CROP POST HARVEST PROGRAMME

Project Title: To determine the potential of *Bacillus thuringiensis*, for the control of a range of insect pests of stored products in the tropics

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FINAL TECHNICAL REPORT

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Summary

The major aim of tbe project was to determine the potential of the insect pathogen *Bacillus thuringiensis* (Bt) for the control of a range of stored product pest species. To identify strains of Bt with activity, previously characterised strains were tested against four major coleopteran storage pest species. New strains of Bt were also isolated from previously unexamined tropical stored product environments and tested for activity. A total of 47 bacterial strains were tested, although disappointingly none were found to cause significant mortality to any of the pest species.
Three commercial Bt products were also tested for activity against two storage moth pests. All three products were active to both pest species with the Bt product Agree of greatest potency. The results confirm the potential of Bt for the control of stored product moth pests, highlighting the use of Agree as an improved Bt product.
A new group of insect toxins produced by the bacterial genera *Xenorhabdus* were also tested for coleopteran activity. Preliminary data on the toxins, has shown them to have activity to *P. truncatus, T. granarium* and *L sericorne*. These results although preliminary, are of interest as this is the first time a class of protein toxins has been shown to have significant activity to this group of coleopteran pests. However, although these toxins may have great potential, unlike Bt, the technology to develop them for use in crop protection would require more detailed strategic research.

Background

Substantial amounts of stored food are lost in the tropics through the action of insect pests. Increasing concerns over human health, pesticide residues and increasing insect resistance drives the need for safe, non-toxic alternatives. One such alternative is the use of the insect pathogenic bacterium *Bacillus thuringiensis* (Bt). Due to the high activity, specificity to target pest species and non-toxicity to humans, strains of *Bt* are widely used in pest control. Annual usage of Bt is approximately 5,000 tonnes, used mainly on field crops for the control of Lepidoptera. Therefore the major aim of this short 6 month project was to determine the potential of Bt for use to control a range of major tropical pest species in stored product environments.

Strains of Bt have been shown to produce a number of insect toxins, which have good activity to pest species belonging to the orders Lepidoptera, Diptera and Coleoptera (Entwistle, et al . In, Bt an Environmental Biopesticide. 1993, pub. Wiley, UK). Recent studies have also shown Bt to be widely distributed in the environment (Bernhard, et al. 1997, Jour. Invert. Pathol.70, 59-68) and to produce a greater range of toxins than was initially thought, highlighting the potential for wider use of these bacteria in pest control. However, although considerable data has been published on the effective use of Bt on fieldcrops, relatively little work has been published on the use of Bt for the control of stored product pest species. The most detailed studies published have been on the lepidopteran pests *Ephestia cautella* and *Plodia interpunctella,* where both species were shown to be effectively controlled with commercial Bt products (Mc Gaughey, 1980. Can. Ent. 112, 327-331). Published studies on the control of coleopteran pest species with Bt crystal toxins are very limited with the most detailed studies by Beagle (1996. Biocontrol Sci. Tech. 6, 15-21) and Mummigiatti *et al* (Proc. 6th Int. Working Conf. on Stored product

Protection. Vol 2) showing strains of Bt had some effect on *Rhyzoertha dominica,* although only at high uneconomic doses.

In recent studies at HRI we have collected and characterised 6000 strains of Bt, isolated from a wide range of environments, including extensive product warehouses in the UK (Meadows *et al* 1992. Appl. Environ. Micro. 58, 1344-1350). All of the strains have been tested against a range of pest species and a number found to have activity to the coleopteran pest species *Phaedon cochleariae, Diabrotica virgifera* and *Lasioderma serricorne*. Although activity to the latter two was found to be low. Using these characterised Bt strains it is planned to selectively test a number of key coleopteran pest species to try and identify strains with potential for use in insect control in tropical stored product environments.

Project purpose

To determine the pathogenicity of a range of coleopteran and lepidopteran active Bt strains to a number of major stored product pest species. The isolation of new Bt strains from heavily infested tropical stored product environments.

Research Activities

Bacterial strains: Strains of *Bacillus thuringiensis* (Bt) were selected for testing from the HRI bacterial culture collection on the basis of known coleopteran activity, to the pest species *Diabrotica virgifera, Lasioderma serricorne* and *Phaedon cochleariae*. The list of strains and strain information is listed in Appendix 1. A *Brevibacillus laterosporus* strain 53694 was also included in the studies as it had been reported (US patent 5055293) to have coleopteran activity.
The commercial products Dipel and Xentari were obtained from the production companies Abbott laboratories (Chicago, USA) and Agree from Novartis (Greensboro,USA). See commercial literature on two of the products in Appendix 8.

Culture and formulation of bacterial strains: All isolates (47) were cultured in a cottonseed flour based, Proflo B4 media. During growth, the cultures were microscopically examined, harvested by centrifugation and formulated by freeze-drying . (Appendix 2)

Sample collection: Collection kits, consisting of an import licence, numbered 10ml tubes, sampling information and recording forms were given to NRI staff to obtain samples of African stored product material.

Bt isolation: All returned samples were examined for the presence of Bt using a simple pasteurisation step and plating method as outlined in Appendix 3. From the resulting bacterial growth, all *Bacillus cereus*/*B. thuringiensis* type colonies were microscopically examined for the presence of parasporal crystals.

Insect cultures: All cultures and coleopteran insect food, were supplied by Lucy Birkinshaw of the Natural Resources Institute (Greenwich University). Insect species and cultural conditions are given in Appendix 4.

Development of insect bioassay systems: Specific screening bioassay systems were developed for each pest species in collaboration with Lucy Birkinshaw (NRI), reflecting the feeding behaviour and biology of each species. See Appendix 5.

Outputs/Results

Sample collection and strain isolation: A new import licence was obtained by HRI, to allow the transfer of stored product material into the UK. From the kits provided, a total of thirty samples were obtained from a range of African stored product environments. From the thirty samples examined, 7 new Bt strains were isolated. As the isolation rate of Bt was lower from these samples than expected, 14 previously un-tested Bt strains isolated from African stored product environments were also cultured and included in the testing programme. (see Appendix 1)

Bioassay design: In collaboration with the Natural Resources Institute, bioassay systems for testing all bacterial preparations against the coleopteran pest species *Callobruchus maculatus, Prostephanus truncatus, Trogoderma granarium* and *Sitophilus oryzae* and the lepidopteran pests *Plodia interpunctella* and *Ephestia cautella* were designed. Due to the different biology and feeding behaviour of the test insects, initial experiments were performed to develop suitable and practical assay systems for each pest species. The assay systems also had to reflect the fact that Bt only acts as a stomach poison and has to be ingested. Therefore, for the three species *C. maculatus, P. truncatus* and *S. oryzae,* where the larval stages only feed protected within the seed an adult feeding bioassay system for each was developed.
(See appendix 5 for detailed assay methods)

Bioassay results: A total of 46 Bt strains and one *B. laterosporus* strain were cultured, formulated and tested for activity against the full range of coleopteran pests. A detailed summary of all bioassay result is given in appendix 6.1. Disappointingly, no strains were identified with significant coleopteran activity at the dose rates used.

Three commercial Bt products were tested for activity against the stored product moth pests *E. cautella* and *P. interpunctella*. Both pest species were susceptible to all three products, with one of the newer products Agree, being the most active. The LC50 values for *P. interpunctella* were 2.93 μg of Bt per grm of diet for Agree, 31.87 for Dipel and 150.8 for Xentari. See Appendix 6.2 for full details.

Contribution Outputs/Conclusions

All the project outputs were achieved. However, no putative coleopteran active Bt strains or the new coleopteran active *B. laterosporus* strain were found to have significant activity to the range of coleopteran pest species tested. This was always a potential possibility (as outlined in the project proposal) as it is widely recognised that Coleoptera are less susceptible to Bt than Lepidoptera or Diptera. A number of Bt strains were also isolated from insect infected African food stores. However when tested, all 21 of the new strains were inactive to *T. granarium* at the dose rates used.

From the combined studies, it can be concluded that none of the 47 bacterial strains tested have immediate practical potential for use in stored product pest control. Particularly as the dose rates used (1mg per grm of food) in all the tests would be considered uneconomic for mixing into grain stores. However, these finding do not preclude the fact that some of the strains may have an effect at higher dose rates and contain genes useful in the construction of transgenic plants, where it is possible to target higher toxin doses to the pest.

Studies to evaluate the use of three commercial Bt products to control *P. interpunctella* and *E. cautella* showed both species to be susceptible to all three Bt products. Laboratory bioassays also showed Agree to be the most active product to *P. interpunctella*, being at least 15 times more active than Dipel and Xentari. These results confirm the potential of Bt for the control of stored product moth pests, highlighting the use of Agree as an improved Bt product. Also, Agree contains a different combination of toxins to Dipel and would be a useful alternate product for use in resistance management.

In addition to the targets and milestones of the project, a group of new insect toxins produced by the bacterial genera *Xenorhabdus* were also tested for activity to a range of stored product pests. Preliminary data on the toxins, has shown them to have activity to *P. truncatus, T. granarium* and *L. sericorne* (see appendix 7). These results although preliminary are of interest as this is the first time a group of protein toxins to have shown significant activity to this group of coleopteran pests. However, although these toxins may have great potential, unlike Bt, the technology to develop them for use in crop protection would require more detailed strategic research.

Acknowledgements

I wish to thank Dr Lucy Birkinshaw of the Natural Resources Institute for her collaboration throughout the duration of the project and Debbie Ellis of HRI for her excellent scientific input and coordination of the project.

Appendix 1

List of strains used in test

Putative coleopteran active and commercially used strains

CODE	ORIGINAL SOURCE	ACTIVITY *Pc*	*Dv*	*Ls*	COUNTRY OF ORGIN
B310	HRI – Bt collection		+		
M549	HRI – Bt collection				Ghana
M817	HRI – Bt collection	+	+		UK
M841	HRI – Bt collection	+	+	+	UK
M859	HRI – Bt collection		+		UK
Bt.t.	Standard strain	+		+	Germany
EG5144	Commercial strain	+	+	+	USA
GC91	Commercial strain				UK
HD-1 1971	Standard strain				USA
HD 200	Dulmage collection*	+			
HD 537	Dulmage collection*	+			USA
HD 867	Dulmage collection*	+			
IPS 82	Standard strain				Israel
M1693	HRI – Bt collection		+		USA
M1774	HRI – Bt collection	+	+		UK
M1853	HRI – Bt collection	+		+	UK
M1896	HRI – Bt collection	+			UK
M2369	HRI – Bt collection		+		USA
M3358	HRI – Bt collection		+		USA
M3452	HRI – Bt collection		+		Iceland
M3551	HRI – Bt collection	+	+		UK
M3604	HRI – Bt collection		+		UK
M3734	HRI – Bt collection		+		UK
M3816	HRI – Bt collection	+			UK
M3820	HRI – Bt collection	+		+	UK
M3829	HRI – Bt collection	+			UK
Brevibacillus laterosporus 53694		+			USA (BGSC, Ohio State Univ)

* Cultures originally from USDA, ARS, Perria, USA
\+ Active >25% mortality

New isolates and un-tested African stored product strains

N1	HRI – Bt collection	New isolate from this study, Zimbabwe, Binga
N2	HRI – Bt collection	New isolate from this study, Zimbabwe, Binga
N3	HRI – Bt collection	New isolate from this study, Zimbabwe, Binga
N4	HRI – Bt collection	New isolate from this study, Zimbabwe, Binga
N5	HRI – Bt collection	New isolate from this study, Zimbabwe, Buhera
N6	HRI – Bt collection	New isolate from this study, Ghana, Nanton
N7	HRI – Bt collection	New isolate from this study, Ghana, Nanton
M4626	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Harare
M4717	HRI – Bt collection	Untested African stored product isolate, Zimbabwe
M4736	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Harare
M4737	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Wedza
M4738	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Wedza
M4740	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Harare
M4742	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Wedza
M4748	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Zimuto
M4840	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Harare
M4842	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Wedza
M4843	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Wedza
M4844	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Wedza
M4895	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Zimuto
M4946	HRI – Bt collection	Untested African stored product isolate, Zimbabwe, Zimuto

Commercial Bt products.

Dipel™ is the most commonly used Bt product and is based upon the serotype 3a 3b strain HD1. It has been used commercially for over 30 years. See enclosed commercial literature (Abbott laboratories USA).

Agree™ is a fairly recent product based upon a transconjugant strain coded GC91. The strain has a different host range and toxin content to Dipel. See enclosed commercial literature (Novartis, Ciba, USA).

Xentari™ is a relatively new product and has a different host range and toxin content to Dipel (Abbott Laboratories, USA).

Appendix 2

Growth of isolates for bioassay

All strains from -70°C stored stock cultures, were first grown for 24 hours at 30°C on nutrient agar plates, for use as seed cultures and to check for contamination.
Bacteria were cultured in Proflo B4 broth (Dulmage et al 1970. J. Invertebr. Pathol.15:15-20). 10.0 Proflo, 2.0 Peptone, 2.0 Yeast Extract, 1.0 $CaCO_3$, 0.3 $MgSO_4$.7H2O, 0.02 FeSO4. 7H2O, 0.02 MnSO4 (g per Lt). To provide bacterial powders for insect bioassay, each strain was grown at 26°C in 4, 250 ml fluted flasks each containing 50 mls of B4 broth. During growth, the cultures were microscopically examined and harvested by centrifugation when 95% of the population had lysed, releasing the spore and crystal, usually after 48 to 72 hours. Culture pellets were then washed by centrifugation and finally resuspended in 30 mls of 5% lactose solution prior to preservation.
To dry the samples, they were first frozen at -20°C and then placed in a pre-cooled Edwards Super Modulyo freeze dryer. The tops of the containers were removed and re-sealed using a tissue and elastic band (this prevents the powder escaping from the container on drying caused by static). The samples were allowed to dry over a two-day period at -60°C. On removal from the dryer a "wafer" of powder is produced which was broken down using a spoon spatula to a fine friable powder. The powder was then transferred to a sterile plastic universal, weighed and sealed with parafilm. All powders were kept at 4°C in a dessicator containing silica gel.

The same method was used for production of all powders.

Appendix 3

Method of Bt isolation from stored product samples

To isolate Bt approximately 0.5 grms of each sample was suspended in 10 mls of sterile de ionised water, and mixed by vigorous vortexing for 1 minutes. After mixing the solid material was allowed to settle for 30 seconds. One ml of the supernatant was then removed and pasteurised at 70ºC for 10 mins in a pre warmed 20ml glass universal bottle to kill non spore-forming bacteria. The heated samples were then plated at two dilutions (neat and 10^{-1}) onto nutrient agar (Oxoid). Plates were incubated at 30°C for 48 hours and the *Bacillus cereus/B. thuringiensis* like colonies microscopically examined. The numbers of such colonies varied considerably from each sample, however a minimum of 20 representative colonies from each sample was examined. Isolates, which had typical *B. cereus* morphology and produced parasporal crystals were classified as *B. thuringiensis*. Only colonies from each sample showing a different crystal morphology, shape or size were stored at -20°C, grown up and used for bioassay.

Appendix 4

Insect stocks

All insect species and food were supplied to HRI by the Natural Resources Institute.

At HRI, all stocks were maintained at 3O°C .

Species	Culture food
Insects maintained at HRI for testing	
Callobruchus maculatus	Cowpea
Prostephanus truncatus	Maize
Trogoderma granerium	Whole meal flour
Sitophilus oryzae	Wheat
Supplied as eggs for bioassay	
Ephestia cautella	Wheat flour : glycerol : yeast
Plodia interpunctella	

Appendix 5

Development and methods used for insect testing

Coleopteran assays

In preliminary tests to ensure adequate coverage or mixture of the Bt powder was obtained in the assays, an initial study was made using the various grains, flour and charcoal powder. Using black charcol powder (being of a similar consistency to the powder) it was possible to visualise the degree of mixing obtained. Coverage of all grain types and mixture into the flour appeared to be consistent throughout the samples when a rolling, twisting motion of the container was used for 120 seconds. Therefore we could ensure thorough mixing of the powder in all the assays.

Callobruchus maculatus

Control of the larval stages of this insect is difficult due to its behaviour: eggs are laid on the surface of the bean and once hatched neonates immediately burrow into the bean where the life cycle is completed. Adults emerge from the hollowed out bean to continue the life cycle. It should also be noted that these adults are short lived and the state of the adults used in the assays unknown. The assay system used was as follows:-

Adults were first collected from stock jars, after treatment with CO_2 to knock them out. They were held in a sealed flask whilst samples were prepared.

For each strain, 5mg of Bt powder was added to 5g of cowpea seed contained in glass universal bottles. Each strain was tested in duplicate. To incorporate powder as uniformly as possible, the universals were rolled and twisted for 120 seconds to achieve a good uniform coverage of the seed. Due to the static state of some of the powders some residue was found to stick to the glass container. However, this was thought to represent less than 10% of the powder added. Universal bottles containing untreated cowpea seeds only were used as controls.

To each universal bottle 10 unsexed adults of unknown age were added. Containers were sealed with a layer of thick paper (kimwipe) tissue and an elastic band.
The assay was incubated at 30°C in the dark for two weeks before adults were removed, recording live and dead individuals. Beans were also assessed for damage and the numbers of eggs laid. The subsequent generation of adults emerging from the seeds were also counted approximately one month later.

Prostephanus truncatus

Control of the larval stages of *P. truncatus* is difficult as development occurs within the grain. Therefore a bioassay using adults was developed along similar lines to that for *C. maculatus.* For each strain, 5mg of *Bt* powder was thoroughly mixed into 100mg of maize flour and added to 5g of maize kernels contained in a glass universal bottle. All tests were performed in duplicate. In a previous experiment it was shown that *Bt* powders did not "stick" very well to the maize kernels, so the addition of the maize flour was used as a carrier. To incorporate the powder as uniformly as possible each container was rolled and twisted for 120 seconds. Experiments using maize kernels plus maize flour only, were set up as controls. To each universal, 10 unsexed adults of an unknown age were added. Universals were sealed with thick tissue (kimwipe) and an elastic band. The assays were incubated in the dark at 30°C for 11 days before the adults were removed and mortality recorded. A further assessment was made after 6 weeks, counting the numbers of developing larvae and adults.

Sitophilus oryzae

Control of the larval stages of *S. oryzae* is difficult as development occurs entirely within the grain. Therefore a bioassay method was used to look at the effect of the various powders on the adults and their subsequent progeny. Therefore the following method was used: -

For each strain, 5mg of Bt powder was added to 5g of wheat grain contained in glass universal bottles. Each strain was tested in duplicate. To incorporate powder as uniformly as possible, the universals rolled and twisted for 120 seconds to achieve a good uniform coverage of the seed. Controls were set up using untreated wheat grain only. To each universal bottle 10 unsexed adults of unknown age were added. Containers were sealed with a double layer of netting and an elastic band. The assay was incubated at 30°C in the dark and the adults removed after 6 days recording mortality. The treated wheat was incubated for a further 8 weeks to determine subsequent development of next generation larvae and adults.

Trogoderma granerium (neonate assay method)

Collection of larvae
To collect neonate larvae, stock jars were sieved through a range of mesh sizes to separate grain, adults, larvae and fine flour etc. The small larvae trapped between 30 and 80 gauge sieves were collected using a fine paintbrush with the aid a binocular microscope.

Assay method
For each strain 20mg of Bt powder was added to 10g of wholemeal flour contained in a glass universal bottle. The bottle was then rolled and twisted for 2-3 minutes to fully incorporate the powder into the insect food. After mixing half the treated flour was poured into another bottle to be used as a replicate of the same treatment. To each bottle 10 small larvae were added. To aid in handling of the larvae, when collected they were placed into a small black weighing boat (to allow the white bodies to be seen on the dark background) and added to the top of the flour. Each universal was sealed with a filter paper secured with sticky tape and incubated at 30°C in the dark.
The assay was assessed for larval survival by counting the number of adults that appeared on the surface of the flour. The numbers emerging from the treatments were compared to controls containing no Bt.

Lepidopteran assays

Plodia interpunctella and *Ephestia cautella*

The assays were performed for both species, by incorporating known amounts of Bt powder into the insect food, which consisted of wholemeal flour, glycerol, yeast and water in the ratio of 12**:**2**:**0.7**:**0.3 volumes. For bioassays, each powder was thoroughly mixed into 1000µl of deionised water, diluted as appropriate and used in place of the water (300µl of powder suspension per 14.7 grms of food) in preparing portions of the artificial food. For each concentration the 15grm portions of food was equally divided into two, 4.5 cm diameter pots into which 10 larvae were added. For neonate assays mortality was recorded after 14 days at 30^{o}C and for 7 day old larval assays they were recorded after 7 days at 30^{o}C.

Bioassay containers and assay set up for coleopteran pests

Bioassays in CT room

Handling room - bioassay set-up

Stored product pest bioassay system

Appendix 6

Bioassay data

6.1 Coleopteran results

	Callobruchus			***Prostephanus***		***Sitophilus***		***Trogoderma***	
	1st gen	2nd gen adults after 6 weeks		1st gen	2nd gen	1st gen	repeat assay		
	eggs	live/dead	eggs	live/dead	adults/larvae	live/dead	live/dead	adults after 8wks	
CODE									
Cont	+++	4/82	++++ h	44/1	7/6	44/6	1/59	22/40	32/40
B310	+	0/53	++++ h	19/0	4/7	16/4	0/20		15/20
M549	+	0/8	+ h	20/0	6/1	16/4	0/20	9/20 *	
M817	-	14/30	++++ h	17/2	2/1	11/9	0/20	8/20	
M841	+	0/38	+++ h	18/2	2/3	10/10	0/19		15/20
M859	++	0/25	++ h	20/0	4/0	12/8	2/18		13/20
Bt t	+	0/32	+++ h	18/0 *	0/0	12/10	0/20	14/20	
Brevi lat	+	0/28	++++ h	20/0	0/2	8/15	0/20	7/20 *	
EG5144	- *	0/0	-	18/1	2/0	12/8	0/22	14/20	
GC91	++	0/17	+++ h	18/2	2/4	19/1	0/20		15/20
HD-1 1971	- *	0/0	-	20/0 *	0/0	8/13	0/20	9/20	
HD-200	+	9/23	+++ h	14/5	7/1	11/9	0/25	6/20	
HD-537	++	0/19	+++ h	20/0	7/0	17/3	1/19		17/20
HD-867	+	1/9	+ h	20/0	2/2	17/1	1/19	8/20	
IPS 82	+	2/52	++++ h	13/5	0/7	11/8	0/20		13/20
M1693	++	0/27	+++ h	19/0 *	0/0	10/11	0/20		14/20
M1774	+	20/42	++++ h	15/4	3/0	11/7	0/20		14/20
M1853	+	0/21	+++ h	20/0	1/0	6/14	2/17	5/20	
M1896	+	0/46	++++ h	19/1	7/0	13/7	0/20		15/20
M2369	+	0/17	+++ h	19/1	3/0	16/4	0/20		16/20
M3358	+	0/33	++++ h	19/0 *	0/0	11/9	1/19		16/20
M3452	+	3/62	++++ h	20/0	1/3	15/5	0/20		17/20
M3551	+	3/20	+++ h	16/0	0/0	14/6	0/20	6/20	
M3604	- *	1/1	+	22/0 *	1/1	12/8	0/20	8/20	
M3734	-	1/14	++ h	15/4	1/2	15/5	0/20	6/20 *	
M3816	+	2/14	++ h	20/0	4/0	9/11	2/19	8/20 *	
M3820	+	5/28	+++ h	18/2	2/4	13/7	1/18	-	-
M3829	+	1/10	+++ h	15/4	1/0	14/6	0/20	8/20	
	NE = no eggs			++++ = >30 eggs					
	+ = 1-5 eggs			h = holes					
	++ = 5-15 eggs			* = repeat					
	+++ = 15-30 eggs								

Repeat Assays									
	Callobruchus			***Prostephanus***		***Trogoderma***			
	0.2mg/g	1mg/g		0.2mg/g	1mg/g	0.4mg/g	2mg/g		
	adults/eggs	adults/eggs		adults/larvae	adults/larvae	adults/pupae	adults/pupae		
CODE									
Cont	20/45	20/45		1/5	1/5	34/1	34/1		
EG5144	32/50	24/32							
HD-1 1971	21/34	13/27		26/11	4/0				
M3604	6/15	10/29		15/14	0/0				
Bt t				23/18	1/0				
M1693				13/11	3/1				
M3358				8/12	0/1				
M549						24/0	23/0		
Brevi lat						22/0	14/11		
M3734						18/1	23/0		
M3816						21/3	23/2		

Callobruchus maculatus

Of the 27 powders tested against *C. maculatus* adults in the initial tests, EG5144, HD-1 and M3604 had a possible effect on adult survival and the number of eggs laid. However, when these assays were repeated, the results were similar to that of the control. It was concluded none of the Bt strains had a significant effect at the doses used. (see above)

Prostephanus truncatus

In the initial tests, 5 strains had a possible effect on adult mortality, resulting in a subsequent reduction in egg laying and larval development. These were Bt.t, M1693, M3358, HD-1, and M3604. However, when these strains were tested again, no significant differences from that of the control were observed in the repeat assays (see above).

Sitophilus oryzae

In the first bioassays no significant differences in adult mortality was observed between the different treatments over the period of exposure to Bt. After removal of the adults, the subsequent development of any progeny was also monitored in the assays. However, in all the treatments and controls no subsequent progeny were found. The assay was repeated using a different batch of adults sent from NRI. However, after two weeks all the adults were dead, in all the Bt treatments and the controls, again with no subsequent development of progeny. Due to the short duration of the project we were not able to conclude why egg laying and subsequent development of larvae was not observed. However, possible reasons are the age of the adults and the source of food as in more recent test some development was observed on maize seed.

Activity of new African stored product Bt strains to *T. granarium*

CODE	*Trogoderma* 1st gen adults/larvae	CODE	*Trogoderma* 1st gen adult/larvae
Cont	7/9	Cont	7/9
N1	4/10	M4895	4/10
N2	6/10	M4738	1/12
N3	8/5	M4740	2/13
N4	6/5	M4742	0/13
N5	9/3	M4748	5/9
N6	6/9	M4840	4/10
N7	7/9	M4842	0/10
M4626	3/13	M4843	4/10
M4717	7/5	M4844	0/10
M4736	0/8	M4846	1/10

Trogoderma granarium

As larval stages are usually the most sensitive to Bt a bioassay was developed using neonate larvae. In the first tests four of the powders showed some possible effect on larval development. The strains were *Brevibacillus laterosporus*, M549, M3734 and M3816. These initial positive results, as for all the species could not be repeated. Therefore, it was concluded that the initial positive results in all the assays were false positives caused by experimental variation, which would be expected in a screening exercise of this scale.

Due to the simplicity and availability of insects, *T. granarium* was also used as the single indicator species to screen the new and the previously unscreened strains from Africa. However, none of them proved to have significant activity. (see above)

6.1 Lepidopteran results

Plodia interpunctella

	Larval stage	LC50ugBt per grm	SE	slope
Agree	neonates	1.723	0.094	3.06
	7 day old	2.93	0.382	1.2
Dipel	neonate	31.87	0.223	2.34
	7 day old	60.04	0.221	1.48
Xentari	7 day old	150.80	0.164	1.35

These preliminary LC50 values show that Agree is the most active of the three commercial Bt products tested, being approximately 15 times more potent than Dipel and 50 times more potent than Xentari.
Although these results confirm the potential of Bt for the control of stored product moth pests it is recommended that further assays are performed to confirm these results, before large scale trials are performed.

Ephestia cautella

	Concentration µg Bt per grm of food	% mortality
Assay1		
Agree	5000	100
	500	100
	50	95
Dipel	5000	100
	500	100
	50	95
Xentari	5000	100
	500	60
	50	50
Control	0	20
Assay 2		
Agree	10	60
	2	50
Dipel	10	75
	2	30
Control	0	5

Using all available larvae it was not possible to obtain detailed LC 50 values, however the larval mortalities obtained confirmed that *E. cautella* are susceptible to all three Bt products, with Agree and Dipel the most active.

Appendix 7

Acivity of two *Xenorhabdus* strains on stored product pests

Lasioderma sericorne (young larvae)

Strain	concentration mg bacteria/g food	% mortality
HRI I73	10	97.5
	1	15.6
HRI H31	10	98.0
	1	18.4

Prostephanus truncatus (adults)

HRI I73	10	100
	10	90

Sitophilus oryzae (adults)

HRI 173	10	0
HRIH31	10	0

Trogoderma granarium (larvae)		effect
HRI I73	10	+++
	1	+
HRI H31	10	+++
	1	+

Appendix 8

Enclosed commercial literature on two of the lepidopteran active Bt products, Agree and Dipel.