Biocharacteristics of *Prostephanus truncatus* attracted to flight traps baited with aggregation pheromone

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Abstract

The biological characteristics of adult *Prostephanus truncatus* (Horn)(Coleoptera: Bostrichidae), arriving at pheromone-baited flight traps, were investigated in Ghana in agricultural and non-agricultural habitats of two different agro-climatic zones in three contrasting seasons. This was done to determine whether the risk posed to stored maize by dispersing *P. truncatus* was likely to vary according to season or the source of beetles. Beetles were trapped live and a record made of sex ratio, weight, longevity and female reproductive potential. Comparisons were made with beetles cultured in the laboratory on maize. Some of the beetles trapped in the wild lived for almost a year, and females continued reproducing for over half a year in the absence of males. This suggests that most beetles were captured very young, that females were already inseminated and that these beetles would certainly live long enough in the wild to have a serious impact in stores. The traps captured a higher proportion of females than males and females were heavier. Although some seasonal and locational differences were detected, there was no evidence that these affects would have a significant impact on the biological potential of the pest in the storage environment. *P. truncatus* dispersing in different seasons and in the different habitats, can be assumed to have roughly equivalent biological potential.

Key words: Pheromone traps, *Prostephanus truncatus*

Running title: Biocharacteristics of *Prostephanus truncatus* attracted to pheromone traps

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1. Introduction

Prostephanus truncatus (Horn) (Coleoptera: Bostrichidae) commonly called the Larger Grain Borer is a destructive pest of maize and cassava (Hodges, 1986; Markham et al., 1991) and was first detected in Ghana in 1989 (Dick et al., 1989). *P. truncatus* is primarily a wood boring insect and natural vegetation is an important reservoir where it is capable of breeding and surviving in the dead wood of some tree hosts (Nang'ayo et al., 1993). Thus *P. truncatus* can be found in areas that are not close to agricultural activity, however their economic importance is felt only when they invade food stores. To monitor *P. truncatus*, traps baited with aggregation pheromone are used. The pheromone is attractive to both male and female beetles and can be synthesised in the laboratory (Cork et al., 1991; Dendy et al., 1991).

The extent of *P. truncatus* attack varies from year to year and some years may be particularly bad (Birkinshaw et al., in press). For this reason, a method is being developed to warn farmers of when there is a high risk of a bad year *P. truncatus* infestation. A central feature in the development of the risk warning system is a study to demonstrate that the number of beetles dispersing, shown by *P. truncatus* pheromone trap catches, is directly related to the number of stores that actually become infested. In order to be able to interpret the significance of a particular pheromone trap catch, it is important to know the biological characteristics of those insects that arrive at the traps. In particular, their reproductive abilities and how long will they live. It is also important to know whether these parameters vary with season or between different types of habitat. An investigation was therefore undertaken in the Volta Region of Ghana of the bio-characteristics of *P. truncatus* arriving at traps in agricultural and non-agricultural habitats. Two different agro-ecological zones were studied, forest-savannah transition and semi-arid woodland in a region where there are two annual maize harvests one in August (major season) and the other in December/January (minor season). *P. truncatus* were trapped at three contrasting times of the year, December when it is hot and dry, May when it is hot and moist and October when it is cool and moist. The captures were then assessed for sex ratio, weight, reproductive potential and longevity and compared against a standard of young beetles reared on maize in the laboratory.

2. Methods

2.1 Study sites and trapping seasons

P. truncatus was trapped at four locations (Table 1) and in three different seasons as follows:

- Season 1 (December/January, 1997-98) - a hot dry period when the Harmattan wind is blowing, with day-time temperatures as high as 37°C and r.h. falling, reaching as low as 25% in January. The minor season maize harvest is in store and, typically, captures of *P. truncatus* are rising fast (Fig. 1)
- Season 2 (May, 1998) - a hot moist period during the major rainy season and end of the minor season storage period when typically *P. truncatus* captures are beginning to rise (Fig. 1). Day-time temperatures reach as high as 34°C and the mean r.h. is typically around 70%.
- Season 3 (October 1998) - a cool moist period during the minor rainy season. The start of the major maize storage period and a time when *P. truncatus* catches have been low for two to three months (Fig. 1). Day-time temperatures reach about 30°C and the mean r.h. is about 70%.

2.2 Trap design and trapping protocol

The traps used for this study were Japanese beetle (JB) type, supplied by Trecé Inc., Salinas, CA., USA. They consisted of a yellow plastic funnel (diam. 15 cm, height 11 cm) with a baffle on top, formed from with four 10 cm vertical vanes in the form of a cross, 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 rains out of the traps and a plastic jar (diam. 10 cm) was attached to the base to collect the captured beetles. In order to retain live beetles, each plastic jar was carefully loaded with 60 glass specimen tubes (diam. 1.3 cm, height 5 cm) held in position by a rubber band. Beetles arriving in the trap would drop into the tubes but could not leave them as *P. truncatus* can not climb glass and the tubes were sufficiently narrow and tall to prevent the beetles from taking flight. Many tubes

captured only single beetles, females so caught would not have the opportunity to mate in the trap.

Five traps were placed in each of the four locations described above. They were fixed to the branches of trees at 1.5 m to 2 m above the ground and at least 300 m apart. A standard pheromone lure, consisting of a plastic capsule holding 2 mg of pheromone blend (Trunc-call 1 and Trunc-call 2) in a ratio of 1:1, was placed in each trap. Traps were set before noon and their contents removed by 08.00h the next day. For every location, specimen tubes with single *P. truncatus* in them were taken from each trap first, then others with two or more beetles. The tubes were loaded with a small quantity of fine maize flour to serve as food and sealed with a plastic top with a small hole in it to serve as ventilation.

2.3 Sexing method

P. truncatus were sexed by checking the form of their clypeal tubercles (Shires and McCarthy, 1976). Sexing was confirmed by examination of the genitalia at the end of the trial (Birkinshaw, 1998). All males (single and/or found with other males or females in tubes) were used in this study and single females and/or those with other females in the same tube, i.e. those that had no opportunity to mate after trapping, were also considered for use. Total numbers of males and females (and difficult to sex beetles) were recorded for each trap. The insects were sexed five days after trapping and the few beetles accidentally damaged were excluded from the study.

2.4 Weighing beetles

Before being placed in culture, captured beetles were weighed to four decimal places on a HR-60 multi-function top loading analytical balance.

2.5 Culturing regime

Twenty-five females that had not had the opportunity to mate at the time of capture, and 25 males were used to prepare single specimen cultures. Each beetle was placed on about 30g of kibbled maize in plastic containers (about 60 cm^3) with tight-fitting lids**.** The maize had been disinfested in a deep freeze for two weeks and milled so that grains were broken into approximately equal halves. The lids of the containers were perforated with circular holes (diam. 5 mm) and covered with micropore tape (Boots PLC, UK) to allow ventilation but prevent beetles escaping. *P. truncatus* develops better on stablised maize grain (Cowley et al., 1980) so a small packet of lead weight (about 27 g), held in a plastic sachet, were placed on top of the kibbled maize in each container. The cultures were arranged on shelving in the laboratory and subject to ambient conditions. Male beetles were checked every eight weeks for their mortality (longevity) and females checked every three weeks. At the same time females were transferred onto fresh kibbled maize; this was earlier enough so that they would not be confused with any F_1 generation which at this time had not yet developed into adults. The F_1 generation was counted sixty-three days after females had first been placed on a sample.

For the control beetles, 25 males and 25 females were selected from 55 day old laboratory cultures initiated with 100 unsexed adult *P. truncatus*. 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 a good chance of mating before being placed in single specimen culture as above.

2.6 Statistical analysis

For most data, differences between variables were tested by one way analysis of variance with differences between treatment means tested by Least Significant Difference. For non-parametric data, Chi-squared (χ^2) tests were used.

3. Results and discussion

3.1 Body weight of beetles

On average females weighed more than males, with females ranging between 3.5 to 3.9 mg and males between 3.3 and 3.6 mg (Table 2). This is likely to be due to the fact that females carry eggs and is borne out by the observation that the total number of offspring from heavier females was greater than from lighter females. There was no significant difference between seasons in the body weights of males ($F = 2.26_{(2,236)}$ $p = 0.88$) or females ($F = 2.26_{(2,251)}$ $p = 0.12$). Body weight was relatively consistent between locations with some suggestion of a locational effect in season one, both males and females from Ho forest were lighter although only in the case of males was the effect statistically significant ($F = 2.91_{(3,86)}$, $p = 0.039$). In season 2, there was also a tendency for females caught in Nkwanta savannah to be heavier than those caught elsewhere but the location effect was not statistically significant ($F = 2.35_{(3,75)}$, $p = 0.08$). There is no evidence that insects with access to village stores or those bred on maize are any heavier than those caught in habitats where maize and cassava are not available.

3.2 Beetle sex ratio

The sex ratio in samples of trapped beetles invariably showed a female bias, in the range of 65-75%, irrespective of trapping location or season (Table 3). On two occasions the proportion of females removed from laboratory cultures was nearly the same as that for males although on one occasion it was unusually high. Analysis of location differences in each season showed no effect in season 1 ($\chi^2 = 2.5$, 3 d.f., $p = 0.47$) or season 2 ($\chi^2 = 4.53$, 3 d.f., $p = 0.21$) but a significant effect in season 3 ($\chi^2 = 8.34$, 3 d.f., $p = 0.04$). No seasonal effect on sex ratio was detected in any location with the exception of Nkwanta savannah where the proportion of beetles caught that were females fell in season 3 ($\chi^2 = 6.79$, 2 d.f., $p = 0.034$). The observed sex ratio of trapped beetles is similar to previous estimates. Scholz et al. (1997) found up to 64% of beetles attracted to natural pheromone were female, even though the beetles at source had a 1:1 sex ratio. Similarly, Hodges et al. (1998) recorded 70-80%

of those attracted to synthetic pheromone were female, even though at the end of the trapping period the sex ratio of beetles in the maize cribs from which many of the trapped beetles had originated was also 1:1. As males are the source of the aggregation pheromone, the high proportion of females attracted is not unexpected. Females may be more receptive than the males and/or males might avoid competition with other males by not approaching a strong sources of pheromone.

3.3 Longevity of beetles

P. truncatus caught during this study remained alive for relatively long periods after capture. Some females were still alive up to a year after they were caught, however, on average they lived for 118.3 days. Mortality was fairly similar between locations and seasons (Table 4) with the exception of female insects caught in Ho forest which exhibited worse than average survival in season one. A detailed profile of female mortality in season two is presented in Figure 2 where the average longevity was 112.8 days. Male longevity was only recorded in season two (Fig. 3) and it was on average somewhat shorter than for females at 98.6 days.

Previous laboratory study of the longevity of *P. truncatus* on maize flour, under optimal development conditions of 32ºC and 80% r.h., showed that females generally outlived males with mean life expectancies of 61 and 45 days respectively (Shires, 1980). The current study confirms the difference between males and females but shows that the beetles can have very much longer life expectancies. The fact that trapped insects had long lives suggests that these beetles were fairly young at the time of dispersal and suggests that perhaps mostly only the young specimens disperse. There were no significant differences between the trapping localities and the laboratory cultured insects. The laboratory culture had been established only about 12 months previously. It is not clear why the cultured insects studied by Shires had such short longevities, perhaps the Nicaraguan strain he investigated had reduced fitness due to inbreeding during long-term culturing. Alternatively, they could have been of a particularly short-lived strain or may have been short-lived due to being maintained at a relatively high temperature.

3.4 Reproductive potential of females

Almost all females caught in traps produced viable offspring without further access to males, i.e. were already mated. The mean numbers of offspring per females varied from 6 to 25 across the habitats and three seasons (Table 5). The highest total number of offspring produced by a single female was 97. Initially, female beetles produced large numbers of offspring, but fecundity gradually declined to negligible levels by 114 days (Fig. 4). The change in numbers of offspring produced were not regular so that, for example in season 2, there was initially a high rate of production followed by a fall in all cases but then a steep rise in three cases and continued fall in two (Fig. 4). For statistical analysis of the data, the laboratory culture results were excluded because they showed particularly low fecundity that would distort comparisons between the field trapped beetles. There is evidence of significant differences between seasons in the number of offspring produced ($F = 14.6_{(2, 251)}$, $p = 0.001$), season 3 being particularly low for all sites. Further, only in season 1 is there evidence of a difference between locations ($F = 3.04_{(3,82)}$, $p = 0.034$) as females from Hohoe village produced significantly smaller numbers of F1 than the other three sites ($p<0.05$).

Most of the female beetles arrived in the traps already mated. Li, (1988) found that female beetles require three matings spaced out throughout their adult life to produce maximum numbers of offspring. However, the current study shows that after mating, female *P. truncatus* are capable of reproducing in the absence of males for up to 196 days and are capable of producing up to 97 offspring. Food stores are therefore at high risk from the dispersing female beetles.

4. Conclusions

Previous studies have suggested that beetles taken from laboratory cultures show a 1:1 sex ratio while of those attracted to pheromone traps 64% or more are female (Scholz et al., 1997; Hodges et al., 1998; Birkinshaw, 1998). Higher proportions of females in trap catches may represent a higher risk of damage to stores.

Dispersing insects caught in traps generally had potentially very long life expectancy. Females were almost all fertile and often capable of reproducing for a very extended period without males. The highest reproductive rates were shown 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 in these cases resulted in rather low values for the total number of F1 produced per female.

If it is assumed that the beetles captured in pheromone traps are an unbiased sample of dispersing beetles, and only in the case of sex ratio is there reason to doubt this, then the results indicate that we can be fairly confident that there isn't any large shift in the bio-characteristics of dispersing insects with time. There are also no consistent differences between non-agricultural vs. agricultural sites or semi-arid sites vs. forest-savannah transition sites. In this case, there is no need to take into account a change in the threat of infestation of insects in different seasons or habitats, beyond the changes in **numbers** of insects caught.

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Figure captions

Figure 1: Mean fluctuations in *P. truncatus* number, as measured by pheromone trap catches, in five villages close to Hohoe (1996-1999). Arrows on the x-axis indicate the times of year that beetles were trapped live to study their bio-characteristics.

Figure 2: Mean longevity of female *P. truncatus* trapped at four location, or extracted from a laboratory culture, during seasons two (May 1998) and kept on maize in single specimen culture

Figure 3: Mean longevity of male *P. truncatus* trapped at four location, or extracted from a laboratory culture, during seasons two (May 1998) and kept on maize in single specimen culture

Figure 4: Mean number of F1/female *P. truncatus* trapped at four locations or extracted from a laboratory maize culture, during season 2 (May 1998). Kept on maize in single specimen culture and moved to fresh culture medium every 21 days.

Figure 1

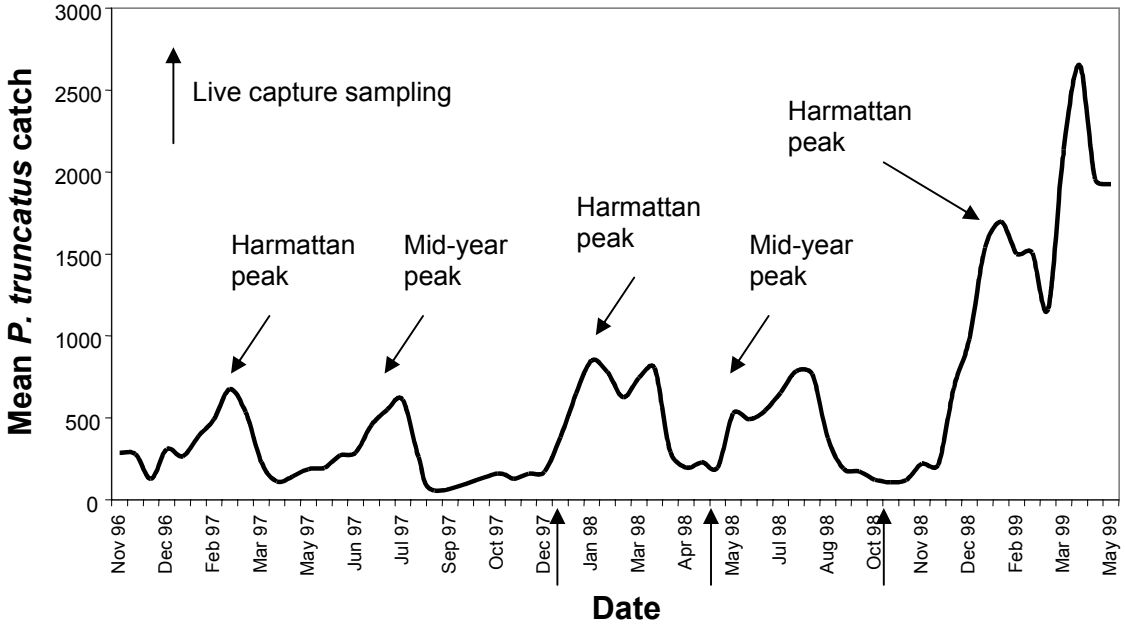
Mean P. truncatus catch
3000
2500
2000
1500
1000
500
0
Live capture sampling
Harmattan peak
Mid-year peak
Harmattan peak
Mid-year peak
Harmattan peak
Nov 96
Dec 96
Feb 97
Mar 97
May 97
Jun 97
Jul 97
Sep 97
Oct 97
Dec 97
Jan 98
Mar 98
Apr 98
May 98
Jul 98
Aug 98
Oct 98
Nov 98
Dec 98
Feb 99
Mar 99
May 99
Date

Figure 2

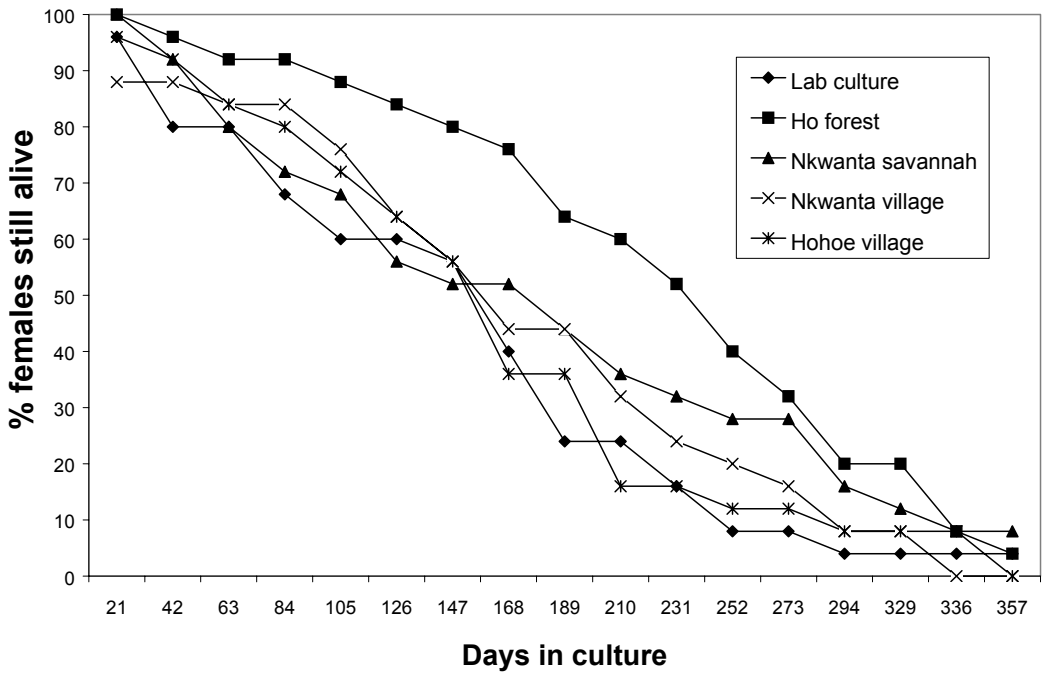
% females still alive
100
90
80
70
60
50
40
30
20
10
0
21
42
63
84
105
126
147
168
189
210
231
252
273
294
329
336
357
Days in culture
Lab culture
Ho forest
Nkwanta savannah
Nkwanta village
Hohoe village

Figure 3

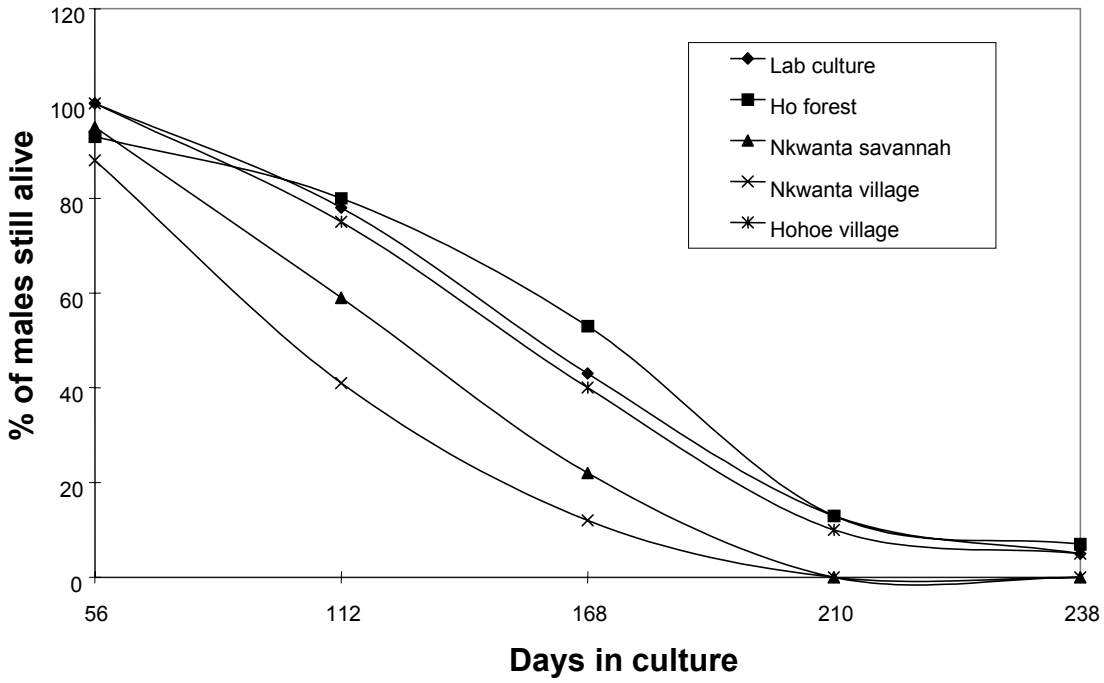
120
100
80
60
40
20
0
% of males still alive
56
112
168
210
238
Days in culture
Lab culture
Ho forest
Nkwanta savannah
Nkwanta village
Hohoe village

Figure 4

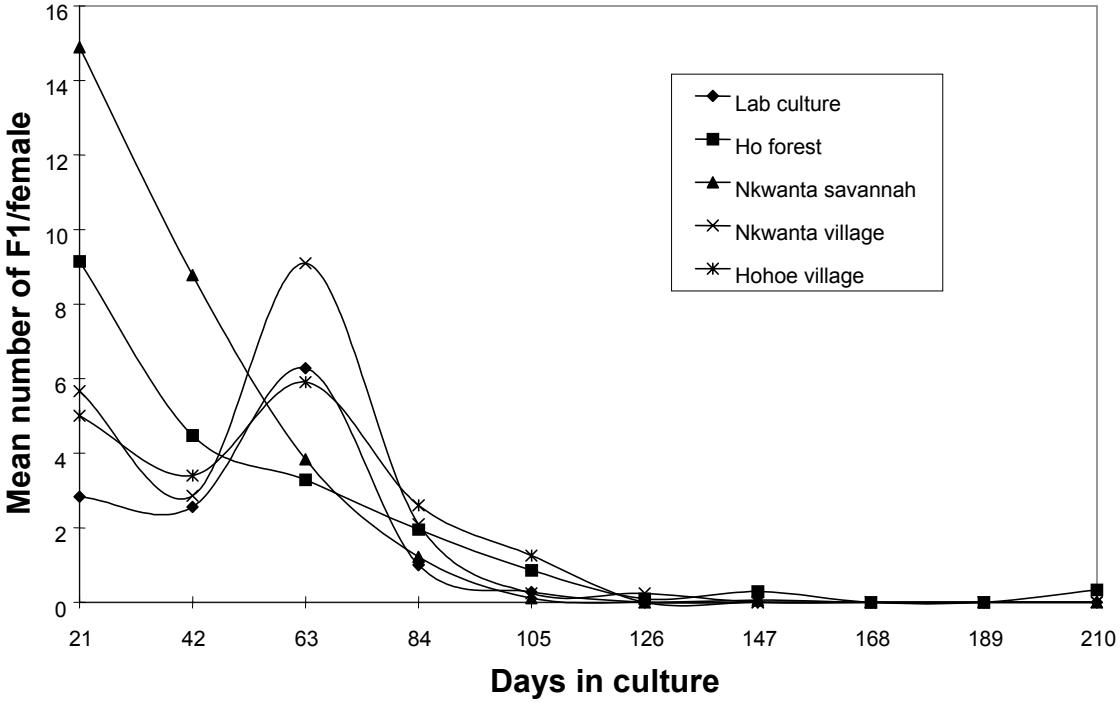
Mean number of F1/female
16
14
12
10
8
6
4
2
0
21
42
63
84
105
126
147
168
189
210
Days in culture
Lab culture
Ho forest
Nkwanta savannah
Nkwanta village
Hohoe village

Table 1: Locations in Ghana of villages and other habitats used for trapping *P. truncatus*

Location or trapping area	**Characteristics of location**
Non- agricultural habitats	
Ho Forest (06° 38.46 N, 00° 28.04 E)	Teak *(Tectona grandis)* plantation with little under growth that includes Acheampong weed *(Chromolaena odorata), Tridax procumbens, Euphobia sp.* and a 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-savannah transition zone.
Nkwanta savannah woodland (08° 17.45 N, 00° 29.58 E)	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) Odomi (07° 16.62 N, 00° 28.66 E) Mempeasem (07° 14.70 N, 00° 28.92 E)	Trapping villages (Mempeasem and Odomi) in Hohoe locality are sited in Forest–savannah transition zone. A lot of maize is stored as sheathed 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) Zongo (08° 16.05 N, 00° 31.12 E) Korantang (08° 15.03 N, 00° 29.52 E)	Two villages (Nkwanta Zongo and Korantang). The villages have dried cassava chips 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.

Table 2: Mean (± sem) body weight of adult *P. truncatus* trapped at various locations in each of three seasons (n = 25)

Location/Season	Female mean weight (mg)	Male mean weight (mg)
Season 1 (Dec. 1997)		
Ho forest	3.49 ± 0.08	3.30 ± 0.10
Nkwanta savannah	3.68 ± 0.09	3.50 ± 0.09
Nkwanta village	3.80 ± 0.10	3.50 ± 0.07
Hohoe village	3.84 ± 0.07	3.60 ± 0.09
Lab. Culture	3.70 ± 0.12	3.50 ± 0.09
Season 2 (May 1998)		
Ho forest	3.58 ± 0.10	3.36 ± 0.09
Nkwanta savannah	3.82 ± 0.12	3.46 ± 0.11
Nkwanta village	3.42 ± 0.12	3.57 ± 0.12
Hohoe village	3.60 ± 0.14	3.39 ± 0.10
Lab. Culture	3.52 ± 0.08	3.13 ± 0.08
Season 3 (Oct. 1998)		
Ho forest	3.80 ± 0.13	3.67 ± 0.09
Nkwanta savannah	3.56 ± 0.11	3.45 ± 0.10
Nkwanta village	3.76 ± 0.10	3.46 ± 0.12
Hohoe village	3.62 ± 0.10	3.59 ± 0.10
Lab. Culture	3.66 ± 0.09	3.68 ± 0.08

Table 3: Sex ratios of *P. truncatus* trapped at different locations, and of beetles extracted from a maize culture, in three different seasons

Location	Number of males	Number of females	Proportion of females
Season 1 (Dec. 1997)			
Ho forest	25	74	0.66
Nkwanta savannah	27	115	0.77
Nkwanta village	39	134	0.71
Hohoe village	33	148	0.78
Lab culture	27	40	0.60
Season 2 (May 1998)			
Ho forest	26	82	0.68
Nkwanta savannah	26	119	0.78
Nkwanta village	28	107	0.74
Hohoe village	25	104	0.76
Lab culture	28	31	0.53
Season 3 (Oct. 1998)			
Ho forest	31	138	0.76
Nkwanta savannah	25	53	0.53
Nkwanta village	25	101	0.75
Hohoe village	29	71	0.59
Lab culture	27	113	0.81

Table 4: Mean and range of longevity of female *P. truncatus* trapped at four location or extracted from a laboratory culture in each of three seasons and maintained on maize in single species culture (n = 25)

Location/season	Mean longevity (days)	Range of longevity (days)
Ho forest		
Season 1	74.6	63 - 280
Season 2	106.3	21 - >357
Season 3	133.0	<21 - <357
Nkwanta savannah		
Season 1	128.1	>63 - >357
Season 2	127.0	21 - >357
Season 3	142.0	<21 - 336
Nkwanta village		
Season 1	97.7	>63 - 336
Season 2	114.7	<21 - 294
Season 3	133.6	21 - 336
Hohoe village		
Season 1	116.0	>63 - >357
Season 2	108.5	<21 - 336
Season 3	128.5	<21 - 336
Lab culture		
Season 1	124.5	>63 - >357
Season 2	106.3	<21 - >357
Season 3	133.1	<21 - <357

Table 5: Reproductive potential of female *P. truncatus* trapped at four locations or extracted from a laboratory culture in each of three seasons, as measured by the mean number of F1 (±sem) and the mean length of the reproductive period (±sem). The females were maintained without males in single specimen culture. (n = 25)

Location/season	Mean total F1	Mean reproductive period (days)
Ho forest		
Season 1	27.62 ± 3.31	61.5 ± 4.0
Season 2	20.43 ± 3.42	79.5 ± 11.5
Season 3	6.04 ± 1.39	17.2 ± 6.2
Nkwanta savannah		
Season 1	24.09± 3.15	36.5 ± 3.4
Season 2	28.89 ± 6.00	56.5 ± 7.5
Season 3	12.48 ± 2.01	21.0 ± 4.3
Nkwanta village		
Season 1	25.27 ± 2.91	57.2 ± 4.1
Season 2	20.19 ± 4.12	60.5 ± 7.2
Season 3	17.73 ± 2.61	28.1 ± 3.5
Hohoe village		
Season 1	15.10 ± 2.75	43.1 ± 5.5
Season 2	18.15 ± 3.45	55.2 ± 7.7
Season 3	12.55 ± 2.69	27.8 ± 4.5
Lab culture		
Season 1	13.13 ± 2.91	27.3 ± 4.0
Season 2	12.94 ± 3.42	43.8 ± 7.8
Season 3	7.76 ± 1.54	33.3 ± 6.2