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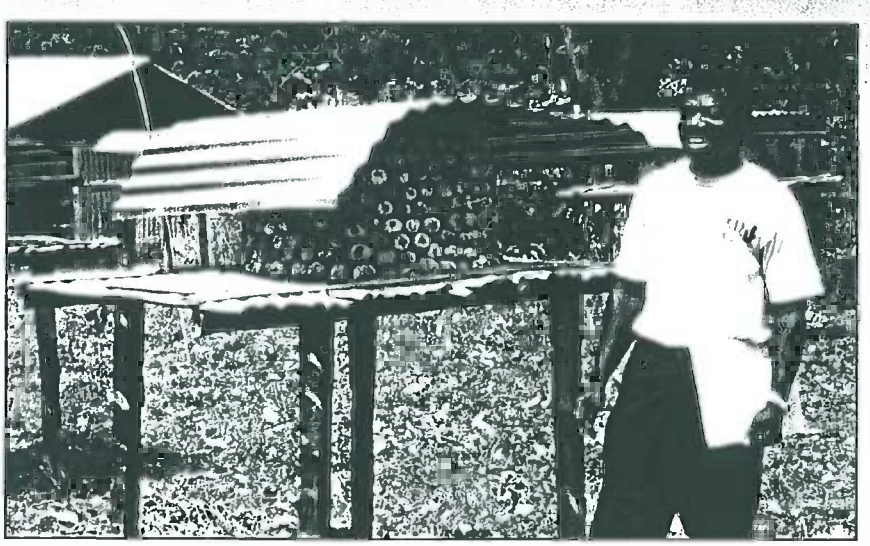
Risk Warning to Farmers of Larger Grain Borer Infestation and Reduced Pesticide Treatment in Farm Maize Stores

Final Technical Report to the Crop Post-Harvest Programme (Project R6684)



Natural Resources Institute

Project R6684

Risk Warning to Farmers of Larger Grain Borer Infestation and Reduced Pesticide Treatment in Farm Maize Stores

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Final technical report to the Crops Post-harvest Programme

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Cover illustration – Experimental Ewe barn at Kpevé (Ghana) used for a study to reduce the amount of pesticide used to protect the maize stored by subsistence farmers

CONTENTS

Acronyms used in the text

DFID	Department for International Development (UK)
EC	Emulsifiable concentrate (an insecticide formulation)
LGB	Larger Grain Borer (*Prostephanus truncatus*)
NRI	Natural Resources Institute (UK)
Tn	*Teretrius nigrescens*

Executive Summary

This report describes the outputs of a project designed to research a package of measures to be implemented in support of improved pest management against the Larger Grain Borer (*Prostephanus truncatus*). Important advances have been made in the development of a risk assessment system that will enable the extension services to notify farmers when their stored produce is at risk. A system based on the numbers of beetles captured in pheromone traps is being proposed for further development and validation. Ancillary studies on the pheromone lure in the traps and on the characteristics of those beetles that come to the traps are also reported.

In parallel with the risk assessment work, the project has investigated opportunities for limiting the amount of pesticide that needs to be applied by farmers to protect their stored maize. Pesticide usage has negative health and environmental impact and is a financial cost to farmers. The project has been able to demonstrate that a high degree of protection can be achieved if stocks of shelled maize grain or maize cobs are only treated with pesticide in their bottom layers. It is suggested that reductions in application of 50% to 80% are achieveable. An important additional benefit of this technique is that it is compatible with biological control by the predator *Teretrius nigrescens*. Further adaptive studies and farmer participatory research are required before the reduced insecticide methodology can be extended.

Impact assessment on *T. nigrescens* is reported that indicates the establishment of this biocontrol agent in Ghana and a close relationship of flight activity of this species and *P. truncatus*. However, the extent to which this predator is reducing infestations of *P. truncatus* is not certain. Further studies are recommended to help interpret the data set that has been collected, in this way suitable recommendations may be formulated to obtain due benefit from this cost free and environmentally sound adjunct to pest management.

Acknowledgement

Our thanks are due to Mr K. Nicol (PHMD, Ghana) for providing facilities for this project at Ho in the Volta Region.

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1. Introduction

The Larger Grain Borer (*Prostephanus truncatus*), or LGB for short, is now a familiar pest of farm-stored maize and cassava in many parts of Africa. LGB has been the subject of much research over the last ten to fifteen years since its invasion into Africa from Meso-America in the late 1970s. Considerable strides have been made in developing appropriate technologies to reduce the losses associated with it. However, further efforts are still required as the pest

- remains a significant threat to subsistence farmers,
- continues to spread, particularly in southern Africa, the Republic of South Africa reported the presence of the pest for the first time in 1999, and
- the use of synthetic pesticides, which is the main defence against the pest, is increasingly being questioned on health and environmental grounds.

Farmers face considerable storage problems caused by insect pests that must be overcome if incomes from agricultural production of durable food crops are to be maximised. In recent years, LGB has become a major pest of stored maize in many African countries, including Ghana. Where this pest occurs weight losses of grain have roughly doubled from around 5% to 10% (Dick, 1989). In Ghana, maize production is about 1 million tonne/annum (1993 FAO figures) and a large percentage of this is stored on the farm. Thus even small reductions in losses of say 0.5 to 1% can give significant saving of grain (5K to 10K tonnes), especially when such savings are accumulated year on year.

The simplest and most effective method to control the pest is to shell maize cobs and to treat the grain with a suitable insecticidal dust. However, in many countries of West Africa, farmers are reluctant to shell their maize, preferring to store it unshelled on the cob. Many farmers in LGB-affected areas of Ghana store cobs until LGB infestation has started. They then shell the cobs and admix insecticide. One of the reasons that farmers, in Ghana and other parts of Africa, respond in this way is that there are considerable year to year variations (Fig. 1.1) in both the severity of attack and even whether there is an attack. Thus farmers can easily be lulled into a sense of security and respond too slowly when infestation does actually occur.

To address these issues, the UK's Department for International Development (DFID) has been supporting the LGB Risk Assessment Project, which is based with the Ghanaian Ministry of Food and Agriculture. This project is pursing a research programme to strengthen integrated pest management (IPM) approaches by

- developing a risk assessment system to enable extension services to predict those years when LGB is likely to be a significant threat to farmers
- assessing the impact of the biocontrol agent *Teretrius* (formerly *Teretriosoma*) *nigrescens*, on LGB populations and their damage in stores in the Volta region of Ghana, and

- pioneering a technique whereby farmers can protect their stored maize by the limiting application to as little as only 20% of the stored commodity

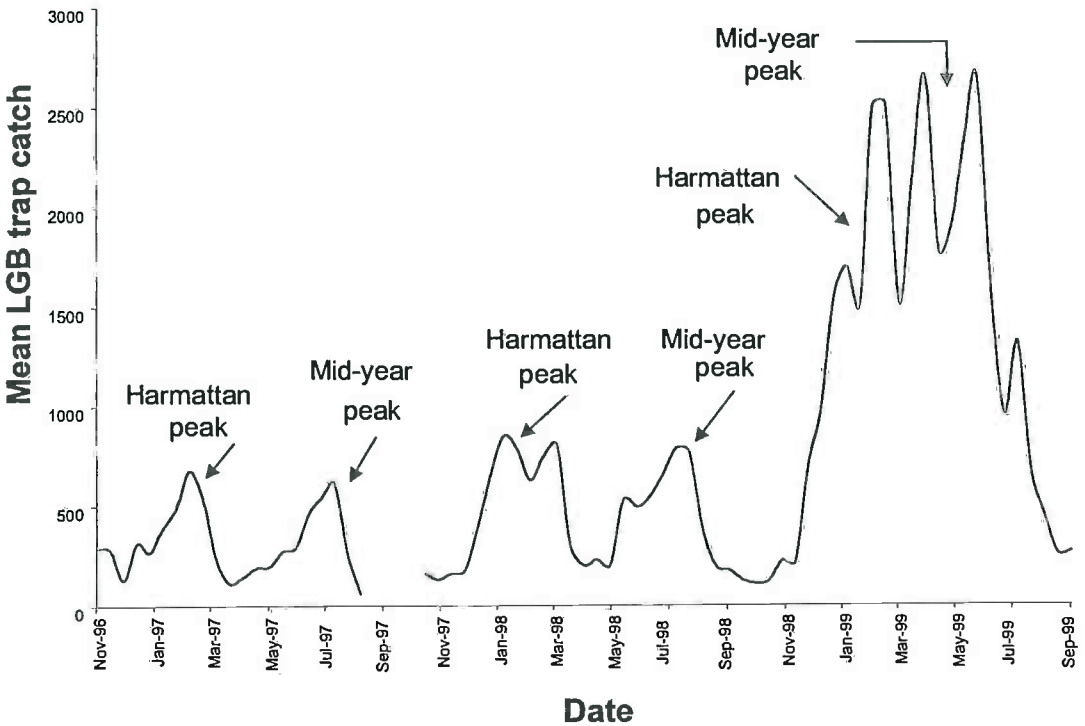

Figure 1.1: Catches of LGB in pheromone-baited flight traps 1996-1999 in villages around Hohoe in the Volta Region of Ghana.

These project components can be seen as a package of interconnected measures (Fig. 1.2). The risk assessment system is to be operated by the extension services and enables them to warn farmers when a 'bad' year for LGB is likely. In this way both extension services and farmers can focus their efforts better. Efforts to establish the impact of the LGB predator, *T. nigrescens* (Tn), which is a self-sustaining cost-free means of pest control, are needed to justify further releases and enable researchers to determine what actions may be needed to improve impact. Finally, the development of methods to reduce the amounts of pesticide used on farm stored food are important on health and environmental grounds but may also lead to a method of pest management that is more compatible with biological control. The biocontrol agent Tn is susceptible to the pesticides used against LGB so that, at present, the pest management methods are mutually exclusive. If grain can be substantially protected against storage pests by treatment of only a small portion of the stock then biocontrol could operated on the small numbers of pests in the untreated portion.

The research results described in this report concern the main project outputs as well as other important support studies. These include

- studies to improve understanding and performance of the pheromone bait, that forms the backbone to the risk assessment system, and
- an investigation of the biological characteristics of the LGB that come to traps. It is essential to know what the biological potential is of the insects that are counted as a 'risk' and whether this risk changes according to habitat or season.

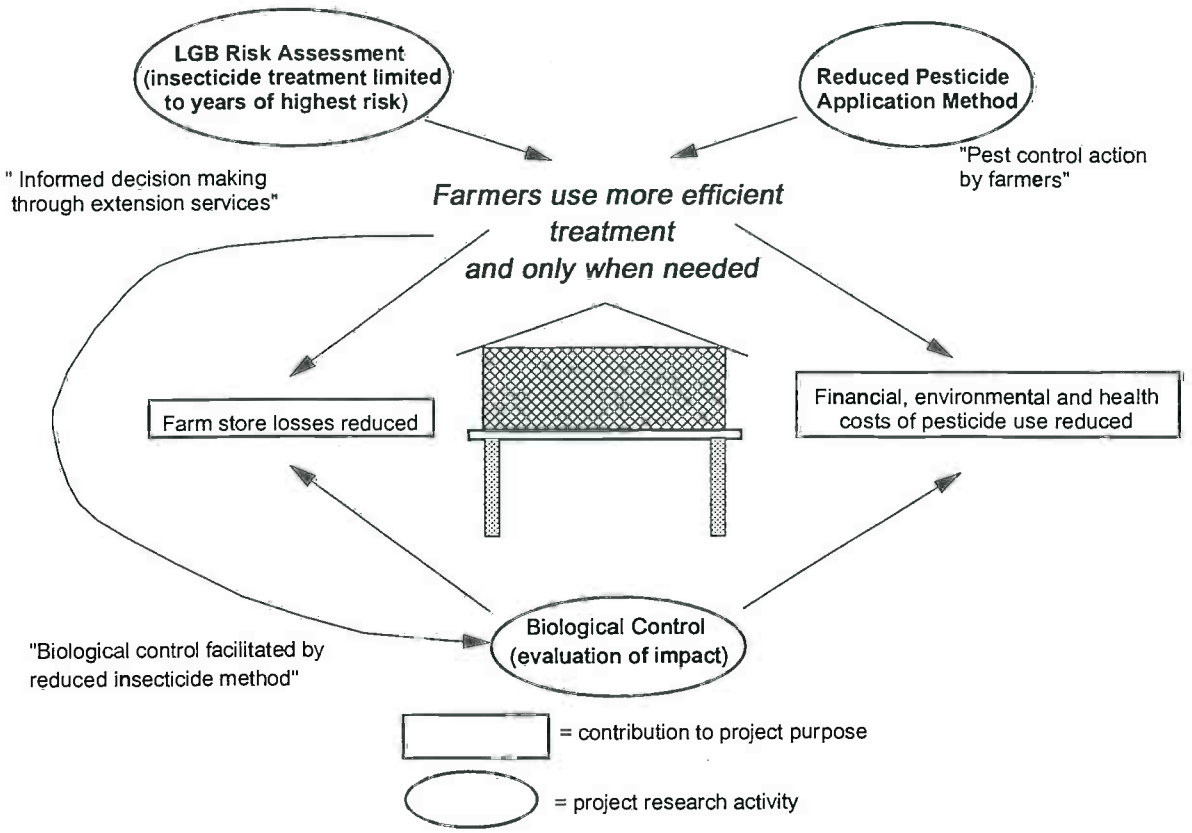

Figure 1.2: Summary of the various elements of the project

Reference

DICK, K. (1988) A review of insect infestation of maize in farm storage in Africa with special reference to the ecology and control, of *Prostephanus truncatus*. Natural resources Institute Bulletin No. 18. Natural Resources Institute, Chatham Maritime, Kent, ME4 4TB, pp 42.

2. Investigations to improve the performance of the pheromone baited capsules used in traps for LGB

Hodges R.J., Farman D., Addo S. and Hall D.R.

Summary

Male *P. truncatus* produce a pheromone that is a blend of two components T1 and T2. Of the two components of the pheromone, T1 is produced in much larger quantities than T2. The abundance of T2 was generally 10%-40% of T1 with an average of 27%. The current standard synthetic lures for LGB consist of polythene capsules holding a 1:1 blend of T1 and T2. Laboratory studies demonstrated that capsules loaded with a 2:1 blend mimic the pheromone released by male beetles more closely than the standard commercial capsule. Field testing of alternative weights and ratios of pheromone components indicate that use of capsules with a 2:1 blend results in higher trap catches. However, although the trap catch would appear to be greater if a 2:1 ratio is adopted it is doubtful that the difference is great enough to justify changing the standard lure.

An initial very high 'flash off' pheromone was observed from plastic capsules stored in foil sachets. The 'flash-off' was largely completed within 3h of exposure and by 24h pheromone output was steady . For typical pheromone trapping programmes, a high initial release of pheromone poses no difficulty. However, for experimental studies, comparing different treatments over short periods of time, it may be important to avoid testing during the 'flash off' period. In these circumstances it is suggested that the pheromone capsules should be aired for one day before being placed in traps.

Background

Adult male *P. truncatus* release a pheromone blend consisting of two components called Trunc-call 1 and Trunc-call 2 (T1 and T2). The pheromone is attractive to both females and males and can be synthesised in the laboratory. To monitor *P. truncatus*, artificial lures are used to bait traps; the lures are prepared by impregnating polythene capsules with synthetic pheromone.

There is strong evidence that LGB can distinguish between the pheromone signals of different males, i.e. that some males produce more attractive signals than others (Birkinshaw, 1998). It is therefore probable that the efficiency of the artificial lure could be improved if the nature of the pheromone blend produced by male LGB was better understood. In order to do this the pheromone output of individual male beetles was investigated to determine

- the total quantities released
- the natural ratio of the components

- changes with time in the amounts released, and
- variation among males in these parameters.

As males may also sometimes be present in groups, the pheromone output of males in groups of two or three was also investigated.

The extent to which the quality of male output is simulated by artificial lures was investigated by monitoring the emissions from polythene capsules. These capsules were treated as they would be under field conditions, i.e. they were kept in foil sachets in a freezer before use. Different blends and different amounts of pheromone were tested. The current standard for artificial lures is a 2mg loading of a 1:1 blend. Compared with a 2:1 blend, this was found to be a relatively poor mimic of the blend released by male beetles. To test whether the 2:1 blend might result in an improved trapping performance, a field trial was undertaken to compare the attractant properties of traps baited with different weights of 1:1 or 2:1 blends.

Compton *et al.,* 1997 suggested that capsules give a 'flash off' of pheromone under typical field conditions which leads to high variation in catch during the first two or three days of trapping resulting in inconsistencies in catch. The 'flash off' was confirmed in the current study when emissions from capsules were measured. To investigate this further, the catch of traps loaded with capsules that had been aired for 24h after removal from a sachet was compared with the trap loaded with unaired capsules.

Methods

Pheromone release from single male P. truncatus

The beetles used were adults of a Ghanaian strain collect from the field in 1996 and cultured on yellow maize in a CTH room at 27°C and 70% r.h. Newly emerged virgin adults, 1-2 days old, were removed from pupal cells. Males were selected according to the form of the clypeal tubercles (Shires and McCarthy, 1976) and placed on wheat flour for two days. Just before the start of the test each male was placed in a single maize grain, this was necessary since males only produce pheromone in the presence of food. To provide the beetles with easy access to the maize, each grain was drilled to give a single blind ending tunnel of 2mm diameter.

The apparatus used to collect pheromone from individual beetles is shown in Figure 2.1. Beetles in maize grains or a control grains without beetles were placed singly in glass vessels of 30 cm^3 capacity (Fisher Scientific, UK – Fig. 2.2) through which air was drawn at a rate of 1000cm^3/min by small electrical pumps. A large glass round bottomed flask (1000 cm^3) was also connected to the system to act as a buffer against pressure variations induced by the pump. The intake air was filtered through activated charcoal to ensure a 'clean' air supply. The output air was passed through filters containing 200mg of 50-80 mesh 'Porapak Q' (Phase Separations,UK) to collect T1 and T2. The air was pumped continuously during the test and Porapak filters changed at intervals of 1, 2 or 3 days over a period of 24 days.

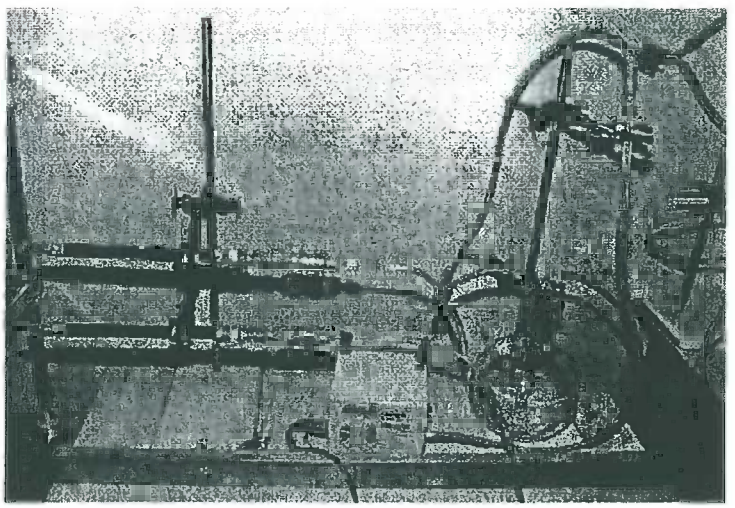

Figure 2.1: Entrainment apparatus used to collect volatiles released by adult male *P. truncatus*. Apparatus arranged here to collect volatiles from two separate beetles.

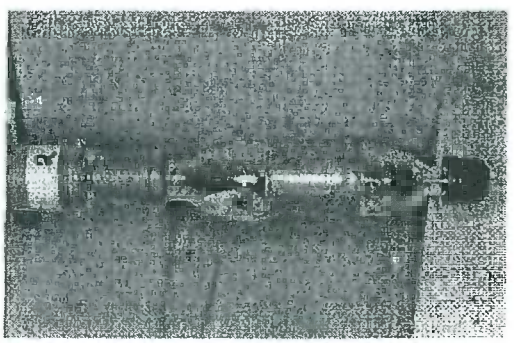

Figure 2.2: Vessel for containing maize grain with adult male *P. truncatus* during entrainment.

To estimate the amounts of pheromone collected on the Porapak, filters were eluted with 750µl of distol grade dichloromethane (Fisher Scientific). Octyl acetate (5µg) was added to each sample as an internal standard. Samples were assayed by capillary gas chromatography, using a 30m x 0.25mm CPWax 52CB column (Chrompack, Netherlands), with helium carrier gas and flame ionisation detection. Pheromone peaks were calibrated against known amounts of pure synthetic standards and identities confirmed using an ion trap detector.

In the first test there were nine beetles and one control. This test was then repeated. The results of the two tests were very similar and for statistical analysis they were combined. There were a total of only 17 observations on the release of pheromone since three sets of data were missing due to mortality and a failure in sexing.

Pheromone release from groups of male P. truncatus

The methods for this were broadly similar for those used for single males. Maize grains were prepared with either one, two or three young males or no males. The males were kept together for five days after which they were transferred to a fresh grain and 24h later each grain placed in the entrainment apparatus. The output air was filtered and volatiles collected for 24h on the second and third days that the beetles were in the entrainment apparatus. Three replicate treatments and one control were run on each of three occasions to give nine replicates of treatments and three controls. The control were included in case a control correction of the measured pheromone output of males was required, this did not prove necessary. The data were analysed by one way analysis of variance.

Pheromone release from polythene capsules

The pheromone components, in pentane solvent, were impregnated into the polythene capsules (9mm O.D. x 23mm with a wall thickness of 1mm, Just Plastics Ltd). Pheromone was collected from the polythene capsules by placing them in the same entrainment apparatus as described for the collection of pheromone from the beetles, the only difference was that when the capsules were to be tested they were entrained for only one hour. Between collections the capsules were held in a wind tunnel at 27°C with a windspeed of 2km/hour.

The capsules tested were loaded with pheromone, sealed into foil sachets and then stored at -18°C. Sachets were removed from the freezer, allowed to warm to room temperature and after 20 minutes the capsules removed from the sachets. They were then placed in the wind tunnel and entrainment for 1h. Two capsules were tested on each of a number of occasions over a period of 15 days. The pheromone output of capsules stored in sachets was compared with those prepared freshly. These were placed in the entrainment apparatus, for the first time, within 1h of preparation, i.e. underwent no storage.

Initally, a 1:1 blend of the two components was tested, with capsules loaded with a total of either 2mg or 4 mg. The 2mg loading is the current standard *P. truncatus* lure. However, as the 1:1 blend was found to provide a relatively poor mimic of the pheromone blend released by the male beetle, a more detailed study was undertaken with a 2:1 blend, with capsules loaded with either 3mg or 6 mg.

Investigation of the response of P. truncatus *to traps baited with lures containing different ratios of the two pheromone components*

As pheromone capsules loaded with the 2:1 blend of T1 and T2 appeared to mimic the outputs from male *P. truncatus* more closely than the conventional 1:1 lures, it was decided to compare the field performance of 1:1 and 2:1 lures. These were loaded with 2mg or 4mg of the 1:1 blend or 3mg or 6mg of the 2:1 blend.

The pheromone traps used for the study were the Japanese beetle (JB) type (Figure 2.3), supplied be Trece Inc., Salinas, CA., USA. They consisted of a yellow plastic funnel

(diam. 15cm, height 11cm) with 4 vertical vanes, in the form of a cross, extending 10cm up from the funnel to give a baffle against which the beetles would fly and then tumble into the funnel to be collected by a plastic jar attached to its base. A sheet of perspex with appropriately cut slits was fitted over the vanes to act as a rain guard. This gave an aperture for the entry of the beetles into the trap of 40mm. Polythene capsules (9 mm O.D. x 23 mm with a wall thickness of 1mm, Just Plastics Ltd) were used as pheromone dispensers. A single capsule was placed in each trap, inserted in a space between the baffles so that it was about 4 cm from the top of the funnel. Before capsules were loaded into traps they were left in open air for two days to ensure that any excess pheromone that might result from condensation within the storage sachet was lost before they were used in the test.

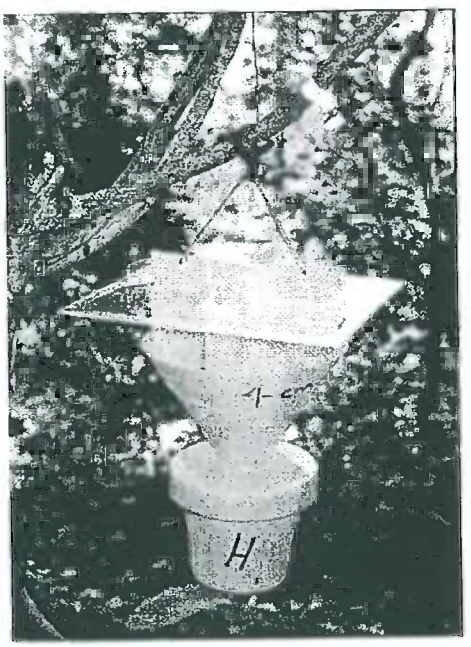

Figure 2.3: Flight trap baited with pheromone for *P. truncatus* with a perspex rain cover

The pheromone traps were placed at least 150m apart along an east/west transect running through teak woodland. The wind direction was from the south or occasionally north and so blew across the line of traps. Traps were hung from trees or bushes at a height of 1.5m to 2m. In the first test, there was a total of 10 trap positions to accommodate two replicates of four treatments and a control. The treatments and control were allocated to the positions according to two randomised Latin squares (Latin rectangle) to give a total of 10 replicates for each treatment over a period of five days. The position of traps was changed daily. The second test was arranged in a similar way to give a further ten replicates per treatment.

For statistical analysis, the differences in trap catch according to treatment were investigated by analysis of variance. The trap catch data were initially transformed log(count+1) in order to meet assumptions underlying analysis of variance but plots of

residuals were better for untransformed data and so analysis was undertaken on this. In all cases the blank (control) treatments attracted very few insects, and because of this lack of variability of response, the control values were omitted from all analyses except in the first experiment where there were a few control captures. The standard error of the difference (SED) between two means was calculated in order to compare treatments; where the difference between two means was at least twice the SED then the means are considered to be significantly different at the 5% level ($p \leq 0.5$).

Investigation of the effect of loading traps with pheromone capsules taken directly from a foil sachet

Four trap sites at the corners of a square with sides of about 200m were chosen in the semi-wooded grounds of an hotel in Ho. Traps were hung at about 2m above ground and loaded with capsules, holding a total of 2mg of the 1:1 blend. The capsules were either taken directly from a sachet recently warmed to ambient temperature or had already been aired for 24h in a laboratory. The capsules were put in place at 15.30h on the first day and trap catch of both *P. truncatus* and its predator *Teretrius nigrescens* collected daily at 08.30h for six days. This exercise was repeated three times with the position of aired and non-aired capsules swapping places each time.

Results

Pheromone released by male P. truncatus

During the first three days that the beetles were on maize no pheromone was detectable (Fig. 2.4). Thereafter, there was a steady output of pheromone components T1 and T2 with a strong correlation between the two in quantity released by the beetles ($r = 0.75$), i.e. those beetle releasing large quantities of T1 also released large quantities of T2. The abundance of T2 in relation to T1 varied considerably between beetles (10.7% - 47% of T2 relative to T1, Table 1). The rate of accumulation of T1 over 24 days varied quite widely between males (Fig. 2.5) although the output of individual males was fairly constant; T2 followed a similar pattern. However, in one case there was a very dramatic release of pheromone, some 70 μg of T1 (Fig. 2.5) and 22 μg of T2 over a period of only two days, followed by little or no further production. There were clear differences between males in the amounts of pheromone that they produced. In one case, T2 could not be detected (Table 1.1, beetle no. 9). It seems possible that the amounts released may have been below the limits of detection and in another case (beetle no. 2) the amounts of both pheromone components detected were very low.

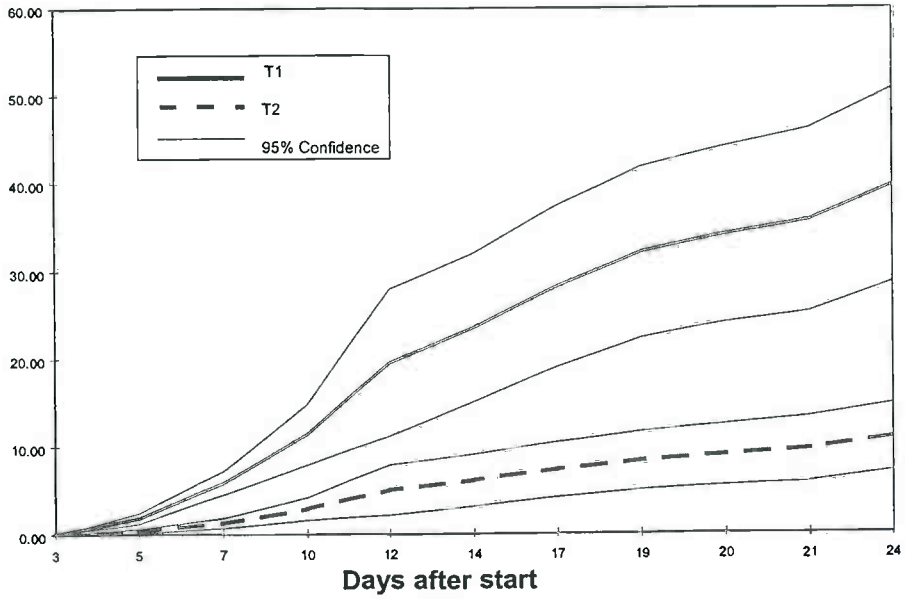

Figure 2.4: Mean cumulative output of T1 and T2 from young male LGB over a period of 24 days

Table 2.1: Total accumulation (μg), over 24 days, of pheromone components T1 and T2 from fifteen male *P. truncatus* and the abundance of T2 as a % of T1

Beetle	T1	T2	% abundance of T2
1	42.05	15.45	36.74
2	2.52	0.27	10.71
3	62.46	9.14	14.63
4	31.24	6.47	20.71
5	69.23	8.40	12.13
6	43.34	10.41	24.02
7	21.03	4.88	21.03
8	74.51	23.43	31.45
9	6.32	0.00	0.00
10	52.10	22.85	43.86
11	38.22	17.98	47.05
12	19.18	3.61	18.83
13	59.82	18.77	31.38
14	44.98	13.93	30.96
15	27.71	7.11	25.65

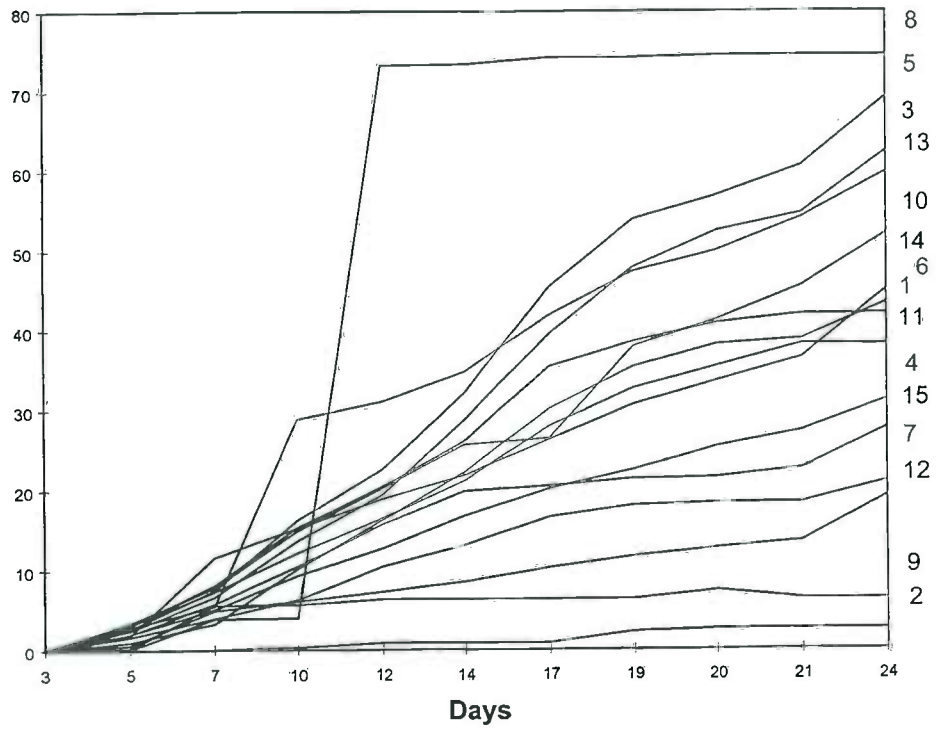

Figure 2.5: The accumulation of pheromone component T1 from each of fifteen young adult male LGB over a period of 24 days (numbers are individual beetles in Table 1.1)

The frequencies with which different males released pheromone blends of different proportions of T1 and T2 are shown in Table 2.2. T2 abundance reached as high as 50% of T1 in some cases while in other less than 10%. At the median abundance the proportion of T2 released was only 24.02 % of T1. Average daily rates for the period between day five and day 24 was 2.08 µg of T1 and 0.57 µg of T2 so that the mean weight of T2 released was 27.4% of T1.

Table 2.2: Frequency distribution of adult male *P. truncatus* that release T2 pheromone at different abundances in relation T1; both pheromone components compared as totals released over 19 days

Range of T2 abundance	Frequency of beetles
0-10%	1
11-20%	4
21-30%	4
31-40%	4
41-50%	2

Pheromone released by groups of male P. truncatus

The outputs of pheromone from males kept as small group or singly on maize grains is shown in Table 2.3. There was no evidence of any significant difference between males kept in groups or singly in the output of T1 ($F_{2,25} = 0.1158$, $p = 0.855$) or T2 ($F_{2,25} =$

0.821 p = 0.44) although there was a trend of decreasing T2 output. The observed ratios of T1 to T2 fall within the typical values observed from single males (Table 2.3).

Table 2.3: Output of pheromone components T1 and T2 (ng/male/day) from male *P. truncatus* retained either singly or in groups of one or two males in a maize grain

	1 male	2 males	3 males
T1	485.8 ± 445.8	558.2 ± 554.2	472.5 ± 466.0
T2	198.3 ± 216.2	156.4 ± 152.0	127.0 ± 131.9
Mean ratio	40.8	28.0	26.8

Pheromone released from polythene capsules

Pheromone outputs from capsules are initially very high (Figs. 2.6- 2.9). The output falls off rapidly and is much reduced by three hours (Fig. 2.6) and relatively low after 24h (Figs. 2.6- 2.9). Thereafter, there is a much more even pheromone output, although the output of T1 has fallen to rather low levels by day 15. By contrast T2 declines more steadily until there is little left after 15 days.

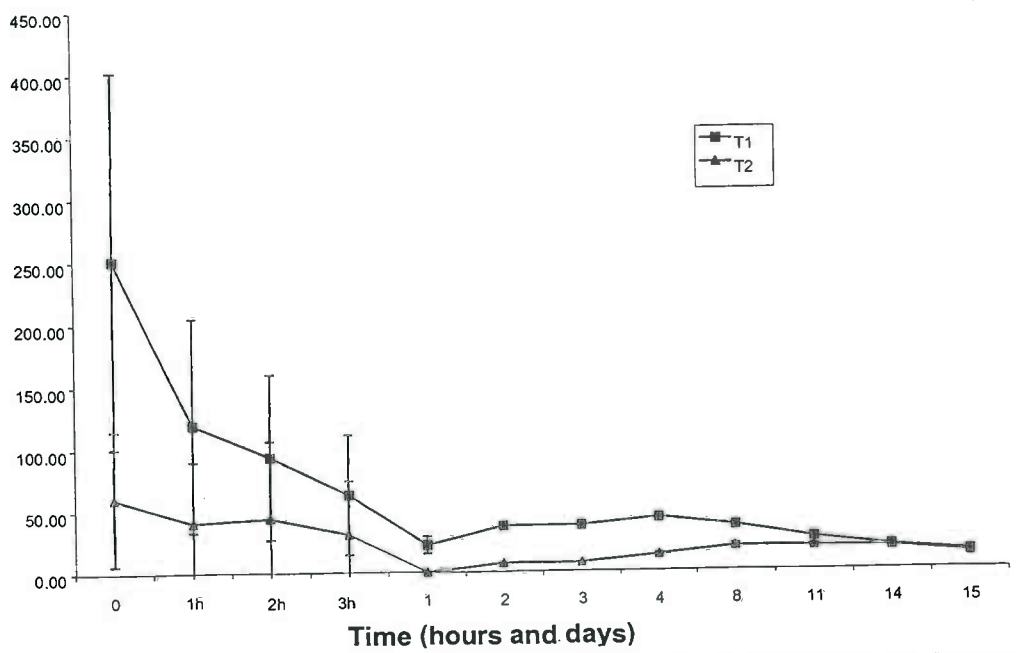

Figure 2.6: Release of *P. truncatus* pheromone from capsules loaded with 1mg of T1 and 1mg of T2 after storage in a sachet for 30 days

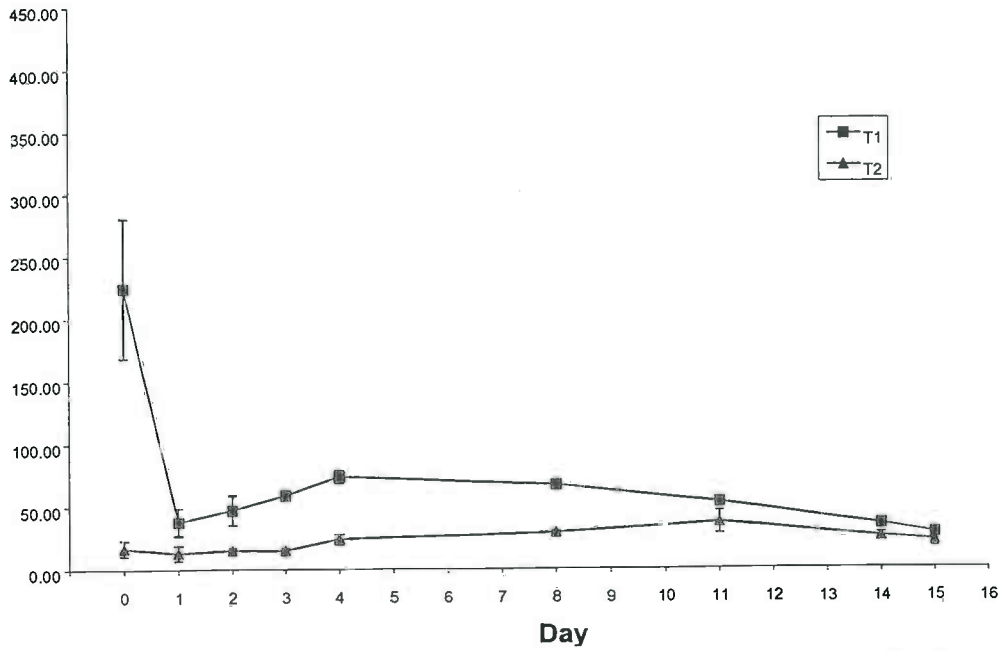

Figure 2.7: Release of *P. truncatus* pheromone from capsules loaded with 2mg of T1 and 2mg of T2 after storage in a sachet for 30 days

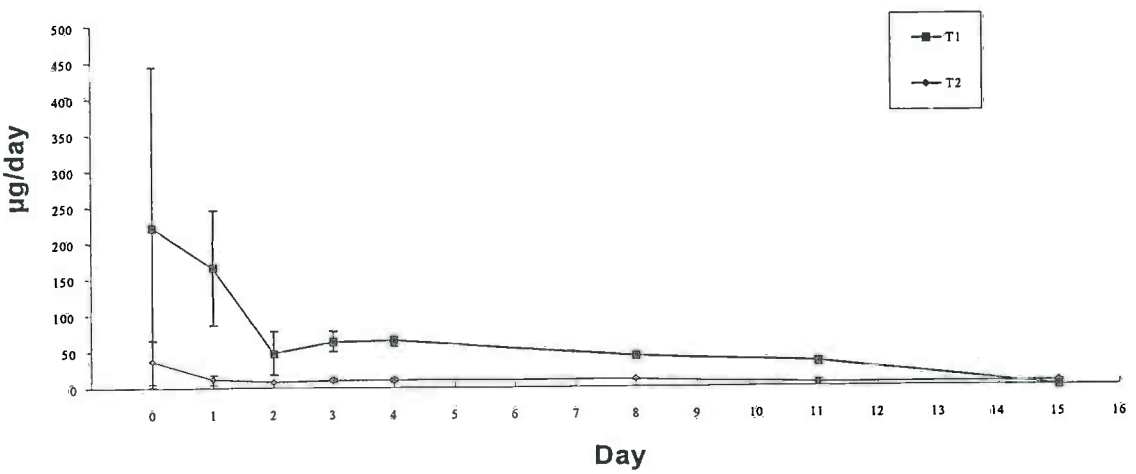

Figure 2.8: Release of *P. truncatus* pheromone from capsules loaded with 2mg of T1 and 1mg of T2 after storage in a sachet for 30 days

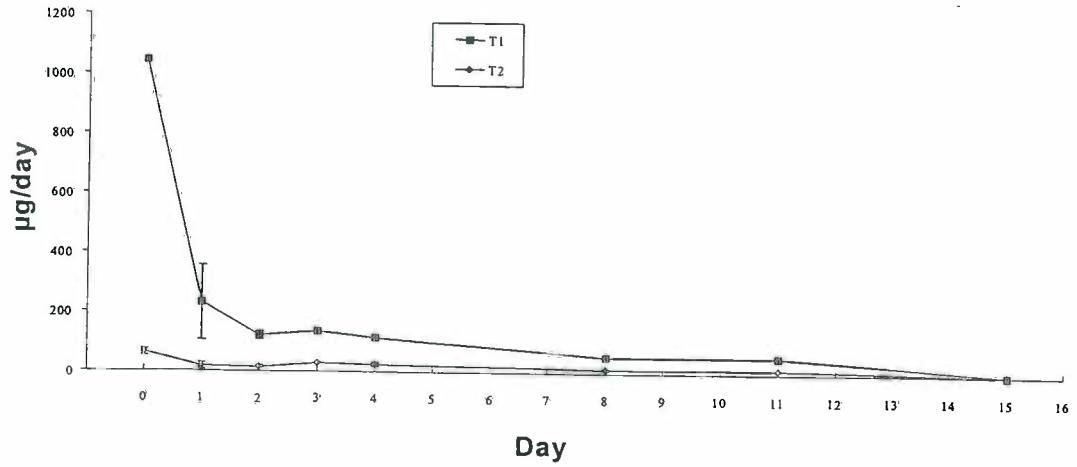

Figure 2.9: Release of *P. truncatus* pheromone from capsules loaded with 4mg of T1 and 2mg of T2 after storage in a sachet for 30 days

The proportion of T2 in the blends released from capsules loaded at ratios of 1:1 or 2:1 of T1:T2 are somewhat different (Table 2.4). With capsules loaded at a ratio of 1:1, the proportion of T2 was high by at least day 8 (40-50%) and had reached 70-95% by day 15. In contrast, capsules loaded at 2:1, i.e. with relatively more T1, showed a much lower ratio over almost the entire period of 15 days and remained much closer to the natural ratio of about 27%.

Table 2.4: The abundance of pheromone component T2 as a % of T1, released from polythene capsules loaded with different weights of these two compounds and stored in a sachet before exposure

Day	Capsule loadings			
	T1 + T2 (1mg + 1mg)	T1 + T2 (2mg + 2mg)	T1 + T2 (2mg + 1mg)	T1 + T2 (4mg+2mg)
0	24.0	7.4	16.2	5.9
1	0	34.1	7.0	12.7
2	18.9	31.3	16.5	11.8
3	18.9	25.7	16.5	21.8
4	30.2	32.5	15.6	21.5
8	52.7	43.0	22.6	24.8
11	73.0	70.2	14.3	28.6
14	94.7	71.1	-	-
15	92.8	76.0	100	0

The pattern of pheromone release from freshly prepared capsules was quite different from those that had been stored in sachets. There was a gradual increase in output for the first two days followed by a gradual fall (Fig.2.10).

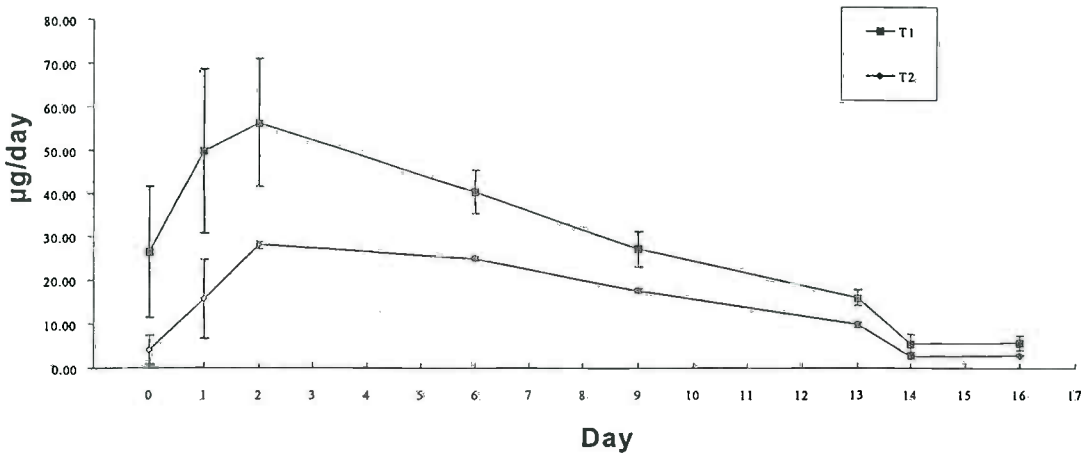

Figure 2.10: Release of *P. truncatus* pheromone from freshly prepared capsules loaded with 1mg of T1 and 1mg of T2

Captures in traps baited with lures containing one of two different pheromone blends
The mean catch achieved by traps loaded with different weights and ratios of the two pheromone components is shown in Table 2.6. Catch differed significantly with treatment ($F_{3,79} = 13.28$, $p = 0.008$). Increasing the proportion of T1 to T2 resulted in higher trap catches. In the case of the capsules loaded with only 1 mg of T2 the difference was not quite statistically significant. However, in the case of lures with 2 mg of T2 there was a strong significant difference. This suggests that not only is catch improved at a 2:1 ratio but that the improvement brought by the addition of T1 is dependant on the absolute loading of T2. In this case T1 may have a more significant role when T2 concentration is high.

Table 2.6: Mean catch of traps baited with lures holding different weights and ratios of the pheromone components T1 and T2

Capsule loading (mg)		Mean trap catch
T1	T2	
1	1	10.3
2	1	12.0
2	2	12.5
4	2	16.08

SED = 1.523

Captures in traps loaded with aired and non-aired capsules

During the first trapping period (first 17h) the catch *P. truncatus* in traps holding non-aired capsules was very high compared with the aired capsules demonstrating the effect of pheromone 'flash-off' (Fig. 2.11). Thereafter, the catch in traps with the two loadings was similar confirming the observation that the flash off is normally completed within the first day after removal from the sachet. The catch of *Teretrius nigrescens* showed a different pattern, the catch in traps loaded with aired or non-aired capsules were more or less identical (Fig. 2.12). This was to be expected since *T. nigrescens* flies during the day while *P. truncatus* flies mainly at dusk. Positioning traps for the first time at 15.30h would have been too late to catch many *T. nigrescens* but ideal for catching *P. truncatus*. As the flash-off is largely completed within 3h, on the following day the pheromone output from aired and non-aired capsules would have been similar.

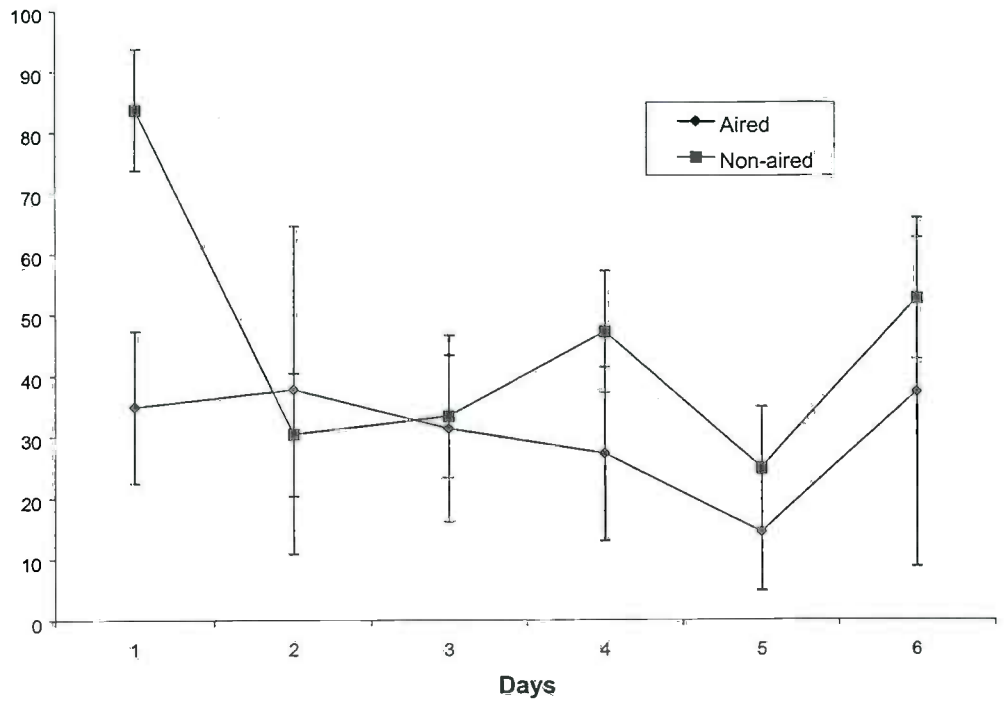

Figure 2.11: Mean catch of *P. truncatus* (± se) in traps loaded with capsule fresh from a sealed foil sachet or with capsules aired for 24h before use.

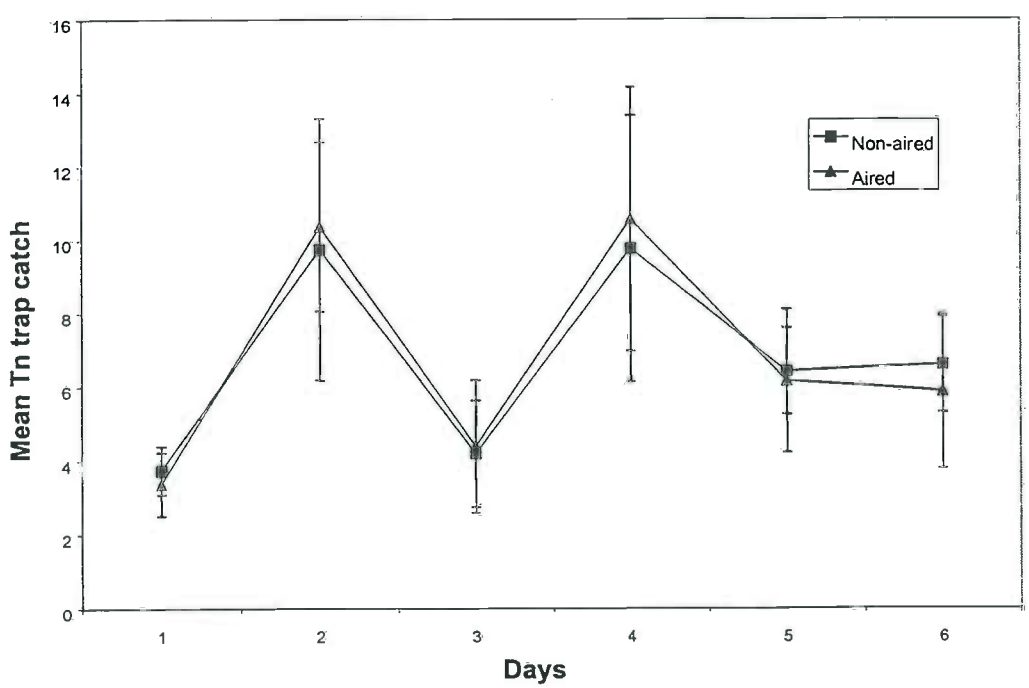

Figure 2.12: Mean catch of *T. nigrescens* (± se) in traps loaded with capsule fresh from a sealed foil sachet or with capsules aired for 24h before use.

Conclusions

Pheromone release from males

When male *P. truncatus* are placed on food they generally appear to produce pheromone for extended periods. After 24 days there was no suggestion that male release of pheromone was in any way declining. During the first three days on food, no pheromone could be detected. However, bioassay studies, have shown that both female and male *P. truncatus* respond to males that have only been present in grain for 24h (Hodges and Dobson, 1998). Clearly during the first three days, production is below detection for GC analysis but well within the range of perception by the beetle. There was generally little day to day variation in male output so that, on the whole, fairly steady accumulations of pheromone was observed in the entrainments – although there were some striking exceptions.

Of the two components, T1 is produced in much larger quantities than T2, despite the fact that tests under field conditions have shown that T2 is much more attractive than T1 (Leos Martinez *et al.*, 1995; Hodges *et al.*, 1998). The ratio of the components varied considerably between beetles although values for the two were strongly positively correlated. On average about four times more T1 was detected than T2, at the extremes either no T2 was detectable (one case) or the abundance of T2 was about 40-50% of T1 (two out of fifteen cases).

Pheromone release from polythene capsules

As expected, the polythene capsules released much greater quantities of pheromone than individual beetles. In the natural pheromone blend, the abundance of T2 was generally 10%-40% of T1 with an average of 27%. The capsules loaded with a 1:1 ratio of the two components (totaling 2mg or 4mg) released a blend with a very high proportion of T2 (40-90%) at least in the second week of the test and much above the typical blend released by males (27%). This deviation may be of some significance as the standard commercial capsule is impregnated with 1mg of T1 and 1mg of T2. In contrast, capsules with either of the 2:1 loadings (3mg or 6mg) released a blend that remained within the 10-40% range for much of the experimental period (Table 4). It may be concluded that capsules holding the 2:1 blend mimic the blend released by male beetles more closely than the standard commercial capsule.

The field testing of alternative weights and ratios of pheromone components indicated that a higher ratio of T1 resulted in higher trap catches. In addition, this advantage was more clearly demonstrated when the absolute values of T2 were higher, suggesting that T1 has more effect at greater T2 concentration. Earlier studies, on the functions of T1 and T2 in isolation and together, have shown that T2 is the major attractant and T1, at least by itself, attracts few beetles. Hodges *et al.* (1998) suggested that the role of T1 may be important where there is extensive exposure to T2 and may modify the response to T2 to facilitate close-range attraction. The current results support these suggestions.

The 'flash off' of pheromone from plastic capsules stored in foil sachets, postulated by Compton *et al.* 1997, has been confirmed both in the laboratory and in the field by comparing the response to capsules taken directly from the sachet with those given a prior airing for 24h. Flash off is largely completed within 3h of exposure and by 24h pheromone output is fairly steady (at least under the conditions of this test). If capsules had been loaded with pheromone and then exposed immediately to the entrainment procedure, the release rate would have been a gradual build up over the first two days followed by a gradual fall. Compton suggested that the effect of 'flash off' lasted over at least the first three days since there was a rapid decline in catch followed by a much longer period of relative stability. Such instability was not apparent in the field test. However, results reported by Compton *et al.* (1997) and Tigar *et al.* (1993) both show a strange oscillation in catch for several days after the start of trapping, this also been observed in some of our trap catches (Fig. 2.12) but this is not necessarily associated with 'flash-off'. The phenomenon is worth further investigation.

For many trapping programmes, pheromone 'flash off' presents no practical problem. However, for experimental studies especially those comparing different treatments over short periods of time, it is important to avoid testing during the 'flash off' period. In these circumstances it is suggested that the pheromone capsules should be aired for one day before being placed in traps. Initial testing is advised to ensure that the airing is having the required affect.

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3. LGB risk assessment

Addo, S., Hodges, R. J., Birkinshaw, L. A, Penne, H., and Gates, J.

Summary

Field experiments have shown that the risk of maize or cassava stored in Ewe-style barns becoming infested with LGB, is significantly related to the numbers of LGB caught in flight traps. Confirmation of this relationship is the first crucial step towards developing an information system that will allow extension services to identify years when risk from LGB damage is particularly high. Quantification of the relationship between trap catches of LGB and barn infestation rates has also shown that cassava is more susceptible than maize.

Introduction

The threat of LGB attack varies between years. This creates a dilemma for farmers. It is costly in both time and other resources to ensure that a harvest is always protected against LGB. However, if a farmer is unprepared in 'bad' years, then he/she is in a very vulnerable position and may lose the bulk of a year's maize or cassava harvest, or be forced to sell early when market prices are low. The aim of this work is to determine if variation in trap catches of LGB can be used to predict the changing risk of barns becoming infested.

P. truncatus is readily trapped in pheromone-baited flight traps. These are known to catch adults from the wider environment and from maize stores, and are currently used by the extension services in Ghana to monitor the spread of LGB across the country. Traps catch the young and fertile dispersing population of LGB (see biocharacteristics of LGB trapped in pheromone traps, this report), and the numbers caught vary a lot between years (see Figures 3.3 and 3.4). Previous research has shown that LGB locate food in stores by chance landings rather than any long distance host location mechanism, (Hodges, 1994). It therefore follows that the years where trap catches are particularly high might also be the years in which farmers have the highest chance of an LGB infestation in their stores.

The risk assessment study was conducted over three years (hereafter referred to as seasons one, two and three). The infestation of purpose-built mini-barns loaded with maize or cassava, the two crops that are most susceptible to LGB damage, was monitored. The first season's data highlighted the possibility that those two commodities may show very different patterns of susceptibility to LGB attack. The experimental design was altered in the second season to enable a clearer comparison to made between the initial infestation rates of maize and cassava. There is concern that the act of trapping out beetles using pheromone traps might influence the frequency with which barns became infested even when spaced 100m apart as they are in the village risk assessment trials. The third season's work (which will not be presented in detail here) has been designed to include a comparison of infestation rates with and without the presence of pheromone traps placed close by.

Methods

Two areas in the Volta Region of Ghana were chosen for the risk assessment study, one was in the forest-savannah transition zone of Hohoe/Jasikan district and the other was in the semi-arid zone of the Nkwanta district. In each study area, five villages were chosen, generally separated by a distance of one or two kilometres. The names and grid references for these villages are shown in Table 3.1.

Table 3.1: Villages names selected for the risk assessment study and grid reference obtained by a GPS

Hohoe/Jasikan		Nkwanta	
Village	map co-ordinates	Village	map co-ordinates
Bowiri Anyinase	07° 21.41 N 00° 27.96 E	Korantang	08° 15.03 N 00° 29.52 E
Bowiri Amanfrom	07° 21.17 N 00° 27.90 E	Nkwanta Zongo	08° 16.05 N 00° 31.12 E
Bowiri Kyirahin	07° 19.82 N 00° 27.68 E	Chala Odomi	08° 19.19 N 00° 32.35
Akpafu Odomi	07° 16.62 N 00° 28.66 E	Gekrong	08° 22.56 N 00° 30.92 E
Akpafu Mempeasem	07° 14.70 N 00° 28.92 E	Keri	08° 23.36 N 00° 31.80 E

In each village, nine mini-barns were constructed, each consisting of a chicken wire cage (with 30cm in diameter round apertures) fixed to a stake (Figure 3.1). The stake was driven into the ground so that the chicken wire cage was about 1.5m above ground level. The mini-barn was covered by a thatch roof leaving the cage in view at the sides. The height above ground and the thatch roof of the barns were designed to simulate, on a small-scale, typical farm stores. The base of the wire cage was lined with black polypropylene against which dust created by any insect boring activity would collect and could be clearly seen. The wire cage had a volume of about 8 litres, and contained stored food, 65 maize cobs (with long, tight husk cover) in the forest-savannah transition or an equal volume of segments of dried cassava chips in the semi-arid zone. Both cassava and maize were fumigated with phosphine prior to being placed in the small barns. The fumigations were undertaken under gas-tight sheet for seven days, using one Gastoxin tablet for approximately every 150 kg of produce.

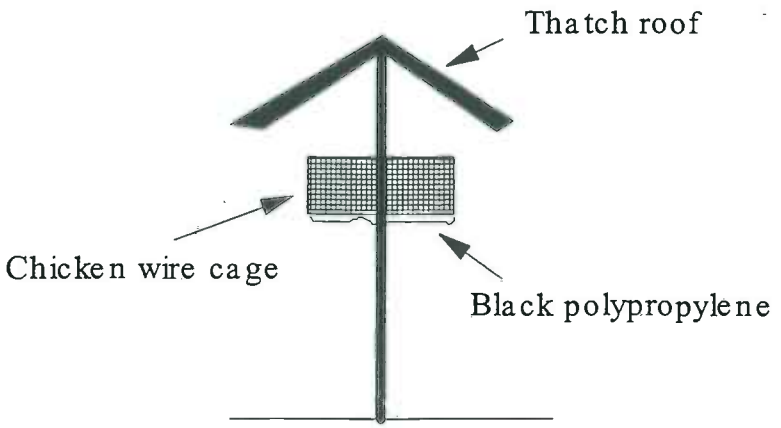

Figure 3.1: Design of experimental mini-barn

In order to monitor the beetles' flight activity, pheromone traps were deployed close to the barns (similar to that shown in Figure 2.3). The traps were the Japanese beetle (JB) type, supplied be Trece Inc., Salinas, CA., USA. They consist of a yellow plastic funnel (diameter 15cm, height 11cm) with 4 vertical vanes, in the form of a cross, extending 10cm up from the funnel to give a baffle against which the beetles would fly and then tumble into the funnel to be collected by a plastic jar attached to its base. A horizontal 38cm-diameter plastic plate was attached to the top of the baffle to keep rain out of the traps.

Four pheromone traps, each loaded with 2mg of pheromone blend (Trunc-call 1 and Trunc-call 2 in a ratio of 1:1), were placed in each village so that all barns were about 100m from at least one trap. One Whatman's No. 1 filter paper treated with 5cm of 10% solution of 25g/l deltamethrin EC and put in the bottom of the plastic collecting jar to kill captured insects and so prevent them from boring out. The layout of traps and barns is shown in Figure 3.2 below:

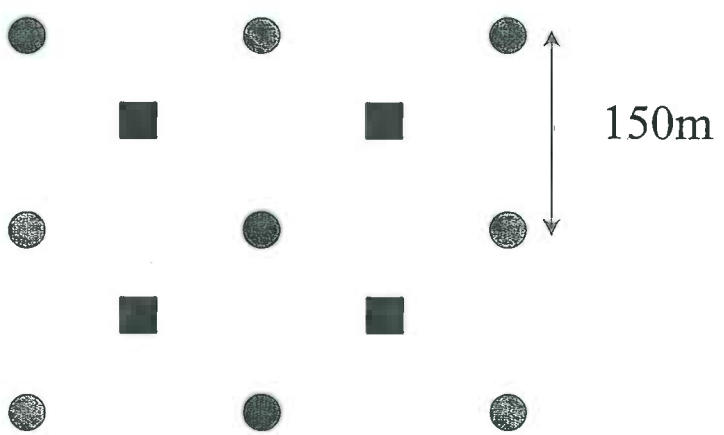

Figure 3.2: Layout of small barns and pheromone traps in villages selected for the risk assessment study. Traps are represented by squares and mini-barns are represented by circles.
NB. Approximately 100m between each trap and its nearest barns.

In the first season's work all barns in Nkwanta were loaded with cassava and all barns in Hohoe were loaded with maize cobs. In the second season four barns in each village were loaded with each commodity and one barn was loaded with a mixture of the two and monitored for infestation in the maize.

Villages were visited every two weeks to collect and count the numbers of *P. truncatus* captured in pheromone traps, to place fresh pheromone capsule in the traps and examine the barns for signs of infestation. Maize cobs or cassava chips were examined individually and if uninfested, returned to the barns. The first signs of infestation were detected as dust on the black sheeting at the base of the chicken wire cage. Once infested, the small barns were cleared of produce and were refilled with fumigated material after fallow period of at least two weeks (sometimes longer when commodity was scarce).

Results

Numbers of insects in traps

Trap catches of LGB are notoriously variable and the data presented here is no exception (see Figures 3.3 and 3.4). Often there are two peaks of flight activity per year (seen clearly in the first two seasons of data in Hohoe). Flight activity in Hohoe has been exceptionally high in 1999 (third season) which is not the case in Nkwanta where flight activity has kept fairly constant between years in most villages, but was exceptionally high in Chala Odomi and Gekrong in season one and Korantang in season two. This large variation in trap catches between locations and between years allows an easier determination of the influence of trap catch on barn infestation rates.

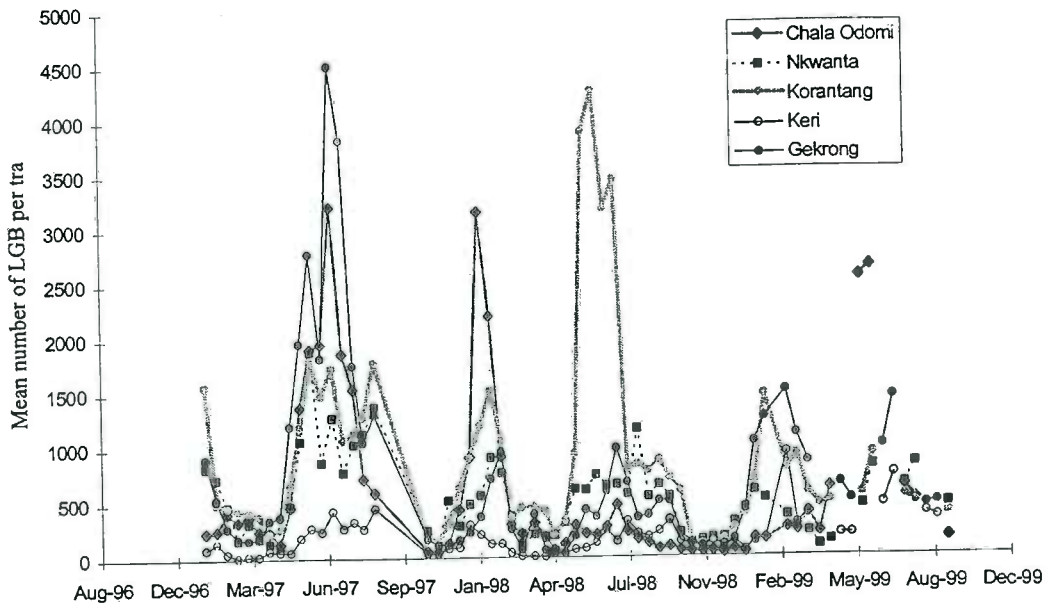

Figure 3.3: Mean number of LGB per trap per two weeks for villages in Nkwanta. NB. Line becomes broken in season three due to traps present/ traps absent comparison

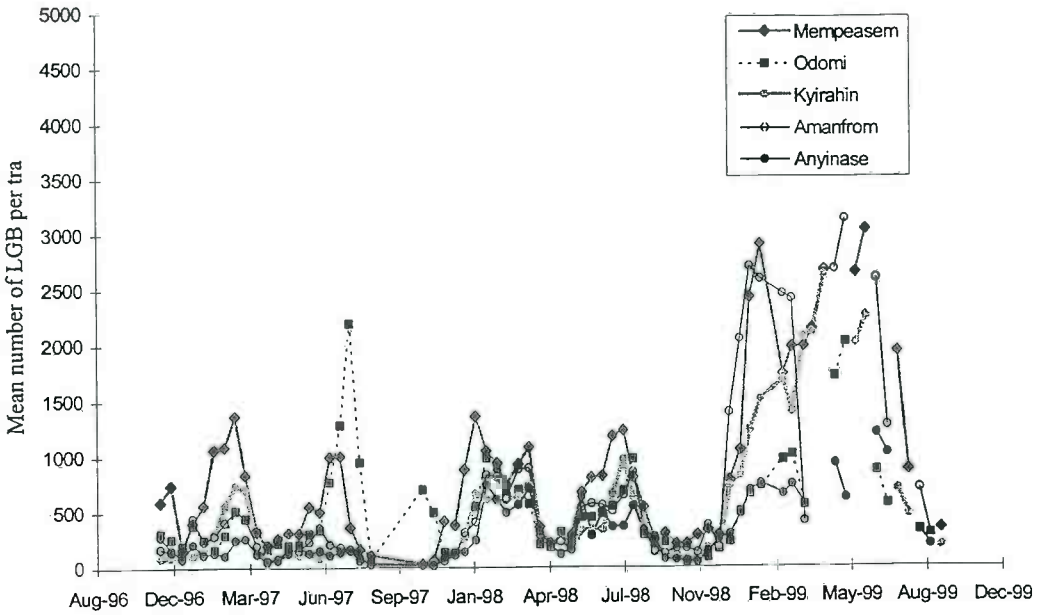

Figure 3.4: Mean number of LGB per trap per two weeks for villages in Hohoe. NB. Line becomes broken in season three due to traps present/ traps absent comparison

LGB infestation of mini-barns season one

- Maize in Hohoe: V. low infestation rates (5 hits)
- Cassava in Nkwanta: 84 hits over entire season

There were too few hits of maize in season one to draw any conclusions. The cassava data obtained in season one is presented alongside comparable data obtained in season two below.

LGB infestation of mini-barns season two

Descriptive statistics

Descriptive statistics for the instances where barns became infested (hereafter referred to as hits), are give for season two in Tables 3.2 and 3.3. Barns loaded with cassava were hit more frequently than barns loaded with maize in all villages except two where they were hit with equal frequency. Cassava barns were hit particularly frequently in the villages in Nkwanta region compared to Hohoe region.

Table 3.2. Summary of mean cumulative LGB associated with barn hits in Hohoe. NB. Figures in parenthesis are standard deviations expressed as a percentage of the mean.

Village	# cassava hits	# maize hits	# mix hits	mean cumulative LGB per cassava hit	mean cumulative LGB for maize hits	mean cumulative LGB for mix hits	mean weeks until hit for cassava	mean weeks until hit for maize	mean weeks until hit for mix
Bowiri Anyinase	2	2	1	2590	1828	796	16	12	8
Bowiri Amanfrom	2	5	1	3969	3414	5368	21	16	28
Bowiri Kyirahin	6	0	0	1676	-	-	11	-	-
Akpafu Odomi	2	2	2	1376	2309	1444	10	14	7
Akpafu Mempeasem	5	2	1	3591	3424	1787	13.6	14	10
Mean (all villages contribute equally)				**2640 (43%)**	**2744 (29%)**	**2349 (87%)**	**14.3 (31%)**	**14 (12%)**	**13.25 (75%)**
Mean (all hits contribute equally)	**3.4**	**2.2**	1	**2580 (67%)**	**2930 (38%)**	**2170 (86%)**	**13.4 (48%)**	**14.5 (20%)**	**12.0 (75%)**

Table 3.3: Summary of mean cumulative LGB associated with barn hits in Nkwanta. NB. Figures in parenthesis are standard deviations expressed as a percentage of the mean

Village	# cassava hits	# maize hits	mean cumulative LGB per cassava hit	mean cumulative LGB for maize hits	mean weeks until hit for cassava	mean weeks until hit for maize
Chala Odomi	8	2	745	701	8.8	8
Nkwanta	11	1	2263	7499	8.8	26
Korantang	11	3	5314	8949	9.8	13.3
Keri	4	0	674	-	13.5	-
Gekrong	6	0	1502	-	11.3	-
Mean (all villages contribute equally)			**2100 (91%)**	**5716 (77%)**	**10.6 (18%)**	**15.8 (59%)**
Season 1 data (all villages contribute equally)			**6016 (50%)**		**15.7 (15%)**	
Mean (all hits contribute equally)	**8**	**1.2**	**2525 (119%)**	**5958 (83%)**	**10.1 (45%)**	**13.7 (52%)**
Season 1 data (all hits contribute equally			**6359 (59%)**		**15.2 (60%)**	

Consistency between years

We have comparable data sets between years for cassava hits in Nkwanta. The mean cumulative LGB (total number of LGB caught during exposure time) recorded before a hit, was over twice as high in the first trial season compared to the second trial season. The variability of the cumulative LGB recorded up until a hit in the second season is about twice that in the first season.

Analytical statistics

Binomial logistical regression was used to determine which out of a number of possible variables significantly contributed to the risk of barns becoming infested. The variables tested were

- Commodity (maize or cassava)
- LGB trap catch
- Age of barn (how long since the barn was loaded with commodity)
- Barn history (the number of infestations recorded in any one barn that season)*
- Village
- Village cluster (Hohoe or Nkwanta)

*Barn history was included as an indirect way assessing if barns were properly cleaned after an infestation being detected before re-loading with fresh commodity.

The following variables were shown to be important

- Commodity
- LGB trap catch
- Cluster

Best model to explain barn hits:			
Barn hit = constant + Commodity + LGB trap catch + Cluster Log- Likelihood = -274.45, d.f. = 3, p-value =<0.0001			
	Coef.	**St. Dev.**	**P-value**
Constant	**-3.828**	**0.271**	**<0.0001**
Commodity	**1.416**	**0.279**	**<0.0001**
LGB trap catch	**0.000499**	**0.000130**	**<0.0001**
Cluster	**-0.711**	**0.250**	**0.004**

It is possible to turn the logistic regression equation around and use it to predict the number of LGB associated with a range of probabilities of barns becoming infested. This was done separately for maize and cassava, including only LGB trap catch as a predicting variable in the model, and is shown graphically in Figure 3.5.

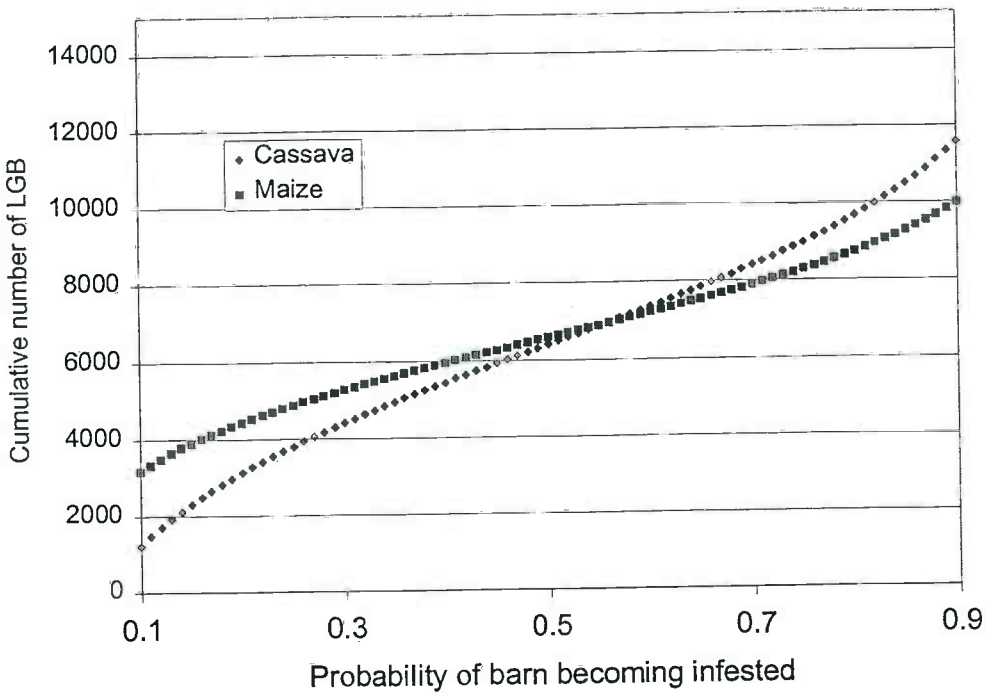

Figure 3.5: Current best estimate of the total numbers of beetles caught in pheromone traps over a period translates into increased risk of a barn becoming infested over that period
NB. The error associated with these curves is currently large

Discussion

The initial hypothesis that the size of trap catches of LGB is a highly significant determinant of the risk of experimental barns becoming infested has proven to be correct. Quantification of the relationship between trap catch and barn infestation risk has begun. We will use the third season's data to increase the accuracy of the model.

The nature of the commodity influences initial infestation rates. One hypothesis that might contribute to an explanation of this difference, is that sheathing cover in maize cobs prevents the entry of some LGB that land on the store, thus delaying initial infestation. The reasons for faster initial infestation of cassava will be explored in the next phase of this project. Barn history was not a determinant of barn infestation, therefore fears that the rigorous cleaning of barns is not sufficient to detect all LGB possibly hiding in the wooden parts of the barn were unfounded. Concerns about residual infestation are a crucial consideration in the case of LGB, since pheromone signalling from single males is enough to attract other insects to a barn.

The challenge is now to validate the system in real farmer's stores and quantify the relationship between trap catches and increase in infestation rates under these conditions. Validation of the model in cassava barns will be conducted in Ghana, and in cob maize stores in Tanzania.

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4. Bio-characteristics of *Prostephanus truncatus* attracted to flight traps baited with aggregation pheromone

Addo S., Birkinshaw L. A., and Awuku M.

Summary

Indicators of the reproductive capacity (and therefore damaging capacity) of dispersing insects caught in flight traps was assessed for three different times of the year in four different locations. Any large differences in these biological characteristics of dispersing insects would need to be accounted for in the risk assessment model.

It was found that most dispersing insects were relatively young and reproductively active and therefore of high threat to stored food. Females were able to reproduce without further access to males implying they were already inseminated at the time of dispersal. No significant differences in the initial rates of F1 production by females were found between seasons and sites, even though some sites were in villages and some sites were in forests away from maize or cassava stores. The results of this study indicate that it is reasonable to assume that the LGB caught in flight traps are of approximately equal threat to stores. No special adjustment to the risk assessment model according to time of year or location is justified.

Background

It is important to know if the populations of LGB that are sampled to indicate risk to stores (those caught in pheromone-baited fight-traps) always have a similar potential to damage stored food. For instance, it may be that high trap catches in locations away from any stores (presumably insects migrating from 'natural' hosts) may be of old beetles of low remaining fecundity. Similarly, insects caught at different times of the year could have different biological characteristics that would influence their threat to stored food. If such differences exist then they would need to be incorporated into any risk assessment model. Knowledge of the biological characteristics of dispersing LGB in different seasons also adds to the ongoing challenge to understand why the numbers of insects caught in flight traps varies with season.

There is no strong evidence of any sex specific difference in response to pheromone signals once male habituation to self signals are accounted for (Birkinshaw, 1998); no documented change in response due to mating status (Boughton and Fadamiro, 1996); and all but the very young and the very old will respond to this signal (Boughton and Fadamiro, 1996).

Bio-characteristics investigated

Beetle size

Fresh weight of trapped beetles was measured as a proxy for condition to assist in interpretation of any changes in female fecundity with season or location.

Sex ratio

Previous studies in Southern Benin have shown that approximately 76.5% of beetles caught in flight traps baited with artificial pheromone were female (Scholz, 1997). This was fairly constant over a year's trapping. If access to mating is not a limiting

factor then a larger proportion of females in traps may represent a greater potential for damage to stores. However, there is some evidence that males of this species provide a relatively large contribution towards the rate of offspring production under some circumstances in this species (Birkinshaw *in prep.*).

Longevity

The longevity of isolated beetles after trapping was measured to determine if the age profile of insects caught varied between season and location. Females produce maximum rates of offspring around the age of three weeks, after which it declines slowly until death (when females have access to males) (Bell and Watters, 1982). Obviously younger insects close to their reproductive peak in fitness would pose a greater threat to stores than old, less fecund insects.

Reproductive potential

It is also particularly important to know the potential initial rates of offspring production when a female arrives at a store and whether they vary with season and/or location. We have continued to monitor the profile of offspring production after capture in the absence of males to quantify the longer term colonising potential of females arriving by chance in stores.

Methods

Study sites

Four locations were chosen for tapping of LGB. The trapping locations/village map co-ordinates are given in Table 5.1. The special characteristics of each study site are given in Table 5.2.

Table 5.1: Study site co-ordinates.

Agricultural areas		**Non-agricultural areas**	
Habitat	map co-ordinates	Habitat	map co-ordinates
a) Nkwanta villages		a) Nkwanta savannah	08° 17.45 N
Korantang	08° 15.03 N		00° 29.58 E
	00° 29.52 E		
Nkwanta Zongo	08° 16.05 N		
	00° 31.12 E		
b) Hohoe village		b) Ho Forest	
Akpafu Odomi	07° 16.62 N		06° 38.46 N
	00° 28.66 E		00° 28.04 E
Akpafu Mempeasem	07° 14.70 N		
	00° 28.92 E		

Table 5.2. Notes on the ecology of trapping sites.

	Characteristics of location
Non-agricultural Habitats	
Ho Forest	Teak *(Tectona grandis)* plantation with few under growths which include Acheampong weed *(Chromolaena odorata), Tridax procumbens, Euphobia sp.* And few trees of odum, *(Chlorophora excelsa)* and silk cotton *(Ceiba pentandra).* Forest is outside the Ho township (about 4 km) and with no maize farm or store. Ho Forest is in the forest- transition zone.
Nkwanta savannah woodland	Vast stretch of savannah grassland (about 8 km from Nkwanta) which is devoid of maize farms and maize stores. Area is interspersed with short woody plants notably *Anogeissus leiocarpus,* (hehe) *Crossopteryx febrifuga,* (ahohoe) *Fagara xanthoxyloides* (xeti), *Baphia nitida* (toti), and *Acacia* sp. The Nkwanta location is situated in the semi-arid region.
Agricultural Habitats	
Hohoe (village)	Trapping villages (Mempeasem and Odomi) in Hohoe locality are sited in the forest –transition zone. A lot of maize is stored as husked cobs in barns standing in the compound. Storage could be up to 8 months. Few trees (notably, *Mangifera indica* (mango), *Azadirachta indica* (neem) and *Acacia* sp.) are present.
Nkwanta (village)	Two villages (Nkwanta Zongo and Korantang) form Nkwanta (village). The villages have dried cassava chips (kokonte) and maize stored either in rooms or in barns. *Mangifera indica* (mango), *Azadirachta indica* (neem) and *Funtumia africana* (kpomi) and *Citrus* sp. are the main woody trees in the villages.

Trap design

The traps used for this experiment were Japanese beetle (JB) type, supplied by Trece Inc., Salinas, CA., USA (see Figure 5.1). They consisted of a yellow plastic funnel (diameter 15 cm, height 11 cm). On top of the funnel were four vertical vanes, in the form of a cross, extending 10 cm up from the funnel to give a baffle against which the beetles would fly and then tumble into the funnel. A horizontal 38 cm-diameter plastic plate was attached to the top of the baffle to keep the rain out of the traps and a plastic jar was attached to the base to collect the captured beetles. Each plastic jar was

loaded with 60 glass specimen tubes (diameter 1.3 cm, height 5 cm) held in position by a rubber band so that beetles that arrive would drop in the tubes. The upright glass tubes enable many of the arriving beetles to be separated singly.

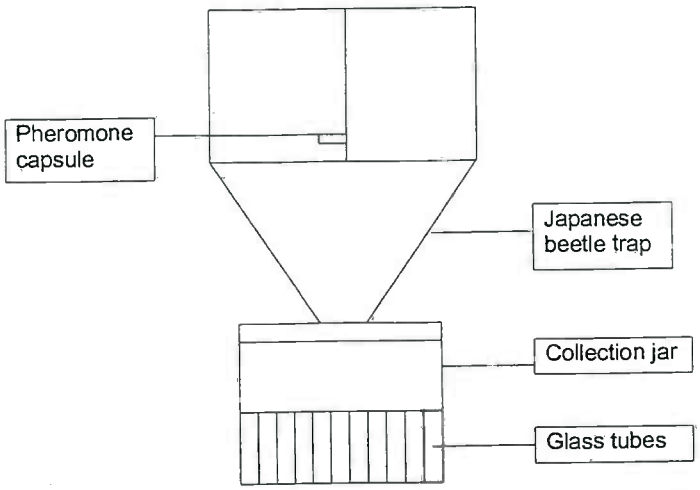


Figure 5.1: Trap design that allows live insects to be kept separate after trapping

Trapping procedure and timing

Five traps were placed in each of the four locations described above at the times indicated in Figures 5.2 and 5.3. Each trap was loaded with 2 mg of pheromone blend (Trunc-call 1 (T1) and Trunc-call 2 (T2) in a ratio of 1:1 and tied onto twigs (which were 1.5 to 2m from ground and at least 300m apart). Traps were set before noon and their contents removed by 8 a.m. the next day. A small quantity of maize flour milled using Laboratory Mill 3303, (Perten instrument, Warrington), (to serve as food) was put into the specimen tubes and sealed with a plastic top that had a small hole in it to serve as air passage. Samples collected were sent to the laboratory for sexing.

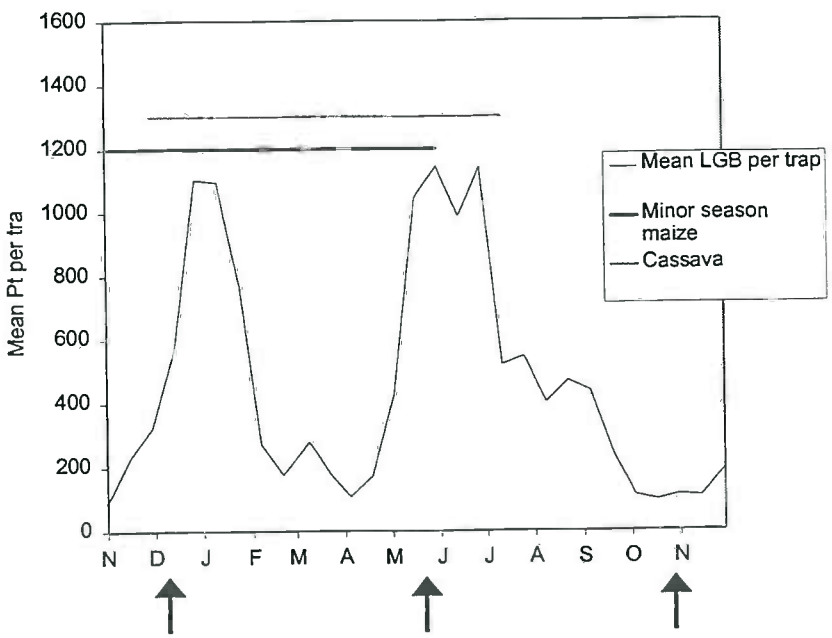


Figure 5.2: Timing of trapping relative to LGB trap catches and the main maize and cassava storage seasons in Nkwanta. N.B. Arrows indicate times of trapping.

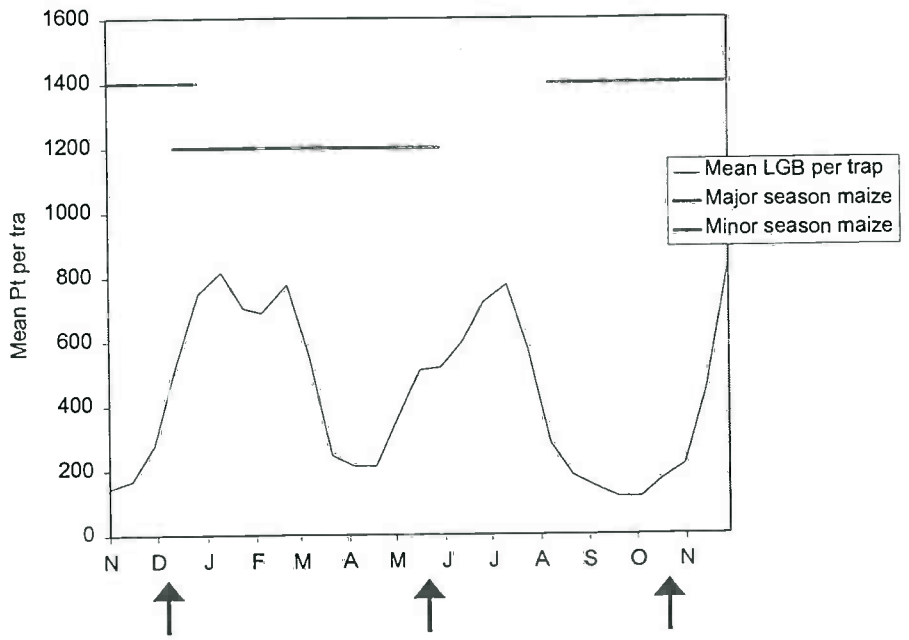


Figure 5.3: Timing of trapping relative to LGB trap catches and the main maize and cassava storage seasons in Hohoe. N.B. Arrows indicate times of trapping.

Sexing method

LGB were initially sexed by checking the form of their clypeal tubercles (Shires and McCarthy, 1976). Sex was confirmed by examining reproductive organs at the end of the trials.

Culturing regime and recording of F1 production

Twenty-five females that had not had the opportunity to mate at the time of capture, and twenty five males were used to prepare single specimen cultures. Each beetle was placed on a small quantity (about 30g) of kibbled maize (that had been sterilised in a deep freezer for 2 weeks) in plastic containers (about 60 cm^3) with tight fitting lid. The lids of the containers were perforated with circular hole (diameter 5 mm) and covered with micropore tape to allow ventilation. A small quantity of lead weights (mean weight 27g) sealed in plastic was placed on the top of the kibbled maize in each tube to stabilise it. All cultures were kept in a laboratory whose conditions were the same as ambient conditions outside. Every three weeks, male and female beetles were checked for their mortality (longevity) and females were transferred onto fresh kibbled maize (although see results for exceptions). The first filial (F_1) generation was counted sixty-two days after captured females were placed on the kibbled maize.

For the control beetles, 25 males and 25 females were selected from laboratory cultures set up 55 days earlier with 100 unsexed adult LGB. One hundred young beetles (4-6 days old, determined by their light brown colour) were selected and placed on kibbled maize for seven days to ensure good chance of mating before being used in single specimen culture.

Parameters recorded

- Fresh weight of newly captured insects
- Sex ratio of all LGB captured

- Longevity of approximately 25 males and 25 females from each treatment
- Profile of F1 production of approximately 25 females from each treatment (including total offspring produced and total time female continued to produce viable offspring)

Results

Beetle size

- As expected, females were generally larger than males (see Figures 5.4 and 5.5).
- There was no significant seasonal effect on body weight for either males or females (see Table 5.3).
- Body weight was relatively consistent between locations with some suggestion of a locational effect in season one, for males and females arising from low weights in Ho forest. Female body weights were also significantly different between locations for season two, where insects caught in Nkwanta savannah were generally heavier than those caught elsewhere.
- There is no evidence that insects with access to village stores are any heavier than those caught in habitats where maize and cassava are not available.

Table 5.3. Summary of statistical analysis for differences between the fresh weights of beetles caught in different locations and seasons. Females and males analysed separately.

FEMALE WEIGHTS	Test	d.f.	Test statistic	p-value
SEASON 1				
Female wt between locations	ANOVA	3+82	F= 3.91	0.012
SEASON 2				
Female wt between locations	ANOVA	3+75	F= 2.35	0.08
SEASON 3				
Female wt between locations	ANOVA	3+84	F= 1.09	0.36
Female wt across Seasons (all locations combined)	ANOVA	2+251	F= 2.26	0.12

MALE WEIGHTS	Test	d.f.	Test statistic	p-value
SEASON 1				
Male wt between locations	ANOVA	3+86	F= 2.91	0.039
SEASON 2				
Male wt between locations	ANOVA	3+67	F= 0.84	0.48
SEASON 3				
Male wt between locations	ANOVA	3+74	F= 1.84	0.15
Male wt across Seasons (all locations combined) NB. Maybe not valid as season 1 has sig. Location effect)	ANOVA	2+236	F= 0.134	0.88

Sex ratio

- The sex ratio of beetles caught in traps between locations was fairly consistent with the exception of a significant difference detected in season 3 (see Figure 5.6).
- The sex ratios are similar to those recorded in Benin (Scholz 1997).
- No seasonal effect on sex ratio was detected in any locations with the exception of Nkwanta savannah where the proportion of beetles caught that were females fell in season three (chi-squared = 6.79, d.f. = 2, p=0.034).

Longevity

LGB caught during this study continue to remain alive for a surprising long time after capture. Some females were still alive up to a year after they were caught, however, in general half the insects caught were dead after about 25 weeks. For a detailed profile of mortality following capture see Figures 5.7 and 5.8. It can be seen that these profiles are fairly similar between locations and seasons with the exception of female insects caught in Ho forest which exhibit better than average survival in season two, yet much worse than average in season three. This pattern is not mirrored in male survivorship.

Reproductive potential

Almost all females caught in traps produced viable offspring without further access to males. The highest total number of offspring produced by a female was 97.

The profiles of F1 production shown in Figure 5.9, illustrate that rates of offspring production per live female decrease from a high initial rate of around eight offspring per 21 days per female to almost nothing after five months of capture. One female still produced live F1 28 weeks after capture. Generally females caught in season three had shorter reproductively active periods following capture than in seasons one or two (see Figure 5.11) which then resulted in lower total numbers of offspring produced (see Figure 5.10). However, note that the mean time that females continued to produce viable offspring and the total numbers of offspring produced by females was also low for the beetles taken from a laboratory culture (control). Therefore, the observed reduction in overall reproductive potential in season three might have arisen from changes in the climatic conditions in the laboratory rather than biological differences between insects caught at different times of the year.

Initial rates of F1 production, however, were not different between seasons (see Table 5.4) and significant locational effects were only demonstrated in season three.

Table 5.4: Summary of statistical analysis of total numbers of F1 produced by females, total time females continued to produce viable F1 and the initial rate of F1 production. NB. The laboratory culture control data is not included in the analysis.

	Test	d.f.	Test statistic	p-value
SEASON 1				
Total F1 between location	ANOVA	82+3	F= 3.04	0.034
Time repro. between location	Kruskal-Wallis	3	$Chi^2 = 21.7$	<0.001
Initial repro. between location	ANOVA	82+3	F= 2.06	0.11
SEASON 2				
Total F1 between location	ANOVA	75+3	F= 1.21	0.31
Time repro. between location	Kruskal-Wallis	3	$Chi^2 = 4.06$	0.26
Initial repro. between location	ANOVA	75+3	F= 1.64	0.19
SEASON 3				
Total F1 between location	ANOVA	84+3	F= 1.52	0.22
Time repro. between location	Kruskal-Wallis	3	$Chi^2 = 11.8$	0.008
Initial repro. between location	ANOVA	84+3	F= 3.93	0.01
ALL LOCATIONS COMBINED				
Total F1 between seasons	ANOVA	251+2	F= 14.6	<0.001
Time repro. between seasons	Kruskal-Wallis	251+2	$Chi^2 = 66.3$	<0.001
Initial repro. rate between season	ANOVA	251+2	F= 0.7	0.50

Trends between parameters

No significant correlation was found between body weight and either total offspring production or initial rate of F1 production in females (see Figures 5.12 and 5.13 respectively). This shows that we cannot use female body weight as a proxy indicator for the potential of that female to produce F1 in the absence of males.

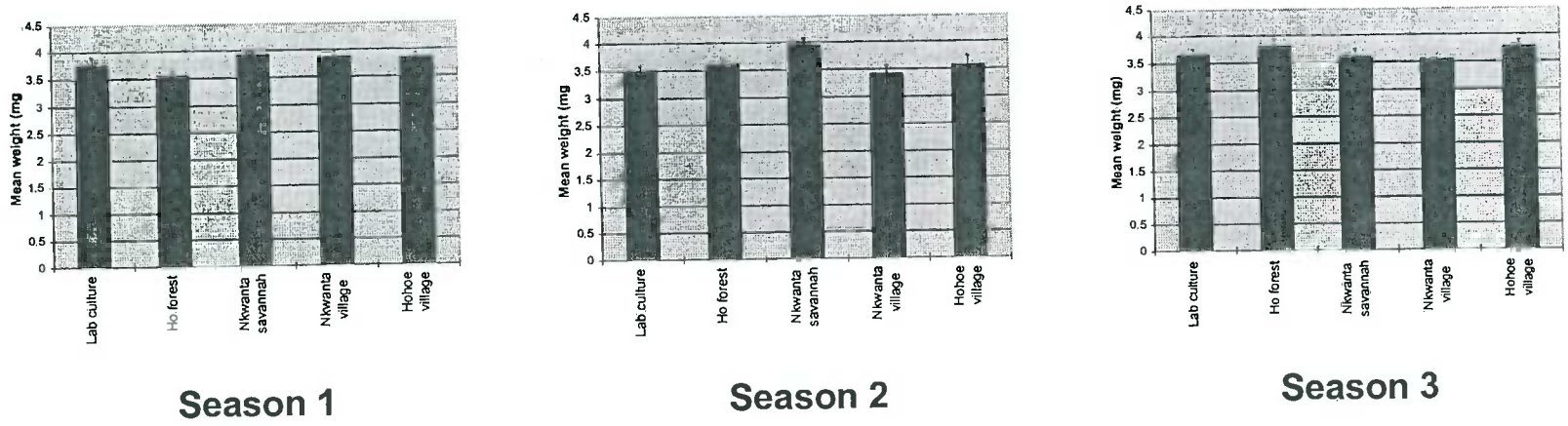


Figure 5.4: Mean weights of females caught in traps

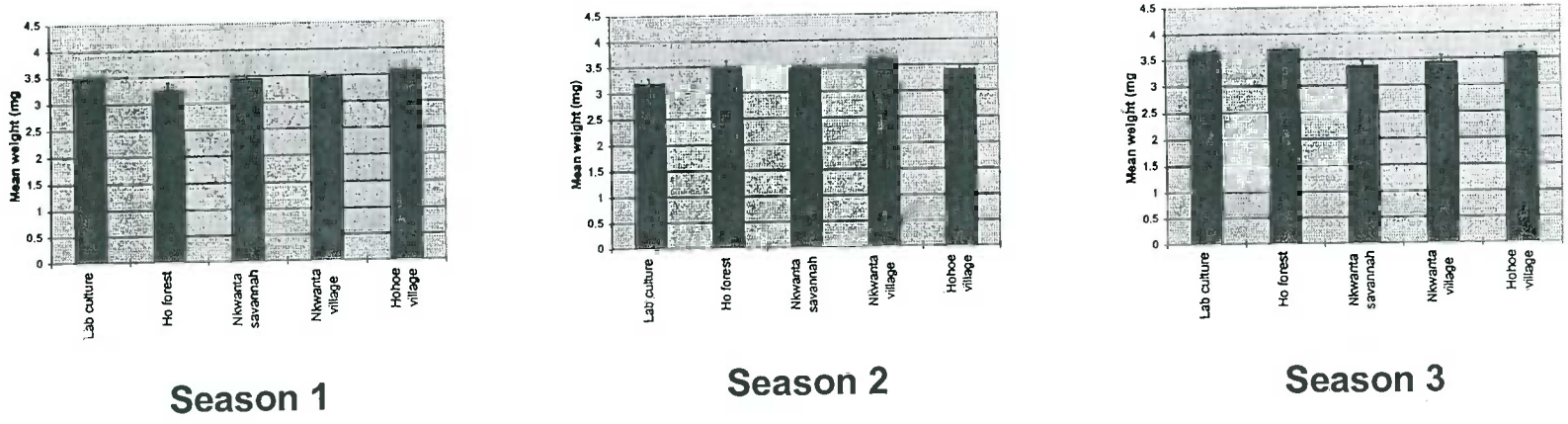
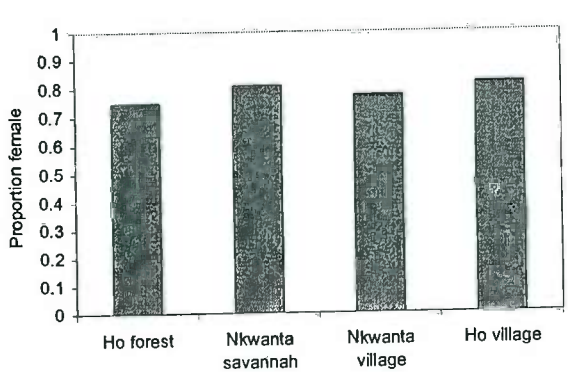
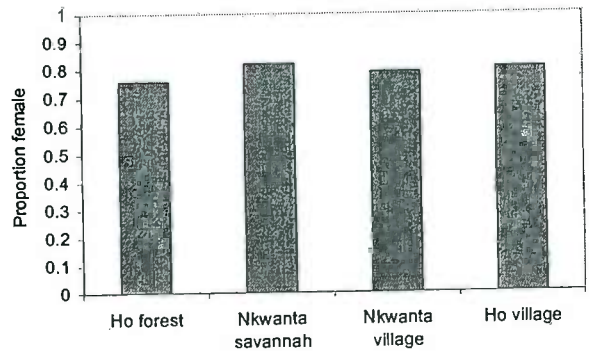


Figure 5.5: Mean weights of males caught in traps



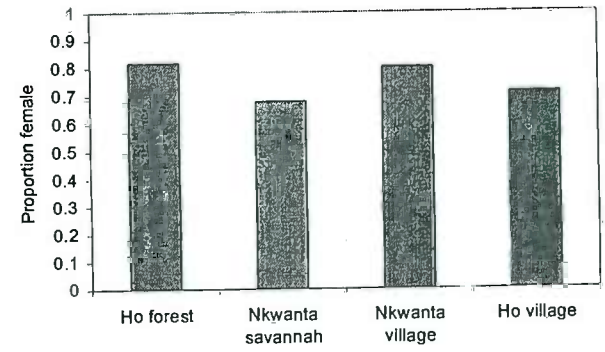
Season 1

Analysis for locational difference:
(chi-squared = 2.51, 3.d.f., p=0.47).



Season 2

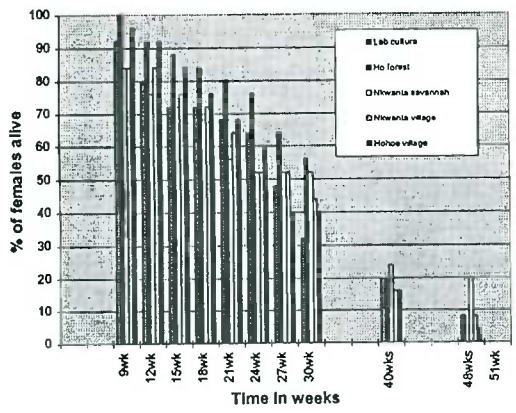
Analysis for locational difference:
(chi-squared = 4.53, 3.d.f., p=0.21).



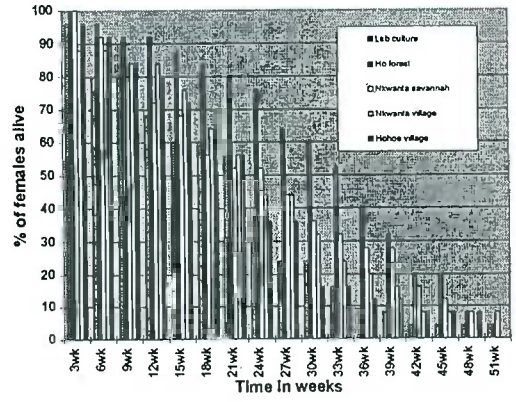
Season 3

Analysis for locational difference:
(chi-squared = 8.34, 3.d.f., p=0.04).

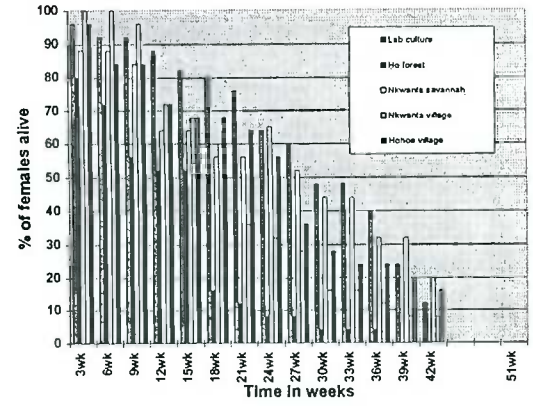
Figure 5.6: Sex ratio of beetles caught in each season. (N ranges from 78 to 181 per bar)



Season 1

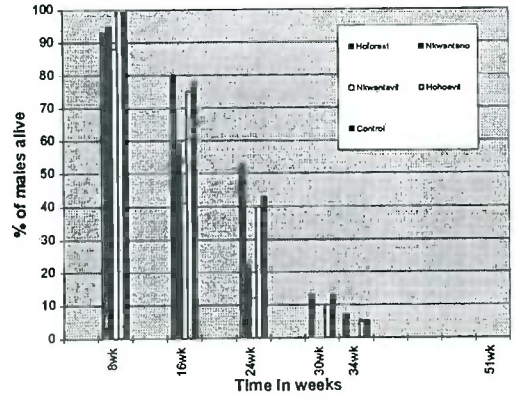


Season 2

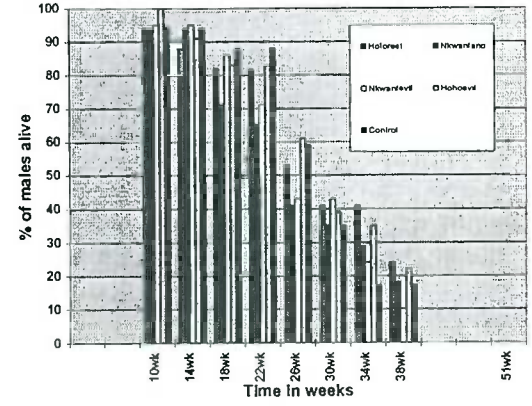


Season 3

Figure 5.7: Survivorship curves for females taken from the time they were caught in traps



Season 2



Season 3

Figure 5.8: Survivorship curves for females taken from the time they were caught in traps

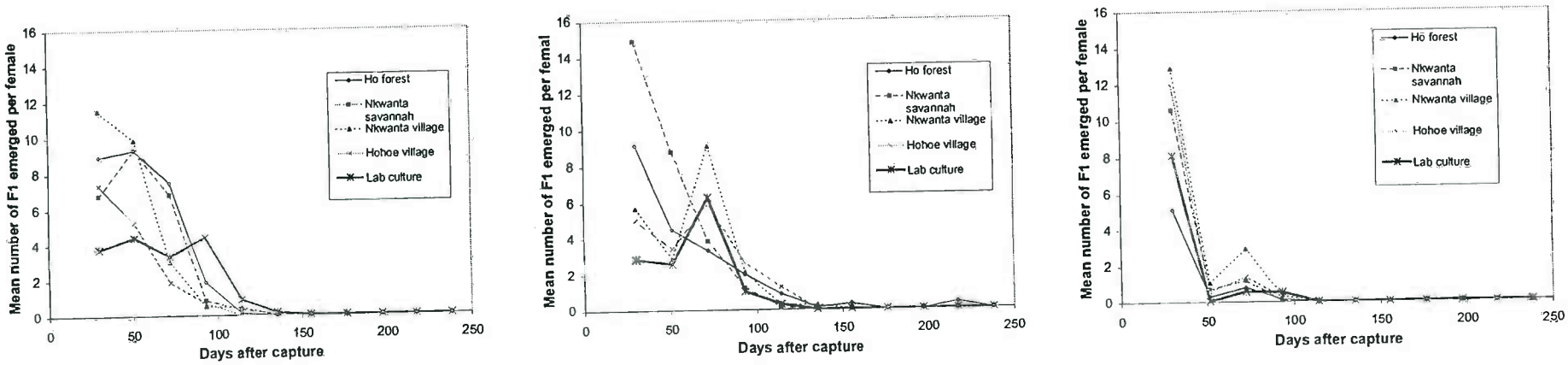
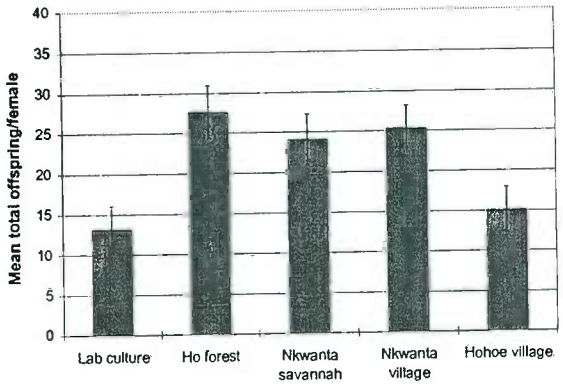
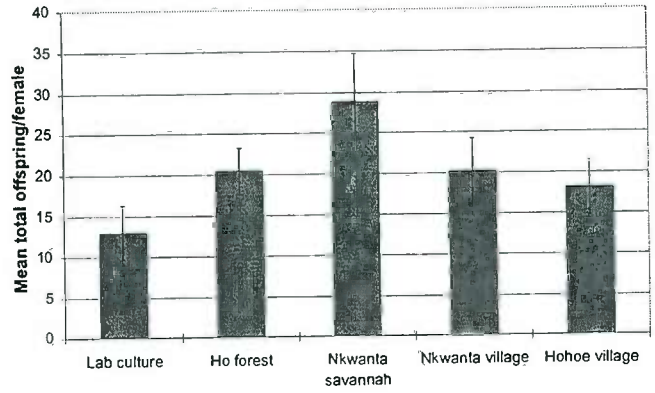


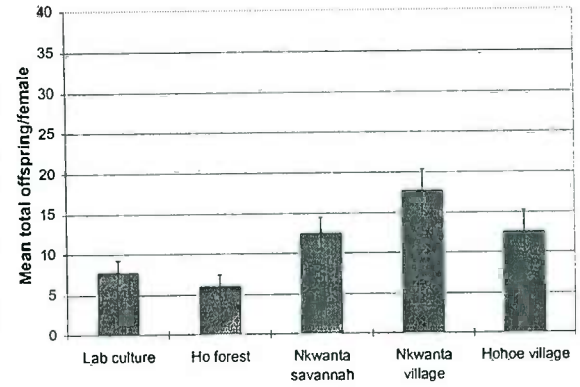
Figure 5.9: Profile of female F1 production with time (calculated per original female)



Season 1

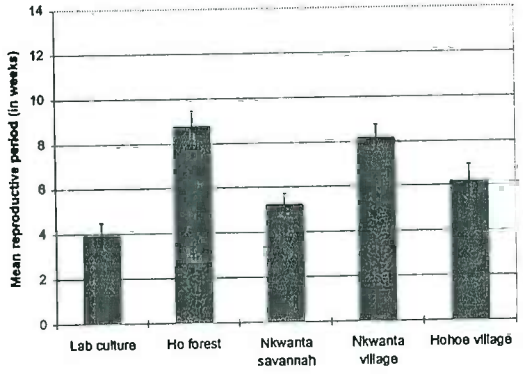


Season 2

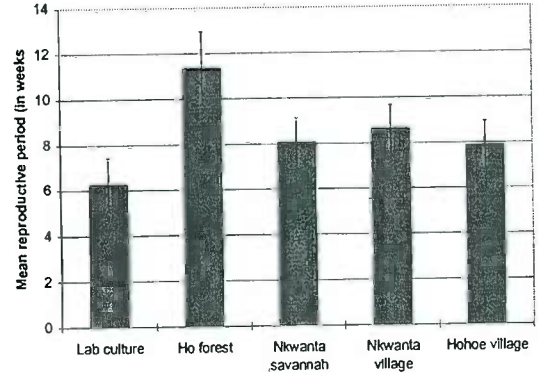


Season 3

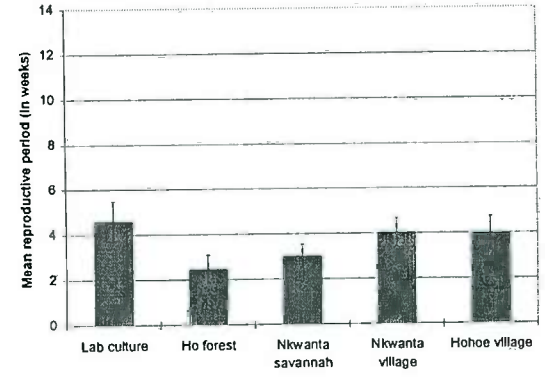
Figure 5.10: Mean total number of F1 produced per female



Season 1



Season 2



Season 3

Figure 5.11: Mean length of time females continued to produce viable offspring after capture

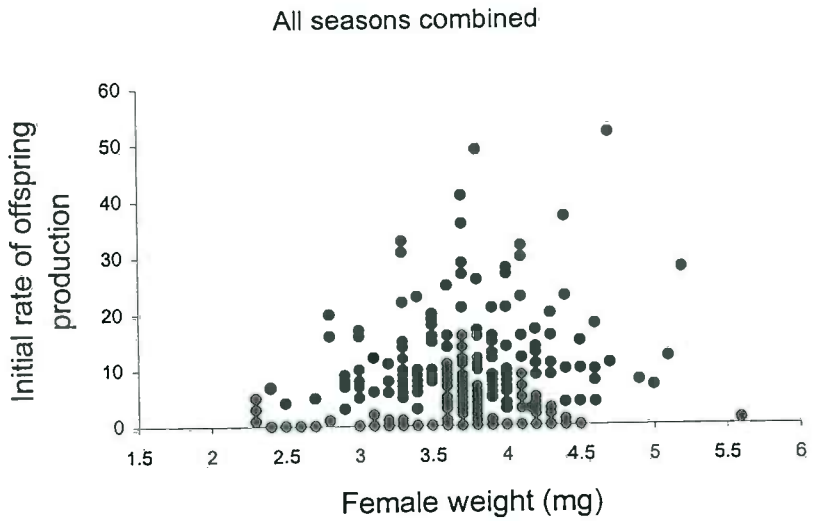


Figure 5.12: Female fresh weight vs. initial rate of F1 production for all locations and seasons combined

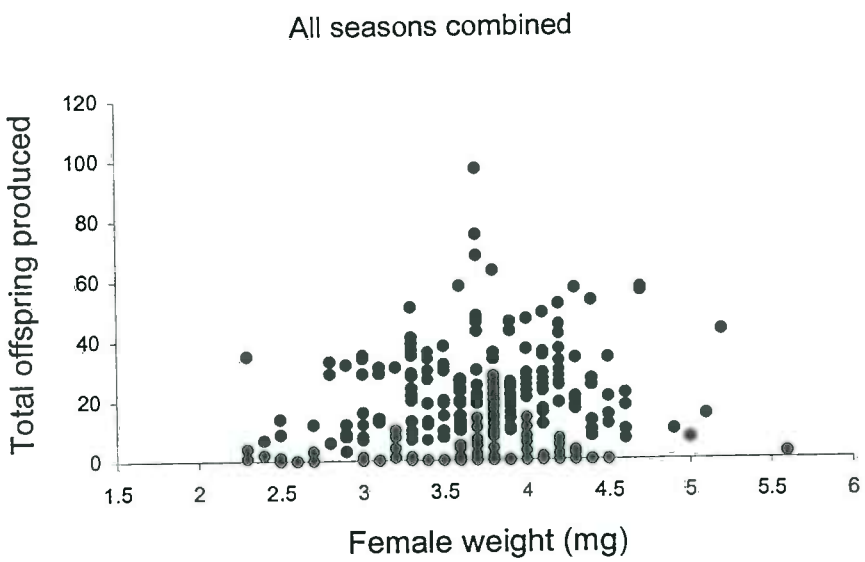


Figure 5.13. Female fresh weight vs. total numbers of F1 produced for all locations and seasons combined

Discussion

Dispersing insects caught in traps generally had high potential life expectancy. Females were almost all fertile and often capable of reproducing for a very extended length of time without males and at highest rates soon after capture i.e. when females would arrive in stores. The threat posed by dispersing insects inferred from the biological characteristics measured in this study, was relatively constant between the seasons sampled, and between different locations. The main exception being some fluctuations in mortality rates between locations that had knock-on effects to the total number of F1 produced per female in these cases. Further study would be required to fully understand this observation, however we can now be fairly confident that there isn't any dramatic shift in the biocharacteristics of dispersing insects with time, and no consistent differences between non-agricultural vs. agricultural sites or semi-arid sites vs. forest transition sites. However it must be stressed that those beetles caught in pheromone traps are not necessarily an unbiased sample of the dispersing population and there is always the chance that shifts in the biocharacteristics of the dispersing population were not represented in the beetles caught in traps.

Given the information from this study, there is no strong justification for a need to incorporate a change in the damaging potential of insects in different seasons, beyond the changes in **numbers** of insects caught that the risk assessment model is based on.

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5. Impact assessment of *Teretrius nigrescens* in the Volta Region of Ghana following its release in 1994

S. Addo, Acquaye K., Agbenyega F., Amekupe S., Asempah D., Awuku M., Birkinshaw L., Bokor G., Compton J., Dekavie R., Gbedevi S., Glah S., Golob P., Jeffries D., Mayebi A., Newell F., Ofosu T., Owoo P., Motte F. and Tettey I.

Summary

Teretrius nigrescens (Tn) (Coleoptera: Histeridae) has established itself well in the Volta region after its release in 1994. It is hoped that Tn will prey on immature LGB to the extent that it will reduce the damaging capacity of this pest in farmer's stores. It is difficult to determine the reasons behind any changes in damage caused by LGB. However, the impact assessment reported here does not indicate a large impact of Tn within the four years since its release. Since Tn is a reported as a success in other countries, we must now determine which situations favour its impact.

Background

Teretrius nigrescens (Tn) (Coleoptera: Histeridae) is a predator of the storage pest *Prostephanus truncatus* (LGB) (see Figure 5.1). Both predator and pest are endemic in meso-America but the pest was accidentally introduced into the African continent in the late 1970's and the predator has subsequently been introduced as a classical biocontrol agent into Togo (1991) (Biliwa *et al.*, 1992), Kenya (1992) (Giles *et al.*, 1995), Ghana (1994) (Compton and Ofosu, 1994), Benin (1992) (Borgemeister *et al.*, 1997), Zambia and Tanzania (Riwa W. pers. Comm.).

The decision to introduce Tn was influenced by laboratory observations on the impact of the predator on *P. truncatus* (Rees, 1991; Helbig and Schulz, 1996). There has been little evidence that *T. nigrescens* is able to reduce the population size of the pest in stores or affect the extent of the losses sustained by farmers (although see Richter *et al.*, 1997). Damage from LGB in stores is variable between years. It is very unlikely that Tn will eliminate LGB in Africa. The hope however, is that Tn will reduce the overall population size of LGB and thereby reduce or even eliminate years when LGB damage is exceptionally high.

Work published during the life of this project, from a study in Benin by GTZ-funded projects, has demonstrated rising numbers of dispersing Tn and reducing numbers of dispersing LGB, but proof of a causal link between these observations is still lacking (Borgemeister *et al.* 1997). The drop in LGB numbers in traps could easily have arisen from changes in farmer practices if a significant proportion of the LGB population is in fact in stored commodity. The Togo/Benin data is interpreted cautiously in the papers, but strongly in a symposium review paper (Nakakita, 1998) and an article in INPhO (see quote in box below), as evidence that Tn "works". On this basis, GTZ and IITA in particular, have gone ahead with a programme of releases in far-flung areas such as Zambia, to which the natural spread of Tn would normally take a long time.

Figure 5.1: *Teretrius nigrescens*, adult and larvae on maize.

'The Larger Grain Borer no longer is a threat to maize famers in the subtropical regions of West Africa. The first worldwide success in biological control of a post-harvest pest, the Larger grain borer, was achieved in the Republic of Benin and in Togo by its natural enemy, the predatory beetle Tn.' (INPHO, 1999).
??

This project aimed to broaden the before/after release comparison to include areas with and without Tn (release and non-release areas) to eliminate the confounding effect of changes in LGB beetle populations with time and changes in farmer practices. Unfortunately, shifts in the grain market resulted in Tn arriving at non-release sites before any meaningful comparison could be made between release and non-release sites. This was almost certainly due to the devaluation of the CFA in February 1994, which led to a reversal of the 'usual' flows of marketed maize from Ghana to Togo. The first catch of Tn in Ghana (at Penyi village, in the south near Lome) was recorded in April 1994 (Compton and Ofosu, 1994). This has meant that the data are only able to demonstrate changes with time.

Male *P. truncatus* produce a volatile pheromone that attracts dispersing male and female LGB. Flying *T. nigrescens* are also attracted to this signal. Artificially-synthesised LGB aggregation pheromone can be used to bait flight traps which can be used to sample the dispersing populations of LGB and Tn very effectively. A proportion of the total LGB population lives in plant hosts away from stores (see Figure 5.2). We have therefore sampled both storage populations and dispersing populations (using pheromone traps) to monitor for impact of Tn.

It is reasonable to assume that the number of beetles captured in flight traps are fairly representative of the numbers of dispersing beetles (although traps may vary in their attractivity between different times of the year). Within any year, seasonal changes in trap catch are unlikely to be accurate representations of changes in the total population of LGB and Tn (since dispersal rates may vary with season), however, **year to year** shifts in the numbers of predator and prey caught in flight traps may reflect shifts in total population size, particularly if there is a consistent trend over many years.

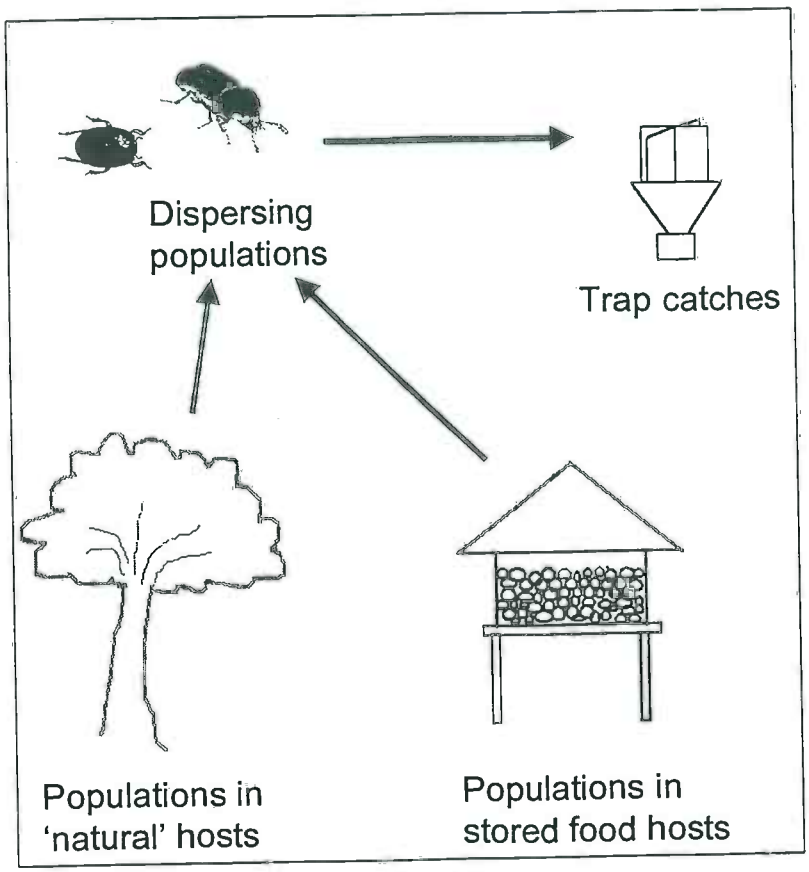


Figure 5.2: Diagram to show which beetles are sampled using traps. Store samples need to be made to directly assess populations in store, yet trap catches can give some indication of changes in the overall population of beetles including those from 'natural' hosts.

Aims

1. Record establishment of Tn in the Volta region
2. Record corresponding changes in LGB abundance in traps and in stores

Methods: Tn release and non-release sites

Tn was released in twenty villages in Volta Region (V/R) in May and again in September 1994, approx. 1000 adult beetles per village on each occasion, using 'free release' from a tray. The release villages were grouped into four clusters of five neighbouring villages each (Papase, Bodada, Ayoma and Peki clusters). These clusters were compared with four clusters of 'non-release' villages (Brewaniase, Hodzo (two villages only), Fodome, and Dzolo), chosen to be broadly similar in maize storage practices and LGB history.

Locations of all the villages, both release and non-release are shown in Figure 5.3

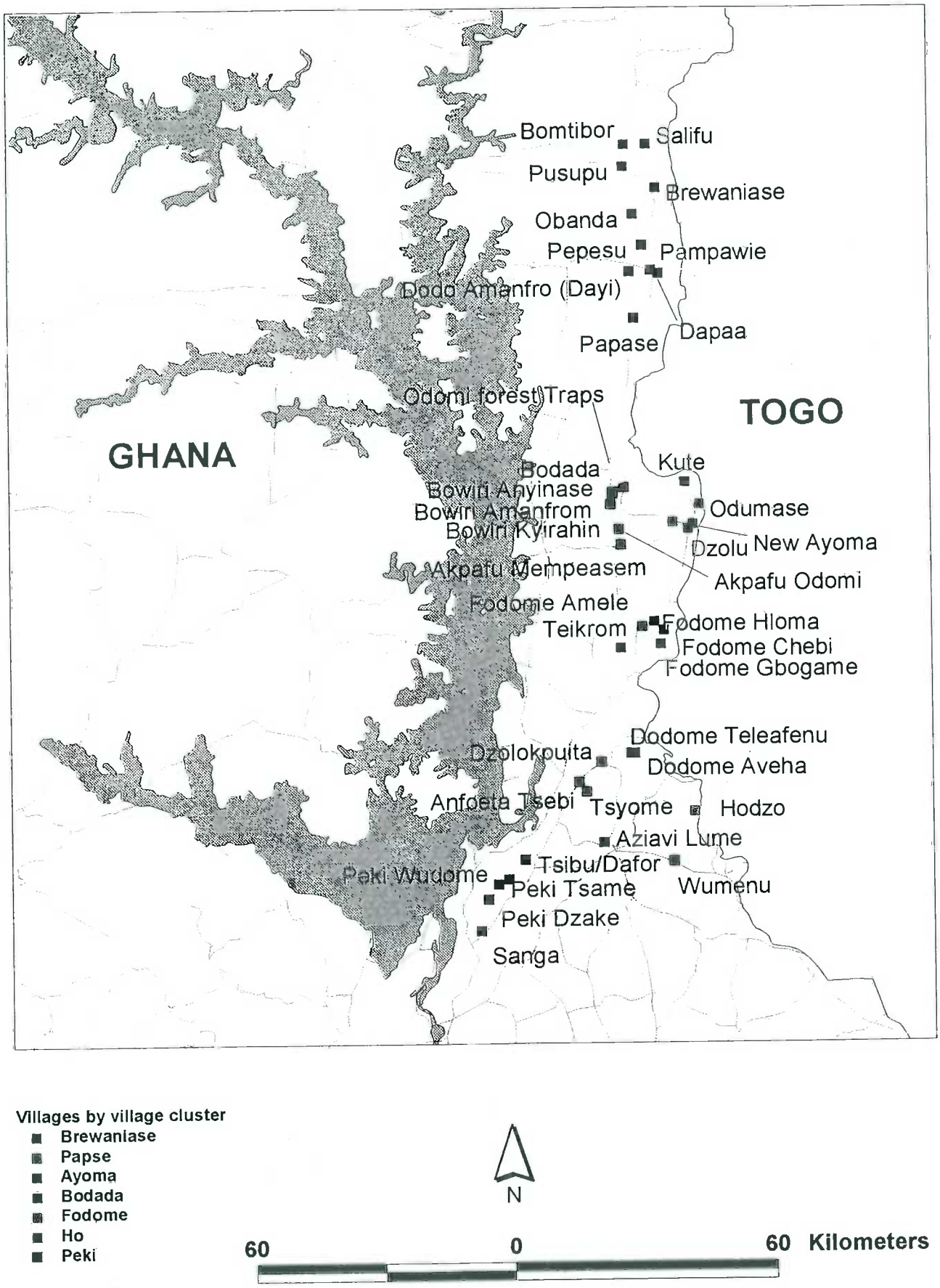


Figure 5.3: The location of study villages in the Volta region of Ghana.

Methods: In storage

Transect walks

Insect populations of predator and prey in the storage environment were assessed during transect walks through all villages in all clusters. These were initially performed monthly.

Enumerators walked in a roughly straight line through each village and sampled stores at random along this line (at least 20 stores in each village). Variables measured using a visual inspection of the external surface of the stores included:

- Percentage of stores with LGB and with serious LGB
- Median time in store to first appearance of LGB
- Length of time maize kept in store.

Cobs were sampled to estimate losses in at least 20 stores in each village. 30 cobs were removed from each store and scored for the presence of LGB, Tn and *Sitophilus spp.* A visual scale was used to estimate losses, (Compton and Sherington, 1999). Each cob was classified into one of six classes, according to the visible damage level. Through calibration of the visual scale with more direct methods of estimation, the scale provides an accurate and rapid method for estimating both weight losses and value losses.

The original protocol for this study was to compare Tn release and non-release villages. Data was collected in 1994/5. Meanwhile, the spread of Tn to both release and non-release villages means that a 'with and without' approach to impact monitoring was no longer appropriate so the transect walks were discontinued.

Extension agent surveys

Extension agents were questioned in 1994, '95 and '97 about maize storage and LGB attack in the villages in which they operate. Although the data is subjective and reliability is variable, extension agent survey data are useful in giving a picture of the region, especially outside the area directly visited by the project team.

Methods: In traps

All eight of the village clusters were included in the flight-population-monitoring programme (see Figure 5.3). Two traps were placed in each village (at least 200m apart). In addition to the village sites, two forest sites were monitored. These were at least 1.5-2 kilometres away from maize producing areas.

Monitoring of the dispersing populations of LGB and Tn started in September/ October 1994 and was continued until March 1999 with a break between September and December of 1996. LGB and Tn populations were sampled using Japanese-style flight-traps (see Figure 2.3) baited with LGB aggregation pheromone (1mg of T1 and 1mg of T2). Traps were emptied and reloaded with a fresh pheromone capsule every two weeks initially, and then every four weeks from December 1996 onwards (with the exception of traps in the Fodome cluster which were emptied every two weeks). The time between reloading was lengthened to allow the monitoring to continue for longer.

Statistical analysis of monitoring data
Repeated measures analysis (using GENSTAT) was used to assess any trends in the data with time, and any short-term correlation between LGB and Tn catch.

Results: In storage

Transect walks

- Tn had appeared, at very low levels, in stores in two of the six clusters sampled by December 1994 and in seven of the eight clusters sampled by March 1995.
- LGB was present in around 20-30% of all stores examined in December 1994 and March 1995, but mostly at economically insignificant levels (<1 insect/cob in December 1994 and <0.06 insects/cob in March 1995).
- In two villages included in the loss study in 1993/4 (Papase and Dzolokpuita), LGB levels dropped drastically between 1993/4 and 1994/5 **(i.e. before the establishment of Tn)**.
- Villages with worst LGB damage (Peki Dzake) have much longer storage periods.
- The first appearance of LGB in the store is very variable, ranging from 1-9 months after storage, implying that immigration into the store may be important.

Extension agent surveys

Extension agent survey data (Figure 5.4) generally indicates that LGB has drastically reduced in importance as a pest in Volta Region over the period 1993-97. The proportion of villages in maize-growing areas in Volta Region where extension agents rated LGB as a 'serious' problem dropped from 31% in 1993 to 13% in 1996 and 6% in 1997.

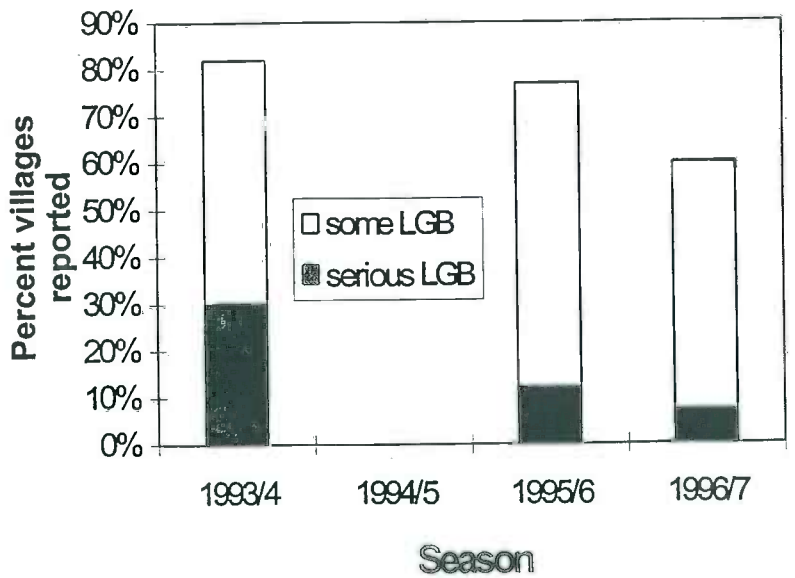


Figure 5.4. Percent of villages with Larger Grain Borer problems as reported in extension staff questionaires.

Extension agents were also asked about changes in storage practice in their villages. In cases where extension agents reported that LGB had been a pest in the past but was no longer a problem, two thirds also reported widespread changes in storage practice. The interesting cases to investigate are those where no change in practices have reportedly taken place, but LGB levels have nonetheless fallen. Mapping these cases showed no clear geographical pattern (e.g. proximity to Tn release areas).

Results: In traps

Descriptive statistics

1. Mean trap catches for all village clusters except Bodada are shown in Figure 5.5. It can be seen that:

- The numbers of LGB caught in flight traps are many times greater than the numbers of Tn caught in the same traps.
- The trap catch of LGB is characteristically erratic with peaks that, at least to some extent, follow a yearly cycle.
- Numbers of LGB in 1996-1997 are particularly low (especially as this is when the trapping period was increased in many of the villages) for three of the village clusters: Fodome, Hodzo and Peki, but increase again in 1997-1998.
- By 1998 the ratio of LGB to Tn was approximately five LGB to every one Tn, with some cases where Tn numbers exceeded LGB numbers in the traps.

2. Taking logs (ln(1+catch) allows easy visual comparison of the changes in LGB and Tn populations with time on the same scale (Figure 5.6). From this plot we can easily see that:

- Tn consistently appeared in traps in **all** clusters except Brewaniase from the start of trapping, which occurred about six months after release, and in Brewaniase (albeit at extremely low levels) about nine months after release.
- Short term (month to month) changes in trap catches for LGB and Tn are often correlated (peaks and crests coincide).

3. To take out short term correlated fluctuations between in the trap catches of LGB and Tn we have plotted the ratio of ln(1+Tn catch)/ln(1+LGB catch) for each village within a cluster (Figure 5.7). From these plots we can see that:

- The proportion of Tn in the trap catches increases with time in all villages (largely arising from an increase in Tn numbers rather than a decrease in LGB numbers. (see Figures 5.5 or 5.6).
- Traps placed within villages of the same cluster give very similar changes in ratio between predator and prey with time.

Analytical statistics

1. Assessing the overall trend in LGB and Tn trap catches with time.

Since the trapping period was switched from 2 weeks to monthly trapping during the monitoring period the data for these two phases was analysed separately (subsequently called first and second data set for 2 weekly and monthly trapping respectively).

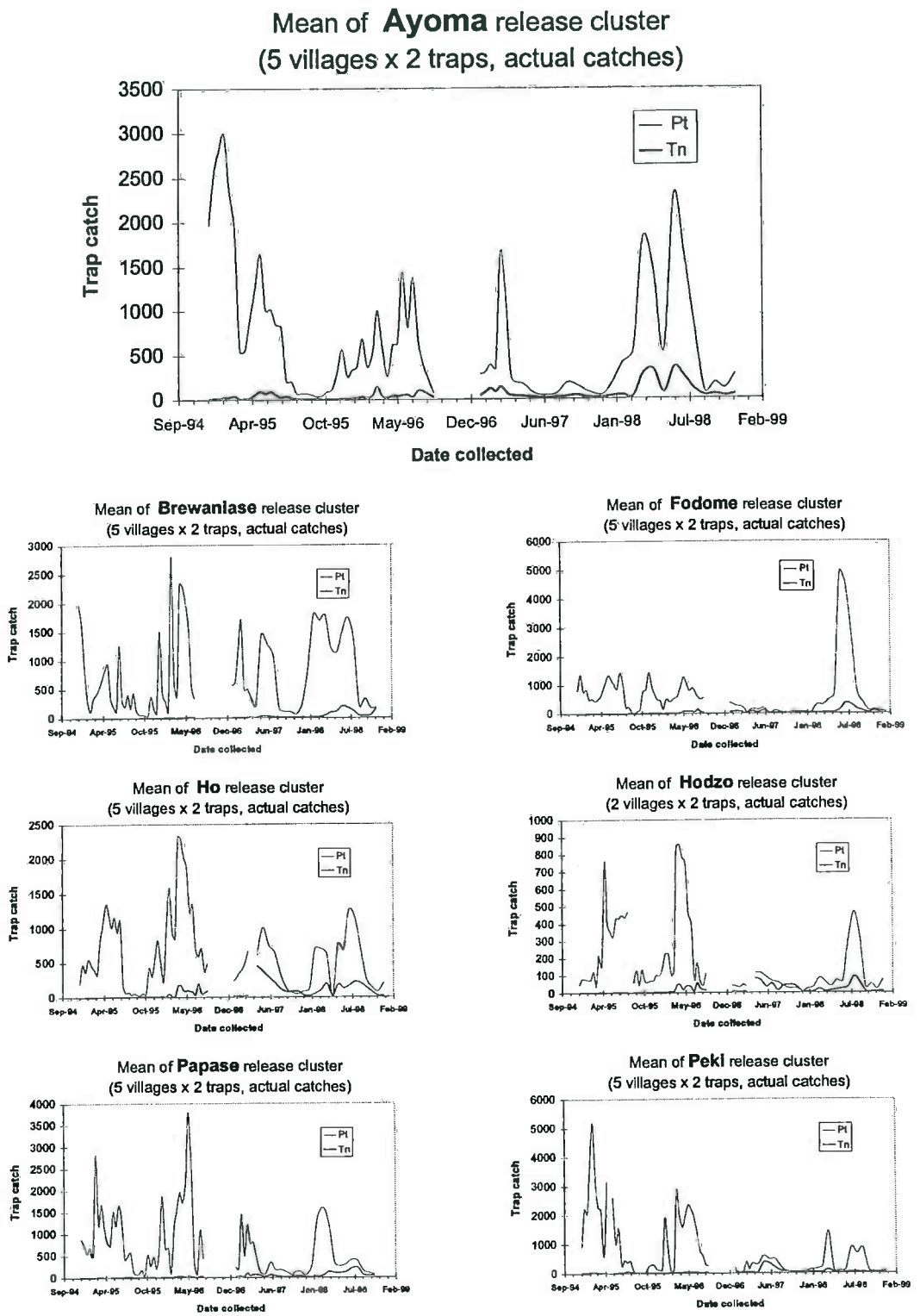


Figure 5.5: Mean trap catches of *P. truncatus* (Pt) and *T. nigrescens* (Tn) against time. Trap catches are for a two week period until December 96 when they become trap catch per four weeks.

NB. The scale on the Y-axis varies between village cluster.

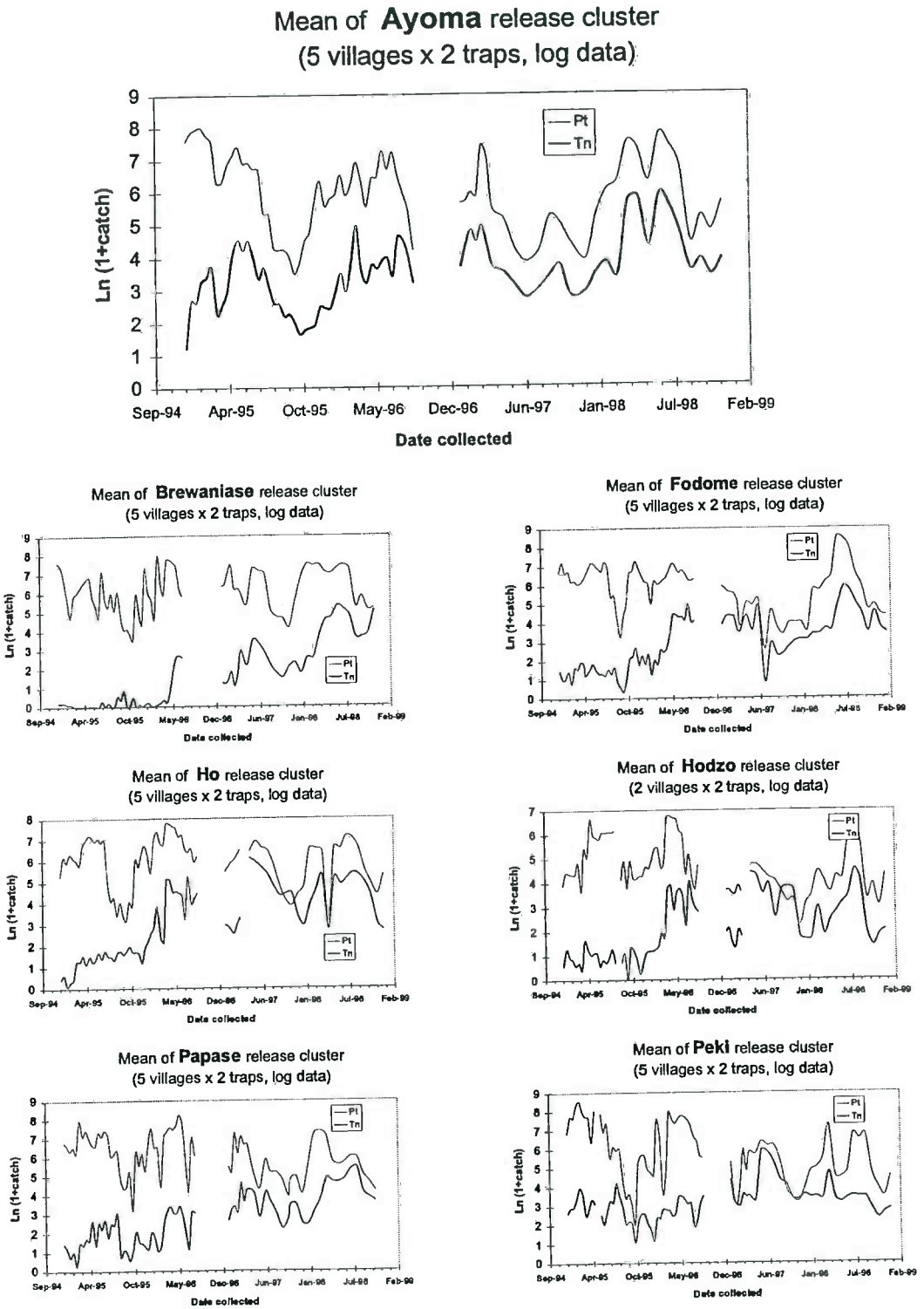
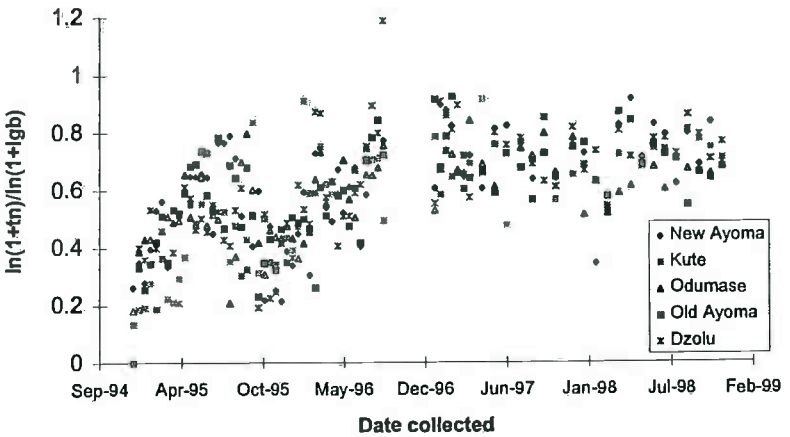
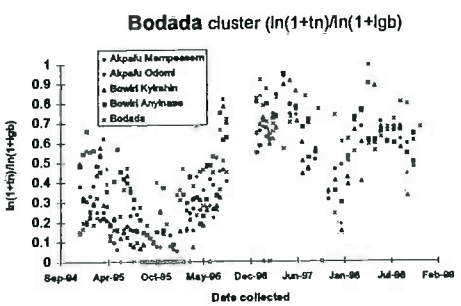
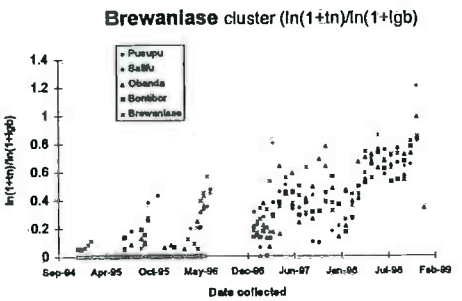


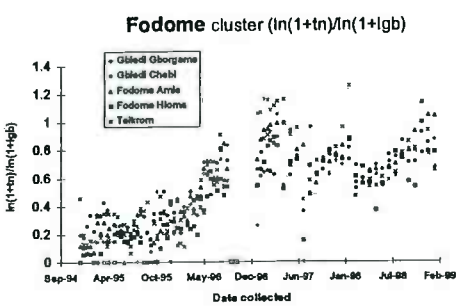
Figure 5.6: Mean Ln(1+trap catch) for *P. truncatus* (Pt) and *T. nigrescens* (Tn) against time. Trap catches are for a two week period until December 1996 when they switch to every four weeks.

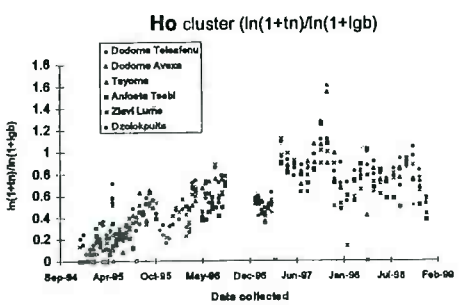
NB. The scale of the Y-axis varies between village cluster.

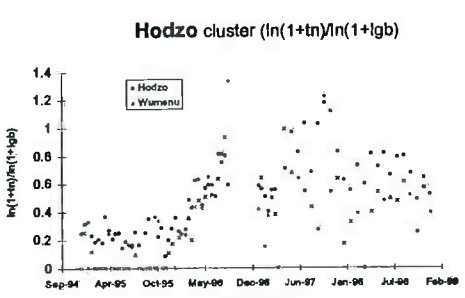


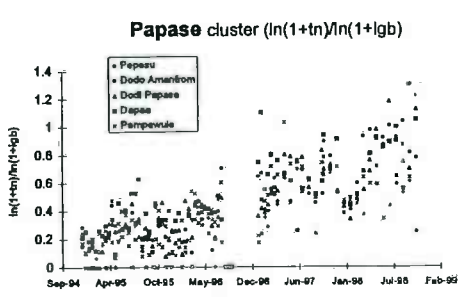












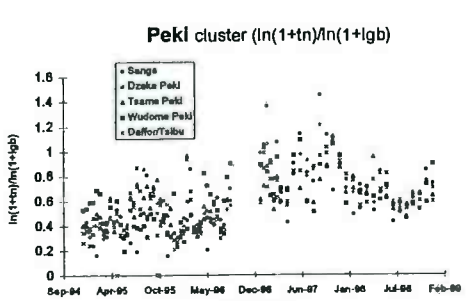


Figure 5.7: (Ln(1+ Tn catch))/Ln(1+LGB catch)) against time for each village within a village cluster.

NB. LGB = Larger Grain Borer = *P. truncatus*

The Y-axis scale varies between village cluster.

Smoothing splines were used to investigate the trends for each set of raw catch data. Smoothing splines are a non-parametric regression technique and the aim is to find a line that reasonably represents the data, rather than achieving a particular significance. The fits were assessed by calculating the percentage of the variance accounted for (the adjusted R^2 statistic). The order of the spline was chosen to ensure that this statistic was at least 30% and unless otherwise stated the models were of order 4. Table 5.1 gives the adjusted R^2 statistic for each of the fits and where appropriate the order.

Cluster	First data set		Second data set	
	Tn catch	LGB catch	Tn catch	LGB catch
Ayoma	32.7%	73.4%	43.2%	39.7%
Bodada	49.0%	69.3%	82.2%[c]	37.8%[c]
Brewaniase	85.9%[a]	35.8%[a]	60.3%	39.6%
Fodome	76.6%[b]	32.4%[b]	41.7%	43.3%
Ho	56.1%	56.4%	42.0%	39.1%
Hodzo	59.0%	38.5%	37.6%[a]	43.3%[a]
Papase	49.0%[a]	35.3%[a]	64.0%	37.8%
Peki	34.8%[c]	61.2%[c]	67.5%[d]	32.1%[d]

[a] order 5, [b] order 6, [c] order 8, [d] order 9

Table 5.1: Percentage variance of the changes in trap catch accounted for by the trend line fitted using smoothing splines.

A straight trend line is inappropriate for the catch data. Since the trends are complex, we have summarised the trend line from each village cluster using the separate opinion of two independent assessors (with graph labels hidden) as either generally rising, falling or neutral **between years**. These subjective opinions are summarised below.

	LGB			Tn		
	Falling	No change	Rising	Falling	No change	Rising
Until Dec. 1996	******	***	*		*****	*****
After Dec. 1996		********	**	**	***	*****

Table 5.2: Independent assessor's subjective opinions of year to year changes in the trend lines created for trap catches of LGB and Tn. NB. Each asterisk represents one opinion of the data from one cluster (each cluster was assessed by two different assessors).

In summary there is some suggestion that Tn numbers rise and LGB numbers fall in the first set of data (over the first 2 years of monitoring after release), however there is no such suggestion for the second set of data (if anything LGB may be increasing in number again). Indeed, if we look at trap catch data gathered for the risk assessment component of this project (which continues into 1999) we can see that in and around

Hohoe this year, LGB catches have risen dramatically despite the presence of Tn (see Figure 3.5).

2. Testing for a correlation between the short-term fluctuations of LGB and Tn. Repeated measures analysis was used to determine the significance of an apparent positive correlation between Tn catch and LGB catch. This analysis is summarised in Table 5.3. Almost all clusters showed a highly significant correlation between short-term variance in trap catches of LGB compared to Tn.

	Until Dec. 1996 (2 week catches)		After Dec. 1996 (monthly catches)	
Cluster	Correlation	p-value	Correlation	p-value
Ayoma	0.15	0.32	0.91	<0.0001
Bodada	0.49	<0.0001	0.42	0.03
Brewaniase	0.14	0.37	0.32	0.09
Fodome	0.15	0.33	0.78	<0.0001
Ho	0.6	<0.0001	0.6	<0.0001
Hodzo	0.48	<0.0001	0.65	<0.0001
Papase	0.61	<0.0001	0.48	0.13
Peki	0.38	0.009	0.37	0.04

Table 5.3. Extent of correlation between the short-term variation of Tn and LGB trap catches assessed using repeated measures analysis.

Discussion

The extension agent survey data gives a picture of a 'wave' of a new pest causing a lot of problems shortly after it reaches an area and then becoming less important after a couple of years. Such a wave is reportedly typical of some other pests even where changes in farmer practice and classical biocontrol are not involved (Roger Day, IIBC, pers. comm.).

Previous work by the LGB project also showed widespread and rapid changes in farmer storage practice in response to LGB. Over 60% of farmers visited in village studies had made at least one change to their storage practices within two years of first experiencing LGB damage (Motte *et al.* 1995, Magrath *et al.* 1996). The most important of these changes is simply shelling maize from LGB-infested stores and either disposing of it quickly or in some cases treating it with chemicals for further storage. Extension agent surveys may tend to overestimate the prevalence of some types of changes, in particular the use of pesticides, as extension agents tend to associate most with richer 'contact' farmers who are more likely to use chemicals, and many extension agents also sell pesticides themselves. It is nevertheless clear that pesticide use on maize has grown dramatically in the region since 1992, when virtually no storage chemicals were available except for cocoa chemicals that some farmers applied to their stored maize. Extension agent surveys rated chemicals (mainly Actellic, Actellic Super and Sumicombi) as progressively easier to obtain in their villages every year. Finally, as LGB generally takes a long time to build up a significant population in stores, storage periods are crucial. A series of poor years and

high prices for maize have meant that most farmers in Volta Region have not kept their maize stocks for long periods in recent years.

Our data set for number of LGB caught in pheromone traps is a good demonstration of the need for caution when interpreting sampling data, which is as characteristically erratic as LGB trap catch data. The generally low LGB numbers caught in traps during the 96/97 season, (even though traps were out for one month at a time during this time) led to the early conclusion that LGB numbers were dropping in a similar fashion to the trend shown in the Borgmeister data (Borgmeister *et al.* 1997). However, we can see that in the fourth year LGB numbers pick up again in many villages even though Tn numbers remain as high as before.

In summary, we have found that the dispersing population of Tn (and probably therefore the total population) has increased and established itself well in the Volta region of Ghana. There is, however, no real evidence that numbers of dispersing *Prostephanus truncatus* have fallen within the four years that we have been monitoring. This study therefore **does not support** the hypothesis that increasing populations of Tn reduce the population of LGB. This information should be considered by decision-makers when deciding on the pros and cons of future Tn releases.

Proposals for possible future work

- Explanation of the significant short correlation between short-term variance in trap catches of LGB compared to Tn. More specifically is this driven by external factors like the influence of climate on dispersal behaviour, or internal factors such as conspecific population density (in the case of LGB) or prey availability (in the case of Tn).
- Determination of which situations favour increased impact of Tn to reduce LGB damage. Situations that could be considered might include:

1. Reduced pesticide methodologies
2. Different commodities
3. Different physical form of the same commodity
4. Different climatic conditions

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6. Limiting the amount of maize grain or maize cobs in a farm store that has to be treated with pesticide

Hodges R.J., Addo S., Birkinshaw L.A., Penne H. and Awuku M.

Summary

If insecticide application to farm stored produce can be reduced there would be considerable health and environmental advantages and farmer expenditure would be lowered. In addition, limitation of pesticide usage on stored food would favour greater impact by biological control since insect bio-control agents are as susceptible to insecticide as the pests.

A means of reducing the amount of insecticide added to farm stored maize, by confining treatment to the bottom layers, was investigated in the laboratory and then in the field. Field study results proved very encouraging and suggest that localised treatment may indeed be a means of reducing pesticide usage by as much as 80%.

Background

The simplest and most effective method of controlling LGB in maize is to shell cobs and to treat the grain with a suitable insecticidal dust to protect against both *P. truncatus* and weevils (*Sitophilus* spp.). The application of pesticide is necessary to prevent substantial losses but this is a considerable expense for subsistence farmers, may pose risks to health and the environment (Ecobichon and Joy, 1993) and is not compatible with the useof the biocontrol agent *T. nigrescens*.

However, treatment of the whole grain bulk may not be necessary. It is known that insects will adopt characteristic distribution patterns within grain bulks (Surtees, 1964) and there have been several reports that *P. truncatus* migrates downwards towards the base in stores containing shelled maize (Verstraeten and Haubruge, 1987; Tierto, 1994) or maize cobs (Wright *et al.*, 1993; Wekesa 1994). This has led to a suggestion that grain bulks may be adequately protected if only the deeper portions of grain are treated with insecticide. A number of ingenious designs for farm stores that would facilitate selective treatment of shelled maize have been suggested (Tierto, 1994), although to date there have been no studies on the efficacy of such treatments. To test whether these would give satisfactory control, a series of laboratory investigations was undertaken to observe the distribution of the beetle in grain at depths typical of farm bulks and also the protection offered by treating portions of the grain at the bottom and the top of the bulk. This was followed by 'on station' field trials in which maize grain or maize cobs were stored with only 50% or 20% of the maize bulk respectively, receiving a pesticide application.

LABORATORY STUDIES

General methods

Laboratory studies were undertaken in a CTH room set at 25°C and 70% r.h. The distribution of insects in maize was observed in grain confined to plastic tubes of 18 cm diameter (Fig. 6.1) and various lengths. The grain was newly purchased

American yellow maize (No. 4 quality) and, except in the first experiment, the grain was placed in a freezer at - 18°C for one week to kill any insects present. The grain was then conditioned in the CTH room, in small lots, for two weeks. The plastic tubes were sealed at the base with a plastic plug, mounted vertically in the CTH room and, once filled, the tops closed with muslin. At the time of filling, four samples of maize were taken and tested for moisture content by drying in a ventilated oven at 130°C for three hours.

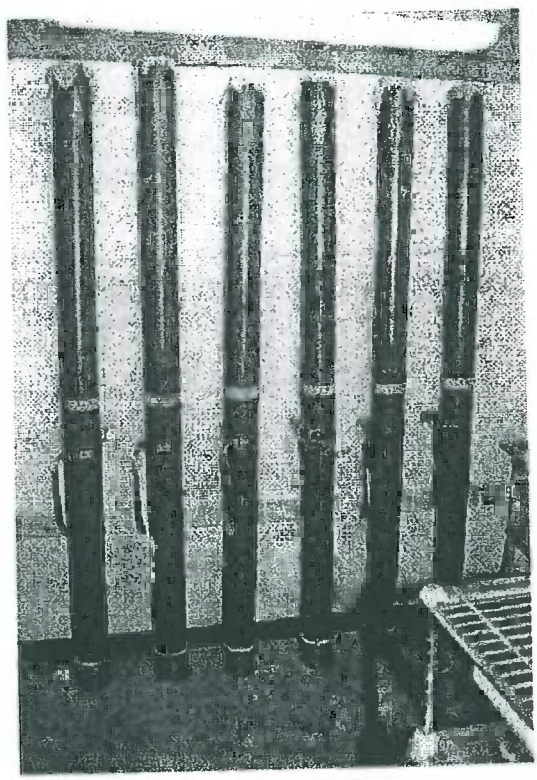


Figure 6.1: Plastic tubes (3m long) used to investigate the distribution of *P. truncatus* in maize

Unless otherwise stated, beetles of mixed age were added to the top of tubes in a manner intended to simulate the arrival of beetles at a food source. The first additions were 2, 4, 8 and 16 males added on each of the first four days. On the fifth and eighth days, 16 females were added, giving a total of 62 beetles. At various intervals after adding the last beetle, depending on the particular study, the distribution of insects was determined by removing maize from the bottom of the tubes in aliquots equivalent to depths of 25cm or 14cm, depending on whether tubes were filled with maize to a depth of 275cm or 126cm respectively. Potentially, this gave 11 or 9 samples/tube although, due to settling and insect damage, sometimes fewer than this were recovered. For maize removal, the tubes were held at an angle of 45° and the plastic plug sealing the base opened gently. Between each extraction, a tube was returned to the upright position to ensure that the grain did not slip between layers as further samples were taken. Unless otherwise stated, insects were removed from these maize samples by sieving for five minutes on an Octogon 200 mechanical sieve (setting 7) then inspected manually for any further beetles which might be extracted.

culture in a glass tank over which a light was suspended. Those beetles flying off from the culture were collected and sexed (Shires and McCarthy, 1976). Where these were insufficient, additional beetles were obtained by sifting the culture.

When grain was to be treated with insecticide, a dilute dust formulation of Actellic Super was admixed at the rate of 1.0g/kg grain. The concentration of active ingredient of this insecticide was confirmed at the Natural Resources Institute by GLC analysis and found to be 0.79% pirimiphos-methyl and 0.26% permethrin. Thus the insecticides were applied at 7.9 ppm and 2.6 ppm respectively.

Differences in grain quality according to treatment were investigated by one-way analysis of variance. The standard error of the difference (SED) between two means was calculated in order to compare treatments; where the difference between two means was at least twice the SED then the means are considered to be significantly different at the 5% level ($p \leq 0.5$).

Results and Conclusions

Depth of penetration into grain bulks

As a preliminary study, to indicate whether or not there was sufficient downward migration of *P. truncatus* to justify testing the efficacy of insecticide treatments towards the base of a grain mass, we investigated the depth to which *P. truncatus* would penetrate in a bulk of grain 275cm deep.

Three plastic tubes were filled to a depth of 275cm with maize with a mean moisture content of 13.6%. Beetles were added to the tops of the tubes, as described in the general methods, and after three weeks the distribution of beetles in the grain was determined. The maize was also infested by *Sitophilus zeamais* Motschulsky that was apparently present as a hidden infestation at the start. This unexpected infestation provided information on the distribution of *S. zeamais* in bulk grain.

After three weeks, only 35% of the *P. truncatus* added to the tubes were recovered. They proved particularly difficult to find in a substantial mass of grain. It is assumed that the efficiency of recovery was the same for all grain samples so that observed differences between samples in the numbers of beetles are true differences in beetle distribution. On average, thirteen percent of the *P. truncatus* penetrated to the full depth of the tube (Table 6.1) and over half penetrated below 75cm from the top. There is clearly a strong downward movement in the grain. The observed distribution of *P. truncatus* was 'patchy' although these patches were more or less random through the bulk (Table 6.1). In contrast, the distribution of *Sitophilus* was much more strongly clustered with about 90% occurring in the lower 45% of grain, i.e. below 1.5m. It is assumed that although the two species were present in the same grain mass their populations did not interact in such a way as to affect their vertical distribution within the tubes. Subsequent testing with *P. truncatus* by itself revealed that this is probably a valid assumption.

Table 6.1: % of adult *P. truncatus* recovered at different depths from maize in plastic tubes and actual numbers of *S. zeamais* recovered

	Depth (cm)	% recovery of *P. truncatus*			Mean % at each depth	Number of *S. zeamais* recovered			Mean % at each depth
		Tube 1	Tube 2	Tube 3		Tube 1	Tube 2	Tube 3	
Top	0 - 25	15	9	4	10.3	0	7	0	0.1
	25 - 50	20	0	17	12.3	0	0	0	0
	50 - 75	35	27	13	25.0	0	20	8	1.9
	75 - 100	15	13.6	0	9.5	0	27	18	3.0
	100 - 125	0	13.6	4	5.9	0	16	14	2.0
	125 – 150	0	9	4	4.3	0	21	10	3.1
	150 – 175	5	18	4	9.0	0	26	19	4.6
	175 – 200	0	4.5	0	1.5	0	41	24	4.4
	200 – 225	0	0	13	4.6	0	>100	30	8.2
	225 – 250	0	4.5	8.6	4.4	0	>100	63	11.0
Bottom	250 – 275	10	0	30	13.3	8	>400	>200	61.7
	No. beetles recovered	20	22	23					

Effect of initial means of access to grain on the final distribution of LGB in the grain mass

It is possible that the means by which *P. truncatus* gain their initial access to a grain bulk may affect the subsequent distribution of this species. To investigate this, male and female *P. truncatus* were added to the grain bulks in the following three ways to simulate three possible modes of entry into the grain bulk.

Simulation of beetles arriving at the top of the bulk - once the tubes had been filled with maize, adult beetles were added to the top in the manner described in the general methods.

Simulation of infestation resulting from the presence of infested residues at the bottom of a grain store - a single large culture of *P. truncatus* was added to the bottom of each tube and the tubes then filled with uninfested maize. The cultures were prepared one week earlier by adding 36 male and 36 female *P. truncatus* to jars containing 500g of maize.

Simulation of an infested crop being added to the store - At the time the tubes were filled with maize, six small cultures of beetles were added at regular intervals. The small cultures were prepared one week earlier with six male and six female *P. truncatus* added to 200g of maize.

Six plastic tubes were filled to a depth of 126cm with maize with an average moisture content of 14.5%. There were two replicates of each treatment and insect distribution was determined eight weeks after starting the test.

The numbers of *P. truncatus* recovered from the maize samples were considerably greater than in the first test (compare Tables 6.1 & 6.2). The reasons for this is that in the second test the beetles had been given sufficient time to reproduce. Irrespective of the initial means of access to the grain mass, beetle distribution in the grain was very similar. In all three cases the observed distribution was much less even than in the first test, with a distinct aggregation towards the bottom; an average of 31% of the beetles were in the bottom 14 cm of grain although only 11% would have been expected if the distribution had been even (Table 6.2). This suggests either that the higher population density of beetles in the shorter tubes of the second test may lead to more aggregation or, probably more likely, there is greater reproductive potential at depth in the grain mass as grain there is more stable which facilitates beetle broing and hence more population growth (Cowley *et al.*, 1980).

These results confirm the observation that beetles added to the top of the grain column migrate downwards and suggest that larger populations develop in the deeper portions of grain. The similarity in the final distribution, irrespective of the initial means of access to the grain, suggests that selective treatment of deeper layers with pesticide may be effective irrespective of the means by which the grain mass becomes infested.

Table 6.2: Mean* (± s.d) numbers of adult *P. truncatus* and cumulative % of beetle at different depths in columns of maize grain eight weeks after beetles were added at the top or bottom of the maize column or added evenly.

Depth of grain	Insects added to top		Insects added to bottom		Insects added evenly	
	Mean no. insects	Mean % at each depth	Mean no. insects	Mean % at each depth	Mean no. insects	Mean % at each depth
0 - 14 cm	4.0 ± 4.2	1.0	36.5 ± 21.9	4.8	4.0 ± 5.7	1.2
14- 28 cm	24.5 ± 28.0	6.0	35.5 ± 20.5	4.7	10.5 ± 14.8	3.1
28 - 42 cm	22.5 ±30.4	5.6	49.5 ± 19.1	6.5	8.5 ± 12.0	3.0
42 - 56 cm	38.0 ± 49.5	9.5	57.5 ± 6.4	7.6	27.5 ± 38.9	8.2
56 - 70 cm	39.5 ± 54.4	9.8	82.0 ± 5.7	10.8	19.0 ± 26.9	5.7
70 - 84 cm	58.0 ± 65.0	14.4	109.0 ± 4.7	14.3	40.0 ± 43.8	12.0
84 - 98 cm	44.0 ± 48.0	10.9	92.0 ± 21.2	12.1	35.5 ± 43.1	10.6
98 - 112 cm	45.5 ± 3.5	11.3	120.0 ± 7.1	15.7	60.0 ± 21.2	17.9
112 - 126 cm	126.0 ± 25.2	31.3	180.5 ± 44.6	23.7	129.5 ± 21.9	38.7

* Mean of two replicates

Protection of grain with insecticide treatment localised at the base of the bulk or at both the base and top of the bulk

The potential for insecticide application to the base of a maize bulk to give long-term protection (15 weeks) against *P. truncatus* was investigated in plastic tubes. These tubes were loaded to a depth of about 126cm with maize grain at a moisture content of 14.1%. The bottom 25cm of grain (20%) or bottom 50cm (40%) were treated with Actellic Super or the grain was untreated (control). Three replicates were prepared of each treatment. In a further test, the potential for localised insecticide application at both the top and bottom of grain bulks to give long-term protection (15 weeks) was investigated in similar plastic tubes loaded with maize grain with a mean moisture content of 14.25%. The bottom 25cm (20%) of nine grain bulks were treated with Actellic Super and all nine had a top screen of treated grain to depths of about 5cm (4.2%), 10cm (8.5%) or 25cm (20%), with three replicates of each depth. There were also three control tubes with only untreated grain.

In both tests, beetles were added to the top of the tubes as described in the general methods. Grain quality was checked after 15 weeks. The grain was removed from the tubes and processed by hand sieving, rather than mechanical sieving, to avoid further grain damage. In the first test, to indicate what percentage of grains was damaged by insects, 100g sub-samples were taken from the bottom three samples and the top two samples of the tubes. In the second test, 100g sub-samples were taken from all sections to assess insect damage and a count and weigh loss assessment undertaken. The weight loss results were averaged across the samples to give a general indication of the extent of losses. Where there was little insect damage some values for weight loss were negative: these were regarded as zero.

In the first test, in which insecticide application was restricted to only the base of the grain bulks, much less grain damage and much less grain dust was observed in the treated bulks than in the control (Table 6.3). The percentage of grain damaged in the 20% treated bulks differed little from those with 40% treated (Table 6.3). There was however strong evidence for differences between treatments in the amount of dust produced ($F=15.78_{2,78}$ $p<0.0001$) with significant differences between the control and treated bulks as well as significantly better grain preservation conferred by 40% treatment than only 20% (Table 6.3).

Table 6.3: Mean* % grain damage and mean weight of dust from grain samples treated with pesticide to different depths, fifteen weeks after 62 beetles were added at the top of the maize column

Depth of grain	**Mean % grain damage ± sd**			**Mean weight of dust (g) ± sd**		
	No insecticide	Insecticide in bottom 20 %	Insecticide in bottom 40%	No insecticide	Insecticide in bottom 20 %	Insecticide in bottom 40%
0-14 cm	73.8 ± 16.8	14.7 ± 3.8	12.0 ± 5.1	126.2 ± 9.5	1.0 ± 1.7	0.6 ± 1.0
14-28 cm	46.8 ± 19.9	29.8 ± 7.1	13.1 ± 5.4	79.5 ± 4.4	28.6 ± 14.7	0.7 ± 1.2
28-42 cm	-	-	-	38.8 ± 8.3	46.9 ± 19.4	6.0 ± 5.2
42-56 cm	-	-	-	56.1 ± 13.0	68.7 ± 9.8	24.0 ± 21.8
56-70 cm	-	-	-	52.8 ± 20.1	56.7 ± 26.1	42.9 ± 12.0
70-84 cm	-	-	-	102.6 ± 17.5	48.2 ± 27.1	42.7 ± 12.3
84-98 cm	80.1 ± 4.4	53.4 ± 6.7	46.8 ± 14.2	126.4 ± 37.2	128.4 ± 43.5	58.9 ± 7.1
98-112 cm	82.4 ± 3.7	47.3 ± 6.3	41.8 ± 17.7	219.1 ± 27.1	120.5 ± 34.2	80.2 ± 34.3
112-126 cm	70.3 ± 21.3	37.6 ± 13.0	37.3 ± 18.5	187.7 ± 49.3	117.7 ± 88.2	38.7 ± 5.8
Mean	70.7 ± 13.2	36.6 ± 7.4	30.2 ± 12.2	109.9 ± 20.7	68.5 ± 29.4	32.7 ± 11.2

*Mean of three replicates - not measured SED between mean values for dust = 13.75

NB Shaded zone is approximate area treated with Actellic Super

For bulks of grain with insecticide treatment at both bottom (20%) and top (4%, 9% or 20%) there was strong evidence of significant differences between the various treatments in the amount of dust produced by insect attack ($F = 24.47_{3,98}$ $p<0.0001$). All treatments differed significantly from the control and the grain columns with a 4% top screen of treated grain had significantly more dust than those with 9% or 20% (Table 6.4). The last two did not differ significantly. The 4% top treatment reduced dust production and grain damage values to about 50% of those experienced in the untreated control. In contrast, 9% and 20% top screens were considerably more effective but differed little with dust production and grain damage about 95% and 66% lower than the control respectively (Table 6.4).

The estimated mean percentage weight loss (±sd) for the untreated control was 9.3 ± 3.6%, but in the treated grain with the 4% top screen this was reduced to 3.6 ± 2.9%. Further reductions to 1.3% ± 1.4 and to only 0.7% ± 0.4 were achieved with the 9% and 20% top screens respectively. It is clear that a top screen of insecticide would be of particular value in protecting against *P. truncatus* infestation gaining access to the grain bulk from the top surface but that this screen would need to extend to a depth of not less than about 10cm.

Conclusions

Laboratory studies, undertaken using long plastic tubes filled with maize, have confirmed that a substantial proportion of the population of both *P. truncatus* and *S. zeamais* will penetrate to considerable depths in bulk maize within a fairly short period (less than three weeks). Application of pesticide to only the lower portions of the grain bulk, the bottom 25cm out of a full depth of 126cm, can reduce attack by *P. truncatus* but the addition of a top screen of about 10 cm depth appears to be important for keeping grain losses low over extended storage periods if insects can gain access to the grain from the top of the bulk. It is of interest to note that grain treated with Actellic Super at the recommended rate, with a confirmed active ingredient, still suffered some insect damage and weight loss. It must be assumed that inadequacies of insecticide admixture allow some insect survival. It would appear that the probability of insects being killed when passing through a treated 'top screen' is proportional to the depth of treated grain. Thus a 5cm treated top layer apparently caused little mortality of the insects that moved through it, while 10cm and 25cm gave progressively greater mortalities. These results suggest that 'on-station' field trials should be undertaken to generate further information on the potential of this technique.

Table 6.4: Mean* % grain damage and mean weight of dust from grain samples treated with insecticide at the bottom of the bulk and to different depths at the top of the bulk, fifteen weeks after 62 *P. truncatus* were added to the top of the bulk

Depth of grain	**Mean % damaged grain ± sd**				**Mean weight of dust(g) ± sd**			
		Bottom 20% of grain treated				Bottom 20% of grain treated		
	Control	+ top 4%	+ top 9%	+ top 20%	Control	+ top 4%	+ top 9%	+ top 20%
0-14 cm	62.7 ± 12.2	21.6 ± 0.06	20.3 ± 4.6	21.9 ± 3.4	83.1 ± 18.1	1.6 ± 1.4	3.7 ± 1.0	3.9 ± 1.8
14-28 cm	44.7 ± 8.5	23.5 ± 2.0	18.5 ± 2.7	19.5 ± 3.1	40.2 ± 10.4	27.8 ± 6.1	7.7 ± 3.8	4.7 ± 0.9
28-42 cm	38.9 ± 3.3	34.4 ± 5.3	22.7 ± 1.4	23.9 ± 1.2	45.3 ± 5.8	49.6 ± 6.5	11.0 ± 7.4	4.5 ± 1.8
42-56 cm	38.9 ± 12.3	36.4 ± 11.0	25.6 ± 8.6	19.2 ± 3.9	65.4 ± 23.6	65.7 ± 20.0	13.9 ± 10.0	3.9 ± 1.5
56-70 cm	60.3 ± 20.4	36.2 ± 2.8	22.5 ± 10.0	17.4 ± 2.1	78.6 ± 31.9	57.0 ± 35.2	16.3 ± 12.9	3.7 ± 0.7
70-84 cm	63.7 ± 30.8	35.5 ± 4.6	23.4 ± 7.9	17.6 ± 1.8	102.4 ± 51.1	63.2 ± 34.6	14.7 ± 12.4	5.0 ± 2.6
84-98 cm	86.6 ± 12.6	35.9 ± 8.6	22.6 ± 10.5	20.2 ± 2.5	191.7 ± 113.2	63.7 ± 29.8	10.4 ± 8.8	3.8 ± 0.5
98-112 cm	82.6 ± 11.1	32.4 ± 5.8	21.7 ± 5.0	18.3 ± 4.1	338.7 ± 119.4	74.2 ± 52.9	8.8 ± 4.3	5.1 ± 1.2
112-126 cm	98.3 ± 14.2	25.6 ± 8.3	19.2 ± 1.5	20.8 ± 1.4	231.0 ± 130.2	23.0 ± 23.5	5.8 ± 5.5	5.9 ± 0.7
Mean	64.1 ± 13.9	31.3 ± 5.4	21.8 ± 5.8	19.9 ± 2.6	130.7 ± 46.6	47.3 ± 23.3	10.2 ± 7.3	5.0 ± 1.3

* Mean of four replicates

SED between mean values for dust = 1.95

NB Shaded zone is approximate area treated with Actellic Super

FIELD STUDIES

Localised Pesticide Treatment to Protect Mud Silos

This study was undertaken to assess the pest control efficacy of limiting pesticide treatment to only 50% of a bulk of maize grain stored in mud silos at Tamale (Northern Region).

Methods

General
Traditional mud silos were constructed from a mixture of soil from termite mounds and grass. The silos were more or less spherical (Fig. 6.2), measuring about 1.5m high and 1.3m wide and when full of maize would hold about 300kg. The silos were supported on three mud legs about 30cm from the ground and sealed with a mud lid that was covered by a conical thatched-roof. Each silo was loaded with 100kg of maize that had recently been fumigated with phosphine. Two discharge ports were made in each silo, one with its bottom edge in line with the height reached by the bottom 30kg of grain. The other, lower, port was located near the silo base. Both ports were sealed with mud and reopened each time a sample was required. Two separate tests were undertaken, in the first grain was removed during the study to simulate farmer consumption, in the second all grain was stored for the duration of the trial, i.e. there was non consumption. To ensure that infestation had the opportunity to start early, each mud silo, whether treatment or control, was seeded with a small portion of *P. truncatus* culture placed at the centre of the grain mass. Thus even in the treated silos, the beetles were placed on untreated grain.

Insecticide treatment
The bottom 30kg of maize (approximately 20cm depth) was treated with Actellic Super at 1g/kg; in addition the top 20kg (approximately 10cm depth) was treated with insecticide in the same manner to provide a 'top screen'. Between the two layers was 'sandwiched' 50kg of untreated grain. The 50kg of treated grain in each silo was thoroughly admixed with 30g of Actellic Super, giving an application rate of 1.8ppm permethrin and 8.4 ppm pirimiphos-methyl.

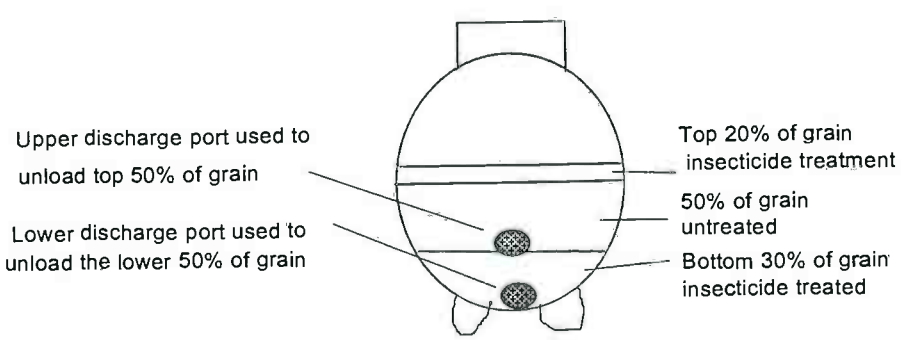


Figure 6.2 - Mud silo showing the distribution of treated maize grain and position of two discharge ports.

Test with simulated farmer consumption

Four silos held treated grain and four held untreated grain. The condition of the grain was observed over a period of 24 weeks. Of the eight silos prepared at the start of the test, two of the controls had to be abandoned due to termite infestation: one at 18 weeks, the other at 22 weeks. Six weeks after the trial was initiated, each silo had 5kg of maize withdrawn by hand from the upper discharge port to simulate grain consumption by a farmer. This procedure was repeated every two weeks up to and including the 22nd week (giving nine samples/silo). Thus a total of 45kg was removed; in the case of the treated silos the grain removed was mainly from the 50kg of grain sandwiched between the top and bottom treated layers, i.e. it would have been mostly untreated grain. The samples were sifted and live and dead insects counted, and four sub-samples of about 50g were assessed for insect damage. At the end of the 22nd week, grain weight loss of two replicate 100g samples from each silo was estimated using the count and weigh method (Adams and Schulten, 1978). In some cases, where insect damage was low, this resulted in a false negative estimate of weight loss: where this happened the weight loss was recorded as zero. Of the six silos remaining at the end of the 24-week period, all the grain at or above the upper port was removed, coned and quartered to give a 2kg sample. In the case of the treated silos nearly all this grain would have been exposed to insecticide treatment. The two 2kg samples were screened for insects, and two sub-samples from each of approximately 100g were taken and weight losses estimated again using the count and weigh method. The remaining grain was then discharged and sampled in the same way from the lower port.

Test with no simulated consumption

A second test was undertaken to observe the extent of damage to grain stored in the same manner as before but which had no grain removed, i.e. no simulated farmer consumption during the storage period.

Four mud silos, two treated and two untreated, were used for this study and were of the same construction as previously. Each was loaded with 100 kg of recently fumigated maize. All replicates of the treatment and control remained sealed for 24 weeks. After 24 weeks, grain was first removed from the upper discharge port as five samples, each of 10 kg. A sub-sample of 1 kg was removed from each sample using coning and quartering. The grain was sampled in the same way from the lower discharge port. The resulting ten 1kg samples were sieved for insects and numbers of live and dead recorded. A count and weigh loss assessment was undertaken on a further two sub-samples of about 100g of grain separated from the 1kg sub-samples by coning and quartering.

Results

With consumption test

In the treated silos, grain damage was on average about 13% lower than the untreated control (Table 6.7). However, a more substantial difference was observed in weight losses suggesting that the damage suffered by treated grain was more superficial.

Weight losses after 22 weeks in treated silos had reached only 1.6% compared with nearly 8% in the control. At the end of 24 weeks, separate estimates were made of the

weight losses at the top and bottom of the silos: again there was a large difference between treatment and control with the treated grain remaining below 2% and the untreated silos suffering at least 9.5% weight loss (Table 6.7). The grain at the top of the silos tended to suffer a small but consistently greater weight loss compared with that at the bottom (Table 6.7). In neither the treated nor control silos were the numbers of *P. truncatus* high and over the whole trial the mean numbers (± sd) of live and dead adults were only 1.40 (± 1.50)/kg and 0.07 (±0.09)/kg in the control and treated silos respectively. Numbers of two other species were broadly comparable between untreated and treated silos: *Sitophilus* sp. (7.50±7.80/kg and 6.40±4.90/kg) and *Tribolium castaneum* (Herbst) (31.80±34.40/kg and 24.80±20.30/kg) respectively. The major difference was in the numbers of *Rhyzopertha dominica* (F.): the untreated silos averaged 31.0(±50.1)/kg over the whole test and had 163.0/kg at the end of the trial while treated silos averaged only 0.6 (±0.8)/kg and had 5.2/kg at the end of the trial.

In view of the much lower weight losses observed in treated silos, it is clear that localised treatment, involving the application of only 50% of the standard insecticidal dust, gives adequate protection of the grain.

Non-consumption test

Treated grain suffered almost no noticeable damage and after six months storage the only insects found on the grain were a few *Tribolium castaneum*, 2.2/kg and 2.1/kg in the two silos. In view of the excellent condition of the grain in the treated silo no weight loss estimate was undertaken. The situation was very different in the control silos, weigh losses were high after 24 weeks and were similar to those recorded in the first test (Table 6.8). Both *P. truncatus* and *R. dominica* established significant populations on this occasion and this test demonstrates both that retaining the untreated grain in the partially treated silos for the duration of storage grain does not lessen the efficacy of this method and that the method works well against both these species.

Table 6.7: Mean[1] % grain damage and mean % weight loss (± s.d.) from grain in mud silos with either no insecticide treatment or treatment confined to top and bottom layers

Weeks after	Untreated silos			Treated silos		
Treatment	% grain damage	% weight loss		% grain damage	% weight loss	
6	1.0 ± 1.08	-		6.0 ± 4.1	-	
10	15.6 ± 11.5	-		3.8 ± 2.5	-	
14	23.3 ± 14.9	-		7.3 ± 4.7	-	
18	19.5 ± 4.5*	-		13.6 ± 4.1	-	
22	28.8 ± 5.3+	7.8 ± 1.5+		13.9 ± 3.6	1.6 ± 1.0	
		Top	Bottom		Top	Bottom
24	36.0 ± 2.6+	14.5 ± 2.4+	9.5 ± 4.0+	20.1 ± 10.5	1.6 ± 2.2	0.84 ± 1.2

[1]Estimates made on four replicate silos except where indicated otherwise *Reduced to three replicates +Reduced to two replicates

Table 6.8: Mean[1] numbers of live insects/kg, grain damage and mean[1] % weight loss of grain stored for six months in two mud silos without any pesticide treatment.

	No. live insects /kg		% grain	Mean % weight
	P. truncatus	*R. dominica*	damage ± sd	loss ± sd
Silo 1	63.3 ± 53.3	52.6 ± 28.5	58.0 ± 19.73	14.93 ± 15.75
Silo 2	53.9 ± 29.4	45.0 ± 20.7	51.88 ± 6.32	9.24 ± 9.8

[1]N = 20

Conclusions

It is clear that under field conditions a pesticide treatment of only 50% of shell grain in a mud silos, 30% treated at the base and 20% treated at the top, has the potential to give very substantial protection the grain. This confirms the results of the laboratory trials using a base treatment and top screen. The weight losses in the partially treated silos, after 24 weeks storage, is no more than would be expected than if 100% of the grain had received treatment with Actellic Super. It would appear that there is no difference in performance whether the untreated portion in the treated silos is consumed during the storage period or left in place for the duration of storage. In addition, the technique appears to offer good protection against both *P. truncatus* and *R. dominica.* Efficacy against *S. zeamais* remains to be established.

If partial grain treatment is to be adopted by farmers, then mud silos with two discharge ports would be useful. The middle discharge port would be used for grain removal until all the middle and upper portions of grain have been removed. Thereafter, the lower discharge port would be used. Currently, in Ghana, mud silos are traditionally emptied by removal of grain from the top. There would therefore have to be some changes in design to accommodate the treatment method. Mud silos with discharge ports are built in other parts of Africa, such as Tanzania, so it may not be too difficult to modify the existing design.

Localised Pesticide Treatment to Protect Maize Cobs Stored in Ewe Barns

This study was undertaken to assess protection conferred on traditional Ewe maize barns when treatments of Actellic Super, dust or emulsion, are confined to the basal layers of these stores. In addition, to determine if any advantage is gained by covering the sides of barns with a plastic sheet to reduce insect immigration.

Methods

Eighteen Ewe barns were constructed at the Kpevé research station in Ghana (Volta Region). The barns were arranged in a 6 x 3 grid with about 2m between them. Each barn consisted of a bamboo platform raised 1m off the ground, with legs fitted with rat guards (Fig. 6.3). On each platform a cylinder of cobs was constructed in the traditional way. The walls of the cylinder were prepared from two layers of cobs with their bases pointing inwards. The central portion of the cylinder was filled with cobs with no specific orientation. Each cylinder was prepared from about 200 kg of cobs of a local variety arranged in ten layers. Prior to the test, cobs were fumigated with phosphine to minimise variation between experimental lots, and an estimate made of weight loss by selecting 100 cobs and subjecting them to a visual loss assessment (Compton and Sherington, 1999).

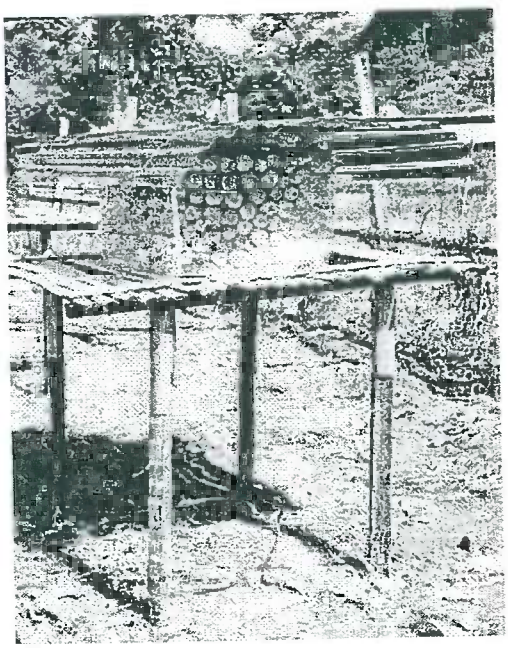


Figure 6.3 – Arrangement of experimental barn

The roof of each barn was prepared from two pieces of corrugated iron sheet resting on four wooden supports (Fig. 6.4). The iron sheets were cut to give an overlap of about 10 cm all the way round the barn. Nine of the barns were given additional protection using a transparent plastic sheet suspended from a cane hoop (Fig 6.4) and hanging down to the bamboo platform.

Attempts were made to increase infestation pressure on the barns. Initially, a glass jar with a sealed mesh top, holding a culture of LGB, was placed under each barn. The intention was that this would attract LGB towards the barns. The incidence of

infestation appeared low so these jars were replaced with other jars containing LGB culture which were open and so would allow LGB to fly out to the barns. Six such jars were placed between barns so that all barns were equi-distant from a jar. The jars were sunk into the ground and provided with a simple rain cover.

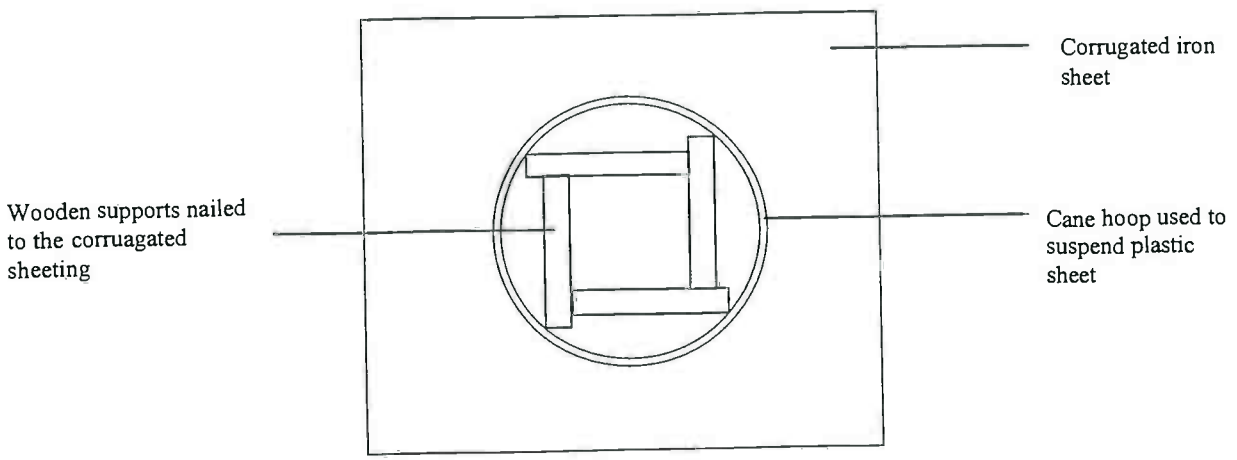


Figure 6.4 – Detail of the underside of the metal roof of the experimental barns

Experimental treatments

The following treatments were allocated randomly to the barns

- Actellic Super dilute-dust treatment
- Actellic Super dilute-dust treatment + plastic sheet
- Actellic Super emulsion treatment
- Actellic Super emulsion treatment + plastic sheet
- No insecticide (control)
- No insecticide (control) + plastic sheet

Actellic Super dilute dust was applied to 24kg of cobs in each of the dust treated barns and confined to the bottom two layers as the barns were built. The cobs were dampened before treatment to aid in construction of the barn. Seventy five grams of dilute dust was sprinkled onto each batch of cobs using a plastic shaker with a small stone inside. The active ingredient in the dust was tested at NRI by GC analysis. It was found to contain 3% permethrin and 14% pirimiphos-methyl, this gave an application rate of 2.25ppm permethrin and 10.5ppm pirimiphos-methyl (on a totla cob weight basis).

Actellic Super emulsion was applied to 24kg of cobs in each of the emulsion treated barns. The cobs were dipped briefly into the emulsion prepared with water. This used about 1.5 litres for each lot of 24kg. The active ingredient in the emulsifiable concentrate was tested at NRI by GC analysis. It was found to contain 16% permethrin and 82% pirimiphos-methyl. The emulsion was prepared by diluting 250g of concentrate in 6 litres of water giving 0.66% permethrin and 3.4% pirimiphos-methyl. This rate is close to that recommended by the manufacturer for application to store and grain surfaces.

methyl. This rate is close to that recommended by the manufacturer for application to store and grain surfaces.

Grain sampling
For the purposes of sampling, grain each barn was divided into three layers, a top (cob layers 9&10), middle (layers 6&7) and bottom (layers 1&2). Sixty cobs were removed from each layer, 30 were selected at random from the wall of the barn and a further 30 from the centre of the barn. The cobs were shelled and the grain thoroughly mixed, keeping grain, from the three layers and from the outside and inside, separate. A 1kg sub-sample from each was then removed by coning and quartering. This sample was sieved for insects. A further two sub-samples each of 100g were then removed using a riffel divider. These samples were subjected to a weight loss assessment using the count and weigh technique. The test for statistical significant differences between treatment means, % weight loss data were transformed to arcsine, subject to one way analysis of variance followed by a least significant difference test.

Results

The weight loss of 100 cobs sampled taken at the start of the trial averaged 1.8% and by the end of 24 weeks most samples from the barns showed increased weight losses (Fig. 6.5). The only possible exception was the EC treated sheet-covered barns where the initial and final loss estimates were very similar. Weight loss data showed significant heterogeneity ($F= 53.6_{5,287}$, $p<0.0001$) and there were significant differences between treatments (Fig. 6.5). The use of a plastic sheet appears to confer some advantage but does not prevent losses increasing. Dust and emulsion treatments were effective and combined with the use of a plastic sheet gave even greater protection.

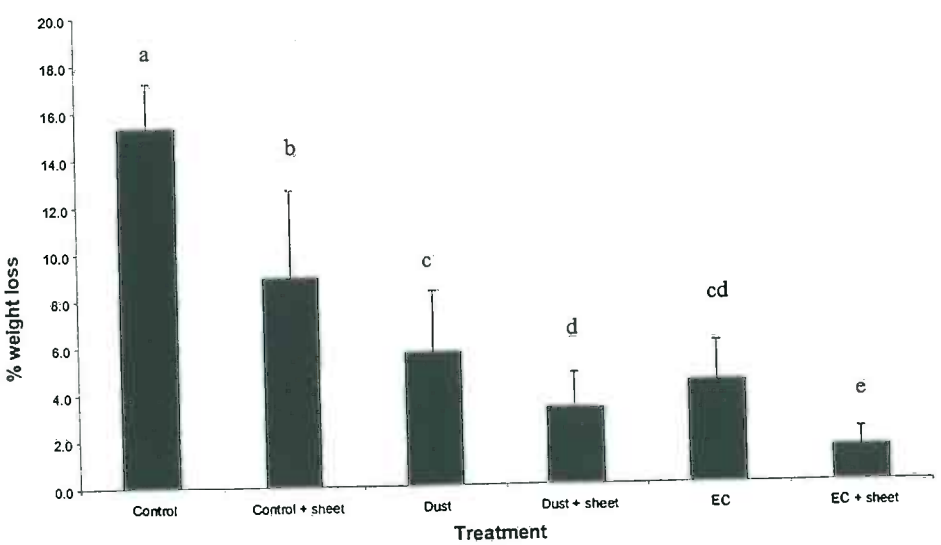


Figure 6.5: Mean weight losses (±sd) from maize cobs stored in Ewe barns for 24 weeks and given various protective treatments. Treatments not coded with any of the same letters are significantly different ($p<0.05$). NB prestorage loss = 1.8%.

Weight losses in maize in relation to where cobs were stored in the barn (top, middle or bottom) and in relation to treatment are shown in Figure 6.6. It is noticeable in the controls that losses are fairly evenly distributed between layers, both inside and outside. Where the sheet was in place on the control, losses appeared somewhat higher towards the base. In the case of barns treated with pesticide, with or without plastic sheeting, considerable protection was conferred on all layers of the barn although basal layers always suffered similar but slightly less damage.

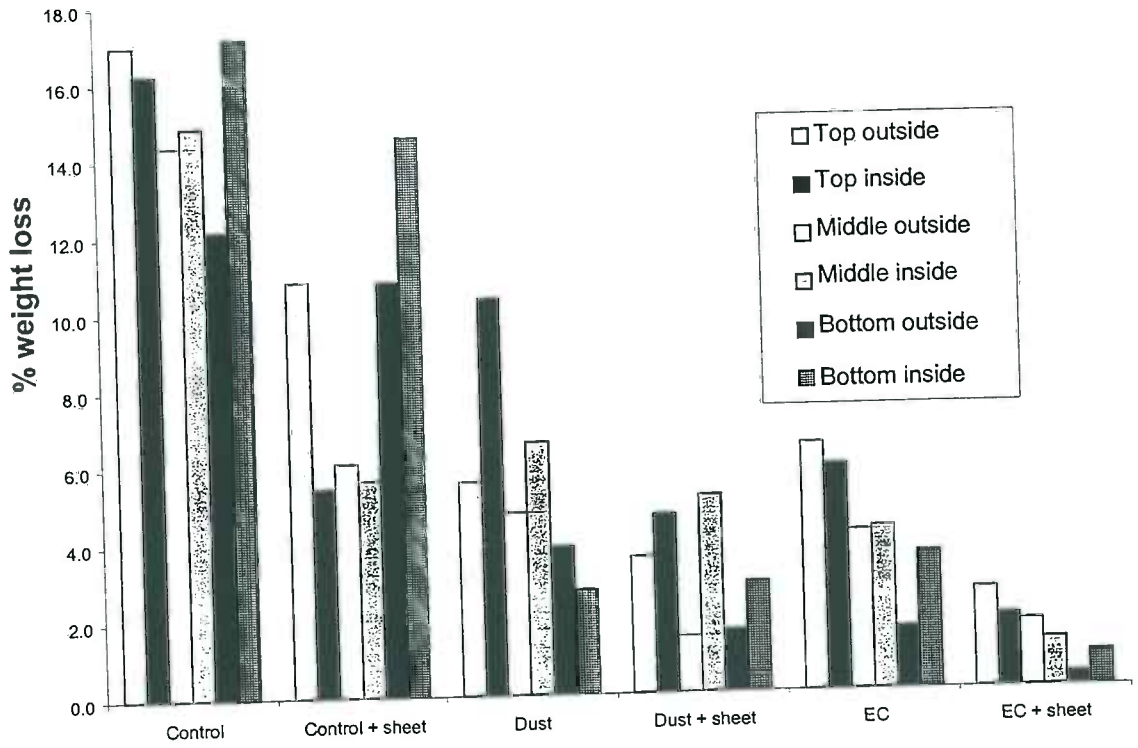


Figure 6.6: % Weight losses from maize cobs stored at three levels in Ewe barns for 6 months and given various treatments

The main species of insect pest observed in the barns were *P. truncatus*, *S. zeamais* and *Tribolium* spp (Fig. 6.7). Numbers of insects were reduced somewhat by the sheet and reduced very substantially by both insecticide treatments. It is noticeable that *P. truncatus* was affected more by the Actellic Super than *S. zeamais*. Small numbers of the predator *Teretrius nigrescens* were also found although these were rarely encountered in the barns treated with insecticide. The distribution of *P truncatus*, *S. zeamais* and *Tribolium* sp altogether in the control and control plus plastic sheet, between the layers of the barns, is roughly even with a tendency for higher numbers at the top and bottom of the barn and no consistent differences between the inside and outside (Figure 6.8). The incidence of insects among treatments and control matches the occurrence of weight losses (compare Figs. 6.5 and 6.7).

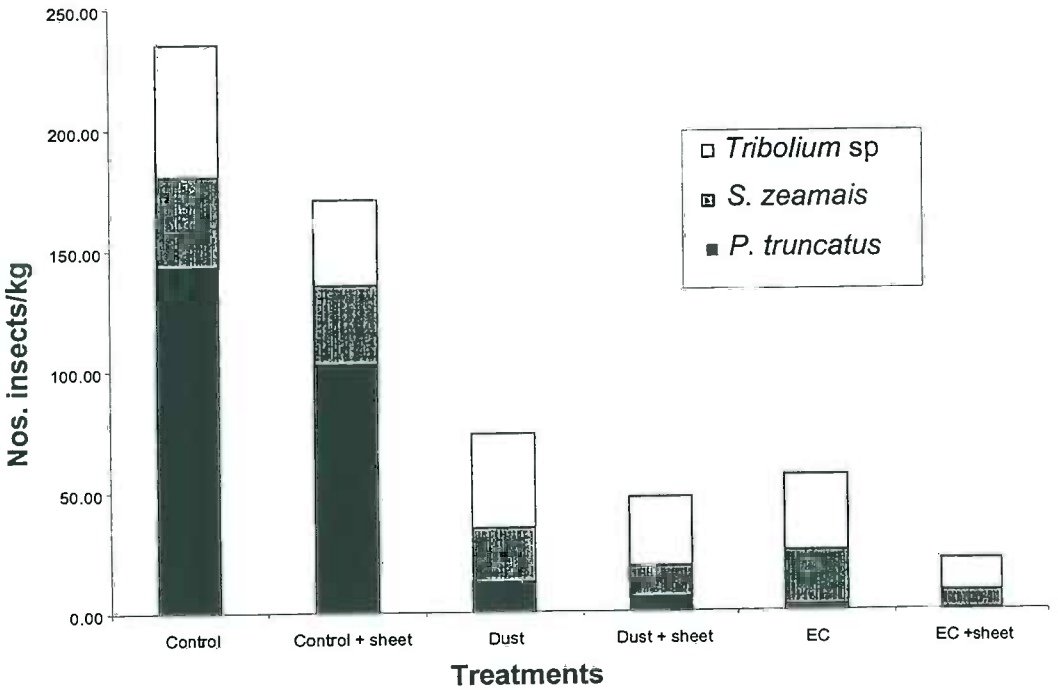


Figure 6.7: Mean total numbers of insects infesting Ewe maize barns after six months storage and subjected to various pest control treatments

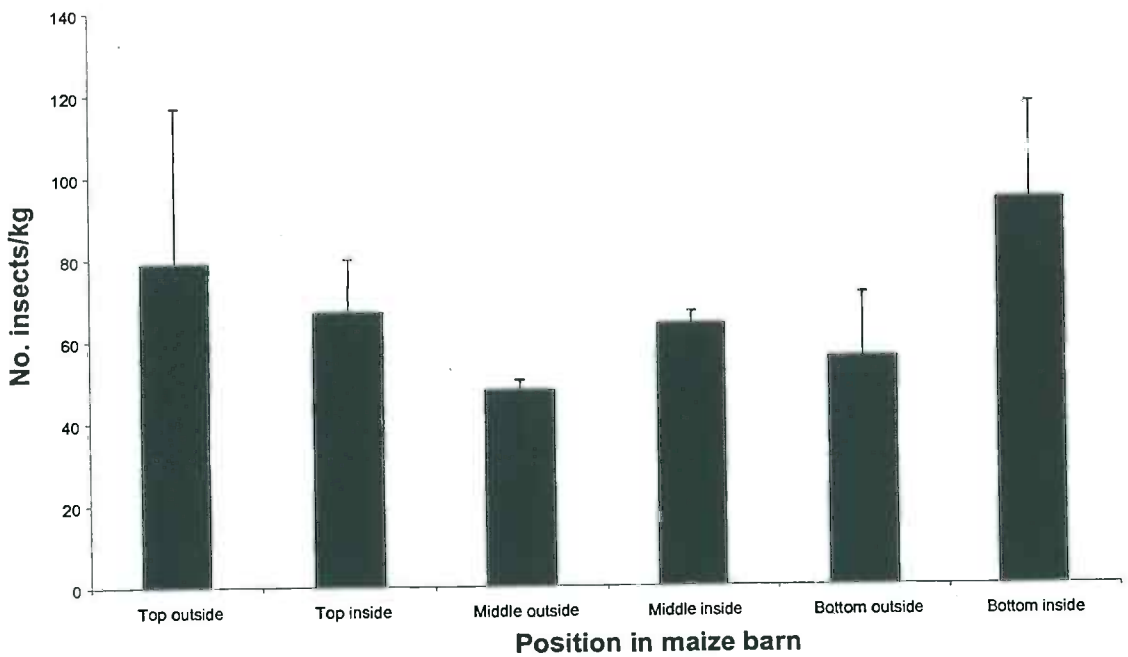


Figure 6.8: Mean total numbers of *P. truncatus, S.zeamais* and *Tribolium* sp/kg (±sd) at various position in maize barns used for control or control plus plastic sheet

The distribution of *P. truncatus* and *S. zeamais* between treatments and the three layers of the barns are shown in Figures 6.9 and 6.10. In all layers of treated stores *P. truncatus* was suppressed to a similar degree although the EC treatment was particularly effective. For *S. zeamais* the suppression was more noticeable in the lower layers. In the control there was quite a strong tendency for there to be much

higher numbers of *S. zeamais* in the bottom outside layer. This was not evident in *P. truncatus*.

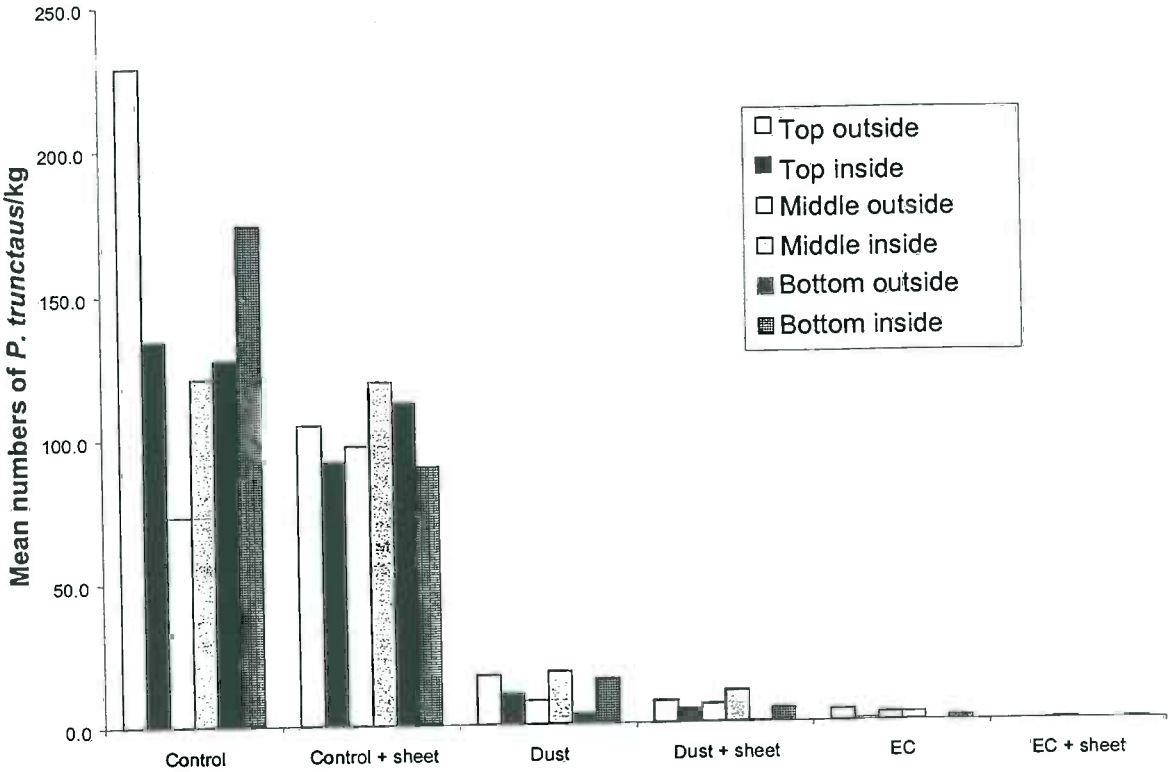
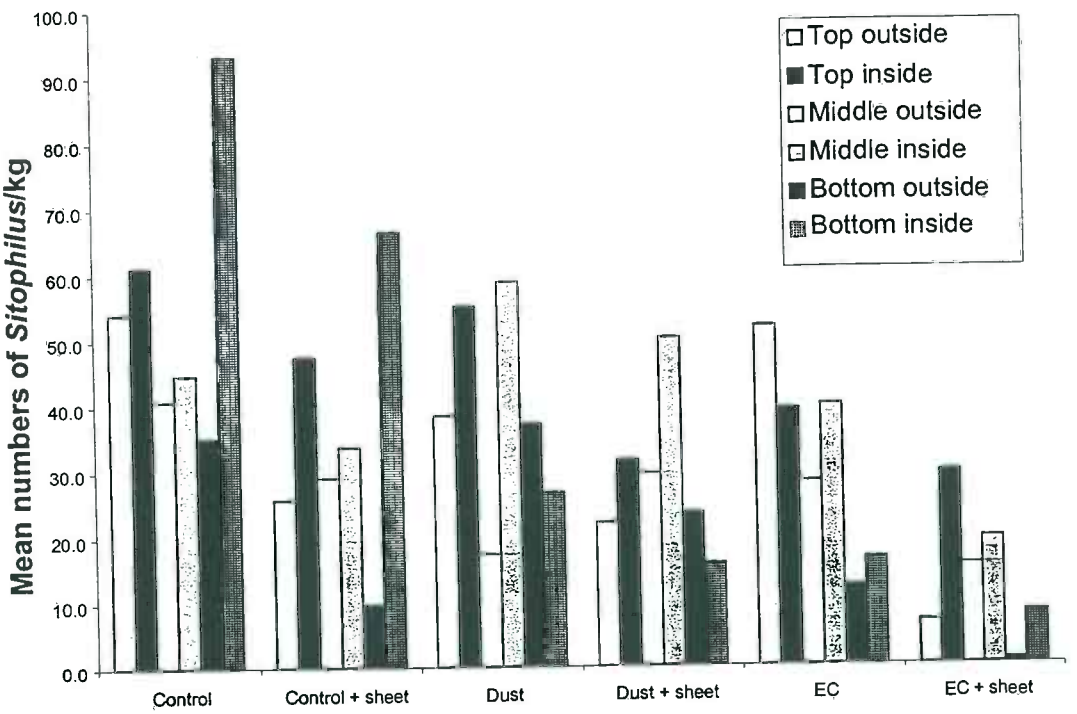


Figure 6.9: Mean numbers of *P. truncatus* extracted from maize cobs stored in Ewe barns for six months and given various treatments



Conclusions

The insecticide treatments of maize cobs limited to the base of Ewe barns (bottom 20% of cobs) provides good protection over a storage period of six months. Weight losses can be reduced from about 15% to around 2-3% (when account is taken of the prestorage weight loss that averaged 1.8%). The addition of a plastic sheet to cover the sides of barns conferred further advantages. In the control, this about halved weight losses, and a similar effect was observed with cobs given either the dust or EC treatments; average losses were reduced to only 0-1.0%, suggesting little or no increase in losses during the course of the test. The advantage of the sheet is probably greatest where there is little or no infestation at the time the barn is constructed and covered with a sheet. This was the case in this test since cobs had been given an initial fumigation with phosphine. Thus under conditions where there is a more substantial infestation at the start of storage the expectation is that the benefit of the sheet would be lower.

The Actellic Super treatment proved rather more effective against *P. truncatus* than *S. zeamais* and damage to the EC treated barns appeared to be almost solely due to *S. zeamais* (Figure 6.7). Never the less the losses in these barns, due to *S. zeamais,* were v*ery* low.

The fact that treatment of the two basal layers confers protection to the entire barn supports the hypothesis that initial insect attack is most prevalent in the base. It follows that a major component of subsequent insect attack is movement of insects from there upwards as the population grows. The fairly even distribution of insects in the barn at the end of the test is presumably a reflection of this upward movement. It might be thought that the use of a corrugated iron roof on these barns could have heated the upper layers of the cobs sufficiently that insects would tend to be driven downwards to be killed on contact with insecticide treatment below. This seems not to have been the case. Temperature measurements with thermocouples placed in the top and other layers of cobs (results not presented here) gave no evidence of any such temperature differential even at mid day when heating would be strongest. In addition, the relatively even distribution of insects observed between the layers of the control adds support to this view. It would be of value to undertake further study of the behaviour of insect in grain stores to shed further light on the typical pattern of attack so that further improvements in the extent and positioning of insecticide treatments can be made. The first step in this process should be to confirm the hypothesis that insects initiate their attack in the basal area by comparing the protection offered when the 20% of treated cobs are at the top, middle or bottom of a barn. Clearly only treatment at the bottom would be expected to confer the advantages described here.

It is of interest to note that in barns given an insecticide treatment, the cobs in the untreated portion of the barn were not much more infested that those in the treated portions. This suggests that the observed weight losses would not have been much lower had all the cobs in the barns been treated. Clearly, limiting the application of insecticide to the bottom 20% of maize barns confers good protection and in barns covered by a plastic sheet, losses are only slight.

Proposals for possible future research

Further studies should be undertaken to test the limits of the reduced insecticide methodology. This should consider interactions between the amount of pesticide required, the extent of infestation pressure that can be resisted and differences in result depending upon the extent of infestation at the start of storage. An initial study on confining treatments to the top, middle or bottom portions of grain stocks would be informative.

Farmer participatory trials are required to determine whether farmers can work with, and benefit from, the methodology. Such trials should be undertaken in Ghana and other countries. However, where storage methods and/or pest complexes are significantly different form those tested in Ghana, preliminary on-station trials will be required to confirm efficacy.

Acknowledgements

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