Potential Use of Liquid Nitrogen Aeration on Wheat Disinfestation

by

Hai Yan Li

A Thesis submitted to
the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Biosystems Engineering
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ABSTRACT

A study was undertaken to investigate the effect of liquid nitrogen aeration on mortalities of adult *Cryptolestes furrugineus*, rusty grain beetles, in a prototype cardboard grain bin equipped with an aeration system. The grain bin was filled with Hard Red Spring wheat and liquid nitrogen was introduced from the bottom of the bin. The survival of both cold acclimated and unacclimated *Cryptolestes furrugineus*, rusty grain beetles, was tested. Caged insects were placed at different heights equally spaced at 50 cm along the grain depth and mortalities were analyzed after aerating the bin with liquid nitrogen. Samples of grain were collected at each layer and were analysed for any changes in moisture content and to determine any loss in germination.

Cold acclimated insects had higher survival than unacclimated insects under similar cooling conditions. In most cases, mortalities of as high as 100% were achieved at the bottom 100 cm of the grain bin for unacclimated insects for most of the trials. Insect survival increased as the distance from the bottom of the grain bin increased. Grain moisture decreased by 1.9% and was considered statistically significant. There was no adverse effect of liquid nitrogen aeration on wheat germination.

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my sincere gratitude to my advisor, Dr. Jitendra Paliwal, for his supervision and constant encouragement during my thesis work.

Thanks are also due to my advisory committee members. I am thankful to Dr. N. D. G. White for providing me with the insect samples and letting me use the facilities of his lab. I thank Dr. D.S. Jayas and Dr. Paul Fields for their valuable suggestions for my experiments.

Mr. Dale Bourns and Mr. Matt McDonald offered me technical assistance that was instrumental to the completion of this project. I thank them for their efforts.

I would also like to take this chance to thank my friend Wenbo who offered me generous emotional support that greatly helped me along the path.

Finally, I would like to thank my parents, to whom I owe everything. Whatever I have achieved in my life is because of their affection and sacrifice.

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1. INTRODUCTION

1.1 Grain Storage Losses

Cereal grains represent a major component of food supply for humans and most domesticated animals in the world. Canada produces 57.8 Mt (million tonnes) of grain annually including durum wheat, winter and spring wheat, oats, barley, flaxseed, canola, soybeans and corn (Canada Grains Council 2003). Canada is the second largest exporter in the world, with more than 50% of the grains produced are exported every year (Muir and White 2001). Grains are usually stored on the farm in Canada and move to terminal elevators for shipment to domestic or export markets. However, consumption of cereal grains by insects, mites, rodents and microbial spoilage or contamination during storage cause large grain losses in term of both quality and quantity. It is estimated that annual losses of cereal grains caused by insects and rodents are about 10% of the total in North America and 30% in Africa and Asia (Hill 1990). Zero tolerance for live insect pests in grain bulks is required by the Canada Grain Act and has earned Canada a worldwide reputation in providing good consistent end-use quality grains and oilseeds each year. Therefore, maintaining good grain quality and preserving grain from infestation is a prime task for the Canadian grain handling and export industry.

1.2 Current Disinfestation Methods and Trends

The various methods to control stored-product insects can be classified into three main categories: biological, chemical, and physical methods.

1.2.1 Biological methods

Insect infestation has been a problem in stored-grain ecosystems since the beginning of human civilization. Interestingly, not only grain-eating insects are found in stored grain,

the habitat-specific and host-specific natural enemies that prey on these pests as their food are also found in this ecosystem. Biological control method refers to the utilization of natural enemies to control stored-product insects. These natural enemies can be classified into three categories: parasitoids, predators and pathogens. Parasitoids are insects with an immature stage that develop on or in the host insects, and ultimately kill the host (Muir and White 2001). The hosts' life cycle, physiology and defenses are important factors that influence the survival rate of these parasitoids. Therefore, many parasitoids are always limited to one or a few closely related host species. Many kinds of predators feed on insects that are usually smaller than themselves. Lady beetles, ground beetles, rove beetles, hover flies and predatory true bugs are some of the important insect predators (Weseloh and Andreadis 1992). Like other animals and plants, insects are infected by pathogens (such as fungi, protozoans, bacteria, and viruses) that cause diseases which may reduce their growth and feeding rate, or kill them.

The earliest biological control method had been implemented to control Mediterranean flour moth using parasitoid wasp *Bracon hebetor* in a warehouse in 1887 in London (Bower et al. 1996). Weseloh and Andreadis (1992) reported that fungus *Entomophaga maimaiga*, a pathogen of the gypsy moth, has been used to control New England gypsy moth populations successfully. Certain parasites, predators and pathogens of storage pests are notable for their potential as biological control agents in stored-grain ecosystems. Many researchers have reviewed and explored the potential use of biological controls in stored-products ecosystems (Arbogast, 1984; Brower and Mullen 1990; Brower et al. 1996). Bower et al. (1996) summarized various beneficial species that have been investigated for stored-grain insect control. However, the control agents are not only

species specific but also unsuitable for dealing with heavy infestation. In addition, implementation costs limit the application of biological controls in many grain storage systems. Therefore, biological controls are not normally considered to be comparable with chemical and physical methods. However, as additional research is conducted and new data become available, new possibilities for biological insect control could arise for specific situations.

1.2.2 Chemical methods

Chemical methods of insect control (such as fumigants and contact insecticides) have been used as a primary tool for storage management for many years. Fumigation is widely used around the world to treat insect infestation of stored grain. The origin of fumigation goes back to 2000 – 1000 BC in Egypt. Fumigation has been used in Canada since 1950. Fumigants act on all life stages of insects by diffusing through spaces between grain kernels as well as into kernels themselves, which enable fumigants to be applied in places where application of contact insecticides is impractical (Harein and Subramanyam 1990). Phosphine and methyl bromide are commonly used as fumigants.

Phosphine (PH_3) is a colourless and tasteless gas with low molecular weight (only 1.2 times heavier than air) which makes it an ideal fumigant for stored grain (Muir and White 2001). It has been used as a fumigant in stored grains and other commodities worldwide for more than 40 years (Chaudhry 1996). The basis of phosphine toxicity towards insects is that the oxidation of phosphine prevents electrons from being transferred from oxygen to the body which eventually becomes lethal for insects and rodents by interrupting their respiration (Chaudhry 1996; Muir and White 2001). Phosphine diffuses quickly, mixes with air readily, and penetrates grain more quickly than any known fumigant, and also

can be aerated immediately to exhaust residual phosphine gas efficiently (Muir and White 2001). However, longer time is needed to eliminate target pests using phosphine compared to other fumigants. Exposure time usually ranges from 3 to 7 d and more time is required as temperature decreases (Muir and White 2001). Phosphine causes serious corrosion problem to copper wire, electronic motors, and switches. Like other fumigants, it is highly toxicity to mammals. Finally several species of stored-product insects have developed widespread resistance causing control failures (Chaudhry 1996).

Methyl bromide (CH_3Br) is an effective fumigant used to control the development of all stages of insects in stored grain (Harein and Subramanyam 1990), is a colourless gas that is packed in liquid form in pressurized steel bottles. Once the container is open, methyl bromide changes into a gaseous state at temperatures above 4°C, i.e., its boiling point. Methyl bromide is odorless, so sometimes it is formulated with chloropicrin to make its detection easy (Muir and White 2001). Its success as a fumigant is mainly because of its ability to penetrate containers in either soil or during shipping (Stamopoulos 2005). Short exposure time of methyl bromide is enough to attain effective fumigation due to its high toxicity and penetration ability in commodities at ambient temperatures and pressures (Muir and White 2001). In addition, there has been little resistance that insects have developed against methyl bromide so far (Stamopoulos 2005), which is a great advantage compared to other fumigants. However, methyl bromide was identified as an atmospheric ozone depleting compound with ozone depletion potential (ODP) of 0.4 in 1991 (Stamopoulos 2005). The depletion of O_3 in the atmosphere allows ultraviolet radiation to reach the earth and become a potential hazard for human beings. For this reason, the amount of methyl bromide used for fumigation is gradually reduced

and it will completely be banned in the near future (Jayas 2000). In fact, methyl bromide has been phased out on January 1, 2005 in developed countries, except for a few exemptions. The phase-out of methyl bromide will take effect in 2015 in developing countries.

Contact insecticides are another widely used chemical methods. Chemicals including bioresmethrin, bromophos, carbaryl, malathion, pyrethrins etc. are currently used to treat stored grain around the world. Contact insecticides are not effective unless insects come into contact with the chemicals directly. They pose little risk to the person who applies these insecticides to stored products because chemicals are handled in non-gaseous state. The residual effect of these insecticides can prevent future migration of insects into the granary, which is an advantage over fumigants. On the other hand, this residual effect can also be a disadvantage because these residual chemicals can contaminate food (Muir and White 2001). Singh et al. (1993) noticed that some insecticides could gradually bind with seeds and not be detected by chemical analysis. These residual insecticides could become biologically active in mammals who consume these seeds.

Malathion is a main residual contact insecticide in Canada which is applied either as liquid or dust. Over 50% mortality of *Tribolium confusum* was achieved in 33 weeks by applying 1.5 g/m^2 malathion to dust-covered concrete floors in terminal elevators (Watters 1970). The resistance to malathion developed by stored-grain pests is widespread due to its frequent use. Therefore, the use of malathion on food grains is not common in Canada and USA, even though it has low mammalian toxicity (Muir and White 2001).

Chemical insecticides are widely used because they are inexpensive and fast-acting. However, chemicals, not only pose health risks to people who apply them, but can also pose risk to humans and animals who consume the chemically treated grain (Muir and White 2001). In addition, the frequent use of chemical insecticides and fumigants helps insects develop resistance, making higher doses of these chemicals necessary. With consumers beginning to become more concerned about insecticide residues in foods, many markets no longer accept grain that has been treated with chemical insecticides. As a result, alternate control methods need to be developed and applied.

1.2.3 Physical methods

Concerns about chemical residues left in foodstuffs, development of insect resistance, and the restricted use of the fumigant methyl bromide in developed countries, have led a movement away from chemical methods to physical methods of disinfestations. Physical controls, one of the oldest methods used by man for controlling insects, are once again gaining a high interest for disinfestations of stored-products. Physical control methods are based on the ecology of stored-product insects, and the realization that biological limitations exist for all insect species. These methods including manipulation of the physical environment, like temperature, composition of atmospheric gases and moisture content; mechanical impact, inert dusts and ionizing radiation, are used to modify the environment to such a degree that it becomes lethal to the stored-products pests.

1.2.3.1 Extreme temperatures

Lately, interest has reverted to modification of grain temperatures in order to protect stored-grain. Temperature between 25 and 33°C with relative humidity of 65-75% are the ideal conditions for most stored-product insects. Outside of these optimal environmental

conditions, the growth and fecundity of insects is reduced and they eventually die in extreme conditions. High temperatures (40-65°C) are lethal to stored-product insects. There are several high temperature methods used in stored-grain, such as fluidized bed heating, microwaves, and high frequency electric fields. Most of these methods are applied to raise the grain a temperature to about 60°C with a short exposure time (i.e. a few seconds) (Locatelli and Traversa 1989). Rapid cooling is recommended after high temperature treatment to reduce the risk of damage to the grain including germination and baking quality. However, all the high temperature methods are still at pilot or laboratory levels except fluidized bed heating.

The low temperature control method has been used either to disinfest stored products or to prevent products from insect infestation. Low temperature control method has several advantages over chemical methods. Unlike the contact insecticides, low temperature control method has no residue left on the products after treatment. There are fewer risks to the operators, whereas safety is a huge concern during the fumigation process (Garry et al. 1989). Low temperature control methods are more suitable to disinfest stored products because they are effective against pesticide resistant populations and against mould growth (Fields 1990).

Turning, ambient air aeration, and chilled air aeration are three low temperature control methods. Turning can break up "hot spots" but leave the mean temperature of grain largely unchanged (Fields 1995). Grain temperature can be brought to the average air temperature by using ambient aeration. However, this method depends heavily on the availability of cold ambient air. Chilled-air aeration has the potential for solving problems in which cold temperature is used to disinfest stored-products, but ambient

temperature is not cold enough (Hunter and Taylor 1980; Fields 1992; Fields and Muir 1995). These warm conditions are commonly found in tropical areas or in temperate regions during summer time. However, due to non-availability and costs associated with refrigeration equipment, extensive use of this technology by the agricultural industry is not practical. New low temperature control methods need to be explored to overcome these weaknesses in order to kill insects economically and effectively.

1.3 Liquid Nitrogen Aeration System as a Viable Alternative

Liquid nitrogen is widely used in the medical and seed storage field for its extremely low temperature. However, little work has been done on the use of liquid nitrogen as a low temperature agent to disinfest stored-products. It is, therefore, of interest to explore how effective liquid nitrogen is as a disinfestations agent. The objectives of this study were:

1. to determine the effect of a liquid nitrogen induced cooling front on grain bulk temperature;
2. to study the effect of liquid nitrogen aeration on mortalities of rusty grain beetle adults in a grain bulk; and
3. to study the effect of liquid nitrogen aeration on grain germination.

2. LITERATURE REVIEW

2.1 Low Temperature Control Methods

2.1.1 Principle of low temperature disinfestation

Environmental temperature determines the body temperature of insects and thereby affects many of their biological processes. In assessing the potential significance of low temperature control methods on mortality of stored-product insects, the ability of insects to survive the physical changes at low temperatures is a major consideration. There are many aspects that contribute to the death of insects at low temperatures.

Insects face the challenge of potential ice formation at sub-zero temperatures. Most insects die when they are frozen. Ice does not form immediately at temperature below zero. Indeed insects begin to supercool when the temperature falls below the melting point of their body fluids (Fig.1). The bold line in Fig. 1 indicates insect body temperature, in relation to the melting point of body fluids, the supercooling point and the temperature at which internal ice forms. The right side of the figure indicates the general temperature ranges for different categories of insect response to low temperature. The ice begins to form until the temperature reaches the limit of supercooling (Solomon and Adamson 1955; David and Richard 1998). Supercooling point, the temperature at which water in the insect crystallizes to ice, occurs at various temperatures after a certain time of exposure which usually varies among species and individuals of a certain species (Salt 1950; Fields 1992). Species, stage of development, gender, temperature, exposure time, relative humidity and acclimation are the main factors that determine the survival of insects at low temperature (Solomon and Adamson 1955; Fields 1992). Another explanation is that when insects are exposed to cold, their movement becomes slower

than at normal temperature. They may be reduced to a chill coma state and all movement ceases and the metabolic rate is reduced to an extremely low level. With long exposures, insects, which escape from the direct impact of internal tissue freezing, will die of starvation if the temperature does not rise before their reserves are exhausted (Solomon et al. 1955; Fields and White 1997). Therefore, for a certain type of insect, exposure temperature and duration are the most important factors that determine their survival rate in cold environment.

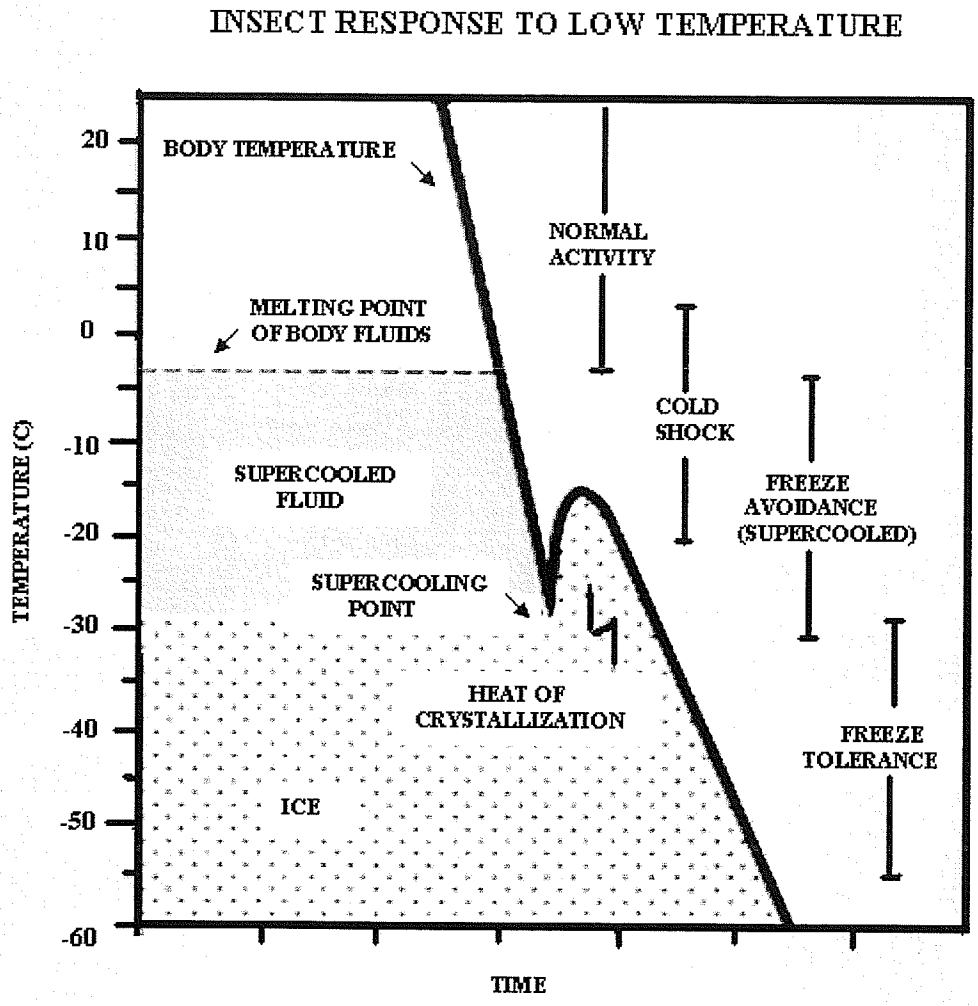

Fig. 1. Response of insects cooled to low temperature (Lee 1989)

2.1.2 Response of stored-product insects to temperatures

Cooling has been used to control stored-product insects for several decades (Fields 1990; Fields 1992; Fields and Muir 1995). Cooling temperature usually ranges from -25 to 25°C. Several researchers have studied and described the effect of low temperatures on stored-product insects (Howe 1965; Lhaloui et al. 1988; Fields 1992). Fields (1992) incorporated the response of insects to temperatures by listing temperatures and times necessary for disinfestations in stored-products as shown in Table 1. The optimal temperature for maximum rate of development of stored-product insects is between 25 and 33°C. Temperatures between 13 and 25°C will slow insect development, whereas insects will eventually die at temperatures between -25 and 5°C (Fields 1992).

Table 1. Response of stored-product insects to temperature (Fields 1992)

Zone	Temperature Range (°C)	Effects
Lethal	-25 to -15	Internal freezing, death in minutes
	-10 to -5	Death in weeks, or months (acclimated)
	5	Death in days (unacclimated), movement ceases
Sub-optimum	13 to 25	Slow population increase
Optimum	25 to 33	Maximum rate of population increase
Sub-optimum	33 to 35	Slow population increase
	35	Maximum temperature for reproduction
Lethal	45 to 50	Death in a day
	50 to 60	Death in minutes

Several studies have examined the cold-tolerance of many insect species under low temperature conditions. Early work by Howe and Hole (1968) revealed that up to 84% mortality was achieved by transferring immature stages of *Sitophilus granaries* (L.) to 15°C after 3-4 wk exposure at 25°C. Brokerhof et al. (1992) found that 99.99% mortality was achieved by exposing the egg stage of *Tineola bisselliella* to -20°C for 15 h. Fields et al. (1998) reported that *C. ferrugineus*, a primary storage pest in Manitoba, had an 90 LT

(lethal time for 90% of the population) of about 40 d at -10°C with full acclimation. Additional work by Fields and White (1997) dealt with the multiplication and survival rate of four insect species (*C. ferrugineus, Cryptolestes pusillus, Tribolium castaneum* and *Rhyzopertha dominica*) under cold temperatures. In the laboratory environment, they found that survival of *C. ferrugineus* was 40% when temperature declined from 25 to 10°C over 10 mo, whereas at 25 declining to 0°C over 10 mo survival was 7%. *Cryptolestes pusillus* and *T. castaneum* did not survive once temperatures were below 10°C, whereas *R. dominica* adults did not survive temperatures below 3°C. No survival of *C. ferrugineus* was observed in granaries that had February temperature of -6.7°C or lower.

These observations demonstrate that, if stored-products are held long enough, low temperature control in granaries will disinfest the product. Currently, there are three low temperature control methods in use: turning the grain, ambient aeration and chilled aeration.

2.1.3 Overview of low temperature control methods in the agriculture industry

2.1.3.1 Turning

Moving grain from one bin to another (Fields 1995) or mixing warm grain with cooler grain (Mason and Strait 1998) can help to eliminate hot spots. As noted by Watters (1991), turning can also be used to achieve insect control to some degree. However, the mean temperature of grain is left largely unchanged after turning. Additional costs associated with turning include loss of quality of grain due to breakage, energy, and labour required to turn the grain (Fields 1995; Mason and Strait 1998). Turning is not an effective way to disinfest grain.

2.1.3.2 Ambient aeration

A stored grain bulk is a man-made ecological system in which interactions among physical, chemical and biological factors determine the deterioration of stored-products. The storage life of grains depends mainly on two factors: temperature and moisture content (White 1995). Aeration is the forced movement of ambient air through a grain bulk to bring the grain to a uniform temperature, usually within a few degrees of the ambient temperature (Fields 1995; Mason and Strait 1998; Jayas and Muir 2002; Navarro et al. 2002). It is one of the safest and most widely used technologies for preserving stored-products without any use of chemicals. Aeration with ambient air can be used to cool the grain to the ambient temperature to reduce or stop its deterioration (Jayas and Muir 2002). For most cereal grain, the intergranular void volume is about 35-55% of the grain bulk volume (Navarro et al. 2002). This porous nature of bulk grain allows forced air to contact almost all grain kernels. The low thermal conductivity of the gain bulk enables maintenance of this modified microclimate once grain is cooled, which is another factor that makes aeration is practically useful. The basic components for aeration system are simply to provide a bin with a fully or partially perforated false floor, or with perforated in-floor or on-floor ducts through which air can be blown into the grain; a fan connected to the duct or plenum system to force the air through the grain; and one or more roof vents for intake or exhaust of the air (Fig. 2) (Jayas and Muir 2002).

Aeration fans can be used to blow air through a grain bulk either by pushing air up from the bottom or by pulling air from the top. However, not all the grain cools at the same time when the fan is switched on. Instead, a cooling front (temperature front) begins to form where the air enters the grain bulk, and the grain is gradually cooled in level as

the cooling front moves forward creating distinct zones of cooled grain, cooling grain, warm grain in the bulk (Muir and White 2001; Jayas and Muir 2002). Navarro et al. (2002) reported that under optimal environments insect population increases rapidly, which may result in the unsaleablility of a commodity. Cooling grain quickly using aeration can reduce the grain temperature below the developmental temperature of insects suppressing insect development. The use of ambient-air aeration to disinfest grain successfully has been reported by several researchers (Navarro 1974; Burges and Burrell 1964; Foster and Mckenzie 1979; Sinicio and Muir 1998). Navarro (1974) studied the possibilities of cooling grain bulk using aeration with ambient air to suppress stored-grain insect development. They aerated a wheat bulk of 1142 tonnes with ambient air for about 22 mon to reduce grain temperature from 32.2 to 10.5°C without any insect development. Sinicio and Muir (1998) studied the effect of ambient aeration on preventing spoilage of wheat stored in round bins and large horizontal storages under tropical and subtropical climatic conditions in Brazil. They found that aeration can successfully prevent wheat from deterioration at a maximum period of 12 mon and 3 mon for round bins and horizontal grain storage structures, respectively. Unfortunately, the use of ambient-air aeration is weather dependent. In some regions cool days or nights are not long enough for the grain to be cooled adequately (Mason and Strait 1998). In a study conducted in the mid-western United States in 1994, Maier et al. (1996) demonstrated that, due to the fact that the ambient temperature was not below the target of 15°C for long time periods, maintenance of grain temperature below 15°C to control *Sitophilus zeamais* sufficiently would have been impossible with ambient aeration. Therefore, ambient aeration is always

perceived as a secondary strategy after chemical treatment for insect control in stored grain, especially for stored wheat (Reed and Harner 1998).

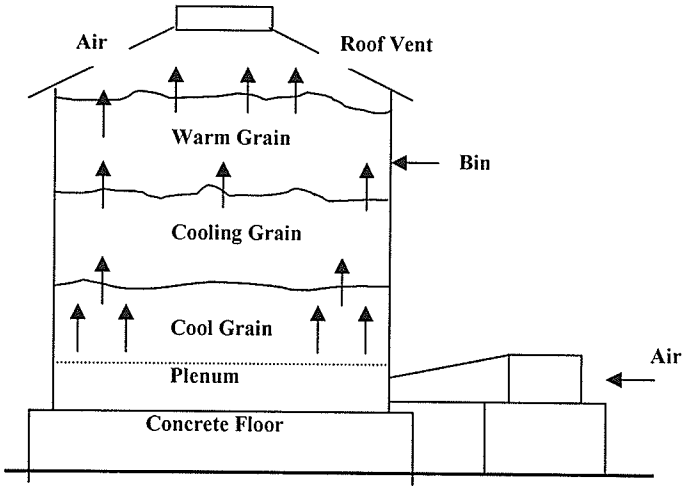

Fig. 2. Components of a typical aeration system and the movement of the cooling front

2.1.3.3 Chilled aeration

When grain temperature could not be sufficiently reduced by ambient aeration, chilled aeration is a viable pest management alternative. In this type of aeration process, ambient air is cooled by an evaporator coil, and then passes through a secondary reheating coil where it is reheated a few degrees to decrease the relative humidity. The chilled air is then blown through the grain bulk via the existing aeration system (Mason and Strait 1998). The major advantage of chilled aeration over ambient aeration is that grain can be cooled by a refrigeration system that controls both the temperature and relative humidity of the airflow independent of ambient air conditions (Mason and Strait 1998). In addition, cold-acclimation of insects can be prevented by cooling grain rapidly under warm weather conditions; as a result, chilled aeration can achieve higher mortality than ambient

aeration, especially during the fall (Mason and Strait 1998). Satisfactory insect control using chilled aeration in Texas was reported by Maier et al. (1992). Mason et al. (1997) and Montross (1999) compared chilled aeration with ambient aeration in granaries, and they found that chilled aeration suppressed insect population more effectively with fewer insects surviving during the treatment.

Various chilling units have been developed and utilized throughout the world, including in Great Britain, United States, Israel and Australia (Maier et al. 1996, Mason et al. 1997; Maier and Navarro 2002). However, major disadvantages of chilling equipment that have not been addressed over decades of evolution are the high capital and operating costs which prevent the extensive use of this technology worldwide.

A thorough review of literature indicates that turning, ambient aeration, and chilled aeration have their own constraints which prevent their extensive use in the stored-grain industry. Turning leaves grain average temperature unchanged and is not lethal for insects. Ambient aeration relies much on weather conditions. The high cost associated with chilled aeration restricts its use in granaries. As a result, an alternative method needs to be developed.

2.2 Overview of liquid nitrogen

2.2.1 Properties of liquid nitrogen

Nitrogen makes up the major portion of the atmosphere (78.03% by volume, 75.5% by weight). Liquid nitrogen can be obtained by compressing nitrogen gas and it is stored in cylinders equipped with safety relief valves. Products can be withdrawn either as liquid or gas. Liquid nitrogen has a boiling point of -195.8°C and is colorless, odorless, non-corrosive, non-flammable, and extremely cold. Although nitrogen is non-toxic and inert,

it should be handled carefully as it can cause suffocation by displacing oxygen and may be fatal.

2.2.2 Use of nitrogen as asphyxiant

Low oxygen atmospheres have been used for preserving stored-product quality as an alternative to chemical fumigation. Blanchard et al. (1996) reported effective control of microbes in freshly prepared diced yellow onions by using a continuous stream of nitrogen containing ($\%O_2/\%CO_2$): 20/0, 2/0, 2/5, 2/10 and 2/15. Williams et al. (1980) studied the effect of nitrogen on controlling insects in wheat within a bolted galvanized iron silo (21.5 t). Oxygen level was reduced to <1% by purging with nitrogen and this level was maintained by a slow nitrogen bleed for 35 d. They found that all adult insects were dead, but some immatures survived due to the short period of exposure. They recommended a high level of gastightness to achieve efficient disinfestations using nitrogen. The cost associated with sealing bins and labor can be very high, up to $19.16 per ton as reported by the authors.

2.2.3 Use of liquid nitrogen based on its low temperature

The use of low temperatures from liquid nitrogen (-196°C) to store various seeds without any adverse effects on germination or seedling development has been reported by several researchers (Lipman 1936; Lockett and Luyet 1951). Barnabas and Rajki (1976) and Crisp and Grout (1984) examined the effect of liquid nitrogen on storage quality of pollen. They found that maize and broccoli pollen after cryopreservation storage remained viable and fertile when kept at -196°C. However, Crisp and Grout (1984) observed that seeds produced by broccoli pollen stored in liquid nitrogen lost germinability rapidly. The result alerts us to be more concerned about the adverse effects that may be caused by low

temperature. One use of liquid nitrogen in the medical area is in cryosurgery. Research has been conducted to treat chromonycosis, a chronic fungal infestation of the skin, using liquid nitrogen. Lubritz and Spence (1973) and Castro et al. (2003) reported cases of chromonycosis cured successfully by cryosurgery with liquid nitrogen.

Lewis and Haverty (1996); Rust et al. (1997) and Taylor et al. (1997) reported the use of low temperatures from liquid nitrogen to kill wood-destroying insects. Little research has been done to investigate the use of liquid nitrogen to disinfest agricultural products. In a report on a specific case, Kovach et al. (2006) used liquid nitrogen to kill chalcid wasps in coriander seeds. They placed seeds above liquid nitrogen for 16 h and concluded that liquid nitrogen was effective in killing all life stages of chalcids without reducing seed germination.

2.2.4 Potential use of liquid nitrogen to disinfest stored-grain

Wide use of liquid nitrogen is based on two principles: low temperature and low oxygen environment. As discussed in Section 1, low temperature environment can be lethal for stored-product insects. Therefore, it can be assumed that liquid nitrogen can be used to disinfest stored-grain.

The use of liquid nitrogen in conjunction with aeration systems to disinfest grain is a promising method. It offers many advantages over chilled aeration. Nitrogen is abundant in the atmosphere as it makes up 75.5% of the atmospheric air by weight. The cost of liquid nitrogen equipment plus the cost to compress nitrogen gas are much lower than chilled aeration equipment. Combining ambient aeration with liquid nitrogen aeration can further reduce the cost. In addition, insects are confronted with the challenge of freezing,

as well as the gradually decreased oxygen level in the environment which will accelerate their death rate.

3. MATERIALS AND METHODS

3.1 System Design

The entire liquid nitrogen aeration system consisted of an insulated cylindrical cardboard grain bin, an aeration fan, a data acquisition system, a temperature control device, a liquid nitrogen tank, and an electronic weighting scale (Fig. 3). The cardboard grain bin was filled with red hard spring wheat (cultivar AC Barrie) grown in Manitoba in 2006. The moisture content of wheat was measured using ASAE standard S352.2 and found to be 11% (wet basis). An aeration fan was used to blow liquid nitrogen into the grain bin more rapidly and uniformly during the experiments and to achieve uniform grain temperature during experiment. The liquid nitrogen tank was placed on an electronic weighting scale and this scale was used to measure the weight loss rate of liquid nitrogen. A temperature control device was connected with the grain bin to control the inflow of liquid nitrogen.

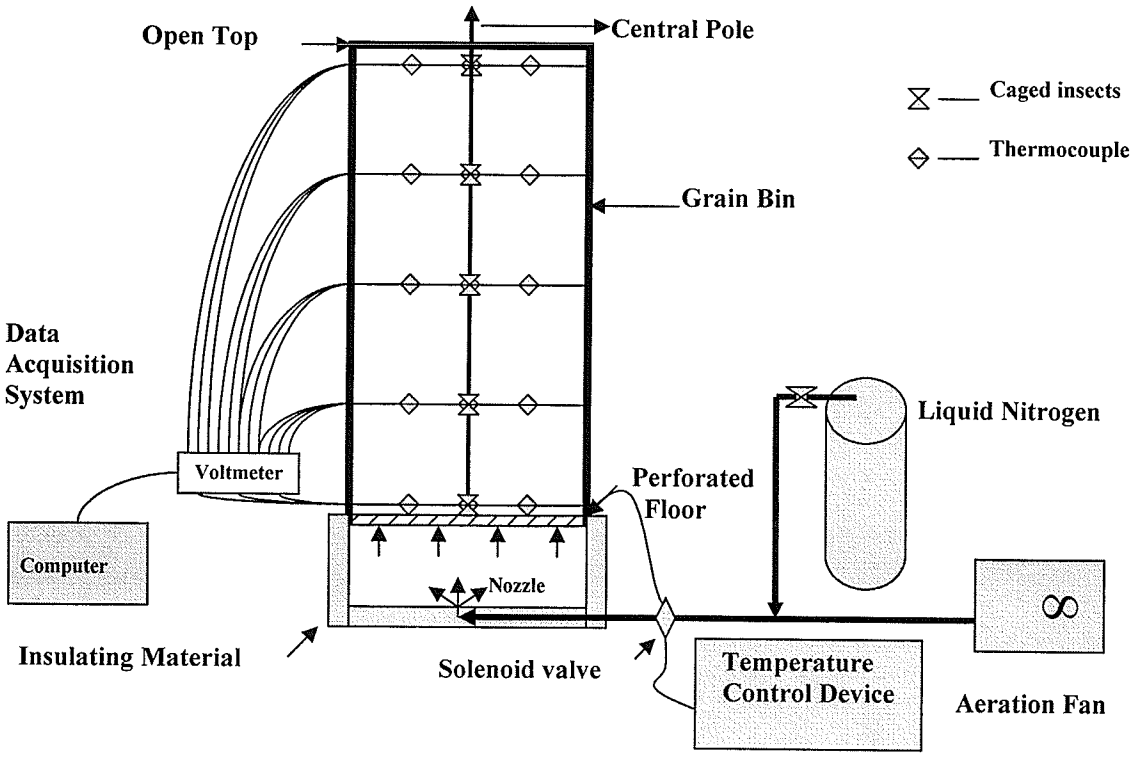

Fig. 3. Schematic of liquid nitrogen aeration system

A data acquisition system was used to monitor and record the temperature changes throughout the grain bin and liquid nitrogen consumption.

3.1.1 Insulated cylindrical cardboard grain bin

The grain bin consisted of two parts: the bin body and its foundation. The body of the bin was cylindrical and made of cardboard. It was 2 m in height and 0.61 m in diameter. The bin space was divided into four vertical zones by five levels of thermocouples. Each level was equally spaced 0.50 m apart from its adjacent level(s) along the vertical direction. Four sampling holes, through which grain samplers containing caged insects (Fig. 4) were inserted into the grain bin, were drilled at heights of 0 m, 0.5 m, 1.0 m, and 1.5 m separately from the bottom of the bin. Because it was convenient to bury the grain sampler in grain sample at the top level, i.e., the level at the height of 2.0 m, no sampling hole was drilled. Plastic tubes with a diameter of 38 mm and rubber stoppers were installed on the entrance of sampling holes. Five pieces of straight copper wire were used to bind the thermocouples to monitor the temperature changes along the grain samplers. The copper wires started from 5 mm below the sampling hole position, ran horizontal to the bin wall, and were fixed to the other side of bin wall. The foundation of the bin was made of wood. It had a square cross section (0.76 m × 0.76 m) and was 0.61 m tall. The surrounding of the foundation was covered by plastic foam for heat insulation. A metal perforated floor was inserted between the bin body and its foundation to form the entire grain bin. The perforated floor, with 1 mm holes on it, allowed air to pass through freely but prevented the wheat kernels from falling through. The nitrogen container was connected with the grain bin through an insulated inlet at the bottom and liquid nitrogen was introduced into the grain bin by the aeration fan. The fan was a single-phase axial fan

with an RPM of 3450 (Northern Blower, model No. L3506, Winnipeg). A solenoid valve was installed on the other side of the insulated inlet pipe. Liquid nitrogen was introduced inside the bin through a set of four nozzles that spread it uniformly into the bin plenum in four different directions perpendicular to one another. Thermocouples were installed in the bin to monitor temperature changes throughout the bin.

3.1.2 Data acquisition and temperature control system

A data acquisition system was connected with the grain bin to monitor temperature changes through the bin. Multiple T-type thermocouples were calibrated using an ice/water bath and used for this experiment. The temperature range which can be measured by these thermocouples was from -80 to 160°C. Three thermocouples were bonded to the copper wire which was mounted along grain sampler direction on each level. One of the three thermocouples was installed in the middle and the other two were installed on the two ends of the copper wire inside the grain bin (Fig. 3). In total, there were 15 thermocouples to monitor temperatures (3 thermocouples × 5 levels). One more T-type thermocouple was used on the perforated floor. This thermocouple was connected with a temperature control device connected with the data acquisition system. The temperature control device was connected to the solenoid valve on the inlet. This valve controlled the on/off state of the solenoid valve and therefore controlled the liquid nitrogen intake from the container. The threshold temperature to control the solenoid valve could be adjusted by pressing the up and down button on the temperature control device. The solenoid valve turned itself off when the temperature reached the threshold and turn back on again when the temperature went below the preset temperature.

3.1.3 Insects and sampling

Rusty grain beetle, *Cryptolestes furrugineus*, is one of the most common and cold-hardy insects of stored grain in Canada (Smith 1970; Fields 1992). In this laboratory study, *C. furrugineus* adults were used to evaluate the potential of liquid nitrogen as a disinfestation method.

To investigate the mortality of insects, 50 adult rusty grain beetles were put in each screen bag with a size of 80 mm × 20 mm. Screen bags containing insects were placed at the five different levels where copper wires carrying the thermocouples were mounted. In order to get insects in and out of the bin easily, a standard grain probe traps (samplers) were used and these screen bags containing insects were put in the traps. Each trap consisted of the following two parts (Fig. 4).

Part A: a cylindrical, perforated plastic probe trap with 3.00 cm internal diameter, and 43.18 cm length, had a flat cap at one end and a pointed piece at the other end which could be screwed and easily inserted in place. The size of holes on the trap, which is smaller than the average grain kernel diameter, prevented grain kernels from getting inside the trap.

Part B: A cable tie was attached to the trap at the flat cap end for convenient removal of the trap from the grain mass.

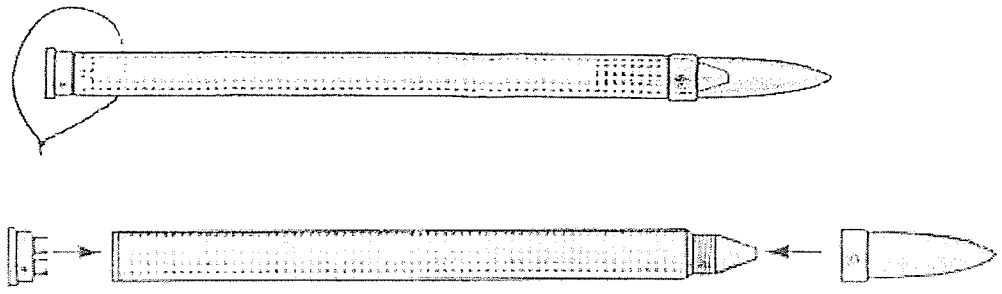

Fig. 4. Modified probe trap (Trece Inc.)

One sampler containing insects in the screen bags was inserted into the grain bulk through the hole at each level prior to each experiment and was pulled out after the experiment. Rubber stoppers were used to seal the plastic tubes. Duct tape with a width of 48 mm was used to seal the rubber stoppers tightly inserted into the plastic hole. The number of live insects was counted before and after each trial to calculate the mortality.

3.2 Preparation of Insect Samples

A culture of *C. ferrugineus* was started from adults, reared on red spring wheat with a moisture content of 12% at 30°C. The grains used for the mass rearing were sieved after 2 mo using 20 μm sieve and adults collected were divided into two groups of mixed age and sex. Group one was used for culturing cold acclimated insects and group two was used as unacclimated insects. Each group was subdivided into five jars and each jar was used for individual experiment trials separately.

The unacclimated adults of *C. ferrugineus* used in the experiments were obtained by sieving daily the jar content with a seive of 20.0 mm from group 1. Cold acclimated adults of *C. ferrugineus* were obtained by sieving daily the jar content with a sieve of 20.0 mm from group 2. Then the insects were held at 15°C for one week then transferred to 10°C for one week before the experiment. All the insects used for the experiment trial were cultured sufficiently before each experiment in order to make sure we had enough insects.

3.3 Methods

3.3.1 Investigation of cooling rate

Cooling rate is important to disinfestation efficiency. Different cooling rates need to be tested by adjusting the flow rate of liquid nitrogen in order to achieve the highest possible

mortality of *C. ferrugineus*. Change of cooling rate was achieved by adjusting the threshold temperature (set temperature at which the solenoid valve shut off the liquid nitrogen) to control how fast nitrogen entered the grain bin. The constraint on the set temperature was that the set temperature must be higher than the temperature at which grain at the bottom of the bin began to freeze.

The effect of different cooling rates was evaluated by two factors. The first factor was how fast the grain at the top of the bin cooled down to 0°C. This was done by using thermocouples within the bin to monitor the grain temperature. The second factor was how many insects were killed during the treatment, especially the insects on the top of the grain bin. This was monitored by counting live and dead insects in cages.

3.3.2 Investigating insect cold acclimation

Acclimation is one of the main factors that determine the survival of insects at low temperature (Solomon and Adamson 1955; Fields 1992). Survival time increases 2 to 10 times with acclimation of insects at low temperatures (Fields 1992; Fields et al. 1998). Several researchers have addressed the vital influence of acclimation on the cold tolerance of insects. Smith (1970), David et al. (1977) and Fields et al. (1998) compared survival rates of acclimated insects with those of non-acclimated insects. Similar results were found among these references which revealed that 50 LT (lethal time for 50% of the population) of acclimated insects are much higher compared to unacclimated ones. For example, Fields et al. (1998) reported that fully acclimated *Sitophilus granarius* had an 50 LT of 40 d at 0°C compared with 12 d without acclimation. In Canada, *C. ferrugineus* is capable of surviving the winter in unheated granaries in the Prairie Provinces, because of its ability to acclimate to low temperature. According to Fields and White (1997), *C.*

ferrugineus in granaries are more cold hardy than laboratory-reared strains. Cold acclimated insects require longer exposure time or even lower temperatures to achieve certain mortality which was often a problem when we use low temperature control methods. Interestingly, the successful use of ice nucleation-active bacteria, ice-nucleating active fungi and surfactants to reduce cold-hardiness of insects has been reported by several researchers (Fields 1990; Lee et al. 1998). Therefore, the survival of acclimated insects was studied in liquid nitrogen aeration treatment. Unacclimated insects and acclimated insects were used separately for several trials under similar low temperature conditions created by using liquid nitrogen. Mortalities of insects among these trials were used to examine the effect of cold acclimation on insect survival rate.

3.3.3 Investigating germination effect

Liquid nitrogen creates a fairly cold environment. Some researchers insist that liquid nitrogen has no significant effect on seed germination (Crisp and Grout 1984; Kovach et al. 2006) while others believe it has a negative impact on seed germination ability (Lockett and Luyet 1951). Therefore, it is vital to examine the effect of liquid nitrogen treatment on grain germination. Samples of grain were collected before and after liquid nitrogen aeration treatment at different heights and analysed for any loss in germination.

Germination of the wheat seeds subjected to liquid nitrogen treatment was assessed by incubating 25 seeds on no. 3 filter paper in a 90 mm diameter Petri dish saturated with 5.5 mL of distilled water. The dishes were placed in a plastic bag to prevent desiccation of the filter paper and kept at 25±1°C with a humidity of 70±5 % for 7 d. On the seventh day the germinated seeds were counted and the germination percentage was calculated (Appendix F).

3.4 Experiment Design

Five experimental trials were carried out to study the three objectives outlined in Section 3.3(Table. 2). For the first two trials, unacclimated insects were used for the first trial and both acclimated and unacclimated insect samples were used for the second trial. Two screen bags, each containing 50 insects, were fixed to the mid-point of each sampler on each level. The two screen bags in trial 2 contained 50 non-acclimated and acclimated insect samples separately. The purpose of trial 2 was to compare the mortalities of cold acclimated insects and unacclimated insects in the same conditions. It was designed to see whether there was any difference and how the difference was between the mortalities of cold acclimated and unacclimated insects. It was later found that the difference was quite large. Therefore, the last three experimental trials were designed. The valve of the liquid nitrogen tank was opened fully in all trials to make sure the liquid nitrogen was supplied at a constant rate. The threshold temperature that controlled the solenoid valve was set at -10°C for the first two trials. After each trial, the grain was sampled from the bottom level of the bin to ensure that no block of frozen wheat was formed. It was assumed that a lower threshold temperature created a harsher cooling condition and hence a threshold temperature of -15°C was used for the last three trials. Even at this conditions, no freezing of wheat samples occurred. For the last three trials, equal number of unacclimated and acclimated insect samples was arranged in the samplers at each level. Of a total of 200 insects placed in each sampler in the second group trials, 100 insects were acclimated and the other 100 were unacclimated insects. The insect samples were arranged in 4 screen bags with two bags containing acclimated insects and two bags

containing unacclimated insects. This group of experiment trials was designed in this way to explain three points:

1. to study mortality rates for both non-acclimated and acclimated insects under the same cooling conditions;
2. to compare effect of lower threshold temperature (-15°C), i.e., harsher cooling conditions, on insect mortality rates and germination rates with the results obtained from the first two trials; and
3. to examine the consistency of experimental results.

Table 2. The threshold temperature and liquid nitrogen aeration duration during 5 trials

Trial #	Threshold temperature set point (°C)	Time of 1st nitrogen tank stopped (h)	Time of 2nd nitrogen tank stopped (h)	Time of aeration fan stopped (h)	Total duration of trial (h)
1	-10	7.80	14.00	15.80	16.00
2	-10	7.60	16.60	25.00	25.00
3	-15	8.30	17.20	17.20 restart at 40 h	48.00
4	-15	10.87	38.60	38.60 restart at 60 h	68.00
5	-15	16.30	28.00	26.00	32.00

A control group of 100 insects (50 non-acclimated and 50 acclimated insects) at each level was placed in the grain bin before running the five experiment trials described above. No liquid nitrogen was introduced into the grain bin for the two consecutive days when the control group was tested in the grain bin for survival rates.

3.5 Procedures and Data Collection

In the preparation step, 50 reared insect samples were counted manually and placed in each screen bag. Care was taken to ensure that selected insects demonstrated good vitality, i.e., reasonable crawling speed. Necessary caution was also taken to use two separate carrying trays for counting non-acclimated and acclimated insects. Wheat germ was placed in each screen bag to ensure there was food for the insects during experiments. After screen bags containing insect samples were properly secured in the samplers, the samplers were carried to the grain bin for loading. A quick examination of the equipment was done to ensure that the experimental setup was in good working order. Then samplers were loaded into grain bin through sampling holes. Rubber stoppers were inserted into the sampling hole openings and sealed with duct tape. The data acquisition system that took measurements from thermocouples began to record temperature and weight readings once the valve on liquid nitrogen container was opened. For the duration of liquid nitrogen injection into the grain bin, each experiment took approximately 16 h to 40 h to finish. During the cooling process, a manual check of the entire experimental system setup was conducted every 3 h. Liquid nitrogen injection was stopped when no further decrease in the weight of nitrogen tank was observed. Dismantling rubber stoppers from sampling holes and removal of samplers from grain bins was conducted 8 h after liquid nitrogen injection stopped. Grain samples were taken through sampling holes at the same time samplers were removed. Samplers and grain samples were then forwarded to the insect control lab for data analysis.

4. RESULTS AND DISCUSSION

4.1 Cooling Patterns Inside Grain Bin

There were three thermocouples located on each of the five levels inside the grain bin. Symbols for data reporting consisted of three digital numbers to describe the position of measurement throughout the grain bin. The first digit stands for the position of level and the second and third numbers stand for the positions of thermocouples at each level. For the locations of the three thermocouples on each level, position 01 meant the position near the sampling hole, position 02 stood for the central position, and position 03 meant the opposite side of the bin from the hole. For example, 101 means the temperature changes near the sampling hole on the bin at the first level. The temperature change curves are shown in Fig. 5 to 7 and Appendix B. The vertical line in these figures denoted the time when the first tank of liquid nitrogen was stopped and the second tank of liquid nitrogen was connected to the grain bin.

In general, cooling patterns at the three measurement locations on the same level were similar while cooling patterns at different levels were quite different. For example, take the temperature change recorded for experiment trial 3. The temperatures at all three measurement locations on the bottom level reach their lowest readings in 11 to 17 minutes after the injection of liquid nitrogen started (Fig. 5). After 37 minutes from the start of the experiment, temperature rose to a value where it gradually stabilized. After 8.3 h the second liquid nitrogen tank was connected. This brought drastic temperature changes, but the temperature stabilized again within 40 min. The temperature stabilized at -22°C to -28°C. When liquid nitrogen was stopped at 16 h, the temperature began to rise at a rate of 0.5°C/h.

There was a dramatic temperature decrease after 1.5 h on level 2 from the start of the experiment (Fig. 6). It took about 3 h before the temperatures stabilized to a value around -10°C. For the temperatures on level 3 (Appendix B.3.3), it took 5.5 h before significant temperature decrease was observed. Temperature readings at all three locations (301, 302, and 303) went down to a value around -10°C. This cooling process took about 8.5 h. In the next 2 h, the temperature began to stabilize until the second tank of liquid nitrogen was depleted. No abrupt temperature changes were observed for the three locations on level 4 (Fig. 7) and temperatures decreased smoothly at a rate of -0.6°C/h. The temperature was not affected when the first tank of liquid nitrogen was depleted. After the second tank of liquid nitrogen was depleted, the temperatures began to increase at a rate of 0.1°C/h.

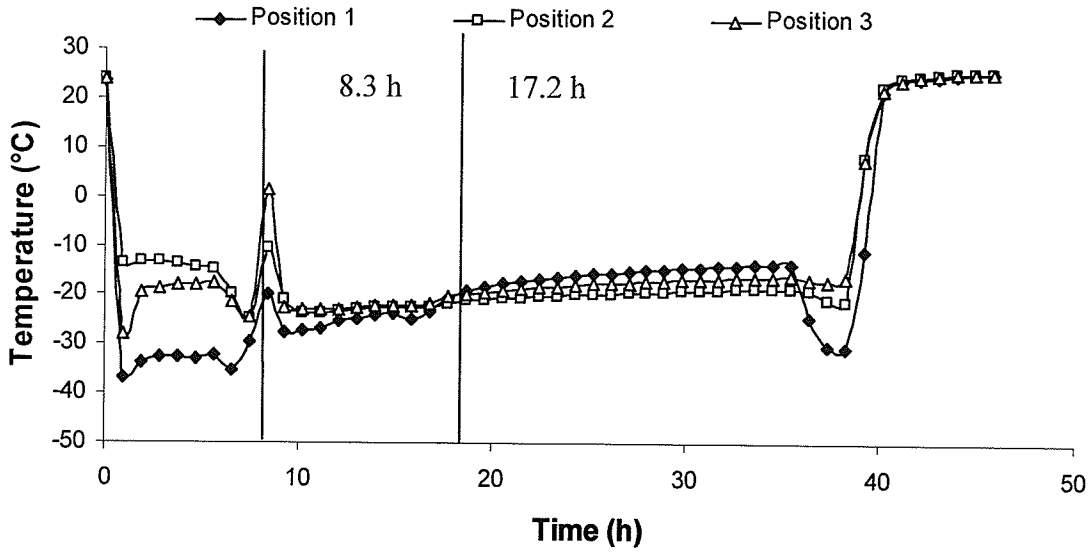

Fig. 5. Temperature change at level 1 inside the grain bin during trial 3

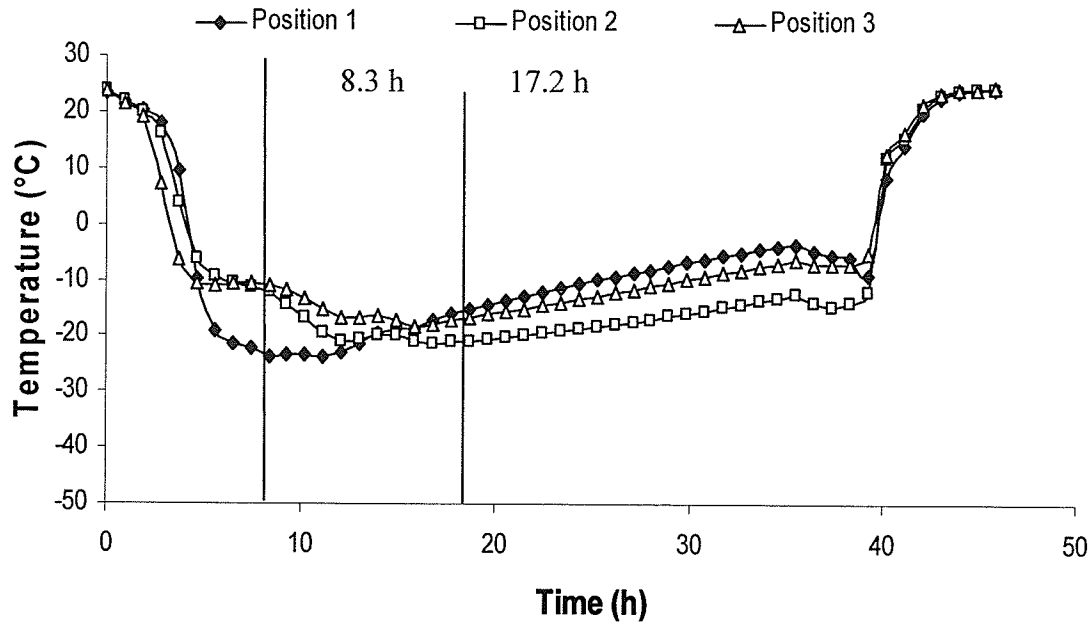

Fig. 6. Temperature change at level 2 inside the grain bin during trial 3

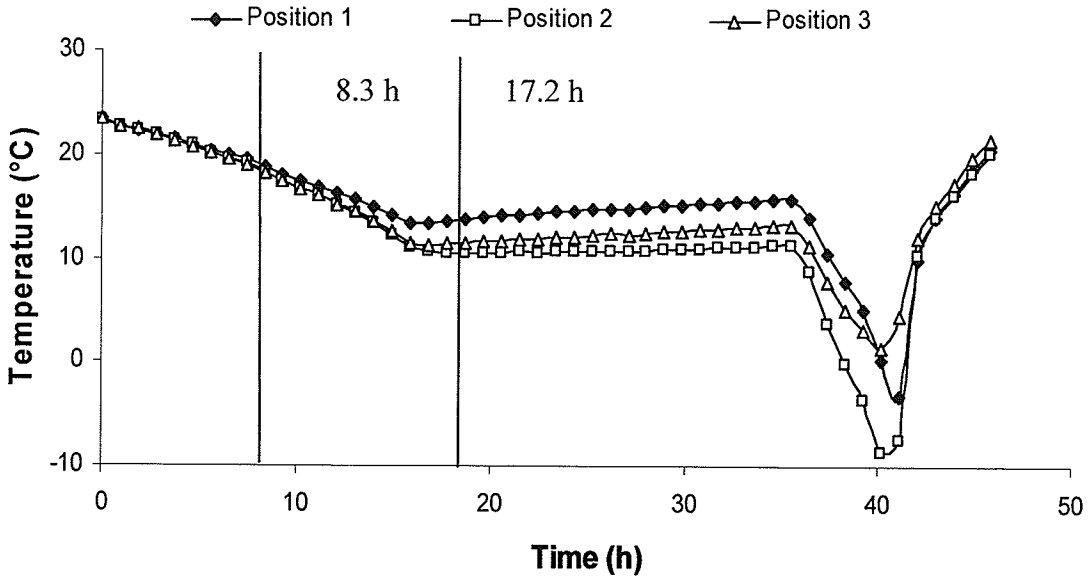

Fig. 7. Temperature change at level 4 inside the grain bin during trial 3

Similar cooling patterns were observed for the other four experimental trials (Appendix B). The temperature at level 1 dropped quickly after the application of liquid nitrogen to the grain bin. For the first three experimental trials, the initial temperature

drop took approximately 16.5 min. The lowest temperatures reached during the initial temperature drop were -28.3°C, -34.8°C, and -41.6°C respectively. For the last two experiment trials, it took 12 minutes for the temperature to drop to the lowest readings. The lowest temperature achieved in trial 4 and 5 were -62.3°C and -72.9°C. This difference in cooling time and lowest achievable temperatures could be explained by the following reason. Before conducting trial 4, mild nitrogen gas leakage was noticed along the seams between the cardboard grain bin and its foundation. Sealant was applied to the seams to cut down the nitrogen loss. This modification resulted in improvement of cooling efficiency of the grain bin system, i.e., a faster initial cooling process with less required time. The pressure difference of each liquid nitrogen tank was also a factor which influenced the grain temperature.

For all five experimental trials, temperature values at the three measurement locations on level 2 began to diverge and decrease sharply after 1.3 to 1.6 h of introducing liquid nitrogen. Except for experiment trial 1, the temperature for the other four trials stabilized 5.5 h on average after the experiment started. Temperature in trial 1 stopped decreasing 2 h after liquid nitrogen application. Though the cooling process was quick for trial 1, the lowest temperatures achieved for the three locations on level 2 were only -3.7°C, -5.9°C, and -14.2°C, respectively. The lowest and average temperature achieved on the second level for the other four trials were much lower (Table C.1 and Table C.2) and therefore took longer cooling time to stabilize. However, it remains unclear as to why temperature could not drop to lower values on level 2 and above in trial 2. A possible reason might be the pressure difference among the liquid nitrogen tanks.

On levels 3, 4 and 5, temperature changes were much more gradual than levels 1 and 2 of the five experiment trials. The lowest achieved temperature and the average cooling temperature are listed in Table C.3 to C.8 (Appendix C). Interestingly, temperature drop was observed on level 5 after the liquid nitrogen supply was disconnected from the foundation of the grain bin while the aeration fan was kept running. This was because the cooling front was still moving upward through the bin even though the liquid nitrogen supply had been cut off. The temperature change rates were -10°C/h to -12°C/h. When both the liquid nitrogen supply and aeration fan were stopped simultaneously, temperature at all levels seemed to maintain well. For example, in experiment trial 4, temperature on the second level changed at a linear rate of 0.5°C/h without aeration.

The concept of effective cooling period (ECP) was defined in this research. Effective cooling period described the time span starting from the moment when temperature measurement at all three locations had dropped and stabilized until the moment when at least two of the temperature values began to rise. This was because the warming up of a single level is signaled by temperature rise in at least two or more locations. For each of the five levels, the cooling period was different and unique in this experiment. The ECP described a cooling period with lowest achievable temperatures with a relatively uniform spatial distribution. This concept was used to explain the mortality of insects.

4.2 Insect Mortality

Test results from the control trial indicated that the survival rates for both unacclimated and acclimated insects were 99.6% when no nitrogen was blown through the bin. For trials 1 to 5, at least 8 h after liquid nitrogen injection stopped, samplers were removed from grain bins and insects were manually counted to calculate mortality. Tables F.1 to

F.5 (Appendix F) show the mortalities obtained at different levels for five experimental trials. In general, mortalities of as high as 100% were obtained from level 1 to level 3 for unacclimated insects for most of the trials. The mortality gradually decreased to about 50% on levels 4 and 5, which were farther away from liquid nitrogen injection position than the first three levels. In the last four trials while using cold acclimated insects, mortalities of 100%, 86%, 100%, and 90% were achieved on the first levels and mortalities of 100%, 57%, 100%, and 100% were achieved on the second levels of the bin. Unlike unacclimated insects, the mortality of cold acclimated insects on levels 3 to 5 dropped from 24% to 4% in all trials except trial 5. In trial 5, mortality dropped from 71 to 25% for the top three levels.

The insect mortality in trial 1 agreed with that in trial 2 for unacclimated insects. In trial 2, when comparing unacclimated insects with acclimated ones, mortality remained the same at the lowest two levels and decreased from 100% to 20% at the third level. On level 4 and 5, the mortality of unacclimated insects was higher than that of acclimated ones. This was because cold acclimated insects were more cold hardy and earlier studies established that their survival rates could increase 2 to 10 times when compared with unacclimated insects at similar low temperature conditions (Fields 1992; Fields et al. 1998; David et al. 1977).

The distribution of temperature and duration of ECP on each level were related to mortality of insects which is shown in Table C.9 (Appendix C). In general, long duration (>2 h) combined with low temperature inside grain bin resulted in high mortality of unacclimated insects. To increase the mortalities of acclimated insects, a lower

temperature was required than the temperature to which the unacclimated insects were exposed. Mortality on level 1 to 5 generally followed this rule.

On level 1, mortalities of 100% were achieved in all experimental trials. On level 2, the combination of low temperature and sufficiently long duration of cooling period resulted in mortalities of 100% for unacclimiated insects and over 90% for acclimated insects except that in trial 4, which had a mortality of 57%. Mortality results in experimental trial 1 and 4 needed to be further explained. In experimental trial 1, the lowest temperature and average temperature were -9.7°C and -4.0°C, which were much higher than those achieved in other trials. The duration (7 h) was also shorter than those in other four trials. However, the condition was sufficiently lethal to unacclimated insects, which had a mortality of 100%. In trial 4, the achieved minimum temperature, average temperature, and duration were -23.2°C, -13.6°C, and 54.0 h. Though the duration (54 h) was long compared to those in other trials, the conditions at the three locations were moderate, i.e., they did not achieve the coldest temperature simultaneously and these conditions at each location did not last long. This indicated an uneven cold temperature distribution, which might be one cause of low mortality of acclimated insects in one screen bag. The other possible reason here was that due to the mixed ages of acclimated insects in trial 4, there might be uncontrolled variation in the cold hardiness of insects, which contributed to the difference in insect mortalities.

On level 3, mortalities of over 98% were achieved in all experimental trials for unacclimated insects. The temperature conditions achieved in experimental trial 1 were characterized by a minimum temperature of -11.5°C, an average temperature of -3.4°C, and duration of 2.7 h. This condition was sufficient to kill the unacclimated insects in

trial 1. Compared with trial 1, the harsher conditions in trial 2 to 5 were effective against unacclimated insects as well. For acclimated insects, temperature conditions achieved on level 3 were less effective as evidenced by comparing the insect mortalities for both levels 2 and 3. Acclimated insect mortalities of 20%, 20%, 23%, and 71% were achieved in trial 2, 3, 4, and 5, respectively. This could be explained by the fact that the ECPs achieved on level 3 were shorter in duration and higher in minimum temperature compared with those achieved on level 2. For example, the temperature condition achieved on level 3 in trial 2 was comparable to that achieved on level 2 in trial 3. However, the duration of ECP was 9.0 h on level 3 in trial 2 and the duration of ECP was 16.8 h on level 2 in trial 3. This difference in duration accounted for the mortality difference of acclimated insects. The mortality result on levels 2 and 3 was 100% and 71% in trial 5. This decrease of mortality could be explained by milder temperature conditions, and shorter duration and higher temperature.

On levels 4 and 5, the mortalities in all five experimental trials decreased greatly because ECPs were greatly reduced in duration and could not achieve sufficiently low temperature. On level 4, the average temperature and minimum temperature achieved in trial 2 were -16.7°C and -25.1°C. However, the duration was 1.5 h. The mortalities for acclimated and unacclimated insects were 6% and 20%. Therefore, this temperature condition was not effective against either acclimated or unacclimated insects. On level 4, the temperature conditions achieved in trials 3, 4, and 5 appeared to be effective against unacclimated insects. This suggests that longer exposure (>2 h) at an average temperature of around 0°C was still helpful in killing unacclimated insects. On level 5, ECP conditions achieved in trials 3 and 5 were somehow effective against unacclimated

insects. The temperature was mild with duration of about 2 h. This suggests that cooling duration was important in deciding the mortality of unacclimated insects.

To analyze the mortalities and examine the consistency of experiment, procedure GLM and Contrast of SAS (2006) were used. For unacclimated insects, mortalities in trial 1 agreed with that in trial 2. As mentioned in Section 3.4, trial 2 was used to compare mortality differences between cold acclimated and unacclimated insects under the same conditions. In addition, there was only one caged insect bag at each level for unacclimated and acclimated insects which was quite different from the other trials with duplicate bags at each level. Therefore, trial 2 was not used for comparisons. In the SAS program, T1, T3, T4, and T5 stand for trials 1, 3, 4 and 5, respectively. T1, T3, T4, T5 were used as a group to analyze the mortalities of unacclimated insects, and T3, T4, and T5 were used as a group to compare mortalities of cold acclimated insects since there were no cold acclimated insects used in trial 1. The experiment was organized as a completely randomized design (CRD). Insects were assigned randomly to each trial. The model chosen for the analysis of variance (ANOVA) was

$$Y_{ij} = \mu + h_i + e_{ij}$$

where Y_{ij} represents the observation on insect mortality of the j th insect sample in the i th trial; μ is the population mean for insect mortality; h_i is the effect of the i th trial; e_{ij} is the error deviation of the j th insect sample in trial i.

Because there were similar insect mortalities at level 1 to 3 throughout five trials, only level 4 and 5 were analyzed and compared for mortality differences for cold unacclimated insects. The comparisons among trials were used to evaluate mortality differences as well as to examine the consistency of the experiment. The hypothesis used

for the GLM procedure was: H_0: $h_i = h_{i'}$, for all $i \neq i'$, i.e. all liquid nitrogen aeration trials have the same effect; H_A: $h_i \neq h_{i'}$, for at least one pair of $i \neq i'$, i.e. at least one liquid nitrogen aeration trial differs from one of the others. The hypothesis used for Contrast procedure was: H_0: $y_{1.} - y_{2.} = 0$, i.e. there was no significant difference between two trials; H_A: $y_{1.} - y_{2.} \neq 0$, i.e. there was significant difference between two trials. SAS results are listed in Appendix D.

Results of levels 4 and 5 from GLM procedure in Table D.1 and Table D.2 show that the value s of Pr > F was 0.5503 and 0.2412, respectively which were greater than 0.05. Therefore, all the trials had the same effects and we can accept H_0. It was concluded that the mortality rate of unacclimated insects on levels 4 and 5 were consistent in the three trials (T3, T4, and T5). The results also indicate that there is no significant difference in unacclimated insect mortalities between levels 4 and 5 in trials 1, 3, 4, and 5. This also can be concluded from Contrast procedure with all the Pr > F value greater than 0.05. We can conclude that the effect of trial 3 to 5 on unacclimated insect mortality rates at levels 4 and 5 is not significantly different from trial 1 even with a harsher cooling condition.

For comparing mortalities of cold acclimated insects, trial 3, 4 and 5 were used in GLM and Contrast procedure. The values of Pr > F for these trials in GLM procedure were 0.5338, 0.0924 and 0.0517, respectively, which were larger than 0.05 (Table D.3, D.5, D.6, Appendix D). Therefore, all the trials had the same effects and we accept H_0. The result tells us that there is no significant difference in all the trials on insect mortality at levels 2, 4 and 5. For level 3, at least one liquid nitrogen aeration trial differs from one of the others since the Pr > F value (0.0208) was smaller than 0.05 in GLM procedure (Table D.4, Appendix D). From Contrast (i), (ii) and (iii), the Pr > F value was 0.7717,

0.0147 and 0.0125, respectively. It indicates that there is no significant difference between trials 3 and 4 (0.7717 > 0.05) on level 3 while the effect of trial 5 on insect mortality was significantly different from the effect of trial 3 and trial 4 on level 3 (0.0147< 0.05 and 0.0125 < 0.05).

4.3 Wheat Moisture Changes

Grain samples were collected by scooping wheat kernels from the sampling hole openings at each level as soon as samplers were removed. Care was taken to get samples from one side, middle and the other side of the bin at each level to minimize the error of the result. The initial wheat moisture content was measured at the control group (Table. G.1). Moisture content of wheat after each experimental trial at the five levels of the bin was measured (ASAE 2003) and is shown in Table G.1 (Appendix G). Average grain moisture content decreased about 1.7%, 1.8%, 1.9%, 1.8%, and 1.8% from levels 1 to 5, respectively for the five trials. To analyze the effect of liquid nitrogen aeration on grain moisture content (whether the changes of moisture content were significant or not), a simple one way ANOVA (Method: Tukey) was used to compare means between control moisture content and the five experiment trials as shown in Appendix E.1- E.5.

In Tukey method, column A stands for the moisture content in control treatment, and columns B, C, D, E and F stand for trials 1, 2, 3, 4 and 5, respectively. Comparison of means was done among control and the 5 trials at each level separately. From Appendix B, it could be seen that there was no significant difference between control trial and trial 1 to 4 (P> 0.05) for level one (bottom of the bin). However, there was a significant difference between control trial and trial 5 (P< 0.001). This was because grain moisture content changes were small at the beginning, but the changes became larger as more

experimental trials were conducted. Therefore, compared with control trial, the decrease of wheat moisture content during first four liquid nitrogen aeration trials was not significant while moisture content decrease (1.7%) in the last trial was statistically significant for level 1. For level 2, there was a significant difference in wheat moisture content between control treatment and trials 2 to 5 ($P< 0.001$). And since P value was greater than 0.05, the moisture content decrease in trial 1 was not considered to be significant. It meant that the decrease in grain moisture from trial 2 was considered to be significantly different from initial moisture content for level 2. For levels 3, 4 and 5, the moisture difference from trials 3 to 5 was significantly different from the initial wheat moisture ($P< 0.001$) while the effect of trials 1 and 2 on moisture changes was not significant ($P> 0.05$). In summary, grain moisture content decreased as more trials were conducted. This might be due to the fact that at least 20 h ambient aeration was carried out after each trial. And these differences in moisture content values became severe from the second or third trial on all levels except level 1. Therefore, for our experiments, liquid nitrogen aeration had a significant drying effect on grain kernels.

4.3 Effect of Liquid Nitrogen Aeration on Germination

To investigate if liquid nitrogen aeration treatment has any adverse effect on the germination of wheat, germination tests were performed before and after each cooling process. Tables H.1 to Table H.5 (Appendix H) show the percentage germination before and after each trial. Germination levels of wheat kernels before and after each cooling process ranged between 97.3% and 100%. Statistical analysis indicates that the difference in the mean values of the two groups in each trial was not large enough to accept that the

effect of cooling process was significant. Therefore, it can be concluded that aeration with liquid nitrogen does not affect the germination level adversely.

5. CONCLUSIONS

The following conclusions can be drawn from this study:

1. As the cooling front moves up, cooling patterns for different levels were different. Temperature distribution on the same level was not uniform. Temperature at the bottom level reached its lowest values within 20 min and quickly rose back and eventually stabilized to a value higher than the lowest temperature. Temperature reduction on other levels lagged behind the temperature drop on their neighboring level directly below them. Quick temperature drops on levels 4 and 5 were observed when liquid nitrogen was depleted at the end of experiments and the aeration fan was kept running. A lower threshold temperature (-15°C) did not improve disinfestation efficiency of liquid nitrogen on mortality of unacclimated insects.
2. Insect mortalities for cold acclimated and unacclimated insects were different under the same cooling conditions. Cold acclimated insects survived better than unacclimated insects. In general, long exposure time (>2 h) is required to achieve effective killing of insects. Low temperature for only a short duration was not effective in killing insects. The insect mortality decreased as levels moved further away from the bottom.
3. Cooling of grain by liquid nitrogen did not affect the germinability of wheat.
4. Grain moisture decreased by about 1.9% after five experimental trials. This may have been a consequence of repeated aeration of the grain.

6. RECOMMDATIONS FOR FUTURE WORK

Based on the review of literature and the current state of low temperature control methods, turning, ambient aeration and chilled aeration have some disadvantages which prevent their wide use in the agricultural industry. Until now, there are few researches on applying of liquid nitrogen aeration on grain disinfestaion. From the result of these 5 trials, we can see that liquid nitrogen does have an effect on insect mortalities and has the potential to be used in grain handling facilities as a new temperature control agent. To make it more effective, recommendations for future research directions are listed as follows:

1. Be stricter in selecting group of insects, for example: the some age range of insects to reduce the variance among treatments.
2. This study showed that temperature distribution was not uniform through out the bin. It would be beneficial to improve the uniformity of the temperature distribution in order to improve the efficiency of liquid nitrogen treatment.
3. Airtightness of grain bin was an important factor in grain aeration process. A better sealing method should be developed to improve the efficiency of cooling in further study.
4. In this study, insect mortalities for cold acclimated and unacclimated insects were different under the same cooling conditions. However, how significant this difference is in a bigger capacity bin should also be studied.
5. Grain moisture decrease was significant in this study. Further study should be conducted to examine the effect of liquid nitrogen aeration on grain moisture changes.

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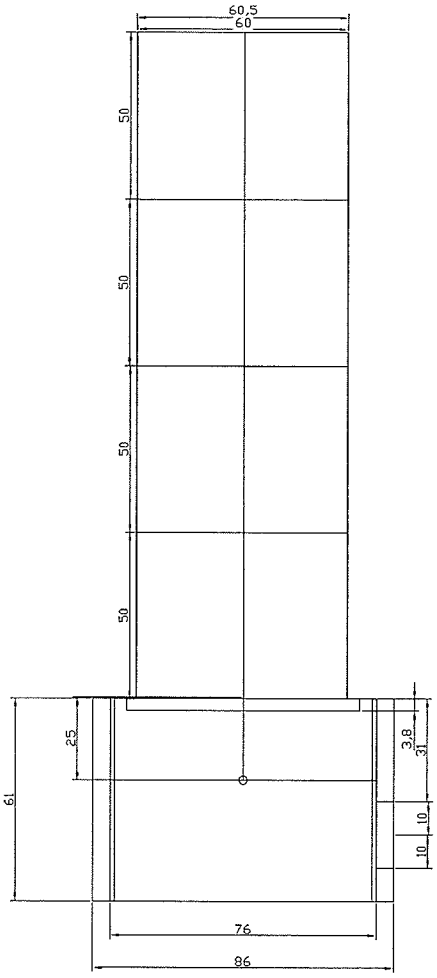
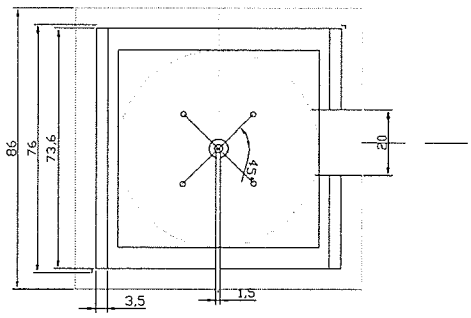
APPENDIX A: MECHANICAL DRAWINGS OF BIN STRUCTURE

Note: All drawings have been prepared in AutoCAD 2006 (Autodesk, Inc, San Rafael, CA). Technical presentation principles, tolerancing and dimensioning rules were referred to general principles set by International Organization for Standardization (1982) and standards approved by Canadian Standard Association (1991). The unit used in these drawings is cm. All drawings in the appendix were rescaled to fit into the page format and do not necessarily reflect the actual scales of the originals.



Side view



Top view

APPENDIX B: TEMPERATURE CHANGES DURING FIVE TRIALS

The first vertical line denotes the time when the first tank of liquid nitrogen was stopped and the second vertical line denotes the time when the second tank of liquid nitrogen was stopped. Each lines indicates the mean temperature changes on each level and the error bar indicates 50% of the standard error of the mean.

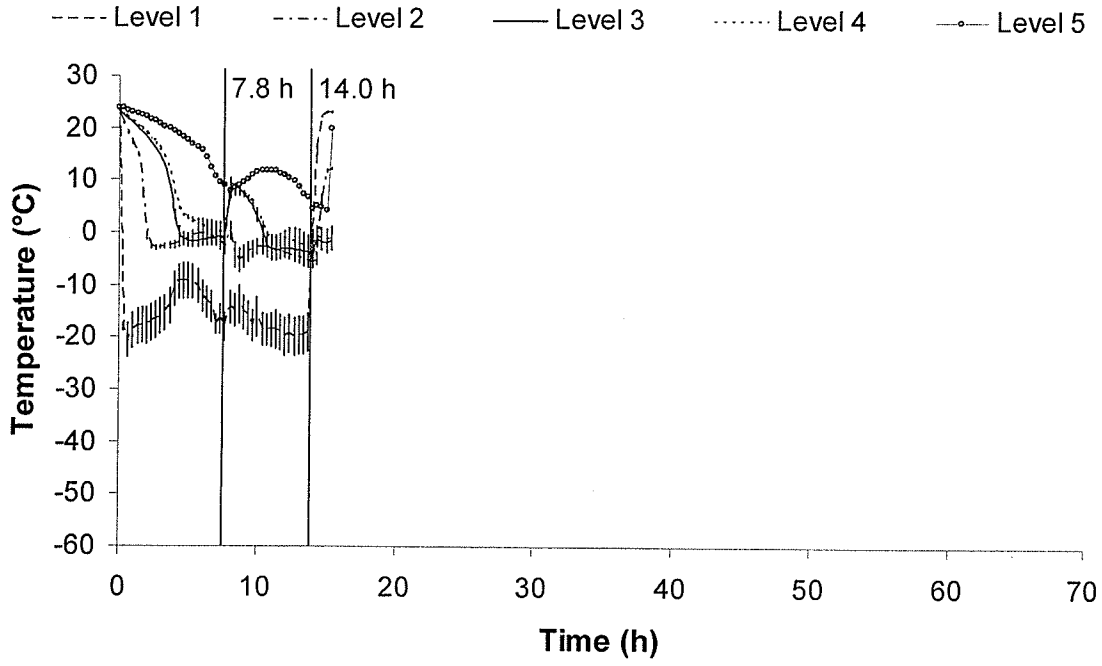


Fig. B.1. Temperature change on level 1 to 5 inside grain bin during trial 1

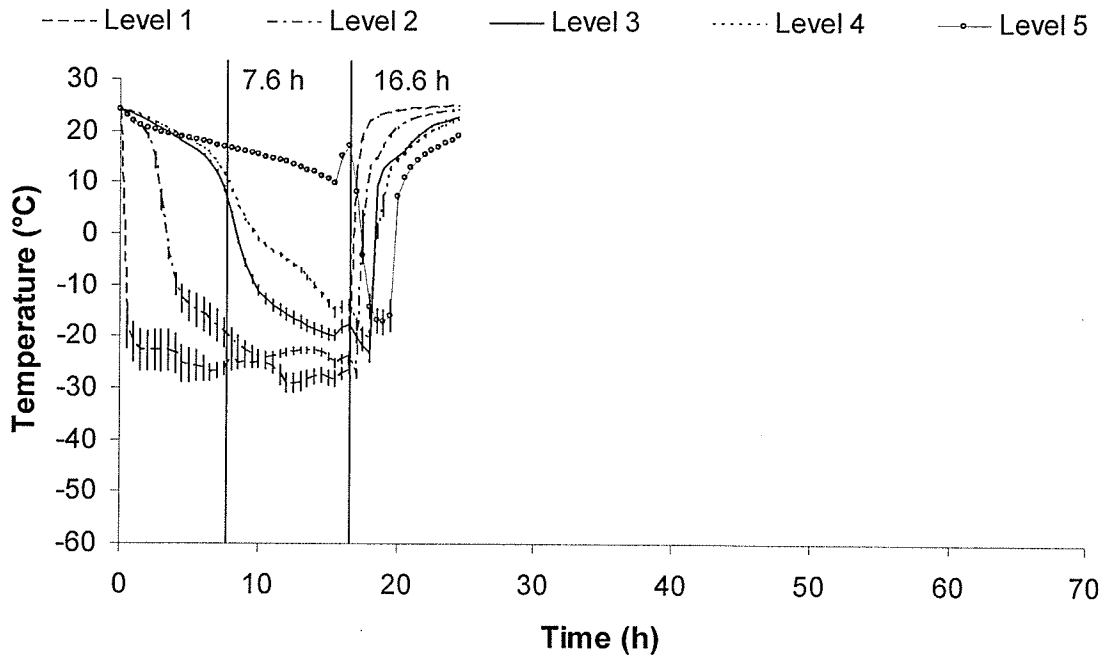


Fig. B.2. Temperature change on level 1 to 5 inside grain bin during trial 2

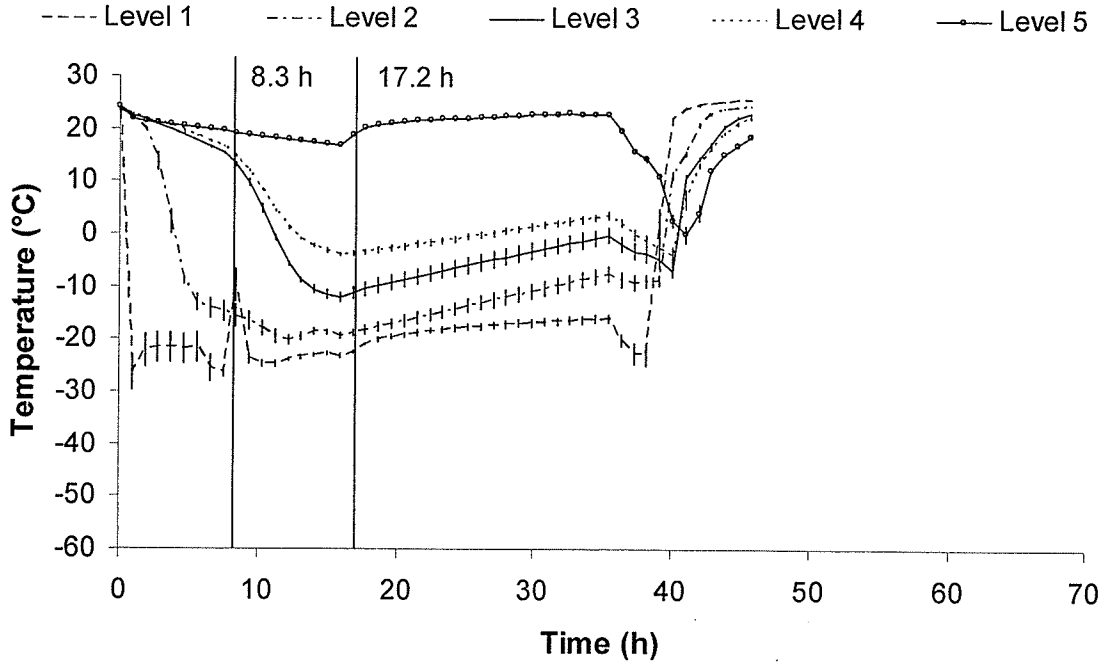


Fig. B.3. Temperature change on level 1 to 5 inside grain bin during trial 3

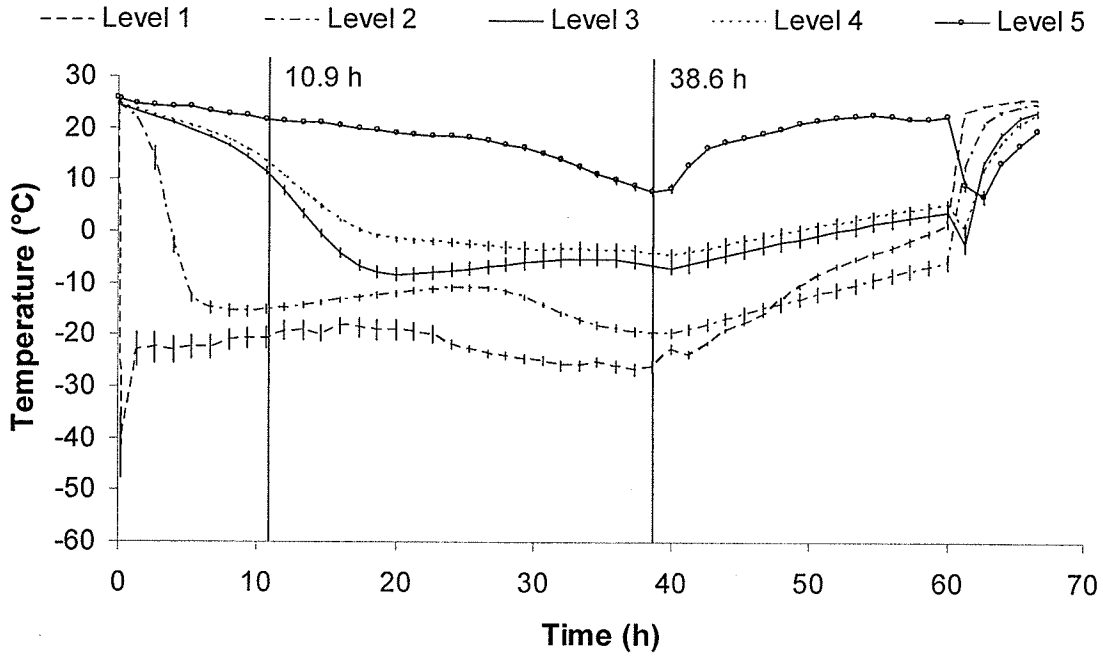


Fig. B.4. Temperature change on level 1 to 5 inside grain bin during trial 4

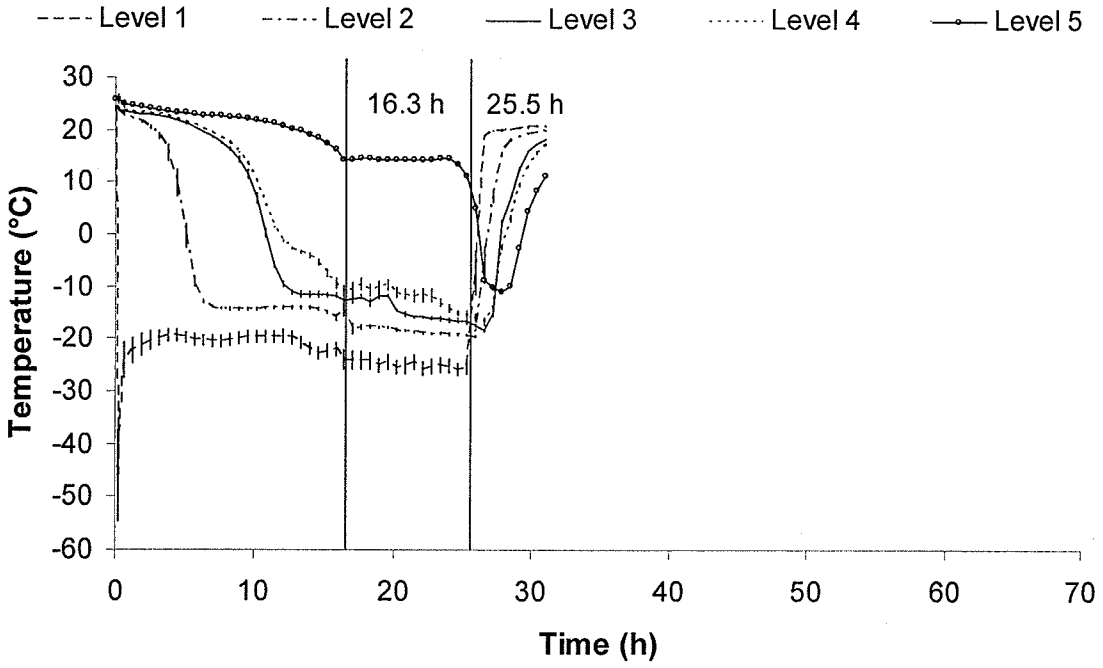


Fig. B.5. Temperature change on level 1 to 5 inside grain bin during trial 5

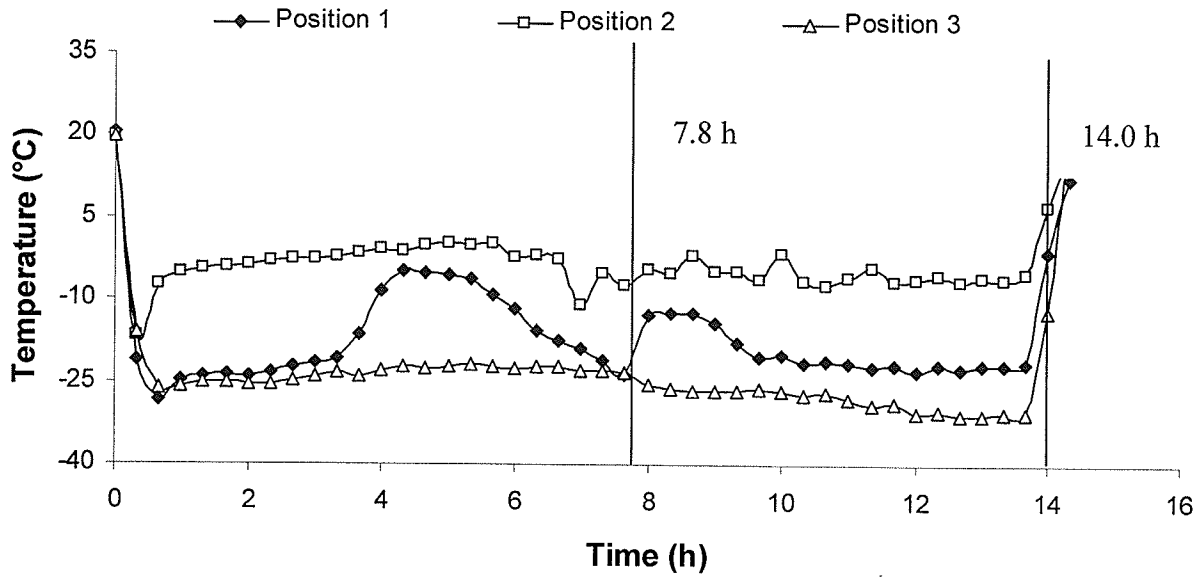


Fig. B.1.1. Temperature change at level 1 inside grain bin during trial 1

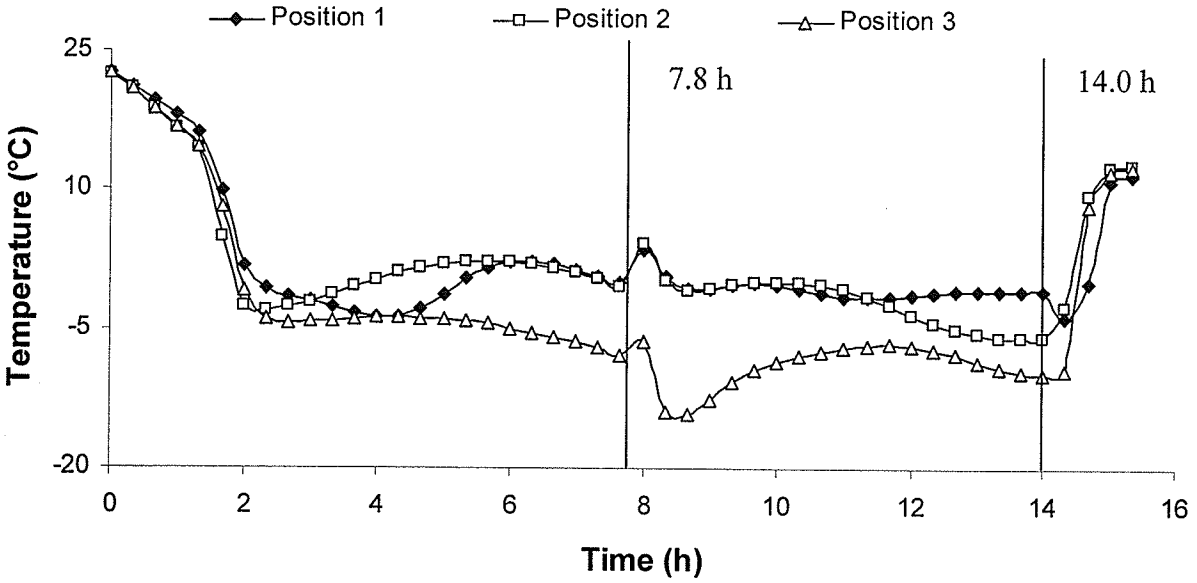


Fig. B.1.2. Temperature change at level 2 inside grain bin during trial 1

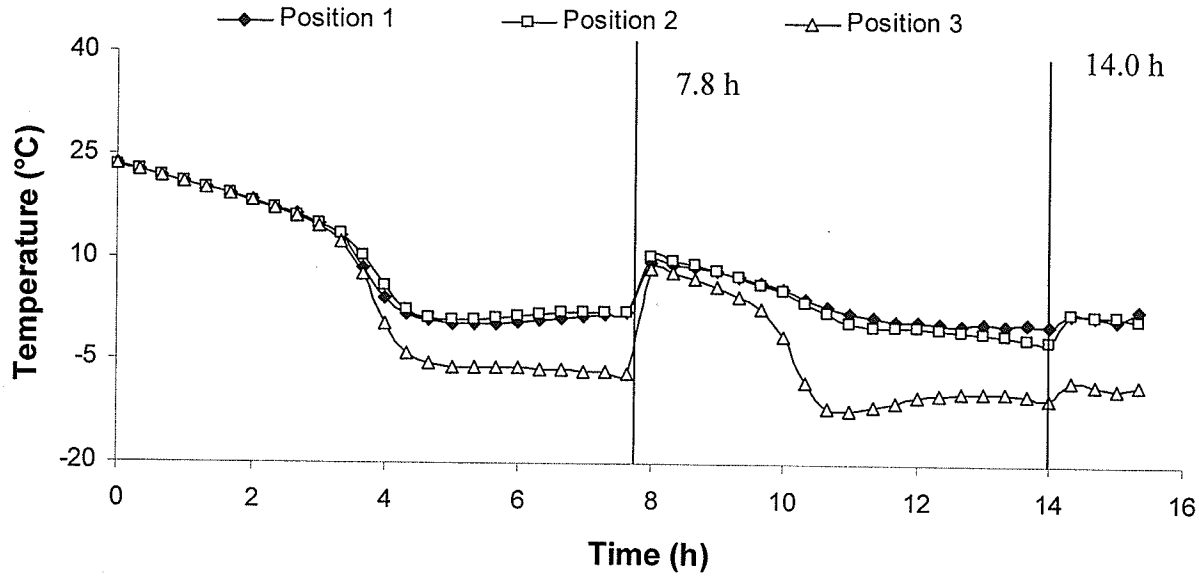


Fig. B.1.3. Temperature change at level 3 inside grain bin during trial 1

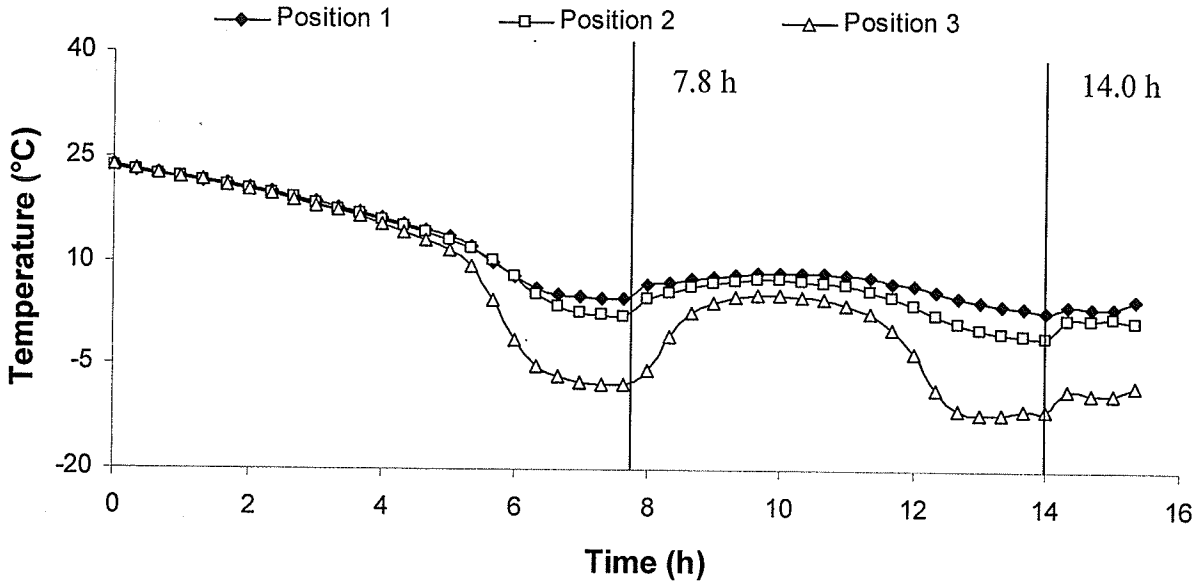


Fig. B.1.4. Temperature change at level 4 inside grain bin during trial 1

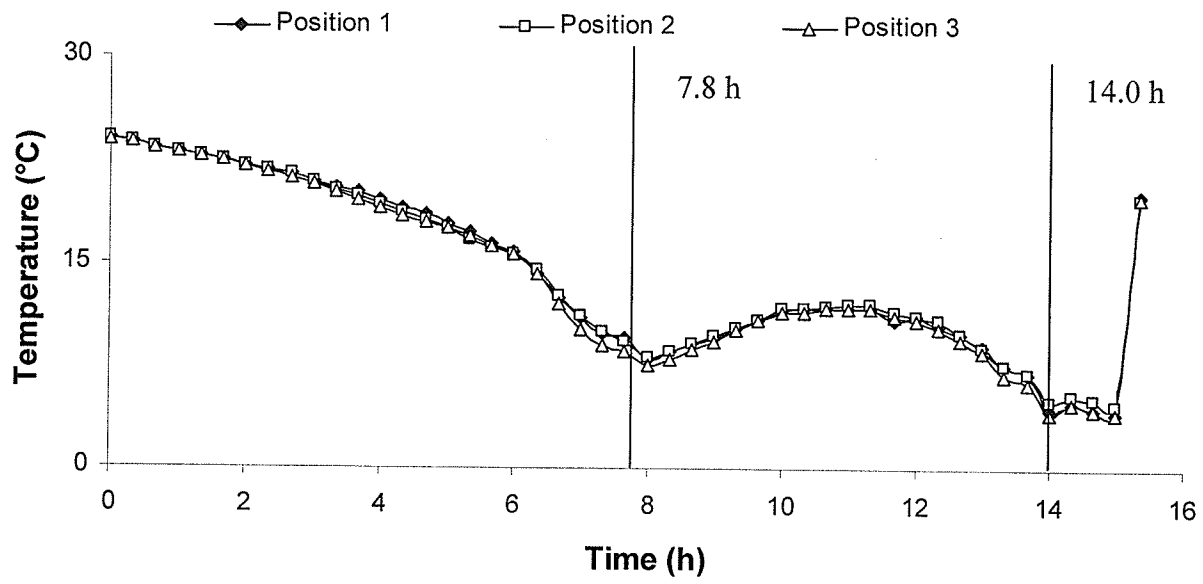


Fig. B.1.5. Temperature change at level 5 inside grain bin during trial 1

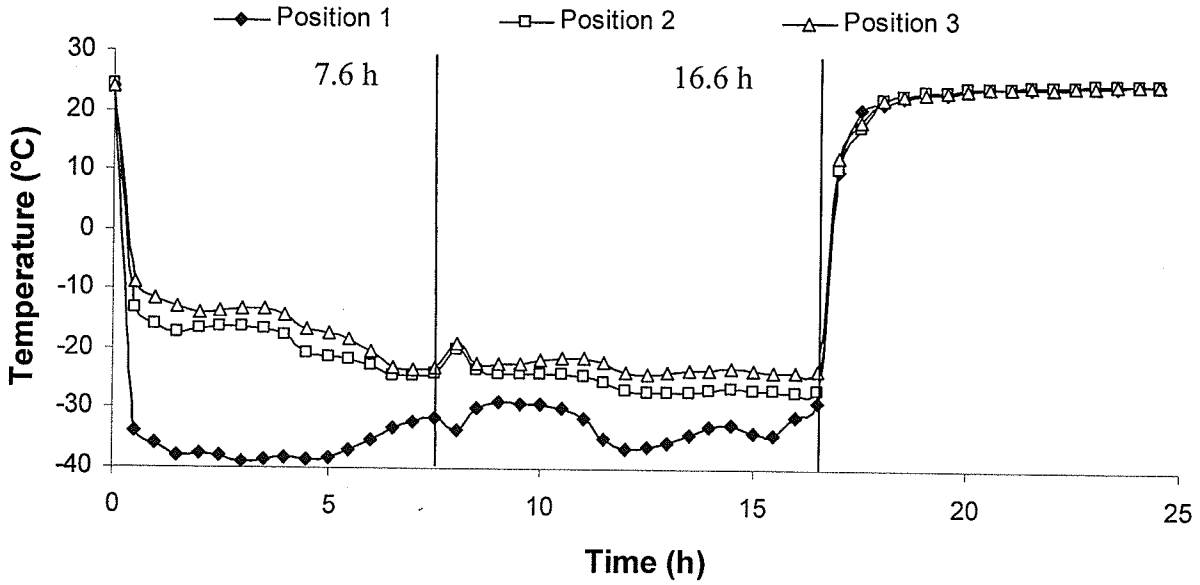


Fig. B.2.1. Temperature change at level 1 inside grain bin during trial 2

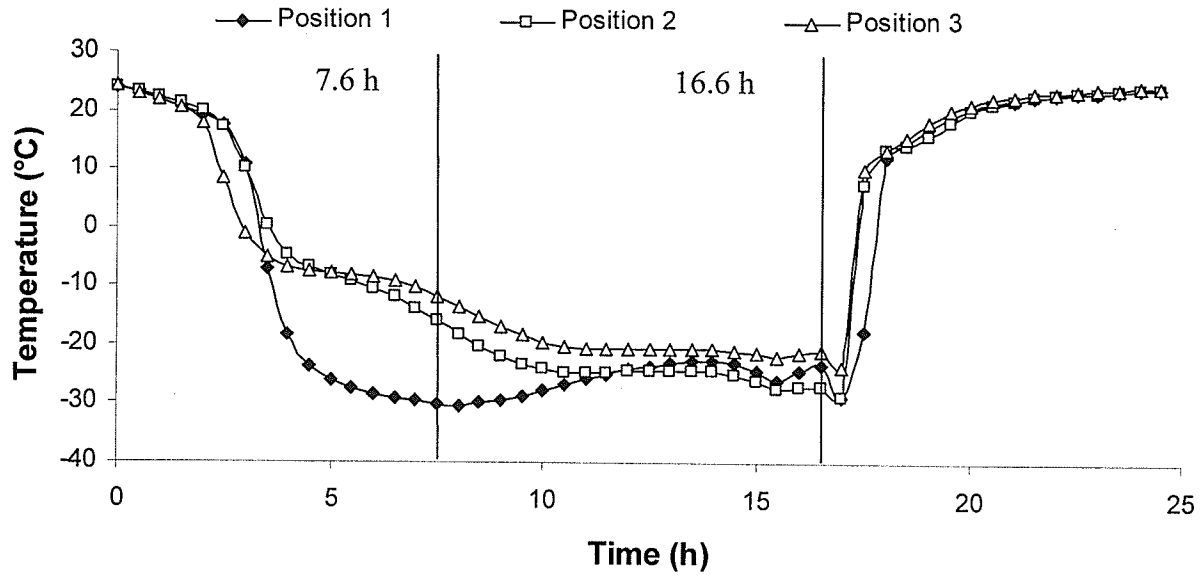


Fig. B.2.2. Temperature change at level 2 inside grain bin during trial 2

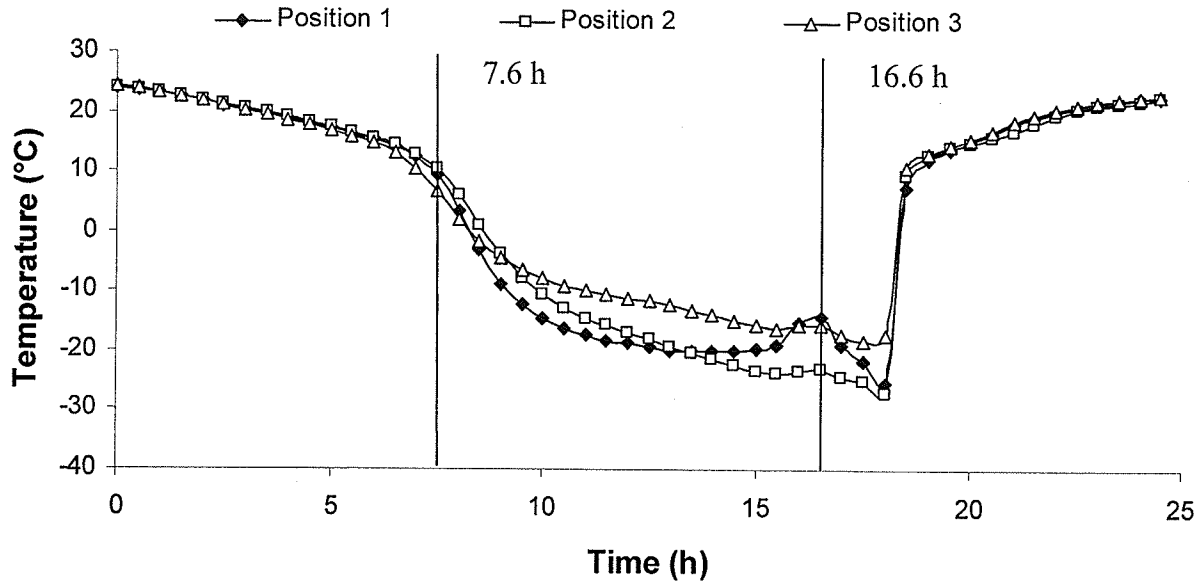


Fig. B.2.3. Temperature change at level 3 inside grain bin during trial 2

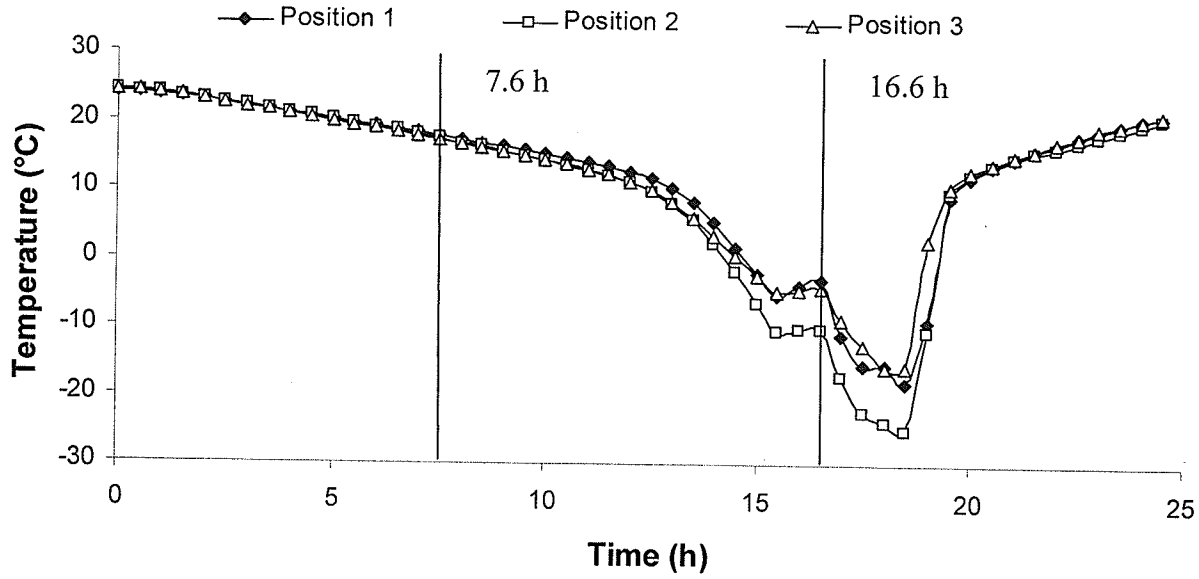


Fig. B.2.4. Temperature change at level 4 inside grain bin during trial 2

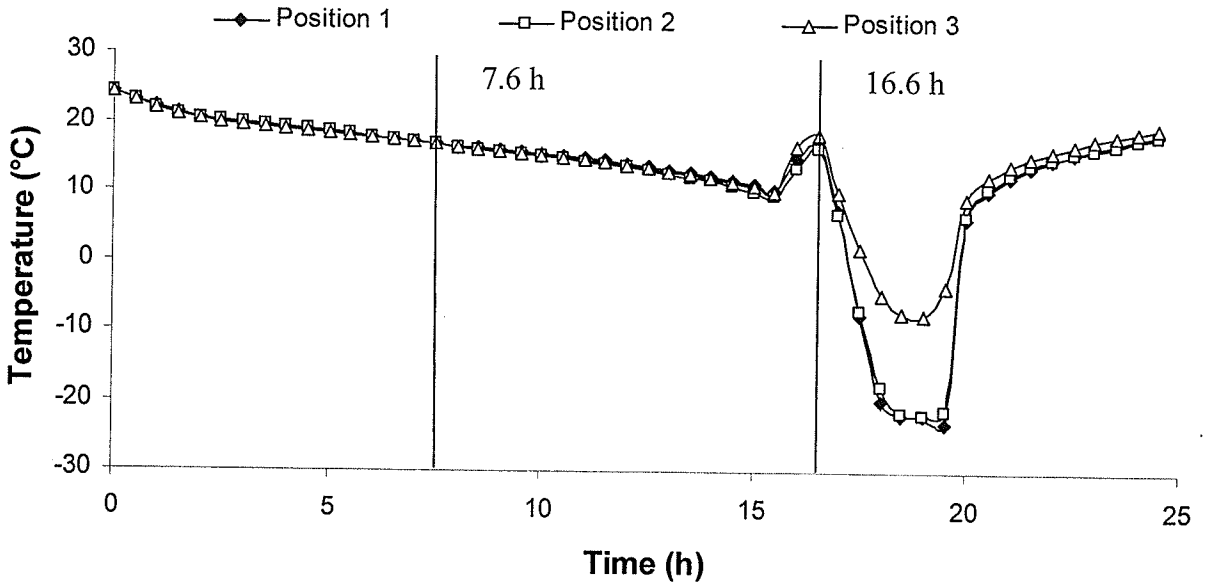


Fig. B.2.5. Temperature change at level 5 inside grain bin during trial 2

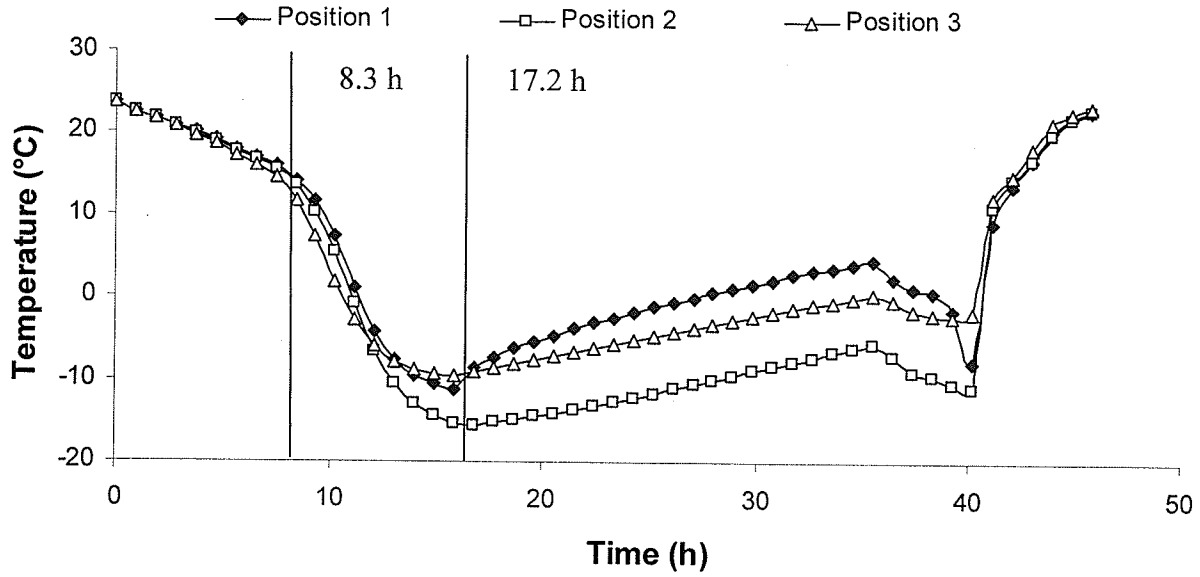


Fig. B.3.3. Temperature change at level 3 inside grain bin during trial 3

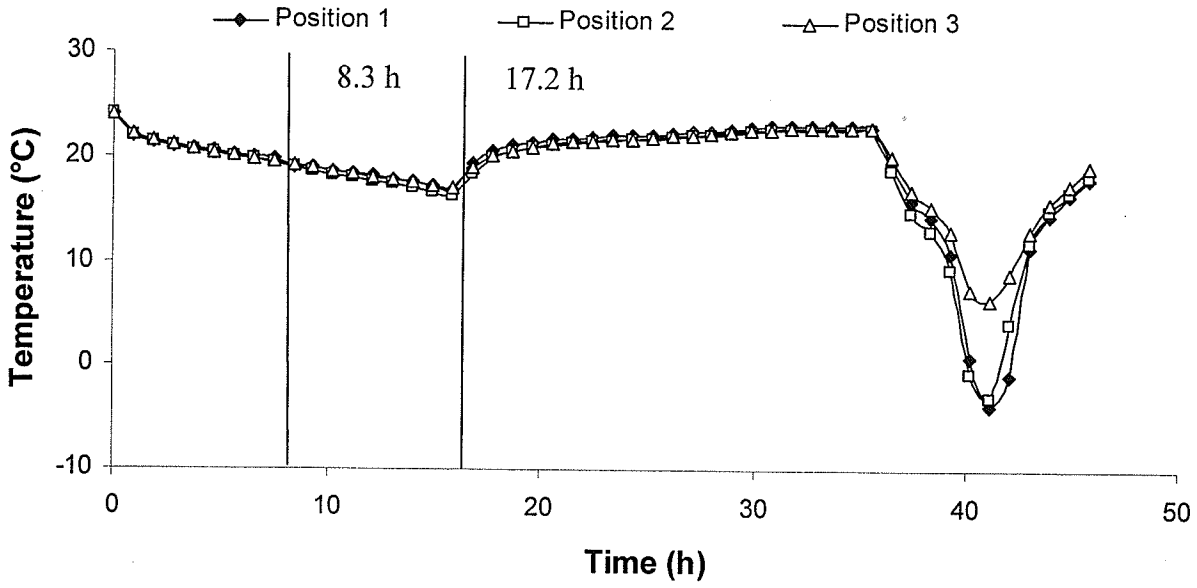


Fig. B.3.5. Temperature change at level 5 inside grain bin during trial 3

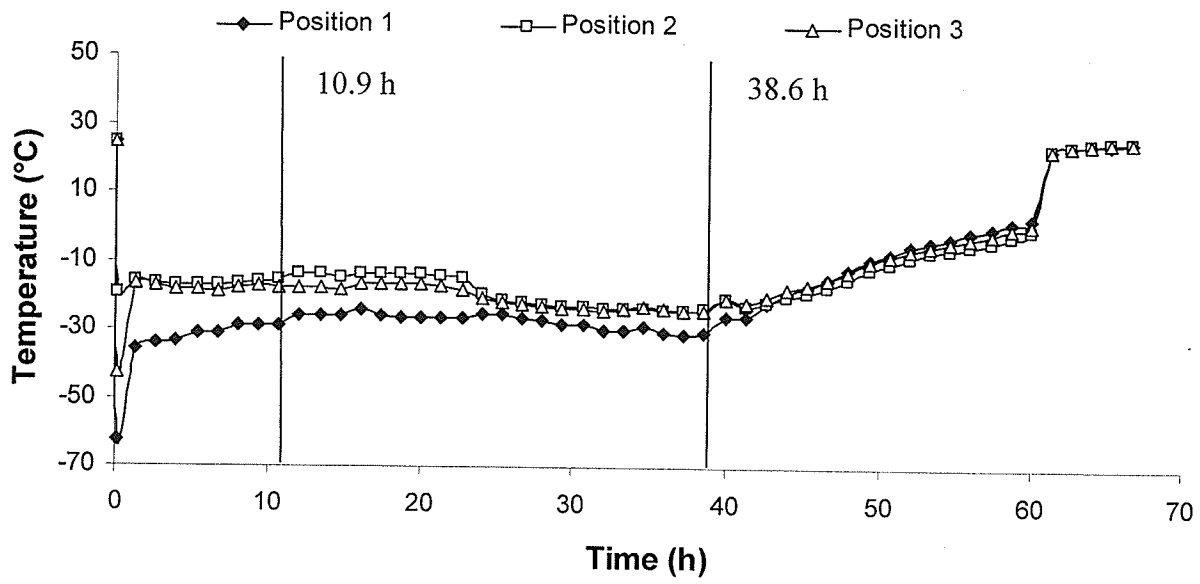


Fig. B.4.1. Temperature change at level 1 inside grain bin during trial 4

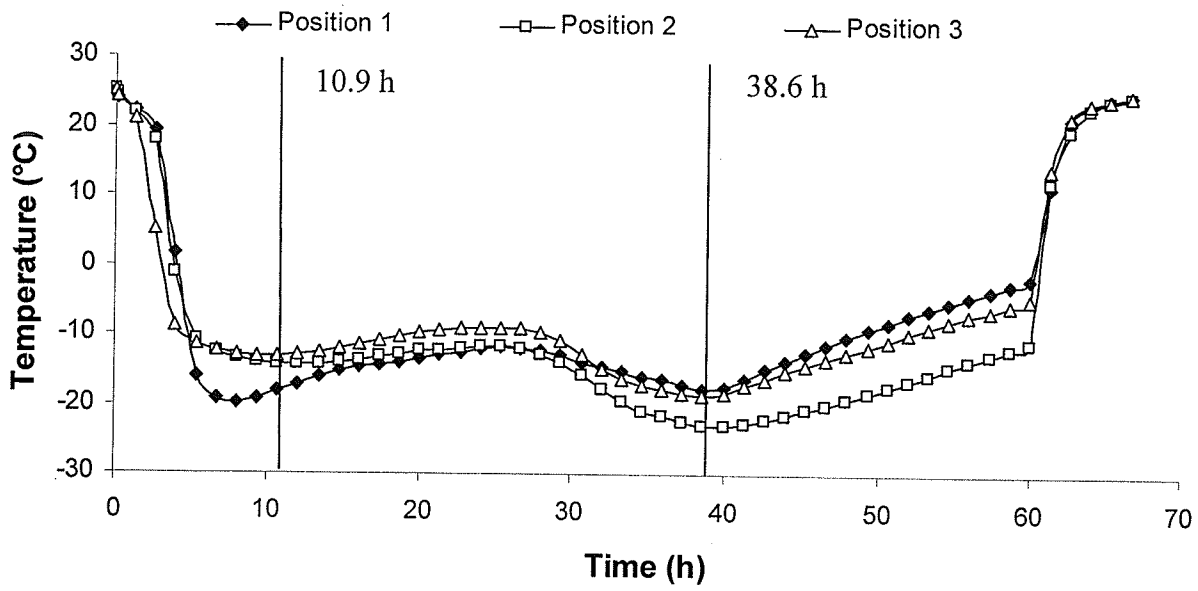


Fig. B.4.2. Temperature change at level 2 inside grain bin during trial 4

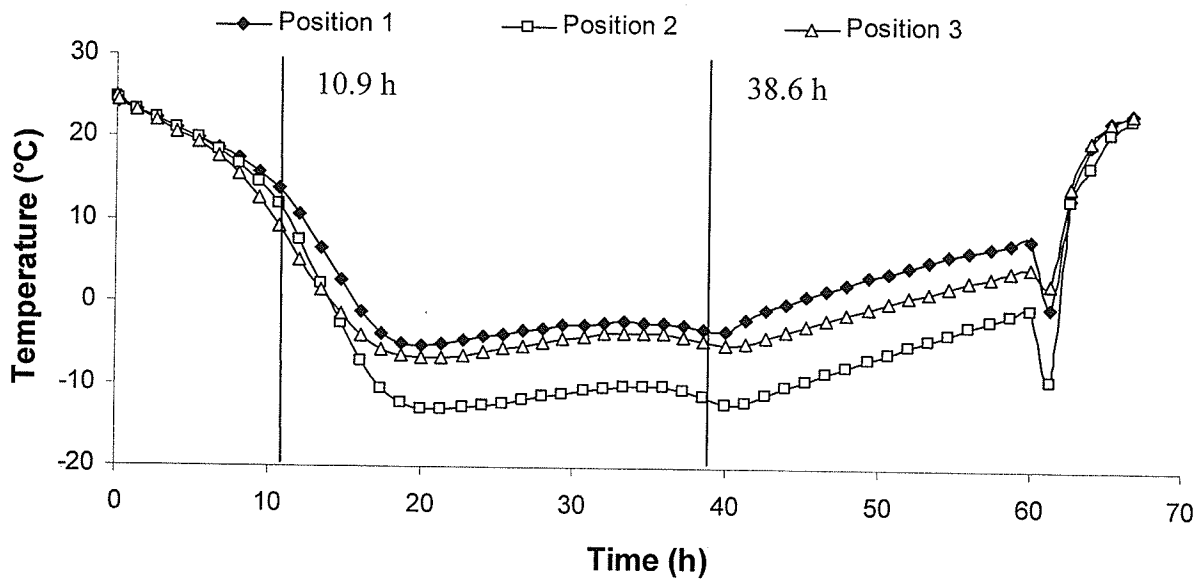


Fig. B.4.3. Temperature change at level 3 inside grain bin during trial 4

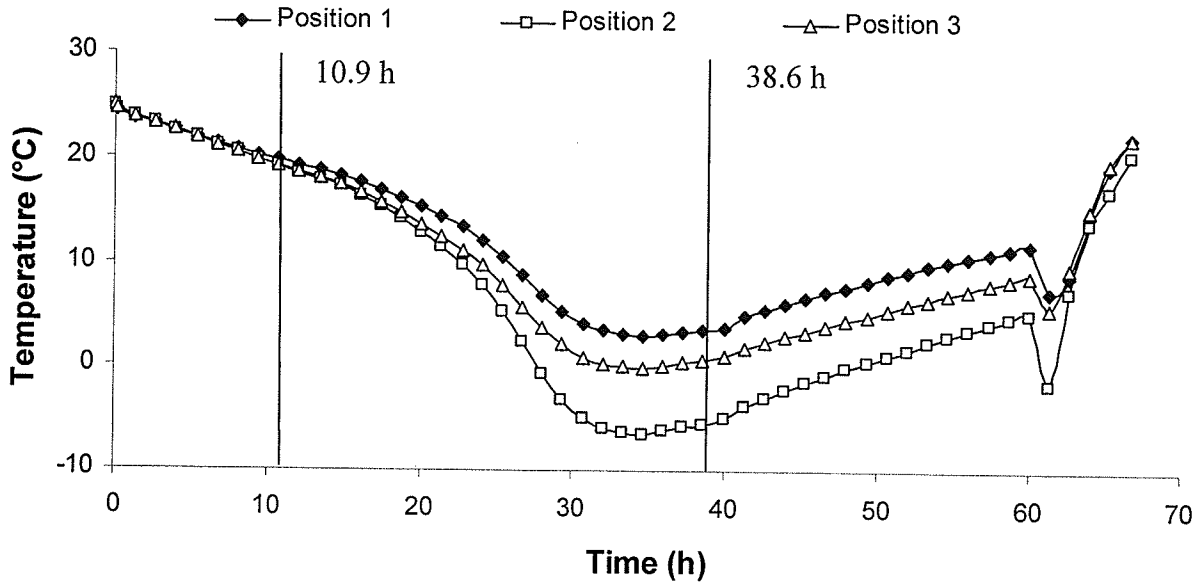


Fig. B.4.4. Temperature change at level 4 inside grain bin during trial 4

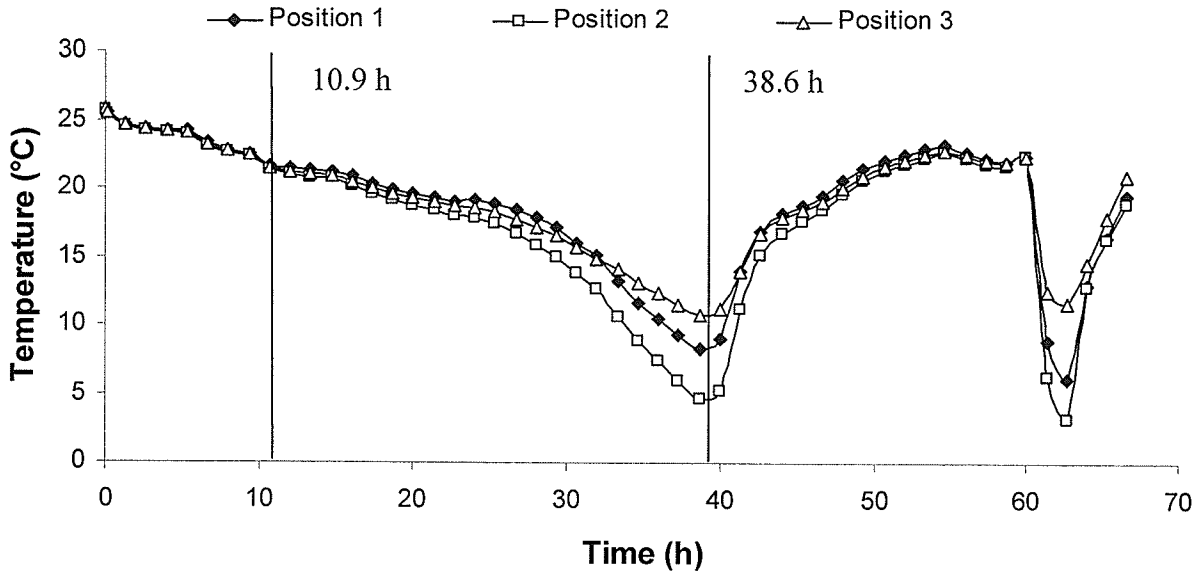


Fig. B.4.5. Temperature change at level 5 inside grain bin during trial 4

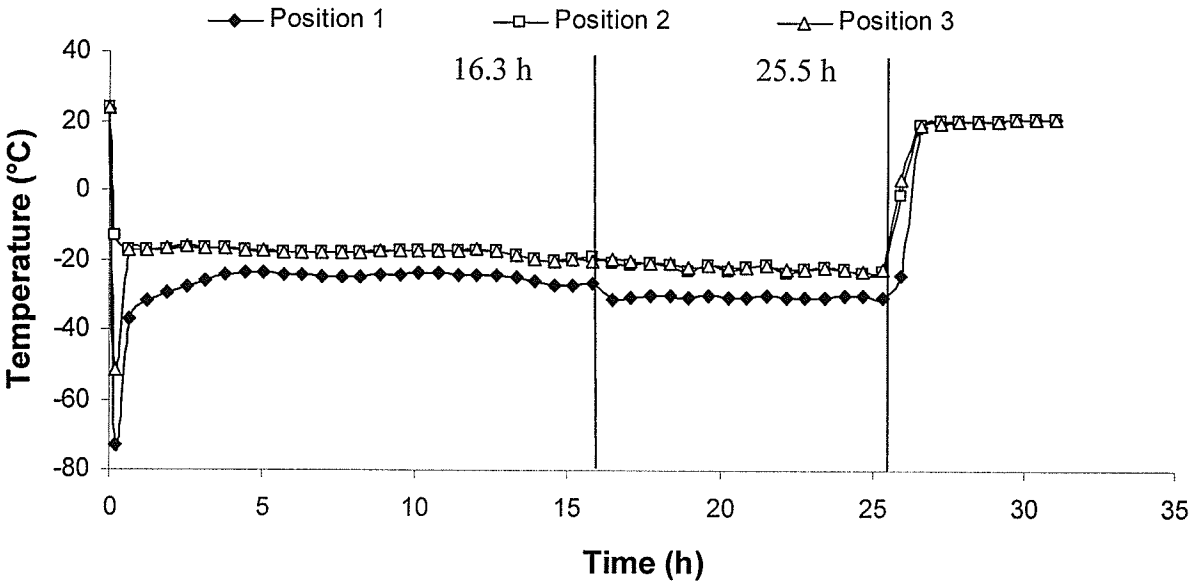


Fig. B.5.1. Temperature change at level 1 inside grain bin during trial 5

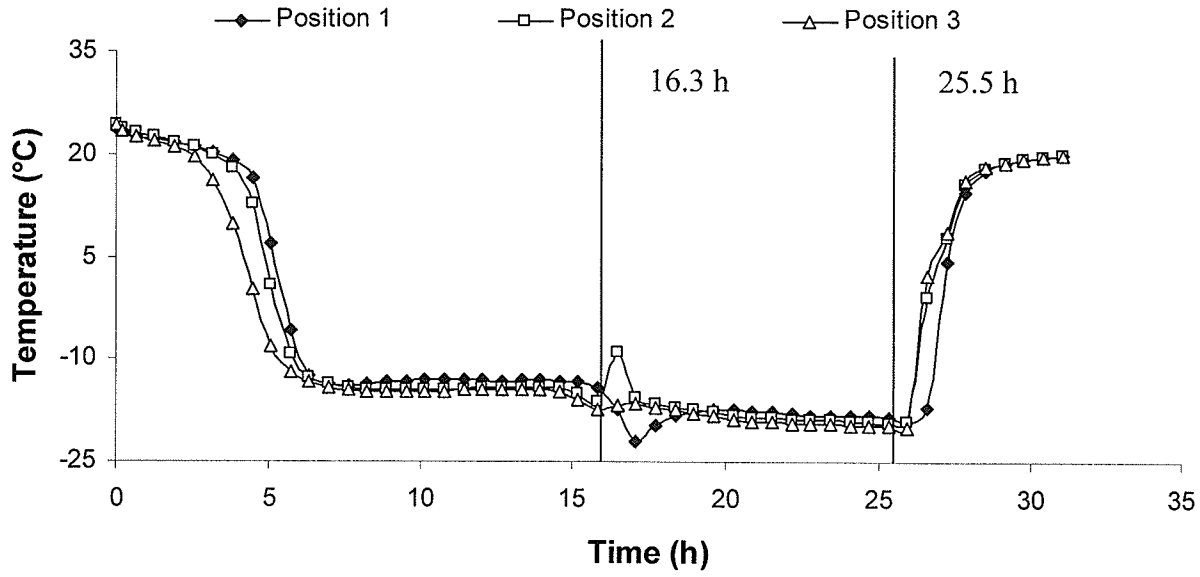


Fig. B.5.2. Temperature change at level 2 inside grain bin during trial 5

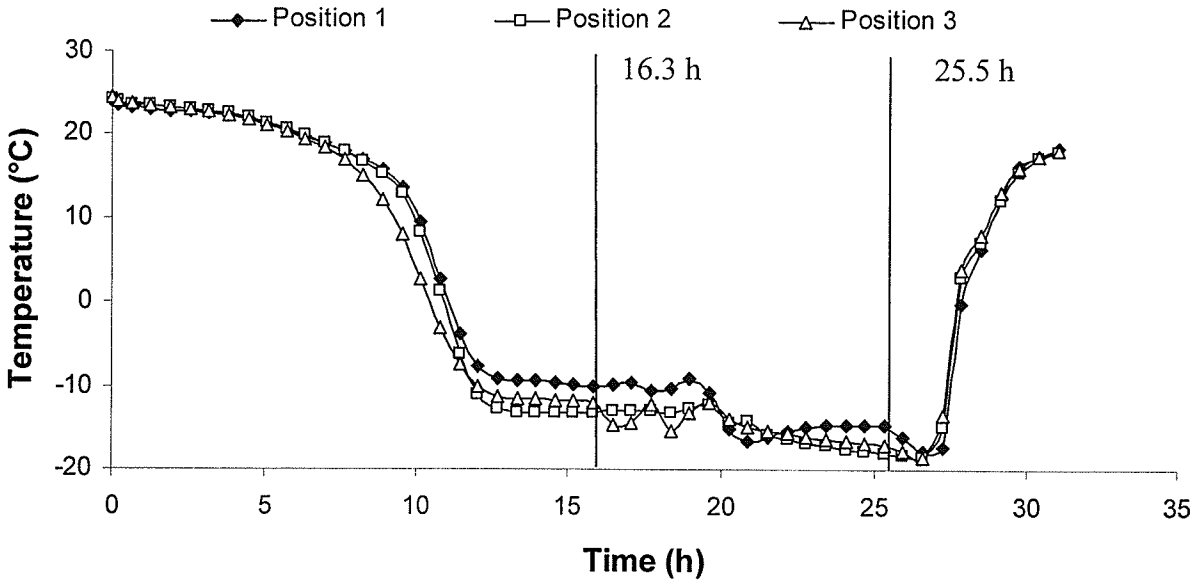


Fig. B.5.3. Temperature change at level 3 inside grain bin during trial 5

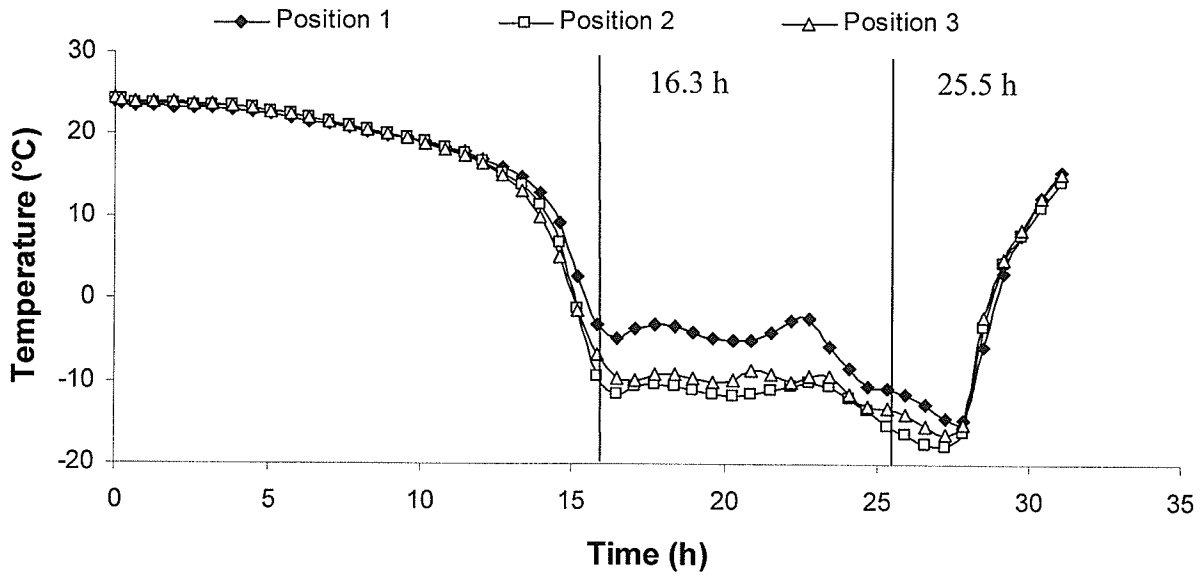


Fig. B.5.4. Temperature change at level 4 inside grain bin during trial 5

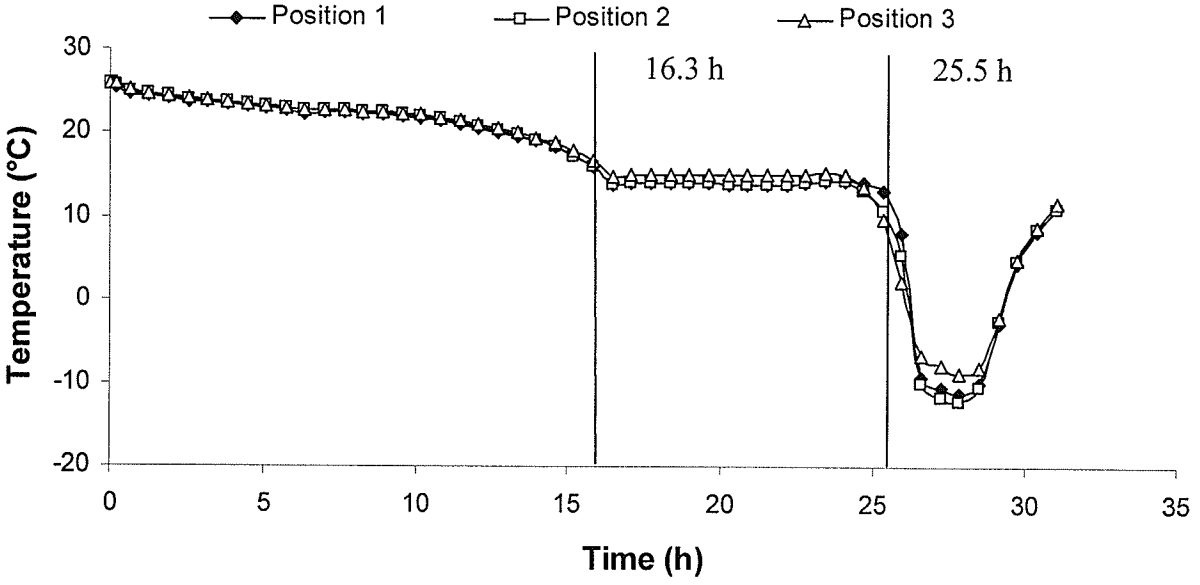


Fig. B.5.5. Temperature change at level 5 inside grain bin during trial 5

APPENDIX C: LOWEST AND AVERAGE TEMPERATURE ACHIEVED AT EACH LEVEL IN FIVE TRIALS

Table C.1. Lowest temperature achieved on level 2 (°C)

Trial #	location		
	left	centre	right
Trial 1	-3.7	-5.9	-14.2
Trial 2	-30.7	-28.9	-23.6
Trial 3	-23.7	-21.0	-18.7
Trial 4	-19.8	-23.1	-18.6
Trial 5	-22.0	-19.3	-19.7

Table C.2. Average temperature achieved on level 2 (°C)

Trial #	location		
	left	centre	right
Trial 1	1.8	1.5	-3.4
Trial 2	-16.3	-11.6	-9.2
Trial 3	-5.3	-8.0	-5.0
Trial 4	-9.1	-11.9	-8.9
Trial 5	-7.3	-7.3	-8.6

Table C.3. Lowest temperature achieved on level 3 (°C)

Trial #	location		
	left	centre	right
Trial 1	0.2	-2.1	-12.2
Trial 2	-20.2	-23.7	-19.2
Trial 3	-11.3	-15.6	-9.7
Trial 4	-5.2	-12.9	-6.8
Trial 5	-17.7	-18.6	-18.5

Table C.4. Average temperature achieved on level 3 (°C)

Trial #	location		
	left	centre	right
Trial 1	6.7	6.6	0.9
Trial 2	-0.7	-0.8	1.6
Trial 3	4.9	-0.5	3.3
Trial 4	4.4	-2.3	2.2
Trial 5	1.0	-0.2	-0.4

Table C.5. Lowest temperature achieved on level 4 (°C)

Trial #	Location		
	left	centre	right
Trial 1	3.0	-0.8	-11.8
Trial 2	-18.1	-25.1	-15.9
Trial 3	-3.3	-7.4	1.3
Trial 4	3.0	-6.4	-0.1
Trial 5	-14.7	-17.8	-16.5

Table C.6. Average temperature achieved on level 4 (°C)

Trial #	location		
	left	centre	right
Trial 1	10.7	9.3	4.3
Trial 2	12.2	10.6	12.3
Trial 3	15.2	12.5	14.1
Trial 4	12.4	7.5	10.5
Trial 5	7.9	5.6	6.2

Table C.7. Lowest temperature achieved on level 5 (°C)

Trial #	Location		
	left	centre	right
Trial 1	4.1	4.7	4.1
Trial 2	-22.9	-21.8	-7.8
Trial 3	-3.7	-3.0	6.3
Trial 4	6.1	3.1	11.6
Trial 5	-11.3	-12.2	-9.0

Table C.8. Average temperature achieved on level 5 (°C)

Trial #	location		
	left	centre	right
Trial 1	12.5	12.5	14.3
Trial 2	12.2	10.6	12.3
Trial 3	18.7	18.4	19.3
Trial 4	12.4	7.5	10.5
Trial 5	15.5	15.4	16.0

Table C.9. Cooling temperatures and duration

	Level 2				Level 3			
	Max (°C)	Min (°C)	Average (°C)	T (h)	Max (°C)	Min (°C)	Average (°C)	T (h)
Trial 1	0.0	-9.7	-4.0	7.0	1.5	-11.5	-3.4	2.7
Trial 2	-4.7	-30.2	-21.0	13.0	-3.9	-27.2	-16.5	9.0
Trial 3	-10.4	-23.9	-17.3	16.8	-2.5	-15.3	-9.7	9.3
Trial 4	-1.9	-23.2	-13.6	54.0	1.0	-13.0	-6.2	28.0
Trial 5	-5.9	-22.0	-16.0	20.3	-3.9	-18.6	-13.5	15.8

	Level 4				Level 5			
	Max (°C)	Min (°C)	Average (°C)	T (h)	Max (°C)	Min (°C)	Average (°C)	T (h)
Trial 1	4.4	-11.8	-2.78	1.0	5.4	4	4.6	1.0
Trial 2	-8.8	-25.1	-16.8	1.5	-3.5	-22.9	-16.0	1.5
Trial 3	7.9	-8.7	0.3	2.8	8.9	-3.7	2.1	2.0
Trial 4	3.8	-6.4	-0.7	8.0	12.4	3.1	8.0	1.3
Trial 5	-2.2	-14.7	-10.1	12.3	-2.0	-12.2	-8.4	2.5

APPENDIX D: SAS RESULT OF INSECT MORTALITIES COMPARISON

Table D.1. Comparisons between T1, T3, T4 and T5 (for Unacclimated insects) at level four

The GLM Procedure

Dependent Variable: mortality

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	246.5000000	82.1666667	0.81	0.5503
Error	4	405.0000000	101.2500000		
Corrected Total	7	651.5000000			

R-Square	Coeff Var	Root MSE	mortality Mean
0.378358	65.98233	10.06231	15.25000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	3	246.5000000	82.1666667	0.81	0.5503

Type 3 Model ANOVA

Dependent Variable: mortality

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	246.5000000	82.1666667	0.81	0.5503

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
(i) T1 vs T3	1	6.2500000	6.2500000	0.06	0.8160
(ii) T1 vs T4	1	81.0000000	81.0000000	0.80	0.4216
(ii) T1 vs T5	1	42.2500000	42.2500000	0.42	0.5535
(iii) T3 vs T4	1	42.2500000	42.2500000	0.42	0.5535
(iiii) T3 vs T5	1	81.0000000	81.0000000	0.80	0.4216
(iiiii) T4 vs T5	1	240.2500000	240.2500000	2.37	0.1983

Table D.2. Comparisons between T1, T3, T4 and T5 (for Unacclimated insects) at level Five

The GLM Procedure

Dependent Variable: mortality

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	509.0000000	169.6666667	2.11	0.2412
Error	4	321.0000000	80.2500000		
Corrected Total	7	830.0000000			

R-Square	Coeff Var	Root MSE	mortality Mean
0.613253	77.89771	8.958236	11.50000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	3	509.0000000	169.6666667	2.11	0.2412

Type 3 Model ANOVA

Dependent Variable: mortality

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	509.0000000	169.6666667	2.11	0.2412

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
(i) T1 vs T3	1	156.2500000	156.2500000	1.95	0.2354
(ii) T1 vs T4	1	36.0000000	36.0000000	0.45	0.5397
(ii) T1 vs T5	1	462.2500000	462.2500000	5.76	0.0744
(iii) T3 vs T4	1	42.2500000	42.2500000	0.53	0.5083
(iiii) T3 vs T5	1	81.0000000	81.0000000	1.01	0.3719
(iiiii) T4 vs T5	1	240.2500000	240.2500000	2.99	0.1586

Table D.3. Comparisons between T3, T4 and T5 (for acclimated insects) at level two

The GLM Procedure

Dependent Variable: mortality

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	506.333333	253.166667	0.78	0.5338
Error	3	974.500000	324.833333		
Corrected Total	5	1480.833333			

R-Square	Coeff Var	Root MSE	mortality Mean
0.341925	43.78089	18.02313	41.16667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	2	506.3333333	253.1666667	0.78	0.5338

Type 3 Model ANOVA

Dependent Variable: mortality

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	506.3333333	253.1666667	0.78	0.5338

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
(i) T3 vs T4	1	272.2500000	272.2500000	0.84	0.4274
(ii) T3 vs T5	1	25.0000000	25.0000000	0.08	0.7995
(iii) T4 vs T5	1	462.2500000	462.2500000	1.42	0.3187

Table D.4. Comparisons between T2 and the mean of T3, T4 and T5 (for acclimated insects) at level three

The GLM Procedure

Dependent Variable: mortality

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	819.0000000	409.5000000	18.34	0.0208
Error	3	67.0000000	22.3333333		
Corrected Total	5	886.0000000			

R-Square	Coeff Var	Root MSE	mortality Mean
0.924379	24.87271	4.725816	19.00000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	2	819.0000000	409.5000000	18.34	0.0208

Type 3 Model ANOVA

Dependent Variable: mortality

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	819.0000000	409.5000000	18.34	0.0208

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
(i) T3 vs T4	1	2.2500000	2.2500000	0.10	0.7717
(ii) T3 vs T5	1	650.2500000	650.2500000	29.12	0.0125
(iii) T4 vs T5	1	576.0000000	576.0000000	25.79	0.0147

Table D.5. Comparisons between T2 and the mean of T3, T4 and T5 (for acclimated insects) at level four

The GLM Procedure

Dependent Variable: mortality

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	102.3333333	51.1666667	9.30	0.0517
Error	3	16.5000000	5.5000000		
Corrected Total	5	118.8333333			

R-Square	Coeff Var	Root MSE	mortality Mean
0.861150	38.03040	2.345208	6.166667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	2	102.3333333	51.1666667	9.30	0.0517

Type 3 Model ANOVA

Dependent Variable: mortality

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	102.3333333	51.1666667	9.30	0.0517

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
(i) T3 vs T4	1	0.25000000	0.25000000	0.05	0.8448
(ii) T3 vs T5	1	81.00000000	81.00000000	14.73	0.0312
(iii) T4 vs T5	1	72.25000000	72.25000000	13.14	0.0361

Table D.6. Comparisons between T2 and the mean of T3, T4 and T5 (for acclimated insects) at level five

The GLM Procedure

Dependent Variable: mortality

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	134.3333333	67.1666667	5.84	0.0924
Error	3	34.5000000	11.5000000		
Corrected Total	5	168.8333333			

R-Square	Coeff Var	Root MSE	mortality Mean
0.795656	58.13426	3.391165	5.833333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	2	134.3333333	67.1666667	5.84	0.0924

Type 3 Model ANOVA

Dependent Variable: mortality

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	134.3333333	67.1666667	5.84	0.0924

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
(i) T3 vs T4	1	1.0000000	1.0000000	0.09	0.7873
(ii) T3 vs T5	1	90.2500000	90.2500000	7.85	0.0678
(iii) T4 vs T5	1	110.2500000	110.2500000	9.59	0.0535

APPENDIX E: MOISTURE CONTENT COMPARISON TEST RESULT

E.1. Result of comparison mean of moisture content among control and 5 trials at level 1 (bottom)

Tukey-Kramer Multiple Comparisons Test

Comparison	Mean Difference	q		P value
Column A vs Column B	0.02000	0.1379	ns	P>0.05
Column A vs Column C	0.06000	0.4137	ns	P>0.05
Column A vs Column D	0.4700	3.241	ns	P>0.05
Column A vs Column E	0.4000	2.758	ns	P>0.05
Column A vs Column F	1.710	11.791	***	P<0.001
Column B vs Column C	0.04000	0.2224	ns	P>0.05
Column B vs Column D	0.4500	2.502	ns	P>0.05
Column B vs Column E	0.3800	2.112	ns	P>0.05
Column B vs Column F	1.690	9.395	***	P<0.001
Column C vs Column D	0.4100	2.279	ns	P>0.05
Column C vs Column E	0.3400	1.890	ns	P>0.05
Column C vs Column F	1.650	9.173	***	P<0.001
Column D vs Column E	-0.07000	0.3891	ns	P>0.05
Column D vs Column F	1.240	6.893	**	P<0.01
Column E vs Column F	1.310	7.283	***	P<0.001

E.2. Result of comparison mean of moisture content among control and 5 trials at level 2

Tukey-Kramer Multiple Comparisons Test

Comparison	Mean Difference	q		P value
Column A vs Column B	0.1700	2.091	ns	P>0.05
Column A vs Column C	0.6100	7.503	***	P<0.001
Column A vs Column D	0.8600	10.578	***	P<0.001
Column A vs Column E	1.190	14.637	***	P<0.001
Column A vs Column F	1.830	22.510	***	P<0.001
Column B vs Column C	0.4400	4.363	ns	P>0.05
Column B vs Column D	0.6900	6.843	**	P<0.01
Column B vs Column E	1.020	10.115	***	P<0.001
Column B vs Column F	1.660	16.462	***	P<0.001
Column C vs Column D	0.2500	2.479	ns	P>0.05
Column C vs Column E	0.5800	5.752	**	P<0.01
Column C vs Column F	1.220	12.099	***	P<0.001
Column D vs Column E	0.3300	3.273	ns	P>0.05
Column D vs Column F	0.9700	9.619	***	P<0.001
Column E vs Column F	0.6400	6.347	**	P<0.01

E.3. Result of comparison mean of moisture content among control and 5 trials at level 3

Tukey-Kramer Multiple Comparisons Test

Comparison	Mean Difference	q		P value
Column A vs Column B	0.2800	2.504	ns	P>0.05
Column A vs Column C	0.4800	4.293	ns	P>0.05
Column A vs Column D	0.8300	7.423	***	P<0.001
Column A vs Column E	1.250	11.179	***	P<0.001
Column A vs Column F	1.910	17.082	***	P<0.001
Column B vs Column C	0.2000	1.442	ns	P>0.05
Column B vs Column D	0.5500	3.966	ns	P>0.05
Column B vs Column E	0.9700	6.994	**	P<0.01
Column B vs Column F	1.630	11.753	***	P<0.001
Column C vs Column D	0.3500	2.524	ns	P>0.05
Column C vs Column E	0.7700	5.552	*	P<0.05
Column C vs Column F	1.430	10.311	***	P<0.001
Column D vs Column E	0.4200	3.028	ns	P>0.05
Column D vs Column F	1.080	7.787	***	P<0.001
Column E vs Column F	0.6600	4.759	*	P<0.05

E.4. Result of comparison mean of moisture content among control and 5 trials at level 4

Tukey-Kramer Multiple Comparisons Test

Comparison	Mean Difference	q		P value
Column A vs Column B	0.1900	2.454	ns	P>0.05
Column A vs Column C	0.3200	4.133	ns	P>0.05
Column A vs Column D	0.7900	10.204	***	P<0.001
Column A vs Column E	1.210	15.629	***	P<0.001
Column A vs Column F	1.780	22.992	***	P<0.001
Column B vs Column C	0.1300	1.354	ns	P>0.05
Column B vs Column D	0.6000	6.248	**	P<0.01
Column B vs Column E	1.020	10.622	***	P<0.001
Column B vs Column F	1.590	16.558	***	P<0.001
Column C vs Column D	0.4700	4.894	*	P<0.05
Column C vs Column E	0.8900	9.268	***	P<0.001
Column C vs Column F	1.460	15.204	***	P<0.001
Column D vs Column E	0.4200	4.374	ns	P>0.05
Column D vs Column F	0.9900	10.310	***	P<0.001
Column E vs Column F	0.5700	5.936	**	P<0.01

E.5. Result of comparison mean of moisture content among control and 5 trials at level 5

Tukey-Kramer Multiple Comparisons Test

Comparison	Mean Difference	q		P value
Column A vs Column B	0.1200	1.500	ns	P>0.05
Column A vs Column C	0.3000	3.749	ns	P>0.05
Column A vs Column D	0.7200	8.997	***	P<0.001
Column A vs Column E	1.150	14.370	***	P<0.001
Column A vs Column F	1.760	21.993	***	P<0.001
Column B vs Column C	0.1800	1.813	ns	P>0.05
Column B vs Column D	0.6000	6.045	**	P<0.01
Column B vs Column E	1.030	10.377	***	P<0.001
Column B vs Column F	1.640	16.522	***	P<0.001
Column C vs Column D	0.4200	4.231	ns	P>0.05
Column C vs Column E	0.8500	8.563	***	P<0.001
Column C vs Column F	1.460	14.709	***	P<0.001
Column D vs Column E	0.4300	4.332	ns	P>0.05
Column D vs Column F	1.040	10.477	***	P<0.001
Column E vs Column F	0.6100	6.145	**	P<0.01

APPENDIX F: INSECT MORTALITIES IN FIVE TRIALS

Table F.1. Insect mortalities inside storage bin after trial 1

level	Mortality (%±S.D.)		lowest temperature (°C)	Time (min) at			
	Unacclimated insect	acclimated insect		below 0°C	below -5°C	below -15°C	below -20°C
1 (bottom)	100±0.00	N/A	-28.3	13.7	7.4	0.3	N/A
2	100±0.00		-14.2	7.5	N/A	N/A	
3	100±0.00		-12.2	N/A	N/A	N/A	
4	33±0.18		-11.8	N/A	N/A	N/A	
5	3±0.01		4.0	N/A	N/A	N/A	

Table F.2. Insect mortalities inside storage bin after trial 2

level	Mortality (%±S.D.)		lowest temperature (°C)	Time (min) at			
	Unacclimated insect	acclimated insect		below 0°C	below -5°C	below -15°C	Below -20°C
1 (bottom)	100±0.00	100±0.00	-38.9	16.0	15.5	12.0	10.5
2	100±0.00	86±0.00	-30.4	14.0	12.5	8.5	6.5
3	100±0.00	20±0.00	-27.2	9.5	8.5	3.5	N/A
4	20±0.00	6±0.00	-25.1	3.5	1.5	0.5	N/A
5	10±0.00	2±0.00	-22.9	2.0	1.0	N/A	N/A

Table F.3. Insect mortalities inside storage bin after trial 3

level	Mortality (%±S.D.)		lowest temperature (°C)	Time (min) at			
	Unacclimated insect	acclimated insect		below 0°C	below -5°C	below -15°C	Below -20°C
1 (bottom)	100±0.00	100±0.00	-36.9	37.3	36.8	32.7	9.3
2	100±0.00	90±0.14	-23.9	34.6	33.8	11.2	N/A
3	98±0.03	20±0.14	-15.6	29.0	7.5	N/A	N/A
4	76±0.14	6±0.06	-8.7	1.0	N/A	N/A	N/A
5	38±0.11	6±0.06	-3.7	N/A	N/A	N/A	N/A

Table F.4. Insect mortalities inside storage bin after trial 4

level	Mortality (%±S.D.)		lowest temperature (°C)	Time (min) at			
	Unacclimated insect	acclimated insect		below 0°C	below -5°C	below -15°C	Below -20°C
1 (bottom)	100±0.00	100±0.00	-62.3	57.0	51.8	46.4	18.0
2	100±0.00	57±0.61	-23.2	56.0	49.0	10.1	N/A
3	100±0.00	23±0.07	-13.0	30.6	2.7	N/A	N/A
4	52±0.11	7±0.01	-6.4	N/A	N/A	N/A	N/A
5	15±0.01	4±0.03	3.14	N/A	N/A	N/A	N/A

Table F.5. Insect mortalities inside storage bin after trial 5

level	Mortality (%±S.D.)		lowest temperature (°C)	Time (min) at			
	Unacclimated insect	acclimated insect		below 0°C	below -5°C	below -15°C	Below -20°C
1 (bottom)	100±0.00	100±0.00	-72.9	25.8	25.2	24.7	10.8
2	100±0.00	100±0.00	-21.9	21.6	20.2	9.5	N/A
3	100±0.00	71±0.04	-18.6	17.1	15.2	5.7	N/A
4	67±0.18	24±0.06	-17.9	13.3	9.5	N/A	N/A
5	46±0.34	25±0.10	-12.2	2.6	1.9	N/A	N/A

APPENDIX G: MOISTURE CONTENT OF WHEAT SAMPLES

Table G.1. Moisture content changes during 5 trials*#

Level	Total moisture content after treatment n (%)				
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
	Mean ±S.D.	Mean ±S.D.	Mean ±S.D.	Mean ±S.D.	Mean ±S.D.
Level 1	9.3±0.5	9.2±0.57	8.8±0.04	8.9±0.20	7.6±0.07
Level 2	9.1±0.03	8.7±0.02	8.4±0.14	8.1±0.04	7.5±0.09
Level 3	9.0±0.44	8.8±0.30	8.5±0.06	8.1±0.03	7.4±0.03
Level 4	9.1±0.03	8.9±0.04	8.5±0.01	8.1±0.03	7.5±0.01
Level 5	9.2±0.01	9.0±0.02	8.6±0.03	8.2±0.09	7.5±0.01

Initial wheat moisture content before trials was 9.3±0.24%
* Weight of wheat sample for moisture content measurement is about 10.0 g

APPENDIX H: WHEAT GERMINATION RATE AFTER TREATMENTS

Table H.1. Number of seeds of Red Spring Wheat germinated of trial 1

No. of seeds used for germination = 25

Position	Sample No.	Total number of seeds germinated					
		Before treatment			After treatment		
		Germination	Mean	S.D.	Germination	Mean	S.D.
Level 1 (bottom)	1	24	24.3	0.58	24	24.3	0.58
	2	25			24		
	3	24			25		
Level 2	1	25	24.7	0.58	25	24.3	0.58
	2	24			24		
	3	25			24		
Level 3	1	24	24.3	0.58	24	24.7	0.58
	2	24			25		
	3	25			25		
Level 4	1	25	24.3	0.58	25	24.7	0.58
	2	24			24		
	3	24			25		
Level 5	1	25	25.0	0.00	25	24.7	0.58
	2	25			25		
	3	25			24		

Table H.2. Number of seeds of Red Spring Wheat germinated of trial 2

No. of seeds used for germination = 25

Position	Sample No.	Total number of seeds germinated					
		Before treatment			After treatment		
		Germination	Mean	S.D.	Germination	Mean	S.D.
Level 1 (bottom)	1	25	24.7	0.58	25	25.0	0.00
	2	25			25		
	3	24			25		
Level 2	1	25	24.7	0.58	25	24.7	0.58
	2	24			24		
	3	25			25		
Level 3	1	24	24.3	0.58	24	24.7	0.58
	2	24			25		
	3	25			25		
Level 4	1	25	24.3	0.58	25	24.7	0.58
	2	24			24		
	3	24			25		
Level 5	1	25	25.0	0.00	25	25.0	0.00
	2	25			25		
	3	25			25		

Table H.3. Number of seeds of Red Spring Wheat germinated of trial 3

No. of seeds used for germination = 25

Position	Sample No.	Total number of seeds germinated					
		Before treatment			After treatment		
		Germination	Mean	S.D.	Germination	Mean	S.D.
Level 1 (bottom)	1	24	24.3	0.58	25	25.0	0.00
	2	25			25		
	3	24			25		
Level 2	1	25	25.0	0.00	25	25.0	0.00
	2	25			25		
	3	25			25		
Level 3	1	24	24.3	0.58	24	24.7	0.58
	2	24			25		
	3	25			25		
Level 4	1	25	24.3	0.58	25	24.7	0.58
	2	24			24		
	3	24			25		
Level 5	1	25	25.0	0.00	25	25.0	0.00
	2	25			25		
	3	25			25		

Table H.4. Number of seeds of Red Spring Wheat germinated of trial 4

No. of seeds used for germination = 25

Position	Sample No.	Total number of seeds germinated					
		Before treatment			After treatment		
		Germination	Mean	S.D.	Germination	Mean	S.D.
Level 1 (bottom)	1	25	25.0	0.00	25	25.0	0.00
	2	25			25		
	3	25			25		
Level 2	1	25	24.7	0.58	25	25.0	0.00
	2	24			25		
	3	25			25		
Level 3	1	24	24.3	0.58	25	25.0	0.00
	2	24			25		
	3	25			25		
Level 4	1	25	24.7	0.58	25	24.7	0.58
	2	25			24		
	3	24			25		
Level 5	1	25	25.0	0.00	25	25.0	0.00
	2	25			25		
	3	25			25		

Table H.5. Number of seeds of Red Spring Wheat germinated of trial 5

No. of seeds used for germination = 25

Position	Sample No.	Total number of seeds germinated					
		Before treatment			After treatment		
		Germination	Mean	S.D.	Germination	Mean	S.D.
Level 1 (bottom)	1	25	25.0	0.00	25	25.0	0.00
	2	25			25		
	3	25			25		
Level 2	1	25	24.7	0.58	25	24.7	0.58
	2	25			24		
	3	25			25		
Level 3	1	24	24.7	0.58	25	25.0	0.00
	2	25			25		
	3	25			25		
Level 4	1	25	24.7	0.58	25	24.7	0.58
	2	25			24		
	3	24			25		
Level 5	1	25	25.0	0.00	25	25.0	0.00
	2	25			25		
	3	25			25		