

TPRI ON-STATION STORAGE TRIAL TO EXPOSE DE TREATED MAIZE GRAIN TO HIGH *PROSTEPHANUS TRUNCATUS* (LGB) PRESSURE

Introduction

Following two field seasons of storage trials in five sites in Tanzanian using diatomaceous earths (DE) as grain protectants (see <http://www.nri.org/de/> for further information), where the DEs effectively protected grain against naturally occurring insect damage for storage periods of >10 months at all sites (with the exception of the Arri village maize trial in 2003/4). The project team feels it would be useful to run an additional on-station trial in which *P. truncatus* are actually artificially introduced to the trial in order to feel secure that in the presence of a 'high' LGB year the DEs would still provide adequate protection for farmers' grain. It should be noted that several laboratory studies with the DEs Protect-It and Dryacide at a range of concentrations have been conducted from 1997 - 2001 in order to understand more about what concentrations of DEs were needed for admixture with grain in field trials in areas with LGB (Stathers, Mvumi & Golob, 2002¹, Stathers, 2003²; Stathers, Denniff & Golob, 2004³). This document describes a trial protocol to 'double' check that the DE rates we are testing are effective against LGB attack even during a 'high' LGB incidence year, the fact that the field trials for the last two seasons have performed so well suggests that the levels of DEs being used are either repelling LGB from entering the DE treated grain sacks or kihenges or kill those LGB that do enter preventing their populations from building up to damaging levels. In one site during the 2003/4 season all the treatments except the Actellic Super dust and the DE treatments were heavily attacked by LGB by the end of the season, even though the sacks were stored directly on top of each other high numbers of LGB had not entered the DE treated sacks.

Maize grain:

Source: Uninfested, non pesticide treated freshly harvested, well dried and winnowed maize, (check and record the %mc).

Quantity: 400 kg of maize required for the trial (so probably wise to purchase 420kg to be sure we have enough in case some of it needs winnowing still)

Note: all the grain must be thoroughly mixed on a clean surface in case it comes from different sources.

Preparing the grain:

Equipment required: 20 new sacks the normal type farmers use to store maize, **not** the small flour bags which have plastic layers inside.

Marker pen to label the bags

Shovel to mix the grain and to admix the treatments

Water to clean shovel and hands etc between admixing the different grain protectants to prevent contamination

Plastic sheeting for admixing grain and protectants on – separate sheets will be needed for each protectant treatment to avoid

¹ Stathers, T.E., Mvumi, B.M. and Golob, P. 2002. Can Diatomaceous Earths have an Integrated Role in Small-Scale Tropical Grain Storage? Proceedings of the conference of the IOBC/WPRS Working Group Integrated Protection of Stored Products, September 2-5, 2001, Lisbon, Portugal. IOBC Bulletin, 25, 245-251.

² Stathers, T., 2003. Combinations to enhance the efficacy of diatomaceous earths against the larger grain borer (*Prostephanus truncatus*). In: P.F. Credland, D.M. Armitage, C.H. Bell, P.M. Cogan and E. Highley (eds.) Advances in Stored Product Protection, Proceedings of the 8th International Working Conference on Stored Product Protection, July 2002, York, UK. CAB International, UK. 925-929.

³ Stathers, T.E., Denniff, M. and Golob, P. 2004. The Efficacy and Persistence of Diatomaceous Earths Admixed with Commodity against Four Tropical Stored Product Beetle Pests. *Journal of Stored Products Research*, 40(1), 113-123.

contamination

String and needle for sewing up sacks

Storage shed

Plastic bags for taking 500 g grain samples (note: 5 treatments * 4 replicates means that 20 samples will be taken for laboratory analysis at each sampling period, including trial set up)

Small wooden platform to prevent grain sacks from being stored on the concrete floor.

Scales for weighing batches of 20 kg of grain

Masks, anyone admixing grain protectants should wear a mask

Grain preparation: If the grain was not winnowed before purchase it must now be winnowed and then all of it should be thoroughly mixed together to try and ensure homogeneity.

Then fill 20 unlabelled sacks with 20 kg of grain each

Experimental design

Treatments: 5

Replications: 4

Layout: 5 treatments each to be replicated 4 times, and then arranged in randomised block design for the course of the trial (see figure 1 below for details of the randomised block design)

Figure 1. Plan for randomised block design layout of the trial

C - 1	B - 1	A - 1	E - 1	D - 1
A - 2	B - 2	E - 2	C - 2	D - 2
E - 3	C - 3	D - 3	B - 3	A - 3
A - 4	D - 4	C - 4	B - 4	E - 4

Note: C - 1 = Replicate 1 of treatment C (Protect-It 0.1%w/w/ plus permethrin)
D - 3 = Replicate 3 of treatment D (Actellic Super dust)

Treatments:

The treatments to be trialled are as follows (please note codes will be used to try and prevent bias during sampling), treatments will be provided by Mr Riwa:

- A - Protect – It 0.1% w/w (20g Protect-It / 20 kg of maize grain)
- B - Protect – It 0.25% w/w (50g Protect-It / 20kg of maize grain)
- C - Protect – It 0.1% w/w **plus** permethrin at 2mg/kg pyrethroid dust (20 g Protect-It **plus** 2g of 2% a.i. permethrin dust / 20kg of maize grain)
- D - Actellic Super dust at recommended applicn. rate (22.22g Actellic Super dust/ 20 kg of maize grain)
- E - Untreated control (20 kg of maize grain)

Weigh out treatments prior to admixing with grain

Mixing grain with the treatments (see pictures below):

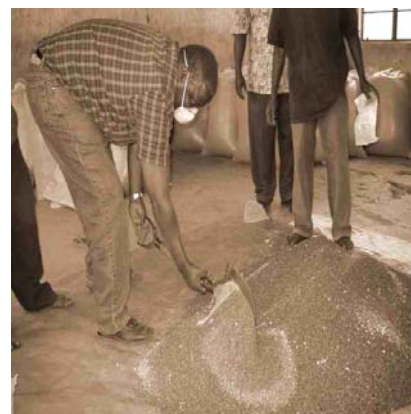
1. Make sure admixture of grain with the treatment is done in a calm sheltered place with no wind and a smooth floor.
2. Sweep the floor and surrounding area to remove dust and debris.
3. Lay down clean plastic sheeting (appropriate to each protectant, DO NOT USE the same piece of plastic for different protectants as this will contaminate the experiment)
4. Make sure shovel is clean
5. Put on mask
6. Empty a randomly chosen pre weighed 20kg sack of grain onto the plastic sheet
7. Sprinkle the correctly weighed quantity of each treatment (see treatments above) in a circle on the surface of the mound of grain
8. Gently use the shovel to move the grain up so that the protectant is covered by grain.
9. Then admix the grain and protectant by gently shovelling the grain to one side to form a new pile of grain (1st mixing).
10. The new pile of grain is then shovelled to the other side to form another pile (2nd mixing)
11. The pile is then shovelled to one side again (3rd mixing)
12. It is at this stage a sample of the admixed grain can be taken using the shovel it should be gently poured into the plastic bag (~500g) – this sample should then be carefully labelled with the treatment and rep code. Note future sampling will be done using a bag spear.
13. Clearly label the empty grain sack with the treatment and replicate number code (e.g. A-1), the date of admixing, and if wished the details of the treatment in Swahili (e.g. 20g za Protect-It kwa 20kg za mahindi) in order to make visiting the trial more interesting for outsiders. Ask someone else to check each label is correct to prevent any mistakes.
14. Gently return the grain to the labelled sack
15. Sew up the sack and place it to one side
16. Repeat the above for each treatment, remembering that although the untreated control grain replicates do not need to be admixed with a protectant, samples of each of them still need to be taken for laboratory analysis.
17. When all sacks have been treated and labelled, they can be carefully transported to the storage facility which should have been cleaned in advance and a large enough platform placed in it to allow the treated sacks to be laid out as per the experimental design above.



Place grain on clean surface



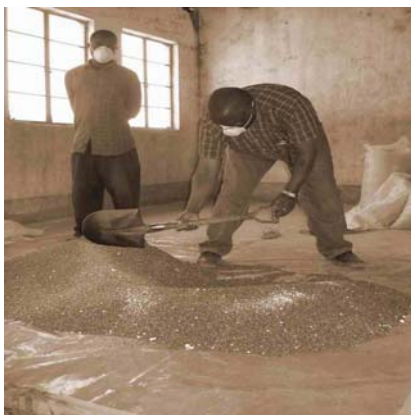
Sprinkle DEs on grain, then gently cover the DE with grain



Mix DEs in by creating a new pile



Mix DEs again into a second pile



Mix again a third time



Return to bag



And, sew up and store

Laboratory analysis:

In order to analyse the efficacy of each treatment over the storage period, regular sampling of each of the sacks is necessary and these samples then need to be taken to the laboratory and carefully analysed.

At each sampling period you will have 20 sample bags of code labelled grain (each containing ~500g).

Make sure the area you are working in is clean.

- Equipment needed:
- Data sheets (see example data sheet at end of this document)
 - Scale for weighing grain sample
 - Stack of grain sampling sieves
 - base (to catch dust and very small insects such as parasitic wasps, psocids etc),
 - small aperture sieve (to catch storage insects such as Sitophilus, Prostephanus truncatus etc),
 - larger aperture sieve (to catch maize grain only)
 - lid (be careful that this does not get full of water)
 - Grain sample divider
 - Trays for counting grains and insects on
 - Forceps for separating insects
 - Glass jars for counting insects into
 - Small plastic boxes for holding insects and weighing sub samples of grain
 - Handheld tally counter
 - Pens for recording data
 - File for safely storing data in
 - Waste bucket for placing analysed grain samples in.

Laboratory analysis procedure:

1. Write sample code and sample dates on top of sample analysis data form (note each sample will have a separate data sheet)
2. Weigh grain sample in bag, and record weight on data sheet
3. Pour sample into top of sieve stack and shake (side to side) the sieve for a couple of minutes to remove any insects from the grains and to enable them to drop down into the middle sieve layer.
4. Place sieve stack on to table for a minute to allow the dust to settle before opening the sieve stack.
5. Weigh the empty bag and record its weight on the data sheet
6. Open the sieve stack and check the bottom (base) layer for insects, if insects are present either pick them out directly from the base sieve and record the number of each species on the data form, or gently pour the dust and insects onto a tray and then separate out the insects and record the number of each species on the data sheet.
7. Weigh the trash/dust and record on the sample sheet, the trash/dust can then be disposed of
8. Pour the insects into a contained, if there are many it is not possible to count them all before they crawl or fly off the tray so pour just a few at a time onto the tray, separate them into the different species and count and record the numbers of each species until all insects in the sample are recorded.
9. Divide the sieved grain in the top layer of the sieve, using the sample divider to obtain three sub samples
10. Pour each sub sample onto a separate tray and then check each grain for storage insect damage, put damaged grains in one corner and undamaged grains in the other corner until the whole sub sample has been checked, then count the number of damaged and undamaged grains and record on the data sheet, then weigh the damaged and then the undamaged grains and record on the data sheet.
11. Do the same for each of the three sub samples.
12. Check the data sheet has been completed correctly, and then return the sample to its bag and place to one side away from the other samples.
13. Trays and forceps should be cleaned between samples.
14. Masks and gloves should be worn for sample analysis.
15. Once all samples have been analysed and all data sheets have been rechecked and carefully filed, the samples can be disposed off.

Data recording

A copy of the data sheet is given on the following page. 20 photo copies of this sheet will be needed at each sampling. Please make sure writing is legible and data sheets are carefully stored in a file.

LGB SEEDING OF DE TREATED MAIZE SAMPLES DATA SHEET

1. Sample code:
2. Sampling date:
3. Date of sampling analysis:
4. Mass of sample (g)
5. Mass of empty bag (g)
6. Mass of trash (g)

7. Number of insects:

Insect species	Live	Dead
Prostephanus truncatus		
Sitophilus		
Tribolium castaneum		
Rhyzopertha dominica		
Cryptolestes ferrugineus		
Sitotroga cerealella		
Corcyra cephalonica		
Plodia interpunctella		
Parasitic wasps		
Teretrius nigrescens		
Oryzaeophilus		
Others (specify):		

8. Damage assessment:

SUBSAMPLE A	Damaged grains	Undamaged grains
Number		
Mass (g)		

SUBSAMPLE B	Damaged grains	Undamaged grains
Number		
Mass (g)		

SUBSAMPLE C	Damaged grains	Undamaged grains
Number		
Mass (g)		

Data recorded by: