50 and 95% mortality.

ANOVA

The differences in SCP due to developmental stage, species and acclimation were tested using a three-way ANOVA. As there was a significant interaction between the factors, two-tailed t-test ($P < 0.05$) was done for the effect of acclimation. Blocking is the process of removing the variability so that the differences in treatments (usually within the same culture) are more pronounced (Krzywinski and Altman, 2014). Ideally, a randomized block design would have been used to test for differences between batches of 20 insects done in each run of SCP apparatus, day to day variation, month to month variation and cohorts of insects. Unfortunately, I did not have the necessary insects to run all treatments with the same cohort of insects, or to spread randomly in runs of each batch of 20 insects. Tentative results are presented here.

To test for difference in adult weight, a two-way ANOVA was run with species and diet as factors. Given there were significant interactions, one-way ANOVA was run and then differences were tested using Tukey's multiple range test.

Figure. 2.1. Device to measure SCP with 20 thermocouples connected to data loggers, which read temperature every 1 second. The polystyrene box was placed into a freezer at -44°C for 1.5 h. The SCP was the lowest temperature before the sudden increase in temperature due to the heat of crystallization.

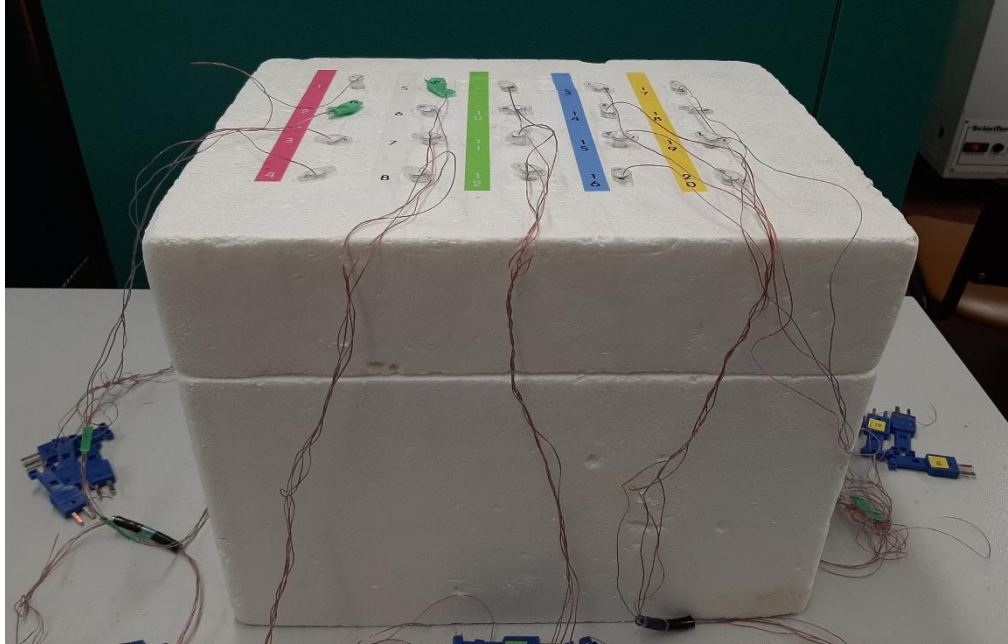
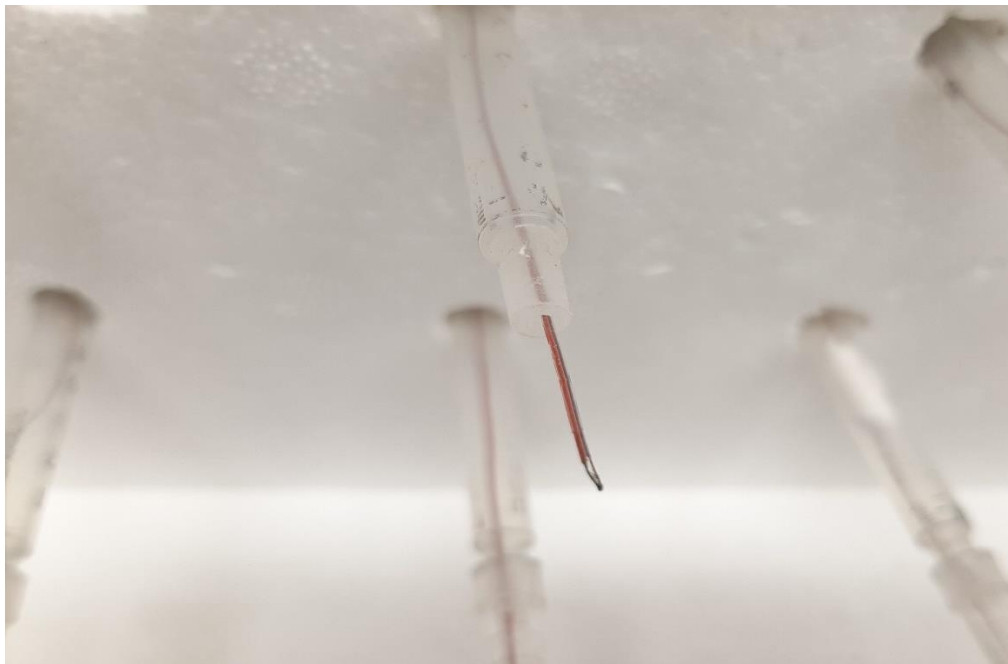


Figure. 2.2. Insects were held in contact with copper-constantan thermocouple wire using petroleum gel and a cap (cap not shown).



Chapter 3: Results

Effects of Developmental Stages, Species and Acclimation on Cold Tolerance

In experiment 1, the non-acclimated *C. ferrugineus* adult, pupa and old larva had similar cold tolerance with LT₅₀ ranging from 27 to 33 h and LT₉₅ ranging from 72 to 136 h. Old larva did take significantly longer to die with an LT₉₅ of 136 h. Egg and young larva tended to die quicker, but I was unable to test for differences because the estimates were done graphically (Table 3.1). For the non-acclimated *C. turcicus*, young larva were the most cold-hardy of all stages with an LT₅₀ of 30 h. Egg and pupa tended to die quicker, but I was unable to test for differences because the estimates were done graphically (Table 3.1). For the non-acclimated *C. pusillus*, all stages had similar cold tolerance with the LT₅₀ being approximately 12 h. There was a trend for *C. ferrugineus* to be more cold-tolerant than *C. turcicus*, which was more cold-tolerant than *C. pusillus* and this was seen in all 3 experiments without and with acclimation.

In experiment 1, the acclimated adult *C. ferrugineus* were more cold-tolerant than the old larva, which were more cold-tolerant than other stages. Trends could only be described, because adult had only 20% mortality at 168 h, whereas the other stages had LT₅₀ of 79 h or less (Table 3.2). For the acclimated *C. turcicus*, using the LT₅₀ to estimate cold tolerance, the adult was the most cold-tolerant stage with an LT₅₀ of 95 h. Pupa, old larva and young larva had similar cold tolerance with LT₅₀ ranging from 14-24 h (Table 3.2). Egg tended to be the least cold-tolerant stage. For the acclimated *C. pusillus*, the adult, pupa and old larva had similar cold tolerance. Young larva was less cold-tolerant at the LT₅₀, but much more cold-tolerant at the LT₉₅, this trend did not repeat itself in the other experiments. The egg of *C. pusillus* tended to be the least cold-tolerant stage.

In experiment 2, the non-acclimated *C. ferrugineus* adult tended to have the highest cold tolerance (LT₅₀ = 115 h), pupa and old larva tended not to be as cold-tolerant as adult and had similar cold tolerance (LT₅₀ ranging from 23-35 h) and young larva and egg were much less cold-tolerant (LT₅₀ ranging from 1-2 h) (Table 3.3). For the non-acclimated *C. turcicus*, adult was the most cold-tolerant followed by pupa. The old larva, young larva and egg had similar cold tolerance with LT₅₀ of approximately 1 h (Table 3.3). For the non-acclimated *C. pusillus*, adult

showed the most cold tolerance with an LT_{50} of 2 h and the other stages tended to have LT_{50} of 0.2 to 1 h.

In experiment 2, the acclimated *C. ferrugineus* adult was more cold-tolerant than the pupa and old larva, which were more cold tolerant than other stages. Trends could only be described, because adults had only 0% mortality at 168 h, whereas the pupae and old larvae had LT_{50} of 60-62 h and young larvae and eggs had LT_{50} of 2-3 h (Table 3.4). For the acclimated *C. turcicus*, using the LT_{50} to estimate cold tolerance, the adult were the most cold-tolerant stage, followed by pupa and old larva which had similar LT_{50} with 10-12 h, then followed by young larva and egg which had LT_{50} of 1-3 h. The LT_{95} followed similar trends except for pupa which had the highest LT_{95} of 652 h, although this is not significantly different from the adult, due to the large confidence intervals. For the acclimated *C. pusillus* the adult, pupa and old larva had similar cold tolerance with a graphical estimation of LT_{50} of 1 h. Young larva and egg tended to be the least cold tolerant stages.

In experiment 3, the durations were adjusted again to better estimate the LT_{50} and LT_{95} . The non-acclimated *C. ferrugineus* adult had the highest cold tolerance with an LT_{50} of 91 h, pupa and old larva had similar cold tolerance (LT_{50} ranging from 11-16 h), and young larva and egg were much less cold tolerant (LT_{50} ranging from 4-8 h) (Table 3.5). The egg LT_{95} was unusually high at 333 h with very wide confidence intervals. For the non-acclimated *C. turcicus*, adult was the most cold tolerant with an LT_{50} of 118 h. The pupa, old larva, young larva and egg had similar cold tolerance with LT_{50} ranging from 2-4 h (Table 3.5). For the non-acclimated *C. pusillus*, adult tended to be the most cold-tolerant stage with an LT_{50} of 7 h, similar to pupa with LT_{50} of 5 h and old larva with LT_{50} of 6 h. Young larva and egg had LT_{50} between 0.2 and 1.7 h.

In experiment 3, the acclimated *C. ferrugineus* adult was more cold tolerant ($LT_{50} = 777$ h) than the pupa and old larva (LT_{50} from 13-28 h), which were more cold tolerant than young larva and egg ($LT_{50} = 3$ h) (Table 3.6). For the acclimated *C. turcicus*, using the LT_{50} to estimate cold tolerance, the adult was the most cold tolerant stage with an LT_{50} of 122 h, old larva were the next most cold-tolerant with LT_{50} of 26 h and pupa, young larva and egg were the least cold-hardy of all stages with an LT_{50} of 0.4-1 h. For the acclimated *C. pusillus*, adult was the most cold-hardy with an LT_{50} of 14 h and the other stages had LT_{50} ranging from 0.2 to 1 h.

Exposing insects to cooling conditions, 18, 10 and 5°C for 1 week/temperature, had varied effects on cold tolerance. The comparisons to data sets are limited to where the probit analysis could estimate the LT₅₀ with confident intervals. In experiment 1, acclimation significantly increased the cold tolerance of *C. ferrugineus* pupa and old larva (but not young larva and egg) and *C. turcicus* adult (Tables 3.1 and 3.2). In experiment 2, acclimation significantly increased the cold tolerance of *C. ferrugineus* adult, pupa and old larva; *C. turcicus* adult and young larva, but not old larva. The cold tolerance of *C. pusillus* adult did not increase with acclimation (Tables 3.3 and 3.4). In experiment 3, acclimation significantly increased the cold tolerance of *C. ferrugineus* adult, but not the pupa, old larva or egg; *C. turcicus* old larva but not adult, *C. pusillus* adult, but not other stages (Tables 3.5 and 3.6). Calculating the ratio of acclimated to non-acclimated LT₅₀ and LT₉₅ gives estimates of how much cold tolerance changed with acclimation (Table 3.7). *Cryptolestes ferrugineus* tended to have increased cold tolerance for adult with LT₅₀, and for all stages except egg. *Cryptolestes turcicus* tended towards increased cold tolerance for old larva. *Cryptolestes pusillus* tended towards increased cold tolerance only for adult stage. The largest increases were seen by *C. ferrugineus* adult (8.5-fold increase) and *C. turcicus* old larva (13-fold increase).

Effects of Diet, Species and Acclimation on Cold Tolerance

In experiments 1-3, the insects were reared on flour and brewer's yeast. However, these insects are found in other foods. In this section results are from experiment 4 to examine the effect on cold tolerance of adults of the three species of *Cryptolestes* reared on three different diets. In general, adults reared on the grain diet had the highest cold tolerance compared to the adults reared on flour and brewer's yeast and flour diet. There were some overlapping of confidence intervals between values of grain and wheat flour + brewer's yeast diet for all three species (Table 3.8).

On the grain diet, *C. ferrugineus* had the highest LT₅₀ followed by *C. turcicus*, and *C. pusillus* had the lowest LT₅₀. The adults reared on flour and brewer's yeast had the next highest cold tolerance for all three species, followed by the adults reared on the flour diet. *Cryptolestes ferrugineus* had the highest cold tolerance compared to other species on flour and brewer's yeast diet with LT₅₀ of 79 h whereas on flour diet it had an LT₅₀ 42 h. *Cryptolestes pusillus* had the

lowest cold tolerance on all three diets. The LT₅₀ of *C. pusillus* adult on the grain, flour and brewer's yeast and flour diets were similar, ranging from 8-11 h, with overlapping confidence intervals (Table 3.8).

In experiment 4, I examined the effect on cold tolerance of adults of the three species of *Cryptolestes* reared on three different diets with cold acclimated adults. In general, adults reared on the grain diet had the highest cold tolerance compared to the adults reared on flour and brewer's yeast and flour diet. There were some exceptions where the confidence intervals overlapped for LT₅₀: *C. ferrugineus* on grain and flour and brewer's yeast diet and *C. turcicus* on flour and brewer's yeast and flour diet (Table 3.9).

On the grain diet, *C. ferrugineus* had the highest LT₅₀ followed by *C. turcicus*, and *C. pusillus* was the least cold tolerant (Table 3.8). The adults reared on flour and brewer's yeast had the next highest cold tolerance for all three species, followed by the adults reared on the flour diet. *Cryptolestes ferrugineus* had the highest cold tolerance on flour and brewer's yeast diet. *Cryptolestes pusillus* had the least cold tolerance of all species on all three diets (Table 3.8).

Comparing non-acclimated vs acclimated insects, there was a consistent increase in cold tolerance with acclimation for both *C. ferrugineus* and *C. turcicus* for all three diets. The increases ranged from 3-fold to 34-fold in cold tolerance (Table 3.10). *Cryptolestes pusillus* had small increases as in grain with 2-fold for LT₉₅ or no increases at all for flour.

Comparing the LT₅₀ values between experiment 3 and 4 for similar rearing conditions and developmental stage (flour and brewer's yeast, adult), for non-acclimated adult there were no differences for *C. ferrugineus* and *C. pusillus*, but *C. turcicus* had higher cold tolerance in experiment 3 compared to experiment 4 (Tables 3.5 and 3.7). For acclimated adult, *C. ferrugineus* showed no differences between experiments 3 and 4, whereas *C. turcicus* and *C. pusillus* had higher cold tolerance in experiment 3 compared to experiment 4 (Tables 3.6 and 3.8). Effects of Developmental Stages, Species, Acclimation and Diet on Supercooling Point

Due to lack of insects and time to rear enough insects at the same time and the time it takes to measure the SCP, it was not possible to run a randomized block design, which would take into account effects of running SCP on different days and months. Thus, these results are

tentative given the limitations of the experimental design. Acclimation did not have an overall effect on SCP ($F = 0.02$, $df = 1$, 1007; $P = 0.877$), species did have an effect ($F = 6.11$, $df = 2$, 1007; $P = 0.002$), as did the life stage ($F = 76.55$, $df = 4$, 1007 $P < 0.001$; three-way ANOVA), all interactions were significant ($P < 0.001$). Least square means for different stages of the species combined are: adult $-22.0 \pm 0.2^{\circ}\text{C}$, pupa $-25.8 \pm 0.2^{\circ}\text{C}$, old larva $-23.1 \pm 0.2^{\circ}\text{C}$, young larva $-24.0 \pm 0.2^{\circ}\text{C}$ and egg $-25.1 \pm 0.1^{\circ}\text{C}$. Pupa of *C. pusillus* had the lowest SCP of $-26.7 \pm 0.1^{\circ}\text{C}$, followed by the egg of *C. pusillus* with SCP of $-26.2 \pm 0.2^{\circ}\text{C}$ and the old larva and pupa of *C. turcicus* with SCP of $-26.1 \pm 0.2^{\circ}\text{C}$ and $-26.1 \pm 0.4^{\circ}\text{C}$. Adults of the three species had the highest SCPs compared to other developmental stages; adult *C. ferrugineus* and *C. turcicus* had statistically similar SCPs of $-21.4 \pm 0.5^{\circ}\text{C}$ and $-21.3 \pm 0.5^{\circ}\text{C}$, respectively. The adult stage of *C. pusillus* had the highest SCP of $-20.1 \pm 0.5^{\circ}\text{C}$ (Table 3.11).

The supercooling points of three species of *Cryptolestes* under acclimated conditions, pupa of *C. turcicus* had the lowest SCP of $-26.7 \pm 0.4^{\circ}\text{C}$, followed by the pupa of *C. pusillus* with SCP of $-25.7 \pm 0.4^{\circ}\text{C}$, the young larva of *C. ferrugineus* with SCP of $-25.5 \pm 0.5^{\circ}\text{C}$, egg of *C. turcicus* with SCP of $-25.4 \pm 0.3^{\circ}\text{C}$ and egg of *C. ferrugineus* with SCP of $-25.1 \pm 0.2^{\circ}\text{C}$, all of which were not statistically different. The old larva of *C. ferrugineus* and *C. pusillus* had the highest SCPs of $-22.2 \pm 0.4^{\circ}\text{C}$ and $-20.5 \pm 0.7^{\circ}\text{C}$ among other developmental stages; adult stage of *C. pusillus* had the highest SCP of $-22.2 \pm 0.4^{\circ}\text{C}$ compared to other developmental stages (Table 3.11). Occasionally, acclimated insects had significantly lower SCP than the corresponding non-acclimated insects: *C. ferrugineus*, young larva and adult; *C. turcicus*, adult; *C. pusillus*, adult. There were also some cases where, unexpectedly, the SCP was higher with acclimated insects: *C. turcicus*, egg and old larva; *C. pusillus*, egg and pupa (Table 3.11).

I also examined the SCP of insects reared on the different diets (Table 3.12). Acclimation did have an overall effect on SCP ($F = 45.0$, $df = 1$, 578; $P < 0.001$; non-acclimated SCP; -22.2 ± 0.2 vs acclimated SCP; -23.6 ± 0.1), species did have an effect on SCP ($F = 6.63$, $df = 2$, 578; $P = 0.001$), diet have an effect ($F = 13.8$, $df = 2$, 578; $P < 0.001$; three-way ANOVA), all interactions were significant ($P < 0.035$). SCP of adults of the three species of *Cryptolestes* reared on three different diets under non-acclimated conditions, *C. turcicus* on flour and brewer's yeast diet had the lowest SCP of $-24.7 \pm 0.4^{\circ}\text{C}$, followed by *C. pusillus* on flour diet with SCP of

$-23.5 \pm 0.5^{\circ}\text{C}$. *Cryptolestes ferrugineus* reared on grain and flour diet had the highest SCPs of $-20.6 \pm 0.4^{\circ}\text{C}$ and $-19.4 \pm 0.4^{\circ}\text{C}$ respectively (Table 3.12).

For acclimated insects, *C. ferrugineus* reared on flour and brewer's yeast diet and flour diet with SCPs of $-24.0 \pm 0.4^{\circ}\text{C}$ and $-24.5 \pm 0.4^{\circ}\text{C}$ and *C. turcicus* reared on flour diet with SCP of $-24.0 \pm 0.4^{\circ}\text{C}$ had the lowest SCP. *C. ferrugineus* reared on grain diet with SCP of $-23.0 \pm 0.4^{\circ}\text{C}$ and *C. pusillus* reared on grain and flour diets with the SCPs of $-23.1 \pm 0.5^{\circ}\text{C}$ and $-23.0 \pm 0.5^{\circ}\text{C}$ had the highest SCP. In general, *C. ferrugineus* and *C. turcicus* showed lower SCP values after acclimation compared to non-acclimated insects, whereas *C. pusillus* did not (Table 3.12).

Effect of Diet on Adult Body Weight

Diet did have an overall effect on weight ($F = 1030.0$, $df = 2, 18$; $P < 0.001$) as did the species ($F = 1537.2$, $df = 2, 18$; $P < 0.001$; two-way ANOVA). There was a significant interaction between diet and species based on the diet present ($F = 339.2$, $df = 4, 18$; $P < 0.001$). The adults reared on flour and brewer's yeast diet had the highest body weight for the three species compared to the other diets. On flour and brewer's yeast and the flour diet, *C. turcicus* had the highest body weight followed by *C. ferrugineus*, and *C. pusillus* with the lowest body weight. There was a similar trend for the adults on the flour diet, *C. ferrugineus* and *C. turcicus* had the next highest body weights of 22.8 ± 0.3 mg and 33.5 ± 0.3 mg respectively. On the grain diet, *C. ferrugineus* had the highest body weight, followed by *C. turcicus*, and *C. pusillus* had the lowest body weight (Table 3.13).

Table 3.1. Experiment 1, the lethal time, LT₅₀ and LT₉₅ (h) of different developmental stages of three *Cryptolestes* spp. at –10°C to estimate cold tolerance without cold acclimation. The insects were held at 30°C, 60% r.h¹.

Species	Stage	LT ₅₀ (h) (95% confidence limits)	LT ₉₅ (h) (95% confidence limits)	Slope ± SEM	Intercept ± SEM	Chi- square	Heterogeneity
<i>C. ferrugineus</i>	Egg	9 ²	22 ²				
	Young larva	16 ²	46 ²				
	Old larva	33 (27-39)	136 (110- 185)	2.7 ± 0.2	–4.1 ± 0.3	70	3
	Pupa	28 (24-32)	72 (62 -89)	4.1 ± 0.2	–5.6 ± 0.4	63	3
	Adult	27 (24-31)	88 (77-106)	3.2 ± 0.2	–4.6 ± 0.4	36	2
<i>C. turcicus</i>	Egg	11 ²	28 ²				
	Young larva	30 (26-34)	91 (79-111)	3.4 ± 0.2	–5.1 ± 0.4	46	2
	Old larva	16 (13-19)	40 (36-47)	4.2 ± 0.5	–5.1 ± 0.8	21	1
	Pupa	13 ²	27 ²				
	Adult	14 (8-19)	86 (70-120)	2.1 ± 0.2	–2.4 ± 0.3	49	2
<i>C. pusillus</i>	Egg	11 ²	23 ²				
	Young larva	12 ²	23 ²				
	Old larva	13 ²	36 ²				
	Pupa	11 ²	35 ²				
	Adult	12 ²	32 ²				

1. There were 50 insects/vial, 5 vials/duration and 5-6 durations for each treatment.

2. Estimated graphically.

Table 3.2. Experiment 1, the lethal time, LT₅₀ and LT₉₅ (h) of different developmental stages¹ of three *Cryptolestes* spp. at –10°C to estimate cold tolerance with cold acclimation².

Species	Stage	LT ₅₀ (h) (95% confidence limits)	LT ₉₅ (h) (95% confidence limits)	Slope ± SEM	Intercept ± SEM	Chi- square	Heterogeneity
<i>C. ferrugineus</i>	Egg	9 ³	31 ³				
	Young larva	7 ³	– ³				
	Old larva	79 (73-85)	320 (272-391)	2.7 ± 0.2	–5.1 ± 0.3	23	1
	Pupa	55 (50-59)	87 (79-102)	8.3 ± 0.9	–14.4 ± 1.6	44	2
	Adult	– ⁴	– ⁴				
<i>C. turcicus</i>	Egg	0.5 ³	– ³				
	Young larva	14 (10-17)	36 (32-42)	4.1 ± 0.7	–4.7 ± 1.0	9	0.4
	Old larva	24 (16-31)	125 (97-184)	2.3 ± 0.2	–3.2 ± 0.3	63	3
	Pupa	17 (9-25)	161 (114-304)	1.7 ± 0.2	–2.1 ± 0.3	74	3
	Adult	95 (82-112)	254 (194-401)	3.9 ± 0.2	–7.6 ± 0.3	168	7
<i>C. pusillus</i>	Egg	11 ³	23 ³				
	Young larva	4 (0.5-10)	306 (175-1139)	0.9 ± 0.2	–0.5 ± 0.3	27	1
	Old larva	15 (11-18)	42 (37-50)	3.7 ± 0.5	–4.4 ± 0.7	26	1
	Pupa	14 (9-17)	36 (32-43)	3.8 ± 0.6	–4.3 ± 0.9	18	1
	Adult	17 (14-20)	51 (46-59)	3.5 ± 0.3	–4.3 ± 0.5	14	1

1. There were 50 insects/vial, 5 vials/duration and 5-6 durations for each treatment.

2. Insects were held at 18, 10 and 5°C for 1 week/temperature.

3. Estimated graphically.

4. 20% mortality at 168 h.

Table 3.3. Experiment 2, the lethal time, LT₅₀ and LT₉₅ (h) of different developmental stages of three *Cryptolestes* spp. at –10°C to estimate cold tolerance without cold acclimation. The insects were held at 30°C, 60% r.h¹.

Species	Stage	LT ₅₀ (h) (95% confidence limits)	LT ₉₅ (h) (95% confidence limits)	Slope ± SEM	Intercept ± SEM	Chi- square	Heterogeneity
<i>C. ferrugineus</i>	Egg	1 (0.4-2)	3 (3-4)	4.0 ± 1.2	–0.5 ± 0.6	10	0.5
	Young larva	2 (1-2)	5 (4-5)	3.9 ± 1.0	–0.9 ± 0.4	9	0.4
	Old larva	23 (20-26)	62 (55-71)	3.8 ± 0.3	–5.2 ± 0.5	24	1
	Pupa	35 (32-37)	68 (63-75)	5.6 ± 0.4	–8.6 ± 0.6	25	1
	Adult	115 ²	163 ²				
<i>C. turcicus</i>	Egg	1 ²	3 ²				
	Young larva	1 ²	3 ²				
	Old larva	1 ²	3 ²				
	Pupa	12 ²	48 ²				
	Adult	14 (11-17)	40 (36-46)	3.7 ± 0.5	–4.3 ± 0.7	5	0.2
<i>C. pusillus</i>	Egg	0.2 ²	0.5 ²				
	Young larva	0.2 ²	0.5 ²				
	Old larva	1 ²	3 ²				
	Pupa	1 ²	3 ²				
	Adult	2 (1-2)	8 (7-10)	2.6 ± 0.3	–0.7 ± 0.2	17	0.7

1. There were 50 insects/vial, 5 vials/duration and 5-6 durations for each treatment.

2. Estimated graphically.

Table 3.4. Experiment 2, the lethal time, LT₅₀ and LT₉₅ (h) of different developmental stages¹ of three *Cryptolestes* spp. at –10°C to estimate cold tolerance with cold acclimation².

Species	Stage	LT ₅₀ (h) (95% confidence limits)	LT ₉₅ (h) (95% confidence limits)	Slope ± SEM	Intercept ± SEM	Chi- square	Heterogeneity
<i>C. ferrugineus</i>	Egg	3 (2-3)	6 (6-7)	4.4 ± 0.5	–2.0 ± 0.3	9	0.4
	Young larva	2 (2-3)	6 (5-7)	4.0 ± 0.5	–1.3 ± 0.3	9	0.4
	Old larva	62 (59-65)	99 (92-108)	8.2 ± 0.8	–14.7 ± 1.4	13	1
	Pupa	60 (53-66)	116 (104-136)	5.8 ± 0.5	–10.2 ± 1.0	39	2
	Adult	_ ³	_ ³				
<i>C. turcicus</i>	Egg	1 ⁴	3 ⁴				
	Young larva	3 (2-3)	9 (8-12)	3.0 ± 0.3	–1.2 ± 0.2	28	1
	Old larva	10 (7-13)	62 (45-103)	2.1 ± 0.2	–2.1 ± 0.3	45	2
	Pupa	12 (10-15)	652 (312-2027)	1.0 ± 0.1	–1.0 ± 0.1	25	1
	Adult	26 (20-32)	485 (339-818)	1.3 ± 0.1	–1.8 ± 0.2	21	1
<i>C. pusillus</i>	Egg	0.2 ⁴	0.5 ⁴				
	Young larva	0.2 ⁴	0.5 ⁴				
	Old larva	1 ⁴	3 ⁴				
	Pupa	1 ⁴	3 ⁴				
	Adult	1 (0.2-1.5)	4 (3-4)	2.8 ± 0.7	0.1 ± 0.4	6	0.2

1. There were 50 insects/vial, 5 vials/duration and 5-6 durations for each treatment.

2. The insects were held at 18, 10 and 5°C for 1 week/temperature.

3. 0% mortality at 168 h.

4. Estimated graphically.

Table 3.5. Experiment 3, the lethal time, LT₅₀ and LT₉₅ (h) of different developmental stages of three *Cryptolestes* spp. at –10°C to estimate cold tolerance without cold acclimation. The insects were held at 30°C, 60% r.h¹.

Species	Stage	LT ₅₀ (h) (95% confidence limits)	LT ₉₅ (h) (95% confidence limits)	Slope ± SEM	Intercept ± SEM	Chi- square	Heterogeneity
<i>C. ferrugineus</i>	Egg	8 (4-13)	333 (114-5054)	1.0 ± 0.1	–1.0 ± 0.1	101	4
	Young larva	4 (3-4)	14 (12-19)	2.8 ± 0.2	–1.5 ± 0.1	52	2
	Old larva	16 (12-20)	59 (51-70)	3.0 ± 0.3	–3.6 ± 0.5	17	1
	Pupa	11 (5-16)	38 (32-47)	3.1 ± 0.5	–3.3 ± 0.7	47	2
	Adult	91 (84-100)	572 (447-786)	2.1 ± 0.1	–4.0 ± 0.3	19	1
<i>C. turcicus</i>	Egg	4 (3-5)	26 (20-39)	2.1 ± 0.1	–1.3 ± 0.1	72	3
	Young larva	3 (2-4)	13 (10-19)	3.0 ± 0.2	–1.2 ± 0.1	66	3
	Old larva	2 (1-2)	8 (7-9)	2.4 ± 0.3	–0.4 ± 0.2	19	1
	Pupa	2 ²	11 ²				
	Adult	118 (107-131)	691 (524-997)	2.1 ± 0.2	–4.4 ± 0.3	17	1
<i>C. pusillus</i>	Egg	1.7 (1.6-1.9)	6 (5-7)	3.2 ± 0.2	–0.8 ± 0.1	34	1
	Young larva	0.2 (0.1-0.3)	0.9 (0.7-1.1)	3.0 ± 0.4	1.8 ± 0.1	44	2
	Old larva	6 ²	14 ²				
	Pupa	5 (4-6)	21 (18-24)	3.0 ± 0.2	–2.1 ± 0.2	31	1
	Adult	7 (5-8)	48 (37-68)	2.0 ± 0.1	–1.6 ± 0.1	41	2

1. There were 50 insects/vial, 5 vials/duration and 5-6 durations for each treatment.

2. Estimated graphically.

Table 3.6. Experiment 3, the lethal time, LT₅₀ and LT₉₅ (h) of different developmental stages¹ of three *Cryptolestes* spp. at –10°C to estimate cold tolerance with cold acclimation².

Species	Stage	LT ₅₀ (h) (95% confidence limits)	LT ₉₅ (h) (95% confidence limits)	Slope ± SEM	Intercept ± SEM	Chi-square	Heterogeneity
<i>C. ferrugineus</i>	Egg	3 (1-6)	198 (93-876)	1.0 ± 0.1	–0.5 ± 0.1	81	3
	Young larva	3 ³	18 ³				
	Old larva	28 (18-37)	163 (125-242)	2.1 ± 0.2	–3.1 ± 0.4	41	2
	Pupa	13 (3-22)	127 (86-290)	1.7 ± 0.2	–1.8 ± 0.3	84	4
	Adult	777 (709-887)	1384 (1077-5000)	6.6 ± 1.3	–19.0 ± 3.7	66	3
<i>C. turcicus</i>	Egg	0.4 ³	3 ³				
	Young larva	1 ³	3 ³				
	Old larva	26 (17-36)	111 (63-673)	2.6 ± 0.3	–3.6 ± 0.4	133	6
	Pupa	1 ³	3 ³				
	Adult	122 (109-139)	943 (663-1542)	1.9 ± 0.2	–3.9 ± 0.3	17	0.7
<i>C. pusillus</i>	Egg	0.2 ³	0.5 ³				
	Young larva	0.2 ³	0.5 ³				
	Old larva	1 (0.1-2)	3 (2-4)	3.4 ± 1.2	–0.03 ± 0.6	8	0.3
	Pupa	2 ³	10 ³				
	Adult	14 (12-16)	257 (195-360)	1.3 ± 0.1	–1.5 ± 0.1	39	1

1. There were 50 insects/ vial, 5 vials/ durations and 5-6 for each treatment.

2. The insects were held at 18, 10 and 5°C for 1 week/temperature.

3. Estimated graphically.

Table 3.7. Comparing cold tolerance of acclimated to non-acclimated insects (data from Tables 3.5 and 3.6).

Species	Stage	LT₅₀ (h) (Acclimation : Non-Acclimation)	LT₉₅ (h) (Acclimation : Non-Acclimation)	Ratio LT₅₀ (Acclimation/Non-Acclimation)	Ratio LT₉₅ (Acclimation/Non-Acclimation)
<i>C. ferrugineus</i>	Egg	3 : 8	198 : 333	0.4	0.6
	Young larva	3 : 4	18 : 14	0.8	1.3
	Old larva	28 : 16	163 : 59	1.8	2.8
	Pupa	13 : 11	127 : 38	1.2	3.3
	Adult	777 : 91	1384 : 572	8.5	2.4
<i>C. turcicus</i>	Egg	0.4 : 4	3 : 26	0.1	0.1
	Young larva	1: 3	3 : 13	0.3	0.2
	Old larva	26: 2	111 : 8	13.0	13.9
	Pupa	1: 2	3 : 11	0.5	0.3
	Adult	122 : 118	943 : 691	1.0	1.4
<i>C. pusillus</i>	Egg	0.2 : 1.7	0.5: 6	0.1	0.1
	Young larva	0.2 : 0.2	0.5 : 1	1.0	0.5
	Old larva	1: 6	3 : 14	0.2	0.2
	Pupa	2 : 5	10 : 21	0.4	0.5
	Adult	14 : 7	257 : 48	2.0	5.4

Table 3.8. Experiment 4, the lethal time, LT₅₀ and LT₉₅ (h) of adult *Cryptolestes* spp. at −10°C to estimate cold tolerance with different diets¹ without cold acclimation. The insects were held at 30°C².

Species	Diet	LT ₅₀ (h) (95% confidence limits)	LT ₉₅ (h) (95% confidence limits)	Slope ± SEM	Intercept ± SEM	Chi- square	Heterogeneity
<i>C. ferrugineus</i>	Grain	104 (95-114)	211 (181-263)	5.3 ± 0.3	−10.7 ± 0.5	91	4
	Flour + BY	79 (65-96)	130 (104-217)	7.6 ± 0.4	−14.4 ± 0.7	482	21
	Flour	42 (36-48)	99 (82-132)	4.3 ± 0.2	−7.1 ± 0.4	129	6
<i>C. turcicus</i>	Grain	55 (47-63)	123 (100-172)	4.7 ± 0.2	−8.1 ± 0.4	191	8
	Flour + BY	26 (25-28)	43 (40-48)	7.7 ± 0.6	−11.0 ± 0.9	24	1
	Flour	26 (25-28)	40 (36-45)	9.3 ± 0.9	−13.1 ± 1.3	25	1
<i>C. pusillus</i>	Grain	11 (10-12)	28 (23-36)	4.1 ± 0.2	−4.2 ± 0.2	120	4
	Flour + BY	9 (8.5-10)	24 (21-28)	4.1 ± 0.2	−4.0 ± 0.2	66	2
	Flour	8 (7-9)	23 (20-30)	3.6 ± 0.2	−3.3 ± 0.2	99	4

1. Insects were reared on one of three diets: Grain (whole wheat kernels, cracked wheat kernels and wheat germ; 90:5:5 proportions); Flour + BY (white flour from wheat and brewer's yeast; 95:5 proportions) and Flour (white flour from wheat).
2. There were 50 insects/vial, 5 vials/duration and 5-6 durations for each treatment.

Table 3.9. Experiment 4 to estimate cold tolerance with different diets¹ with cold acclimation² the lethal time, LT₅₀ and LT₉₅ (h) of adult *Cryptolestes* spp.³ at –10°C.

Species	Diet	LT ₅₀ (h) (95% confidence limits)	LT ₉₅ (h) (95% confidence limits)	Slope ± SEM	Intercept ± SEM	Chi-square	Heterogeneity
<i>C. ferrugineus</i>	Grain	760 (713-824)	1488 (1261-1937)	5.6 ± 0.5	–16.2 ± 1.3	47	2
	Flour + BY	695 (603-828)	4427 (2855-9078)	2.0 ± 0.2	–5.8 ± 0.5	38	2
	Flour	237 (219-255)	448 (412-497)	6.0 ± 0.3	–14.2 ± 0.9	36	2
<i>C. turcicus</i>	Grain	153 (128-187)	1879 (1136-3979)	1.5 ± 0.1	–3.3 ± 0.2	46	2
	Flour + BY	77 (70-83)	141 (129-159)	6.2 ± 0.4	–11.8 ± 0.9	48	2
	Flour	86 (76-94)	174 (157-200)	5.4 ± 0.4	–10.4 ± 0.9	47	2
<i>C. pusillus</i>	Grain	18 (16-20)	54 (46-65)	3.5 ± 0.2	–4.4 ± 0.3	58	2
	Flour + BY	12 (10-13)	29 (26-35)	4.2 ± 0.3	–4.5 ± 0.3	63	2
	Flour	8 (7-9)	23 (21-27)	3.5 ± 0.2	–3.2 ± 0.2	40	1

1. Insects were reared on one of three diets: Grain (whole wheat kernels, cracked wheat kernels and wheat germ; 90:5:5 proportions); Flour + BY (white flour from wheat and brewer's yeast; 95:5 proportions) and Flour (white flour from wheat).

2. The insects were held at 18, 10 and 5°C for 1 week/temperature.

3. There were 50 insects/vial, 5 vials/duration and 5-6 durations for each treatment.

Table 3.10. Comparing cold tolerance of acclimated to non-acclimated adults reared on different diets¹ (data from Tables 3.8 and 3.9).

Species	Diet	LT₅₀ (h) (Acclimation Vs. Non-Acclimation)	LT₉₅ (h) (Acclimation Vs. Non-Acclimation)	Ratio LT₅₀ (Acclimation Vs. Non-Acclimation)	Ratio LT₉₅ (Acclimation Vs. Non-Acclimation)
<i>C. ferrugineus</i>	Grain	760 : 104	1488 : 211	7.3	7.1
	Flour + BY	695 : 79	4427 : 130	8.8	34.1
	Flour	237 : 42	448 : 99	5.6	4.5
<i>C. turcicus</i>	Grain	153 : 55	1879 : 123	2.7	15.3
	Flour + BY	77 : 26	141 : 43	3.0	3.3
	Flour	86 : 26	174 : 40	3.3	4.4
<i>C. pusillus</i>	Grain	18 : 11	54 : 28	1.6	2.0
	Flour + BY	12 : 9	29 : 24	1.3	1.2
	Flour	8 : 8	23 : 23	1.0	1.0

1. Insects were reared on one of three diets: Grain (whole wheat kernels, cracked wheat kernels and wheat germ; 90:5:5 proportions); Flour + BY (white flour from wheat and brewer's yeast; 95:5 proportions) and Flour (white flour from wheat).

Table 3.11. The supercooling points, SCP (°C) of different developmental stages of three *Cryptolestes* spp. The insects were non-acclimated to cold, being held at 30 °C, 60% r.h. or acclimated by being held at 18, 10 and 5°C for 1 week/temperature.

Species	Stage	Non-Acclimated		Acclimated	
		SCP (°C) (mean \pm SEM) ¹	N	SCP (°C) (mean \pm SEM) ¹	N
<i>C. ferrugineus</i>	Egg	-25.4 \pm 0.5 ² a	44	-25.1 \pm 0.2a	63
	Young larva	-23.4 \pm 0.4 ² a	33	-25.5 \pm 0.5b	22
	Old larva	-22.4 \pm 0.4a	48	-22.2 \pm 0.4a	43
	Pupa	-24.6 \pm 0.3a	24	-25.1 \pm 0.4a	16
	Adult	-21.4 \pm 0.5 ² a	37	-23.7 \pm 0.4b	42
<i>C. turcicus</i>	Egg	-25.8 \pm 0.1 ² a	54	-25.4 \pm 0.3b	69
	Young larva	-23.9 \pm 0.4 ² a	48	-24.4 \pm 0.7a	20
	Old larva	-26.1 \pm 0.2a	40	-20.5 \pm 0.7b	26
	Pupa	-26.1 \pm 0.4a	16	-26.7 \pm 0.4a	22
	Adult	-21.3 \pm 0.5 ² a	53	-23.4 \pm 0.4b	30
<i>C. pusillus</i>	Egg	-26.2 \pm 0.2 ² a	51	-22.4 \pm 0.4b	35
	Young larva	-23.1 \pm 0.3 ² a	48	-23.0 \pm 0.5a	19
	Old larva	-23.0 \pm 0.6a	27	-24.4 \pm 0.7a	15
	Pupa	-26.7 \pm 0.1a	40	-25.7 \pm 0.4b	26
	Adult	-20.1 \pm 0.5 ² a	41	-22.2 \pm 0.4b	28

1. Within a row, means that are significantly different have different letters (two-tailed t-test, $P < 0.05$).

2. Insects from experiment 3, the rest of the insects were from experiment 2.

Table 3.12. The supercooling points, SCP (°C) of adult *Cryptolestes* spp. The insects were non-acclimated to cold, being held at 30 °C, 60% r.h. or acclimated by being held at 18, 10 and 5°C for 1 week/temperature¹.

Species	Diet	Non-Acclimated		Acclimated	
		SCP (°C) (mean ± SEM) ²	N	SCP (°C) (mean ± SEM) ²	N
<i>C. ferrugineus</i>	Grain	−20.6 + 0.4a	31	−23.0 + 0.4b	37
	Flour + BY	−22.9 + 0.5a	26	−24.0 + 0.4a	45
	Flour	−19.4 + 0.4a	19	−24.5 + 0.4b	49
<i>C. turcicus</i>	Grain	−21.2 + 0.5a	30	−23.4 + 0.4b	35
	Flour + BY	−24.7 + 0.4a	29	−23.7 + 0.3a	44
	Flour	−22.9 + 0.4a	31	−24.0 + 0.4b	46
<i>C. pusillus</i>	Grain	−21.9 + 0.6a	29	−23.1 + 0.5a	22
	Flour + BY	−22.4 + 0.5a	30	−23.5 + 0.3a	29
	Flour	−23.5 + 0.5a	34	−23.0 + 0.5a	30

1. Insects were from experiment 4 and reared on one of three diets: grain diet (whole wheat kernels, cracked wheat kernels and wheat germ (90:5:5 proportions); white flour from wheat and brewer's yeast (95:5 proportions) and white flour from wheat.

2. Within a row, means that are significantly different have different letters (two-tailed t-test, $P < 0.05$).

Table 3.13. The bodyweight of 100 adults of *Cryptolestes* spp. on three different diets¹.

Species	Diet (Weight/100 adults, mg \pm SEM) ²			<i>F</i> -value	<i>P</i>
	Grain	Flour + BY	Flour		
<i>C. ferrugineus</i>	20.5 \pm 0.3Ca	27.7 \pm 0.3Bc	22.8 \pm 0.3Bb	147.6	<0.001
<i>C. turcicus</i>	15.9 \pm 0.6Ba	38.0 \pm 0.1Cc	33.5 \pm 0.3Cb	1057.9	<0.001
<i>C. pusillus</i>	14.1 \pm 0.1Ab	19.9 \pm 0.4Ac	11.0 \pm 0.3Aa	258.7	<0.001
<i>F</i> -value	80.8	1164.8	1407.7		
<i>P</i>	<0.001	<0.001	<0.001		

1. Insects were reared on one of three diets: grain diet (whole wheat kernels, cracked wheat kernels and wheat germ (90:5:5 proportions); white flour from wheat and brewer's yeast (95:5 proportions) and white flour from wheat.

2. Within a row, means that are significantly different have different lowercase letters, within a column, means that are significantly different have different uppercase letters (One-Way ANOVA, Tukey's MRT, $N=3$, $df = 2, 18$).

Chapter 4: Discussion

Effect of Developmental Stage

In this study, in general for all non-acclimated insects, adult was more cold tolerant than the immature stages. Specifically for *C. ferrugineus*, adult had the highest cold tolerance, pupa and old larva had similar cold tolerance and young larva and egg were much less cold tolerant. Smith (1970) found that non-acclimated *C. ferrugineus* adult and older larva were more cold-hardy than pupa, young larva and egg. For the non-acclimated *C. pusillus* in this study, adult, pupa and old larva had similar cold tolerance and the young larva and egg were less cold tolerant. Williams (1954b) determined the cold tolerance at 2°C for 60 hours of the different developmental stages of *C. pusillus* (*Laemophloeus minutus* (Oliver) is synonymous with *C. pusillus*). The mortality of the last instar larva (fourth) and pupa were 7% and 2% only compared to that of the 1st, 2nd and 3rd larval instars and adult (38, 16, 20 and 26% respectively), and the egg was the least cold tolerant stage (63% mortality). There are no previous studies on the stage-specific cold tolerance of *C. turcicus*, this study showed that the adult was the most cold-hardy stage for unacclimated insects.

For acclimated insects, generally in this study, adult was the most cold tolerant of all stages, followed by old larva then pupa, and the egg and young larva were the least cold tolerant stages. The differences in cold tolerance between stages became more pronounced with acclimation. For example, in cold-acclimated *C. ferrugineus* the older larva and adult had a large difference in LT₅₀ (28 h vs 777 h) compared to non-acclimated insects (16 h vs 91 h). This is the first laboratory study to examine the effect of acclimation on cold tolerance of all life stages of *C. ferrugineus*, *C. pusillus* and *C. turcicus* in a comparative aspect. Fields (1990) showed that *C. ferrugineus* larva and adult stages increased their cold tolerance when acclimated and that the adult is more cold tolerant than young larva and old larva, and the egg did not survive acclimation.

Abdelghany and Fields (2017) examined stage-specific survival in grain bulks of 300 kg infested with all stages of *C. ferrugineus* as the grain temperature decreased from 30°C to -5°C over a 3-week period. In contrast to my experiments and previous laboratory studies (Fields, 1990), Abdelghany and Fields (2017) found that the immature stages were more cold-hardy than

the adult stage. This is concerning, because Abdelghany and Fields (2017) simulated bin conditions better than the laboratory studies and hence raises the question if the laboratory studies are a good predictor of overwintering mortality in bins. Due to large grain bulks, the temperature decline occurred over several days, so acclimation could have occurred and there was a range of temperatures in the grain bulk (Abdelghany and Fields (2017)). *Cryptolestes ferrugineus* is capable of increasing cold tolerance with an exposure of a few hours to cool temperatures (Burks and Hagstrum, 1999). However, as immature stages are less mobile than adults, the temperatures that the different life stages experienced would be different but there were also some differences. I can think of three possible reasons for the difference seen. Firstly, I tested cold tolerance at -10°C , whereas in the bins used by Abdelghany and Fields (2017) there was a range of cold temperatures reaching only a low of -5°C . Smith (1970) found that for non-acclimated *C. ferrugineus*, the old larva was more cold tolerant than adult at -12°C , but adult and old larva had similar survival at -6°C and 2°C . So, the different low temperatures could be the reason for different survival rates of the different stages. Secondly, the acclimation schedules were different between my experiment and Abdelghany and Fields (2017) and this could effect the cold tolerance of the different stages. Thirdly, my experiments with larva were run in flour and brewer's yeast. Larva could be damaged more than adult when shaken out of the flour and brewer's yeast due to differences in their cuticles. Abdelghany and Fields (2017) were working with natural infestation, so the late instar larva would be inside the kernel protected from any damage due to shaking. Also, the grain in my experiments were shown to increase the cold tolerance of adult and may do so for larva.

There are several possible experiments to determine why there are differences in results between Abdelghany and Fields (2017) and my laboratory studies and others (Fields, 1990; Smith, 1970). Treat the life stages with the same declining temperatures as measured in Abdelghany and Fields (2017) in the laboratory using the flour and brewer's yeast media to determine if the temperatures are responsible for the differences. Test immature insects reared in wheat instead of flour and brewer's yeast to determine if the diet is responsible for the differences. This is complicated because the exact number of insects in the grain is difficult to determine. X-rays could be used to count the insects in the grain before treatment (Haff and Slaughter, 2004) and then the number of adults emerged used to estimate survival after cold treatment. Experiments could be designed with different amounts of disturbance for extracting

egg, larva, pupa and adult stages from flour to determine if that could be a mortality factor that effects the stages differently. Finally, the experiments could be done with *C. ferrugineus*, *C. pusillus* and *C. turcicus* to see if all species are affected in the same manner using the same diet from the grain bins to rear the life stages of all three species of *Cryptolestes*.

The possible reasons for the differences in results compared to other studies might be due to the use of different laboratory strains and laboratory simulation conditions as acclimation happens over different durations when the temperature reduces gradually in the bins while the durations were fixed for 7 days at each acclimation temperature in the laboratory.

It is very common within the stored-product insects that one life stage will be more cold tolerant other stages (Fields, 1992). The following insects have adult as the most cold-hardy stage: *S. paniceum* (Abdelghany et al., 2010), *T. confusum* (Nagel and Shepard, 1934), *S. granarius* (David et al., 1977) and *C. ferrugineus* (Abdelghany et al., 2010; Smith, 1970). For some stored-product insects, immature stages were more cold-tolerant than adult. *Ephestia kuehniella* have pupa as the most cold-hardy stage (Andreadis et al., 2012) but other studies found the larva to be the most cold-hardy stage (Salt, 1953). *Rhyzopertha dominica* (David et al., 1977), *T. granarium* (Wilches et al., 2017), *Trogoderma variabile* Ballion (Coleoptera: Dermestidae) (Abdelghany et al., 2015) and *P. interpunctella* (Fields and Timlick, 2010; Naemullah et al., 1999) have larva as the most cold-hardy stage. It is interesting to note that there are very few examples of stored-product insects that have egg as the most cold-hardy stage like *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae) (Athanassiou et al., 2018).

Effect of Acclimation

In general, in this study exposure to cool temperatures increased cold tolerance but there were varying degrees of increases in cold tolerance depending on the life stages and species. Adult showed more increase in cold tolerance than the other stages, old larva also increased in cold tolerance with acclimation, but the other stages did not. In the diet experiment, the cold tolerance of *C. ferrugineus* increased the most (6 to 9-fold), followed by *C. turcicus* (3-fold) and then by *C. pusillus* (1 to 2-fold). Smith (1970) showed that by acclimating *C. ferrugineus* adult at 15°C for 14 days increased the LT₅₀ by 5-fold. Fields (1992) showed an increase in cold tolerance of adult *C. ferrugineus* using different acclimation schedules by approximately 3 to 5-

fold. He showed that longer acclimation durations that ranged between 15 and 5°C produced greater cold tolerance. Fields (1990) showed increases in cold tolerance by approximately 8-fold for *C. ferrugineus* adult that overwintered in grain bins. Even a brief exposure to cold, 4°C for 2 h, (Burks and Hagstrum, 1999) increases the cold tolerance of *C. ferrugineus* adult by 9-fold. These studies gave similar increases in the cold tolerances as this study.

The differences in results of my experiments and published papers might be due to the differences in treatment temperature and acclimation times. The work by Fields (1990) with natural temperature reductions found in grain bins, compares closely with my studies using 18, 10 and 5°C for 21 days in the laboratory. This suggests that the estimates for what to expect for increases in cold tolerance with acclimation for *C. turcicus* and *C. pusillus* under natural conditions would be similar to what I saw in my laboratory experiments. The stages that were the most cold tolerant when non acclimated, adult and old larva, were also the stages that increased their cold tolerance the most in response to cold acclimation. In the same trend, the species that were the most cold tolerant were also the species that were the most able to increase their cold tolerance in response to acclimation.

Acclimation increased the cold tolerance in stored-product insects by approximately 2 to 10-fold as in *C. ferrugineus*, *E. kuehniella*, *P. interpunctella*, *T. molitor*, *R. dominica*, *O. surinamensis*, *T. castaneum*, *S. oryzae* and *S. granarius* (Burks and Hagstrum, 1999; Fields, 1992). For example, adult *S. granarius* increased cold tolerance by 3 to 4-fold (Fields et al., 1998). *Tribolium confusum* and *O. surinamensis* different developmental stages showed a mixed response to acclimation (Athanassiou et al., 2019), as I saw in my study. *Tribolium confusum* and *O. surinamensis* held at 15°C for 7 d had 4-fold increase in adult survival at low temperatures compared to non-acclimated insects. *Tribolium confusum* pupa did not acclimate. The larvae of *T. confusum* and *O. surinamensis* had a mixed response to acclimation. *Tribolium confusum* egg stage had increased cold tolerance when pre-exposed to cool temperatures but *O. surinamensis* had mortality caused by being at 15°C for 7 d (Athanassiou et al., 2019).

Effect of Food

There was a significant effect of the diet on cold tolerance. The grain diet had the highest cold tolerance compared to the adults reared on flour and brewer's yeast and flour diet. There are

some studies on the effect of diet on growth, development time and preference (Applebaum, 1969; Borzoui et al., 2015; Fedina and Lewis, 2007; Shaurub and Abou Gharsa, 2012). However, there is no work that has been done on the effect of diet on stored-product insect cold tolerance. Flour and brewer's yeast is the most commonly used diet in experiments on *Cryptolestes* spp. (Smith, 1965; Smith, 1970) because it makes it easier to extract different development stages.

There have been a couple of studies on the effect on diet on *Cryptolestes* biology. White et al. (1995a) showed that *C. ferrugineus* and *C. pusillus* populations survived better on wheat than on maize at 30°C; this was attributed to the low nutritional quality of maize compared to wheat. The study also found that interspecific competition suppressed the populations of both species compared to the populations with no interspecific competition. For mixed populations, *C. ferrugineus* did better at 35°C and *C. pusillus* did better at 20°C. Interspecific and intraspecific competition was not studied in my experiments but studying the cold tolerance of the three species when present together can be a useful aspect to understand the effect of competition on cold tolerance and survival.

Cryptolestes turcicus and *C. pusillus* when reared on different diets at 30°C, wheat and cracked food was preferred compared to soybean, wheat, whole corn, peanuts and rice as they were unable to penetrate the seed coat of intact grains (LeCato, 1974). These different diets may also affect cold tolerance. Chang and Loschiavo (1971) tested the development of *C. turcicus* on artificial diets with different concentrations of fungi and found that *C. turcicus* developed the fastest on infested flour and flour containing fungi. Although not examined in my experiments, the presence of other micro-flora and fauna in diet (mostly above 70% r.h.) can influence the survival and development of *Cryptolestes* spp. and many other species of stored-product insects like *S. granarius* (Agrawal et al., 1957; Agrawal et al., 1958), *T. confusum* (Van Wyk et al., 1959) and *O. mercator* (Sinha, 1965). It would be interesting to test if feeding on mouldy grain would increase the cold tolerance of *Cryptolestes* spp. When comparing the experiment 3 and experiment 4 (Diet experiment), *C. pusillus* showed increased cold tolerance in experiment 3 when acclimated and *C. turcicus* showed increased cold tolerance in experiment 3 under both acclimated and non-acclimated conditions.

The presence of food materials act as ice-nucleating agents causing rapid crystallization of the insect body fluids when exposed to cold temperatures. Hence, the insects empty their gut contents when starting to acclimate to lower temperatures before the temperatures reaches lower extremes in winter. Coleman et al. (2015) found that feeding the adult blowfly *Calliphora vicina* Robineau-Desvoidy (Ditera: Calliphoridae) with diet consisting of sugar, water and meat decreases the rapid cold hardening capacity and also increases the lower threshold temperature for insect activity compared to the adults fed a diet of only water and sugar; a protein-based diet decreases cold tolerance and survival while carbohydrate-based diet increases the cold tolerance and survival. This study is a case where the type of diet can influence the cold tolerance of insects. However, it would not be the case in my study as exposure to low temperature, -10°C , was well above the SCP of the insects.

Cryoprotectants concentrations increase with acclimation in *C. ferrugineus* (Fields et al., 1998). In other insects these cryoprotectants are shown to be produced from fat stores in the insect fat body (Joanisse and Storey, 1995). Grain may provide better nutrients, allowing *Cryptolestes* to better produce cryoprotectants needed to survive cold temperatures. Future studies could examine the effect of diet on cryoprotectants, such as proline and trehalose (Fields et al., 1998).

Effect of Species

In general, *C. ferrugineus* is the most cold tolerant, *C. turcicus* has slightly lower cold tolerance and *C. pusillus* was much more cold-susceptible. This is similar to what has been reported in the literature. According to the laboratory study by Smith (1965), *C. ferrugineus* is more cold told tolerant than *C. turcicus*. According to Bahr (1978) and Howe (1965b), *C. pusillus* is more susceptible to cold than *C. ferrugineus* where mortality occurred at 0°C after a short exposure. The experimental conditions such as temperature, r.h. and food may be different in literature but similar results were obtained from this study. Bishop (1959) found that *C. ferrugineus*, *C. turcicus* and *C. pusillus* showed extreme variations in cold tolerance; *C. pusillus* had 100% mortality and *C. ferrugineus* and *C. turcicus* had 12 and 8% mortality, respectively, after several months of cold exposure in the bins. He also found that *C. ferrugineus* was less tolerant to cold than *C. turcicus* when tested before acclimation and was more tolerant after prolonged exposure.

When tested for the influence of diet, *C. ferrugineus* was more tolerant to cold when reared on all three diets, followed by *C. turcicus* and *C. pusillus*. The pattern of cold tolerance remained the same as when the species were tested for cold acclimation on all three diets and SCP with the order of cold tolerance from highest to lowest being *C. ferrugineus* > *C. turcicus* > *C. pusillus*.

Supercooling Points

SCP was affected by the species and developmental stage and acclimation. In general, acclimation was associated with lower SCP, both in the experiments with the different stages and in the experiments with the different diets. Smith (1970) also showed that SCP of adult *C. ferrugineus* was lower due to acclimation changing from -17.9°C to a low of -20.2°C after being held at 15°C for 4 weeks. This increase in acclimation duration also increased the cold tolerance; LT_{50} at -12°C changed from 2 d to 30 d. A similar example can be found in *T. variabile*, old larva had a SCP of -16.4°C when not acclimated and -25.1°C when acclimated (Abdelghany et al., 2015). *Trogoderma granarium* larva had a SCP of -14.4°C when not acclimated and -24.3°C when acclimated in diapause (Wilches et al., 2017). SCP of egg and pupa were lower than the other stages, this could be because these stages do not feed and food can be a source of ice nucleators (Fields and McNeil, 1988; Somme, 1982). For example, in *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) SCP for the various stages are: egg (-24°C), larva (-5 to -16°C), puparium (-18°C) and adult (-12°C) (Ring, 1972).

There was a statistically significant difference in SCP due to diet, but the aggregated means were less than 1°C apart, so biologically there is probably not much significance given the wide range of temperatures that insects can experience in the grain bins (Fields and White, 1997). The SCP of non-acclimated adult for *C. ferrugineus* in this study ranged from -19.4 to -22.9°C and for *C. pusillus* it ranged from -20.1 to -23.5°C which is lower than what was reported in the literature. Smith (1970) observed a SCP of -17.9°C for *C. ferrugineus*, Fields (1992) observed a SCP of -17.0°C for *C. ferrugineus* and -14.0°C for *C. pusillus*. Burks and Hagstrum (1999) measured the SCP of non-acclimated adult *C. ferrugineus* at -20°C and with a short acclimation of 4 h, adult had a slightly lower SCP at -21°C . There are a number of factors that effect SCP (Somme, 1982). The flour or brewers yeast used in my experiments, may have

had fewer ice nucleators than in previous studies. The moisture content of the diet may have been less than other studies, as desiccation has shown that it may lower SCP.

Control Using Low Temperatures

Recommended temperatures and durations to control the different life stages of *Cryptolestes* spp. are given. Without acclimation, for example through the months of June until August, the insects can more easily be controlled with low temperature. The problem with this approach is that cooling the grain artificially (Maier and Navarro, 2001) is expensive and slow. Ambient air (Jayas and White, 2003) cooling of grain is more affordable, but then this only becomes available in the fall, when the cool grain will have allowed some acclimation of the insects. *Cryptolestes turcicus* being the most tolerant when not acclimated required 691 h (29 d) to reach 95% mortality followed by *C. ferrugineus* which required 572 h (24 d) to reach 95% mortality at -10°C . The more probable case is to assume that the insects will be acclimated, in which case, the adult of *C. ferrugineus* which require 1384 h (58 d) to reach 95% mortality followed by *C. turcicus* and *C. pusillus* which requires 943 h (39 d) and 257 h (11 d) to reach 95% mortality should be used to guide durations needed. These recommendations are in agreement with the recommendations to control *C. ferrugineus* given by the Canadian Grain Commission: -5 , -10 , -15 and -20°C for 12, 8 (1344 h), 4 or 1 weeks respectively. Additional work is needed to estimate the durations needed at temperatures above the SCP to control different insects. This additional work could then be used to build a model (Jian et al., 2007) to predict overwintering mortality of the different species. This model could then be verified with overwintering experiments in grain bins.

The SCP provides a temperature at which insects die instantaneously. There are only slight variations for the lowest SCP for all stages for a given species and -27°C would be cold enough to freeze any of the life stages, acclimated or non-acclimated for all three species of *Cryptolestes*.

Recommendations and Conclusions

As many other studies did, this study did not consider the interspecific competition between the three species when present together. In particular, *C. ferrugineus* and *C. pusillus* may be found in the same grain bin (White et al., 2011). All these experiments used vials

containing one species and developmental stage to test cold tolerance. Studies with interspecific competition could change the prediction for cold related mortality in grain bins.

The use of ice-nucleating agents has demonstrated to be an effective way to increase the supercooling point of stored-product insects and specifically in *C. ferrugineus* (Fields, 1990). The ice-nucleating bacteria, *P. syringae* reduced the SCP and hence the cold tolerance of unacclimated adult *C. ferrugineus* decreased from -17°C to -8.1°C (Fields, 1990). Research on cold hardiness using ice-nucleating bacteria to control *C. pusillus* and *C. turcicus* may prove as useful resource for pest management strategies.

The temperatures used in the experiment are simulated cooling of grain in the fall and they may not be the exact temperatures the bins are exposed to in the fall and winter with changing weather patterns, bin sizes and aeration. With global warming and climate change increasing temperatures may cause significant changes in the overwintering survival of insects and so, more consideration should be given to cold tolerance studies in the future according to the changing climatic conditions.

Rapid cold hardening has shown to be present in stored-product insects like *C. ferrugineus*, *O. surinamensis*, *R. dominica*, *S. oryzae* and *T. castaneum* (Burks and Hagstrum, 1999). The temperature profile of grain is quite variable due to aeration, bin size and weather. Further work with all three species of *Cryptolestes* could increase the accuracy of prediction for low temperature mortality. Given there is considerable variation in cold tolerance due to diet, acclimation, species, stage, it would be interesting to determine if these factors also effect the level of cryoprotectants, as seen with *C. ferrugineus* and *S. granarius* (Fields et al., 1998).

This thesis has demonstrated differences in the effect of three different species of *Cryptolestes*, their life stages, acclimation and diet. Effective reduction in the population of *Cryptolestes* can be achieved by implementing cold temperature treatment with IPM and clean grain handling practices. More work needs to be done on the different acclimation temperatures/ regimes and durations of exposure, validating the laboratory experiments in the field and modeling, testing different cereals and using artificial diets with known compounds can help to find the relationship of diet and cold tolerance of the three species of *Cryptolestes*.

Appendices

Appendix 1. Durations at –10°C for insects that were held under non-acclimation and acclimation conditions for the three species of *Cryptolestes* and different stages, reared on flour and brewers’ yeast (95:5).

Species	Stage	Durations at –10°C (h)					
		Experiment 1		Experiment 2		Experiment 3	
		Non-acclimation	Acclimation	Non-acclimation	Acclimation	Non-acclimation	Acclimation
<i>C. ferrugineus</i>	Egg	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36
	Young larva	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36
	Old larva	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168
	Pupa	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 36, 48, 60, 72	0, 24, 48, 72, 120, 168
	Adult	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 168, 336, 504, 672, 840
<i>C. turcicus</i>	Egg	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36
	Young larva	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36
	Old larva	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36
	Pupa	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36
	Adult	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 3, 6, 12, 24, 36	0, 24, 48, 72, 120, 168
<i>C. pusillus</i>	Egg	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 0.5, 1, 1.5, 2, 2.5, 3	0, 0.5, 1, 1.5, 2, 2.5, 3	0, 0.5, 1, 1.5, 2, 2.5, 3	0, 0.5, 1, 1.5, 2, 2.5, 3
	Young larva	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 0.5, 1, 1.5, 2, 2.5, 3	0, 0.5, 1, 1.5, 2, 2.5, 3	0, 0.5, 1, 1.5, 2, 2.5, 3	0, 0.5, 1, 1.5, 2, 2.5, 3
	Old larva	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36
	Pupa	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36
	Adult	0, 24, 48, 72, 120, 168	0, 24, 48, 72, 120, 168	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36

Appendix 2. Durations at –10°C under non-acclimation and acclimation conditions for different diets, Experiment 4.

Species	Diet*	Durations at –10°C (h)	
		Non-acclimation	Acclimation
<i>C. ferrugineus</i>	Grain	0, 24, 48, 72, 120, 168	0, 168, 336, 504, 672, 840
	Flour + BY	0, 24, 48, 72, 120, 168	0, 168, 336, 504, 672, 840
	Flour	0, 24, 48, 72, 120, 168	0, 168, 336, 504, 672, 840
<i>C. turcicus</i>	Grain	0, 3, 6, 12, 24, 36	0, 24, 48, 72, 120, 168
	Flour + BY	0, 3, 6, 12, 24, 36	0, 24, 48, 72, 120, 168
	Flour	0, 3, 6, 12, 24, 36	0, 24, 48, 72, 120, 168
<i>C. pusillus</i>	Grain	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36
	Flour + BY	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36
	Flour	0, 3, 6, 12, 24, 36	0, 3, 6, 12, 24, 36

*Grain diet consisting of whole wheat kernels, cracked wheat kernels and wheat germ (90:5:5, proportions); wheat flour and brewer's yeast (BY) diet (95:5, proportions) and straight wheat flour diet.

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