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Comparative survival of *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Sitophilus zeamais* (Coleoptera: Curculionidae) under large-scale hermetic grain storage conditions

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Abstract

We compared the responses of Prostephanus truncatus (Horn) and Sitophilus zeamais Motschulsky to bio-generated modified atmospheres under hermetic field conditions. GrainPro CocoonsTM of 20-tonne capacity were loaded with bagged maize grain at three different sites. Twenty unsexed adult insects of each species were introduced into separate glass jars containing maize grain and the jars were closed using perforated lids to confine the insects in the jars during storage. The jars were placed at three elevations (Top, Middle and Bottom) inside each cocoon, with five position replicates at each level. Oxygen levels inside the cocoons were measured during the duration of the trials. Destructive sampling was carried out with one Cocoon being opened after 4 (Cocoon A), 8 (Cocoon B), and 12 mo (Cocoon C). The oxygen depletion in the cocoons was proportional to initial insect infestation levels in the stored grain. The lowest oxygen levels recorded were: 3.0% after 123 d in Cocoon A, 3.7% after 143 d in cocoon B and 1.5% after 14 d in Cocoon C. There was an increase in insect numbers in the glass jars with time. Fecundity at the experimental termination was higher for S. zeamais as compared to P. truncatus and there were significant differences (P < 0.05) between the two adult species for both insect numbers and grain powder except in Cocoon C. However, insect feeding-induced grain powder was high in glass jars containing P. truncatus. The highest live insect numbers and grain powder were recorded in Cocoon B for both species, and no live insects were recorded in Cocoon C at the end. The results showed that the efficacy of hermetic storage was hinged upon airtightness and rapid oxygen depletion to effectively suppress insect pest development and insect-induced damage, and to maintain grain quality.

Keywords: Bio-generated modified atmospheres, GrainPro CocoonTM, Oxygen depletion, Hermetic storage, Insect feeding-induced grain powder, Insect fecundity

Introduction

Storage insect pests control strategies in durable commodities have been centred on the use of synthetic pesticides. However, use of this curative method has faced challenges which include but are not limited to development of resistance among targeted species (Gautam et al., 2016), environmental pollution, and user and consumer health hazards (Damalas and Eleftherohorinos, 2011). Growing concerns and increased awareness on deleterious effects of synthetic pesticides on humans and the environment are influencing use of alternative control methods. This has seen an increase in research aligned to biorational control methods (Stevenson et al., 2012), and other non-chemical storage insect pest control options (Navarro, 2012).

Modifying atmospheric gaseous composition in grain storage structures or containers is another non-residual pesticide-free insect pest control option that is being promoted globally as an alternative to synthetic pesticides (Villers et al., 2006; Mvumi et al., 2013). Mode of action (Jay et al., 1971; Murdock et al., 2012; Ahn et al., 2013), performance and limitations (Jayas and Jeyamkondan, 2002; Chigoverah and Mvumi, 2016), types of modified atmospheres (MAs) and suitable facilities are well-documented (Navarro, 2012). Efficacy of MAs is hinged on low gas permeability of storage media which prevent replenishment of depleted oxygen and retention of produced carbon dioxide (Villers et al., 2006). GrainPro Cocoons are among the most common flexible structures being used for storing various dry agricultural commodities by applying the different types of MAs; bio-generated MAs, gas-hermetic fumigation, and vacuum-hermetic fumigation (Villers et al., 2006).

Insect species exhibit variable responses when exposed to MAs, hence it is important to understand the response of individual species to storage conditions to develop with efficient management strategies. Response of various storage insect pest species under hypoxic, hypercarbic and anoxic conditions are well-documented (Jay et al., 1971; Mitcham et al., 2006; Ahn et al., 2013). However, studies on the response of storage insect pest species of economic importance in sub-Saharan Africa like *Prostephanus truncatus* (Horn) (Coleoptera: Brostrichidae) are scarce, especially at commercial level. Moreover, knowledge gaps exist on the comparative responses of stored-maize pest species under bio-generated modified atmospheres. It is in this context that a comparative evaluation of the response of stored-maize insect pest species; *P. truncatus* and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) to bio-generated modified atmospheres was conducted under hermetic field conditions using GrainPro Cocoons.

Methodology

Trial sites and duration

The study was conducted at three Grain Marketing Board (GMB) depots in Zimbabwe; Marondera (mean annual temperature 16.7°C and rainfall 900 mm), Bindura (mean annual temperature 19.4°C and rainfall 847 mm) and Cleveland (mean annual temperature 18.4°C and rainfall 831 mm).

Maize grain and test insects

Freshly harvested untreated hybrid maize grain was supplied by GMB from deliveries made by farmers to the strategic grain reserve agency. The hybrid varieties used in the trial were Pioneer PHB 30G19 in Marondera, Seed Co SC727 in Bindura, and a mixture of varieties in Cleveland whose identity could not be ascertained.

Unsexed 7 to 14 d old adult *P. truncatus* and *S. zeamais* insect species were obtained from an existing culture in the Entomology Laboratory, Department of Crop Science at the University of Zimbabwe. The age of the insect species was determined during laboratory rearing as outlined by Tefera et al. (2010). The existing colony was monitored daily, and emerging insects were transferred over 10 consecutive days into separate jars containing clean disinfested grain until the required numbers were obtained. Insects which emerged on the same day were put in the same glass jar. The emerged insects were then kept under room conditions $(27 \pm 3^{\circ}C)$ and insects which had emerged 7–14 d from the date of the first emergence were used in the trial.

Treatment layout

The trial evaluated the response of two storage insect pest species (S. zeamais and P. truncatus) to bio-generated atmospheres under hermetic field conditions. GrainPro Cocoons™ made from flexible polyvinyl chloride (PVC) with a capacity of 20 tonne were installed; one unit at each of the three GMB depots. A Cocoon consists of a top and bottom section which are brought together and sealed using a double-grooved gas-proof zipper. Each Cocoon was loaded to capacity with maize grain bagged in 50 kg polypropylene bags as described by Chigoverah et al., (2018). During loading, two 450 mL glass jars, each containing 200 g of sterilised maize grain, were artificially infested with 20 unsexed adult S. zeamais and the other jar with 20 unsexed adult P. truncatus. The jars were placed next to each other at each of the three levels: Top, Middle and Bottom layers. At each of the three levels, the pair of glass jars were placed at the four corners and at the centre. Thus, glass jars were placed at 15 positions in each Cocoon. The lids of the jars were perforated to allow free air circulation while restricting movement of insects in and out of the closed jars. Bags were stacked to recommended height and the dimensions of a fully loaded Cocoon were 4.4 x 3.4 x 2 m³ (Length \times Width \times Height). A configured data logger (EL-USB-2, Lascar, Whiteparish, Wiltshire, UK) for measuring temperature and relative humidity was placed at each of the three levels before closing the Cocoons. Each Cocoon was closed by bringing the bottom and top sections together and sealing with the gas-tight zipper.

Sampling and sample analyses

An oxygen analyser (GPO₂-HH, GrainPro Inc, Subic Bay, Philippines) was used for monitoring oxygen levels in the cocoon from the day of setting up and subsequently; daily for the first two weeks after which readings were taken twice per week until the level reached less than 3%. Oxygen monitoring was also carried out at irregular intervals thereafter as a way of monitoring airtightness of the Cocoons.

The glass jars containing artificially infested maize grain were collected at termination of the trial after 4 mo in Marondera, 8 mo in Bindura and 12 mo at Cleveland, Harare. The grain in the collected jars was weighed and sieved (3 mm aperture) to separate whole grain and adult insects. The number of adult insects were counted (live and dead) and the grain powder from the jars was then weighed and recorded.

Data entry and analyses

Data were entered into Microsoft Excel and were subjected to normality tests using the Shapiro–Wilk test and transformed using log (X + 1) for insect count data and arcsine square root for percentage insect-induced grain powder following failure to meet assumptions of normality. GenStat 14 was used to subject the data to ANOVA and the LSD test (P<0.05) was used to separate treatment means to show statistically significant differences.

Results and discussion

Conditions inside installed Cocoons

There was a decrease in oxygen levels in all the Cocoons (Fig. 1). The rate of oxygen depletion was rapid at Cleveland where the lowest oxygen reading of 1.5% was recorded after 14 d. In Marondera, the lowest reading of 3.7% was recorded after 112 d storage and the lowest reading in Bindura was recorded after 143 d storage (Chigoverah et al., 2018). The rate of oxygen depletion was directly proportional to level of insect infestation since insects were known to be the major consumers of oxygen in hermetically stored dry grain (Ahn et al., 2013). An increase in oxygen levels in Cocoons after attaining the lowest value was attributed to low biotic activity which creates a positive oxygen balance resulting in replenishment since rate of oxygen uptake would be less than the ingress oxygen. However, the rapid increase in oxygen readings after 92 d storage at Cleveland was due to an opening that was observed during routine inspections which are recommended given the importance of an airtight seal of the closed Cocoon.

The average conditions in the Cocoons were $19.0 \pm 1.9^{\circ}$ C and $56.3 \pm 0.4\%$ RH, $25.0 \pm 1.2^{\circ}$ C and $53.9 \pm 0.4\%$ RH, and $21.2 \pm 0.6^{\circ}$ C and $53.9 \pm 1.3\%$ RH at Marondera, Bindura, and Cleveland; respectively. The sun-filtering shade pitched over the Cocoon regulated the effect of ambient conditions. The shade reduced the effect of diurnal temperature changes which led to relatively low temperatures during the duration of the trial.



Fig. 1. Oxygen levels inside Cocoons A (Marondera), B (Bindura) and C (Cleveland) installed at three GMB depots (Chigoverah et al., 2018).

Insect Activity

There was an increase in insect numbers for both *P. Truncatus and S. zeamais* in Cocoon A in Marondera and Cocoon B in Bindura, while there were no changes in the total numbers in the Cocoon installed at Cleveland (Table 1). There were no live insects in Cocoon C. This could be attributed to rapid oxygen depletion in Cocoon C where readings of less than 3% were observed after 9 d storage. Previous studies have reported increased insect mortality under hypoxia and/or hypercarbia (Alvindia et al., 1994; Murdock et al., 2012).

The slow rate of oxygen depletion in Cocoon A and B supported insect development leading to an increase in insect numbers in the glass jars stored in the respective Cocoons. The glass jars in the two Cocoons (A and B) had considerable numbers of live *S. zeamais* with more than 130 live adults in glass jars in the Cocoon in Bindura after 8 mo storage. On the other hand, the rate of multiplication of *P. truncatus* in glass jars stored in Cocoon A and B was far less than that of *S. zeamais*. The results showed that *P. truncatus* was relatively more susceptible to modified atmospheres compared to *S. zeamais*. Previous studies have also reported the ability of *S. zeamais* to tolerate hypoxic conditions (Cao et al., 2010). However, literature also reported the lethal result of *P. truncatus* to specified modified atmospheres.

	Cocoon A		Cocoon B		Cocoon C	
Insect	(4 Mo)		(8 Mo)		(12 Mo)	
Species	Total	Live	Total	Live	Total	Live
S. zeamais	$50\pm15.9^{\text{b}}$	19 ± 11.2	380 ± 104.6^{b}	134 ± 52.7	$20\pm0.0^{\rm a}$	0.0
P. truncatus	24 ± 2.7^{a}	0.0	80 ± 17.0^{a}	2 ± 1.2	20 ± 0.0^{a}	0.0
ANOVA	$F_{1.58} < 0.01$	-	$F_{1.58} < 0.01$	-	$F_{1.58} > 0.05$	-

Table 1. Comparative mean insect numbers (±SEM) in glass jars placed in GrainProCocoons at three Grain Marketing Board depots (n = 15).

*Means within a column for each site were compared and separated using LSD test (P<0.05) and different alphabetical letters indicate significant differences

Insect feeding-induced grain powder

Initially there was no grain powder in the glass jars. Insect feeding-induced grain powder was recorded in all the glass jars at termination. The glass jars in Cocoon B had the highest amount of grain powder followed by Cocoon A and Cocoon C which had the least values (Table 2). The trend could be attributed to the total insect numbers in the respective jars. Insect feeding-induced grain powder is highly correlated to insect population (Chigoverah and Mvumi, 2016). Even though there were significant numbers of insects in the glass jars, the amount of powder produced was low in relation to the numbers. This could be attributed to the antifeedant effect of MAs (Murdock et al., 2012). Glass jars containing *P. truncatus* had the most grain powder at all sites despite having lower insect numbers than those recorded in glass jars containing *S. zeamais*. While for other storage insect pests, feeding accounts for most grain losses, *P. truncatus* adult tunnelling accounts for as much as four times as the destruction due to both larval and adult feeding activities (Demianyk and Sinha, 1988). The results showed that under efficient bio-generated modified atmospheres, the severity of insect-induced damage could be suppressed thereby reducing storage losses.

Table 2. Comparative mean insect feeding-induced grain powder (± SEM) in
glass jars containing artificially infested maize grain placed in
GrainPro Cocoons at three Grain Marketing Board depots (n = 15).

	Marondera	Bindura	Cleveland
	(4 Mo)	(8 Mo)	(12 Mo)
Insect Species	Grain powder (g/kg)	Grain powder (g/kg)	Grain powder (g/kg)
S. zeamais	7.2 ± 3.22^{a}	88.5 ± 32.58^a	$0.5\pm0.12^{\rm a}$
P. truncatus	$22.7\pm4.11^{\text{b}}$	96.2 ± 37.23^a	$1.6\pm0.20^{\text{b}}$
ANOVA	$F_{1.58} < 0.01$	$F_{1.58} > 0.05$	$F_{1.58} < 0.01$

*Means within a column for each site were compared and separated using LSD test (P<0.05) and different alphabetical letters indicate significant differences.

Conclusions

The study showed that the two primary storage insect pests were relatively susceptible to biogenerated modified atmospheres under field commercial conditions. The results support the promotion of GrainPro Cocoons in African countries where *P. truncatus* and *S. zeamais* are pests of economic importance. However, there is need to generate more data on the response of *P. truncatus* under different types of MAs (bio-generated, gas-hermetic fumigation and vacuumhermetic fumigation). There is also a need to ascertain durability of Cocoons under incoming *P. truncatus* infestation rather than just focussing on resident infestation.

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