

**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**

BY

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DEDICATION

To my wife, Shamiso and sons, Kutenda and Mufaro, I will always cherish your support and endurance.

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ABSTRACT

This study determined the occurrence and importance of *Prostephanus truncatus* (Horn), the larger grain borer (LGB) (Coleoptera: Bostrichidae) in relation to other storage insect pests in farm-stored maize grain samples taken from various communal households located in eight districts of Mashonaland Central and Mashonaland East provinces in Zimbabwe. The study also evaluated inherent resistance of selected maize varieties to LGB and the susceptibility of the pest to currently registered grain protectants. Surveys showed that LGB was present in all the eight districts within the two provinces, and was recovered from 22.6 % of the total samples collected. LGB was found to be most abundant in Murewa district where it represented about 90 % of all the insect pests that were recovered from stored maize grain samples. *Sitophilus zeamais* was the most frequently occurring insect pest, and was recovered from almost all (92%) the grain samples collected. Laboratory evaluation of 25 maize varieties and breeding lines showed varying levels of susceptibility. Significant differences ($P < 0.05$) were observed in apparent grain weight loss, dust production and progeny counts. There were no significant differences ($P > 0.05$) among the varieties in LGB parent and progeny mortality. The relative susceptibility of the varieties was compared using the selection index and showed that six hybrids and one open pollinated variety were resistant whilst the remaining 18 varieties were susceptible to LGB. The seven resistant varieties (CKPH09002, CKPH09004, CKPH08010, CKPH08043, ZM309, CKPH09001 and CZH0913) showed considerably low apparent weight loss and frass production after a 95-day exposure to *P. truncatus*, suggesting that they contain traits that confer resistance to the pest. The study showed that host plant resistance can be used as an important component of an integrated pest management strategy against LGB. In laboratory evaluations of five currently registered grain protectants for efficacy against LGB, significant differences ($P < 0.05$) in LGB mortality among the treatments were noted after two days of exposure to insecticide-treated grain. Actellic Gold Chirindamatura Dust[®] (1.6 % pirimiphos methyl + 0.36 % thiamethoxam) was

the fastest killer, achieving 83 % mortality after two days of exposure whilst Actellic Super Chirindamatura Dust[®] (1.6 % pirimiphos methyl + 0.3 % permethrin) gave the lowest insect kill (9.5 %). No significant differences ($P > 0.05$) were, however, observed after seven days of exposure. The results of the laboratory evaluation suggest that all the test formulations are efficacious against LGB when treatments are still very fresh. Completely different results may, however, be obtained when the treatments age. The results and implications of these studies are discussed in relation to storage pest management.

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CHAPTER ONE

INTRODUCTION

1.1 Background

The larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) is endemic to meso-America and is a serious pest of farm-stored maize and cassava (Hodges, 1986; Markham *et al.*, 1991; Golob, 2002). It was accidentally introduced to Africa between the late 1970s and early 1980s and is currently the most serious insect pest of stored maize on the continent (Stewart-Jones *et al.*, 2006). In Africa, *P. truncatus* was first identified as the pest causing severe losses in stored maize in Tanzania in 1981 (Golob and Hodges, 1982). A separate introduction occurred in West Africa through Togo (Harnisch and Krall, 1984). From these two points of introduction, *P. truncatus* then spread throughout sub-Saharan Africa (Kalivogui and Muck, 1991; Pike *et al.*, 1992). Its occurrence in Zimbabwe was officially reported in 2010 (Anonymous, 2010). The pest's known crop hosts are dried maize, cassava and sweet potato, but it is understood to breed only in maize and cassava (Boxall, 2002). *Prostephanus truncatus* causes sporadic damage to stored maize grain and dried cassava. Damage due to the pest can be as high as 36–40 % within six months and 70–80 % within four months, in maize and cassava, respectively (Giles and Leon, 1974).

1.2 Justification

Rwegasira (2003) observed that the environmental conditions of Zimbabwe are similar to other countries where *P. truncatus* had successfully invaded and established. It was therefore postulated that Zimbabwe was prone to *P. truncatus* invasion. Nyagwaya *et al.* (2010) further observed that *P. truncatus* was distributed across most parts of Zimbabwe.

Existing control measures for stored product insect pests include good storage hygiene, chemical pesticides, biological control and host plant resistance (Golob, 2002). Chemical control is the most commonly used and most effective control measure at the farm level. However, the high cost of synthetic insecticides, pesticide resistance and environmental contamination justifies the need for pest management methods that are effective, affordable and safe for humans and the environment. This has therefore directed research to the development of alternative control strategies such as the use of resistant maize varieties. Although an integrated approach to control *P. truncatus* has been proposed, the use of host plant resistance is practically missing in this approach (Kumar, 2002). Many maize varieties are being developed for adaptation to a wide range of environments including resistance to pathogens and field pests. There is more need for work to test the susceptibility of maize varieties to storage insect attack (Cortez-Rocha *et al.*, 1990). Information on the relative tolerance of maize cultivars to storage insect pests would be essential in the design of a sustainable control programme for LGB.

Besides the above, there is also need to establish the current status of *P. truncatus* and its relative importance as a storage pest in Zimbabwe. A survey of farmer grain storage practices

and its occurrence in farm-stored maize grain will assist in determining the relative importance of *P. truncatus* in the selected maize producing districts. Furthermore, there is need for a continuous evaluation of registered grain protectants for efficacy against *P. truncatus* so as to monitor insecticide resistance development. Resistance and cross resistance of stored-product insect pests to permethrin, deltamethrin, and other pyrethroids has been reported (Heather, 1986). Undocumented claims of inefficacy of registered grain protectants by farmers have also been reported (Nyagwaya, 2008).

1.3 Objectives

1.3.1 Overall Objective

The overall objective of the study was to explore the occurrence of *P. truncatus* in farm-stored maize grain across selected maize-producing areas of Zimbabwe and the potential of using resistant maize cultivars for its management.

1.3.2 Specific Objectives

The specific objectives of the study were to:

- (i) determine the diversity of storage insect pests including the status of *P. truncatus* in Zimbabwe,
- (ii) evaluate the efficacy of registered chemical grain protectants against *P. truncatus*, and
- (iii) evaluate the relative susceptibility of different maize cultivars and breeding lines to *P. truncatus*.

1.4 Hypotheses

The following hypotheses were tested:

- (i) There are no significant differences in the diversity of stored-product insect pests across different maize producing districts in Zimbabwe.
- (ii) Different chemical grain protectants do not differ significantly in their toxic effect on *P. truncatus*.
- (iii) Different maize cultivars do not differ significantly in their susceptibility to *P. truncatus*.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of Maize in Zimbabwe

Maize is the staple food crop of Zimbabwe and is grown by about 90 % of the people. Stored maize grain is susceptible to infestation by primary and secondary pests as well as fungi. Since independence in 1980, Zimbabwe rose to being a net regional exporter of maize except during drought years. Prior to the land reform programme in 2000, the smallholder farming sector contributed a peak of about 66 % of the country's maize production. A strong negative trend in national maize production was experienced in the country since 2001 (Anonymous, 2008). This was partly due to the structural changes precipitated by land tenure changes and also due to the economic deterioration as well as reduced profitability of maize production. Biotic constraints, among them, pests and disease also contributed to the declining trend in maize production (FAO, 2010).

2.2 Insect Pests of Stored Maize

Primary pests of stored maize grain include *Sitophilus zeamais*, *P. truncatus* and *Sitotroga cerealella*, while secondary pests include *Plodia interpunctella*, *Ephesia cautella*, *Tribolium castaneum* and *Cryptolestes ferrugineus*. The Larger Grain Borer, *P. truncatus* only became a major pest of stored maize grain in Africa from the 1980s after its introduction from the Americas. It is primarily a wood boring pest that is pre-adapted to infest only certain stored products specifically maize and cassava, *Manihot esculenta* (Hodges *et al.*, 1998). It has recently been reported in Zimbabwe (Nyagwaya *et al.*, 2010) and poses a serious threat to

food security in the country. The adults of *P. truncatus* bore into the maize grains, making holes by their tunneling action and generating large quantities of dust.

Prostephanus truncatus can be distinguished from other insect pests using characteristic features. It has a large pronotum that protects the head during tunneling and provides strong support for the mandibular muscles. The body length of *P. truncatus* adults ranges from 2 to 3.5 mm, and width from 1 to 1.5 mm (Nansen and Meikle, 2002). The colour is black to dark brown. The tip of the abdomen is square when viewed from above or below. There is a ridge marking the junction of the side and tip of the elytra (Figure 2.1).

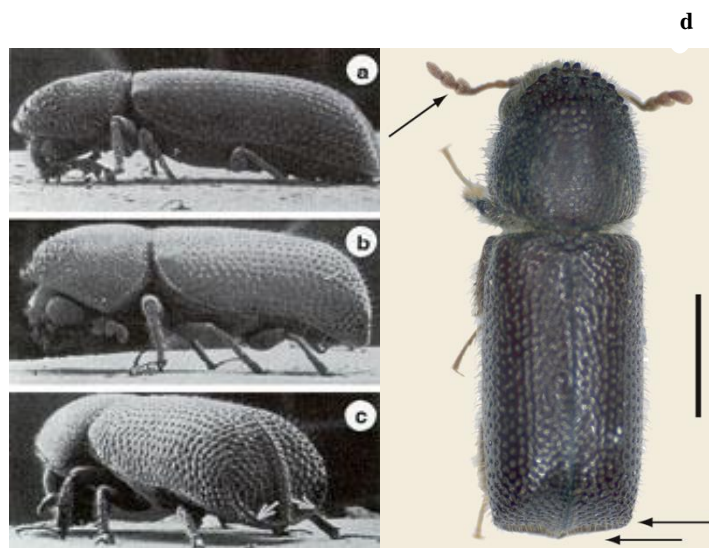


Figure 2.1 **a.** Lesser grain borer. **b.** Larger grain borer lateral view **c. & d.** Larger grain borer dorsal view (characteristic truncated posterior). (Source: Anonymous, 2011)

2.3 Current Management Strategies for *P. truncatus*

Considerable progress in crop protection has been achieved through the use of synthetic pesticides, but a host of their negative effects has been recorded on human health and the environment (Plantegenest *et al.*, 2007). The continued use of chemical insecticides could result in loss of efficiency due to rapid selection of resistance in targeted insect pests. Organophosphorous insecticides such as pirimiphos methyl or fenitrothion are highly efficacious against the common storage insect pests such as *S. zeamais* and *S. cereallela*, but not against *P. truncatus* (Golob *et al.*, 1985; Anonymous, 2011). Synthetic pyrethroids such as permethrin, deltamethrin or fenvalerate are more effective against *P. truncatus* but less toxic to the other storage insect pests (Borgemeister *et al.*, 2003). Fumigation using Phostoxin® (aluminium phosphide) is also effective in controlling the larger grain borer. Effective management of the storage pest complexes where LGB is present therefore requires the use of insecticide formulations that contain at least a pyrethroid and an organophosphate. However, the need for high technical expertise, high costs and the high risk of use associated with chemical insecticides is a challenge to the resource-constrained smallholder farmers.

Terestrius nigrescens (Lewis) (Coleoptera: Histeridae) has been identified to be an effective *P. truncatus* natural enemy (Omondi *et al.*, 2011). It has been introduced in Togo, but its establishment has not yet been ascertained. Shelling of grain soon after harvesting is, to some extent, effective in the management of *P. truncatus*. However, this approach is not consistent with smallholder farmer practices.

Prostephanus truncatus is sporadic both in time and space (Nyagwaya *et al.*, 2010). Pest incidence may be insignificant for several years and then suddenly increase in a ‘bad’ year. Also, variability exists in the severity of infestations, even among close-standing stores. Given this scenario, it is difficult to introduce practices that require significant investment by poor farmers. It is in this context that IPM approaches may help smallholder farmers. It is inevitable that IPM for the protection of smallholder farm stores will be more limited than its relatively sophisticated counterpart for the protection of large-scale crop production. Smallholder farmers generally lack the resources and flexibility to alter pest management at will, but if offered acceptable options and a means of decision making so that the proposed action is cost effective in relation to the risk of losses, then a more sustainable and cost effective approach is possible (Birkinshaw *et al.*, 2002).

2.4 Biology and Ecology of *P. truncatus*

Prostephanus truncatus can infest the maturing maize crop as well as the harvested crop and persists in storage. It occurs in different habitats, including forests (Nang’ayo *et al.*, 1993). The beetle has managed to establish itself as a serious pest in both the hot, dry conditions of western Tanzania, the hot, humid conditions of Togo and up to an altitude of 2 200 m in Mexico, suggesting that it has the potential to spread to all areas where maize is grown (Hodges, 1986). Laboratory studies have shown that the beetle is able to reproduce on a variety of wood species and this has led to the conclusion that it evolved as a wood borer (Hodges *et al.*, 1998). *Prostephanus truncatus* is not attracted from a long range by the odours of stored food products, and thus it exploits food products by sporadic invasion. Once

established in a new habitat, *P. truncatus* may also invade stored grain ecosystems from which it could be extremely difficult, if not impossible to eradicate. Upon invasion of stored grain, it causes enormous damage compared to other primary pest species such as *S. zeamais*.

Primary host selection is by the “land and choose” principle (Hodges *et al.*, 1998). Once a male has found an appropriate food source, it releases an aggregation pheromone which attracts both females and other males. This pheromone consists of two components, 1-methylethyl (*E*)-2-methyl-2-pentenoate and 1-methylethyl (*E,E*)-2,4-dimethyl-2,4-heptadienoate called Trunc-call 1 and Trunc-call 2 (T1 and T2), respectively (Cork *et al.*, 1991). Male *P. truncatus* probably produces the aggregation pheromone primarily to attract females as potential mates and other males respond opportunistically (Hodges *et al.*, 1999, 2002). Large aggregations may occur in response to this chemical communication signal. Laboratory studies have shown that pheromone production is low in recently emerged males, rises to a peak when beetles are around three weeks old, and then declines steadily until death (Cork *et al.*, 1991). Females produce a non-volatile chemical signal (female factor) which temporarily switches off male aggregation pheromone production (Birkinshaw and Smith, 2000). Synthetic aggregation pheromone has been shown to attract individuals from considerable distances provided the concentration of pheromone is sufficiently high (Farrell and Key, 1992). The continued production of pheromone probably assists in locating suitable breeding sites (Hill *et al.*, 2002). Female *P. truncatus* can distinguish between the pheromone signals of different males and some males are more attractive than others (Birkinshaw and Smith, 2000). Presently, the means of assessing infestations in stores is by manual sampling

of the produce and placement of synthetic pheromone traps in the natural forests (Meikle *et al.*, 1998, 2000).

Prostephanus truncatus can live at least six months when reproducing (Shires, 1980). Optimum conditions for development on maize are 32 °C and 70-80 % relative humidity, under which the life cycle can be completed in 24-25 days (Hodges, 1986). At 27 °C and 70 % relative humidity, the intrinsic rate of natural increase, r is 0.73 and 0.7-0.8/week on maize cobs and shelled grain, respectively (Bell and Watters, 1982). Adult females lay up to 430 eggs in batches of 20, and covered with fine maize dust, within the grain in blind ended chambers bored at right angles to the main tunnel. The pre-oviposition period is 5-10 days and oviposition reaches a peak at 15-20 days (Bell and Watters, 1982; Li, 1988). The larvae feed on the inside and complete their development mostly within the grains, with three larval instars (Subramanyam *et al.*, 1985). The mean development period under optimum conditions is 3 days for eggs, 13.2 days for larvae (3 instars), 3.9 days for pre-pupae, and 2.4 days for pupae (Demianyk and Sinha, 1988). The last instar larva of *P. truncatus* pupates either within the grain or in the surrounding dust. Females have a mean survival time of 61 days while that for males is 45 days (Shires, 1980; Bell and Watters, 1982). Adult dispersal is predominantly by flight, reaching a peak 8-12 days post-eclosion, and the adults usually eventually congregate on the same new host in response to male-produced aggregation pheromone (Hodges, 1986). Adult flight is initiated by temperatures of 25–30 °C. Population densities and food quality also influence initiation of flight.

X-ray studies have shown that *P. truncatus* feeds predominantly inside the grain (Hodges, 1986). The beetle favours softer maize cultivars on which it causes greater damage and weight loss, also producing more progeny than on the harder flint varieties (Demianyk and Sinha, 1988). It has also been demonstrated that *P. truncatus* causes more damage on maize stored on the cob than shelled grain (Bell and Watters, 1982; Hodges, 1986). This is probably because *P. truncatus* easily inserts the hooked spines at the end of the first pair of tibiae in spaces between the grains on the cob to anchor its body. Infestations may begin in the field when grain moisture content is above 40 % and continue throughout storage (Shires, 1977). The most prominent form of damage is through feeding and elevation of grain moisture content due to accelerated respiration resulting in creation of a favourable environment for postharvest fungi (Demianyk and Sinha, 1988).

In smallholder maize storage systems, *P. truncatus* frequently occurs as part of a pest complex. However, *P. truncatus* is more tolerant of low grain moisture contents than the other insect pest species (Hodges, 1982). In rural maize storage systems, *P. truncatus* infestations are characterized by high levels of infestation, accompanied by significant grain losses, from the fourth month of storage (Henckes, 1992). The population growth rate of *P. truncatus* declines as weight loss increases, and becomes negative above 50 % weight loss. It is usually highly aggregated at very low population densities during the first few months of storage (Meikle *et al.*, 2000). A devastating *P. truncatus* outbreak during the storage season can be established from population densities as low as one beetle per 10 kg grain at harvest (Meikle *et al.*, 1998).

2.5 *Prostephanus truncatus* Frass/Dust Production

The most outstanding feature of *P. truncatus* infestation in grain is frass (dust) production which also helps to protect oviposited eggs. The quantity of frass produced by *P. truncatus* during feeding is a reliable indicator of its degree of infestation and damage (Von Berg and Biliwa, 1990). There is a close correlation between frass production and dry matter loss and the total number of *P. truncatus* (Von Berg and Biliwa, 1990; Osipitan *et al.*, 2011). In laboratory bioassays, Stewart-Jones *et al.* (2006) observed *T. nigrescens* to congregate in maize grains mixed with dust and frass from *P. truncatus*. Frass from *P. truncatus*-infested and damaged commodities is infected by bacteria and fungi, and the microbial count from the damaged commodities correlates positively with weight of frass, percentage damage and percentage weight loss (Osipitan *et al.*, 2011). The measurement of frass in infested grain can be used to estimate cultivar susceptibility to *P. truncatus*.

2.6 Grain Resistance to Storage Pests

Maize kernels are in a state of dormancy and any damage inflicted by storage pests is terminal and tolerance has no use in stored grain. Evaluation of resistance to stored grain insects therefore focuses on measuring antixenosis. Plant resistance to insects is the relative amount of heritable qualities possessed by a plant or its materials which influence the ultimate degree of damage done by the insects (Ahmed and Yusuf, 2007). Stored grain resistance is the ability of a certain crop variety to produce grains that maintain better quality than other cultivated varieties following long storage under similar insect populations (Mbata, 1987). Research has shown that there is significant variation among maize genotypes in resistance to storage insect

pests (Giga and Mazarura, 1991; Arnasson *et al.*, 1994). Three resistance mechanisms have been studied and found to be important bases for grain resistance to maize insect pests, and these are antibiosis, non-preference and tolerance (Arnasson *et al.*, 1994). Kogan and Ortman (1978) proposed the term “antixenosis” to replace the term “non-preference” which in the context of the current study is a feature of the grain, which discourages insects from colonizing, feeding and oviposition or a combination of the three.

Several factors lead to the production of resistance against infestation by storage insect pests (Ahmed and Yusuf, 2007). The tightness of the glumes in unmilled rice serves as a physical barrier working against penetration by insects. Well-fitting and tight-sheathing leaves of the husks covering a maize cob may reduce infestation by *Sitophilus* spp. in the field and in store. Seed hardness is thought to make insect penetration more difficult thus providing protection. Adult insects consume more of mealy endosperms than vitreous endosperms (Demianyk and Sinha, 1988). Therefore, oviposition and adult emergence rates are higher in mealy grain than in vitreous grain whilst insect life span is shorter in hard than in soft grain.

The secondary chemistry and other biochemical and physical characteristics of maize cultivars confer resistance to insect pests. Certain chemical compounds may also inhibit oviposition and the development of insects on seeds (Gatehouse *et al.*, 1979; Dobie, 1986; Mbata, 1987). The quantity and quality of nutritional constituents influence fecundity of females, the development period of the pre-imaginal instars and the rate of adult emergence (Dobie, 1986). In maize, chemical factors such as amylose influence grain resistance to insect attack. Phenolic compounds, total amino acids, ether extracts, ferulates, diglycerides, free

sterols, sugar content, p-coumaric acid and dichloromethane are among some of the chemical factors influencing cereal grain resistance to storage pests (Serratos *et al.*, 1987). Santiago and Malvar (2010) observed that dehydrodiferulate cross-links are involved in maize defence mechanisms against insects such as the European, Mediterranean and tropical corn borers and storage pests including *S. zeamais*. These phenolic acids are found in highest concentration in the pericarp and cell walls of the endosperm and their concentration correlates strongly with hardness of the grain.

The International Maize & Wheat Improvement Centre (CIMMYT) has more than 14,000 maize accessions from all over the world (Kumar, 2002). When 105 Caribbean land races were infested with *P. truncatus* in the laboratory, 19 showed resistance to the pest (Kumar, 2002).

2.7 Methods of Screening Maize Varieties for Resistance to Storage Insect Pests

Methods for screening resistant grain varieties involve infesting grains artificially with the storage insect under investigation and evaluating the suitability of the test variety for oviposition, duration of development of the insect on the varieties, adult emergence pattern and calculating the susceptibility indices from these observations (Jackai *et al.*, 1985). Losses sustained by the different varieties are then compared. Occasionally, particular attention is given in these studies to assessing the effects of relative humidity and the associated variable grain moisture content on pest population dynamics. These parameters have helped in identifying resistant varieties (Mbata, 1986; Mbata *et al.*, 1988; Vowotor *et al.*, 1997).

2.8 Sampling for Stored Grain Pests

The occurrence of insect pests in stored grain is an important global setback for grain farmers, bulk grain handlers and distributors (Elmoultie *et al.*, 2010). Bulk grain commodities need to be inspected in order to detect pests, reduce the risk of their presence and estimate their damage to stored products. There is need to choose the correct method of sampling so as to make accurate estimates of insect pest infestation and damage (Stejskal *et al.*, 2008). Microclimatic variations within stored grain cause insect pests to be heterogeneously distributed throughout the grain storage system (Stejskal *et al.*, 2008). Sampling methods should therefore account for the heterogeneous distribution of insects in bulk grain.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Spectrum of Storage Insect Pests and Status of *P. truncatus* in Farm-Stored Maize Grain

Grain samples were collected from 178 households within eight districts (Rushinga, Bindura, Mutoko, Murewa, Goromonzi, Seke, Marondera and Wedza). Forty-eight of the samples were collected from two districts (Rushinga and Bindura) in Mashonaland Central province and the remainder from six districts in Mashonaland East province (Mutoko, Murewa, Goromonzi, Seke, Marondera and Wedza). The areas from which the samples were collected represent some of the major maize producing areas of Zimbabwe and areas where LGB has been previously reported (Nyagwaya *et al.*, 2010). Initially, at least 25 samples were collected per district; however, some of the samples were discarded due to missing information, leaving 17-30 samples per district. A household was selected regardless of whether LGB was previously reported or not. The samples were collected from farm-stored maize between mid-September and Mid-October 2011. Sampling was done with the assistance of extension officers from the Department of Agricultural Technical and Extension Services (AGRITEX). Grain samples of about 200-500 g each were taken from the grain store per household. For each sample, the date of sampling, name of area, farmer's full name, type of landholding, form in which maize was stored and whether the maize was treated or not was recorded on a sample information sheet. The samples were taken to the University of Zimbabwe for extraction of storage insect

pests. The samples were sub-sampled further using a sample divider so as to come up with a standard 100 g sample.

3.2 Susceptibility of *P. truncatus* to Currently Registered Chemical Dust Formulations in Zimbabwe

The experiment was carried out in the Entomology Laboratory, Department of Biological Sciences, University of Zimbabwe. The experiment was arranged in a randomized complete block design (RCBD) with four replicates. Maize grain of several mixed varieties was obtained from CIMMYT, sieved to remove extraneous matter and then deep frozen (about -4 °C) for 21 days to kill any live stages that could be present before treatment. About 50 g maize grain were placed in 300 ml glass jars and pre-equilibrated to experimental conditions of 27 ± 1 °C in a constant temperature room. The grain protectants (Table 3.1) that were tested in the experiment were bought from retail shops in Harare. The application rate was per manufacturer's recommendations and the amount applied was weighed using an electronic balance and thoroughly admixed with test grain by shaking and tumbling the jar for one minute. The control was untreated.

Table 3.1 Treatments investigated in the study

Trade Name	Active ingredients	Application rate (g/50 kg of grain)
Untreated Control	-	0
Shumba Super Dust [®]	fenitrothion (1%) + deltamethrin (0.13%)	25
Actellic Super Chirindamatura Dust [®]	pirimiphos-methyl (1.6%) + permethrin (0.3%)	25
Actellic Gold Chirindamatura Dust [®]	pirimiphos-methyl (1.6%) + thiamethoxam (0.36%)	25
Hurudza Grain Dust [®]	fenitrothion (1.7%) + deltamethrin (0.05%)	25
Chikwapuro Grain Protectant [®]	pirimiphos-methyl (2.5%) + deltamethrin (0.1%)	20

Twenty unsexed adult *P. truncatus* (aged about three weeks) from laboratory-maintained cultures were introduced to each treated jar and the jars were then sealed using perforated lids. Insect mortality was recorded at two and seven days after treatment by separating the contents into grain and insects and then recording the cumulative counts of dead insects. Confirmation of insect mortality was done by pricking the insect under a microscope where necessary. After mortality determination, the contents of the jar were replaced excluding dead insects.

Data were adjusted for the untreated control mortality using Abbott's (1925) formula:

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

Corrected mortality data were arcsine-transformed before being subjected to a one-way ANOVA. Where significant differences were detected, treatment means were separated using Tukey-Kramer HSD test.

3.3 Relative Susceptibility of Different Maize Cultivars and Breeding Lines to *P. truncatus*

Prostephanus truncatus cultures were established from infested grain collected in the Harare area. These cultures were maintained on maize grain. Twenty maize cultivars and breeding lines were obtained from local breeding programmes (CIMMYT-Zimbabwe, Pioneer Hi-Bred, Pannar, Seed Co and Pristine Seeds) and five from CIMMYT Kenya breeding programme (Table 3.2).

Table 3.2 Maize varieties and breeding lines used in the experiment

Treatment no.	Treatment code	Type of variety	Source
1	ZM309	Open Pollinated Variety	CIMMYT
2	ZM523	Open Pollinated Variety	CIMMYT
3	ZM627	Open Pollinated Variety	CIMMYT
4	ZM521	Open Pollinated Variety	CIMMYT
5	Pan53	Hybrid Variety	Pannar
6	Pris601	Hybrid Variety	Pristine Seeds
7	CZH0928	Breeding line	CIMMYT Zimbabwe
8	CZH0837	Breeding line	CIMMYT Zimbabwe
9	CZH0616	Breeding line	CIMMYT Zimbabwe
10	CZH0524	Breeding line	CIMMYT Zimbabwe
11	CZH01008	Breeding line	CIMMYT Zimbabwe
12	CZH0913	Breeding line	CIMMYT Zimbabwe
13	SC403	Hybrid Variety	Seed Co
14	SC513	Hybrid Variety	Seed Co
15	SC627	Hybrid Variety	Seed Co
16	CKPH09001	Breeding line	CIMMYT Kenya
17	CKPH09002	Breeding line	CIMMYT Kenya
18	CKPH08043	Breeding line	CIMMYT Kenya
19	CKPH09004	Breeding line	CIMMYT Kenya
20	CKPH08010	Breeding line	CIMMYT Kenya
21	P2859W	Hybrid Variety	Pioneer Hi-Bred
22	30G19	Hybrid Variety	Pioneer Hi-Bred
23	3253	Hybrid Variety	Pioneer Hi-Bred
24	30D79	Hybrid Variety	Pioneer Hi-Bred
25	30B50	Hybrid Variety	Pioneer Hi-Bred

Except for Pioneer cultivars, all the cultivars and breeding lines were grown at CIMMYT Harare Station and Muzarabani Sub-station, sib-pollinated and then harvested to obtain genetically pure commercial maize grain. The dry maize cobs were shelled and dried to about 12.5 % moisture content prior to use. Before the start of the laboratory experiment, grain samples were sieved to remove dust and foreign matter before sealing 1-2 kg of grain of each cultivar in a plastic bag. The bags were then put in a freezer for two weeks to disinfest the grain. Grain was inspected as clean and undamaged and then equilibrated for 31 days at 26 °C

before exposure to the insects. Twenty unsexed newly-emerged *P. truncatus* adults were placed in each jar containing 100 g of each maize variety. Jars were sealed using perforated lids. The experiment was conducted in a constant temperature room at 26 °C and 70 % relative humidity. Seeds of each variety without *P. truncatus* were kept under similar conditions and served as controls. The treatments were arranged in a randomized complete block design with three replications.

After 30 days, the jars were opened and the parental adults removed and mortalities recorded. Jars were resealed and returned to the experimental conditions for a further 65 days. After this period, jars were opened, the content separated into grains, insects and dust using 4.7 and 1.0 mm sieves. Six susceptibility parameters were measured on each maize variety or breeding line: (i) parent insect mortality, (ii) progeny F1 mortality, (iii) progeny F1 emergence, (iv) apparent grain weight loss, (v) weight of dust produced by feeding LGB and (vi) weight of adult progeny. The weight of dust produced due to *P. truncatus* feeding on the maize grain was expressed as a percentage of the initial grain weight.

Apparent weight loss was determined by measuring the weight of grain for each variety at the end of the emergence of the F1 progeny and then subtracting it from the initial grain weight of the sample. Percentage difference was calculated and recorded as the apparent weight loss. The number of dead parent insects was expressed as a percentage of initial population, whilst adult production was the total number of adult F1 progeny in each variety or breeding line

In order to determine the resistance reaction of the experimental varieties and breeding lines to *P. truncatus*, the susceptibility parameters (parent mortality, number of live F1 progeny, F1 progeny mortality, apparent weight loss and % dust) were integrated and a selection index calculated by summing the ratios between values and overall mean and dividing by 5 (number of parameters) as described by Tefera *et al.* (2011). Varieties or breeding lines with selection index values less than 0.8 were regarded as resistant and those with an index greater than 0.8 as susceptible (Bergvinson *et al.*, 2004; Tefera *et al.*, 2011).

Data on percentage parent mortality and percentage F1 mortality progeny counts were arcsine-transformed whilst data on adult progeny counts were log-transformed. Apparent weight loss, dust production and transformed percentage parent mortality and progeny counts were then analyzed using one-way ANOVA. Where significant differences ($P < 0.05$) were found, Tukey-Kramer HSD test was used to separate the means.

CHAPTER FOUR

RESULTS

4.1 Relative Abundance of Stored Grain Insect Pests

Table 4.1 shows relative abundance (%) of different stored product insect pests in maize grain from eight districts. Six insect pest species were recovered from the maize grain, namely, *P. truncatus*, *Sitophilus* spp., *Rhyzopertha dominica*, *Tribolium castaneum*, *Plodia interpunctella* and *Sitotroga cerealella*. *Sitophilus* species were the most abundant across the eight districts. Interestingly, even though *P. truncatus* is a new pest of stored grain in Zimbabwe, it occurred in all the eight study districts and was the third in terms of relative abundance. *Rhyzopertha dominica* and *S. cerealella* were relatively low in abundance across the eight districts. *Prostephanus truncatus* was observed in all the districts, but was most abundant in Murewa (89.8 %), followed by Rushinga district (32.1 %). *Prostephanus truncatus* was least abundant in Bindura district where it constituted about 0.6 % of the total number of insects.

Table 4.1 Relative abundance (%) of stored product insect pests across different districts in Mashonaland East and Central provinces

Insect species	Bindura		Goromonzi		Marondera		Murewa		Mutoko		Rushinga		Seke		Wedza		Means	
	n	%	N	%	n	%	n	%	n	%	n	%	N	%	n	%	n	%
<i>P. truncatus</i>	2	0.6	8	4.7	18	4.1	1976	89.8	9	4.3	129	32.1	44	0.8	9	4.8	18	17.7
<i>Sitophilus</i> spp.	341	94.2	143	83.0	396	90.4	183	8.2	146	69.8	177	43.9	90	58.1	143	77.6	66	65.7
<i>R. dominica</i>	0	0.0	0	0.0	2	0.4	3	0.2	0	0.0	29	7.7	6	3.9	0	0.2	2	1.6
<i>T. castaneum</i>	11	3.0	2	1.1	8	1.7	22	1.0	1	0.2	15	3.7	10	6.5	6	3.0	3	2.5
<i>P. interpunctella</i>	8	2.3	19	11.1	14	3.1	16	0.7	53	25.2	51	12.7	3	2.2	24	12.9	9	8.8
<i>S. cerealella</i>	0	0.0	0	0.0	1	0.3	0	0.7	1	0.5	1	0.2	2	1.0	3	1.4	1	0.5
No. of samples	27		17		30		21		25		21		18		19		22	

4.2 Susceptibility of *P. truncatus* to Registered Dust Formulations of Grain Protectants

Table 4.2 shows the percentage mortality of *P. truncatus* after exposure to five grain protectants. There were significant differences ($P < 0.05$) in insect mortality after two days of exposure to the grain protectants. The highest mortality of 82 % was observed on grain treated with Actellic Gold Chirindamatura Dust[®] whilst Actellic Super Chirindamatura Dust[®] gave the least mortality of 9.5 %. When the insects were left on treated grain for seven days, no significant differences ($P < 0.05$) were observed among the treatments. Mortality at 7 days ranged from 73.7 % (Actellic Super Chirindamatura Dust[®]) to 100 % (Chikwapuro[®]).

Table 4.2 Percentage insect mortality after 2 and 7 days of exposure to different grain protectants

Treatment	2 days	7 days
Shumba Super [®]	39.0 ab	93.3 a
Actellic Gold Chirindamatura Dust [®]	82.0 c	83.8 a
Chikwapuro [®]	66.8 bc	100 a
Hurudza [®]	37.0 ab	93.3 a
Actellic Super Chirindamatura Dust [®]	9.5 a	73.7 a
Mean	39.1	74.0

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

4.3 Relative Susceptibility of Different Maize Cultivars and Breeding

Lines to *P. truncatus*

Table 4.3 shows mean susceptibility parameter values for the breeding lines and varieties. There were significant differences ($P < 0.0001$) among test hybrids in apparent weight loss. The least apparent weight losses of 5.4-8.2 % were observed on entries CKH09002, CKPH08043, CKPH09004, CKPH08010, ZM309, CZH0913 and CZH0837. Entries 3253, 30B50, CZH0928 and SC513 had the highest apparent weight losses of 29.8, 27.3, 26.8 % and 24.2 %, respectively. The breeding lines and varieties also differed significantly ($P < 0.0001$) in % dust production. The varieties' pattern of dust production due to *P. truncatus* damage was similar to that of apparent weight loss with entry CKPH09002 producing the least amount of dust and entry 3253 producing the most. There were no significant differences among the varieties in mean parent and progeny mortality ($P > 0.05$). The mean number total F1 progeny counts differed significantly ($P < 0.05$) among the cultivars.

Table 4.3 Susceptibility parameters measured on 25 maize breeding lines and varieties

Entry	% Apparent weight loss	% Dust	% Parent mortality	F1 progeny counts	% F1 mortality progeny counts
ZM309	7.9 de	8.5 bcde	25.0 a	69.0 abc	13.0 a
ZM523	13.6 abcde	11.3 abcde	18.3 a	54.3 abc	23.7 a
ZM627	13.1 bcde	10.8 abcde	18.3 a	54.7 abc	24.0 a
ZM521	15.1 abcde	12.6 abcde	21.7 a	74.7 abc	15.4 a
Pan53	20.4 abcde	16.3 abcde	15.0 a	96.3 ab	21.8 a
Pris601	20.0 abcde	16.0 abcde	25.0 a	90.3 abc	22.4 a
CZH0928	26.8 abc	21.1 ab	15.0 a	132.7 a	19.4 a
CZH0837	8.2 de	6.9 cde	40.0 a	27.0 abc	26.1 a
CZH0616	23.3 abcd	18.3 abcd	20.0 a	94.3 ab	24.0 a
CZH0524	18.2 abcde	14.7 abcde	25.0 a	70.0 abc	22.6 a
CZH01008	14.2 abcde	12.3 abcde	20.0 a	53.0 abc	32.9 a
CZH0913	7.5 de	6.7 cde	21.7 a	56.3 abc	26.0 a
SC403	21.5 abcde	16.7 abcde	13.3 a	81.3 abc	34.6 a
SC513	24.2 abcd	19.7 abc	13.3 a	113.7 a	19.9 a
SC627	17.9 abcde	14.2 abcde	16.7 a	72.0 abc	21.4 a
CKPH09001	10.4 cde	8.2 bcde	16.7 a	42.0 abc	30.5 a
CKPH09002	5.4 e	4.3 e	15.0 a	24.7 c	32.1 a
CKPH08043	5.9 e	5.1 de	25.0 a	25.7 c	22.0 a
CKPH09004	5.7 e	5.0 e	20.0 a	21.3 bc	35.8 a
CKPH08010	6.3 e	5.3 de	20.0 a	30.7 abc	26.3 a
P2859W	10.8 bcde	7.5 cde	46.7 a	41.7 abc	28.9 a
30G19	18.1 abcde	13.7 abcde	28.3 a	59.0 abc	33.9 a
3253	29.8 a	23.6 a	23.3 a	90.7 a	34.2 a
30D79	16.3 abcde	12.6 abcde	20.0 a	63.0 abc	26.5 a
30B50	27.3 ab	21.3 ab	18.3 a	110.0 a	26.8 a
Mean	15.5	12.5	21.7	65.9	25.8

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

All the locally bred and commonly grown maize hybrids that were used in the experiment had selection indices higher than 0.8 and therefore considered to be susceptible to *P. truncatus*. Pioneer hybrid variety 3253 was the most susceptible with a selection index of 1.52 whilst the two widely grown Seed-Co varieties, SC513 and SC403 had fairly high indices of 1.25 and 1.18, respectively.

Table 4.4 Selection Index (SI) and reaction of breeding lines and varieties to *P. truncatus*

Entry	Selection index	Reaction
ZM309	0.78	Resistant
ZM523	0.87	Susceptible
ZM627	0.86	Susceptible
ZM521	0.94	Susceptible
Pan53	1.12	Susceptible
Pris601	1.19	Susceptible
CZH0928	1.37	Susceptible
CZH0837	0.87	Susceptible
CZH0616	1.25	Susceptible
CZH0524	1.09	Susceptible
CZH01008	0.98	Susceptible
CZH0913	0.78	Resistant
SC403	1.18	Susceptible
SC513	1.25	Susceptible
SC627	1.00	Susceptible
CKPH09001	0.78	Resistant
CKPH09002	0.60	Resistant
CKPH08043	0.64	Resistant
CKPH09004	0.68	Resistant
CKPH08010	0.65	Resistant
P2859W	1.04	Susceptible
30G19	1.16	Susceptible
3253	1.52	Susceptible
30D79	0.99	Susceptible
30B50	1.40	Susceptible

CHAPTER FIVE

DISCUSSION

Both *S. zeamais* and *P. truncatus* occurred in almost all the study districts. The occurrence of *P. truncatus* in all the study districts and in 22.6 % of the sampled households suggests that even though the pest is regarded to be new to Zimbabwe, it is now of major economic importance in the country. These findings also confirm previous work by Nyagwaya *et al.* (2010) who found *P. truncatus* to occur in most maize growing parts of the country.

Even though *P. truncatus* occurred in all districts, the highest numbers were recorded in Murewa. The Lepidopteran pest species were difficult to sample using the methods that were employed in this study. If the samples were to be incubated, adult moths could have emerged from eggs in sampled grain and this could have given different abundance values. However, since the focus was on *P. truncatus*, the samples were not incubated.

The sporadic nature of *P. truncatus* and its availability in alternative hosts suggests that even though low numbers of the pests were recovered from sampled grain, it may actually be abundant in unsampled stores and in the forest. It has previously been observed that a devastating outbreak can establish from population densities as low as one beetle per 10 kg of stored maize grain (Meikle *et al.*, 1998). This implies that even where small numbers of the pest are found, the pest should be regarded to be of economic importance and control measures should be implemented.

Actellic Gold Chirindamatura Dust® and Chikwapuro® were the best treatments at 2 days post exposure and were not significantly different from each other. After 7 days of exposure, Actellic Super Chirindamatura Dust®, Hurudza® and Shumba Super® were as equally effective as Actellic Gold Chirindamatura Dust® and Chikwapuro® even though only Chikwapuro® gave 100% mortality at this time. In general, the failure by Chikwapuro®, Actellic Super Chirindamatura Dust®, Hurudza® and Shumba Super® to achieve a kill as high as that of Actellic Gold Chirindamatura Dust® within two days of exposure implies that these formulations may not be relied upon to prevent establishment of LGB in grain stores. Golob *et al.* (1985) observed 100 % mortality when LGB was exposed to 10 ppm pirimiphos-methyl for up to 3 weeks but the pest developed tolerance very quickly. Golob *et al.* (1985) also observed LGB to develop resistance to permethrin. Pirimiphos-methyl and permethrin are the active ingredients in Actellic Super Chirindamatura Dust®. Chinwada (unpublished) also observed the breakdown of Shumba Super® after about six months of storage post-treatment, with affected farmers having to re-treat with Hurudza®. Hurudza® was first registered in Zimbabwe in 2011 and trial data used for its registration (Chinwada, unpublished) actually gave highly effective protection for an entire 48 weeks. Actellic Gold Chirindamatura Dust® was registered in 2010 and trial data submitted for its registration showed it to be as effective as Hurudza® (Chinwada, unpublished). In the Actellic Gold Chirindamatura Dust field trials, grain in the Actellic Super Chirindamatura Dust treatment was so heavily damaged by LGB that the concerned farmers asked for the treatment to be terminated after 40 weeks of storage (Chinwada, unpublished). In a recent bioassay conducted using maize which was admixed with Actellic Gold Chirindamatura Dust® in October 2010

and retained in a granary up to end of January 2012, 70% LGB adult mortality was recorded after 7 days of exposure to a sample of the treated grain (Chinwada, unpublished).

The study revealed that there was considerable variation in susceptibility levels of the maize breeding lines and varieties with respect to apparent grain weight loss, dust production and insect progeny emergence, suggesting an inherent ability of certain varieties to resist *P. truncatus*. Based on selection indices (Bergvinson *et al.*, 2004; Tefera *et al.*, 2011) of the different varieties, six hybrid lines and one open pollinated variety were found to be resistant to *P. truncatus*. The remaining 18 varieties were susceptible to the pest. There appeared to be a close correlation between dust production and apparent weight loss among the resistant varieties, giving credibility to the findings of Von Berg and Biliwa (1990) and Osipitan *et al.* (2011) who observed that the amount of dust produced by feeding LGB is a good indicator of the grain's susceptibility to the pest. The existence of resistance to insect pests by grain has also been reported by previous workers (Serratos *et al.*, 1987). Working with CIMMYT germplasm, Kumar (2002) and Tefera *et al.* (2011) observed that some of the germplasm had inherent resistance to LGB, a pattern which seems to be confirmed by this study.

Resistance of grain to insect attack has been shown to be correlated with physical factors such as kernel hardness and low moisture content (Garcia-Lara *et al.*, 2004). However, since *P. truncatus* is a wood boring pest, grain hardness is less likely to be a major resistance parameter. Biochemical parameters such as phenolics, amylose and sugar contents could be the most important resistance factors (Serratos *et al.*, 1987). However, the determination of biochemical properties of the varieties was beyond the scope of this study. The significant

differences observed in apparent weight loss, frass production and total progeny counts among the different varieties was probably due to non-nutritional biochemical parameters of the varieties. These have been found to be correlated with resistance through mechanical resistance (cell wall bound hydroxycinnamic acids) and antixenosis (phenolic acid amides) in the pericarp and aleurone layer, respectively (Arnasson *et al.*, 1994). There were no apparent differences among the varieties with respect to parent and progeny mortality, agreeing with the findings of Dobie (1974) and Makate (2007). It appears that susceptibility of the varieties to LGB was a function of the ability of a particular variety to support feeding by the pest rather than its ability to support oviposition. This was supported by studies in Coombs and Porter (1986) and Makate (2007). Parent mortality on the test varieties might not be a good indicator of susceptibility, because under laboratory conditions, adult beetles have been observed to survive without food for more than 10 days (Abraham, 1991). The resistant CIMMYT breeding lines CKPH09002, CKPH09004, CKPH8010, CKPH08043, ZM309, CKPH09001 and CZH0913 showed high progeny mortality values compared to the other test varieties. This suggests that reduced progeny survival and establishment also reduced the LGB populations and the resultant grain damage and loss. Abraham (1991) observed that the degree of damage during storage depends upon the number of emerging adults and their survival and grain that permits fast and elevated levels of adult survival will experience more damage.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study confirmed the presence of *P. truncatus* in Murewa, Mutoko, Marondera, Goromonzi, Seke, Rushinga and Bindura. The pest was most abundant in Murewa and least abundant in Bindura. Relative occurrence and abundance of LGB is not influenced by insect pest species diversity in the storage system. The study further revealed that the test maize varieties differed in their susceptibility to LGB. Eight of the varieties were shown to be resistant as depicted by their low selection indices, apparent weight loss and frass production. The maize varieties used in this work contain traits for resistance to LGB. These varieties can be stored for relatively longer periods of time or they can be a source of resistance in local maize breeding programmes. The efficacy of Chikwapuro[®], Shumba Super[®], Hurudza[®], Actellic Super Chirindamatura Dust[®] and Actellic Gold Chirindamatura Dust[®] against LGB over seven days of exposure to freshly-treated maize grain did not differ significantly. However, since this was a bioassay limited only to freshly-treated grain, the results may not be reflective of what happens throughout a whole storage season.

6.2 Recommendations

The confirmed occurrence of LGB in farm-stored grain in the smallholder farming sector is a threat to food security in this sector where food production is already a challenge. It is important that farmers' grain handling and storage practices be reviewed so that farmers are

able to manage this devastating pest and minimize grain losses. The responsible government agencies need to employ measures to monitor LGB occurrence and movement patterns. The existence of some resistant varieties suggests the need for breeding programmes to focus on exploiting this germplasm to develop varieties that are resistant to the pest. Varying levels of effectiveness of currently registered grain protectants further suggest the need for continual evaluation of grain protectants against the pest so that their effectiveness against LGB is ensured. There is also need for field testing of the maize varieties and grain protectants in different agro-ecological regions, different storage systems and also over time to confirm the laboratory findings of this study.

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