Temporal Population Dynamics of Stored Maize Insect Pests and Efficacy of Safer Grain Protectants in the Smallholder Sector of Zimbabwe

By

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DECLARATION

I hereby declare that this thesis is my own original work and has not been submitted for a degree in any other university.

Honest Machekano	**Date**

We as supervisors confirm that the work reported in this thesis was carried out by the candidate under our supervision. The thesis was examined and we approved it for final submission.

Dr. B.M. Mvumi	**Date**
Dr. P. Chinwada	**Date**

DEDICATION

To my loving parents, my supportive wife and children, who patiently waited long hours for dad when he was óalways busy with his samplesô as Tino would call it.

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ABSTRACT

The overall objective of the study was to establish the efficacies of different inert dusts alone and in combination with safer levels of a synthetic pyrethroid or a bio-pesticide as grain protectants. Parallel on-station ecological studies at the Institute of Agricultural Engineering (IAE), Harare, were conducted to establish sources of primary infestations that are responsible for most grain damage incurred seasonally. Results from both were integrated to advocate for the combination of the use of safe grain protectants with grain handling practices that minimise or eliminate resident infestations. The efficacies of new food grade imported diatomaceous earths (DEs) (A2 and A3), imported new DE (MN51), registered imported DE (Protect-It®) and local raw DE (Chemutsi®) combinations with spinosad (Spintordust® 0.125%) and reduced quantities of deltamethrin were tested against *Prostephanus truncatus* (Horn), *Sitophilus zeamais* (Motsch.) and *Tribolium castaneum* (Herbst) under controlled conditions of 27±1^{o}C and 60±5% relative humidity. Shumba Super® dust (fenitrothion 1% + deltamethrin 0.13%) applied at 0.5 g/kg and untreated grain were used as positive and negative control treatments, respectively. Cumulative mortality data were recorded at 7, 14 and 21 days, while F1 progeny emergence was recorded at 49 (*S. zeamais*) and 70 (*P. truncatus* and *T. castaneum*) days after treatment. Protect-It® gave significantly higher ($P < 0.001$) insect mortalities than all the other DEs across all test species. Chemutsi was equally efficacious against *S. zeamais* with significant differences ($P < 0.001$) from other DE treatments except Protect-It®. In combination treatments, except for spinosad 0.5 mg/kg + Chemutsi 0.05% w/w that gave low mortality, there were no significant differences between all the treatments and the positive control (Shumba Super® dust 0.5 mg/kg) on all the three test insects. There were no significant differences in *P. truncatus* F1 progeny emergence across all the DE treatments. However, there were significant differences ($P < 0.001$) between Deltamethrin 0.05 mg/kg + Chemutsi 0.1% (w/w) and other cocktail treatments in *S. zeamais* progeny emergence. Out of the 31 laboratory-tested materials, Protect-It® 0.1% (w/w), Spintordust® 1 mg/kg, Spintordust® 0.3 mg/kg + Protect-It® 0.1% (w/w), Deltamethrin 0.05 mg/kg + Protect-It® 0.08% (w/w), Deltamethrin 0.05 mg/kg + Chemutsi 0.1% (w/w), Deltamethrin 0.1 mg/kg + Chemutsi 0.08 % (w/w), Deltamethrin 0.1 mg/kg + Protect-It® 0.08 % (w/w), Spintordust® 0.5 mg/kg + Chemutsi® 0.05 % w/w were further tested for 24 weeks at IAE . These treatments were compared with Shumba Super® 0.5 g/kg and untreated control. Grain damage and weight loss in the untreated control were significantly higher ($P < 0.001$) compared to all other treatments. On-farm trials were conducted in Musami Communal Area, Murehwa district, using five best combination treatments selected from the laboratory bioassay results. The treatments were: Chemutsi 0.08% (w/w) +

Deltamethrin 0.1 mg/kg, Chemutsi 0.05% (w/w) + Spinosad 0.5 mg/kg, Chemutsi 0.1%(w/w) + Deltamethrin 0.05 mg/kg, Protect-It® 0.05% (w/w) + Deltamethrin 0.1 mg/kg, Protect-It® 0.1 % (w/w) and were compared with Chikwapuro® 0.4 g/kg (pirimiphos-methyl 2.5% w/w + deltamethrin 0.1% w/w) and untreated control. At 24 weeks after treatment, grain damage (22.5 %) and weight loss (1.96 %) in the untreated control were both significantly higher ($P < 0.001$) than in all other treatments. *Sitophilus zeamais* was the most abundant pest species observed at all the on-farm trial sites. In the ecological studies, bulk grain was used to determine the critical source of infestation in stored maize. Two sets of 150 kg of grain were fumigated and placed in clean re-plastered granary compartments; one was completely sealed, while the other was left open. The same was repeated using un-fumigated grain. Resident pest infestations caused significantly higher grain damage ($P < 0.001$) in all the unfumigated treatments than the fumigated ones. Damage was more pronounced at top and middle levels in unfumigated closed granaries but was higher at the bottom in unfumigated open ones. High *S. zeamais* populations were observed at top levels of unfumigated open granaries, though it manifested heavily on all unfumigated treatments as well as fumigated open ones. Trash per kg of grain was significantly higher ($P < 0.001$) in all unfumigated treatments where *P. truncatus* was recorded in high populations. The results of the study showed that local and imported DEs can be combined with deltamethrin and spinosad to replace the organophosphate component of the synthetic grain protectants currently available commercially. Resident insect infestation causes more damage and at a faster rate than incoming infestation. Opening or closing the granary is of little importance when the grain is initially free of resident infestation. The elimination or avoidance of resident infestation in harvested maize grain retards insect pest build-up, delays damage and loss, and hence saves resources, time and reduces the risk of pesticide poisoning. Further investigations need to lengthen the study period to a whole storage season (about 40 weeks) to establish if the results obtained at 24 weeks will still hold.

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INTRODUCTION

1.1 Background

The global human population has increased from about 3 billion to over 7 billion in the last five decades (Anonymous, 2013), implying an average increase of about 80 million people per annum. The population of sub-Saharan Africa and that of Zimbabwe in particular, now at 12,973,808 people (Zimstat, 2012) has not been spared. This rapid population growth has also resulted in a concomitant increase in food demand, especially for cereal staples like maize (*Zea mays*) in Zimbabwe. The increased demand has been exacerbated by the dwindling yields year after year due to climate change. The shortage of food has greatly increased the necessity for effective methods of storage and preservation of the little grain that is harvested without increasing the burden on the already degraded natural environment (Tefera, 2012). Since yields recorded after harvest represent the maximum value in terms of inputs and expended human effort (Bond, 1974), it is important that much more effort and resources be directed towards post-harvest handling and stored product protection (than to continue increasing productivity) to prevent loss of stored grain.

The control of insect pests in stored maize grain in Zimbabwe has largely been based on curative chemical methods (Mvumi and Stathers, 2003). Consequently, there has been a wide array of pesticides produced for this purpose. In Zimbabwe six registered grain protectants are currently available on the market. All these pesticides are mainly based on organophosphates and synthetic pyrethroids. Continuous use of organophosphate-based and pyrethroid-based chemicals in cereal grain storage meant for human or livestock consumption poses a serious environmental and health hazard due to residues (Nhachi and Kasilo, 1996; Kamanyire and Karalliedde, 2004; Kolacnski and Curtis, 2004). There is also high level of pollution of human blood with organophosphate and organochlorine residues in Zimbabwe (Khoza *et al.*, 2003) and consequent chronic and sub-chronic symptom manifestations due to these residues (Nhachi, 2001). Farming communities are liable to prolonged exposure to these agrochemicals because of inadequate education, training and uncoordinated occupational health and safety systems in the country (Khoza *et al.*, 2003, Magauzi *et al.*, 2011). Though such incidences were first reported 35 years ago in Zimbabwe (Hayes *et al.*, 1978), chronic and sub-chronic organophosphate poisoning cases are still evident in local hospitals today (Nhachi, 2001). However, such incidences are on the decrease in the developed countries because of declining availability and use of such chemical compounds in these countries (Aarderma, 2008). It is feared that this will make organophosphate and organochlorine poisoning

treatment even more difficult in developing countries when the developed countries cease to produce the treatment drugs (Aarderma, 2008). There is a huge global drive towards the reduction and possible elimination of chemical pesticides. This is partly evidenced by various conferences, symposia, books and journal publications by different organisations and individuals on this subject matter (Mvumi and Stathers, 2003).

Applying chemicals when they are not needed adds unnecessary costs. However, not applying chemicals when they are needed results in heavy losses and huge costs (Adam *et al*., 2006). Many grain protectant pesticides have been developed (Snelson, 1987), but these alone have proved inadequate given that 83% out of the total post-harvest losses occurring to small scale farmers in Zimbabwe are due to insect pests (ZIMVAC, 2011). Therefore, there is need for a combination of many grain protection techniques ranging from correctly applied safe chemicals, change in farmer behaviour towards pests, manipulation of pest ecological environments to the physical prevention of grain from insect pest infestation; all packaged into an integrated pest management (IPM) approach. Though many IPM methods of processing and handling maize grain to minimize infestations have been developed, grain losses to storage pests continue to be incurred (Bond, 1974). Therefore, new, safe and low cost grain protection methods need to be implemented.

Post-harvest losses in maize grain in the tropics have largely been caused by the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), the larger grain borer (LGB), *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae), Angoumois grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) and the rust red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) (Mvumi and Stathers, 2003; Nyagwaya *et al.*, 2010; Tefera *et al*., 2011). *Prostephanus truncatus* is by far the most destructive insect pest in sub-Saharan Africa (Hodges, 1986). In Zimbabwe, first reports of the occurrence and damage by the bostrichid were in the 2006/07 storage season (Nyagwaya *et al.*, 2010). Plant Protection Research Institute (PPRI) later acknowledged its occurrence in Midlands and Mashonaland provinces in March 2010. This pest is a threat to food security in the country due to its aggressive nature and the extensive damage that it causes within a short period (Hodges, 1986; Tefera *et al*., 2011).

Development of an IPM system for management of stored grain insect pests requires the understanding of the ecology of the major post-harvest pests previously mentioned. Various environmental factors influence post-harvest pestsô distribution and proliferation. Since IPM strives to concentrate on developing compatible chemical and non-chemical arsenals against storage insect pests in proper sequence and timing (Harein, 1974), it becomes important to

establish whether the most devastating source of infestation is from resident insects (brought in from the field at the time of storage), or in-coming infestation when the grain is initially stored free of pests. This will enable farmers to precisely target and time grain treatments with safe grain protectants as well as take adequate measures in suppressing the major sources of infestations. Work towards the above will also establish whether elimination of resident infestation prior to storage is crucial or not.

The current study was aimed at developing grain protectants that do not have toxic residual effects to humans, animals and the environment. A major component of such grain materials were local and imported diatomaceous earths (DEs); the former are readily available and cheap but their efficacy needs to be ascertained first. The major thrust of the study was to remove the toxic organophosphate component in commercial pesticides and replace with safe materials like the inert dusts and bio-pesticides. The research also sought to establish the efficacy of such materials under controlled laboratory conditions, on-station natural environment and on-farm typical smallholder grain storage conditions. The on-farm trial gave an in depth understanding of existing storage practices necessary for designation of appropriate post-harvest technologies that will fit perfectly into the existing framework and thus can readily be adopted by the farming community.

1.2 Justification

About seventy per cent of Zimbabweôs population live in the communal areas, and make a living out of agricultural production on degraded marginal lands (ZIMVAC, 2011) with low chances for other sources of income. The unreliable rainfall patterns observed in the last few years (ZIMVAC, 2011) and the seasonality of maize production versus its inelastic demand (Nukenine *et al.*, 2010) in an agriculture-dependent economic environment where poverty is worsening, have resulted in low staple maize yields year after year (Pasipanodya, 2012). Although low yield problems due to poor seasons are often the reason for food insecurity in Zimbabwe (ZIMVAC, 2011) and Africa at large, they are not responsible for the 14-36% of the harvested yield that fails to reach the consumer year after year (Tefera, 2012).

Zimbabweôs annual per capita maize grain requirement is 153 kg (Hassan *et al*., 2001). In the face of population increase (Zimstat, 2012, Geohive, 2013), the per capita supply has decreased though the area under maize cultivation has been concurrently increasing (ZIMVAC, 2011). Though this per capita decrease has been attributed to economic and climatic reasons, it has been exacerbated by post-harvest losses; especially that portion of grain that is eaten by insect pests (Tefera, 2012).

The provision of food has always been a challenge to mankind, but the challenge this time has become tougher in the face of competition from various species of insect pests on small quantities of finally harvested grain.

The United States and Europeôs stance on pesticides in grain protectants is focusing on new developments and bans on organophosphates and pyrethroids. The obvious question is what Africa will do if Europe bans these products since most of these ingredients are imported from the latter. Environmental safety, human and animal health has resulted in diminishing support and confidence level in chemical pesticides. The mandatory reviews of all pesticides in relation to public health and the environment has resulted in withdrawal of some previously very important pesticides (Mvumi and Stathers, 2003). There is also a very narrow range of acceptable pesticides in food (Mvumi and Stathers, 2003); therefore there is an urgent need for new, effective and safe alternative grain protectant materials to fill the void.

Farmers are not fully aware of the dangers of pesticides (Magauzi *et al.*, 2011). Apart from using the grain before the safety cut-off period of the synthetic chemicals, 50% out of the 83% of farmers that use synthetic insecticides in Zimbabwe apply them incorrectly (Mvumi *et al.*, 1995). This has resulted in several cases of poisoning (Nhachi, 2001; Khoza *et al.*, 2003; Magauzi *et al.*, 2011). These cases also have economic implications in terms of hospital bills and unproductive time. The cases can be avoided if non-toxic inert dusts and bio-pesticides are made available to the farmers. Besides, farmers can save their hard-earned income as a locally mined inert dust will be far much cheaper than the industrially manufactured synthetic pesticides.

Most of the previous work done to investigate insect pest control using safe grain protectants (inert dusts, botanicals and bio-pesticides) has been conducted under laboratory conditions (Stathers *et al.*, 2000). In the laboratory, researchers tend to deal with a single species at a time, the bottle environments at controlled temperature and humidity are too confined, most of the environmental factors are often held constant. Besides, laboratory-reared insects may be weaker than field insects from different sources and histories in the natural environment. Therefore, the call for further tests of safe grain protectants on-station and on-farm is driven by the fact that in nature, insects live in complex environments governed by multiple factors (Campbell *et al.*, 2006).

Prostephanus truncatus is now endemic in Zimbabwe. This pest was reported to be causing serious damage in Mashonaland and Midlands provinces which are the countryôs major maize-producing belts (Nyagwaya *et al.*, 2010, Mashavakure, 2012). Many of the crop varieties

developed over the last five decades produced high yields but poor storage characteristics. Over 95% of maize varieties succumb to maize weevils (Komen *et al*., 2008) and 100% of the varieties are attacked by *P. truncatus* (Golob *et al*., 1998), though research on resistant varieties is still on-going (Kumar, 2002; Mwololo *et al*., 2010; Mashavakure, 2012). Therefore, there is need to quickly search for more efficacious protectants as most of the currently registered grain protectants in the country were registered before the presence of the pest was declared. The vulnerability of the bag storage system, the lack of resistant varieties and the tolerance of *P. truncatus* to available pesticides leaves maize grain seriously vulnerable. There is therefore urgent need for a holistic grain protection approach that includes technologies like inert dusts that are persistent and whose mode of action is unlikely to face tolerance or resistance.

In Zimbabwe, 86% of the farmers traditionally cut and stoke their maize, the maize is then left to dry in the field first before being de-husked and dried in open pole or pole and fence/thatch traditional cribs (Mvumi *et al.*, 1995). This provides suitable breeding ground especially for *P. truncatus* (which thrives on unshelled cobs) and *S. zeamais* in the field well before the maize is shelled and stored. After shelling, Nyagwaya *et al*. (2010) reported that about 25% of the farmers store their maize as bulk grain in open granaries, over 70% bag the grain and store the bags either in storerooms, living quarters or in granaries. All these storage systems and the extended post-harvest handling period increase chances of infestation by these and other storage pests resulting in heavy resident infestation that is likely to build up later in the store.

The evaluation of grain damage by incoming infestation compared to resident (field infestations) will establish the importance of shortening the handling and open drying period. There is not much literature in this regard and the results of the current study may open avenues for further investigations to establish a solid postharvest Integrated Pest Management (IPM) package for smallholder farmers. Such pest preventative practices will not only save costs but also reduce the level of exposure to grain handlers and the residual quantities of toxic chemicals in maize grain and the environment.

Overall, prevention of postharvest losses will result in more food for the farmers and their families, more grain for sale during the off-season at attractive prices thus making available income for basic needs and thus, improving the living standards of the farming communities. Non-farming communities will also benefit from abundant and thus cheaper food. Abundant grain on the market will result in improved quality grain trade due to competition. This may expand to export and

ultimately improve the countryôs trade reputation, food security and general international recognition.

1.3 Objectives

1. To determine the efficacy of new imported and local DEs on three major storage pests of maize under laboratory conditions.
2. To determine the synergistic effects of a local raw DE (Chemutsi) and an imported enhanced DE (Protect-It®) in cocktail combinations with a bio-pesticide (spinosad) and reduced pyrethroid (deltamethrin) on three major storage pests of maize under laboratory conditions.
3. To further test the best performing laboratory treatments under on-station conditions.
4. To determine the relative efficacies of five laboratory-screened treatments under on-farm storage conditions.
5. To assess the differences in grain damage and storage pest population build up between resident (field) infestation and re-infestation (incoming pests).

1.4 Hypotheses

1. Imported and local diatomaceous earths (DEs) are efficacious on three major storage pests of maize.
2. There is no difference in efficacy of the cocktail combinations of local raw and imported enhanced DE with bio-pesticides and minimal pyrethroids on three major storage pests of maize in the laboratory.
3. The best performing laboratory treatments will perform well under on-station conditions.
4. The five best performing laboratory treatments will retain this level of performance under on-farm conditions.
5. There is no difference in grain damage and pest population build up between grain exposed to resident (field) infestation only and that exposed tore-infestation (incoming pests) only.

Chapter 2

LITERATURE REVIEW

2.1 Insect Pest Spectrum of Stored Maize

2.1.1 The maize weevil

The maize weevil, *Sitophilus zeamais* (Plate 1) is a cosmopolitan weevil with a protruded head that forms a snout or a proboscis. At the end of the snout is a pair of mandibles or jaws. Adults are usually reddish brown to black in colour. It is distinguishable by its elbowed, eight-segmented antennae and four yellowish to reddish brown oval plates on its elytra and separation from its close relative *Sitophilus oryzae* can only be done through microscopic dissections and genitalia examination (Haines, 1991; Khare, 1994; Tefera *et al*., 2010). The weevils use their elongated snouts for boring into grain; females do the same to dig a shallow hole on grain to lay one egg in each hole. Up to four eggs can be laid singly in a single kernel (400-500 eggs in its lifetime). The eggs are covered by secretions from the female weevil that solidify to form an egg plug. The eggs hatch after one week at 25^{o}C. Upon hatching, the larva burrows into the grain and constructs a winding tunnel that enlarges as it grows. The larva has four instars and the last instar is C-shaped, dirty and legless. It pupates and subsequently the adult weevil emerges by biting out a circular hole on the grain. The sex ratio of emerged adults is 1:1. Female lays most of the eggs in the first five weeks of emergence from the pupae and generally lives longer. At low (< 20^{o}C) or higher temperatures (> 32^{o}C), *S. zeamais* does not breed. (Arthur and Throne, 2003: Tefera *et al*., 2010).

Plate 1. The maize weevil, *Sitophilus zeamais.*

Weevil damage results in direct loss of grain quantity (weight loss) of 12-20% and up to 70% has been recorded in sub-Saharan Africa (Boxall, 2002). Loss of quality through caking and the

introduction of fungi such as *Aspergillus flavus* through hot spots has been recorded (Tefera *et al*., 2010).

2.1.2 The Larger Grain Borer (LGB)

Prostephanus truncatus (Plate 2) is a beetle of a wood-boring family originating from meso-America. It was accidentally introduced into Africa through maize aid to Urambo refugee camp, Tabora, Tanzania in the late 1970s and in Togo in the early 1980s (Dunstan and Magazini, 1981; Krall, 1984). The adult beetle is 2-3.5 mm long, 1-1.5 mm wide and dark brown in colour. It has a cylindrical body shape and when viewed from above, the rear of the insect is square shaped. The thorax bears a row of teeth on its upper front edge and the head is turned down underneath the thorax so that it cannot be seen from above. The deflexed head, the strong mandibles and the cylindrical body shape of *P. truncatus* are typical features of wood-boring insects. The larger pronotum protects the head during tunnelling and provides strong support for the mandibular muscles. The male produces an aggregation pheromone that brings together both males and females to a food source (maize or cassava) where mating and subsequent egg laying takes place in the feeding tunnels (Tefera *et al*., 2010).

Plate 2. The larger grain borer (LGB), *Prostephanus truncatus.*

Prostephanus truncatus is a long-lived species with extended oviposition period and rapid larval development. Potential full lifespan can be as long as 12 months during which period adults continue to feed and infest the host. A single adult can destroy the energy equivalent of five maize kernels in its lifetime (Anonymous, 2009). Optimum conditions for development on maize are

32°C and 70-80% relative humidity (RH). The total developmental time from egg to adult at 70% RH ranges from 25 days at 32°C to 118 days at 18°C (Anonymous, 2009; Tefera *et al*., 2010).

Oviposition occurs 5-10 days after adult emergence, peaking at 15-20 days (Hodges, 1986). Eggs are laid in batches of 20 inside blind-ended tunnels in grain and covered with finely chewed dust. Larvae hatch from the eggs after 3-7 days. The larvae are white, fleshy, C-shaped and sparsely covered with hairs and have three pairs of legs. There are three larval instars inside the food source and these last for about 16 days before pupating. The last larval instar forms a pupal case from frass bound with larval secretions within the grain or surrounding grain dust. The adult emerges after 5-7 days (Anonymous, 2009; Tefera *et al.*, 2010). Females live longer (mean survival time of 61 days) than males (mean survival time of 45 days) (Shires, 1980).

Until relatively recently, *P. truncatus* was only considered to be of quarantine importance in Zimbabwe as it was not yet present in the country (Nyagwaya *et al*., 2010). Official confirmation of its presence was made in early 2010 by the Plant Protection Research Institute (PPRI) under the Ministry of Agriculture. It is capable of reducing maize in stores by up to 41.2% after 8 months and up to 80% losses in untreated grain stored in traditional structures have been recorded in tropical countries (Boxall, 2002). Detection of larger grain borer in export maize consignments at national border inspections results in stiff penalties such as return or destruction of the consignment, treatment (fumigation) or complete ban.

Though LGB is a strong flier, its slow rate of spread through normal flight activity suggests that maize trade has been chiefly responsible for its widespread occurrence near roads and rail trade routes (Farrell, 2000; Nyagwaya *et al.*, 2010). The international grain trade in drought years and the pestôs ability to survive and breed outside storage environments have limited the success of control campaigns in Africa (Hodges, 1986). These survival mechanisms enable the pest to continue spreading into all favourable agro-climatic conditions and food sources in Africa (Farrell, 2000).

The larger grain borer is capable of sustained long flights (25 km in 45 hours). This enables dispersal by flight. Flight activity of LGB peaks at 8-12 days post-enclosion. Favourable temperature (25-30°C), food quality and population densities are important factors determining flight initiation (Hodges, 1986; Anonymous, 2009). The pest has a tendency to aggregate on the same host due to the aggregation pheromone produced by the males which attract both sexes (Hodges, 1986). Thus, just like *S. zeamais*, infestation begins in the field prior to harvest through

exploratory holes at the base of the cob when the moisture content of the maturing maize may well be above 40%. These holes are later abandoned at 1-2 cm depth and the insects then penetrate through the apex of the cob (Anonymous, 2009). These field-initiated infestations continue to grow in maize cobs/grain throughout storage especially if control measures are inadequate.

Maize stored on the cob suffers more damage than shelled maize because the spaces between grains on the cob give *P. truncatus* an opportunity to properly anchor its body for strong support to chew the holes (Hodges, 1986). Maize and cassava are the major hosts while dried sweet potato, sorghum, rice, millets teak seeds and jowa seeds are minor hosts (Hodges, 1986; Anonymous, 2009).

In Ghana, Addo *et al.* (2010) reported that all farmers could recognise *S. zeamais* and acknowledge having it in their stores, but 15% could not recognise LGB ten years after its arrival. It is now also ten years since Rwegasira *et al.* (2003) reported the potential invasion of Zimbabwe by LGB, yet farmers still can neither recognise it nor its grain damage symptoms. Unlike in Ghana where 74% and 55% of the male and female farmers, respectively, changed their storage systems due to pressure from LGB infestation and NGO training (Addo *et al.*, 2010), farmers in Zimbabwe have not been well informed and do not recognise the danger posed by LGB. Research is now on-going to assess the efficiency of metal silos under local conditions and the potential for their subsequent adoption by the farmers in order to cope with LGB. Nevertheless, this technology will need complementation from safe grain protectants, store hygiene and other post-harvest IPM practices.

The level of damage caused by *P. truncatus* in a short period, for example, > 40% in just 3 months (Boxall, 2002), indicates that control measures need to be implemented at the onset of the grain storage especially if the grain will be stored for over 30 days (Tefera *et al.*, 2011). Grain is reduced to powder (Plate 3B) with a sour taste that is unsuitable for human consumption. Tefera, (2011) reported 67.1% grain weight loss, producing 52.8% powder/flour (compared to 1.2% powder and 6.9 grain weight loss by *S. zeamais*) in maize damaged by LGB in 90 days in laboratory experiments.

Plate 3. The difference between (A) Undamaged maize grain and (B) LGB damaged grain

2.1.3 The rust red flour beetle

The rust red flour beetle, *T. castaneum* (Plate 4) is of Indo-Australian origin but is now cosmopolitan in most warmer climates including sub-Saharan Africa. It got its name from its reddish colouration and is mainly found in wheat grain and wheat flour in mills and groceries and occurs in maize as a secondary pest (Mason, 2003). A total of 350-400 eggs are deposited (a few per day) by the adult female directly onto flour, in store cracks and crevices, on wheat or attached by a sticky substance to the surface of containers or stores. The eggs are oblong, initially colourless, later turning white. They hatch in 3-5 days at 32-35^{o}C to produce a yellowish white larva with three pairs of thoracic legs that burrow into flour or enter grain through existing cracks or holes. There are on average about 7-8 larval instars, but range from 5-11 depending on food, temperature, humidity and the individual insect (Park, 1934; Mason, 2003). Larvae hide in their food away from light. It is the last instar larvae that move to the food surface to pupate. The pupae are naked (not covered in puparia) and white, with appendages not fused to the body (as in other pupae). External genitalia on the pupae are distinct and can be used to separate males from females. The larvae and pupae can survive in grain with moisture content as low as 8% (Mason, 2003).

Plate 4. The rust red flour beetle, *Tribolium castaneum.*

Development from egg to adult takes 41.8 days at 25^{o}C and 24 days at 35.5^{o}C days. At 70% relative humidity and temperatures between 35-37.5^{o}C, development can be completed in 19-20 days. Male adults can be distinguished morphologically from their female counterparts by a setiferous patch on the posterior side of their fore femur (Park, 1934; Mason, 2003).

The rust red flour beetle is a secondary pest of maize. Its presence in grain, mealie-meal, flour or any food processing facility is a significant concern because of spoilage through larvae, dead adults and frass. Flour or other food products where insects are detected can be deemed adulterated (Dowdy and Fields, 2002). Exposure of *T. castaneum* and *T. confusum* to a diatomaceous earth marine formulation Protect-It® at a rate of 0.5 mg/cm^2 at 27^{o}C and a relative humidity of 40-75% showed that *T. castaneum* was more susceptible to the diatomaceous earth (Arthur, 2000). *Tribolium castaneum* succumbed to Protect-It®, with 61% mortality being recorded after 15 days at a concentration of 0.5%. Lower Protect-It® rates gave lower mortalities (Motti and Awaknavar, 2009). Mvumi *et al.* (2006) tested five African DEs (including two Zimbabwean) against *S. zeamais*, *T castaneum* and *R. dominica* and recorded significant mortalities at rates as high as 5,000 ppm (0.5% w/w).

2.2 Grain Protectants

2.2.1 Diatomaceous Earths

Diatomaceous earths (DEs) are geological deposits consisting of fossilised skeletons of various species of siliceous fresh water or marine unicellular algae called diatoms (Quarles and Winn, 1996). Diatoms are a very diverse group of plant species abundant in all aquatic and terrestrial environments (Korunic, 1998). These fossilised sedimentary layers originated about 20 million years ago in the lakes and seas of the Eocene and Meocene epochs (Quarles and Winn, 1996; Korunic, 1998). Different diatoms extracted silicon from water to produce hydrated amorphous silica skeleton. When they died, the tiny diatom shells sunk to the bottom and formed thick layers over centuries. The layers fossilised and compressed into a soft chalky rock called diatomaceous earth (Korunic, 1998).

After quarrying, crushing and milling, a fine, light dust is obtained containing porous particles with abrasive and lipid absorption properties (Korunic, 1998). Diatomaceous earths DEs as mined have 50% moisture content, the remaining 50% of dry matter is made up of 86-94% amorphous silicon dioxide and the remaining 6-14% is made up of inorganic salts and oxides. Other elements that have been detected in various DEs include Aluminium (Al), Magnesium (Mg), Zinc (Zn),

Phosphorus (P), Calcium (Ca), Nickel (Ni), Manganese (Mn), Sodium (Na) and Iron (Fe) but their contribution to the insecticidal activity of various DEs has not been reported (Quarles and Winn, 1996; Korunic, 1998; Fields and Korunic, 2000). Processing reduces the moisture content to 2-6%, crystalline silica to 2-7%, pH values to 4.4-9.2 and the mean aggregate particle size to between 10 and 50 µm (Korunic, 1998).

DEs are extremely stable and do not react with other substances, the grain or the environment. DEs have no smell, but vary in colour from white grey to yellow red depending on composition. They are insoluble in water and are neither flammable nor explosive. The oral lethal concentration (LC_{50}) of silicon dioxide in DEs is 3,160 mg/kg which shows very, very low mammalian toxicity. The Environmental Protection Agency (EPA) of the United States classifies DEs as ñGenerally Recognised As Safe" (GRAS) (Korunic, 1998; Fields and Korunic, 2000). Korunic (1998) and Kostyukovsky *et al*. (2010) reported that as a non-chemical natural original product, DE is used in toothpastes and paints, as a filter media in swimming pools and fish tanks, liquor clarification, separation and filtration of commercial oils and fluids. It is also used in foodstuffs, additive in spices, artificial sweeteners and baby powders (Nikpay, 2006; Kostyukovsky *et al*., 2010).

The DE mode of action as an insecticide was first discovered in 1931 as the ñZacher Effectò by Zacher and Kunike (Korunic, 1997). The main modes of action include sorption of the lipid layer of the cuticle resulting in desiccation and death, abrasive effect on the cuticle and the digestive tract, suffocation due to limited amount of oxygen as a result of huge quantities of dust and general avoidance of DE-treated grain (repellence) (Korunic, 1997). These effects work in combination. DE efficacy depends on physical properties of the diatoms that include high proportion of amorphous silicon dioxide (SiO_2), uniform particle size (< 10 µm), high oil sorption capacity and very little clay and other impurities (White *et al.*, 1966; Korunic, 1997).

Inert dusts disrupt the epicuticle by mechanical abrasion and absorption of lipids and insects become more vulnerable to desiccation once they lose the protection of the water-proof layers. Any DEs with oil-absorbing capacity is a potential insecticide. However, the absorbing capacity, size of particles, uniformity of particles, pH, and purity of formulation affects its efficacy (Korunic, 1998). The efficacy of DEs is also affected by temperature, relative humidity and target insect species (Arthur, 2001; Nikpay, 2006) and the commodity being treated. Nikpay (2006) reported high efficacy on small grains such as wheat than on maize and legume grain, therefore, the commodity to be treated is key in determining the concentration of DEs required for effective pest control.

Diatomaceous earths are probably the most efficacious natural dust used as insecticides. Heavy doses of DEs were discovered to give better protection to grain over a period of 3 years than a cocktail of organophosphates and synthetic pyrethroids (White *et al*., 1966). On treated grain, dust particles are picked up and trapped onto insect bodies during normal movement. More dust is picked up by insects that have rough and/or hairy (setaceous) cuticular surfaces. The dust begins to abrade the cuticle, adsorbing the wax and the insects desiccate and die (Korunic, 1998; Nikpay, 2006). The first commercial DE became available in 1960 (Korunic, 1994). To date, numerous DEs including Diasource®, Perma Guard®, Dryacide®, Insecto®, Silisico® and Protect-It® have been registered in different countries throughout the world.

The use of synthetic pesticides is under global scrutiny due to chemical environmental contamination, atmospheric ozone depletion, potential carcinogenic residues in commodities, exposure of users, pest resistance due to narrow range of acceptable chemical grain protectants and general consumer aversion to chemicals (Fields and Korunic, 2000; Mvumi and Stathers, 2003). Diatomaceous earths are the best alternatives due to their low mammalian toxicity, chemical stability and non-effect on non-target insects (Fields and Korunic, 2000). The implementation of DEs technology in developing countries is, however, still being constrained by their scarcity, product ownership and lack of awareness (Mvumi and Stathers, 2003). A wide spectrum of stored product pests have been shown to succumb to different types of DEs (Arthur, 2000; Mewis and Ulrichs, 2001; Athanassiou *et al.*, 2007; Kostyukovsky *et al.*, 2010). However, *T. castaneum* adults are fairly tolerant to a range of DEs compared to their larval stages (Arthur, 2002; Kostyukovsky *et al.*, 2010). This insect species is far less susceptible to DEs than any other stored product pest (Nikpay, 2006).

DEs are, however, not without their own weaknesses. Physical and morphological characteristics affect their efficacy. Small-bodied insects with large surface area to volume ratio are more sensitive than large-bodied insects (Korunic, 1997; Nikpay, 2006). Hairy insects pick more dust per unit time and unit area and thus experience more cuticular damage that insects without setae. Insects with thin cuticles/wax layers suffer high mortality than those with thick cuticles/wax layers, for example Lepidopterans suffer more than Coleopterans (Korunic, 1997). Grain with large particle size mix less effectively with the inert dusts than small grain and thus high mortalities of insects are achieved in small than large grains. Since the mode of action of all DEs is mostly water loss, insect mortalities tend to be low on grain with high moisture content or grain stored in highly humid environments. In the same vein, fluid-feeding insects quickly recover the

lost water and can thus survive the DEs (Korunic, 1997, 1998). In this regard, the mixing of DEs with spinosad and deltamethrin in this research is a strategy meant to counter the weaknesses of DEs. The humidity in the natural environment under local conditions under which the DEs are going to be tested is low enough to enable the DEs to work perfectly except under extreme weather conditions which are rare. The insects being tested are all non-fluid feeding Coleopterans with hard cuticles that are expected to quickly respond to the abrasion effect of the DEs.

2.2.2 Spinosad

Spinosad is a natural product derived from the fermentation products of a soil-dwelling actinomycete, *Saccharopolyspora spinosa* (Actinomycetales: Actinomycetaceae) (Subramanyam *et al*., 2007; Hertlein *et al*., 2011). Spinosad is a white crystalline solid that is soluble in water but breaks down on leaf surfaces (Salgado, 1998). Dust and liquid suspension concentrate formulations have been made and used as grain protectants, but the latter is mainly recommended by the World Health Organisation (WHO) for larvicidal mosquito control (Subramanyam *et al.*, 2007; Hertlein *et al.*, 2011). Though it is mainly a contact insecticide, spinosadôs ingestion activity is also superior (Hertlein *et al.*, 2011). It is a broad spectrum bio-pesticide effective against all stages of several species of stored product insect pests at a labelled rate of 1 mg/kg (Subramanyam *et al*., 2007; Hertlein *et al*., 2011).

Spinosad contains two insecticidal metabolites called Spinosyn A and Spinosyn D at the ratio of 85:15% in the final commercial product (Hertlein *et al*., 2011). These fermentation products (metabolites) are the active ingredients. The mode of action of spinosad is acting on the nicotinic acetylcholine and gamma amino butyric acid (GABA) receptors sites of the central nervous system. Insects die of muscle contractions, tremors, hyper-excitation and paralysis (Salgado, 1998). This mode of action is unique and enables spinosad to control insect pest strains resistant to other grain protectants (Hertlein *et al*., 2011).

The U.S Environmental Protection Agency approved the use of spinosad as a grain protectant at 1 mg (a.i) /kg in January 2005 (Subramanyam, 2006). At this rate, spinosad exhibits low mammalian toxicity and persists for 6-12 months with minimal loss in insecticidal activity (Thompson *et al.*, 2000). These attributes make spinosad a very important grain protectant. Spinosad is also effective in killing adult *Rhyzopertha dominica*, *Cryptolestes ferrugeneus* and *Oryzaephilus surinamensis* but is ineffective against *T. castaneum.* However, F1 progeny numbers were suppressed on spinosad-treated grain for all the test insects including *T. castaneum* (Subramanyam *et al*., 2007).

A single application of 1 mg a.i/kg of grain is effective in managing stored grain insects for over six months (Subramanyam *et al.*, 2007). Huang and Subramanyam (2007) reported that in maize treated with spinosad at rates of 1-2 mg/kg, there was 98% mortality of seven storage pests (including *S. zeamais*) in just 12 days. At 0.5 mg/kg, spinosad completely suppressed egg to larval survival of these pests; therefore spinosad is worth testing in maize storage pest complex under local conditions.

Spinosad is registered by Dow Agro Sciences® (Indianapolis, Indiana, U.S.A) as Spintordust® (0.125% spinosad) and in Africa, it is registered as a grain protectant and has been marketed and distributed by Lachlan Kenya Ltd, Kenya since 2003 (Mutambuki *et al*., 2012). Since then, it has been registered in more than 15 African countries (Hertlein *et al.*, 2011) but not in Zimbabwe. Since both the DEs and spinosad have very low mammalian toxicity and have been tested and approved in other countries as safe grain protectants, it is worth combining these two together and test them for synergistic efficacy against maize storage pests for prospective adoption by smallholder farmers in Zimbabwe.

2.2.3 Organophosphates and pyrethroids

Some of the commercially available synthetic grain protectants currently available in Zimbabwe include Hurudza Grain Dust® (fenitrothion 1.7% w/w + deltamethrin 0.05% w/w), Shumba Super Dust® (fenitrothion 1.0% w/w + deltamethrin 0.13% w/w), Actellic Super Chirindamatura Dust® (pirimiphos-methyl 1.6% w/w + permetrin 0.3% w/w, Chikwapuro® (pirimiphos-methyl 2.5% w/w + deltamethrin 0.1% w/w); Ngwena Yedura® (pirimiphos-methyl 2.5% w/w + deltamethrin 0.2% w/w) and Actellic Gold Chirindamatura Dust® (pirimiphos-methyl 1.6% w/w + thiamethoxam 3.6% w/w). Phosphine (Aluminium phosphide 56% w/w + inert ingredients 44% w/w) is the most commonly available fumigant and is used as a tablet formulation in seed houses and commercial storage facilities.

It is important to note that the most widely used organophosphate grain protectants in Zimbabwe are fenitrothion ($C_9H_{12}NO_5PS$) and pirimiphos-methyl ($C_{11}H_{20}N_{30}3PS$). The common synthetic pyrethroids in use are deltamethrin ($C_{22}H_{19}Br_2NO_3$) and permethrin ($C_{21}H_{20}Cl_2O_3$). Actellic Gold Chirindamatura Dust® is the only grain protectant that uses a nicotinoid (thiamethoxam) in place of a synthetic pyrethroid. Syngenta Agrochemicals recently registered Actellic Gold Chirindamatura Dust® to replace Actellic Super Chirindamatura Dust® which is no longer effective against *P. truncatus*.

Permethrin and deltamethrin are broad spectrum synthetic pyrethroids generally used as insecticides, acaricides, pharmaceuticals and repellents. Their mode of action is by interfering with sodium ion (Na^+) channels to disrupt neuron functions in the central nervous system. This results in muscle spasms that culminate into paralysis and death. They are mainly effective through contact and stomach poisoning. Pirimiphos-methyl is a special broad spectrum organophosphate mainly reserved for grain protection. Its mode of action is by phosphorylation of the acetylcholine esterase enzyme of the tissues. This results in the accumulation of acetylcholine at the cholinergic neuro-effector junctions, a condition known as muscarinic effect which results in death (Golob *et al.*, 2002). Fenitrothion is a contact organophosphate with acaricidal properties. Its mode of action is similar to that of pirimiphos-methyl.

Pirimiphos-methyl has a lower rate of degradation from grain. Its residues remain biologically active over extended periods (more than 12 months) (Snelson, 1987). In Zambia, pirimiphos methyl applied at 4 mg/kg on maize grain, degraded by less than 30% over a 7-month storage period at grain moisture content between 9.8 and 12.2% (Snelson, 1987). Ong *et al.* (1994) observed 0.9 mg/kg residues of fenitrothion on maize applied at 12 mg/kg 36 weeks after treatment and 1.1 mg/kg of pirimiphos-methyl applied at 6 mg/kg to maize grain 30 weeks after treatment. It is important to note that 30-36 weeks are equivalent to 8-9 months, which is the length of the normal storage season for smallholder farmers in Zimbabwe.

Organophosphate poisoning has been reported in Zimbabwean hospitals (Hayes *et al.*, 1978; Nhachi, 2001; Khoza *et al.*, 2003, Magauzi *et al.*, 2011). Toxicological effects of chronic ingestion are difficult to determine in man. However, experiments carried out with small mammals have shown changes in haematology and enzyme activity. Specifically, pirimiphos methyl has been shown to cause chronic increase in alkaline phosphatase, which is a symptom of acute hepatitis (Golob *et al.*, 2002) indicating that some of the hepatitis cases reported in Zimbabwean hospitals may not necessarily be viral. The same organic compound also causes a reduction of lymphocytes and monocytes (without showing any sign of weight loss in tested individuals) which are essential for the effective operation of the immune system (Golob *et al.*, 2002).

Deltamethrin and permethrin are synthetic analogues of natural pyrethrum. Pyrethrum is derived from oily extracts of the pyrethrum flower *Tanacetum cinerariaefolium*) (= *Chrysanthemum cinerariifolium)*. This natural product has broad spectrum insecticidal activity and low mammalian toxicity. Pyrethrum is made up of six products: pyrethrins 1 and 2, cinerins 1 and 2 and jasmolins 1 and 2. However, this compound breaks down quickly on exposure to sunlight and some insects

can detoxify it when received in small quantities (Golob *et al*., 2002). This rapid degradation of pyrethrum necessitated the need to develop synthetic analogues that are photo-stable and cannot be detoxified by insects, hence the introduction of synthetic permethrin and deltamethrin in grain protectants. Ong *et al.* (1994) observed 0.1 m/kg residues of deltamethrin and 0.6 mg/kg of permethrin on maize applied at 1 mg/kg and 6 mg/kg at 36 and 30 weeks after treatment, respectively. This shows that though very efficacious, these chemical protectants do not come without toxicological, safety and environmental risks (Golob *et al.*, 2002). Though there has been extensive research on the fate and toxicity of pesticides, there are still research gaps (Damallas and Eleftherohorinos, 2011) that pose uncertainty in predicting the long term health and environmental effects of these pesticides.

Most chemical pesticide end users in developing countries have poor knowledge of the risks associated with their use including the importance of using the correct application methods and rates as well as taking the necessary precautions during pesticide application (Damallas and Eleftherohorinos, 2011). In a national postharvest survey of more than 2,000 farmers carried out in Zimbabwe, Mvumi *et al.* (1995) discovered that 50% of the farmers used synthetic insecticides incorrectly. Observations and outcomes from farmer training sessions during the course of this research revealed that Mvumi *et al*.ôs (1995) observations still apply today, almost two decades later.

Commercial synthetic grain protectants on the market in Zimbabwe are in dust formulations (for ease of application). The formulations are easily inhaled by workers and farmers during application. This is exacerbated by the fact that most rural households do not have protective clothing and are not aware of widely available alternatives like the use of a clean cloth to cover the mouth and nose instead of a commercial dust mask. Various circumstances (funerals, community gatherings, etc.) often compel farmers to use treated grain before the pesticide has broken down to acceptable levels of residues. Besides, very few farmers bother to record pesticide application dates to enable accurate calculation of the needed number of days before the grain is safe for human consumption.

Zimbabwe, like other developing countries, lacks rigorous legislation that regulates the use of insecticides. Extensive testing is needed to correctly and properly verify the manufacturerôs claimed efficacy and safety levels. These processes are often too expensive and the required equipment, expertise and other resources are often lacking. More so, there is a chance that if

poorly remunerated, the authorities become prone to bribery by corrupt pesticide manufacturers resulting in untested chemical products possibly finding their way onto the market.

Other problems associated with synthetic pesticides include the acquired ability of some insects to survive insecticide treatments. This arises through natural selection of the most resistant individuals of a population through continuous pesticide use. This is called resistance. Though there have been numerous reports of synthetic insecticide resistance on most grain protectants (Subramanyam and Hagstrum, 1995), to date resistance in stored grain insects to DEs has not been reported (Korunic, 1998). Synthetic insecticides are broad-spectrum and thus attack non-target species. This translates into ecosystem imbalance and food web disruption that may ultimately affect human health and other animals and insects (Damallas and Eleftherohorinos, 2011). Advanced campaigns for pesticide-free food in the U.S and Europe makes the future of these pesticides very uncertain. In 1996, tougher standards for reviewing all registered grain protectants were set though the Food Quality Protection Act of 1996 of the United States. Eight years later, the sale and/or distribution of Reldan® (Chlorypyriphos-methyl 6 mg a.i/kg) were banned (Subramanyam, 2006). The major question which stands out is whether Africa, and Zimbabwe in particular, have other options if one morning the entire chemical commercial grain protectants are no longer manufactured.

2.5 Ecological Studies

The key to successful grain storage is to make conditions unfavourable for the survival of stored grain insect pests. This technique is based on an understanding of the ecology and behaviour of the respective pests. This understanding must be at an appropriate spatial and temporal scale for the pest species and the environment (Campbell *et al*., 2006). This is so because the niche occupied by any pest influences ecological processes such as population dynamics, movement patterns and spatial distribution. Insect population dynamics when well-known significantly contribute to the success of post-harvest pest control (Mvumi *et al.*, 2002), especially in IPM programs. Studies on the ecology of most storage pests have been done in laboratories giving results that are thus limited in scope of application.

Pest populations in grain stores differ with depth but the position of the grain in relation to sides and middle of the store has no effect on pest populations (Mvumi *et al.*, 2002). Although *S. cerealella* ecology on sorghum is relatively well studied, its biology, population dynamics and pest potential on maize in sub-Saharan Africa is little known (Mvumi *et al.*, 2002; Hansen *et al.*, 2004).

In Slovenia, seasonal dynamics of *Ephestia kuehniella*, *P. interpunctella* and *S. cerealella* from March to December showed peak trap catches in June and the first half of July with a second peak occurring in mid-September. These peak trap catches show generational emergence (Trdan *et al*., 2010) that could be used to schedule control measures. Studies of *P. truncatus* population dynamics have shown that diet, aggregation pheromone release, the environment (day length, temperature, humidity and their interactions and attraction to plant volatiles significantly affected *P. truncatus* flight activity (Hill *et al.*, 2002).

Analysis of the distribution of insects is important in understanding the ecology and post-harvest pest complex systems. Knowledge of the nature of distribution of the major species (*P. truncatus* and *S. zeamais*) can give clues on how these species interact with their environment (Vowotor *et al.*, 2005). The protection of stored grain from insect damage with minimum use of pesticides requires the understanding of the storage environments and their interactions with different pest species. The ecology experiment described in this study focused on determining the pest population dynamics *in vivo* (inside granaries) where the initial infestation is eliminated (fumigated) or left intact (unfumigated).

Chapter 3

GENERAL MATERIALS AND METHODS

3.1 Research Sites

The research was conducted at three sites. Preliminary laboratory bioassays for screening of best performing DEs and their combinations with spinosad and deltamethrin were conducted in a controlled temperature and humidity (CTH) room at the Department of Biological Sciences, University of Zimbabwe. On-station experiments for further evaluation of selected best performing treatments were conducted at the Institute of Agricultural Engineering (IAE) (17°45'S and 31°10'E with 750-1,000 mm annual rainfall), Hatcliffe, Harare. The IAE is a research Institution under the Ministry Agriculture, Mechanisation and Irrigation Development. The institute mainly focuses on farm mechanisation and post-harvest research. Researcher-managed on-farm experiments were conducted in Murehwa district in Wards 13 and 28. This district is located in Natural Farming Region IIa, receiving 750ï 1,000 mm annual rainfall and is one of the high maize-producing communal areas of Zimbabwe. Co-ordinates for the IAE and every host farmer homestead were recorded using Eterex Garmin® GPS meter.

3.2 Insect Collection and Rearing

The room was first thoroughly cleaned with Jik® (calcium chloride) and powdered soap and allowed to dry. The walls were later wiped using mutton cloth dipped in 70% ethanol. The experimental conditions in the CTH room were set at 27 ± 1^{o}C and 60±5% relative humidity. Temperature was automatically controlled using electrical heating bars, a fan and a thermostat while humidity was recorded on ZEAL® hygrometer and maintained manually using concentrated sodium chloride solution in open containers such as shallow bowels and trays (Dowdy and Fields, 2002; Mvumi *et al.,* 2006). These temperature and humidity conditions were monitored and recorded for 14 days before insects were introduced. Insects were reared in glass jars (1 and 2 L capacity). Founder populations of *P. truncatus* and *S. zeamais* were obtained from infested shelled and unshelled untreated maize grain obtained from Crop Breeding Institute, Department of Research and Specialist Services (Harare) and from laboratory colonies maintained in both the Departments of Crop Science and Biological Sciences at the University of Zimbabwe. Starter colonies of *T. castaneum* were obtained from infested ground maize samples that were at the Chemistry and Soil Research Institute of the Department of Research and Specialist Services as well as laboratory colonies kept at the two University departments previously mentioned.

Maize grain for insect rearing was obtained from CIMMYTôs Mid-Altitude Maize Research Station, Harare. The grain consisted of a mixture of breeding lines bulked together. The grain was first disinfested by deep-freezing for 14 days. The grain was then removed from the freezer and equilibrated to experimental conditions for 14 days prior to use in insect culturing.

Sitophilus zeamais was distinguished from *S. oryzae* by dissection to observe internal genitalia (Haines, 1991). Ten male adults were identified from each initial population by examining the rostrum (the male one is stout and rough while the female one is thin and smoother) (Haines, 1991). These were then dissected under a microscope to examine the genitalia. The head and the legs were first removed to immobilise the insect. The headless cadaver was pinned lying on its dorsum so that the venter of the abdomen faced upwards, clearly exposing the abdominal segments. A sharp needle was then used to slowly and softly prick across the abdominal segments to open the abdomen to view the internal genitalia. The apex of the aedegus of the male *S. zeamais* has two longitudinal depressions which are missing in *S. oryzae* (Haines, 1991). A similar number of females from each population were also dissected to further check the identity of the *Sitophilus* species. The Y-shaped sclerites of *S. zeamais* have sharper and thicker horns than those of *S. oryzae* (Haines, 1991). These observations were complemented by other morphological features such as body size, colourations and food source from which the population was sieved to further confirm the identification and raising of a *S. zeamais* pure culture.

For rearing pure cultures, insects were picked up individually so as to avoid mites, natural enemies and general contamination. Culture jars were placed on shelves in the CTH room and arranged according to insect species. The jars were clearly labelled with the date of culture, insect species, rearing medium, bottle code and a provision for recording adult sieving dates (Tefera *et al.*, 2010). The adult insects were sieved off every 14 days and introduced to new maize grain. Emergence of adults was checked every week, targeting 7-21 day old insects for the laboratory experiments.

3.3 Research Timing and Storage Facilities

Laboratory experiments were conducted from December 2011 to July 2012. On-station (grain protectants and resident versus incoming infestation) as well as on-farm experiments were set up at the beginning of September 2012. This beginning of the storage season was considered to be the proper time since farmers had finished shelling all their maize. The rationale was to match the study cycle with the farmersô storage season. The on farm trials were meant to enable the treatments to face the real problem pests under conditions in farmersô stores. The timing was also

meant to enable evaluation of these test materials under farmersô actual storage environments. Store types for the farmers hosting the on-farm trials were the uniform modern asbestos-roofed 3-4 roomed ôbedroomô houses mainly used as grain stores currently. The traditional raised store house has been abandoned due to grain theft in drought years (Nyagwaya *et al.*, 2010).

3.4 Experimental Designs

Laboratory bioassays were conducted under uniform conditions, and the experimental jars were placed on laboratory benches of the same height therefore a Completely Randomised Design (CRD) was used. A Randomised Complete Block Design with three blocks was used for on-station (tests of grain protectants and resident versus incoming infestation experiments) while four blocks were used for the on-farm experiment. Each block contained a full set of all the treatments. All these designs were run in Genstat 14.1 to randomise the treatments. Each specific bag number occupied the same specific position (in relation to the doors and windows) in each block (granary/farmerôs store) but would not necessarily carry the same treatment.

3.5 Sampling Frequency and Techniques

In laboratory bioassays, counts of dead and live insects were taken at 7, 14 and 21 days after treatment (DAT). The contents of each jar were returned after counts of dead and live insects were made. All the on-station and field experiments were sampled at 4-week intervals. The sample size was about 1 kg for all the field experiments. On-farm and on-station grain protectant trials were sampled using bag-sampling probes. The resident versus incoming insect infestation experiment was sampled using a multi-compartmentalised brass sampling spear.

3.5 Data Records

General data collected included the following:

- grain sample weight,
- number of dead and live insects of each species found in the sample,
- weight of chaff (dust and frass) due to insect activity,
- identities of natural enemies,
- number and weight of insect-damaged kernels,
- grain moisture content,
- % grains damaged, and
- % weight loss.

Percentage grains damaged was determined using the formula

$$\% \text{ grains damaged} = \frac{\text{number of damaged grains}}{\text{total number of grains}}$$

Percentage weight loss was determined using the Count and Weigh method (Boxall, 2002):

$$\% \text{ weight loss} = \frac{W_u - W_d}{W_d(N_u + N_d)} \times 100$$

Where W_u = weight of undamaged kernels

N_d = number of damaged kernels

W_d = weight of damaged kernels

N_u = number of undamaged kernels

Chapter 4

LABORATORY EVALUATION OF DIFFERENT DIATOMACEOUS EARTHS AND THEIR COMBINATIONS WITH SPINOSAD AND DELTAMETHRIN

4.1 Introduction

The combination of spinosad and diatomaceous earths (DEs) can potentially replace the commonly used combination of organophosphates and synthetic pyrethroids. The use of synthetic pesticides is under global scrutiny due to chemical environmental contamination, atmospheric ozone depletion, potential carcinogenic residues in commodities, exposure of users, pest resistance and general consumer aversion to chemicals (Fields and Korunic, 2000; Mvumi and Stathers, 2003).

Diatomaceous earths are slow in action (Korunic, 1997, 1998). Combination of DEs with reduced quantities of deltamethrin results in high insect mortalities as deltamethrin provides the initial knockdown that is lacking in DEs. Korunic and Rozman (2010) observed synergism between different combinations of deltamethrin and DEs against *S. zeamais*, *R. Dominica* and *T. castaneum*. *Tribolium castaneum* has been found to tolerate both spinosad and DEs (Korunic *et al*., 1997; Arthur 2000, 2002); therefore deltamethrin is introduced in the cocktails mainly to control *T. castaneum.*

Little information is available in literature on the effectiveness of combinations of spinosad and DEs. Experiments carried out by most researchers focused on either spinosad alone (Williams *et al*., 2003; Subramanyam, 2006; Huang and Subramanyam, 2007; Athanassiou *et al*., 2008; Hertlein *et al.*, 2011; Mutambuki *et al.*, 2012) or DEs alone (Korunic, 1998; Arthur, 2000; Mewis and Ulrichs, 2001; Dowdy and Fields, 2002; Nikpay, 2006; Athanassiou *et al.*, 2007; Kostyukovsky *et al.*, 2010). However, DEs have been tested in combination with botanicals (Nukenine *et al.*, 2010; Khakame *et al*., 2012) and deltamethrin (Korunic and Rozman, 2010).

The main objective of the current study was to evaluate the relative efficacies of 31 treatments, to come up with the most efficacious combinations of grain protection materials that could be used for further tests under research-managed on-station and on-farm grain storage conditions. A secondary objective of this experiment was to evaluate three new Canadian DEs in the laboratory and compare them to a local and a commercial one.

4.2 Materials and Methods

4.2.1 Treatment materials

Spinosad (Spintordust®), a product of Dow Agro Sciences (Indianapolis, Indiana, U.S.A) (Subramanyam *et al*., 2007), was supplied by Lachlan Kenya Ltd, Kenya, where the product is already registered as a grain protectant. Protect-It® was obtained from Canada while ñChemutsiò (mined in Chemutsi area, Zambezi Valley) was supplied by Chemplex (Pvt) Ltd, Zimbabwe as raw DE in clods. It was then ground and sieved through BS410/1986 250 µm sieve (Endecotts Ltd, UK) to obtain a very fine powder. Deltamethrin (99%) was supplied by Chemplex (Pvt) Ltd while Shumba Super® (standard; fenitrothion 1.0% w/w + deltamethrin 0.13% w/w) was supplied by Ecomark (Pvt) Ltd, Zimbabwe. MN51 is also an enhanced and pure DE prepared by Ep Minerals, Reno, Nevada, USA. This new DE is under efficacy tests and has not yet been registered in any country. This DE contains < 5% moisture content, 73.6% SiO_2, 7.8% Al_2O_3, 1.8% FO_2O_3, 5.6% CaO, 0.3% MgO and 2.3% other oxides. A3 comprises a DE and food grade substances, silica gel, sesame seeds and pyrethrins while A2 contains DE, silica gel and sesame seed. These three formulations are ñGenerally Recognised As Safeò (GRAS) by the USA Environmental Protection Agency (EPA) (Korunic, 2012 personal communication). The two enhanced food grade DEs were applied at different rates to different insect species (Table 1) according to recommendations by the authority of the formulation, Dr. Z. Korunic, who facilitated the delivery of all the new DEs; (MN51, A2 and A3) from the Diatom Research and Consulting Inc. Canada (Korunic, 2012 personal communication).

4.2.2 Experiment 1: Laboratory bioassays to determine the relative efficacies of different DEs

New 750 ml jars lids were punctured uniformly (± 20 holes) using a 3-inch wire nail in such a way that free air movement was possible but insect escape was difficult because of the louvered nature of the punctures. Circular pieces of newsprint paper were placed on the underside of each lid to exclude mites and prevent escape of highly mobile insects like *S. zeamais.* For each insect species, 16 treatments, each replicated four times were randomised in a Completely Randomised Design (CRD) (GenStat version 14.1) producing 64 bottles (experimental units). A summary of the treatments is presented Table 1. The untreated control was the 16^{th} treatment.

Table 1. Table of different diatomaceous earths and their application rates on three insect species

Insect species	DE type	Rates (%w/w)				
P. truncatus	Protect-It®	-	-	0.06	-	0.1
	MN51	0.02	0.04	0.06	0.08	0.1
	A2	0.025	0.03	0.035	-	-
	A3	0.015	0.02	0.025	-	-
	Chemutsi	-	-	0.06	-	0.1
S. zeamais	Protect-It®	-	-	0.06	-	0.1
	MN51	0.02	0.04	0.06	0.08	0.1
	A2	0.02	0.025	0.03	-	-
	A3	0.010	0.015	0.02	-	-
	Chemutsi	-	-	0.06	-	0.1
T. castaneum	Protect-It®	-	-	0.06	-	0.1
	MN51	0.02	0.04	0.06	0.08	0.1
	A2	0.03	0.035	0.04	-	-
	A3	0.015	0.02	0.025	-	-
	Chemutsi	-	-	0.06	-	0.1

- not tested at that application rate

4.2.3. Experiment 2: Laboratory bioassays to evaluate the relative efficacies of cocktail combinations of DEs, spinosad and deltamethrin

Different cocktail combinations (Table 2) were evaluated for their efficacies against *P. truncatus, S. zeamais* and *T. castaneum*. For experiments involving *S. zeamais* and *P. truncatus*, treatments were made on 100 g of clean sterile whole maize grain in 375 ml Consol® glass jars. In the case of *T. castaneum* (secondary pest) experiments, maize was first crushed using a blender and then passed through 1 mm Endecotts BS410/1986 sieve. The sieved product was then mixed with whole grain to achieve 1% w/w. The grain was then admixed in the treatment jars with the different cocktail combinations of DEs, spinosad and deltamethrin (Table 2). Accurate measurement of minute quantities of deltamethrin needed for 100 g of grain were not possible, so grain was treated in 1 kg quantities (for all the treatments that needed that level of deltamethrin) then the different quantities of the DEs were added after separating the deltamethrin-treated grain into 100 g portions.

After adding various treatments, the jar contents were mixed by vigorous shaking continuously for 120 seconds. Fifty unsexed 7-21 day old adult insects (Dowdy and Fields, 2002, Khakame *et al.*, 2012) were then introduced to each jar with treated grain. The jars were then labelled appropriately.

Table 2. Cocktail combinations of spinosad, deltamethrin and diatomaceous earths in the cocktail experiment

Treatment	Application rate
Untreated control	0.00% (w/w)
Shumba Super®	0.50 g/kg
Spinosad (Spintordust®)	1.00 mg/kg
Spinosad 0.8 mg/kg + Protect-It®	0.03% (w/w)
Spinosad 0.8 mg/kg + Chemutsi	0.03% (w/w)
Spinosad 0.5 mg/kg + Protect-It®	0.05% (w/w)
Spinosad 0.5 mg/kg + Chemutsi	0.05% (w/w)
Spinosad 0.3 mg/kg + Protect-It	0.10% (w/w)
Spinosad 0.3 mg/kg + Chemutsi	0.10% (w/w)
Deltamethrin 0.05mg/kg + Chemutsi	0.10% (w/w)
Deltamethrin 0.05 mg/kg + Protect-It®	0.10% (w/w)
Deltamethrin 0.1 mg/kg + Chemutsi	0.08% (w/w)
Deltamethrin 0.1 mg/kg + Protect-It®	0.08 (w/w)
Deltamethrin 0.1mg/kg + Chemutsi	0.05% (w/w)
Deltamethrin 0.1 mg/kg + Protect-It®	0.05% (w/w)

4.2.4 Data records

Jars were sieved off at 7, 14 and 21 days and dead and live insects were counted and recorded. An insect was deemed dead when none of its appendages moved after being pricked three times with a sharp needle. Percentage mortalities were then calculated. *Sitophilus zeamais* progeny (F1) counts were made at 49 days while that of *P. truncatus* and *T. castaneum* were done at 70 days (Dowdy and Fields, 2002).

4.2.5 Data analysis

Percentage mortality data for Experiment 2 were corrected for control mortalities using Abbottôs formula (Abbott, 1925) because the mean mortality in the untreated control was Ò 10% for this experiment. This is the assumption of Abbottôs formula (Abbott, 1925). The Fittedï Value plot of residuals in Genstat was used to test for normality. All progeny emergence data were not normally distributed and therefore were transformed by $\log_{10}(x+1)$. The data were then subjected to one way analysis of variance (ANOVA) in GenStat Release 14.1 software to determine any significant differences between the treatments. Where the *F*-ratio was significant ($P < 0.05$), treatment means were separated by Tukey-Kramer HSD test.

4.3 Results

4.3.1 Laboratory evaluation of diatomaceous earths

Table 3 summarises cumulative mortality results for *P. truncatus*, *S. zeamais* and *T. castaneum* after 7, 14 and 21 days of exposure to different DEs. *Prostephanus truncatus* showed no significant differences (P = 0.071) in mean mortality between the untreated control and all the other treatments at 7 days after treatment (DAT) except MN51 0.1% w/w. The untreated control had the lowest mean mortality (8%) while MN51 0.1% w/w had the highest (40.5%). Similarly, after the same period of exposure, *T. castaneum* had the lowest mortality in the untreated control and highest mortality in Protect-It® 0.1% w/w (42%). There were no significant differences between most of the DE treatments and the untreated control except Protect-It® 0.1% w/w (42%). At 7 DAT, Protect-It® 0.1% w/w gave the highest mortality to *S. zeamais* (59.5%) though this was not significantly different from that caused by Chemutsi 0.1% w/w, MN51 0.08% w/w, A3 0.015% w/w, A2 0.025 w/w and Protect-It® 0.06% w/w. Except for Protect-It® 0.1% w/w, there were no significant differences (P > 0.05) in *T. castaneum* mortality between the untreated control and all the DE treatments.

At 14 DAT, only A2 0.035% w/w (46.5 %) and Protect-It® 0.06% w/w (45.5%) gave *P. truncatus* mortality that was significantly higher than that in the untreated control (15%). For *S. zeamais*, there were no significant differences in mortality given by Chemutsi 0.1% w/w (49%), A3 0.015% w/w (44.5%), Protect-It® 0.1 w/w (81%), Protect-It® 0.06% w/w (52.5%) and MN51 0.1% w/w (44.5%). In the case of *T. castaneum*, mortality in Protect-It® 0.1% w/w (73%), Protect-It® 0.06 w/w (34%) and MN51 w/w (35.5) was significantly higher than that in the untreated control.

At 21 DAT, *P. truncatus* mortalities given by MN51 0.08% w/w (61.5%), A3 0.02% w/w (64.5%), Protect-It® 0.1% w/w, Protect-It® 0.06 w/w and MN51 0.1% w/w (68.5%, 64 and 65.5%, respectively) were similar and were also the only ones that were significantly different from the untreated control. After the same period of exposure, *S. zeamais* mortality was significantly higher than that in the untreated control in seven treatments only: Chemutsi 0.06% w/w, Chemutsi 0.1% w/w, MN51 0.08% w/w, A3 0.015% w/w, Protect-It® 0.06% w/w, Protect-It 0.01% w/w and MN51 0.1% w/w. Despite numeric differences, these seven treatments were not significantly different from each other. In the case of *T. castaneum*, the two rates of Protect-It® 0.06% w/w and 0.01% w/w) and MN5 0.1% w/w were the only treatments that gave mortality significantly higher than the untreated control. However, Protect-It® 0.1% w/w was the most effective, giving 91% mortality.

4.3.2. Laboratory evaluation of cocktail combinations of diatomaceous earths with spinosad and deltamethrin

Corrected mortalities given by different cocktail combinations of DEs with spinosad (Spintordust®) are summarised in Table 4. At 7 DAT, the combination of deltamethrin 0.1 mg/kg + Chemutsi 0.05 % w/w gave the lowest mortality (44.4%) against *P. truncatus* which was significantly lower than the positive control (Shumba Super®) (73.3%). However, this deltamethrin-Chemutsi combination was not significantly different from deltamethrin 0.05 mg/kg + Chemutsi 0.1% w/w and deltamethrin 0.1 mg/kg + Chemutsi 0.08% w/w combinations. Shumba Super® was out-performed by Spinosad 0.8 mg/kg + Protect-It® 0.03% w/w and spinosad 0.5 mg/kg + Chemutsi 0.05% w/w (99.5 and 98.9, respectively). For *S. zeamais*, mortalities at 7 DAT ranged from 74.1% (deltamethrin 0.05 mg/kg + Chemutsi 0.01% w/w) to 100% (Shumba Super®) with the former being the only treatment that was significantly lower than the positive control. In the case of *T. castaneum*, all treatments performed equally as well as Shumba Super® except for Spinosad 0.05 mg/kg + Chemutsi 0.05% w/w and spinosad 0.3 mg/kg + Chemutsi 0.1% which gave significantly lower mortalities after the same period of exposure.

Table 3. Effects of different diatomaceous earths on cumulative mortality (%) of three insect pest species

Treatment (w/w)	7 days			14 days			21 days		
	‡ *Pt*	*Sz*	*Tc*	*Pt*	*Sz*	*Tc*	*Pt*	*Sz*	*Tc*
Untreated control	8.0±2.7a	3.5±1.0a	1.5±1.0a	15.0 ±0.6a	8.0±0.6 a	3.0 ±1.3a	31.5±2.4a	10.5±0.5a	7.0± 1.3a
A3 0.025%	12.5±1.8ab	-	18.0± 5.3a	29.0± 10.1ab	-	22.0 ±5.6abc	46.5±5.6ab	-	30.5±5.7abc
Chemutsi 0.06%	12.5±3.8ab	16.0± 12.7ab	5.5±3.0a	30.5±2.8ab	28.5±14.2abc	11.5±9.0abc	41.0 ±0.8ab	54.5±12.7bcdef	15.5±3.9ab
MN 51 0.06%	13.0±2.8ab	17.0± 7.1ab	3.0 ±1.0a	35.0 ±5.8ab	27±6.6abc	18.5±4.4abc	54.0 ±4.2ab	50.0 ±4.1abcdef	21.5±4.8ab
MN51 0.04%	15.0±3.9ab	10.0 ±1.7ab	3.0 ±1.9a	28.5±3.9ab	13.0± 1.8abc	14.5±4.6abc	45.0 ±3.3ab	24.5±5.0abcd	21.5±6.7ab
Chemutsi 0.1%	15.0±3.9ab	36.5±1.6abc	5.5±1.0a	28.0± 6.5ab	49.0 ±1.3bcd	19.5±3.9abc	44.0 ±4.2ab	88. 5±6.0f	23.0± 4.9abc
A2 0.03%	17.0±2.1ab	12.0± 4.5ab	2.0± 2.4a	32.0± 1.9ab	14.0±4.8 abc	10.0±2.6 ab	48.5±7.9ab	21.5±2.2abc	16.5±4.8ab
MN51 0.08%	17.5±1.9ab	27.0 ±4.0abc	11.0±11.1 a	41.5±5.3ab	39.5±5.9abc	16.0 ±8.5abc	61.5±5.7b	61.0± 5.8cdef	20.0 ±8.8ab
A3 0.015%	18.5±3.3ab	41.5±4.1bc	8.0± 2.0a	28.5±3.4ab	44.5±10.0abcd	17.5±4.4abc	53.5±5.3ab	53.0 ±12.6bcdef	18.0 ±1.5ab
MN51 0.02%	19.5±5.5ab	7.5±0.6ab	1.0± 2.0a	34.0±9.0 ab	12.0± 1.0abc	9.0±3.8 a	54.5±10.2ab	24.0± 3.1abc	12.0±2.9 a
A2 0.025%	19.5±3.9ab	33.0 ±9.2abc	-	36.0±8.4 ab	36.5±11.5abc	-	52.5±7.7ab	45.5±10.4abcde	-
A3 0.02%	21.0±2.9ab	10.5±3.4ab	1.5.0± 0.8a	44.0± 4.9ab	15.0 ±4.0abc	7.0±3.8 a	64.5±3.5b	26.0± 6.2abcd	20.0± 4.0ab
A2 0.035%	22.0±1.3ab	-	11.0 ±5.0a	46.5±2.5b	-	18.0 ±4.3abc	58.0 ±3.5ab	-	28.0 ±4.3abc
Protect-It® 0.1%	25.5±3.6ab	59.5±2.2c	42.0±1.5 b	37.5±7.6ab	81.0 ±0.5d	73.0± 2.4d	64.0±4.8 b	89.5±2.9f	91.0± 7.0d
Protect-It® 0.06 %	26.0±4.7ab	41.5±16.4bc	19.0 ±5.7ab	45.5±6.4b	52.5±18.5cd	34.0± 6.3bc	68.5±5.8b	64.0 ±16.4def	39.5±6.4bc
MN51 0.1%	40.5±1.7b	22.0±6.7 ab	23.0 ±2.2ab	44.5±2.9ab	44.5±6.5abcd	35.5±2.2c	65.53.4±b	68.0± 8.8ef	48.0±1.7 c
A2 0.02%	-	11.0 ±7.1ab	-	-	11.5±6.8ab	-	-	19.5±4.6ab	-
A3 0.01%	-	12.0± 6.6ab	-	-	12.5±4.4abc	-	-	22.0 ±3.5abc	-
A2 0.04%	-	-	11.0 ±3.0a	-	-	18.5±2.2abc	-	-	21.5±2.4ab
$F_{15,48}$	1.71	55.12	5.56	2.07	6.52	11.69	3.53	10.29	16.22
P	0.071	< 0.001	< 0.001	0.029	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CV%	59.9	61.9	88.8	33.7	52.3	47.0	20.4	34.5	36.2

Means within a column followed by the same letter are not significantly different (Tukey, $P < 0.05$).

- Not tested.

ÿ Pt ï *Prostephanus truncatus*; Sz ï *Sitophilus zeamais*; Tc ï *Tribolium castaneum*

Table 4. Cumulative corrected mortalities (%) of *P. truncatus*, *S. zeamais* and *T. castaneum* adults exposed to grain treated with combinations of DEs, deltamethrin and spinosad.

Treatment (w/w)	7 days			14 days			21 days		
	Pt	***Sz***	***Tc***	***Pt***	***Sz***	***Tc***	***Pt***	***Sz***	***Tc***
Shumba Super 0.5g/kg	73.3±6.0 bc	100±0.0 b	99.0 ±1.0b	100±0.0 a	100 ±0.0 b	99.5± 0.5b	100 ±0.0 a	100 ±0.0 a	99.5 ±0.5b
Spinosad (Spintordust) 1mg/kg	97.3±1.5 cd	97.5±1.0 b	85.4 ±4.0ab	100± 0.0 a	99.5± 0.5b	85.4 ±3.0ab	100 ±0.0 a	99.5 ±0.5a	100 ±0.0 b
pinosad 0.8mg/kg + Protect-It® 0.03%	99.5± 0.5d	97.0± 1.7b	83.8± 6.3ab	100± 0.0 a	100 ±0.0 b	1000.0 ± b	100 ±0.0 a	100± 0.0 a	98.9 ±1.0b
Spinosad 0.8mg/kg + Chemutsi 0.03%	97.3 ±1.5cd	98.5±1.0 b	86.9± 3.7ab	100± 0.0 a	100±0.0 b	98.0 ±21.9ab	100 ±0.0 a	100±0.0 a	98.9± 1.0b
Spinosad 0.5mg/kg + Protect-It® 0.05%	96.3±2.4 cd	97.5± 1.5b	90.4± 5.6ab	100± 0.0 a	100 ±0.0 b	99.0 ±1.0b	100 ±0.0 a	100±0.0 a	98.9 ±1.0b
Spinosad 0.5mg/kg + Chemutsi 0.05%	98.9± 0.6d	98.5 ±0.5b	74.2± 5.3a	100 ±0.0 a	98.4± 0.5b	83.6 ±21.9a	100 ±0.0 a	99.5± 0.5a	92.6 ±3.5a
Spinosad 0.3mg/kg + Protect-It® 0.1%	96.3 ±1.3cd	97.5±1.0 b	86.4± 1.3ab	100 ±0.0 a	99.5±0.5b	99.0 ±1.0b	100 ±0.0 a	100± 0.0 a	98.9 ±1.0b
Spinosad 0.3mg/kg + Chemutsi 0.1%	97.3 ±0.5cd	97.5± 0.5b	77.8±9.5 a	100± 0.0 a	98.9±0.6 b	96.9± 4.2ab	100 ±0.0 a	98.9± 0.6a	98.4 ±1.5ab
Deltamethrin 0.05mg/kg + Chemutsi 0.1%	66.3± 11.1ab	74.1± 5.6a	90.9± 2.1ab	100 ±0.0 a	90.4± 1.9a	100±0.0 b	100 ±0.0 a	97.8 ±1.4a	100 ±0.0 b
Deltamethrin 0.05mg/kg + Protect-It® 0.1%	97.3± 1.0cd	98.0±1.4 b	100± 0.0 b	100±0.0 a	100± 0.0 b	100± 0.0 b	100 ±0.0 a	100 ±0.0 a	100± 0.0 b
Deltamethrin 0.1mg/kg + Chemutsi 0.08%	60.4± 5.4ab	97.0±1.3 b	99.0± 1.0b	99.5 ±0.5a	100 ±0.0 b	100 ±0.0 b	99.6 ±0.5a	100 ±0.0 a	100 ±0.0 b
Deltamethrin 0.1mg/kg + Protect-It® 0.08%	93.6± 3.5cd	99.5 ±0.5b	100± 0.0 b	100 ±0.0 a	100± 0.0 b	100 ±0.0 b	100 ±0.0 a	100 ±0.0 a	100 ±0.0 b
Deltamethrin 0.1mg/kg + Chemutsi 0.05%	44.4± 5.3a	96.5± 1.3b	90.9 ±3.4ab	100 ±0.0 a	98.9± 0.6b	100±0.0 b	100 ±0.0 a	99.5 ±0.5a	100±0.0 b
Deltamethrin 0.1 mg/kg + Protect-It® 0.05%	84.5 ±7.5bcd	99.0±0.6 b	100± 0.0 b	100 ±0.0 a	100±0.0 b	100 ±0.0 b	100± 0.0 a	100 ±0.0 a	100±0.0 b
$F_{13,42}$	12.85	12.33	4.18	1.00	15.41	7.06	1.00	1.50	2.48
P- value	<0.001	<0.001	<0.001	0.468	<0.001	<0.001	0.468	0.157	0.013
CV%	11.5	3.8	9.2	0.3	0.3	3.4	0.3	1.0	2.5

ÿ Pt ï *Prostephanus truncatus*; Sz ï *Sitophilus zeamais*; Tc ï *Tribolium castaneum*

Means within a column followed by the same letter are not significantly different (Tukey-Krammerôs HSD test $P < 0.05$).

At 14 DAT (Table 4), *P. truncatus* mortality though lowest in the (deltamethrin 0.1 mg/kg + Chemutsi 0.08% w/w combination (99.5%), was not significantly different from that given by all the other treatments which were 100%. In the case of *S. zeamais*, only deltamethrin 0.05 mg/kg + Chemutsi 0.1% w/w gave mortality (90.4%) which was significantly lower than that in the positive control. For *T. castaneum*, mortality given by spinosad 0.5 mg/kg + Chemutsi 0.05% w/w (83.6%) was the only one which was significantly lower than that given by Shumba Super (99.5%). However, the level of mortality given by spinosad 0.5 mg/kg + Chemutsi 0.05% w/w was similar to that given by spinosad alone (Spintordust®) 1 mg/kg, spinosad 0.8 mg/kg + Chemutsi 0.03%w/w and spinosad 0.3 mg/kg + Chemutsi 0.1% w/w.

At 21 DAT, there were no significant differences in mortality of either *P. truncatus* or *S. zeamais* among the different treatments, with mortality falling in the range of 97-100%. In the case of *T. castaneum*, only spinosad 0.05 mg/kg + Chemutsi 0.05% w/w gave mortality (92.6%) which was significantly lower ($P = 0.013$) than that of Shumba Super® (99.5%). The rest of the treatments were not significantly different from the positive control and from each other.

4.3.3 F1 Progeny emergence on DEs

Table 5 shows the numbers of F1 adult progeny that emerged from different DE treatments. For *P. truncatus*, the average number of F1 adult progeny emerged ranged from 6.8 (MN51 0.04% w/w) to 39.8 (Untreated control) and Chemutsi 0.1% w/w and there were no significant differences among all these treatments. *Sitophilus zeamais* had the lowest F1 progeny count of 11.5 in the positive control (Protect-It® 0.1% w/w), and a highest count of 151.3 in the untreated control. However, F1 progeny counts in the untreated control were not significantly different from A3 0.025% w/w, MN51 0.02% w/w, A2 0.02% w/w and A3 0.01% w/w. The rest of the treatments had F1 progeny emergence counts that were not significantly different from Protect-It® 0.01% w/w. Unlike the other two species, *T. castaneum* F1 progeny emergence was generally low across all treatments ranging from 0.0 (Protect-It® 0.1% w/w) to 37 (A2 0.03% w/w). Although there were some significant differences between some treatments, none were significantly different from the untreated control.

Table 5. Number of F1 adult progeny of *P. truncatus, S. zeamais* and *T. castaneum* emerging from grain admixed with different diatomaceous earths

Treatment (w/w)	*P. truncatus* (70 DAT)	*S. zeamais* (49 DAT)	*T. castaneum* (70 DAT)
Untreated control	39.8±11.3 a	151.3±18.1 f	22.0 ±7.7ab
A3 0.025%	29.3± 4.0a	111.0±16.8 cdef	1.5± 1.5a
Chemutsi 0.06%	10.5±3.3 a	35.0± 1.7a	24.8± 10.1ab
MN 51 0.06%	11.3±6.6 a	53.0±18.9 abc	17.8± 3.7ab
MN51 0.04%	8.5 ±2.5a	70.0 ±2.9abcde	13.8 ±6.3ab
Chemutsi 0.1%	39.8±2.9 a	19.0 ±3.3a	19.8 ±5.4ab
A2 0.03%	24.0±4.8 a	62.3± 6.3abcd	37.0±5.6 b
MN51 0.08%	16.0 ±2.6a	62.3±5.7 abcd	26.0 ±10.5ab
A3 0.015%	17.5 ±5.8a	74.8± 8.3abcde	3.8±3.3ab
MN51 0.02%	14.3± 6.8a	127.5 ±16.2ef	26.0 ±5.6ab
A2 0.025%	15.8± 8.4a	69.8±16.7abcde	-
A3 0.02%	15.5± 6.8a	-	20.0± 2.2ab
A2 0.035%	15.8 ±4.7a	-	16.3± 8.0ab
Protect-It® 0.1%	9.8±14.5 a	11.5 ±14.5a	0.0±0.0 a
Protect-It® 0.06 %	30.3 ±11.9a	46.0 ±23.7abc	8.3± 1.0ab
MN51 0.1%	6.8± 3.2a	42.8± 9.0ab	15.0 ±12.4ab
A2 0.02%	-	106.5±6.4 bcdef	-
A3 0.01%	-	119.0± 6.5def	-
A2 0.04%	-	-	13.3± 8.9ab
$F_{15,48}$	1.44	9.64	5.13
P-values	0.169	<0.001	<0.001
CV%	31.90%	1.10%	35.80%

Means within a column followed by the same letter are not significantly different (Tukey-Krammerôs HSD test, $P < 0.05$.

4.3.4 F1 Progeny emergence on different cocktails of DEs with spinosad and deltamethrin

Table 6 shows the patterns of F1 adult progeny emergence within different cocktail combinations of DEs, spinosad and deltamethrin. In the case of *P. truncatus*, only the untreated control had significantly higher F1 progeny numbers (55.3) compared to the rest of the treatments which did not differ significantly ($P = 0.093$) among themselves (with average progeny ranging from 0.0 to 2.8 insects). For *S. zeamais*, the number of F1 progeny that emerged was significantly higher in the untreated control (174.3) than in all the other treatments. Compared to the positive (Shumba Super®), only spinosad 0.3 mg/kg + Protect-It® 0.1% w/w had significantly lower number of *S. zeamais* adult progeny emergence. *Tribolium castaneum*, shows that the numbers of emerging F1 progeny were all significantly lower ($P < 0.001$) than in the untreated control (18.5). However, there were no significant differences among all the combination treatments and the positive control.

Table 6. Numbers of F1 adult progeny of emerging from grain admixed with cocktail combinations of DEs, spinosad and deltamethrin

Treatment (w/w)	*P. truncatus* (70 DAT)	*S. zeamais* (49 DAT)	*T. castaneum* (70 DAT)
Untreated control 0%	55.3 ±10.9b	174.3± 25.5d	18.5 ±3.8b
Shumba Super 0.5 g/kg	2.3±2.7 a	27.5 ±5.1bc	0.5± 0.5a
Spinosad (Spintordust®) 1 mg/kg	0.3±0.3 a	14.3 ±2.5ab	0.0 ±0.0a
Spinosad 0.8 mg/kg + Protect-It® 0.03%	0.3±0.3 a	16.0 ±2.3abc	3.5 ±2.9a
Spinosad 0.8 mg/kg + Chemutsi 0.03%	0.3±0.3 a	12.5±0.5 ab	1.0± 0.0a
Spinosad 0.5 mg/kg + Protect-It® 0.05%	0.0±0.0 a	13.3±1.3 ab	2.3 ±1.0a
Spinosad 0.5 mg/kg + Chemutsi 0.05%	0.3±0.3 a	14.8± 1.0ab	2.8±1.4 a
Spinosad 0.3 mg/kg + Protect- It® 0.1%	0.3±0.3 a	12.5 ±1.5a	3.8±1.9 a
Spinosad 0.3 mg/kg + Chemutsi 0.1%	0.0±0.0 a	13.8 ±2.5ab	3.8 ±0.9a
Deltamethrin 0.05 mg/kg + Chemutsi 0.1%	12.3±6.9 a	32.0 ±3.5c	2.3± 2.3a
Deltamethrin 0.05 mg/kg + Protect-It® 0.1%	0.0± 0.0a	23.3 ±1.4abc	3.0±2.1 a
Deltamethrin 0.1 mg/kg + Chemutsi 0.08%	0.8± 0.5a	26.0 ±4.1abc	0.0 ±0.0a
Deltamethrin 0.1 mg/kg + Protect-It® 0.08%	8.0± 7.7a	23.3 ±2.3abc	1.3±1.3a
Deltamethrin 0.1 mg/kg + Chemutsi 0.05%	0.5± 0.3a	22.8±4.4 abc	0.5± 0.5a
Deltamethrin 0.1 mg/kg + Protect-It® 0.05%	0.0±0.0 a	24.0±2.9 abc	0.0± 0.0a
$F_{14, 42}$	1.72	20.51	6.59
P-values	0.093	<0.001	<0.001
CV%	118.7	9.5	81.8

Means within a column followed by the same letter are not significantly different (Tukey-Kramerôs HSD test, $P < 0.05$).

4. 4 Discussion

There was low *P. truncatus* mortality at 7 DAT confirming reports by Korunic and Rozman (2010) that DEs are slow in action and need a long exposure period. *Sitophilus zeamais* succumbed fairly quickly to the DEs than *P. truncatus* and *T. castaneum*. *Tribolium castaneum* was the least affected, succumbing only to the positive control, enhanced DE (Protect-It® 0.1% w/w). This indicates that *T. castaneum* is comparatively more tolerant to different DEs than the other two insect species, at least after an exposure period of 7 days.

Sitophilus zeamais proved to be very susceptible to most DEs. MN51 and Chemutsi were both not significantly different from each other at rates ranging from 0.06 ï 0.1% w/w. The highest mortality of 68.5% at 21 DAT for a devastating pest like *P. truncatus* was very low at farm situations; the surviving 31.5% insects can do great damage. Boxall (2002) postulated that with *P. truncatus*, very low counts of about 1 insect/kg at the beginning of the season suffice to cause very high infestations and serious losses at the end of the storage season.

At 0.1% w/w, raw local DE, Chemutsi, new DE MN51 and enhanced registered Protect-It® were not significantly different from each other on *S. zeamais*. This shows huge potential for local DE Chemutsi in the control of *S. zeamais* which is, and has been, cosmopolitan in all communal areas of Zimbabwe, causing serious grain damage since time immemorial. On the other hand, apart from Protect-It® 0.1% w/w, all the other treatments were not effective against *T. castaneum*. In practical terms, less than 48% pest control in grain stores does not economically reduce level of damage, spoilage and weight loss on stored grain. This corresponds with findings by Korunic *et al*. (1997), Arthur (2000, 2002), Mvumi *et al.* (2006), Kostyukovsky *et al.* (2010) and Mohale *et al*. (2010). Combining the DEs with spinosad and deltamethrin at different rates may counter the DE weaknesses against this and other pests.

On *P. truncatus*, varying rates of spinosad and Chemutsi (spinosad 0.8 mg/kg + Chemutsi 0.03% w/w and spinosad 0.3 mg/kg + Chemutsi 0.1% w/w) did not change the results as both independently gave a mean mortality of 100% at 21DAT. Spinosad alone (1 mg/kg) and deltamethrin 0.05 mg/kg + Protect-It 0.1% w/w) also gave the same level of mortality against *P. truncatus* as early as 7 DAT. Thus far, the results showed that numerous combinations of safer grain protectant options are available and very efficacious against the most devastating and most feared pest, *P. truncatus*. This pest had slightly higher untreated control mortality than the other two species. This may have been exacerbated by the need to break the kernels to count the insects inside kernels at each of the three assessments (i.e. 7, 14 and 21 DAT).

Failure by all DEs to give 100% mortality is a clear indication that though they are safe to use and quite efficacious, they need supplementation. The results showed that there was synergy in the efficacy and mode of action of DEs against all the three insect species when combined with spinosad and deltamethrin. Thus, the combination of the DEs with spinosad and deltamethrin unlike the pure DE treatments exhibited a quick mode of action against all insect species including the notorious *P. truncatus* and the DE-tolerant *T. castaneum*. This synergy countered the negative effects of each material when applied alone and combined the different modes of action: chemical (deltamethrin/spinosad) and physical (DEs). Desiccated insects became weak and therefore failed to resist the toxic effects of the active ingredients of deltamethrin (Korunic and Rozman, 2010) or spinosad.

Farmers are free to choose combinations that are cheaper and readily available to them, but emphasis is put on spinosad as it is a safer bio-pesticide as opposed to deltamethrin, which is a synthetic pyrethroid. It was also interesting to note that there was very high *P. truncatus*

mortality (96.3%) at 7 DAT from the combination of spinosad 0.5 mg/kg (half the label rate) + Protect-It® 0.05% w/w (half the label rate) which was not significantly different from that of spinosad 0.3 mg/kg (less than a third of the label rate) + Protect-It® 0.1% w/w (full label rate). The latter would be the best combination for the farmers as lower rates for spinosad translate into lower costs. Spinosad is currently packaged by Agro Sciences in USA at 0.125% a.i. composition and is thus expensive compared to locally mined DEs packaged at Ò90% a.i. If approval for local registration of spinosad is eventually granted, its manufacturers are encouraged to pack it in small affordable packets for small scale farmers.

There was general tendency by *P. truncatus* to tolerate deltamethrin as exhibited by relatively low mean mortalities on all deltamethrin combinations as opposed to spinosad ones (Table 4). *Prostephanus truncatus* seemed to succumb quickly to spinosad than to deltamethrin, indicating that spinosad may be the best product to use in the face of the new grain enemy. Unlike *P. truncatus*, *S. zeamais* had slightly lower mean mortalities on spinosad combinations than on deltamethrin ones. This indicates that whereas *P. truncatus* tolerates deltamethrin, *S. zeamais* tolerates spinosad. Just like *S. zeamais, T. castaneum* is slightly tolerant to spinosad (Mutambuki *et al*., 2012) than to deltamethrin. It can also be noted that for the latter species, deltamethrin-DE cocktails suppress F1 progeny emergence more than spinosad-DE cocktails. This may be a result of a quick knockdown effect achieved by deltamethrin on *T. castaneum* thus reducing the time for the introduced insects to mate and oviposit before they die (Arthur and Throne, 2003).

Prostephanus truncatus F1progeny emergence was completely suppressed by spinosad 1 mg/kg + Protect-It® 0.05 % w/w, spinosad 0.03 mg/kg + Chemutsi 0.1 % w/w, deltamethrin 0.05 mg/kg + Protect-It® 0.1 % w/w and deltamethrin 0.1 mg/kg + Protect It® 0.05 % w/w. Though these treatments were not significantly different from the positive control (Shumba Super®) (2.8 average number of emerged insects), they have proved to be able to maintain complete suppression of *P. truncatus* F1 adult progeny emergence up to 70 DAT and 49 days for *S. zeamais*.

High mortalities caused by the cocktail combinations consequently resulted in adults dying before oviposition as exhibited by very low F1 progeny emergence for most of the cocktail treatments. It is interesting to note that the positive control (Shumba Super®) had significantly higher number of *S. zeamais* F1progeny than Spinosad 0.3 mg/kg + Protect- It® 0.1% w/w,

revealing that these registered pesticides are not necessarily the best at controlling internally feeding infestations.

Chapter 5

ON-STATION EVALUATION OF VARIOUS GRAIN PROTECTANTS AGAINST STORED MAIZE INSECT PESTS

5.1 Introduction

Though laboratory testing for economic selection of potential pesticides destined for subsequent further field tests or adoption by farmers is mandatory, there are a series of variables that can alter the correlation between the laboratory and field data (Harein, 1974). These may include the effect of competition for food and space from other species, presence of natural enemies and super natural enemies, natural environment, effect of treatments on other post-harvest pest species that were not tested in the laboratory and possible length of period of effectiveness of the treatment materials. In light of this, it was found necessary to evaluate the efficacy of the nine best laboratory performing treatments on-station. On-station environment closely mimics on-farm conditions unlike the laboratory environment where the insects and the treatment materials are confined in glass jars.

Sometimes, laboratory data alone can be misleading unless verified by real life conditions. This is difficult to achieve in the laboratory without the labour of having to collect and rear individual colonies of the insects under artificial conditions. Though it was not the objective of this study, the effect of grain protectants on non-target species in the natural environment achieved on-station is important. High mortalities of non-target and beneficial insects is contrary to the principles of sound post-harvest IPM apart from disturbing the natural balance of the ecosystem.

On-station experiments enable the assessment of the efficacy of the treatment materials to the naturally occurring insect species. This broadens the research in terms of species spectrum tested as well as the populations (large numbers of insects from natural infestations). In other words, the treatments were exposed to the vagaries of the environment. The objective of this study was to test the efficacy of the best performing grain protectants from laboratory bioassays under on-station conditions that mimic the farmersô storage conditions.

5.2 Materials and Methods

5.2.1 Site, granaries and timing

The experiment was carried out in granaries at the Institute of Agricultural Engineering (IAE), Hatcliffe, Harare. The granaries had compartments (60 cm width x 60 cm length x 165 cm height/depth) specifically constructed for post-harvest research purposes(Plate 5). These granaries are an improvement of the pole and mortar traditional granaries (*ó'hozi'*) that used to be very common in communal areas of Zimbabwe. The modifications made to replace poles with cast concrete and the stone base with bricks were meant to reduce labour, conserve the environment and create a complete seal against rodents. However, the basic structure, the thatch and compartmentalisation is still the same.

A B

Plate 5. The model granaries at the Institute of Agricultural Engineering, Harare, used to test the grain protectants: Outside (A) and inside (B) of each granary.

The experiment was conducted from August, 2012 to February, 2013. This timing was deliberate to target the smallholder farmerôs normal grain storage season. This was important to expose the treatments to a typical farmer storage environment in terms of insect pest activity and the climate.

5.2.2 Grain source and preparation

About 1.1 tonnes of shelled and clean maize grain (hybrid variety SC 637) were obtained from IAE. The shelled grain was first thoroughly winnowed prior to use. Nine out of the 31 treatments tested in the laboratory bioassays that were most efficacious with high potential of local availability were selected for the on-station tests. Positive (Shumba Super® dust) and negative (Untreated) controls were also added to make a total of 11 treatments (Table 7). Seventy-five kilogrammes (75 kg) of the prepared grain were weighed out and placed on clean 200 µm plastic sheets for each of the treatments. Pre-measured quantities of the various

treatment materials were applied separately onto each 75 kg heap before the grain was thoroughly admixed with the treatment using shovels. After mixing, the grain was divided into three 25 kg pockets which were then labelled and allocated to the three different blocks (granaries).

Table 7 On-station experiment treatments.

Treatment no.	Treatment (w/w)
1	Untreated Control 0%
2	Shumba Super® dust 0.5 g/kg
3	Protect-It® 0.1%
4	MN51 0.1%*
5	Spintordust® (Spinosad) 1 mg/kg
6	Spintordust® (Spinosad) (0.3 mg/kg) + Protect-It® 0.1%
7	Deltamethrin 0.05 mg/kg + Protect-It® 0.08%*
8	Deltamethrin 0.05 mg/kg + Chemutsi 0.1%
9	Deltamethrin 0.1 mg/kg + Chemutsi 0.08%
10	Deltamethrin 0.1 mg/kg +Protect-It® 0.08%
11	Spintordust® (Spinosad) 0.5 mg/kg + Chemutsi 0.05%

*These treatment materials were laboratory-prepared and are not yet commercially available on the market Except for Shumba Super®, these treatment materials are registered and available commercially in other countries.

The experiment was set up as a Randomised Complete Block Design (RCBD) of 11 treatments with 3 blocks/replicates (granaries). For each block, each of the 11 treatments was randomly allocated a bag (plot) number. The six compartments in each granary were not enough for the 11 bags, so the granary passages and space on top of compartmentsô ceiling was used as well. Each bag number occupied the same position in each of the three granaries throughout the duration of the study but would not necessarily carry the same treatment (Fig 1). The 11 positions in the granaries were predetermined on the basis of maximum distance each bag would have from the next one to avoid influence of one treatment on the other.

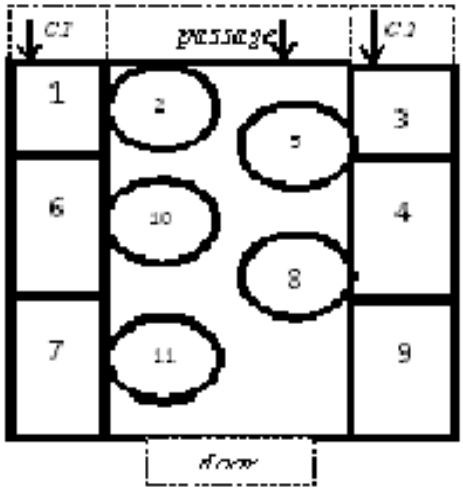

Figure 1. Bag positions in each block (granary)

- C1- ceiling top 1, C2- ceiling top2
- 1 to 11 positions occupied by bags 1 ï 11 in each granary.

5.2.4 Sampling frequency and sampling techniques

Baseline samples were taken soon after treatment. The bags were then sampled at 4-week intervals for a period of 24 weeks. The sample size was estimated to 1 kg measured using a Super Samson Salter® scale. Hollow Seedburo® grain metal probes measuring 30 cm (length) and 2.5 cm (rear diameter) with blunt rear and sharp front ends were used to extract the grain sample. To obtain a composite sample representative of the whole bag at each sampling, grain samples were drawn from the bags diagonally, horizontally and vertically at the middle on both sides of the bag (Figure 2). Sampling holes marked at the previous sampling session were avoided at the following session to reduce the effect of sampling on insect behaviour but could be used during other sampling sessions. Immediately after sampling, the moisture content of the grain in each sampling bag was measured using a digital moisture meter (DICKEY-john®) before securely tying each bag for further sample processing.

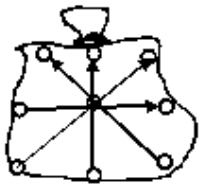

Figure 2.Sampling positions on a treated bag.

5.2.5 Data records

In the laboratory, each sample mass was weighed and then sieved to separate insects and chaff. All dead and live insects were counted and recorded separately and according to their species while the chaff was weighed. The insects and chaff-free grains were then placed in freezers to stop further insect development. The samples were then assessed for insect damage. Each sample was divided into four quarters (sub-samples) using an Endecotts® riffle divider. One sub-sample was discarded and the other three were assessed for damage and weight loss (%) using the Count and Weigh Method (Boxall, 2002). With this method, the insect-damaged grains were separated, distinguishing those with 1 hole only, 2 holes only and Ó3 holes and the completely undamaged grain. Each category was weighed separately and the grains then counted using a Numigral Seed Counter, model NUM 1 series 172799.

5.2.6 Data analysis

The data on % weight loss and % grain damage were subjected first to One Way Analysis of Variance (ANOVA). Product moment correlation coefficient (**R**) and the co-efficient of determination ($\mathbf{R}^2$) were then calculated in Excel. Correlation coefficient (**R**) was used to determine the strength of the relationship between weight loss and live insect numbers, grain damage and moisture content as well as weight loss and quantity of chaff, while $\mathbf{R}^2$ was used to determine how much each of the factors contributed to damage and weight loss.

5.3 Results

There were no significant differences ($P > 0.05$) in grain damage among all the treatments from 0 weeks (baseline) to 8 weeks after treatment (Table 8). From 12-24 weeks, significant differences among treatments were noted. However, these differences only came about because of the untreated control which had significantly higher damage than all the other treatments.

The same trend was observed for grain weight loss for the same time period (Figure 3). Significant treatment differences in weight loss ($P < 0.001$) were noticed from 12 through to 24 WAT. Weight loss in the Shumba Super®-treated grain remained around zero. Though all the other treatments began to show slight increases in weight loss from 12 weeks, there were no significant differences among all the treatments except the untreated control.

Table 8. Effects of different treatments on % grain damage during 2012/13 storage season in on-station experiments (n=3)

Treatment	*0 weeks*	*4 weeks*	*8 weeks*	*12 weeks*	*16 weeks*	*20 weeks*	*24 weeks*
Untreated control 0% (w/w)	2.1a	1.6a	6.2a	8.3b	25.6b	34.0b	61.2c
Shumba Super 0.5g/kg	1.7a	1.5a	1.3a	1.2a	1.8a	3.3a	2.1a
Protect-It 0.1% (w/w)	1.8a	1.7a	2.7a	1.8a	2.7a	5.4a	7.0ab
MN51 0.1% (w/w)	3.0a	2.3a	2.6a	2.8a	4.1a	6.1a	9.2ab
Spinosad 1mg/kg	2.6a	2.5a	2.3a	2.4a	2.8a	4.8a	5.5ab
Spinosad 0.3mg/kg +Protect-It 0.1% (w/w)	2.7a	2.1a	2.9a	2.6a	2.9a	4.4a	3.7ab
Deltamethrin 0.05mg/kg +Protect-It 0.08% (w/w)	1.7a	1.7a	2.3a	1.9a	3.1a	4.6a	3.9ab
Deltamethrin 0.05mg/kg + Chemutsi 0.1% (w/w)	2.3a	2.2a	2.4a	2.1a	3.8a	6.0a	13.4b
Deltamethrin 0.1mg/kg + Chemutsi 0.08% (w/w)	2.2a	2.3a	2.8a	2.9a	3.6a	5.0a	8.3ab
Deltamethrin 0.1mg/kg + Protect-It 0.05%	2.2a	2.1a	2.3a	2.5a	2.2a	5.1a	3.1ab
Spinosad 0.5mg/kg +Chemutsi 0.05%	2.4a	1.6a	2.5a	3.2a	3.2a	5.5a	7.8ab
$F_{10,32}$	1.28	1.58	1.44	15.71	20.98	63.60	58.66
P-value	0.303	0.184	0.234	<0.001	<0.001	<0.001	<0.001
CV%	5.2	7.6	23.3	5.3	15.3	15	11

Means within a column followed by the same letter are not significantly different (Tukey-Kramerôs HSD test, $P < 0.05$).

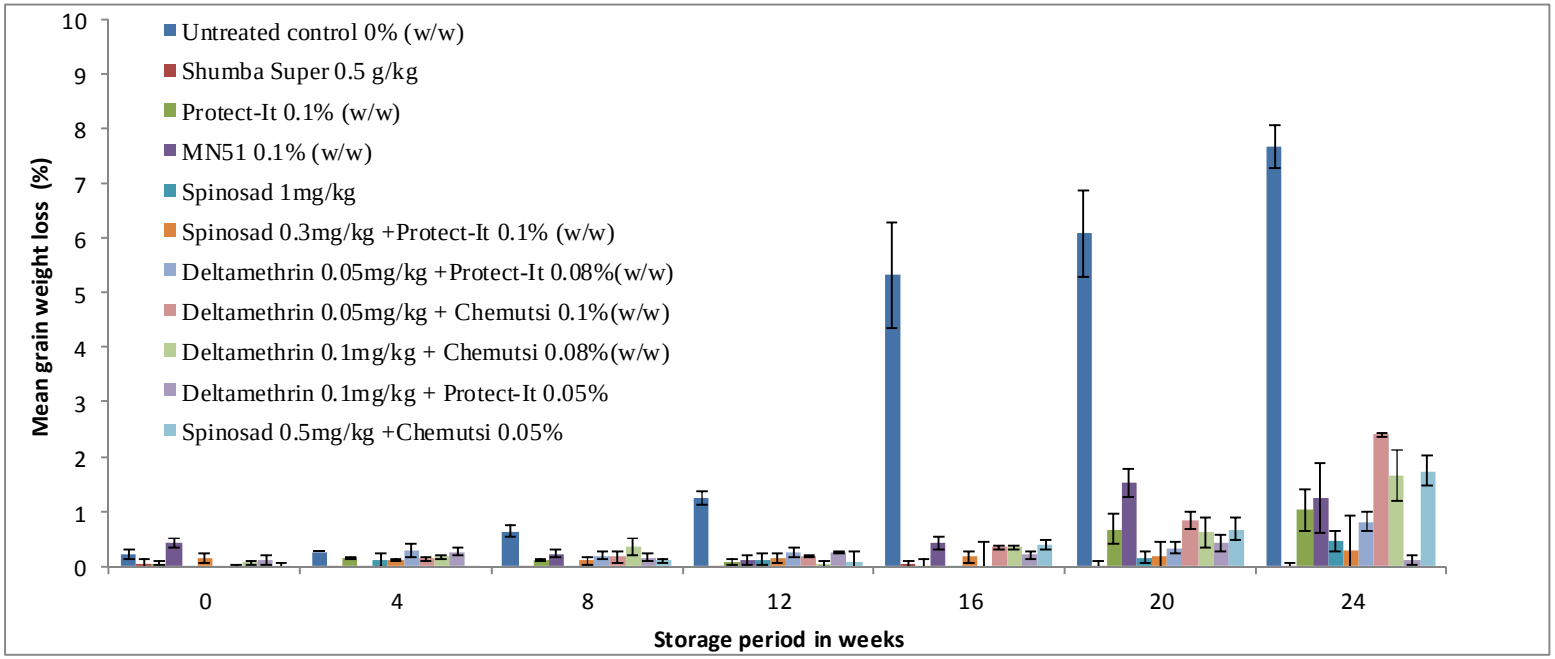

Figure 3. Mean grain weight loss (± SEM) over a 24 week storage season (n=3).

Figure 4 shows the general trend in grain moisture content over the 24 week storage season. Grain in the untreated control maintained the highest moisture content until week 16 when the damage level began to increase to levels where the hollowed kernels cannot hold moisture anymore. Thus at week 24, the untreated control has the lowest moisture content. Shumba Super® and Spinosad®-treated grain generally had higher moisture content than all the other treatments containing DEs earths throughout the storage season. The moisture content for these two treatments increased over and above that of the untreated control between week 16 and week 20 for the first time throughout the duration of the experiment.

There was a decrease in moisture content from around 12% to below 10% at week 12 for all the treatments. Treatments that consistently showed low moisture content were those with high quantities of DEs like Protect-It® 0.1% w/w and Spinosad 0.3 mg/kg + Protect-It® 0.1% w/w.

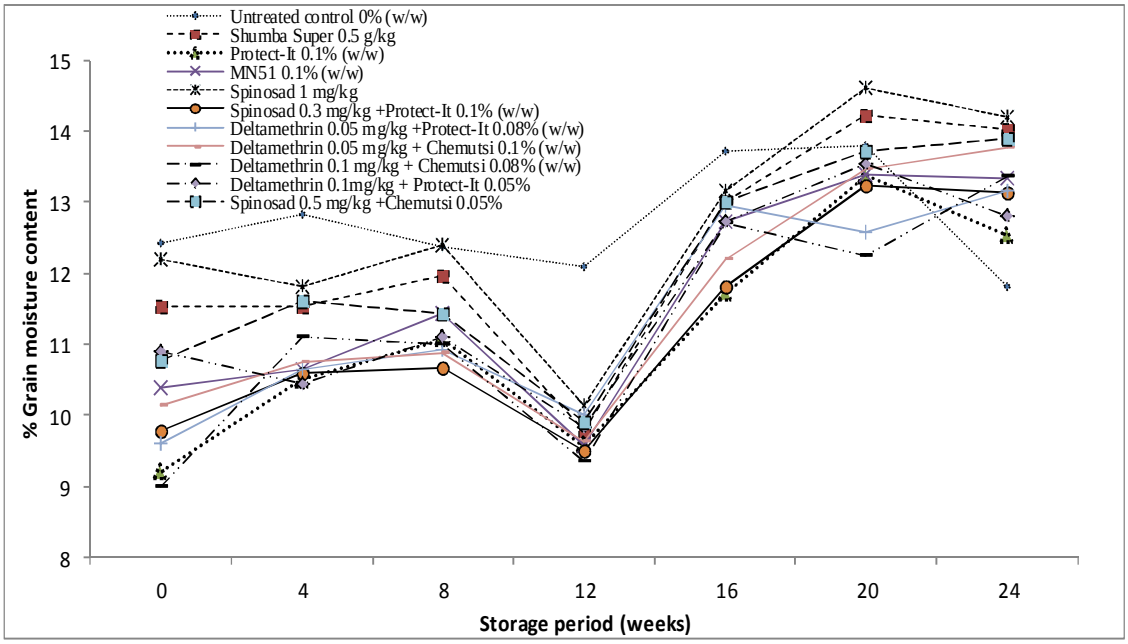

Figure 4. Changes in grain moisture content over a 24 week storage season (n=3).

The Product Moment Correlation Co-efficient, **R**, between number of live insects and the level of damage was 0.7561 (Table 9). Its corresponding co-efficient of determination, $\mathbf{R}^2$ of 0.5718 showed that live insects contributed about 57% of the observed damage. The moisture content of the grain ($\mathbf{R}^2$ = 0.051398) contributed about 5% to damage while the number of dead insects ($\mathbf{R}^2$ = 0.28622) contributed about 28%. It is assumed that dead insects fed on the grain before they died. On the other hand, $\mathbf{R}^2$ of 0.5927 between the number of live insects

and weight loss meant that about 59% of variability observed in grain weight loss was due to insect activity while a low 4.19% was contributed by grain moisture content.

Table 9. Correlation co-efficients between grain damage, weight loss and other parameters

Compared parameters	Product Moment Correlation Coefficient (R)	Coefficient of determination (R^2)
Number of live insects and % grain damage	0.7561	0.5718
Grain damage and grain moisture content	0.2267	0.0513
Number of live insects and weight loss	0.7699	0.5927
Number of dead insects and % grain damage	0.5349	0.2862
Weight loss and grain moisture content	0.2047	0.0419

Table 10. Number of live insects and trash (g/kg) 24 weeks after treatment

Treatments	Live insects/kg	Trash (g/kg)
Untreated control	150.65c	28.55b
Shumba Super 0.5g/kg	1.00ab	2.65a
Protect-It 0.1% (w/w)	66.61c	5.56a
MN51 0.1% (w/w)	43.31abc	6.20a
Spinosad 1mg/kg	34.11abc	2.80a
Spinosad 0.3mg/kg +Protect-It 0.1% (w/w)	0.30a	3.57a
Deltamethrin 0.05mg/kg +Protect-It 0.08%(w/w)	0.60a	8.12ab
Deltamethrin 0.05mg/kg+ Chemutsi 0.1%(w/w)	31.03abc	5.13a
Deltamethrin 0.1mg/kg + Chemutsi 0.08%(w/w)	32.47abc	4.33a
Deltamethrin 0.1mg/kg + Protect-It 0.05%	1.56ab	2.65a
Spinosad 0.5mg/kg +Chemutsi 0.05%	34.83bc	3.73a
$\boldsymbol{F}_{10, 20}$	4.61	2.25
P-value	<0.001	<0.001
CV%	28.5	13

Means within a column followed by the same letter are not significantly different (Tukey-Kramer HSD test, $P = 0.05$)

In terms of the number of live insects/kg, only Shumba Super®, spinosad 0.3 mg/kg + Protect-It® 0.1% w/w, deltamethrin 0.05 mg/kg + Protect-It® 0.08% w/w and deltamethrin 0.1 mg/kg + Protect-It® 0.05% had significantly lower numbers compared to the untreated

control (Table 10). However, insect damage in the untreated control produced 2-3 times as much trash as in the other treatments.

5.4 Discussion

Low levels of grain damage and weight loss observed in the first 12 weeks are chiefly a result of low pest populations at that time. It has been demonstrated that significant insect pest populations and subsequent grain damage and weight loss occur from around storage week 16 (Stathers *et al.*, 2000). The significant differences in both grain damage and weight loss between the untreated control and the other treatments from 12 weeks onwards indicate that all the treatments were effective in protecting the grain for the duration of storage. It may be worthwhile to test these materials for extended periods like 32-48 weeks to determine when the potency begins to wane (Khakame *et al.*, 2012).

The Product Moment Correlation Co-efficient (R) between number of live insects and the level of damage was a fairly strong one for observations of data for the whole trial period. The changes in weight loss which was noted from 0-24 WAT were not consistent. These inconsistencies could have been caused by damage being concentrated on, or insect preference for bigger and heavier grain kernels whose weight difference was not enough to be reflected in the Count and Weigh grain weight loss formula. This is the reason why negative weight loss values were recorded for most treatments in the early stages. This is one of the weaknesses of the conventional Count and Weigh Method (Compton *et al.*, 1998).

A drop in grain moisture content observed at 12 WAT is suspected to be a result of very dry environment and low humidity experienced in November when the 12 WAT samples were collected. This explanation could be plausible considering the sudden increase in grain moisture content once the rains began to fall from 16 WAT onwards (December and January). Once the rains commenced, grain absorbed moisture from the humid environment resulting in high grain moisture content. At 24 WAT (February), the moisture content began to drop gently as a result of extended dry spells that occur annually around that time of the year.

All DE treatments and their combinations generally had low moisture content than non-DE treatments. Spinosad, Shumba Super® and untreated grain showed consistently higher moisture content than all the other treatments. This is because DEs are themselves very dry and absorb moisture from the grain (Korunic, 1998). The untreated control showed that

despite the humid environment, at certain levels of damage above 30%, the grain fails to absorb moisture from the atmosphere.

The low insect numbers observed in all deltamethrin + Protect-It®-treated samples suggest that there is a strong synergy between these two materials both in terms of efficacy and repellence effects (Korunic and Rozman, 2010). Pure DEs (Protect-It® 0.1% w/w and MN51 0.1% w/w) recorded quite a significant numbers of live insects/kg, reflecting their slow action, low repellence effect and low efficacy when used alone (Korunic, 1997).

CHAPTER 6

ON-FARM EVALUATION OF GRAIN PROTECTANTS AGAINST STORED MAIZE INSECT PESTS

6.1 Introduction

In rural areas of Zimbabwe, maize production is mainly subsistence and on-farm storage forms a greater part of the traditional practices (Mvumi *et al.*, 1995; Nyagwaya *et al.*, 2010). Every household dedicates a greater part of their cultivated land, labour and other inputs to maize production for income and food security reasons (ZIMVAC, 2011). However, having successfully produced and harvested, significant grain losses continue to occur in storage due to insect pest damage.

Successful treatments tested by researchers with the farmers in their existing storage systems, on their favourite maize varieties and against their common problem pests produce credible results that may be easily understood and readily adopted by the farmers. There is very little information in Zimbabwe on the efficacy of diatomaceous earths (DEs) on post-harvest pests in smallholder farm stores. Most of the bioassays conducted so far have been laboratory-based and focused on acute effects of the DEs on adult insects (Stathers *et al.*, 2000). Efficacy of cocktail combinations of DEs and bio-pesticides or reduced quantities of deltamethrin on mixed pest populations in farm stores has not received enough attention. Current national pesticide regulations in Zimbabwe restrict the registration of DEs as grain protectants (Mvumi *et al.*, 2006). Data on the potency and efficacy of test grain protectants evaluated under the farmerôs circumstances must be obtained for the research results to be more credible and have an impact on both policy-makers and farmers.

Farmers in wards 13 and 28 of Murehwa district reported harvesting enough maize to last them through each storage season after exchanging surplus for weeding labour, firewood, cash and other needs (Murehwa farmers, 2012 personal communication). However, substantial quantities of grain are fed to livestock every year, having been heavily damaged by insects to levels unfit for human consumption (Murehwa farmers, 2012 personal communication). This presents a portion of lost potential income, and in poor seasons, where there is no surplus, such level of pest damage heavily contributes to serious grain shortages at household, community and ultimately at national level. The main objective of this experiment was to test the efficacy of five most promising treatments based on laboratory experiments under typical farmersô storage conditions.

6.2 Materials and Methods

6.2.1 Sites and timing

The sites chosen for the on-farm trials are located in Murehwa district in Wards 13 and 28. This part of the district lies within Natural Farming Region IIa and receives 750ï 1,000 mm rainfall annually. The wards were identified through collaboration with extension staff and Community Technology Development Trust (CTDT) project officers. Four farmers were identified to host the on-farm trials. The criteria for host farmer selection was a history of good farming practices and high yields (so that the farmer would not be short of grain before the end of the storage season and be tempted to use trial grain), similar storage facilities, experience with researcher-managed trials, character, attitude of the farmer towards research and accessibility of the homestead.

The trials were set up on the 6th of September, 2012 when all the farmers had completely finished harvesting, shelling and were storing their grain. This timing was deliberate in order to synchronise the experiment period with the farmersô normal storage season. The purpose was to expose the treatments to the natural spectrum of insect pests and environmental conditions that the farmers face in their normal storage seasons.

6.2.2 Grain, tests for pre-treatment and store types

One-and-half tonnes of maize grain (SC 627 variety) from the 2011/2012 harvest were used in the study. The grain was procured from a local farmer in ward 28. A preliminary sample was taken from each grain bag from top, mid and bottom. These samples were tested for moisture content and for traces of insecticide (to check if the grain was never treated; as that would influence the outcome) by placing grain in a jar and introducing 50 unsexed 7-14day old adult *S. zeamais* and observing mortalities after 7 days. The tests confirmed that the grain had sufficiently dried (12.3ï 14.5% moisture content) and had no prior chemical treatment (< 10% mortality observed in 7 days).

Currently, the common grain store types used in Zimbabwe are the asbestos-roofed 3-4 roomed houses that farmers use as bedrooms. Nyagwaya *et al.* (2010) pointed out that there was a paradigm shift from the granary bulk storage system to bagged bedroom storage over the last decade and half due to a variety of reasons. Thus, the asbestos-roofed bedroom houses were used as store types at all the four sites in this study. The grain was stored in bags and placed on wood, wood and brick or just brick dunnage, depending on what was available at

the host farmerôs homestead. Each bag was placed at least 15 cm from the next bag to avoid influence of one treatment on the adjacent ones.

6.2.3 Treatments and experimental layout

The five most efficacious treatments selected from the laboratory experiments were used (Table 11). All the grain (1.5 tonnes) was removed from the original bags and poured onto one heap on a cleaned floor surface and mixed thoroughly by shovelling grain from the bottom to the top repeatedly around the heap. This was done to create grain uniformity in terms of kernel types, sizes and moisture content. About 200kg (4 × 50 kg bags) were poured on to clean thick plastic sheets. Laboratory-weighed and packed sachets of the treatment materials equivalent to 200 kg of grain were applied onto the heap of grain. Clean shovels (previously washed with water and allowed to sun-dry) were then used to thoroughly mix the grain with the chemicals (Plate 6).

Table 11. Treatments used in on-farm trials.

Treatment no.	Treatment materials
1	Untreated Control
2	Chikwapuro® 0.4 g/kg (positive control)
3	Chemutsi 0.08% (w/w) + Deltamethrin 1 mg/kg
4	Chemutsi 0.05% (w/w)+ Spinosad 0.05 mg/kg
5	Chemutsi 0.1% (w/w)+ Deltamethrin 0.05 mg/kg
6	Protect-It® 0.05% (w/w) + Deltamethrin 0.1 mg/kg
7	Protect-It® 0.1% (w/w)

A B

Plate 6. Applying the dust treatments: on to the heap of grain (A) and admixing (B)

After mixing, the treated grain was weighed out and packed back into 4 x 50 kg bags and arranged according to their blocks until all the treatments were completed. The bags were then delivered to each of the four host farmers, each receiving 7 x 50 kg bags, representing the seven treatments. Each host farmer acted as a block, with block allocation to the farmers being done randomly. All the bags were appropriately labelled.

The experiment was laid out as a Randomised Complete Block Design (RCBD). GenStat Release 14.1 was used to randomise and allocate the treatments to blocks and bags. Each bag was labelled with bag number, treatment number and block number. One label was placed in the grain inside the bag, another was fastened to the string after sewing, and the bag itself was labelled on both sides to ensure easy and positive identification.

6.2.4 Storage and sampling

Baseline samples (approximately 1 kg) were taken from each bag before sewing, using Hollow Seedburo® grain metal probes. The bags were transported according to their block/replication numbers. In the houses, the bags were placed on dunnage made of planks/poles placed on bricks or on bricks alone (Plates 7A and B). The bags were placed 25-30 cm apart to reduce the influence (insect repellence and mortality) of one treatment on the adjacent ones.

Plate 7. Grain bags on different types of dunnage (A) brick and timber and (B) brick alone.

Sampling was done at 4-week intervals for 24 weeks (6 months) using the same equipment and method described in chapter 5. Grain withdrawn from each bag was placed in plastic sampling bags (28 cm × 50 cm and approximately 75-100 µm thick) which were then labelled. At each sampling, the bags were turned round to access all sides and previously

pierced points were avoided. Soon after sampling, the moisture content of the grain was recorded on site.

6.2.6 Sample analysis and data records

Sample analysis and data records were similar to those described in chapter 5.

6.2.7 Data analysis

Four-week interval data on weight loss (%), grain damage level (%) were subjected to one-way ANOVA. Prior to analysis, % damage and weight loss data were first tested for normality using fitted-value plots in GenStat. The scatter plots showed defined patterns and the data was then transformed using arcsine-square root transformation (Fowler *et al.*, 1998). Analysis was then performed on transformed data.

6.3 Results

Table 12 summarises treatment mean differences for grain damage at 0 weeks (baseline) and 24 weeks. There were no significant differences (P = 0.557 and P = 0.36) in grain damage among all the treatments at 0 and 4 weeks after treatment respectively. At 8 weeks after treatment, the positive control (0.37%) is not significantly different from Spinosad 0.5 mg/kg + Chemutsi 0.05% (w/w) (0.74%) and Deltamethrin 0.1 mg/kg + Protect-It® 0.05%(w/w) (0.69%), otherwise, the rest of the treatments are significantly different (P = 0.036) from the positive control including the untreated control. Twelve to twenty four weeks after treatment, there were no significant differences between the positive control (Chikwapuro® 0.5 g/kg) and any of the other treatments except the untreated control. Damage on all treated grain at 24 WAT ranged from 0.67% (Chikwapuro® 0.5 g/kg) to 1.74% (Deltamethrin 0.05 mg/kg + Chemutsi 0.1% w/w). Unsurprisingly, the untreated control had the highest grain damage (22.47%) and was the only treatment that was significantly different (P < 0.001) from all the others 24 weeks after treatment. Table 12 also shows the change in mean damage for each treatment. The untreated control had the highest change in grain damage (22.29%) from 0 to 24 weeks compared to 0.32% for the positive control (Chikwapuro®).

Table 12. Mean % grain damage (±SEM) from 0-24 weeks after treatment (n=4).

Treatment	Storage period (weeks)						
	0	4	8	12	16	20	24
Untreated control	0.20±0.06a	0.14±0.02a	0.82±0.06b	2.02±01.14b	5.46±2.52b	10.27±0.98b	22.48±6.32b
Chikwapuro® 0.4g/kg	0.35±0.08a	0.35±0.04a	0.37±0.13a	0.11±0.04a	0.99±0.21a	0.81±0.11a	0.67±0.13a
Deltamethrin 0.1 mg/kg +Chemutsi 0.08% (w/w)	0.27±0.02a	0.50±0.04a	0.80±0.04b	0.32±0.11a	1.54±0.39a	2.19±0.33a	1.59±0.39a
Spinosad 0.5 mg/kg +Chemutsi 0.05% (w/w)	0.28±0.08a	0.23±0.11a	0.74±0.06ab	0.40±0.11a	1.38±0.13a	1.53±0.20a	1.43±0.15a
Deltamethrin0.05 mg/kg + Chemutsi 0.1% (w/w)	0.37±0.03a	0.50±0.02a	0.80±0.14b	0.42±0.02a	1.38±0.22a	1.32±0.13a	1.74±0.16a
Deltamethrin 0.1 mg/kg + Protect-It® 0.05%(w/w)	0.39±0.15a	0.27±0.04a	0.69±0.06ab	0.27±0.06a	1.32±0.21a	0.69±0.06a	0.96±0.07a
Protect-It® 0.1% (w/w)	0.24±0.03a	0.24±0.09a	0.78±0.06b	0.32±0.08a	1.69±0.20ab	0.78±0.09a	1.12±0.11a
$F_{6, 18}$	0.84	2.91	2.91	2.38	3.71	11.89	25.97
P- value	0.557	0.36	0.036	0.072	0.014	<0.001	<0.001
CV (%)	29.2	18.9	8.5	68.1	36.3	24.8	12.9

Means within a column followed by the same letter are not significantly different (Tukey-Kramerôs HSD test, $P = 0.05$)

Grain weight loss showed a similar pattern to that of grain damage (Table 13). There were no significant differences among all the treatments 0 to 12 week after treatment. At 16WAT, the untreated control (0.65%) was only significantly different (P = 0.018) from the positive control, Chikwapuro® 0.5 g/kg (0.24%) and Deltamethrin 0.1mg/kg + Protect-It® 0.05% (w/w) (0.17%). Otherwise, there were no significant differences between the untreated control and the rest of the treatments at that sampling period. At 20 and 24 WAT, only the untreated control was significantly different ($P < 0.001$) from the rest of the treatments, otherwise, there were no significant differences among all the treatments. At 24 WAT, weight loss ranged from 0.148% (Chikwapuro®) to 0.4705% (Deltamethrin 0.05 mg/kg + Chemutsi 0.1% w/w).

Table 14 shows that *P. truncatus*, *T. castaneum*, *S. cerealella*, and *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) and a parasitoid *Anisopteromalus calandrae* (Howard) were observed in the samples and recorded. *Sitophilus zeamais* was the most abundant insect pest species and occurred at all the homesteads. *Anisopteromalus calandrae* was only found in the untreated control with almost equal number of dead and live adult insects. However, the numbers for most insect species were very low and some of them were found already dead in almost all the cases. *Sitophilus zeamais* live adults were noticeably higher (18.26 insects/kg of grain) in the untreated control at week 24 (Table 14). High numbers of dead adult *S. zeamais* (162.7 insects/kg) were also recorded at this time. However, in the treated grain, populations remained very low. All the other treatments successfully suppressed insect pest populations (Table 14) to very low levels up to 24 weeks.

Table 13. Mean (%) grain weight loss (±SEM) from 0 to 24 weeks after treatment (n=4).

Treatment	Storage period in weeks						
	0	4	8	12	16	20	24
Untreated control	0.02±0.024a	0.03±0.01a	0.08±0.11a	0.14±0.05a	0.65±0.08b	1.02±0.05b	2.00±0.251b
Chikwapuro® 0.4g/kg	0.04±0.006a	0.09±0.02a	0.04±0.02a	0.03±0.02a	0.24±0.07a	0.17±0.02a	0.15±0.023a
Deltamethrin 0.1mg/kg +Chemutsi 0.08% (w/w)	0.04±0.018a	0.06±0.01a	0.08±0.04a	0.04±0.03a	0.30±0.10ab	0.66±0.12b	0.28±0.035a
Spinosad 0.5mg/kg +Chemutsi 0.05% (w/w)	0.02±0.019a	0.04±0.01a	0.12±0.06a	0.01±0.04a	0.27±0.04ab	0.20±0.03a	0.28±0.080a
Deltamethrin0.05mg/kg + Chemutsi 0.1% (w/w)	0.07±0.034a	0.08±0.04a	0.08±0.08a	0.07±0.01a	0.29±0.11ab	0.25±0.05a	0.47±0.020a
Deltamethrin 0.1mg/kg + Protect-It® 0.05%(w/w)	0.04±0.017a	0.03±0.01a	0.04±0.04a	0.05±0.02a	0.17±0.01a	0.20±0.02a	0.25±0.045a
Protect-It® 0.1% (w/w)	0.01±0.014a	0.05±0.01a	0.15±0.03a	0.06±0.02a	0.35±0.09ab	0.25±0.07a	0.24±0.094a
$F_{6, 18}$	0.88	1.33	1.07	1.9	3.48	13.74	20.23
P-value	0.527	0.293	0.416	1.36	0.018	< 0.001	<0.001
CV%	0.6	1.4	7.5	2.9	6.1	6	5.1

Means within a column followed by the same letter in a column are not significantly different (Tukey-Kramerôs HSD test, $P = 0.05$)

Table 14. Numbers (per kg of grain) of dead and live insects (±SEM) in each treatment 24 weeks after treatment

Treatment	*S. zeamais*		*T. castaneum*		*P. truncatus*		*S. cerealella*		*R. dominica*		*A. calandrae*	
	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
Untreated control 0% w/w	18.3±7.5	162.7±78.5	0.2±0.0	2.0±0.9	0±0.0	0.18±0.0	0±0.0	4.10±1.0	0±0.0	0±0.0	2.8±0.3	2.5±1.6
Chikwapuro® 0.5g/kg	0.0±0.0	2.3±1.0	0.0±0.0	1.0±0.3	0±0.0	0±0.0	0±0.0	4.2±1.0	0±0.0	0±0.0	0±0.0	0±0.0
Deltamethrin 0.1mg/kg + Chemutsi 0.08% (w/w)	6.7±5.3	0.7±0.2	0±0.0	0±0.0	0±0.0	0±0.0	1.8±1.8	3.7±3.9	0±0.0	0±0.0	0±0.0	0±0.0
Spinosad 0.5 mg/kg + Chemutsi 0.05% (w/w)	1.8±0.9	7.9±1.5	0±0.0	0±0.0	0±0.0	0±0.0	0.9±0.9	0±0.00	0±0.0	0±0.0	0±0.0	0±0.0
Deltamethrin 0.05 mg/kg + Chemutsi 0.1% (w/w)	4.3±0.0	5.6±3.0	0±0.0	0±0.00	0±0.0	0±0.0	0.7±0.7	0±0.0	0±0.0	0.21±0.2	0±0.0	0±0.0
Deltamethrin 0.1mg/kg + Protect-It® 0.05 %(w/w)	0.0±0.0	0.7±0.2	0±0.0	0±0.0	0±0.0	0±0.0	0.8±0.8	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Protect-It® 0.1% (w/w)	0.0±0.0	0.6±0.3	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0

Since *S. zeamais* was the most abundant species found in this area, a line graph showing the development of the observed population densities over the duration of the experiment was plotted (Fig 5). Data labels were only placed on interesting points.

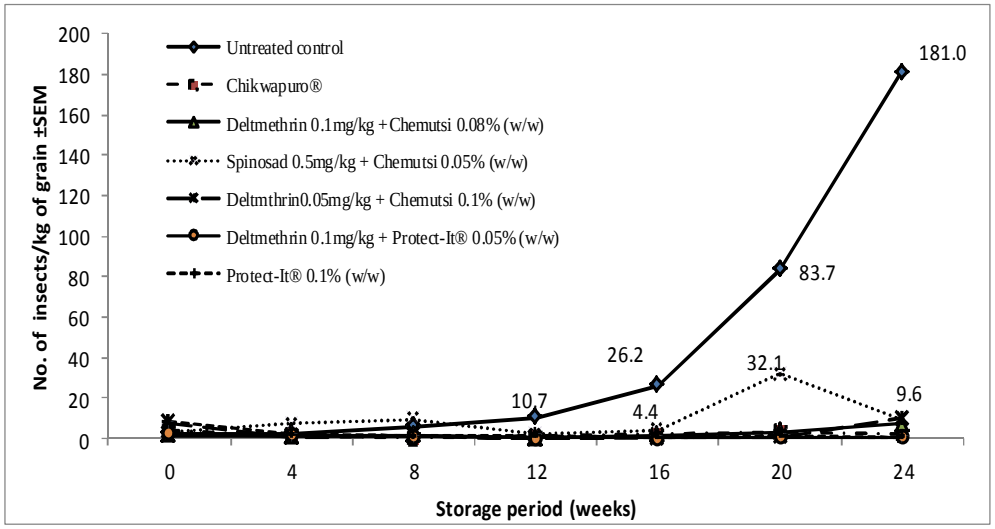

Figure 5. *Sitophilus zeamais* total population trend in different treatments over a 24 week storage season (n = 4).

Sitophilus zeamais numbers started to rise rapidly starting from 16 weeks after treatment in the untreated control (Figure 5). However, in the treated grain, populations remained very low throughout storage. There was a sudden population increase in spinosad 0.5 mg/kg + Chemutsi 0.5% w/w. The increase was caused by dead *S. zeamais* observed in that treatment at that time at one of the (blocks) homesteads, but the insects were assumed to have died before causing any damage. All the other treatments successfully suppressed insect pest populations up to 24 weeks.

The trend observed in *S. zeamais* populations on spinosad 0.5 mg/kg + Chemutsi 0.05% w/w showed that there was a relatively early appearance of insects in this treatment, reaching an average of at 8 weeks. The population then dropped to 2.0 insects/kg at week 16 before rising again to 32.1 insects/kg at week 20 only to drop drastically to 9.6 insects/kg at 24 weeks.

6.4 Discussion

It is difficult to pick a single treatment that performed better than the others as all the test treatments performed as well as the positive control. This is in agreement with the findings by Stathers *et al.* (2000) in Buhera District where, after a 40 week trial period, there were no significant differences between the positive control and Protect-It® at 0.1% and 0.2% w/w.

Chemutsi is a raw DE just mined and crushed without any form of enhancement or further processing. Its performance in combination with deltamethrin or spinosad compared to the performance of similar Protect-It® combinations is a sign of its potential efficacy. It remains to be researched whether this performance would increase if the period of exposure is extended beyond 24 weeks. This is important because Chemutsi is locally available and can be exploited and improved for the benefit of the farmers at a possibly low cost. If enhanced, improved and approved, Chemutsi has the potential to replace the toxic organophosphate component in commercial pesticides. This would then reduce farmersô heavy dependence on synthetic chemical pesticides (Mvumi *et al.*, 1995). This will not only make the grain protectant safe, but possibly affordable too.

Generally, pest population densities, damage and weight loss levels recorded in all treatments were quite low. This could be attributed to the short length of the research period (24 weeks) versus a complete storage period of at least 32 weeks. With the exception of the untreated control, low pest populations recorded in all the other treatments was due to the repellent effects of DEs (Korunic, 1998) and general avoidance of treated grain by insects.

Sitophilus zeamais was the dominant pest species in this study and similar to observations by Stathers *et al.* (2000), increased pest densities became apparent from week 16 at which time differences in damage between treated and untreated grain was becoming pronounced. It can therefore be deduced that when grain is harvested, it takes about 16 weeks for pest populations to build up to levels high enough to cause significant damage. On spinosad 0.5 mg/kg + Chemutsi 0.05% w/w, the *S. zeamais* population trend can possibly be explained in terms of suspected delayed efficacy and selective effect on adult insects of this treatment combination (Korunic, 1998). It is suspected that when the adults emerge they have ample time to move, damage the grain and oviposit before they die. In the process, they pick a lot of treatment dust which eventually kills them. The eggs later hatch and immobile larvae begin to feed inside kernels where they are safe from the effect of the treatment dust and develop to the pupal stage. The pupa develops into an adult that is mobile and picks treatment and dies, the cycle then continues. Though Stathers *et al.* (2000) also reported the presence of *S. cerealella*, the sampling techniques used in this study were not appropriate for capturing the evasive moths. Besides, samples were not incubated to determine *S. cerealella* populations from emerging progeny.

Despite claims by farmers in Murehwa that current commercial grain protectants are not very efficacious against pests in their area, it was demonstrated that Chikwapuro® worked perfectly as a positive control. It is suspected that farmers do not follow label instructions, do not measure the grain or the pesticide properly and ultimately do not adequately mix the grain and the protectant. This was also confirmed by reports and outcomes during post-harvest training workshops held with farmers during the course of this study. In a national post-harvest survey, Mvumi *et al.* (1995) reported that farmers regard the need to measure pesticide dust as impractical and academic. Though measuring cups are provided, they are just used as tools to transfer (not to measure) the grain protectant just like they do with spoons and other household utensils.

CHAPTER 7

GRAIN DAMAGE AND POPULATION DYNAMICS RESULTING FROM RESIDENT AND INCOMING INSECT PESTS

7.1 Introduction

Stored product insect pests ecology has not been accorded the systematic scientific investigation it deserves, but effective stored product IPM from any perspective requires one to understand insect pest bionomics. Adequate knowledge about their ways of life and development in areas where they cause damage enables optimal choice of treatment types in time and space (Trdan *et al*., 2010). This will enable effective suppression of pest populations to acceptable levels and reduce the cost and risk associated with chemical pesticides (Campbell and Arbogast, 2004).

Post-harvest research that involves periodic sampling from the field provides information on insect pest species composition and their relative abundance. However, they do not really reveal the spatial and temporal distribution and population densities of the associated insect species (Hagstrum, 1984). Data from periodic field samples do not also give the life history components of population growth and often do not measure the factors responsible for such population changes (Hagstrum, 1984).

Studies on the ecology of most storage pests of maize have been done in laboratories giving results that are thus limited in scope of application to the farm situation. Farmer-managed stores have very diverse spectra of species, complex levels of inter-, and intra-specific competition, environmental conditions and the presence or absence of natural enemies that influence field ecological studies (Mvumi *et al.*, 2002). Therefore a study of the ecology of the maize pest complex on-station is meant to mimic farmer conditions as much as is possible. Besides, Mvumi *et al*. (2003) also acknowledged that there are very few studies that have investigated the ecology of pest complexes on shelled stored maize which this experiment seeks to investigate. The results of these ecological studies will hopefully contribute towards improved grain protection tactics for integration into existing IPM programs in stored product protection. At the moment, lack of information on pest populations is hampering the development of alternative pest management tools and effective implementation of IPM programs in stored product protection.

Knowledge of the climatic data in specific geographical locations that correspond to certain pest populations is important in the development of pest abundance prediction models. These models can then be used to develop pest monitoring systems and response mechanisms related to the prevailing climate (Kasambala and Chinwada, 2011). Farmers have information about the climatic environment within which they operate; when the relationship between the climate and pest abundance is scientifically established, simplified and presented to them, they will be able to foretell pest outbreaks and prepare accordingly.

In Southern Malawi, Kasambala and Chinwada (2011) researched on a model with the potential to predict *P. truncatus* abundance basing on climatic factors. Research towards development of such a model has not been conducted in Zimbabwe. *Prostephanus truncatus* is an aggressive but fairly recent pest whose populations have not stabilised in any geographical location of Zimbabwe as established by differences in trap catches by Nyagwaya *et al.* (2010) and Mashavakure (2012). However, *S. zeamais* is cosmopolitan and has been observed in abundance since time immemorial, causing serious grain damage.

The main objective of this experiment was to determine whether it is the resident (field infestation) or incoming (re-infestation), that causes more grain damage and weight loss than the other. The study was also aimed at determining the trends in populations of different insect pest species over a storage season both on pest-free (fumigated) and on field-infested (unfumigated) grain. In addition, the study also investigated population dynamics in bulk grain damage in relation to granary depth and associated pest species.

7.2 Materials and Methods

7.2.1 Granary preparation

The experiment was carried out at the Institute of Agricultural Engineering (IAE) in the granaries already described in Chapter 5. Three granaries were selected, repaired, thoroughly cleaned and re-plastered using clay and small amounts of cow-dung (to prevent the clay from cracking), as per typical farmer practice.

7.2.2 Treatments

One tonne of shelled maize (SC 637 hybrid variety) was fumigated using phosphine tablets (Phostoxin®, Detia-Degesch GmbH, Aluminium phosphide 56% w/w + inert ingredients 44% w/w) at a rate of 10 tablets/ton of shelled maize grain. Fumigation was done in a metal silo of

volume 2.395 m^3. The metal silo was placed on a strong iron bench and loaded with the grain. Ten tablets were applied to the grain at different levels (3 at the bottom, 4 at the middle and 3 at the top). This was achieved by driving a metal pipe to the desired level and then dropping the tablet through the metal pipe. The spouts of the metal silo were then immediately closed using custom-made tight fitting lids followed by extensively wrapped with packaging tape to make the silo air tight.

About 900 kg of the fumigated grain were weighed and separated into six portions of 150 kg each. These portions were loaded into granary compartments (Plate 8) in three granaries (blocks). Each granary had two compartments loaded with the fumigated grain, immediately after loading one compartment was closed and sealed completely while the other was left open. The closed compartments (Fumigated Closed and Unfumigated Closed) were fitted with tightly closing doors whose surfaces were then plastered using clayey soil (Plate 8) to make a continuous seal with the wall plastering. The same was repeated with un-fumigated grain.

Plate 8. Closed (A) and Open (B) granary compartments. The open compartments were not fitted with doors (B) to allow insects to move in and out and outside the granary.

The grain treatments are shown in Table 15.

Table 15. Grain treatments.

Grain treatment	Entrance
Fumigated	Open
Fumigated	Closed
Not fumigated (unfumigated)	Closed
Not fumigated (unfumigated)	Open

7.2.3 Sampling frequency

After every four weeks, grain samples were withdrawn using a sampling spear. The spear was dipped vertically inside the grain whilst it was closed, it was then opened when its tip touched the bottom, before being shaken to enable grain to enter, then it was closed. The sampling pattern in each granary compartment was as shown in Figure 6. The depth of the grain (45 cm) in each compartment enabled sampling to be conducted from the top, middle and bottom positions of the granary. Grain sampled from each level was packed and labelled separately. This was meant to enable observation of the differences in grain damage and pest densities and distribution between the top, middle and bottom layers of stored grain. Samples from each point per level were bulked to make a composite sample of size approximately 1 kg.

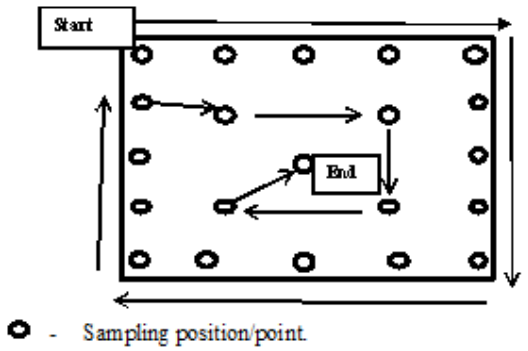

Figure 6. Sampling positions and pattern in a granary

7.2.4 Data records

For each grain sample, moisture content was measured using a Dickey-john® digital moisture meter. The weight of each sample was first weighed and then sieved to remove insects and chaff (trash). The weight of sieved chaff was also measured and converted to g/kg of grain. All insect pest species, including natural enemies, found in each sample were identified and their numbers recorded.

Insect-damaged and undamaged grains were separated, counted and weighed. This was achieved by dividing each sample into four equal sub-samples using a riffle sample divider. A sample was first poured out from the sample bag into the riffle divider to produce two equal sub-samples. These were each further divided in the same manner to produce a total of four equal sub-samples. Grain from three sub-samples were each poured out into white plastic trays and examined for insect damage. The fourth sub-sample was not considered. Data from the three sub-samples were averaged to give a sample average for damage and weight loss. Trash weight and insect counts were done for the entire sample.

7.2.5 Data analysis

Data on grain damage (%) was arcsine square rootï transformed before being analysed. Data on grain weight loss (%) and amount of trash or chaff (produced by insect feeding) were analysed without any transformation. Data on insect numbers for each species were $\sqrt{(x + 1)}$ -transformed. All the data were then subjected to one way analysis of variance (ANOVA).Where the *F*-ratio was significant ($P < 0.05$), means were separated by Tukey-Kramerôs HSD test.

7.3 Results

Table 16 shows the trends in grain damage in different treatments over a 24 week storage period. At the start of the trials (0 WAT), all unfumigated treatments had significantly higher ($P < 0.001$) grain damage than fumigated ones. At 4 WAT, only the top and middle levels of fumigated open (FO TOP and FO MID) treatments were significantly different from the unfumigated closed middle (UFC MID and UFC BOT) and bottom layers as well as unfumigated open top and bottom layers (UFO TOP and UFO BOT). The rest of the treatments were not significantly different from each other.

At 8 WAT, grain damage (%) in all fumigated treatments, the top levels of unfumigated closed and unfumigated and open (UFC TOP and UFO TOP) were not significantly different from each other, however, all these treatments had grain damage significantly lower ($P < 0.001$) than the middle and bottom layers of unfumigated closed (UFC MID and UFC BOT) as well as the middle and bottom layers of unfumigated open (UFO MID and UFO BOT) treatments.

At 12 WAT, grain damage in all fumigated treatments was not significantly different from each other. All unfumigated treatments however showed significantly higher ($P < 0.001$) grain damage than in all the fumigated treatments. The unfumigated treatments did not show any significant differences among themselves except the fumigated closed bottom (UFO BOT) which was significantly higher ($P < 0.001$) than the top and middle layers of unfumigated open (UFO TOP and UFO MID) treatments.

At both 16 and 20 WAT, all fumigated treatments were not significantly different from each other. Likewise, all unfumigated treatments also did not show any significant differences among themselves. However, there were significant differences ($P < 0.001$) between the fumigated and the unfumigated treatments despite sampling depth and status of the granary compartment door (i.e, being open or closed).

At 24 WAT the top levels of unfumigated closed and unfumigated open (UFC TOP and UFO TOP) showed significantly higher ($P < 0.001$) grain damage than the rest of the treatments. All the other treatments were not significantly different from each other. After the same period of exposure, trash (dust) weight was significantly higher ($P < 0.001$) in the bottom levels of the unfumigated closed and open (UFC BOT and UFO BOT) treatments than in all the other treatments. The top layer of fumigated grain (FO TOP) had the least trash quantities (2.57 g/kg) that was significantly different from the bottom layers of fumigated open and closed grain (FO BOT and FC BOT), all layers of unfumigated closed (UFC TOP UFC MID and UFC BOT) as well as the bottom layer of unfumigated (UFO BOT) treatment.

Table 16. Grain damage (%) by resident and incoming infestation on maize grain sampled at different depths of the granary over a 24-week storage period.

Treatment	0 weeks	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks	Trash (g/kg) at 24wks
Fumigated Closed Top	0.55±0.08a	0.66±0.21abc	0.46±0.03a	0.52±0.16a	0.69±0.40a	2.33±0.64a	12.17±3.34a	2.57±0.44a
Fumigated Closed Middle	0.55±0.08a	0.69±0.23abc	0.43±0.11a	0.46±0.13a	0.77±0.17a	2.39±0.75a	10.035±3.24a	8.28±2.43ab
Fumigated Closed Bottom	0.55±0.08a	0.34±0.01ab	0.51±0.09a	0.80±0.20a	1.41±0.33a	3.05±0.45a	10.41±1.92a	13.35±1.57b
Fumigated Open Top	0.48±0.15a	0.39±0.24a	0.47±0.10a	0.52±0.17a	0.94±0.57a	7.93±0.58a	30.09±16.23ab	8.21±1.42ab
Fumigated Open Middle	0.48±0.15a	0.24±0.02a	0.60±0.21ab	0.46±0.16a	0.79±0.42a	6.01±2.56a	20.13±10.23ab	8.61±0.43ab
Fumigated Open Bottom	0.48±0.15a	0.48±0.07abc	0.69±0.05ab	0.54±0.11a	2.38±1.18a	5.68±1.51a	18.47±6.56ab	14.12±0.82b
Unfumigated Closed Top	1.61±0.45b	1.22±0.37abcd	1.92±0.97abc	11.15±4.37bc	14.33±5.65b	33.23±9.12b	49.82±15.77b	8.62±a2.53b
Unfumigated Closed Middle	1.61±0.45b	1.68±0.45bcd	2.96±0.31cd	12.44±2.70bc	18.55±2.64b	29.24±4.51b	37.41±9.74ab	12.07±1.16b
Unfumigated Closed Bottom	1.61±0.45b	2.94±0.58d	2.52±0.57cd	17.68±1.35c	21.23±2.97b	26.46±0.58b	28.97±2.92ab	24.68±2.99c
Unfumigated Open Top	1.67±0.24b	1.91±0.55cd	1.98±0.12bcd	8.93±2.89b	17.87±0.07b	27.23±7.15b	47.43±9.54b	7.88±0.44ab
Unfumigated Open Middle	1.66±0.24b	1.52±0.47abcd	2.54±0.66cd	8.59±1.34b	17.37±3.30b	22.15±3.60b	40.01±8.16ab	10.04±2.15ab
Unfumigated Open Bottom	1.66±0.24b	1.91±0.13cd	3.87±0.53d	14.02±0.49bc	21.29±5.94b	25.18±1.62b	35.63±3.89ab	25.05±1.64c
$\boldsymbol{F}_{11, 35}$	20.87	2.90	14.32	35.82	38.64	20.81	4.66	15.03
***P*-value**	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
CV (%)	20.1	12.4	12.7	13.2	19.6	18.9	22.2	6.6

Means within a column followed by the same letter are not significantly different (Tukey- Kramerôs HSD test, $P = 0.05$)

Table 17 shows grain weight loss on fumigated and unfumigated grain treatments from 0-24 WAT at different depths of sampling. At both 0 and 4 WAT, there were no significant differences ($P > 0.05$) among all the treatments. At 8 WAT, the bottom layer of unfumigated open (UFO BOT) had significantly higher ($P < 0.002$) grain weight loss than the rest of the treatments. At 12 WAT, all the unfumigated treatments, whether open or closed were not significantly different from each other. Some negative weight losses were obtained in some treatments (FC BOT and FO MID).

At 16 WAT, weight loss ranged from 0.03% (FO TOP) to 3.61% (UFO MID). The top and middle layers of unfumigated closed (UFC TOP and UFC MID) were not significantly different from each other and the rest of the fumigated treatments. Apart from the unfumigated closed top and middle layers, the rest of the unfumigated treatments had significantly higher ($P < 0.001$) grain weight loss than the rest of the fumigated treatments. However, the bottom layer of fumigated open (FO BOT) was not significantly different from all the unfumigated treatments except the middle layer of the unfumigated open (UFO MID) treatment.

At 20 WAT, only the middle and top layers of fumigated closed (FC MID) and fumigated open (FO TOP), respectively, had grain weight loss that was significantly ($P < 0.001$) lower than the top layer of unfumigated closed (UFC TOP). The rest of the treatments were not significantly different from each other.

At 24 WAT, only the top layer of fumigated open (FO TOP) had significantly lower ($P = 0.004$) grain weight loss (0.28%) than the middle layers of unfumigated open and closed (UFO MID and UFC MID) treatments as well as the bottom layer of unfumigated open (UFO BOT). The rest of the treatments had weight losses that were not significantly different from each other.

Table 17. Grain weight loss (%) by resident and incoming infestation on maize grain sampled at different depths of the granary over a 24-week storage period.

Treatment	0 weeks	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	24 weeks
Fumigated Closed Top	0.13±0.48a	0.07±0.07a	-0.00±0.01a	0.03±0.01ab	0.08±0.10a	0.30±0.04abc	0.323±1.05abc
Fumigated Closed Middle	0.13±0.48a	0.02±0.01a	0.00±0.03a	0.09±0.07abc	0.10±0.02a	-0.12±0.12a	0.975±0.26ab
Fumigated Closed Bottom	0.13±0.48a	0.06±0.04a	0.06±0.03a	-0.06±0.08a	0.05±0.05a	0.31±0.13abc	1.62±0.14abc
Fumigated Open Top	0.06±0.36a	0.04±0.02a	0.01±0.02a	0.01±0.03ab	0.03±0.05a	0.13±0.29ab	0.28±1.37a
Fumigated Open Middle	0.06±0.36a	0.02±0.01a	0.09±0.04a	-0.06±0.07a	0.12±0.06ab	0.25±0.29abc	1.47±1.34abc
Fumigated Open Bottom	0.06±0.36a	-0.03±0.02a	-0.04±0.01a	0.034±0.04ab	0.26±0.07abc	0.75±0.49abcd	3.73±1.00abc
Unfumigated Closed Top	0.16±0.06a	0.02±0.05a	0.01±0.06a	1.71±1.05abcd	2.66±1.66abcd	2.62±0.73cde	4.29±0.975abc
Unfumigated Closed Middle	0.16±0.56a	-0.07±0.08a	0.39±0.22a	2.13±0.57bcd	2.97±0.71abcd	2.41±1.03bcde	5.27±2.08c
Unfumigated Closed Bottom	0.16±0.56a	0.09±0.08a	0.11±0.11a	2.60±0.88d	3.49±1.30cd	3.55±0.808e	3.59±1.45abc
Unfumigated Open Top	0.01±0.07a	-0.01±0.17a	0.03±0.06a	1.51±0.92abcd	3.45±0.68cd	1.28±0.26abcde	2.50±1.49abc
Unfumigated Open Middle	0.01±0.067a	-0.08±0.13a	0.07±0.12a	1.06±0.56abcd	3.61±0.87d	1.76±0.83abcde	4.59±1.54bc
Unfumigated Open Bottom	0.01±0.07a	-0.05±0.14a	0.55±0.04b	2.21±0.18cd	3.43±1.38bcd	2.87d±0.51e	5.01±0.75c
$\boldsymbol{F}_{11, 35}$	1.19	0.43	4.22	6.37	6.73	7.02	4.07
***P*-value**	0.348	0.923	0.002	< 0.001	< 0.001	< 0.001	0.004
CV (%)	22.3	762.6	106.7	65.7	55.7	40.9	26.5

Means within a column followed by the same letter are not significantly different (Tukey-Kramerôs HSD test, $P = 0.05$)

Table 18 shows that there were no significant differences in insect numbers among the treatments across all insect species except *S. zeamais*. At baseline (0 WAT), the maize weevil had significantly higher ($P < 0.001$) numbers in all unfumigated treatments than in fumigated ones. In fact, no insects were present in any of the fumigated treatments. However, for *P. truncatus*, there was an average of 0.34 insects/kg of grain for all levels of unfumigated open grain with all the treatments showing no significant differences among themselves.

Table 18. Number of different insect species recorded at 0 weeks after treatment

Treatment	*Sz*	*Pt*
Fumigated Closed Top	0.00±0.0a	0.00±0.0a
Fumigated Closed Mid	0.00±0.0a	0.00±0.0a
Fumigated Closed Bottom	0.00±0.0a	0.00±0.0a
Fumigated Open Top	0.00±0.0a	0.00±0.0a
Fumigated Open Mid	0.00±0.0a	0.00±0.0a
Fumigated Open Bottom	0.00±0.0a	0.00±0.0a
Unfumigated Closed Top	27.93±4.5b	0.00±0.0a
Unfumigated Closed Mid	27.93±4.5b	0.00±0.0a
Unfumigated Closed Bottom	27.93±4.5b	0.00±0.0a
Unfumigated Open Top	23.97±3.0b	0.34±0.3a
Unfumigated Open Mid	23.97±3.0b	0.34±0.3a
Unfumigated Open Bottom	23.97±3.0b	0.34±0.3a
$F_{11, 35}$	45.6	1
P-value	< 0.001	0.477
CV (%)	18.1	10.5

Means within a column followed by the same letter are not significantly different (Tukey-Kramerôs HSD test, $P = 0.05$)

ÿ Sz = *Sitophilus zeamais*, Pt = *Prostephanus truncatus*, Tc = *Tribolium castaneum*, Sc = *Sitotroga cerealella*, Cf = *Cryptolestes ferrugeneus* and Ac = *Anisopteromalous calandrae*

Table 19 shows the numbers of different insect species in maize samples at different sampling depths at 4 weeks after treatment. The bottom layer of unfumigated closed (UFC BOT) had significantly higher ($P < 0.001$) numbers of *S. zeamais* adults compared to the top layer of unfumigated closed (UFC TOP) as well as all the fumigated treatments. Otherwise, there were no significant differences among the treatments. Besides *S. zeamais*, no other pest species were recorded (Table 19) in the different treatments.

Though *Anisopteromalous calandrae* ð a natural enemy of *S. zeamais* ð was detected in the middle layers of unfumigated closed (UFC MID), other treatments did not have it.

Table 19. Number of different insect speciesÿ recorded at 4 weeks after treatment

Treatment	*Sz*	*Pt*	*Tc*	*Sc*	*Cf*	*Ac*
Fumigated Closed Top	0.6±0.5a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.0±0.0a	0.00±0.0a
Fumigated Closed Middle	0.7±0.5a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.0±0.0a	0.00±0.0a
Fumigated Closed Bottom	2.2±1.1ab	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.0±0.0a	0.00±0.0a
Fumigated Open Top	0.7±0.5a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.0±0.0a	0.00±0.0a
Fumigated Open Middle	2.5±0.2abc	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.0±0.0a	0.00±0.0a
Fumigated Open Bottom	1.8±1.4ab	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.0±0.0a	0.00±0.0a
Unfumigated Closed Top	7.8±2.4abcd	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.0±0.0a	0.00±0.0a
Unfumigated Closed Middle	15.3±3.7cde	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.0±0.0a	0.83±0.6a
Unfumigated Closed Bottom	28.4±1.4e	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Unfumigated Open Top	18.2±3.7de	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Unfumigated Open Middle	13.4±1.8bcde	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Unfumigated Open Bottom	24.9±5.1de	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
$F_{11, 35}$	12.89	-	-	-	-	
P-value	< 0.001	-	-	-	-	0.477
CV (%)	26.7	-	-	-	-	600

Means within a column followed by the same letter are not significantly different (Tukey-Kramerôs HSD test, $P = 0.05$)

ÿ Sz = *Sitophilus zeamais*, Pt = *Prostephanus truncatus*, Tc = *Tribolium castaneum*, Sc = *Sitotroga cerealella*, Cf = *Cryptolestes ferrugeneus* and Ac = *Anisopteromalous calandrae*

Table 20 shows the number of pest insects and *A. calandrae* recorded within the different treatments at 8 WAT. *Sitophilus zeamais* numbers were significantly higher ($P < 0.001$) at all layers (sampling depths) of unfumigated closed treatments (UFC TOP, MID and BOT) as well as the bottom layer of unfumigated open (UFO BOT) than the middle and top levels (UFO MID and UFO TOP) of unfumigated treatments. Except for the latter two treatments, all unfumigated treatments had significantly higher ($P < 0.001$) numbers of *S. zeamais* than the fumigated ones.

Prostephanus truncatus, *T. castaneum* and *S. cerealella* showed very low insect numbers that had no significant differences ($P = 0.477$ for all) across all the treatments. *Anisopteromalous calandrae* also showed the same trend, with insects mainly being

detected in the unfumigated treatments, but there were no significant differences (P = 0.17) among all the treatments.

Table 20. Number of different insect speciesÿ recorded at 8 weeks after treatment.

Treatment	*Sz*	*Pt*	*Tc*	*Sc*	*Cf*	*Ac*
Fumigated Closed Top	0.26±0.2a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Closed Middle	0.31±0.2a	0.00±0.0a	0.00±0.0a	0.000.0a	0.00±0.0a	0.00±0.0a
Fumigated Closed Bottom	0.35±0.3a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Open Top	0.54±0.2a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Open Middle	1.52±0.8a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Open Bottom	2.98±0.0ab	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Unfumigated Closed Top	24.2±6.1c	0.51±0.4a	0.00±0.0a	1.31±1.3a	0.00±0.0a	1.18±0.9a
Unfumigated Closed Middle	20.72±4.1c	0.00±0.0a	0.00±0.0a	1.68±1.0a	0.00±0.0a	1.73±0.7a
Unfumigated Closed Bottom	29.09±5.4c	0.00±0.0a	0.37±0.3a	0.00±0.0a	0.00±0.0a	0.39±0.3a
Unfumigated Open Top	14.60±4.2bc	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.27±0.2a
Unfumigated Open Middle	17.74±4.8bc	0.00±0.0a	0.00±0.0a	0.31±0.3a	0.00±0.0a	3.75±1.5a
Unfumigated Open Bottom	31.42±4.4c	0.78±0.6a	0.00±0.0a	0.00±0.0a	0.00±0.0a	3.93±1.9a
$F_{11, 35}$	18.47	1	1	1	-	2.87
P-value	< 0.001	0.477	0.477	0.477	-	0.17
CV (%)	24.4	15.6	7.5	27.7	-	32.5

Means within a column followed by the same letter are not significantly different (Tukey-Kramerôs HSD test, P = 0.05)

ÿ Sz = *Sitophilus zeamais*, Pt = *Prostephanus truncatus*, Tc = *Tribolium castaneum*, Sc = *Sitotroga cerealella*, Cf = *Cryptolestes ferrugeneus* and Ac = *Anisopteromalous calandrae*

At 12 WAT, *S. zeamais* had significantly higher (P < 0.001) numbers in all unfumigated treatments than in fumigated ones except unfumigated open bottom layer (UFO BOT) (Table 21). All the fumigated treatments were not significantly different from each other. Likewise, all unfumigated treatments were also not significantly different from each other. In the case of *P. truncatus* and *T. castaneum*, there were no significant differences (P > 0.05) in insect numbers among all the treatments. *Sitotroga cerealella* had significantly higher (P = 0.011) numbers at the top layer of unfumigated closed (UFC TOP) than all the other treatments except the top and bottom layers of unfumigated closed (UFO TOP and UFO BOT). *Anisopteromalous calandrae* had generally low numbers in all fumigated treatments than unfumigated ones. However, only the top and middle layers of unfumigated open (UFO TOP and UFO MID) treatments had significantly higher (P = 0.002) *A. calandrae* numbers than all the

fumigated treatments but there were no significant differences among all the unfumigated treatments.

Table 21. Number of different insect speciesÿ recorded at 12 weeks after treatment

Treatment	*Sz*	*Pt*	*Tc*	*Sc*	*Ac*
Fumigated Closed Top	2.65±1.1a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Closed Middle	4.75±0.4a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Closed Bottom	2.98±0.8a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Open Top	4.92±1.5a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Open Middle	7.01±2.6ab	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Open Bottom	5.76±1.0ab	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Unfumigated Closed Top	68.96±17.0c	0.49±0.4a	0.89±0.4a	1.31±0.1b	3.24±1.6ab
Unfumigated Closed Middle	109.15±14.7c	0.60±0.5a	2.22±1.3a	0.00±0.0a	2.56±2.0ab
Unfumigated Closed Bottom	105.65±18.5c	0.00±0.0a	0.00±0.0a	0.00±0.0a	5.81±2.9ab
Unfumigated Open Top	108.65±33.0c	0.00±0.0a	0.00±0.0a	0.39±0.3ab	17.58±10.2b
Unfumigated Open Middle	69.8±6.3c	1.11±0.5a	0.00±0.0a	0.00±0.0a	17.28±1.7b
Unfumigated Open Bottom	62.66±16.3bc	0.77±0.6a	1.47±1.1a	0.77±0.6ab	8.36±1.5ab
$F_{11, 35}$	13.1	1.56	1.42	3.15	4.18
P-value	< 0.001	0.179	0.23	0.011	0.002
CV (%)	30.8	18.4	3.02	14.8	51.9

Means within a column followed by the same letter are not significantly different (Tukey-Kramerõs HSD test, $P = 0.05$)

ÿ Sz = *Sitophilus zeamais*, Pt = *Prostephanus truncatus*, Tc = *Tribolium castaneum*, Sc = *Sitotroga cerealella* and Ac = *Anisopteromalous calandrae*

At 16 WAT, the highest number of *S. zeamais* was observed at the bottom layer of unfumigated closed (UFO BOT) treatment with 135.57 insects/kg, which was significantly different ($P < 0.001$) from all the other treatments except the middle and top layers of unfumigated open (UFO MID and TOP) treatments as well as the unfumigated closed middle layer (Table 22). *Prostephanus truncatus* and *C. ferrugeneus* showed no significant differences in ($P = 0.065$ and 0.25 respectively) among all the treatments. *T. castaneum* only showed significantly higher ($P = 0.003$) insect numbers in the bottom layer of open unfumigated grain (UFO BOT) than in all the other treatments. After the same period of exposure, *S. cerealella* only showed significantly higher ($P = 0.016$) populations in the middle and bottom layers of unfumigated closed (UFC MID and BOT) than all the other treatments. *Anisopteromalous calandrae* however showed significantly higher ($P < 0.001$) populations only in the top layer of open unfumigated (UFO TOP) treatment than all the fumigated treatments. However, this treatment did not significantly differ from all

the other unfumigated treatments despite sampling depth and compartment entrance status.

Table 22. Numbers of different insect speciesÿ recorded at 16 weeks after treatment.

Treatment	*Sz*	*Pt*	*Tc*	*Sc*	*Cf*	*Ac*
Fumigated Closed Top	0.45±0.4a	0.00±0.0	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Closed Middle	5.60±1.3a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Closed Bottom	3.71±1.2a	0.00±0.0a	0.37±0.3a	0.00±0.0a	0.00±0.0a	0.00±0.0a
Fumigated Open Top	11.23±1.6ab	0.00±0.0a	0.00±0.0a	3.97±3.1a	0.00±0.0a	0.00±0.0a
Fumigated Open Middle	15.26±3.8abc	0.00±0.0a	0.00±0.0a	0.00±0.0a	0.00±0.0a	1.35±0.5ab
Fumigated Open Bottom	15.20±9.5ab	0.00±0.0a	0.63±0.5a	0.00±0.0a	0.00±0.0a	1.89±1.5ab
Unfumigated Closed Top	52.67±15.7bcd	0.00±0.0a	2.46±1.4ab	14.91±6.8b	0.00±0.0a	4.24±1.6abc
Unfumigated Closed Middle	67.84±10.2de	0.00±0.0a	7.79±2.6ab	11.41±3.8b	11.88±4.9a	10.76±1.8bc
Unfumigated Closed Bottom	135.57±13.6e	0.52±0.4a	8.56±5.3ab	0.42±0.3a	7.56±5.9a	2.44±1.9abc
Unfumigated Open Top	49.13±10.3bcd	0.46±0.4a	1.37±1.1a	4.18±1.9a	0.00±0.0a	15.57±4.5c
Unfumigated Open Middle	60.96±7.5cde	0.00±0.0a	8.62±4.1ab	3.06±1.9a	8.43±6.5a	8.74±1.0abc
Unfumigated Open Bottom	77.54±11.8de	1.13±0.5a	23.70±10.7b	0.00±0.0a	9.47±7.3a	3.16±2.5abc
$F_{11, 35}$	16.56	2.12	4.03	2.91	1.38	5.05
P-values	< 0.001	0.065	0.003	0.016	0.25	<0.001
CV (%)	24.6	15.1	51	57	78.3	41.2

Means within a column followed by the same letter are not significantly different (Tukey- Kramerôs HSD test, $P = 0.05$)
ÿ Sz = *Sitophilus zeamais*, Pt = *Prostephanus truncatus*, Tc = *Tribolium castaneum*, Sc = *Sitotroga cerealella*, Cf = *Cryptolestes ferrugeneus* and Ac = *Anisopteromalous calandrae*

At 20 WAT, only the bottom layer of unfumigated closed (UFC BOT) with a mean of 140.39 insects/kg was significantly different ($P < 0.001$) from all the fumigated treatments (Table 23). The top layer of fumigated closed (FC TOP) had the lowest number (6.58) of insects/ kg of grain which was significantly different from both unfumigated closed bottom (UFC BOT) and unfumigated open top (UFO TOP). All the other treatments were not significantly different from each other. Notably, fumigated treatments did not show any significant differences among themselves, likewise, all unfumigated treatments did not also show any significant differences among themselves.

For *P. truncatus*, *S cerealella* and *C. ferrugeneus*, there were no significant differences ($P > 0.05$) in insect numbers/kg across all the treatments. After the same period of exposure on *T. castaneum*, only two unfumigated treatments (UFO BOT and UFC BOT) had significantly higher ($P < 0.001$) numbers of insects than all the unfumigated

treatments. No insects were observed in the top layer of fumigated closed (FC TOP) treatment and very few (0.32) mean insects/kg) were observed in middle layer of the same treatment (FC MID). These two treatments had significantly lower insect numbers than all the unfumigated treatments except unfumigated closed top and middle layers (UFC TOP and MID) as well as the top layer of unfumigated open (UFO TOP). The rest of the treatments were not significantly differences from each other. *Anisopteromalous calandrae* had significantly higher ($P < 0.001$) numbers at the bottom layers of the unfumigated open (UFO BOT) grain than all the other treatments.

Table 23. Number of different insect pest species at 20 weeks after treatment.

Treatment	*Sz*	*Pt*	*Tc*	*Sc*	*Cf*	*Ac*
Fumigated Closed Top	6.58±0.4a	0.00±0.0a	0.00±0.0a	0.92±0.7a	0.00±0.0a	0.00±0.0a
Fumigated Closed Middle	9.87±2.1ab	0.00±0.0a	0.323±0.3a	0.65±0.5a	0.00±0.0a	0.00±0.0a
Fumigated Closed Bottom	14.32±4.4ab	0.00±0.0a	3.34±1.5ab	0.44±0.3a	0.00±0.0a	1.33±1.0a
Fumigated Open Top	17.60±4.1ab	0.00±0.0a	0.93±0.7ab	3.41±2.6a	0.00±0.0a	0.00±0.0a
Fumigated Open Middle	14.43±2.5ab	0.43±0.3a	1.53±0.6ab	2.57±2.0a	0.00±0.0a	0.78±0.6a
Fumigated Open Bottom	22.21±3.2ab	0.00±0.0a	1.45±1.1ab	0.45±0.4a	0.40±0.3a	0.00±0.0a
Unfumigated Closed Top	63.99±8.7abc	0.32±0.2a	1.27±0.6ab	3.07±2.4a	0.62±0.5a	0.31±0.2a
Unfumigated Closed Middle	83.23±18.9abc	0.37±0.3a	5.34±1.5abc	7.44±3.2a	0.90±0.7a	0.00±0.0a
Unfumigated Closed Bottom	140.39±37.5c	1.9±50.6a	14.36±1.0c	1.58±0.8a	2.36±1.8a	0.00±0.0a
Unfumigated Open Top	89.89±10.1bc	0.00±0.0a	5.70±1.8abc	9.63±3.6a	0.69±0.5a	1.03±0.8a
Unfumigated Open Middle	45.27±21.3abc	0.00±0.0a	10.22±3.4bc	2.81±1.1a	0.00±0.0a	2.47±1.3a
Unfumigated Open Bottom	75.78±22.1abc	4.86±2.9a	17.73±6.6c	0.00±0.0a	1.85±1.4a	9.32±1.8b
$F_{11, 35}$	5.7	2.26	8.43	1.54	0.95	6.38
P-value	< 0.001	0.05	< 0.001	0.19	0.51	< 0.001
C.V. (%)	33.9	34.9	29.6	51.5	29.2	31.9

Means followed by the same letter in a column are not significantly different (Tukey- Kramerôs HSD test, $P = 0.05$)

ÿ Sz = *Sitophilus zeamais*, Pt = *Prostephanus truncatus*, Tc = *Tribolium castaneum*, Sc = *Sitotroga cerealella*, Cf = *Cryptolestes ferrugeneus* and Ac = *Anisopteromalous calandrae*

At 24 WAT, only the top level of fumigated closed (FC TOP) with 30 insects/kg had significantly lower ($P < 0.001$) numbers of *S. zeamais* than all the unfumigated treatments (Table 24). Though all the unfumigated treatments generally had higher numbers of insects/kg of grain than fumigated ones, they were not significantly different among themselves and the rest of the treatments except the top layer of fumigated open (FO TOP). For *P. truncatus, S. cerealella* and *A. calandrae,* there were no significant differences ($P > 0.05$) in insect numbers/kg across all the treatments. On the other hand, *T. castaneum* numbers ranged from 1.29 (FC TOP) to 52.29 (UFC

BOT), with the latter having significantly higher ($P < 0.001$) insects/kg of grain than all the other treatments except the bottom layers of fumigated open and unfumigated open (FO BOT and UFO BOT) as well as the middle layer of unfumigated middle (UFO MID). All the other treatments were not significantly different from each other. *Cryptolestes ferrugeneus* seemed to have been mainly concentrated at the bottom layers with unfumigated open (UFO BOT) at 79.78 insects/kg being significantly higher ($P = 0.018$) than the top and middle layers of all the fumigated treatments and all the top layers of the unfumigated ones.

Table 24. Number of different insect speciesÿ recorded at 24 weeks after treatment.

Treatment	*Sz*	*Pt*	*Tc*	*Sc*	*Cf*	*Ac*
Fumigated Closed Top	30.00±13.9a	0.00±0.0a	1.29±0.5a	2.16±1.0a	0.00±0.0a	2.90±0.2a
Fumigated Closed Middle	39.70±13.1ab	0.00±0.0a	1.57±0.6a	15.16±11.7a	0.00±0.0a	3.60±0.3a
Fumigated Closed Bottom	38.70±7.8ab	0.00±0.0a	9.78±3.9ab	0.00±0.0a	4.62±3.6ab	4.49±1.8a
Fumigated Open Top	87.90±14.2ab	0.00±0.0a	0.00±0.0a	32.87±16.9a	0.00±0.0a	1.55±0.7a
Fumigated Open Middle	41.30±10.5ab	0.00±0.0a	5.47±3.9ab	1.92±1.1a	0.00±0.0a	0.00±0.0a
Fumigated Open Bottom	49.80±4.9ab	0.77±0.6a	14.86±8.0abc	3.08±1.3a	5.47±4.2ab	4.24±2.1a
Unfumigated Closed Top	172.40±39.6b	0.70±0.5a	6.65±3.6ab	23.03±16.8a	0.00±0.0a	3.49±2.3a
Unfumigated Closed Middle	189.70±21.6b	1.87±0.4a	13.13±4.4abc	28.34±14.6a	5.46±4.2ab	1.21±0.9a
Unfumigated Closed Bottom	201.80±15.0b	5.08±3.9a	52.29±8.7c	2.36±0.9a	38.41±27.8ab	1.05±0.5a
Unfumigated Open Top	235.80±111.3b	1.08±0.8a	2.4±0.8a	11.49±4.7a	0.00±0.0a	7.12±3.6a
Unfumigated Open Middle	179.00±72.8b	0.30±0.2a	10.8±4.6ab	8.72±1.8a	8.47±4.1ab	3.02±2.3a
Unfumigated Open Bottom	190.80±51.7b	13.97±9.3a	34.94±5.3bc	1.33±0.6a	79.78±41.2b	3.24±1.7a
$F_{11, 35}$	4.84	1.71	6.23	1.13	2.83	0.83
P-value	< 0.001	0.137	< 0.001	0.386	0.018	0.61
C.V (%)	5.2	61.1	42	79.4	94.7	54.9

Means followed by the same letter in a column are not significantly different (Tukey-Kramerôs HSD test, $P = 0.05$)

ÿ Sz = *Sitophilus zeamais*, Pt = *Prostephanus truncatus*, Tc = *Tribolium castaneum*, Sc = *Sitotroga cerealella*, Cf = *Cryptolestes ferrugeneus* and Ac = *Anisopteromalous calandrae*

7.4. Discussion

Open granaries generally suffered higher damage than closed ones, significant differences were, however, noted mainly in the unfumigated granaries. Though under 50%, the unfumigated closed and unfumigated open top surface samples recorded the highest damage. Trash was high mainly at the bottom layers of unfumigated granaries especially those with high *P. truncatus* populations. Fumigated bottom and open as well as unfumigated closed middle layers of granaries showed no significant differences in trash produced per kilogramme of grain though none of them recoded LGB except the unfumigated closed middle.

The significant differences observed in grain damage between the fumigated and unfumigated treatments showed that resident infestations play a major role in grain damage and grain weight loss. This coupled with the low insect numbers in all fumigated closed and open compartments meant that incoming insects, though they should be prevented, do not play a key role in population build up in initially pest-free grain.

The source of infestation for all the fumigated closed compartments is not clear. Possibilities are that grain was infested in transit from the fumigation site to the granaries, or the re-plastering in granaries was not thorough enough to block resident insects in cracks and crevices inside the granary compartments. It is also possible that grain was infested during the short periods when these compartments were opened for sampling. Resistance of these species to the fumigant aluminium phosphide cannot also be ruled out (Daglish *et al.*, 2010). Benhalima *et al.* (2004) reported detecting phosphine resistance by *S. oryzae* in Morocco and acknowledged receiving similar reports from many other countries due to the overuse of the fumigant.

The trend of *S. zeamais* populations showed that all the unfumigated treatments initially started off with higher infestation levels than the fumigated treatments. The populations remained fairly stable for the first 8 weeks, and began to show rapid increases from week 12, where higher numbers were observed at the middle and the bottom than the top layers of the granaries. In the unfumigated open environments,

there were consistently higher *S. zeamais* populations at the top from around week 16. This agrees with reports by Mvumi *et al.* (2002) that *S. oryzae* (closely related to *S. zeamais*) in sorghum is consistently concentrated at the top levels. In the unfumigated closed environments, higher populations were observed consistently in the middle layers from as early as 8 weeks. The bottom layers interestingly recorded high populations interchangeably between the unfumigated closed and the unfumigated open. It is also interesting to note that there were very low populations in fumigated grain whether it was kept closed or open especially in the first 16 weeks. This shows that incoming infestations take time to build up as compared to resident ones.

In open granaries, *S. zeamais* populations started to increase significantly at 16 weeks and were mainly concentrated at the top grain layers. In the closed granaries, the populations were higher at the bottom and middle layers. Campbell *et al.* (2006) explained that inside and outside grain storage structures, insects have patchy spatial and temporal distributions around the food source. This is because of their high mobility on stored grain. Another possible explanation is that when the granary is open, insects are attracted to light and concentrate at the top layers. This enables them to communicate with the outside environment by voluntary in and out movements. There was a general decrease in *S. zeamais* populations from week 12 to 20 in the unfumigated grain in both closed and open environments at all three sampling layers. The decrease was, however, more pronounced in the unfumigated open making it attributable to increases in the activities of the parasitoid *A. calandrae* and probably other natural enemies.

Prostephanus truncatus did not occur in large numbers especially in the fumigated treatments, but where it occurred, it was mainly found at the bottom layers, confirming reports by Vowotor *et al.* (2005) that the bostrichid favours bottom layers. It is postulated that bottom levels provides pressure from the grain above and *P. truncatus* manipulates this pressure to anchor its hind legs and bore into compacted maize kernels in straight lines (Vowotor *et al.*, 2005). *Prostephanus truncatus* population trends showed that it began to appear at 8 weeks in unfumigated open granaries in middle layers though at low populations. In the fumigated open granaries, it appeared much later (16 weeks) but began to multiply rapidly thereafter. The bottom layers in unfumigated open compartments recorded the highest numbers (13.96 insects/kg) for

this species. This suggested that the population developed from incoming rather than resident infestation as less than 1.0 insect/kg was recorded in fumigated closed granaries throughout the duration of the study.

Although there were fluctuations in numbers, *S. cerealella* was mainly detected in top level samples. Mvumi *et al*. (2002) also reported the same vertical distribution. These fluctuations are attributable to the mothôs short life span and migration of the adult as well as high numbers of natural enemies (spiders and lizards) in open granaries. Highest populations were obtained in closed dark environments possibly due to lack of natural enemies and chances of migration. *Sitotroga cerealella* was recorded in high populations from as early as 8 weeks in unfumigated environments. Peak populations (14.91insects/kg) were recorded in the top layers at 16 weeks but rapidly dropped in week 20 in unfumigated open environments. At 24 weeks, a record high *S. cerealella* population of 32.86 insects/kg was recorded at the top layers. This shows *S. cerealella*ôs ability to invade new territories as a primary pest, and almost always appearing as the first pest on clean undamaged grain.

Unlike grain damage that became apparent at 8 weeks, grain weight loss attained significant figures around week 12, but nevertheless, failed to attain a two-figure level by the time of last sampling. The marginal rise attained from 12 weeks at all levels in unfumigated granaries is suspected to be a result of high moisture content due to high humidity caused by heavy rains received at the time of sampling (December).

Chapter 8

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

8.1 General Discussion

The new imported unregistered diatomaceous earths (DEs) A2 at 0.02% w/w and MN51 at 0.1% w/w were observed to be equally efficacious on *P. truncatus* as the registered DE Protect-It® (0.06% w/w). However, the latter at its registered rate (0.1% w/w) has consistently proved to be highly efficacious across all the test insect pest species. This is consistent with work by Stathers *et al.* (2000) and Mvumi *et al.* (2006) where Protect-It® was the most efficacious DE against *S. zeamais, T. castaneum* and *R. dominica*. The local DE, Chemutsi, was very efficacious against *T. castaneum* and *S. zeamais* but was tolerated by *P. truncatus*. This result is likely to be more of the attribute of the pest than the DE itself. Testing five African DEs on three pests, Mvumi *et al.* (2006) reported that Chemutsi ranked second in efficacy after Protect-It® against *S. zeamais* and *T. castaneum*, and fifth against *R. dominica* which is in the same family with *P. truncatus*. Chemutsi can thus be used in pure form in areas were *S. zeamais* and *T. castaneum* are the dominant problem pests.

The new imported food grade DEs MN51, A2 and A3 were very efficacious on *P. truncatus* but the latter two were tolerated by *S. zeamais*. None of A2 and A3 managed to give 50% mortality on *S. zeamais*. MN51 was, however, almost equally efficacious as Protect-It® especially at the top three application rates (0.06%, 0.08% and 0.1% w/w)). This new DE was also very effective on the tolerant *T. castaneum.* Further studies conducted on-station showed that MN51 and Protect-It® performed equally when grain damage and grain weight loss were the basis for comparison. Low insect numbers (dead and live) recorded in the samples of these DE treatments on-station over 24 weeks showed that the physical presence of DEs on grain has repellence effects (White *et al.*, 1966) on insect pests.

Tribolium castaneum was susceptible to none of the imported and local (Chemutsi) DEs except Protect-It®. In contrast with findings by Mvumi *et al.* (2006), who found Chemutsi to be effective on *T. castaneum*, this study produced different results. This could be attributable to the application rates, though Mvumi *et al.* (2002) argued that

the high levels of impurities in local DEs reduce their efficacy and their insecticidal value, it could be increased if they could be accessed in purer form. The maximum Chemutsi rate used in this study was 0.1% w/w whereas Mvumi *et al.* (2006) used rates as high as 0.5% w/w. On the other hand, Arthur (2002) and Kostyukovsky *et al.* (2010) reported that *T. castaneum* was relatively tolerant to most DEs on the market.

Diatomaceous earthsô efficacy on post-harvest insect pests was observed to increase with time. This was evidenced by the increase in mortality from day 7, 14 to 21 across all the test insect species in this study. These results agree with earlier work done by Karnataka (2009) who recorded the highest *T. castaneum* mortality (72%) mortality on Protect-It® after 21 days.

The cocktail combinations of DEs (Protect-It® and Chemutsi) with deltamethrin and spinosad were generally very efficacious on all the test insect pests. *Prostephanus truncatus* was less susceptible to all DE combinations containing deltamethrin. However, the tolerance was a bit more pronounced on Chemutsi-deltamethrin combinations than on Protect-It®ï deltamethrin ones especially in the first seven days. Although spinosad-DE combinations were also initially tolerated by *S. zeamais*, all treatments recorded about100% mortality at 21 days after treatment. *Tribolium castaneum* tolerated spinosad-DE combinations more than *S. zeamais*. The results showed that none of the spinosad-DE combination gave 100% mortality on *T. castaneum* 21 days after treatment. This confirms recent reports by Mutambuki *et al.* (2012) that, though *P. truncatus* succumbed to all tested levels of spinosad, *T. castaneum* mortality on spinosad was only 11% after 24 weeks at a high rate of 1.4 ppm (a.i.).

Synergistic effects on insect mortality exist between DEs combined with either spinosad or deltamethrin (Korunic and Rozman, 2010) as also observed in this study. When mortalities attained by DEs on their own and the time taken to achieve them is compared to that of the combinations, it can be deduced that cocktail combinations of these materials have enhanced efficacy and attain high mortalities in a short period of time. This reduces the time available for the pests to mate and oviposit (Arthur and Throne, 2003) and thus subsequent generations are inhibited. This was also proven in this study by very low F1 progeny emergence recorded in all cocktail combinations as opposed to very high ones in the pure DEs experiment.

In all deltamethrin-DE combinations, *S. zeamais* F1 progeny emergences were not significantly different from those of Shumba Super® which also contains deltamethrin. Deltamethrin, whether combined with an organophosphate (e.g. in Shumba Super®), or with DEs, seems to fail to control internal feeders and insects developing inside grain kernels. Thus, the ability to successfully suppress F1 progeny emergence observed in spinosad-DE cocktails can therefore be solely attributed to spinosad. In this regard, according to the evidence from this experiment and literature, spinosad suppresses *S. zeamais* and *P. truncatus* progeny more than deltamethrin when combined with the same DEs. Spinosad therefore, becomes a better option than deltamethrin where availability and cost are not limiting factors. This is important as the effectiveness of any grain protectant is not only measured in adult insect mortality but also in progeny suppression. The latter is the most important criterion as it measures the ability of the protectant to prevent future infestations (Daglish, 1998; Arthur and Throne, 2003).

Conversations with farmers in Murehwa district during the course of this research revealed that farmers admix grain with pesticides using bare hands, exceed recommended application rates when insect densities are high, and also use laundry dishes for mixing grain with pesticides rather than plastic sheets and open spaces. Treated grain is eaten or sold in emergency situations even when the post-treatment safety period is still far off. Most farmers believe that the bulk of grain protectants sold in their local shops are either expired or counterfeit or simply ódo not workô. This leads to serious over-application of pesticides on grain. Some farmers have even reverted to the use of illegal fumigants, applying the fumigants on grain stored in bedrooms where the family sleeps every night, resulting in serious cases of poisoning, illness and hospitalisation. The same behaviour was observed by Mvumi *et al.* (1995). Magauzi *et al.* (2011) believed the problems highlighted above to be a result of inadequate training and poor occupational health and safety systems. The risks of pesticide toxicity are perceived as theoretical or routine rhetorics of researchers and extension staff. Even some lead farmers, who are well aware of the harmful effects of pesticides, often lack the motive to translate this awareness into their daily practices. Thus, though this research has availed safer and healthy options for grain protection, there is still need for a nationwide awareness campaigns on the potential hazards and dangers of synthetic pesticides. Policymakers need to enable registration of diatomaceous earths as grain protectants in the country. Pesticides manufacturers need to invest in the refinement

and enhancement of the local diatomaceous earth, Chemutsi, to replace synthetic organophosphate in the current commercial grain protectants.

8.2 Conclusions

The local DE Chemutsi and imported new DE MN51 are as efficacious as the imported registered DE Protect-It® against *S. zeamais* and *P. truncatus*. Deltamethrin combinations with Protect-It® and Chemutsi are efficacious on *T. castaneum.* On the other hand, spinosad combinations with both Chemutsi and Protect-It® are more effective against *S. zeamais* and *P. truncatus*. Overall, the synergy of Protect-It® and spinosad or deltamethrin is stronger than that of Chemutsi, though the difference has no practical significance.

There is enhanced efficacy and synergy between the DEs (Chemutsi and Protect-It®) and spinosad and reduced amounts of deltamethrin. Chemutsi and Protect-It® are capable of replacing the organophosphate component in commercial synthetic grain protectants for human, animal and environmental safety.

Resident (field) insect pest infestation is the major source of infestation responsible for the greatest damage and huge populations that are observed in stored grain. Although the arrival of *P. truncatus* has shifted research focus from *S. zeamais*, the latter remains a key pest in terms of abundance and contribution to annual losses through grain damage.

8.3 Recommendations

The following recommendations are made:

- Zimbabweôs local DE deposits need to be exploited. The DEs should be purified, enhanced and registered as grain protectants. This has a potential for high availability, low price and quick adoption if the current research site (i.e. Murehwa) is used as the pilot area for launching the new product.
- It is recommended that policymakers and players in the agrochemical industry in Zimbabwe consider safer alternatives to grain protection. This can be achieved by harnessing the DE technology, allowing its registration and

combining it with bio-pesticides or reduced pyrethroids in high grade technical formulations at industrial level.

- In places where the dominant pest species are *S. zeamais* and *T. castaneum*, deltamethrin-based DE cocktails should be used; and where *P. truncatus* is dominant, spinosad-based DE cocktail preparations are more effective.
- Farmers are encouraged to eliminate resident/field infestation before the grain is stored. This can be achieved by centralised fumigation points, or early harvesting and shelling and timely treatment.
- Awareness in post-harvest grain handling, proper use of grain protectants and Integrated Pest Management practices for smallholder farmers is a critical need. This is not only going to reduce losses prior to and during storage, but also foster grain handling practices that reduce chances of infestation.

9. References

Aardema, H., Meertens, J.H., Liqtenberg, J.J., Peters-Polman, O.M., Tulleken, J.E. and Zijlstra, J.G. 2008. Organophosphate pesticide poisoning: cases and developments. *The Netherlands Journal of Medicine* **66** (4): 149-53.

Adam, B.D., Phillips, T.W. and Flinn, P.W. 2006. The economics of IPM in stored grain. Why donôt more grain handlers use IPM? In : I. Lorini,I. B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J. P. dos Santos, J. D. Biagi, J. C. Celaro, L. R. D'A. Faroni, L.de O. F. Bortolini, M. R. Sartori, M. C. Elias, R. N. C. Guedes, R. G. da Fonseca, V. M. Scussel (Eds.), *Proceedings of the 9th International Working Conference on Stored Product Protection*, 15 to 18 October 2006, Campinas, São Paulo, Brazil. pp 3-12.

Addo, S., Birkinshaw, L.A. and Hodges R. 2010. Ten years after the arrival in Ghana of the Larger Grain Borer. Farmerôs responses and adoption of IPM strategy. *International Journal of Pest Management* **48** (4): 315-325.

Anonymous, 2009. Diagnostic methods for Larger Grain Borer, *Prostephanus truncatus*. Plant Biosecurity Toolbox. http://www.padil.gv.au.pbt. Accessed 28 March, 2013.

Anonymous, 2013. Global population (http://www.google.co.zw/url=/publicdata/ . Accessed 13 March, 2013.

Arthur, F.H. 2000.Impact of food sources on Red and Confused Flour Beetles (Coleoptera: Tenebrionidae) exposed to diatomaceous earths in stored product and quarantine entomology. Grain Marketing and Production Research Centre. Manhattan.

Arthur, F.H. 2002. Survival of *Sitophilus oryzae* on wheat treated with diatomaceous earths: Impact of biological and environmental parameters on product efficacy. *Journal of Stored Products Research* **38**: 305-313.

Arthur, F.H. and Throne, E.J. 2003. Efficacy of diatomaceous earths to control internal infestations of rice weevil and maize weevil (Coleoptera: Curculionidae). *Journal of Economic Entomology* **96** (2): 510-518.

Athanassiou, C.G., Kavallieratos, G.N., Chintzoglou, J.G., Peteinatos, G.G., Boukouvala, C.M., Petrou, S.S. and Panoussakis, C.E. 2008. Effect of temperature and commodity on insecticidal efficacy of Spinosad dust against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Journal of Economic Entomology* **101** (3): 976-981.

Athanassiou, C.G., Kavallieratos, N.G. and Meletsis, C.M. 2007. Insecticidal effects of three diatomaceous earths against three stored product beetle species on wheat and maize. *Journal of Stored Product Research* **43**, 330-334.

Benhalima, H., Chaudhry, M.Q., Mills, K.R. and Price, N.R. 2004. Phosphine resistance in stored product insects collected from various facilities in Morocco. *Journal of Stored Products Research* **40**: 241-249

Bond, E.J. 1974. Future needs and developments for control of stored product insects. In : Brady, E.U.; Brower, J.H.; Hunter, P.E.; Jay, E.G.; Lum, P.T.M.; Lund, H.O.; Mullen, M.A.; Davis, R. (Eds.), *Proceedings of the 1st International Working Conference on Stored-Product Entomology*, 7-11 October 1974, Savannah, Georgia, USA. pp 317 ï 322.

Boxall, R.A. 1986. A critical review of the methodology for assessing farm-level grain losses after harvest. Report of the Tropical Development and Research Institute. U.K.

Boxall, R.A. 2002. Damage and losses caused by larger grain borer *Prostephanus truncatus*. *Integrated Pest Management Reviews* **7**: 105ï 121.

Campbell, J.F, Chingôoma, G. P, Toews, M.D and Ramaswamy, S.B. 2006. Spatial distribution and movement patterns of stored product insects. In : I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J. P. dos Santos, J. D. Biagi, J. C. Celaro, L. R. D'A. Faroni, L.de O. F. Bortolini, M. R. Sartori, M. C. Elias, R. N. C. Guedes, R. G. da Fonseca, V. M. Scussel (Eds.), *Proceedings of the 9th International Working Conference on Stored Product Protection*, 15 to 18 October 2006, Campinas, São Paulo, Brazil. pp 361-370.

Campbell, J.F. and Arbogast, R.T. 2004.Stored product insects in flour mill population dynamics and response to fumigation treatments. *Entomologia Experimentalis et Applicata* **112**: 217-225.

Compton, J.F., Floyd, S.,Ofosu, A and Agbo, B.1998. The Modified Count and Weigh Method: An Improved Procedure for Assessing weight Loss in Stored Maize Cobs. *Journal of Stored Product Research* **34** (4): 277-285.

Daglish, G.J. 1998. Efficacy of six grain protectants applied alone or in combination against three species of Coleoptera. *Journal of Stored Product Entomology* **34** (4): 263-268.

Damallas, C.A. and Eleftherohorinos, I.G. 2011. Pesticide exposure, Safety Issues and Risk Assessment Indicators. *International Journal of Environmental Research and Public Health* **8**: 1402-1419.

Dowdy, A.K. and Fields P. G. (2002). Heat combined with diatomaceous earth to control the flour beetle *Tribolium confusum* (Coleoptera: Tenebrionidae) in flour mill. *Journal of Stored Product Research* **38**: 11-12.

Dunstan, W.R and Magazini, I. A. 1981.Outbreaks and new records, Tanzania. The Larger Grain Borer in Stored Products. *FAO Plant Protection Bulletin* **29**: 80-81

Farrell, G. 2000. Dispersal, phenology and predicted abundance of the Larger Grain Borer in different environments. *African Crop Science Journal* **8** (3): 337-343.

Fields, P and Korunic, Z. 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored product beetles. *Journal of Stored Products Research* **36**: 1-13

Fowler, J., Cohen, L., Jarvis, P. 1998. Practical Statistics for Field Biology. 2nd *Edition*.John Wiley and Sons, Chichester.

Geohive, 2013. Zimbabwe General information. (http://www.geohive.com/cntry/ zimbabwe.aspx, accessed 2 March 2013).

Golob, P., Farrell, G. and Orchard, J. 2002. (Eds.). *Crop Postharvest: Science and Technology. Volume 1: Principles and Practices*. Blackwell Science. Iowa.

Golob, P., Marsland. N., Nyambo, B., Mutambuki, K., Moshy, A., Kasalile, E.C., Birkinshaw, L. and Day, R. 1998. Coping strategies employed by farmers against larger grain borer in East Africa. In: Jin, Z.; Liang, Q.; Liang, Y.; Tan, X.; Guan, L. (Eds.), Proceedings of the 7th International Working Conference on Stored-Product Protection, 14-19 October 1998. *Proceedings of the 7th International Working Conference on Stored Product Protection.* Beijing, China. pp 1772 ï 1781.

Hagstrum, D.W. 1984. The population dynamics of stored product insect pests. Insect attractants, behaviour and basic biology research laboratory. Agricultural Research Service. Florida. USA.

Haines, C.P. (991. (Ed.). *Insects and Arachnids of Tropical Stored Products. Their Biology and Identification*. A training manual. Natural Resources Institute. U.K.

Hansen, L.S., Skovgard, H. and Hell, K. 2004. Life table study of *Sitotroga cerealella* (Lepidoptera: Gelechiidae), a strain from West Africa. *Journal of Economic Entomology* **97** (4): 1484-1490.

Harein, P.K .1974. Evaluation of pesticides for control of stored product insects.Department of Entomology, Fisheries and Wildlife, University of Minnesota. U.S.A.

Hassan, R.M., Mekuria, M. and Mwangi, W.M. 2001. Maize breeding research in eastern and Southern Africa. *Current status and impacts of past investments and private sector 1966-1997.* CIMMYT. Mexico

Hayes, M.M., van der Westhuizen, N.G. and Gelfand, M. 1978. Organophosphate poisoning in Rhodesia. A study of clinical features and management of 105 patients. *Southern African Medical Journal* **54** (6): 230-234.

Hertlein, M.B., Thompson, G.D., Subramanyam, B. and Athanassiou, C.G. 2011. Spinosad: A new natural product for stored grain protection. *Journal of Stored Products Research* **47**: 131-146.

Hill, M.G., Borgemeister, C. and Nansen, C. 2002. Ecological studies on the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and their importance for integrated pest management. *Journal of Biomedical and Life Sciences* **7** (2): 201-221.

Hodges, R.J. 1986. The biology and control of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrychidae). A destructive storage pest with an increasing range. *Journal of Stored Products Research* **22**: (1) 1-14

Huang, F. and Subramanyam, B. 2007. Effectiveness of Spinosad against seven major stored grain insects on corn. *Journal of Insect Science* **14**: 225-2230.

Kamanyire, R. and Karalliedde, L. 2004. Organophosphate toxicity and occupational exposure. *Journal of Occupational Medicine* **54**: 69-75.

Karnataka, J. 2009. Efficacy of diatomaceous earth formulations (Protect-It) admixed with flour for controlling *Tribolium castaneum* (Herbst). *Journal of Agricultural Sciences* **22**: (1) 224 ï 225.

Kasambala, T. and Chinwada, P. 2011. Modelling the occurrence of *Prostephanus truncatus* (Coleoptera: Bostrichidae) in Southern Malawi. *Journal of Stored Products and Postharvest Researc*h **2** (4): 72-78.

Khakame, S.K., Likhayo, P., Olubayo, F.M and Nderitu, J.H. 2012.Effect of grain moisture content and storage time on efficacy of inert and botanical dusts for control of *S. zeamais* in stored maize. *Journal of Post-harvest and Stored Products Research* **3** (10): 145-151.

Khare, B. P. 1994. *Stored grain pests and their management*. Kalyani Publishers. New Delhi.

Khoza, S., Nhachi, C.F.B., Chikumo, O., Murambiwa, W., Ndudzo, A., Bwakura, E. and Mhonda, M. 2003. Organophosphate and organochlorine poisoning in selected horticultural farms in Zimbabwe. *The Journal of Applied Science in Southern Africa* **9** (1): 7-15

Kolancski J.H. And Curtis C.F. 2004. Chronical illness as a result of low level exposure to synthetic pyrethroid insecticide: a review of the debate. *Journal of Food and Chemical Toxicology* **42**: 697-706.

Komen, J.J., Mutoko, C.M, Wanyama, S.C., Rono, N. and Mose, L.O. 2008. Economics of post-harvest maize grain losses in Trans-nzoia and Uasin-gishu districts of North-west Kenya. KARI. Nairobi.

Korunic, Z and Rozman, V. 2010. Synergistic mixture of diatomaceous earth and deltamethrin to control stored grain insects. In: M. O. Carvalho, P. G. Fields, C. S. Adler, F. H. Arthur, C. G. Athanassiou, J. F. Campbell, F. Fleurat-Lessard, P. W. Flinn, R. J. Hodges, A. A. Isikber, S. Navarro, R. T. Noyes, J. Riudavets, K. K. Sinha, G. R. Thorpe, B. H. Timlick, P. Trematerra, N. D. G. White(Eds). *Proceedings of the 10th International Conference on Stored Product Protection*, 27 June ï 02 July 2010. Estoril. Portugal. pp 894-899.

Korunic, Z. 1994. Diatomaceous earths as a natural insecticide. *Proceedings of ZUPP. Novi. Vinodolski-Zagreb, Croatia*. pp 136 -148.

Korunic, Z. 1997. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. *Journal of Stored Products Research* **33** (3): 219-229.

Korunic, Z. 1998 Diatomaceous earths: A group of insecticides. *Journal of Stored Products Research* **34** (23): 87-97.

Kostykovsky, M., Trostanestsky, A., Menasherov, M., Yasinov, G. and Hazan, T. 2010. Laboratory evaluation of diatomaceous earth against main stored product insects. In: M. O. Carvalho, P. G. Fields, C. S. Adler, F. H. Arthur, C. G. Athanassiou, J. F. Campbell, F. Fleurat-Lessard, P. W. Flinn, R. J. Hodges, A. A. Isikber, S. Navarro, R. T. Noyes, J. Riudavets, K. K. Sinha, G. R. Thorpe, B. H. Timlick, P. Trematerra, N. D. G. White (Eds). *Proceedings of the 10th International Conference on Stored Product Protection*, 27 June ï 02 July 2010. Estoril. Portugal. pp 701 ï 704.

Krall, S. 1984. A new threat to farm level storage in West Africa: Prostephanus truncatus (Horn) (Coleoptera: Bostrychidae). *Journal of Tropical Production Information* **50**: 26-31.

Kumar, H. 2002. Resistance in maize to the larger grain borer, *Prostephanus truncatus* Horn (Coleoptera: Bostrichidae). *Journal of Stored Products Research* **38**: 267-280.

Magauzi, R., Mabaera, B., Rusakaniko, S., Chimusoro, A., Ndlovu, N., Tshimanga, M., Shambira, G., Chadambuka, A. and Gombe, N. 2011. Health effects of agrochemicals among farm workers in commercial farms of Kwekwe district, Zimbabwe. *Pan-African Medical Journal* **9**: 26.

Mashavakure, N. 2012. Pest status of the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) in Zimbabwe and an assessment of inherent susceptibility of selected maize varieties to the pest. MSc thesis, University of Zimbabwe.

Mason, L.J. 2003. Red and Confused Flour Beetles. Grain Insect Pest Fact Sheet. Department of Entomology. Perdue University.

Mewis, I. and Ulrichs, C. 2001. Action of armorphous diatomaceous earths against different stages of the stored product pests: *Tribolium confusum*, *Tenebriomolitor*, *Sitophilus granarius* and *Plodia interpunctella*. *Journal of Stored Products Research* **37**: 153-164.

Mohale, S., Allottey, J. and Siame, B.A. 2010. Control of *Tribolium confusum* J. duVal by diatomaceous earth (Protect-It®) on stored groundnuts (*Arachis hypogea*) and *Aspergillus flavus* link spore dispersal. *African Journal of Food, Agriculture, Nutrition and Development* **10** (6): 2678-2694.

Motti, P. and Awakanavar, J.S. 2009. Efficacy of diatomaceous earth formulations of Protect-It® admixture with flour for controlling *Tribolium castaneum*. *Journal of Agricultural Science* **22**(1): 224-225.

Mutambuki, K., Ngatia, C.M., Mbugua, J.N. and Likhayo, P. 2012. Evaluation on the efficacy of spinosad dust against major storage insect pests. *Journal of Stored Products and Post-harvest Research* **3** (2): 19-23.

Mvumi, B.M. and Stathers, T. 2003. Challenges of grain protection in Sub-Saharan Africa.The case of diatomaceous earths. *Food Africa Internet Based Forum*: (http://foodafrica.nri.org/security/cameroon/abstracts/BrightonMvumi.pdf). Accessed 12 December 2012.

Mvumi, B.M., Giga, D. P. and Chiuswa, D.V. 1995. The maize (*Zea mays*) post production practices of small holder farmers in Zimbabwe: findings from surveys. *Journal of Applied Science in Southern Africa* **1**: 115-130.

Mvumi, B.M., Golob, P., Stathers, T.E., and Giga, D.P. 2002. Insect population dynamics and grain damage in small farm stores in Zimbabwe with particular reference to *Sitotroga cerealella* (Olivier) (Coleoptera: Gelechiidae). In: Credland, P.F.; Armitage, D.M.; Bell, C.H.; Cogan, P.M.; Highley, E. (Eds.), *Proceedings of the 8th International Working Conference on Stored Product Protection,* 22-26 July 2002, York, UK. pp 151-168.

Mvumi, B.M., Stathers, T.E., Kaparadza, V., Mukoyi, F., Masiiwa, P., Jowah, P. and Riwa, W. 2006. Comparative insecticidal efficacy of five of five raw diatomaceous earths against three tropical stored grain Coleopteran pests: *Sitophilus zeamais*, *Rhyzopertha dominica* and *Tribolium castaneum*. In: I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J. P. dos Santos, J. D. Biagi, J. C. Celaro, L. R. D'A. Faroni, L.de O. F. Bortolini, M. R. Sartori, M. C. Elias, R. N. C. Guedes, R. G. da Fonseca, V. M. Scussel (Eds.), *Proceedings*

of the 9th International Working Conference on Stored Product Protection, 15 to 18 October 2006, Campinas, São Paulo, Brazil. pp 868-876.

Mwololo, J.K., Mugo, S., Okori, P., Tadele, T. and Munyiri, S.W. 2010. Genetic diversity for resistance to larger grain borer in maize hybrids and open pollinated varieties in Kenya.Second Ruforum Biennial Conference, Entebbe, Uganda.

Nhachi, C.F. (2001). Organophosphate poisoning and management update. *The Central African Journal of Medicine* **47** (5): 134-136.

Nhachi, C.F.B. and Kasilo, O.M.J. (Eds). 1996. Pesticides in Zimbabwe. Toxicity and health implications. University of Zimbabwe Publications. Harare.

Nikpay, A. 2006.Diatomaceous earths as alternatives to chemical insecticides in stored grain. *Journal of Insect Science* **13**: 421-429.

Nukenine, E.K., Goudoungou, A.W., Adler, C. and Reichmuth, C. 2010. Efficacy of diatomaceous earths and botanical powders against the maize weevil *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) on maize. In: M. O. Carvalho, P. G. Fields, C. S. Adler, F. H. Arthur, C. G. Athanassiou, J. F. Campbell, F. Fleurat-Lessard, P. W. Flinn, R. J. Hodges, A. A. Isikber, S. Navarro, R. T. Noyes, J. Riudavets, K. K. Sinha, G. R. Thorpe, B. H. Timlick, P. Trematerra, N. D. G. White(Eds). *10th International Conference on Stored Product Protection*, 27 June ï 02 July 2010. Estoril. Portugal. pp 881 ï 887.

Nyagwaya, L.D.M., Mvumi, B.M. and Saunyama, I.G.M. 2010. Occurrence and distribution of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). *International Journal of Tropical Insect Science* **30** (4): 221-231.

Ong, S.H., Rahim, M. and Sulaiman, Z. 1994. Persistence of grain protectants in maize. In: Highley, E.; Wright, E.J.; Banks, H.J.; Champ, B.R. (Eds.), Stored Product Protection, Proceedings of the 6th International Working Conference on Stored-Product Protection, 17-23 April 1994, Canberra, Australia. pp 828-829.

Park, T. 1934. Observations on the general biology of the flour beetle *Tribolium castaneum*. *Quarterly Review of Biology*. *JSTOR* **1**(1): 36-54.

Pasipanodya, T. 2012. Agriculture and Food Monitoring System- National Early Warning Unit.Food and Agriculture Organisation (FAO) Zimbabwe. *Agriculture Co-ordination Working Group Journal* **68.**

Quarles, W. and Winn, P. 1996. Diatomaceous earths and stored product pests. *IPM Practitioner* **18**: 1-10.

Rwegasira, M.G., Jowah, P. and Mvumi, B.M. 2003.The potential invasion areas by larger grain borer in Zimbabwe. *African Crop Science Conference Proceedings* **6**:254-259.

Salgado, V.L., Sheets, J.J. Watson, G.B. and Schmidt, A.L. 1998. Studies on the mode of action of spinosad: the internal effects concentration and the concentration of dependence of neural excitation. *Journal of Pesticide Biochemistry and Physiology* **60**: 103-110.

Shires, S. W. 1980. Life history of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) at optimum conditions of temperature and humidity. *Journal of Stored Products Research* **16**: 147-150

Snelson, J.T. 1987. *Grain Protectants*. Australian Centre for International Agricultural Research. Canberra.

Stathers, T., Mvumi, B.M., Chigariro, J., Mudiwa, M. and Golob, P. 2000. Grain storage pest management using inert dusts. Final Technical Report Crop Post Harvest Program (CPHP). Natural Resources Institute (NRI). Greenwich. UK.

Subramanyam, B. 2006. Performance of spinosad as a stored grain protectant. In: I. Lorini, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J. P. dos Santos, J. D. Biagi, J. C. Celaro, L. R. D'A. Faroni, L.de O. F. Bortolini, M. R. Sartori, M. C. Elias, R. N. C. Guedes, R. G. da Fonseca, V. M. Scussel (eds.), *Proceedings of the 9th International Working Conference on Stored Product Protection*, 15 to 18 October 2006, Campinas, São Paulo, Brazil. pp 250-257.

Subramanyam, B. and Hagstrum, D.W. 1995. (Eds.). *Integrated management of insects in stored products*. Marcel and Dekker Inc. New York.

Subramanyam, B., Toews, D.M., Klein, E.I., Maier, E.D., Thompson, D.G. and Pitts, T.J. 2007. Evaluation of Spinosad as a grain protectant on three Kansas farms. *Journal of Crop Protection* **26**: 1021-1030.

Tefera, T. 2012. Postharvest losses in African maize in the face of increasing food shortage. *The Science, Sociology and Economics of Food Production and Access to Food* **4** (2): 267-277.

Tefera, T., Mugo, S. and Lakhayo, P. 2011. Effects of insect population density and storage time on grain damage and weight loss in maize due to weevil *Sitophilus zeamais* and the larger grain borer *Prostephanus truncatus*. *African Journal of Agricultural Research* **6**: 2249-2254.

Tefera, T., Mugo, S., Tende, R. and Likhayo, P. 2010. Mass rearing of stemborers, maize weevil and larger grain borer insect pests of maize. CIMMYT. Nairobi. Kenya.

Thompson, G.D., Dutton, R. and Sparks, T.C. 2000. Spinosad - a case study: an example from natural products discovery programme. *Pest Management Science* **56**: 696-702.

Trdan, S., Kac, M., Vidrih, M. and Laznik, Z. 2010. Seasonal dynamics three Lepidopteran stored grain pests in Slovenia. In: In: M. O. Carvalho, P. G. Fields, C. S. Adler, F. H. Arthur, C. G. Athanassiou, J. F. Campbell, F. Fleurat-Lessard, P. W. Flinn, R. J. Hodges, A. A. Isikber, S. Navarro, R. T. Noyes, J. Riudavets, K. K. Sinha, G. R. Thorpe, B. H. Timlick, P. Trematerra, N. D. G. White(Eds). *10th International Conference on Stored Product Protection*, 27 June ï 02 July 2010. Estoril. Portugal. pp 197-202.

Vowotor, K.A., Meikle, W.G., Ayertey, J.N. and Markham, R.H. 2005. Association between the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and the maize weevil, *Sitophilus zeamais* (Motschulsky)(Coleoptera: Curculionidae) in maize stores. *Journal of Stored Products Research* **41**: 498-512.

White G.D., Berndt,W.L., Schesser, J.H. and Wilson, J.L. 1966. Evaluation of 4 inert dusts for the protection of stored wheat in Kansas from insect attack. USDA, ARS Report No. **51** (8): 22.

Williams, T., Valle, J. and Vinuella, E. 2003. Is the naturally derived insecticide Spinosad compatible with insect natural enemies? *Journal of Bio-control Science and Technology* **13** (5): 459- 475

Zimbabwe Statistics (Zimstat). 2012. Statistics at a Glance. www.zimstat.co.zw. Accessed 6th October, 2013.

Zimbabwe Vulnerability Assessment Committee (Zimvac). 2011. Rural Livelihoods Assessment July 2011 Report. Food and Nutrition Council, Harare, Zimbabwe.