# INSECTICIDE SUSCEPTIBILITY STATUS OF Prostephanus truncatus (HORN) AND Sitophilus zeamais (MOTSCHULSKY) IN ZIMBABWE AND ASSESSMENT OF ENHANCEMENT OF GRAIN PROTECTION THROUGH INCORPORATION OF EUCALYPTUS LEAVES

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A thesis submitted in partial fulfillment of the requirements of the degree of MASTER OF SCIENCE IN TROPICAL ENTOMOLOGY



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#### **DECLARATION**

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#### **DEDICATION**

This work is dedicated to my family especially, my wife Lorita and daughter Takudzwa Valerie. Their love, encouragement and support inspired me in all my endeavours.

#### **ACKNOWLEDGEMENTS**

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#### **ABSTRACT**

Sitophilus zeamais Motschulsky and Prostephanus truncatus (Horn) are the two most destructive post-harvest insect pests of maize worldwide. Bioassays were carried out to determine the efficacy of commercially available dilute dust grain protectants in controlling S. zeamais and P. truncatus under laboratory conditions. Efficacy parameters tested included adult insect mortality, population growth rate and grain damage caused in Actellic Super Chirindamatura Dust®-treated grain. The products tested were: Hurudza® (fenitrothion 1.7% + deltamethrin 0.05%), Shumba Super Dust<sup>®</sup> (fenitrothion 1.0% + deltamethrin 0.13%), Actellic Super Chirindamatura Dust<sup>®</sup> (pirimiphos-methyl 1.6% + permethrin 0.3%), Chikwapuro<sup>®</sup> (pirimiphos-methyl 2.5% + deltamethrin 0.1%), Ngwena Yedura<sup>®</sup> (pirimiphos-methyl 2.5% + deltamethrin 0.2%) and Actellic Gold Chirindamatura Dust® (pirimiphos-methyl 1.6% + thiamethoxam 0.36%). These products were evaluated against four and five populations each of *P. truncatus* and *S. zeamais*, respectively, collected from Headlands, Murehwa, Bindura, Zvimba and Masvingo. Laboratory cultures for both P. truncatus and S. zeamais, provided courtesy of University of Zimbabwe Biological Sciences Department were also exposed to the dilute dust insecticides. None of the laboratory strains (both P. truncatus and S. zeamais) had been subjected to any regular insecticide selection pressure specifically designed for its control for more than five years on whole maize grain. Although all the grain protectants were very effective against S. zeamais and were not significantly different among themselves in their effects on adult insect mortality, some differences were noted with respect to P. truncatus. The level of efficacy was also dependent on the population of *P. truncatus* tested. Actellic Super Chirindamatura Dust<sup>®</sup> was the least effective (11.6-34.6% mortalities) against P. truncatus while Actellic Gold Chirindamatura Dust® managed to control both P. truncatus and S. zeamais, achieving 100% mortalities across all populations tested. While Hurudza® and Shumba Super Dust® were very effective against the Bindura and "laboratory" LGB; the two products seemed not to be effective against the Headlands and Murehwa LGB populations. The reproductive performance of *P. truncatus* and *S. zeamais* as well as resultant grain damage in maize treated with half recommended rate of Actellic Super Chirindamatura Dust® were assessed. Damage due to S. zeamais averaged 39-93% in untreated grain; however, the weevil could not successfully establish in grain treated with half recommended rate of Actellic Super Chirindamatura Dust®. Prostephanus truncatus successfully established in both treated and untreated grain and caused damage of 49.4-83.8% and 56.3-87.8% in treated and untreated grain, respectively over a storage period of 10 weeks. For both P.

truncatus and S. zeamais, progeny numbers were positively correlated with grain damage. The toxic and repellent effects of grain admixed with gum tree (Eucalyptus citriodora) leaf powder (10% w/w) on P. truncatus and S. zeamais were evaluated under laboratory conditions. Untreated control and the conventional insecticides were used as negative and positive controls, respectively. The efficacy of the synthetic insecticides was better when applied singly than when combined with E. citriodora leaf powder. With the exception of Actellic Super Chirindamatura Dust®, all conventional insecticides significantly reduced the number of adult insect progeny. The protective effects of gum tree leaf powder alone against P. truncatus and S. zeamais was low since it achieved mortalities below 11%. However, its high repellent properties (up to 68.9%) against P. truncatus and S. zeamais show that it can be used in combination with conventional dilute dust insecticides so as to enhance pest management through repellency.

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#### **CHAPTER 1**

#### INTRODUCTION

#### 1.1 Background to the Study

Curculionid beetles of the genus *Sitophilus* are among the most widely distributed and destructive primary insect pests infesting farm-stored maize and other cereals in the warmer parts of the world (Taylor, 1971). In rural Africa, smallholder farmers' woes as a result of losses caused by the weevils *Sitophilus zeamais* Motschulsky and *Sitophilus oryzae* (L.) on maize have been worsened by the introduction in the late 1970s of a neotropical pest, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) onto the continent (Hill *et al.*, 2002; Vowotor *et al.*, 2004).

Commonly called the Larger Grain Borer (LGB), *P. truncatus* has been known for many years as a pest of farm-stored maize in Central America and the extreme south of the USA, but its accidental introduction into Africa in the late 1970s has drawn attention on it. This attention has been mainly due to the levels of damage it has shown itself capable of outside its native range (Nang'ayo *et al.*, 2002). As well as attacking maize, LGB is a serious pest of dried cassava, both of which are staple food crops in Africa. In the absence of stored crops, the natural vegetation is an important reservoir of *P. truncatus*, where it is capable of breeding and surviving in the dead wood of some tree hosts, some of which are actually used in the construction of grain stores (Nang'ayo *et al.*, 1993; Richter *et al.*, 1997; Nang'ayo *et al.*, 2002).

The apparent absence of any natural enemies and competitors has allowed LGB to expand rapidly and become the most important pest of stored maize and cassava in sub-Saharan Africa (Giga & Canhao, 1992). This contrasts markedly with the situation in its native land where it is unimportant and controlled biologically by its indigenous natural enemies and parasitoids (Hodges, 1982).

Despite the existence of stringent LGB-specific phytosanitary measures in international trade (Tyler & Hodges, 2002), the bostrichid is now established in nearly all of sub-Saharan Africa including Zimbabwe (Addo *et al.*, 2002; Meikle *et al.*, 2002; Cugala *et al.*, 2007; Gueye *et al.*, 2008; Nyagwaya *et al.*, 2010; Kasambala & Chinwada, 2011). In Zimbabwe, the presence of LGB was officially confirmed in 2010 (The Herald, 2010) even though the government had been alerted to the presence of the beetle in some districts some four years previously.

The favourable climatic conditions and poor storage systems in Africa often favours growth and development of these stored-grain pests, resulting in considerable crop losses. For example, losses as high as 40% were reported on stored maize due to *P. truncatus* and *S. zeamais* (Meikle *et al.*, 1998). In Africa, where subsistence grain production supports the population, such grain losses may be substantial (Golob & Tyler, 1994). In addition to grain weight loss, pests of stored grain also cause secondary fungal infection, resulting in a reduction in seed vigour, quality and commercial value.

Since the introduction of *P. truncatus* into Africa, stored grain pest management approaches have also undergone many changes. Although insecticidal control is still the most widely used (e.g. Giga *et al.*, 1991; Dales & Golob, 1997; Benhalima *et al.*, 2004), other strategies whose efficacy has been studied include use of plant products (e.g. Obeng-Ofori *et al.*, 1998; Ogendo *et al.*, 2003; Nenaah & Ibrahim, 2011), inert dusts (e.g. Stathers *et al.*, 2002; Athanassiou *et al.*, 2007; Vardeman *et al.*, 2007), hermetic storage (e.g. Quezada *et al.*, 2006) and host-plant resistance (Kumar, 2002; Mwololo *et al.*, 2012).

There is often a misconception that other insecticides, especially botanicals, are safer than synthetic products (Odeyemi *et al.*, 2008). This, however, is not often the case. For instance, nicotine extracted from tobacco is one of the most effective botanicals for pest control yet it is highly toxic to mammals and can readily be absorbed through the eyes, skin and mucous membrane (Car *et al.*, 1991). Cranshaw (1992) also noted that *Derris, Lonchocarpus* and several leguminous plants are widely used as broad spectrum insecticides; they are extremely toxic to aquatic life and show some level of toxicity to mammals. Nevertheless, neem and Chinaberry plant extracts effectively control diamondback moth with no negative effects (Charleston *et al.*, 2005). Detailed information on mammalian toxicity and residual effect should be provided before botanicals are used as insecticides (Odeyemi *et al.*, 2008). Integrated pest management (IPM) is essentially a holistic approach to pest control, aimed at optimising the use of two or more methods for the management of pests (Dent, 2000; Odeyemi *et al.*, 2008).

#### 1.2 Justification

In sub-Saharan Africa, where grain is stored on-farm for household food security, storage insect pests cause substantial damage to stored grain. These insect pest infestations cause great losses given their low Economic Injury Levels (FAO, 1996). Weevils, for example, can cause losses to grain in storage, either directly, through consumption of the grain, or indirectly, by

producing 'hot spots', causing migration of moisture, and thereby making grain more suitable for other pests (Longstaff, 1986).

Historically, when synthetic chemical pesticides, mostly the organochlorine group came into widespread use in the 1940s, they promised an era of abundant agricultural yields (Rugumamu *et al.*, 2011). In some parts of sub-Saharan Africa such as Zimbabwe, the majority of farmers (≥75%) rely on imported synthetic insecticides to control these pests (Mvumi & Stathers, 2003). In Zimbabwe, the effectiveness of several organophosphorus-pyrethroid insecticides that were proactively registered against *P. truncatus* in the 1990s has become questionable as farmers from several provinces have reported major losses due to the beetle in insecticide-treated stored maize. There is therefore a need to re-evaluate the efficacy of all stored grain insecticides available on the local market as well as registering new ones, preferably with new active ingredients or modes of action. Control failures are likely to increase unless some replacement insecticide can be found.

Studies and observations made so far strongly indicate that loss of effectiveness of some of the grain protectants is a result of insecticide resistance development. This has been worsened by smallholder farmers who often underdose their grain when they are either unable or unwilling to pay for enough insecticide to give a complete treatment. This has a negative effect as the pests become resistant to the insecticides, hence farmers come to distrust insecticide use and subsequently suffer unnecessary grain losses. This negatively impacts the grain market as it reduces the supply of better quality grain and so limits the potential for exports. Large grain handling depots such as the Grain Marketing Board (GMB) and seedhouses have also not been spared as the pests have developed resistance to the fumigant phosphine.

There are mixed responses to the effectiveness of these dilute dust insecticides. Some grain protectants which work very well in one province have lost trust in another province. It would appear that the incidences of insecticide resistance are not the same throughout the country, possibly indicating that different populations of stored grain insect pests differ in their susceptibilities to the insecticides. There is therefore need to manage insecticide resistance. Failure to control stored grain insect pests may lead to national food insecurity. This also poses problems for farmers who plant saved grain (i.e. open pollinated varieties).

It is estimated that between 2010 and 2015, maize demand is projected to grow at an annual rate of 2.6% (FAO, 2009; Anankware, 2012). Obeng-Ofori (2008) suggested that this increase in demand could easily be met by reducing storage losses which could save 40% of the current

production rather than expanding the hectare in an unsustainable manner. Insect attack constitutes a major cause of losses of stored maize in the tropics. Recently, it has been reported that 9% of postharvest losses are due to insect and mite infestation worldwide; suggesting a need to make strenuous effort to control them (Vachanth *et al.*, 2010).

#### 1.3 Objectives

#### 1.3.1 General objectives

The main objective of the study was to determine if the incorporation of *Eucalyptus citriodora* in maize grain-insecticide admixtures would augment control of *P. truncatus and S. zeamais* – the major primary pests of stored maize in Zimbabwe.

#### 1.3.2 Specific objectives

- 1) To determine the relative susceptibilities of different populations of *P. truncatus* and *S. zeamais* to currently registered dilute dust insecticides.
- 2) To determine *S. zeamais* and *P. truncatus* survival and progeny production and associated damage in grain treated with suboptimal rates of Actellic Super Chirindamatura Dust®— a grain protectant which clearly has lost effectiveness against the latter.
- 3) To assess the repellent effects of *Eucalyptus citriodora* leaf powder against *P. truncatus* and *S. zeamais*.
- 4) To assess the overall enhancement of grain-insecticide admixtures against *P. truncatus* through the incorporation of *E. citriodora* leaf powder.

#### 1.4 Hypotheses

- 1) All registered dilute dust insecticides are effective against different geographic populations of *P. truncatus* or *S. zeamais* in Zimbabwe.
- 2) Survival and progeny production by *P. truncatus* and *S. zeamais* in suboptimally-treated and untreated grain is the same.
- 3) Eucalyptus citriodora has significant repellent effects on P. truncatus and S. zeamais.
- 4) The incorporation of *E. citriodora* leaves in grain-insecticide admixtures significantly lowers *P. truncatus* survivorship.

#### **CHAPTER 2**

#### LITERATURE REVIEW

#### 2.1 Economic Importance of Postharvest Pests

Insect pests are a great threat to the production of maize in Africa. The Maize weevil (*S. zeamais*), Rust-red flour beetle (*Tribolium castaneum* Herbst), Angoumois grain moth (*Sitotroga cerealella* Oliv.) and the larger grain borer, *P. truncatus* (Horn) are among the most economically-important postharvest primary insect pests of maize in Africa (Warui *et al.*, 1990). Almost all the insect pests of stored grains have a remarkably high rate of multiplication within one season, they may destroy the grain and also leave behind undesirable odours and flavours (Neupane *et al.*, 1991).

For the control of these insect pests the chemical control methods dominate, but the extensive use of these synthetic insecticides and fumigants led to some serious problems including development of insecticide resistance, toxic residues in food, toxicity to consumers and increasing cost of application (Sighamony *et al.*, 1990). The uncontrolled use of these synthetic pesticides also led to hazards for the environment and consumers due to residual property (White, 1995). That is why there is an urgent call to develop safe alternatives that are of low cost, convenient to use and environmentally friendly (Ribeiro *et al.*, 2003)).

Pest infestation to grain cause loses in grain weight during storage. Infested grain normally has less weight and it is a disadvantage to farmers and grain dealers where grain is sold in accordance to weight (Giga, 1993). The presence of insects in grain samples will cause cash discounts, as the grain will be assigned a lower value on the market. Post-harvest insect pests also induce direct damage on stored produce resulting in reduction in the quality i.e. nutritional value, seed viability and commercial losses (Hill, 1987).

#### 2.2 Biology of *Prostephanus truncatus*

The larger grain borer, *P. truncatus* (Plate 1) was first described by Horn in 1878 who named it *Dinoderus truncatus*. It is 3-4 mm long, cylindrical and dark in colour. The flattened ends of the wing and the ridges give *P. truncatus* a very square-cut end, thus distinguishing it from other bostrichids known to attack stored products. These bostrichids are *Rhyzopertha dominica* (lesser grain borer) and *Dinoderus* spp. The large pronotum protects the head during tunnelling and provides strong support for the mandibular muscles (Nansen & Meikle, 2002).

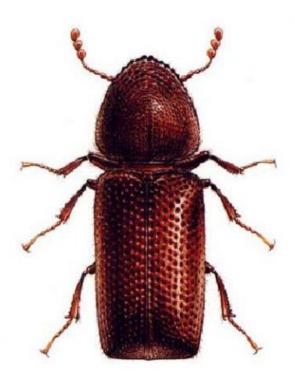


Plate 1. The Larger Grain Borer, Prostephanus truncatus

*Prostephanus truncatus* is a long-lived species with an extended oviposition period and a relatively rapid larval development stage. Its development pattern is similar to the closely related *R. dominica*, which is also from the same insect family. *Prostephanus truncatus* has a potential life span of several months, during which adults continue to feed and infest the host. A single adult *P. truncatus* can destroy the energy equivalent of five corn kernels (Demianyk & Sinha, 1988).

According to Obeng-Ofori (2008), *P. truncatus* can tolerate dry conditions and can breed on maize of 9% moisture content. Its ability to develop in grain of very low moisture content may be one reason for its success. Optimum conditions for development on maize are 32°C and 70-80% relative humidity, and under these conditions, the life cycle can be completed in 24-25 days (Hodges, 1986). Adults bore into maize grains making neat round holes and generating large quantities of dust as they tunnel from grain to grain. After mating, adult females lay most eggs within the grain in blind ended chambers bored at right angles to the main tunnel. Eggs are laid in batches of 20 and are covered with finely chewed maize dust. Oviposition begins 5-10 days after adult emergence, reaching a peak at 15-20 days (Bell & Watters, 1982).

The mean development period of *P. truncatus* under optimum conditions for eggs is 3.0 days, for larvae (3 instars) 13.2 days, prepupae 3.9 days, and pupae 2.4 days (Demianyk & Sinha,

1988). The potential level of infestation is high with life time egg production in laboratory cultures varying between 50 and over 400 (Hodges, 1986; Bell & Watters, 1982). The last larval instar constructs a pupal case from frass stuck together with a larval secretion, either within the grain or in the surrounding dust. Females tend to outlive the males, with a mean survival time of 61 days for females and 45 days for males (Shires, 1980; Bell & Watters, 1982).

#### 2.3 Biology of *Sitophilus zeamais* (Motschulsky)

The maize weevil, *S. zeamais* (Plate 2), is a primary storage pest that starts to infest the ripening maize crop in the field when the grain moisture content is still 50–55% (Adedire 2001; Ojo and Olomoyo, 2012). Infestations initiated on the standing crop may further develop in storage as the grain dries whether stored as cobs or bulk grain. The maize weevil may also infest other cereals if the moisture content is moderate or high.

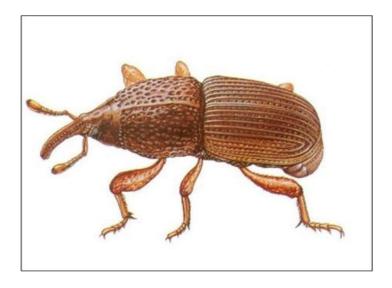


Plate 2. The maize weevil, Sitophilus zeamais

Infestation by *S. zeamais* starts with the female laying eggs into the grain. The female drills a hole into the kernel, deposits the egg, and then secretes a mucilaginous plug to enclose the egg as the ovipositor is withdrawn. The plug rapidly hardens, leaving a small raised area above the seed surface, which provides the only external evidence that the kernel is infested. Tunnels and chambers bored in the grain and are thus not normally seen (Anankware *et al.*, 2012). Eggs, which may be laid anywhere in the kernel, are laid throughout most of the adult life, although 50% may be laid in the first 4-5 weeks. Each female may lay up to 150 eggs. Sometimes, more than one egg may be laid in a single grain but it is rare for more than one larva to develop to maturity because of cannibalism (Longstaff, 1981). Not all excavated holes

are used for oviposition; some are abandoned and others are expanded into feeding holes (Campbell, 2002).

There are four larval instars all of which remain within the grain. Immediately upon hatching, the first instar feeds by burrowing through the tissues of the grain. At the end of the fourth instar the larva uses a mixture of frass and larval secretion to close off the end of the burrow, to form a pupal cell. Under normal developmental conditions, weevil larvae allow their frass to accumulate around them inside the grain in which they are feeding. However, if the carbon dioxide level exceeds 5%, the fourth instar larva makes a small hole in the grain and ejects much of the frass. The larva then assumes a prepupal form for a short period before transforming into the pupa (Longstaff, 1981).

When the adult has developed, it remains inside the grain for several days before emerging, with the time varying with temperature (Longstaff, 1981). During this time, its cuticle hardens and matures. The adults emerge by eating their way through the testa causing rugged exit holes resulting in an insect damaged grain (Arthur &Throne, 2003). Females move to a surface above the food to release a sex pheromone. Males are attracted to this pheromone for mating (Mason, 2003). Egg-adult development time averages 36 days (range 33-45 days) at  $27 \pm 1^{\circ}$ C and  $69 \pm 3\%$  RH (Sharifi & Mills, 1971).

#### 2.4 Grain Protectants

#### 2.4.1 Organophosphates and pyrethroids

Protectants, which are applied as liquids or dusts directly to the grain stream, are designed to provide long term protection. There are a range of protectant chemicals with various efficacies. However, none will control all pest species, so a mixture of two is usually applied to the grain (Lorini *et al.*, 2006). The commercially available synthetic grain protectants in Zimbabwe include Hurudza<sup>®</sup>, Shumba Super Dust<sup>®</sup>, Chikwapuro<sup>®</sup>, Ngwena Yedura<sup>®</sup>Actellic Super Chirindamatura Dust<sup>®</sup>, Actellic Gold Chirindamatura Dust<sup>®</sup>, and Phosphine fumigation tablets used in seed houses and commercial storage facilities.

The most widely used organophosphate grain protectants in Zimbabwe have the active ingredients fenitrothion ( $C_9H_{12}NO_5PS$ ) and pirimiphos-methyl ( $C_{11}H_{20}N_{30}3PS$ ). In Chikwapuro<sup>®</sup>, Ngwena Yedura<sup>®</sup>, Actellic Super Chirindamatura Dust<sup>®</sup> and Actellic Gold Chirindamatura Dust<sup>®</sup>, the organophosphate in the formulation is pirimiphos-methyl while in Hurudza Grain Dust<sup>®</sup> and Shumba Super Dust<sup>®</sup>, the organophosphate is fenitrothion. The

common synthetic pyrethroids in the dust formulations are deltamethrin ( $C_{22}H_{19}Br_2NO_3$ ) and permethrin ( $C_{21}H_{20}Cl_2O_3$ ). With the exception of Actellic Super Chirindamatura Dust<sup>®</sup> and Actellic Gold Chirindamatura Dust<sup>®</sup>, all the other four grain protectants have the synthetic pyrethroid deltamethrin in the formulation. Actellic Super Chirindamatura Dust<sup>®</sup> has permethrin while Actellic Gold Chirindamatura Dust<sup>®</sup> has thiamethoxam (a nicotinoid) as its second active ingredient.

Permethrin is a broad spectrum synthetic pyrethroid generally used as an insecticide, acaricide, a pharmaceutical and a repellent. Its mode of action is by interfering with sodium ion (Na<sup>+</sup>) channels to disrupt neuron functions in the central nervous system. This results in muscle spasms that culminate into paralysis and death. It is 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 acetycholine at the cholinergic neuro-effector junctions, a condition known as muscarinic effect which results in death. Fenitrothion is a contact organophosphate with acaricidal properties. Its mode of action is similar to that of pirimiphos-methyl. It is, however, effective against both crop and grain pests.

#### 2.4.2 Insecticide susceptibility

Insecticide resistance is an evolutionary response to insecticides and, as such, important for environmental biomonitoring and for pest management. According to WHO (2012), knowledge of pest susceptibility to pesticides to changing trends of resistance and their operational implications are basic requirements to guide pesticide use. This information provides the basis for selecting pesticides, for ascertaining continued susceptibility to and efficacy of pesticides in use.

#### 2.4.3 Use of botanicals

Chemical insecticides have been used extensively in grain storage facilities to manage stored products insect pests (Kim *et al.*, 2012). Although the dependence on insecticides like organophosphates and pyrethroids and gaseous insecticides such as methyl bromide and phosphine are effective means of controlling the coleopteran pests, negative effects owing to their repeated use for decades have fostered environmental and human health concerns (Kim *et al.*, 2012). The use of botanicals is seen to be an effective alternative and suitable for smallholder farmers for preserving stored grain from insect damage.

Plants are known to possess secondary chemical compounds which are used as a part of the plant's defense against plant-feeding insects and other herbivores (Lupina and Cripps, 1987). Some of such plant products affect nerve axons and synapses e.g. pyrethrins, nicotine and picrotoxinin; muscles e.g. ryanodine; respiration e.g. rotenone and mammein; hormonal balance; e.g. juvenile and molting hormone analogues and antagonist; reproduction and behaviour e.g. attractants, repellents and antifeedants (Bell *et al.*, 1990). Botanical pesticides represent an important potential for integrated pest management programs in developing countries as they are based on local materials (Bekele *et al.*, 1997). Plant materials with insecticidal properties provide small scale farmers with chemicals that are locally and readily available, affordable, relatively less poisonous and less detrimental to the environment for pest control (Talukder & Howse, 1995).

Traditionally, different parts of the neem tree and other plant leaves have also been used as food grains protectants at farm level (Jilani & Ahmad, 1982). The plant species that have been investigated are frequently those used locally, within individual countries, as culinary spices or in traditional medicine. Currently, only products from a few plant species have found widespread use as insecticides and in commercial production. These include rotenone from *Derris elliptica* and *Lonchocarpus* species, pyrethrum from *Chrysanthemum cinerariaefolium* and azadirachtin from neem.

Kis-Tamas (1990) proposed that prospective plants with desirable characteristics for use in pest control would probably be that the plant is perennial, easy to grow and not expensive to produce. The plant should also show no potential to become weeds or host for plant pathogens and should, if possible, offer complementary economic uses. In addition, the insecticidal product should effectively control the range of pests encountered in local storage situations, be safe to use, pose no environmental hazard, be easy to extract, formulate and use with available skills (Kis-Tamas, 1990).

In a study conducted by Bhulyah (1988) to evaluate whole dried leaves and powdered *Vitex negundo* L. (langundi) leaves, it was found that whole dried leaves checked *S. zeamais* population for 90 days, while 5% of the leaf powder reduced fecundity of adult female weevils. Javier and Morallo Rejesus (1982) reported that ground black pepper used against weevils was as effective as malathion and residually toxic for 2-4 months against *Oryzaephilus surinamensis* L., *R. dominica* and *Tribolium castaneum*. Ground products of some spices such as *Piper guineense* caused significant mortality of weevils while *Afromonum melequata* and

*P. guineense* were repellent to *S. zeamais* (Udo, 2005). There was also significant reduction in damage caused by the weevils as well as a reduction in progeny production.

Eucalyptus (Myrtaceae) is one of the most cultivated tree genera in the world with more than 700 species. Various biological properties have already been attributed to the genus Eucalyptus, among them insecticidal activity against beetles (Haouelet al., 2010), repellent action against Phlebotomuspapatasi (Yaghoobi-Ershadi et al., 2006) and larvicidal activity on culicids (Cheng et al., 2009). Besides, Eucalyptus essential oils are used for medicinal and pharmaceutical purposes (Dellacassaet al., 1990; Nicole et al., 1998; Cimanga et al., 2002). Firdissa and Abraham (1999) reported that treatment with leaves from Eucalyptus globules, Schinese molle, Datura stramonium, Phytolacca dodecandra and Lycopersicum esculentum caused high adult S. zeamais mortality.

#### 2.5 Insect Rearing

Insect rearing is critical in the raising of insect populations of more or less uniform age groups and characteristics to be used for bioassays. Rearing is done under an environment of constant temperature and humidity since most insects have optimum temperature ranges for development. Optimum rearing conditions for *S. zeamais* and *P. truncatus* have been reported as  $28\pm1^{\circ}$ C and  $65\pm5\%$  relative humidity (Yann & Ducomm, 2009; Tefera *et al.*, 2010).

The simplest rearing containers are glass or strong plastic jars. These colony jars are covered with lids with filter paper, 60 mesh brass screen and metal rings. The mesh screen prevents escape of adults while the filter paper stops larval stages from moving out as well. Each jar should be well labelled; the labels are affixed on the outside of the jar (Tefera *et al.*, 2010). The grain should be of 12-14% moisture content (adjusted by adding water or drying) and be of pest-susceptible maize variety. Only untreated grain, whose history is well known can be used; this has to be frozen for two weeks to sterilize it (Yann &Ducomm, 2009). Proper sanitation procedures should be followed to avoid colony contamination by micro-organisms or other (unwanted) insects. Records of insect sources, rearing room conditions, grain source and colonies available should be kept (Tefera *et al.*, 2010). *Prostephanus truncatus* and *S. zeamais* can be reared on whole shelled maize grain or unshelled cobs.

#### **CHAPTER 3**

#### GENERAL MATERIALS AND METHODS

#### 3.1 Location

The experiments were conducted in the laboratory at the Department of Biological Sciences, Faculty of Science, University of Zimbabwe.

#### 3.2 Source and Physical Characteristics of Maize Grains

Clean, healthy dry grains of 30G19 PHB PIONEER HI-BRED maize variety bought from a farmer in Bindura were used for both rearing of insects and the studies. The maize grains were checked visually for damage and then deep-frozen for two weeks to kill hidden infestations. The maize grains were then kept at an ambient temperature of  $27\pm2^{\circ}$ C and  $65\pm5\%$  relative humidity in the laboratory for moisture equilibration for three weeks before use in the experiments.

#### 3.3 Test Insects

The initial stock cultures of *P. truncatus* were originally obtained from naturally-infested maize sampled from farm granaries in Bindura, Murehwa, and Headlands. For *S. zeamais* starter cultures were from insects sampled from infested maize in Murehwa, Headlands, Zvimba and Masvingo.

The laboratory cultures for both *P. truncatus* and *S. zeamais* were provided courtesy of Biological Sciences Department, University of Zimbabwe. These strains have been maintained for more than five years on whole maize grain and were used as the standard/susceptible populations in the experiments. Insects from these laboratory strains had not been subjected to any insecticide selection pressure during this period of rearing.

All insect cultures were reared in a constant temperature and humidity (CTH) room. Culture jars were cleared labelled with date of culture, insect species, and area of origin of the insect species/strain.

#### 3.5 Preparation of Botanical Materials

Leaves of *Eucalyptus citriodora* were obtained from the Forestry Commission head office in Harare. Leaves were air-dried at room conditions for seven days. The dry leaves were then

milled into powder using Christy & Norris Junior laboratory mill and passed through 1x1 mm mesh.

#### 3.6 Insecticides and Grain Treatments

The insecticides used for the study were bought from a hardware shop in Harare. The application rates were as per manufacturers' recommendations (Table 1). The amounts of grain and insecticide were weighed out using an electronic balance. The insecticides were added to grain in glass jars and thorough admixture was achieved by shaking and tumbling the jar for one minute. The control had untreated grain.

Table 1. Trade names, active ingredients and recommended application rates of the commercial dilute dust insecticides that were used in the study.

Product Trade name	Active ingredients	Application rate	
		(g/50 kg of grain)	
Shumba Super Dust®	fenitrothion (1%) +	25	
	deltamethrin (0.13%)		
Ngwena Yedura®	pirimiphos-methyl (2.5%) +	20	
	deltamethrin (0.2%)		
Actellic Super	pirimiphos-methyl (1.6%) +	25	
Chirindamatura Dust®	permethrin (0.3%)		
Actellic Gold	pirimiphos-methyl (1.6%) +	25	
Chirindamatura Dust®	thiamethoxam (0.36%)		
Hurudza Grain Dust®	fenitrothion (1.7%) +	25	
	deltamethrin (0.05%)		
Chikwapuro Grain	pirimiphos-methyl (2.5%) +	20	
Protectant®	deltamethrin (0.1%)		

#### 3.7 Experimental Design and Data Analysis

Unless otherwise stated, a Completely Randomised Design (CRD) was adopted in all the experiments. All data were subjected to analysis of variance (ANOVA) in STATA 11. Where significant differences were detected (i.e. P < 0.05), treatment means were separated using least significant difference (LSD).

#### **CHAPTER 4**

# RELATIVE SUSCEPTIBILITIES OF DIFFERENT POPULATIONS OF S. ZEAMAIS AND P. TRUNCATUS TO CURRENTLY REGISTERED GRAIN PROTECTANTS

#### 4.1 Introduction

Maize is a very important staple for many growing populations in most parts of the world. Its importance has rapidly increased as it is used as food and fuel for human beings, feed for livestock, poultry and as an industrial raw material. Its demand in the region is increasing relatively with the increasing populations, urbanization, changing diets and availability of new varieties. With this in mind, it is very essential to achieve sustainable production and to preserve the produce for increasing future needs.

There are some major threats to the harvested produce from stored grain insect pests such as *S. oryzae*, *S. zeamais*, *T. castaneum*, *P. truncatus* and *R. dominica* (Anankware, 2012). Among these pests, *S. zeamais* and *P. truncatus* are the most damaging primary insect pest species of stored maize. Anankware (2012) also noted that infestation by these insect pests accounts for between 20 and 50% of post-harvest losses in maize, thus threatening food security.

The Larger Grain Borer causes extensive damage to maize in storage and it leads to serious losses to many resource-poor farmers who store grains on farm for use as food and seed without any chemical protectants. The maize weevil is also known to cause huge post-harvest losses and quality deterioration and is reported to be a major obstacle to achieving food security in developing countries (Rouanet, 1992). Initial infestations of maize grain occur in the field just before harvest and insect pests are carried to the store where the population builds up rapidly (Adedire & Lajide, 2003).

Although the use of synthetic chemical insecticides is the most important curative measure in stored product pest control (Obeng-Ofori, 2010), indiscriminate use of different conventional insecticides by farmers and marketers has led to the development of resistance and a resurgence in some insect pests (Anankware, 2012). Almost all the economically important stored product insect pests throughout the world are resistant to most of the insecticides commonly used to protect commodities against insect infestation and damage (Subramanyam & Hagstrum, 1996). For instance, *S. oryzae* is one of the post-harvest pests which developed resistance to deltamethrin (Ceruti & Lazzari, 2003). Ecological variations in the resistance

status of different insect pests to diverse insecticides have been observed by various researchers (Jermannaud, 1994; Shelton *et al.*, 2000; Pereira *et al.*, 2006). Insecticide resistance and the consequent losses of food arising from failure of chemicals to control pests have caused economic losses of several billion dollars worldwide each year (Elzen & Hardee, 2003).

Damage to grain caused by *S. zeamais* and *P. truncatus* includes reduction in nutritional value, germination, weight and commercial value (Yuya *et al.*, 2009). Observations made so far strongly indicate loss of effectiveness of some of the grain protectants as farmers from several provinces of Zimbabwe have reported major losses due to *P. truncatus* in insecticide-treated stored maize. Nyagwaya *et al.* (2010) confirmed the presence of *P. truncatus* in five districts of Mashonaland West and Mashonaland Central provinces of Zimbabwe.

The present study was undertaken to assess the efficacy of currently registered dilute dust protectants against different Zimbabwean populations of *S. zeamais* and *P. truncatus*.

#### 4.2 Materials and Methods

Two hundred grammes of maize grains were separately admixed with various insecticides in 350 ml glass jars. The treatments were as follows:

- 1. Untreated control
- 2. Hurudza® 25 g/50 kg grain
- 3. Shumba Super Dust®- 25 g/50 kg
- 4. Actellic Super Chirindamatura Dust® 25 g/50 kg
- 5. Chikwapuro<sup>®</sup> 20 g/50 kg
- 6. Ngwena Yedura® 20 g/50 kg
- 7. Actellic Gold Chirindamatura Dust® 25 g/50 kg

For each insect species and population, three replicates were used. These treatments were left for eight weeks before being infested. Thirty unsexed adult insects were introduced into each jar. Mortality counts were taken at day 7 post-infestation. An insect was considered dead if it did not respond to three probings of a blunt needle. Percentage mortality data were corrected for untreated control mortalities using Abbott's (1925) formula:

Corrected treatment mortality = 
$$\frac{(\% \text{ mortality in treatment} - \% \text{ mortality in control})}{(100 - \% \text{ mortality in control})} \times 100$$

In the case of *P. truncatus*, data were analysed by ANOVA as a factorial design comprising four geographic populations and seven grain protectants. For *S. zeamais*, data were analysed as a factorial design of five geographic populations and seven grain protectants. Prior to analysis, % data were transformed by arcsine.

#### 4.3 Results

There were no significant differences (P > 0.05) in *S. zeamais* mortality among the different populations. 100% mortality was recorded across all the five populations. In the case of P. *truncatus*, there were significant effects of population ( $F_{3,72} = 18.68$ , P < 0.05), treatments ( $F_{5,72} = 165.04$ , P < 0.05) and population × treatment interaction ( $F_{15,72} = 4.08$ , P < 0.05) (Table 2). Mortality due to Chikwapuro<sup>®</sup>, Actellic Gold Chirindamatura Dust<sup>®</sup> and Ngwena Yedura<sup>®</sup> was highest across all the geographic strains and there were no significant differences among the three products. Actellic Super Chirindamatura Dust<sup>®</sup>, Shumba Super Dust<sup>®</sup> and Hurudza Grain Dust<sup>®</sup> achieved the least mortalities against the Bindura, Headlands and Murehwa LGB populations. However, these three protectants significantly controlled the susceptible laboratory strain achieving above 70% mortality.

Table 2. Effects of different 8-week old treatments on percentage mortality (mean  $\pm$  SE) of *Prostephanus truncatus* at 7 days post-infestation

Treatment	Insect population			
	Bindura	Headlands	Laboratory	Murehwa
Shumba Super Dust®	54.3±0.04a	36.0±0.26a	70.2±0.24a	38.6±0.06a
Chikwapuro®	100±0.00b	97. 7±0.37b	98.8±0.09b	97.7±0.12b
Actellic Super Chirindamatura Dust®	34.6±0.22a	11.6±0.21a	71.4±0.32a	26.1±0.13a
Actellic Gold Chirindamatura Dust®	100±0.00b	$100 \pm 0.00b$	$100 \pm 0.00b$	100±0.00b
Ngwena Yedura®	100±0.00b	100±0.00b	$100 \pm 0.00b$	100±0.00b
Hurudza Grain Dust®	65.4±0.15a	$33.7 \pm 0.16a$	82.1±0.08ab	57.9±0.11a

Means within a column followed by the same letter are not significantly different (P = 0.05)

#### 4.4 Discussion

The results obtained in the present study demonstrated the susceptibility of *S. zeamais* and *P. truncatus* to the currently registered grain protectants in Zimbabwe. The results revealed that *S. zeamais* was more susceptible to the insecticides than *P. truncatus*. Normally, organophosphates compounds like pirimiphos-methyl are effective against *S. zeamais* but not

sufficiently effective against *P. truncatus* (Golob *et al.*, 1990; Richter *et al.*, 1997). The results are consistent with these findings.

According to laboratory studies by Golob *et al.* (1985) and Wohlgemuth *et al.* (1993), *P. truncatus* is more susceptible to pyrethroids (e.g. deltamethrin and permethrin), although they cannot be used against *S. zeamais*. Until recently, deltamethrin was known to satisfactorily suppress LGB (Golob *et al.*, 1985). Therefore, products which combine an organophosphate and a pyrethroid such as Actellic Super Chirindamatura Dust<sup>®</sup> were developed to control both pests (Richter *et al.*, 1997). In contrast to those earlier findings, the present study clearly indicated that *P. truncatus* has become tolerant to some of the 'cocktail' insecticides.

The current results correspond to what Golob and Hanks (1990) observed when they sprayed cobs with insecticides such as permethrin or permethrin in a mixture with pirimiphos-methyl. They realized that a small population of *P. truncatus* was able to develop on treated maize in many cases. However, Golob *et al.* (1985) observed that permethrin applied at 2.5 ppm was effective against *P. truncatus* over a period of eight months. In the present study, the recommended rate of Actellic Super Chirindamatura Dust® gave poor control of *P. truncatus* across all the populations.

More than three decades ago, permethrin applied alone used to provide effective control against storage insect pests (FAO and WHO, 1980). Also, at the time pyrethroid-organophosphate "cocktails" were introduced for stored-grain protection, a cocktail containing 0.3% permethrin and 1.6% pirimiphos was then able to provide effective control of postharvest insect pests (e.g. Golob *et al.*, 1991). However, synthetic pyrethroids were already known to select for resistance in insect populations very readily (e.g. Denholm *et al.*, 1983) and therefore development of resistance by *P. truncatus* is the most probable reason of observed loss of effectiveness of Actellic Super Chirindamatura Dust® in the current studies. This result probably indicates a need to increase application rates of the active ingredients in the organophosphate-pyrethroid cocktails. The future application rates of permethrin could be raised above the present 1 mg/kg grain level, though probably not above 2 mg (FAO and WHO, 1980).

Studies on loose grain in Togo revealed that the combination of pirimiphos-methyl and deltamethrin in concentrations of 7.5 and 0.25 ppm, respectively, as well as 5 and 0.5 ppm resulted in best protection of bagged grain for nine months of storage (Richter *et al.*, 1998). This is consistent with the results of the present study. Chikwapuro<sup>®</sup> which is comprised of

pirimiphos-methyl (2.5% m/m) and deltamethrin (0.1% m/m) and Ngwena Yedura<sup>®</sup> which has pirimiphos-methyl in the same concentration and 0.2% m/m deltamethrin, both managed to contain all populations of *S. zeamais* and *P. truncatus*. However, considering that the treatments were just eight weeks old at the time insects were introduced, the current results are not indicative of absence of resistance to Chikwapuro<sup>®</sup> and Ngwena Yedura<sup>®</sup> by LGB as farmers all over Zimbabwe are currently reporting poor control of the bostrichid by the two protectants as well as Shumba Super<sup>®</sup> (P. Chinwada, personal communication).

Results of the current study may also indicate that *P. truncatus* is now resistant to Actellic Super Chirindamatura Dust<sup>®</sup>. This is supported by very low LGB mortalities in Murehwa, Headlands and Bindura and a relatively high mortality against the susceptible laboratory strain. Actellic Gold Chirindamatura Dust<sup>®</sup>, which Syngenta recently registered to replace Actellic Super Chirindamatura Dust<sup>®</sup>, was quite effective as it gave 100% mortality across all the populations of *S. zeamais* and *P. truncatus*. This level of control was due to the replacement of permethrin by thiamethoxam.

#### **CHAPTER 5**

## INSECT PROGENY PRODUCTION AND GRAIN DAMAGE IN GRAIN TREATED WITH SUBOPTIMAL RATES OF ACTELLIC SUPER CHIRINDAMATURA DUST®

#### 5.1 Introduction

Post-harvest maize insect pests are a major constraint to food security and income generation in sub-Saharan Africa because of significant yield losses and grain quality deterioration (Tang et al., 2008; Tefera et al., 2011). Sitophilus zeamais and P. truncatus, the Angoumois grain moth (Sitotroga cereallela) and S. oryzae are the most economically important postharvest primary insect pests of maize in sub-Saharan Africa (Tefera et al., 2011). For crops in storage, insect infestations cause great losses given their low Economic Injury Levels (FAO, 1996). In recent years, however, the over-reliance and use of chemical insecticides in crop pest control programmes around the world has resulted in environmental damage, pest resurgence, pest resistance to insecticides, and lethal effects on non-target organisms (Tang et al., 2008).

Resistance is the ability in individuals of a species to withstand doses of toxic substances that would be lethal to the majority of individuals in a normal population (Obeng-Ofori, 2010). This means that the target pests are no longer controlled by the originally recommended application rate of an insecticide. The incidence of insecticide resistance is a growing problem in stored product protection and this has been reported in at least 500 species of insects and mites (Talukder, 2006). The development of cross- and multi-resistant strains in many important insect species is a concern all over the world (Zettler & Cuperus, 1990).

The rate of evolution of resistance depends on several factors. In general, the rate of selection for resistance increases with increase in the dose, coverage, frequency of application, and persistence of an insecticide (Obeng-Ofori, 2010). Resistance may be suspected under the following circumstances: (i) if higher doses are required to achieve a constant mortality of insects, (ii) if there is a significant decrease in insect susceptibility to a fixed amount of the insecticide, (iii) if it takes longer to obtain a fixed mortality of insects, and (iv) if the mortality of field populations of a species frequently exposed to insecticides is significantly less than mortality of the same species that has little or no insecticide exposure.

The most effective way to delay the development of resistance is to use an integrated pest management (IPM) approach which emphasizes on the use of non-chemical methods and

selective insecticide treatments. Obeng-Ofori (2010) also recommended that resistance management strategy for stored-product insects should rely heavily on non-chemical methods because of the limited number of safe insecticides available to practitioners. Monitoring of resistance is important for making resistance management decisions and diagnostic tests that distinguish between resistant and susceptible individuals must be used instead of doseresponse tests. The aim of this study was to investigate population growth rates of *P. truncatus* and *S. zeamais* in Actellic Super Chirindamatura Dust®-treated shelled grain and estimate maize grain damage thereof during single infestations.

#### **5.2** Materials and Methods

### 5.2.1 Rearing and selection of Actellic Super Chirindamatura Dust®-resistant insect pests

Initial stocks of *P. truncatus* and *S. zeamais* were obtained from cultures gathered at the onset of this project. About 72 samples of fresh 200 g treated/untreated maize grains were weighed out and placed into Kilner jars. The treatments were as follows:

- i. single infestations of Actellic Super Chirindamatura Dust®-treated grain by parental adults of *P. truncatus* and *S. zeamais*; and
- ii. single infestations of untreated maize grains (controls) by parental adults of *P. truncatus* and *S. zeamais*.

Fifty individuals of *S. zeamais* or *P. truncatus* adults were separately introduced into jars containing either of the above two treatments. The insects were picked individually for rearing of pure insect cultures to avoid contamination. The jars were arranged in a completely randomized design with four replications per treatment. After two weeks, the parents were removed from the test samples which were then left undisturbed for further 10 weeks. The total number of adult progeny was recorded at the end of this period. Grain damage was also assessed on treated and untreated grains at 10 weeks post-infestation. Insects were sieved from grains and the following was recorded: total number of grains, number of damaged grains (i.e. grains with characteristic holes) and number of undamaged grains. Percentage grain damage was then calculated as follows:

$$\% Damage = \frac{Number of Damaged Grains}{Total Number of Grains} \times 100$$

#### 5.2.2 Data analysis

Product moment correlation coefficient was checked for adult progeny numbers and grain damage using the Pearson method. Data on the number of adult progeny each species after 10 weeks and the damage caused during single infestations of shelled maize were analysed by ANOVA.

#### 5.3 Results

There were significant differences ( $F_{1,23} = 26.51$ , P < 0.05) in the damage caused by different populations of LGB. Adult progeny numbers and grain damage (Figs. 2 and 3) from Bindura P. truncatus significantly differed from the Headlands, Murehwa and Laboratory populations. This population had the lowest adult LGB progeny numbers, and hence the lowest grain damage. Although no significant differences ( $F_{1,23} = 1.80$ , P > 0.05) were detected when progeny numbers in treated and untreated grain were considered within the same population, significant differences were noted between populations.

Pearson product moment correlation coefficient showed a high positive correlation (r = 0.911508) between LGB adult progeny numbers and grain damage caused by the bostrichid across all the populations. A scatter plot (Figure 1) drawn confirmed this high positive relationship, i.e. progeny number was directly proportional to grain damage caused.

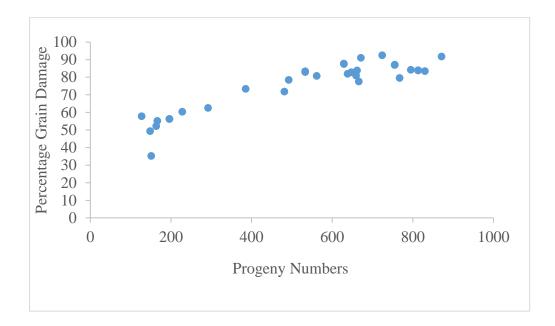


Figure 1. Scatter plot of percentage grain damage against number of *Prostephanus truncatus* adult progeny

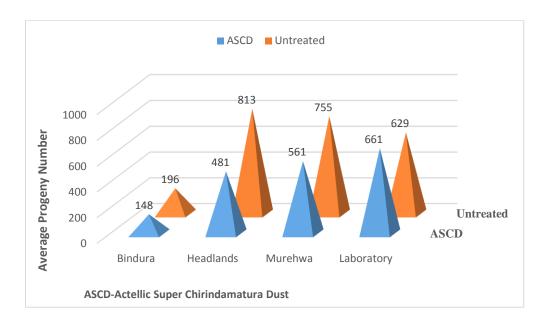


Figure 2. Average progeny number produced by 50 *Prostephanus truncatus* adults from four geographical areas.

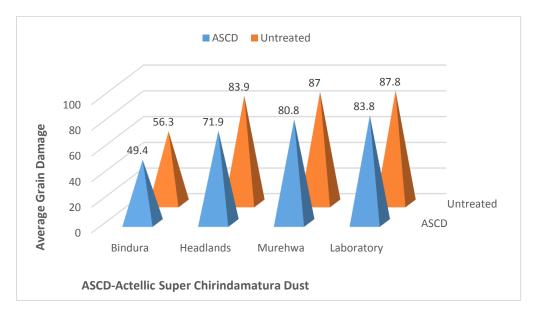


Figure 3. Average grain damage caused by *Prostephanus truncatus* adults (progeny) on untreated and Actellic Super Chirindamatura Dust<sup>®</sup>-treated grain.

Sitophilus zeamais adults could not survive in grains treated with suboptimal rates of Actellic Super Chirindamatura Dust<sup>®</sup>. However, in the case of untreated grain, there were significant differences ( $F_{4,19} = 19.66$ , P < 0.05) in the number of adult progeny produced and the associated grain damage among the different geographic populations (Fig. 4 and 5). The Zvimba strain produced the highest number of adult progeny, ten-fold the original 50 individuals. Like LGB, there was a significant positive correlation (r = 0.961805) between the

adult progeny numbers and grain damage due to *S. zeamais* infestation. The headlands *S. zeamais* had the least number of progeny produced and hence the lowest grain damage.

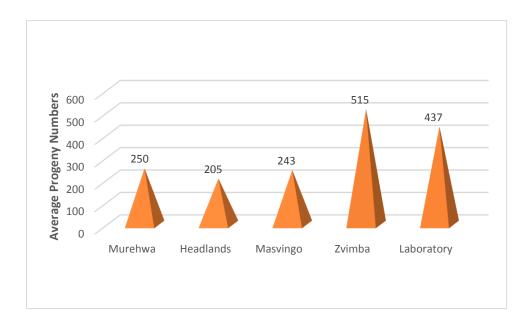


Figure 4. Average number of progeny produced by *Sitophilus zeamais* in untreated maize grain.

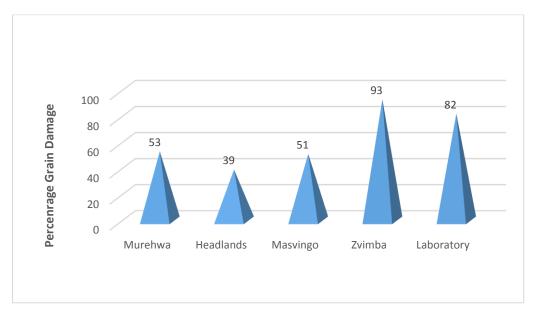


Figure 5. Average grain damage caused by *Sitophilus zeamais* in untreated maize grain.

#### 5.4 Discussion

This study demonstrated the comparative maize grain damage and final insect population for *P. truncatus* and *S. zeamais* over a storage duration of 10 weeks. Tefera *et al.* (2011) reported that the final insect densities, grain damage, dust produced and weight loss for *P. truncatus* 

exceeded that of *S. zeamais*. The population build up was fast for the two insects, particularly for *P. truncatus*, therefore, significant damage and losses can result.

The current study showed that a small initial population of *P. truncatus* in stores at the beginning of the season suffices to give high progeny numbers and grain damage levels at the end of the storage period. Although Adda *et al.* (2002) rightly pointed out that estimated pest densities based on laboratory experiments were always higher than those obtained from onfarm assessments, it should be borne in mind that on-farm assessments lack accuracy than laboratory assessments. This is likely to be a result of the difficulties of getting a truly representative sample when conducting on-farm assessments.

In the present study, mean grain damage of between 49.3-87.8 and 39-93% were recorded after 10 weeks for *S. zeamais* and *P. truncatus*, respectively. Damage caused by the two insect pests reduces the quality of the commodity, making it totally unfit for consumption as reported by Golob and Hodges (1982) and Hodges *et al.* (1983). Reports from Tanzania showed that farmers growing improved maize varieties susceptible to pest attack suffered storage losses averaging 17.9% after six months and 41.2% after eight months (Keil, 1988). Surveys in Togo, West Africa, showed a mean rise in maize storage losses from 7 to over 30% during 6-9 months of storage (Pantenius, 1988). The grain damage reported in the present study for *P. truncatus* is much higher than earlier reported. This could be attributed to the susceptibility of the hybrid used and conducive rearing conditions (27±2°C and 65±5% R.H.) of the CTH room. The rapid population growth also contributed to the extensive grain damage incurred.

Considering the rate at which LGB and the maize weevil multiply, it is apparent that the magnitude of maize losses in storage at the smallholder level in Zimbabwe is now quite substantial losses since the introduction of *P. truncatus* into the country. Individual farmers were reported to have lost up to 50% of the value of their maize to *P. truncatus* attack in the Volta Region of Ghana (Magrath *et al.*, 1996). Losses in the market value of maize infested by only *Sitophilus* spp. were 5-10% while value losses ranged 15-45% for maize damaged by *P. truncatus*. This was equivalent to monetary loss of about 5% of average total household income (Magrath *et al.*, 1996, 1997).

In conclusion, the fast population growth rate and the associated high grain damage by *P. truncatus* and *S. zeamais* indicate that sound control measures should be considered at the onset of grain storage. In fact, considering that *P. truncatus* was able to multiply normally in

grain treated with Actellic Super Chirindamatura Dust®, even though suboptimal rates were used, may indicate that the bostrichid has now developed resistance to the product.

### **CHAPTER 6**

# ASSESSMENT OF ENHANCEMENT OF GRAIN PROTECTION THROUGH INCORPORATION OF *EUCALYPTUS CITRIODORA* LEAVES IN GRAIN/INSECTICIDE ADMIXTURES

### 6.1 Introduction

Maize is one of the most important cereal crops in Zimbabwe and is subjected to both quantitative and qualitative losses due to infestation by a number of insect species in the field as well as in storage. Maize is widely grown by smallholder farmers who contribute about 50% to the national output (Rukuni *et al.*, 2006). Losses due to insect infestation are the most serious problem in grain storage, particularly in developing countries where poor sanitation and use of inappropriate storage facilities permit insect attack. Prevention of food loss with the concept of "a grain saved is a grain produced" should be seriously taken into consideration by all concerned (Chomchalow, 2003) as this is necessary to ensure a continuous supply at stable prices (Talukder, 2005).

The control of stored-grain insect pests is primarily achieved through the use of synthetic insecticides and fumigants (Adane *et al.*, 1996; Faruki *et al.*, 2005; Moharramipour, 2007). However, the widespread and constant (mis)use of these synthetic insecticides have promoted faster evolution of resistant forms of pests, destroyed natural enemies, turned formerly innocuous species into pests, harmed other non-target species and contaminated food (Zettler & Cuperus, 1990; Subramanyam & Hagstrum, 1996; Ribeiro *et al.*, 2003; Afful *et al.*, 2012). There is therefore need to synergize control strategies available in an effort to reduce chemical applications in the context of developing an Integrated Pest Management (IPM) strategy.

Many plant volatile essential oils and their constituents have been shown to possess potential as alternatives for the management of stored product insect pests and these are ecologically safe and biodegradable (Cosmi *et al.*, 2009). The active compounds present in these botanicals are specific to particular insect groups and not to mammals and many of them are not dangerous to humans (Isman, 2000). It is well known that secondary plant metabolites may act as kairomones, allomones, stimulants or deterrents of insect feeding and oviposition, and as antifeedants, insecticides and insect hormone mimics (Nawrot *et al.*, 1986). For the past three decades, many plant allelochemicals including nicotine, pyrethrins, azadirachtin and rotenoids have been isolated, characterised and developed as commercial insecticides (Talukder, 2006).

Mishra et al. (2012) reported that essential oils from Eucalyptus globulus and Ocimum basilicum have repellent properties against T. castaneum and S. oryzae.

In Zimbabwe, a number of plant-based protectants are readily available for use as grain protectants to reduce weevil damage. These include leaves of Colophospermum mopane (Murdock & Kitch, 1997) and Tagetes minuta (Parwada et al., 2012). These provide sustainable and degradable pesticides to resource-poor farmers. Stoll (2000) reported the use of wood ash from fires as grain protectant in other parts of Africa. Eucalyptus, which is one of the most cultivated genera in the world, has various biological properties attributed to it, among them insecticidal activity against beetles (Brito et al., 2006), repellent action against P. papatasi (Yaghoobi-Ershadi et al., 2006) and larvicidal activity on culicids (Cheng et al., 2009). The manipulation of natural product chemicals, such as insect attractants, repellents, stimulants, antifeedants and arrestants which are normally encountered by insects may fulfil the required criteria (Talukder, 2006). Haouel et al. (2010) investigated the chemical composition and assessed the fumigant toxicity of essential oils of Eucalyptus camaldulensis and E. rudis against the date moth Ectomyelois ceratoniae in storage as alternatives to methyl bromide. Results suggested that E. rudis and E. camaldulensis essential oils could be used as an alternative to the synthetic fumigant in postharvest treatment programme for the control of E. ceratoniae.

The primary objective of this study was to assess the enhancement of the protective effects of dilute dust insecticides against LGB and the maize weevil through the incorporation of *Eucalyptus citriodora* leaves in grain-insecticide admixtures. This would be particularly important in an IPM approach, particularly at the smallholder level where under-dosing is common. Taking into account the propensity of insecticide resistance development by stored-grain insect pests, the incorporation of plant by-products in grain-insecticide admixtures may also be an effective resistance management strategy. *Eucalyptus citriodora* was chosen owing to the strong smell from its leaves in its natural state. Although not stated on the label, the grain protectant Hurudza® has gum tree leaf extract incorporated in the formulation so it may owe its effectiveness to a combination of insecticidal activity and repellency.

### **6.2** Materials and Methods

### **6.2.1** Repellency studies

Leaves of *E. citriodora* were obtained from Forestry Commission of Zimbabwe. These were air-dried at room conditions for a week and then stored in khaki bags. The dry leaves were

then milled into powder using a Christy & Norris Junior laboratory mill and passed through a sieve of 1x1 mm pore size.

A choice test arena consisting of two 250 ml plastic jars tightly fitted at their rims to the base of a 21 cm  $\times$  32 cm rectangular flat-bottomed basin (Plate 3) was set up to evaluate repellent effects of powdered *E. citriodora* on *P. truncatus* and *S. zeamais*. The mouth of each jar was therefore flush with the flat base of the basin. These plastic jars were placed equidistant from the centre of the basin.



Plate 3. Setup of choice test of repellency of *Eucalyptus citriodora*.

One hundred grammes of maize grain were weighed out and admixed with powdered E. citriodora leaves (10% w/w), or a combination of insecticide or powdered E. citriodora leaves. Some grain was also left untreated so as to act as the control. Various paired combinations of treated/untreated grain were then placed in the jars in the arena. The treatment combinations were as follows:

- 1. Untreated grain (control) *versus E. citriodora* leaf powder
- 2. Untreated (control) *versus* Hurudza<sup>®</sup>
- 3. Hurudza (control) *versus* Hurudza® + *E. citriodora* leaf powder
- 4. Actellic Gold Chirindamatura Dust<sup>®</sup> (control) *versus* Actellic Gold Chirindamatura Dust<sup>®</sup> + *E. citriodora* leaf powder

The major reason for having an "Untreated control vs Hurudza®" combination in the treatment list is that Hurudza® has *Eucalyptus* oil extract incorporated in the formulation. Fifty *P. truncatus* or *S. zeamais* adults previously deprived of food for 48 hours were separately released at the centre of the basin and left to wander to grain of their choice. A

nylon mesh was placed over the top part of the basin to prevent the insects from escaping, but allowing free movement of air at the same time. The number of insects that settled in the 'control'  $(N_c)$  and treated  $(N_t)$  jars were recorded after 24 hours of exposure. Also recorded was the number of undecided insects (i.e. those found neither on treated nor untreated grain). Each repellency arena was replicated four times.

Percent repellency (PR) values were computed using the formula below:

$$PR = \frac{N_c - N_t}{N_c + N_t} \times 100$$

where:

PR = Percent Repellency

 $N_c$  = insect numbers present in control jar

 $N_t$  = insect numbers present in treated jar

Index of repellency (IR) was also calculated by following formula:

$$IR = \frac{2N_t}{N_c + N_t}$$

The repellency index was classified as: values < 1, repellency; 1, neutral; and > 1, attractant (Padin *et al.*, 2013). Percent repellency data were analyzed by ANOVA after arcsine transformation. Negative PR values were treated as zeros.

### 6.2.2 Studies to determine if incorporating *E. citriodora* leaves enhances the effectiveness of grain protectants

Two hundred grammes of grain were weighed out and admixed with each of six commercially available dilute dust insecticides (applied at the manufacturers' recommended rates). Air-dried *E. citriodora* leaves were then chopped up and mixed with some of the insecticide-treated grain. The gum leaves were applied at a rate of 10% w/w. However, some insecticide treatments were applied with no addition of plant leaves. The full list of treatments was as follows:

- E. citriodora leaves alone
- Actellic Super Chirindamatura Dust
- Actellic Gold Chirindamatura Dust
- Hurudza

- Chikwapuro
- Ngwena Yedura
- Shumba Super
- Actellic Super Chirindamatura Dust + *E. citriodora* leaves
- Actellic Gold Chirindamatura Dust + *E. citriodora* leaves
- Hurudza + E. citriodora leaves
- Chikwapuro + *E. citriodora* leaves
- Ngwena Yedura + *E. citriodora* leaves
- Shumba Super + *E. citriodora* leaves

Once all jars had been set up with the various treatments, 30 unsexed *P. truncates* adults were introduced into each. These insects were obtained from a colony that had been deliberately cultured for 12 weeks on grain treated with a suboptimal dosage of Actellic Super Chirindamatura Dust® (12.5 g/50 kg instead of 25 g/50 kg). The use of insect survivors from an Actellic Super Chirindamatura Dust® treatment (now considered to be resistant) was to see if such insects would be killed by subsequent re-exposure to the same or different chemical treatment alone or applied in combination with *E. citriodora* leaves. This practice simulates the re-treatments which farmers conduct when they find their grain infested with *P. truncatus* despite having initially treated it at the time of storage. Due to a repellent effect, the inclusion of *Eucalyptus* leaves in the admixture would probably enhance effectiveness of such retreatment through making the insects restless and therefore pick up more insecticide deposits during their wandering.

The design of the experiment was a completely randomised factorial of 13 treatments and four factors (LGB populations). For each treatment and LGB population, there were four replications. Replicated control treatments consisting of untreated grain infested with insects from each population were also set up so as to be able to correct for mortalities in the 13 treatments. At seven days post-infestation, the contents of each jar were sieved and the number of dead insects were counted and recorded. Insects were considered dead if they did not respond to gentle probing by a blunt needle. Percentage mortality data (corrected for untreated control mortality) was then determined. Arcsine-transformed percentage mortality data was then subjected to ANOVA in STATA 11.

### 6.3 Results

### **6.3.1** Repellency studies

There were no significant differences in repellency of P. truncatus adults ( $F_{3,15} = 1.24$ ; P >0.05) among the different treatment combinations; however, significant differences were observed on S. zeamais ( $F_{3.15} = 4.05$ ; P < 0.05). Significant differences were also noted between treatments of each combination, e.g. insects preferred to infest untreated controls than treatments which contained Eucalyptus leaf powder. When the LGB adults were put in the test arena, they remained motionless for quite some hours before a few started moving around. Unlike LGB, maize weevils moved their antennae to and fro as soon as they were released into the test arena. They gradually approached the materials by the sides of the basin and moved from one end to the other until they stopped on grain of their choice. By the end of the 24 hours most of them had already made their choice and settled down. A few of weevils were undecided by the end of the exposure period as compared to the bostrichids. All the treatments containing Eucalyptus leaf powder had a repellent effect on both P. truncatus adults and S. zeamais in the choice arena (Tables 3 and 4). After 24 hours, E. citriodora leaf powder produced the highest repellency against P. truncatus and S. zeamais (IR = 0.3 and 0.6, respectively). Incorporating E. citriodora in Actellic Gold Chirindamatura Dust® increased the repellent effect of the product as evidenced by an IR of 0.3 against S. zeamais and 0.6 against P. truncatus. Hurudza<sup>®</sup> was repellent to both species (IR = 0.8 for both species). However, the incorporation of E. citriodora leaf powder slightly lowered its repellency.

Table 3. Effect of *Eucalyptus citriodora* leaf powder and its combination with Hurudza and Actellic Gold Chirindamatura Dust on repellency of *Prostephanus truncatus* adults.

Treatment	Percentage	Index of
	Repellency	Repellency
Untreated vs E. citriodora leaf powder	33.1±11.	0.6
Untreated vs Hurudza	23.6±9.4	0.8
Hurudza vs Hurudza + E. citriodora leaf powder	52.0±16.0	0.6
AGCD§ vs AGCD§ + E. citriodora leaf powder	$38.8 \pm 16.1$	0.6

<sup>§</sup> Actellic Gold Chirindamatura Dust ‡ IR: <1 repellency; 1 neutral; >1 attractant.

Table 4. Effect of *Eucalyptus citriodora* leaf powder and its combination with Hurudza and Actellic Gold Chirindamatura Dust on repellency of *Sitophilus zeamais* adults.

Treatment	Percentage	Index of	
	Repellency	Repellency	
Untreated vs E. citriodora leaf powder	68.9±3.8a	0.3	
Untreated vs Hurudza	31.0±15.7bc	0.8	
Hurudza vs Hurudza + E. citriodora leaf powder	39.4±4.2abc	0.6	
AGCD§ vs AGCD§ + E. citriodora leaf powder	67.4±3.4abcd	0.3	

<sup>§</sup> Actellic Gold Chirindamatura Dust

## 6.3.2 Studies to determine if incorporation of eucalyptus leaves enhances effectiveness of grain protectants

There were significant population ( $F_{3, 156} = 4.14$ , P < 0.05), treatment ( $F_{12, 156} = 52.10$ , P < 0.05) and population × treatment interaction ( $F_{36, 156} = 2.08$ , P < 0.05) effects. From Table 4 it can be deduced that E. citriodora produced the least mortalities across all the geographic populations. Actellic Gold Chirindamatura Dust® achieved the highest mortalities ranging from 92.8-100%. Further analysis revealed that E. citriodora leaf powder had significant variances with all other treatments except with Actellic Super Chirindamatura Dust®, Actellic Super Chirindamatura Dust® + E. citriodora, Ngwena Yedura® + E. citriodora and Shumba Super® + E. citriodora where no significant variation was shown. This trend was also portrayed across all the geographic strains. It was also noted that most of the synthetic insecticides' efficacies against the bostrichids decreased when used in combination with the E. citriodora leaves.

<sup>†\*</sup>Values followed by the same letter in a column do not differ significantly (P < 0.05).

<sup>‡</sup> IR: <1 repellency; 1 neutral; >1 attractant.

Table 5 Effects of *Eucalyptus citriodora*, six commercially registered insecticides and their combinations on % mortality of *Prostephanus truncatus* from different geographic areas

Treatment‡	Bindura	Headlands	Laboratory	Murehwa
E. citriodora leaf powder	10.7±0.12a	5.9±0.16a	0.0±0.00a	4.0±0.13a
ASCD	32.1±0.11a	17.8±0.27a	1.2±0.09a	19.3±0.06a
AGCD	100±0.0b	$100.0 \pm 0.0 b$	95.2±0.14b	92.8±0.16b
Hurudza	70.2±0.42bc	75.0±0.34bcg	90.5±0.19bc	71.1±0.26bc
Chikwapuro	64.3±0.16bc	83.3±0.30bcd	94.0±0.16bcd	86.7±0.27bdc
Ngwena Yedura	70.2±0.18bdc	86.9±0.33be	91.7±0.18bcde	89.2±0.22bcde
Shumba Super	$30.9 \pm 0.26 abd$	53.6±0.30b	72.6±0.34bcdef	73.5±0.34bcdef
$ASCD + E.\ citriodora\ leaves$	16.6±0.27abd	$10.7 \pm 0.34a$	11.9±0.23a	25.3±0.15a
AGCD + E. citriodora leaves	58.3±0.17abde	$82.1 \pm 0.20 bf$	67.8±0.09bcdefg	78.3±0.29bcdefg
Hurudza+ E. citriodora leaves	20.2±0.36abde	21.4±0.21a	55.9±0.09bfh	22.9±0.02abfh
Chikwapuro + <i>E. citriodora</i> leaves	19.0±0.44abd	17.8±0.35ab	46.4±0.13cfhi	30.1±0.09cfhi
Ngwena Yedura + E. citriodora leaves	25.0±0.22abcde	9.5±0.26ab	14.3±0.07agd	18.1±0.10agd
Shumba Super $+ E$ . $citriodora$ leaves	15.4±0.17abde	9.5±0.10ab	8.3±0.18age	42.2±0.16age

Means within a column followed by the same letter are not significantly different (P = 0.05)

<sup>‡</sup> ASCD – Actellic Super Chirindamatura Dust; AGCD – Actellic Gold Chirindamatura Dust

### 6.4 Discussion

Inyang and Emosairue (2005) observed that different insects respond differently to active materials in their environments. However, in some instances, different insect species respond similarly to a common stimulus. Repellents are desirable chemicals which offer grain protection by driving away insect pests from the treated materials through stimulating olfactory or other receptors (Talukder, 2006). When organisms are repelled from feeding materials, they are less likely to feed on them (Inyang & Emosairue, 2005). The results of this study revealed the effects of combining *E. citriodora* with conventional insecticides as an alternative way of managing stored product insect pests. This is consistent with literature by Idoko and Adebayo (2011).

Eucalyptus citriodora is strikingly pungent even without being squashed, and it is possible that because of this attribute, maize grain treated with the leaf powder significantly repelled *P. truncatus* and *S. zeamais* under laboratory conditions. Results suggest that if the *S. zeamais/P. truncatus* adults had choice, they would avoid materials treated with the *E. citriodora* leaf powder. Generally, percentage repellency values were higher on insecticides combined with *E. citriodora* leaf powder or *E. citriodora* alone. Brito *et al.* (2006) reported that *Eucalyptus* spp. contains insecticidal properties. Yaghoobi-Ershadi *et al.* (2006) reported repellent properties of *Eucalyptus* against *Phlebotomus papatasi*.

According to Tuncer and Aliniazee (1998), starved insects would naturally feed on the only food source available. This was not the case with *P. truncatus*; most of them remained stationary at the centre of the arena instead of moving toward a food source. However, this was not the case with *S. zeamais* which became very mobile the very minute the insects were released.

It was also noted Hurudza<sup>®</sup> scored an index of repellency of 0.8 against both LGB and *S. zeamais* and this was clearly due to the *Eucalyptus* oil in the formulation. According to Eziah *et al.* (2013), the overall repellent action of a leaf powder against insects is an indication of the presence of chemicals in the plants that stimulate or cause the insects to make oriented movement away from the source stimulus. The significant repellent activity against *S. zeamais* and *P. truncatus* observed in this exercise therefore suggests that there exist good potential for the use of *E. citriodora* as a traditional grain protectant against storage insect pests. Indeed, fresh gum tree leaves are used in some traditional grain stores in some parts of Zimbabwe (P. Chinwada, personal communication). Since the treatments with repellent properties reduce

grain damage caused by the insects in the stored grains (Udo, 2011; Eziah *et al.*, 2013), it is expected that the use of botanicals will boost food security in areas where investment in synthetic pest control is uneconomic.

In the study to determine if the incorporation of *E. citriodora* leaf powder can enhance the effectiveness of grain protectants, it emerged that the efficacy of the botanical against LGB was comparable to some of the synthetic insecticides i.e. Actellic Super Chirindamatura Dust<sup>®</sup>, and to Ngwena Yedura<sup>®</sup> and Shumba Super<sup>®</sup> in combination with *Eucalyptus*. Actellic Gold Chirindamatura Dust<sup>®</sup> achieved 100% mortality against LGB across all the geographic populations. However, its efficacy against LGB significantly decreased when the same concentration of the treatment was used in combination with *E. citriodora* leaf powder. This scenario was common across most of conventional insecticides when administered in combination with *E. citriodora* leaf powder. The apparent reduced mortality was to be expected as the botanical repelled the insects away from the treated grain.

When a preliminary experiment was conducted using intact *E. citriodora* leaves, most of the LGB adults were found hiding i.e. they were not free to move, but forced to shelter at the bottom of the jars, away from the layer of *E. citriodora* leaves at the top and vice-versa if the leaves were at the bottom. These insect pests were usually found motionless though not dead, hence reducing the chances of picking up the contact insecticides when moving around. This is mainly because the botanical under study has significant repellent effects.

From this study, it can be seen that if farmers are to re-treat grain due to detected presence of live insects in the commodity, there may be need to change the chemical product in order to prevent or delay insecticide resistance development in the target pests. Re-treating the grain with Actellic Super Chirindamatura Dust<sup>®</sup> proved ineffective as evidenced by very low mortalities it caused. On the other hand, Actellic Gold Chirindamatura Dust<sup>®</sup> achieved 100% mortality against Bindura and Headlands populations. When applied singly, the remaining insecticides produced high mortalities (above 50%) against LGB which had survived suboptimal rates of Actellic Super Chirindamatura Dust<sup>®</sup>.

Field evidence shows that only rarely does chemical application kill all the pests, and the few which survive during successive generations develop slight genetic differences from the main stock of the insect species which become biotypes, usually giving serious problems as they develop resistance to the chemicals (Hill, 1987; Golob, 2002). Benbrook (1996) pointed out that genetic resistance to pesticides in pest populations and outbreaks of new pest problems

when broad-spectrum insecticides remove natural checks and balances have led to escalating dependence on pesticide use with no real decline in pest-induced crop losses.

Two insecticides can be used sequentially, as mixtures, in rotation or as mosaics (some areas treated with the first insecticide and other areas with the second insecticide) in a bid to manage resistance. Applying insecticides in rotation is generally the preferred method because susceptible pest genotypes generally have a reproductive advantage over resistant ones in the absence of an insecticide. The frequency of susceptible genotypes may increase during the periods when an insecticide is not used.

Other methods of managing resistance include use of insecticides only under good hygiene conditions; ensuring that dosage and application method are correct. Avoiding using insecticides on a calendar basis but only when it is necessary can also discourage development of resistance. Practitioners must also avoid increasing the amount of insecticide (i.e. increasing concentration) as it promotes further resistance. This approach is also uneconomical and not permitted because of stipulations of maximum residue limits in food products. It has been suggested that the risk of resistance be incorporated into pesticide registration requirements and that resistance management be used as justification for the registration of insecticide mixtures (Obeng-Ofori, 2010).

### **CHAPTER 7**

### GENERAL CONCLUSIONS AND RECOMMENDATIONS

### 7.1 Conclusions

This study revealed the following:

- 1. that there are some currently registered grain protectants, particularly Actellic Super Chirindamatura Dust®, which are no longer efficacious against some populations of *P. truncatus*;
- 2. that sub-optimal rates of insecticide by smallholder farmers may be responsible for ineffectiveness of insecticides in grain storage;
- 3. that when re-treating infested grain, farmers should use insecticides with a different mode of action or of chemical composition different from the predecessor;
- 4. that incorporation of *E. citriodora* leaf powder in insecticide/grain admixtures is unlikely to increase the insecticidal properties of the conventional insecticides in post-harvest pests, especially with regards to LGB;
- 5. that *E. Citriodora* leaf powder significantly repels both *P. truncatus* and *S. zeamais*; and
- 6. that the most practical use of *E. citriodora* leaf powder is as a repellent to deter insect infestations thus reducing potential grain damage.

### 7.2 Recommendations

From the results of the study, several recommendations can be made.

- 1) There is need for further local studies with as many plant species as possible so as to determine their usefulness in stored-product primary insect pest management, especially at the smallholder level.
- 2) The Ministry of Agriculture, through the Pesticide Registration Office, must now be proactive and always conduct regular insecticide susceptibility tests on the major primary and secondary pests associated with stored grain. The information gleaned from such work will make it possible for the Pesticide Registration Office to know in advance which grain protectants to de-register when companies renew registrations for such products. The current scenario whereby many of the currently registered products available on the local market are clearly no longer efficacious against LGB is worrying as this threatens national food security.

- 3) Agrochemical companies themselves must start emulating the example done with Hurudza® by incorporating repellent plant extracts in their dust formulations. Given the shortened shelf lives of insecticides due to development of insecticide resistance, it has become clear that stored grain protection can no longer rely on insecticidal effects alone but on a combination of insecticidal and repellency effects.
- 4) Lastly, small plantations of *E. citriodora* must be established at the village or household level in the rural areas so as to offer a ready source of leaf powder which may be used for enhanced grain protection against primary insect pests. However, further studies with *E. citriodora* leaf powder applied at a higher rate should be conducted.

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