

VARIETAL ROLE IN THE MANAGEMENT OF THE LARGER GRAIN BORER, *PROSTEPHANUS TRUNCATUS* (HORN) IN STORED MAIZE

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ABSTRACT

The study determined the amount of haemolymph vitellogenin (Vg) of the Larger Grain Borer (LGB), *Prostephanus truncatus* (Horn) vitellogenic females reared on different maize varieties. The varieties were ZM 521, ZM 421, ECAVL1-DLN, WEEVIL A, LOCAL 1 and LOCAL 2. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS - PAGE) was conducted on the insect haemolymph. F₁ LGB adults from each variety were used to determine Indices of Susceptibility (IS) of the varieties to the insect pest attack. Percentages of Vg from female insects and IS were in the order ZM 521 > ZM 421 > ECAVL1-DLN > WEEVIL A > LOCAL 1 > LOCAL 2. Weight losses of the varieties after F₁ emergence were recorded. Findings showed that the IS and the weight losses were highest in ZM 521 and lowest in LOCAL 2. ANOVA indicated significant differences in the IS, weight losses and Vg levels of insects among the varieties. IS and Vg were positively correlated. Varying Vg levels reflect reproductive capacity of *P. truncatus* and therefore used to identify resistant maize grains. It is concluded that resistant varieties play a great role in stored maize pest management by reducing LGB populations below economic injury levels (EIL).

INTRODUCTION

Poor storage practices have been cited to be among major causes of food loss in many regions of Tanzania. Losses due to stored pests are currently estimated to be between 35 and 40% (UNIDO 2004). According to KILIMO/GTZ (1996) crop pest infestations deprive farmers of significant parts of their production yearly. Ravnborg 2004 estimated that 10 - 40% of the world's gross agricultural production is damaged by pests. Insect pests cause greater losses of maize crop particularly in storage where it is kept for a relatively longer time for future utilization.

Maize, *Zea mays* (L.) is an increasingly important cereal crop grown and stored in almost all ecosystems in Sub-Saharan Africa (Abate *et al.* 2000). It is a staple food and cash crop and various varieties are grown in almost all the regions of Tanzania. The major producing areas include Morogoro, Iringa, Mbeya, Ruvuma and Rukwa regions. Nutritional and physical statuses of maize varieties are reported to contribute to varying levels of their resistance to insect pests

attack. These attributes are advocated for consideration in the maize breeding for pest management (Dent 2000).

Principal causes of stored maize crop losses today are insect pest species belonging to various families including, Curculionidae, Bostrichidae, Gelechiidae, Pyralidae, Tenebrionidae. The common pests species are *Sitophilus spp.*; *Sitotroga cerealella* (Olivier); *Ephestia*; *Tribolium spp* and the Larger Grain Borer (LGB), *Prostephanus truncatus* (Horn), an alien causing devastating damage to stored maize, (Haines 1999, Borgemeister 2001, Dhliwayo and Pixley 2003, Dhliwayo *et al.* 2005, Rugumamu 2005). The biology and infestations of the insect pests were reviewed by among others, Hodges *et al.* (1983), Hill (1987) and Borgemeister (2001).

Storage problems of maize have become more acute because of the great damage caused by the LGB which became established in Tanzania in the early 1980s. The insect feeding habit has incapacitated some rural household storage technologies

(Rugumamu 2003). This LGB is confirmed as a serious stored grain pest in many African countries including Tanzania. In this regard, an evaluation of effects of some more maize genotypes to this pest shed light on the designing of appropriate pest management strategies in stored maize.

The ensuing losses in stored maize impact negatively the national food security in particular and the economy in general (FAO 1991). It is reported in the National Strategy for Growth and Reduction of Poverty (URT 2005) that poverty remains overwhelming in rural areas where about 87% of the population live. Initiatives to reduce the maize losses in rural storage systems where livelihoods are based on agriculture will contribute to the reduction in the spread and depth of poverty.

Control of insect pests by some farmers in Tanzania include, application of industrial pesticides such as permethrine; pirimiphos-methyl, an organophosphorus (Kilimo/GTZ 1996) but this strategy has had several shortcomings. It was reveal that the use of chemicals not only increases the production costs but also pose health hazards to both producers and consumers and the ecosystem where the pests develop biotypes resistant to the chemicals (Elwells and Maas 1996, Dent 2000). This study propagates the cultivation and storage of more resistant maize varieties to insect infestations and damage as a better management technology to stored products pests.

One of the important characteristics of resistant crop varieties is to lower pest population density and damage symptoms compared to susceptible varieties. This is a very important component of Integrated Pest Management, an approach that combines all economically, technically and ecologically applicable technologies to keep pest populations below those causing economic injury while minimizing unwanted side effects of the applied measures

(Neuenschwander *et al.* 2003). In many respects as Bhatia (1976) correctly reports, crop breeding for pest resistance is the most ecologically desirable insurance measure to be employed in pest management programmes. The time factor is quite considerable in that a breeding programme is slow to conduct, and the cost may be exorbitant, but the end results could be a very long-term pest control. Indices of susceptibility (IS) and weight losses of crop varieties are among the methods currently used to identify maize varieties which are resistant to insect attack (Bhatia 1976, Dobie 1974).

In this study the determined vitellogenin (Vg) levels in F₁ vitellogenic females *P. truncatus* heamolymph reared on different varieties was used to identify resistance of a variety to the insect attack. Vitellogenin is a precursor of yolk components thus its varying amounts in the insects reflect the insect reproductive potential when fed on a maize variety (Hans-Jorg 1993, Kostal 1993, Raikhel and Snigirevskaya 1998). Increasing evidence reveals that in many insects the process of ovarian maturation that contribute most immediately to egg load also respond to variation in host quality (Aluja *et al.* 2001). This study investigated the varietal role in the management of *P. truncatus* in stored maize.

MATERIALS AND METHODS

Maize Samples

The maize varieties were obtained from Mkambarani village through purchase of local varieties (LOCAL 1 and LOCAL 2) from farmers. The improved varieties namely, ZM 521, ZM 421, ECAVL 1 - DLN, WEEVIL A were supplied by agricultural extension staff who cultivated them for various other experiments. Mkambarani was one of the villages which were reported by the regional authority to experience frequent outbreaks of insect pests of stored maize in Morogoro Region. Participatory Rural Appraisal (PRA 1991) approach using focus group discussions

(FGD) was employed in collecting data on the insect pests and maize varieties.

Investigation of Index of Susceptibility and Weight Loss of the Maize to LGB

Pure cultures of *P. truncatus* from Ilonga Agricultural Research Institute were used in the determination of vitellogenin levels, indices of susceptibility, weight losses and hence identification of varying resistance of the maize varieties. Adults were introduced in jars with maize (moisture content 11.84% to 12.75%) for egg deposition and were removed after three weeks. The emerging insects were collected daily for seven days (parent stock 1-7 days old). The sexes of the insect were determined according to the method by Shires and McCarthy (1976).

Samples of maize varieties, free of infestation were equilibrated for 7 days at 27^o - 32^oC and relative humidity of 70 - 80%. Insects were conditioned to the maize varieties for one week by infesting eight 30 g samples of each variety with 12 females and 6 males i.e the ratio of F:M = 2:1 (Dobie 1974). Six replicates of the surviving insects from each variety were placed in fresh 30g samples of the respective varieties contained in ventilated glass bottles. The controls were set. These parent insects are at the beginning of peak oviposition and were removed from test samples after 14 days. Test replicates were kept for 7 days after which the emerged F₁ were removed, counted and recorded for the determination of vitellogenin levels as well as the calculation of IS of each cultivar according to the formula by Dobie (1974). Weight losses were determined by the, sample-weight method after applying corrections for the percentage moisture changes derived from the controls.

Determination of Vitellogenin Levels in *P. truncatus* Female Haemolymph

Vitellogenin levels in haemolymph of F₁ vitellogenic females of *P. truncatus* one 1 - 2 weeks old were assessed as an indicator of the insect reproduction potential after

attacking a variety of maize and hence weight loss of the stored maize crop. Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis of haemolymph sampled from aorta of twenty females and males was run in order to determine the separated vitellogenin amounts (Brewer *et al.* 1974). Samples dissolved in Phosphate Buffered Saline, pH 7.2 added to equal volumes of blue sampling buffer solution were centrifuged at 10,000g for five minutes at 5°C and cooled on ice after being boiled in water bath for 3 minutes; they were then stored at -20°C for further experimentation. Ten µl aliquots of the samples were loaded on gels in six replicates. Coomassie Brilliant Blue R - 250 was used for staining. The various protein bands were identified after destaining and scanning. The bands that appeared in females' and not in males' lanes were identified as vitellogenin (Fleig 1997, Raikhel and Snigirevskaya 1998). Determination of varying amounts as percentage of vitellogenin in the vitellogenic F₁ *P. truncatus* haemolymph was carried out.

Data Analysis

Kruskal-Wallis tested the difference in vitellogenin levels among vitellogenic females reared on different maize varieties; the IS of varieties to *P. truncatus* as well as the weight losses among the maize varieties after insect infestation. Multiple Comparisons followed the analysis of variance. Relationships between vitellogenin levels and IS were tested (Gomez and Gomez 1984, Fowler *et al.* 1999).

RESULTS

Index of Susceptibility of the Maize Varieties

The mean number of F₁ *P. truncatus* from the different varieties varied from 36 ± 0.89 to 132 ± 0.78 and were in the order ZM 521> ZM 421> ECAVL1-DLN > WEEVIL A > LOCAL1> LOCAL 2 (Fig.1). The numbers from the six varieties were significantly different, $\chi^2 = 45.55$, 5 P < 0.05. Multiple comparisons showed

significant differences in the insect infestations between the different maize varieties at $P < 0.05$. The IS of maize varieties to *P. truncatus*, a function of emerged F_1 adult numbers and their median

developmental periods were found to vary significantly $\chi^2 = 48.35$, $P < 0.05$. Variety ZM 521 exhibited the highest IS while the local variety, LOCAL 2 had the lowest IS (Table 1).

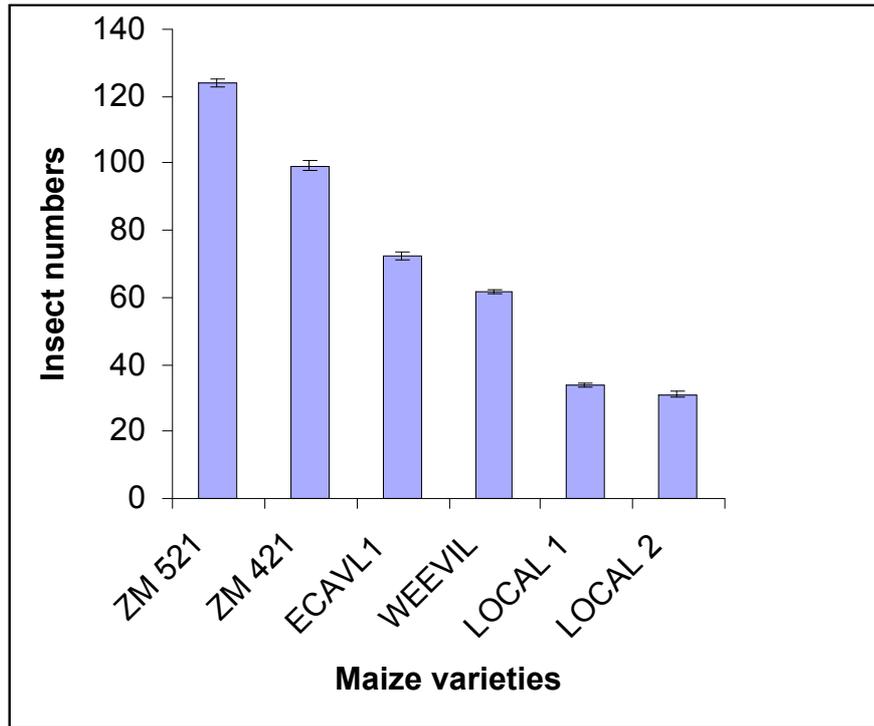


Figure 1: *P. truncatus* populations from the different maize varieties at F_1

Table 1: Mean indices of susceptibility and weight losses (%) of the maize varieties to *P. truncates*

Variety	Index of Susceptibility	Weight Loss (%)
ZM 521	14.18 ± 0.131	14.75 ± 0.007
ZM 421	12.47 ± 0.214	12.89 ± 0.063
ECAVL1-DLN	10.94 ± 0.052	9.50 ± 0.004
WEEVIL A	10.17 ± 0.113	8.65 ± 0.032
LOCAL 1	8.21 ± 0.065	7.00 ± 0.007
LOCAL 2	7.91 ± 0.152	6.89 ± 0.001

Weight Losses of the Maize Varieties after Insect Infestations

After emergence of F_1 *P. truncatus*, the maize samples had lost weight in the

following decreasing order ZM 521 > ZM 421 > ECAVL1-DLN > WEEVIL A > LOCAL 1 > LOCAL 2 (Table 1). There was a significant difference among the weights

lost by the varieties, $\chi^2 = 46.36$ at $P < 0.01$. Correlation test showed a positive relationship between weight losses and the IS of the varieties to *P. truncatus*, $r = 0.91$.

Vitellogenin Levels in Female *P. truncatus* Reared on Different Maize Varieties

Different percentage levels of haemolymph vitellogenin were exhibited by F_1 vitellogenic *P. truncatus* females which fed on the different maize varieties. The highest amount of vitellogenin was recorded in females raised on variety ZM 521 while the

lowest was observed in insects from LOCALS 1 and 2. Mean percentages varied as shown in figure 2: ZM 521 > ZM 421 > ECAVL1-DLN > WEEVIL A > LOCAL 1 > LOCAL 2. Kruskal-Wallis indicated a significant difference among vitellogenin levels at $P < 0.05$, 5. Multiple comparisons showed significant differences between vitellogenin means at $P < 0.05$. There was a positive correlation, $r = 0.96$ ($P < 0.05$) between vitellogenin percentages in *P. truncatus* haemolymph and the IS of the different maize varieties to *P. truncatus*.

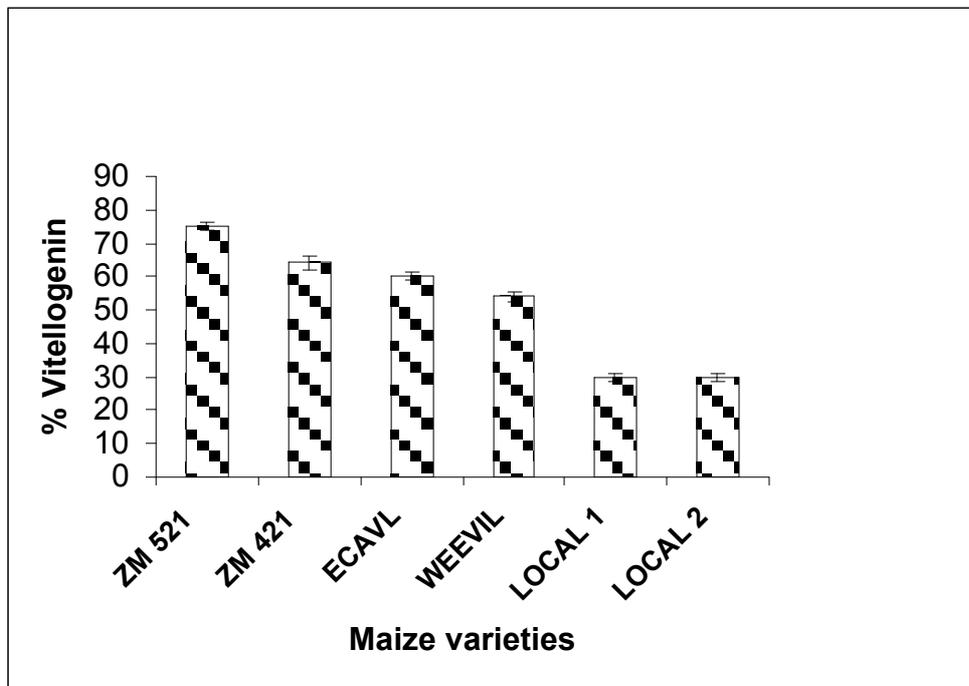


Figure 2: Amount of vitellogenin in *Prostepanus truncatus* females reared on different maize varieties

DISCUSSION

Effect of the maize varieties to *P. truncatus* was different as reflected by the IS of the maize varieties to the insect and the weight lost by the different varieties. The parameters were used to indicate the trend of varietal effect to the insect attack and

correlated positively with the vitellogenin percentages in the tested insects.

***P. truncatus* Infestations and Weight Losses of the Maize Varieties**

Insect pest numbers from the varieties resulting in the varying IS corresponded

positively with vitellogenin levels and weight losses of the different maize varieties to *P. truncatus*. The lowest number of F₁ *P. truncatus* from LOCAL 2, the longest developmental period of the insects in this variety as well as the least weight loss show that the variety has the lowest IS and hence the most resistant. Variety ZM 521 with the highest IS had most F₁ individuals emerging within the shortest developmental period and lost the greatest weight. These results support observations by Dobie (1974) and Rugumamu (2005) who reported that susceptibilities of the maize varieties studied and other cereals to the cosmopolitan *Sitophilus zeamais* and *P. truncatus* attack were variable.

Prostephanus truncatus adults and larvae are serious pests as they cause damage culminating in great maize losses basically because of their relentless feeding habit, the presence of biting–crushing mandibles as well as their hypognathous type of head orientation (Chapman 1998, Hill 1987, van Emden 1999). The comparatively lower losses recorded in this study could be attributed to the fact that shelled maize is known to be relatively less attacked by *P. truncatus* and therefore recommended for storage (Hodges *et al.* 1983, Golob 1988, 2002).

Haemolymph Vitellogenin Levels in *P. truncatus* Vitellogenic Females

Adult females that fed on the different maize varieties had varying levels of vitellogenin in their haemolymph. Insects which were reared on ZM 521, the most infested variety had the greatest amount of vitellogenin in their haemolymph while those reared on the most resistant variety, LOCAL 2 had the lowest amounts of vitellogenin.

It is reported that the greater the amount of vitellogenin in an insect the greater the reproductive success of that insect feeding on a specific nutrient (Chapman 1998, Richards and Davies 1977 and Rees 1977). This fact can be explained by the amount of

food available to the insect. It follows therefore that ZM 521 provided adequate nutrition in the shortest period to the insect pest for their vitellogenesis. Wheeler (1996) acknowledged that oogenesis is a nutrient limited process and that female insects are known to reproduce when richly provisioned with nutrients. It is further, acknowledged that the nutritional requirements of an insect affect growth, development and reproduction.

The findings with vitellogenin levels correspond positively with the trend of the indices of susceptibility of the maize varieties to *P. truncatus* and the weight losses. In this regard it is revealed that local maize varieties are more resistant than the improved varieties. It is, however, reported that there is a scarcity of local varieties of crops in Tanzania despite the information that about 90% of the seeds used by rural farmers are local varieties (Guardian 2006). Such a scarcity results in increased insect pest infestations and ultimately the local gene pool for the perpetuation/production of more resistant local varieties will no longer be available and hence a great setback to the smallholder farmers in this country. Given the 20 % agricultural growth target by 2010, (Guardian 2006) the stakeholders should access knowledge on seed storage and sustainability. The availability of local varieties could contribute to the local source of rural agricultural success.

CONCLUSIONS

On the basis of the findings, the effectiveness of *P. truncatus* management technology is revealed by employing the females haemolymph vitellogenin quantities as reflected by the IS and the weight losses of the different maize varieties to the pest. The vitellogenin parameter which is a measure of reproduction potential, ultimately serves to identify and select more resistant varieties of maize. Resistant varieties have best storage characters and hence play a great role in the on-farm storage

pest management by reducing insect pest populations below economic injury levels.

The results corroborate that each maize variety exhibits a particular level of infestation by *P. truncatus*, the varying IS, amount of weight loss and these relate positively with quantities of haemolymph vitellogenin in F₁ vitellogenic females, an indicator of insect reproduction potential. It follows therefore that low indices of susceptibility of resistant varieties culminate to small quantities of vitellogenin in parent insect pests fed on the varieties.

For the foreseeable future, agriculture will continue to be the mainstay of the Tanzanian economy. Given that programmes for breeding crop varieties require enormous investment including time, it could be advisable that in the meantime, there should be collaboration of farmers, entomologists and Farmer Field School trainers (FFSt) through Agriculture Extension Services to minimize insect pest infestations by strengthening the cultivation and storage of the available resistant varieties of maize and other crops.

In the light of these informative results, further research is recommended in the search for more components for IPM at storage level such as testing efficacy of indigenous storage technologies in various regions in this country coupled with establishment of varying resistance of more maize varieties available to smallholder farmers.

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