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## Optimising pheromone lures and trapping methodology for *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae)

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### Abstract

Male *Prostephanus truncatus* release an aggregation pheromone, attractive to both sexes, consisting of two components, Trunc-call 1 (T1) and Trunc-call 2 (T2). Synthetic T1 and T2 are used as the lure in flight traps to catch the beetle; the standard commercial lure is a polythene capsule loaded with 2 mg of 1:1 mixture of the compounds. However, laboratory comparison of 1:1 and 2:1 loadings showed that capsules with the higher T1 ratio release a blend that is closer to the mean natural ratio. Field testing of alternative weights and ratios of pheromone components demonstrated that capsules with the 2:1 mixture lured more beetles, improving catch by 17–29%. This improvement is probably not great enough to justify a change in practice for routine monitoring in situations where the presence of the pest is already well known. But there may be potential for adopting the new ratio in situations where higher trap sensitivity is required.

The lures were supplied in sealed foil sachets and when they were first exposed a very high ‘flash-off’ of pheromone was observed. The flash-off was largely completed within 4 h of exposure and by 24 h pheromone output was more or less steady. For experimental studies comparing different treatments over short periods of time, it may be important to avoid testing during this flash-off period. In these circumstances, pheromone capsules should be aired for at least 1 day before being placed in traps.

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*Keywords:* *Prostephanus truncatus*; Pheromone; Flight trapping

### 1. Introduction

*Prostephanus truncatus* (Horn) is an important pest of farm stored maize and dried cassava in Africa and Central America (Hodges, 1986; Markham et al., 1991). Once a male has reached a

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suitable food source, an aggregation pheromone is released that is attractive to both females and males (Dendy et al., 1991; Leos-Martinez et al., 1995; Hodges and Dobson, 1998). The pheromone consists of a blend of two components, 1-methylethyl (*E*)-2-methyl-2-heptenoate and 1-methylethyl (*E,E*)-2,4-dimethyl-2,4-heptadienoate (Cork et al., 1991; Dendy et al., 1991) given the trivial names Trunc-call 1 and Trunc-call 2 (T1 and T2), respectively; the major attractant in the blend is T2 (Leos-Martinez et al., 1995; Hodges et al., 1998). Synthetic pheromone is used as a lure in traps to monitor the pest for phytosanitary purposes and biological research. Lures are prepared by impregnating polythene capsules with synthetic pheromone and the lures available commercially are loaded with 2 mg of a mixture of T1 and T2 in a 1:1 ratio.

The ratio of pheromone components in a pheromone blend is well known to have a strong affect on its attractiveness in Lepidoptera (Roelofs, 1980; Linn and Roelofs, 1989) and in Coleoptera such as bark beetles (Wood, 1982). Blend ratios in *P. truncatus* pheromone lures have received some attention; mixtures were better than the components alone but the results were otherwise statistically inconclusive (Dendy et al., 1989, 1991). Hence, a 1:1 ratio has been adopted for the standard commercial lure.

Pheromone traps for *P. truncatus* loaded with this standard lure are commonly used in Africa (Richter and Biliwa, 1990; Fandohan et al., 1992; Pike et al., 1992; Hodges et al., 1996). They catch not only the pest but are also attractive to its predator *Teretrius nigrescens* (Lewis) (Coleoptera: Histeridae) (formerly called *Teretriosoma nigrescens*) (Rees et al., 1990; Boeye et al., 1992). *Prostephanus truncatus* is amongst the most widely trapped of storage pests but only with recent data on individual male pheromone outputs (Hodges et al., 2002) has it been possible to investigate how well the pheromone lure mimics the natural blend. Measurement of the pheromone released by individual males shows that there is inter-male variation in the amounts and ratios of pheromone components released, although the daily average release of T1 and T2 has been observed to be 1.9 and 0.5 µg, respectively (Hodges et al., 2002). Thus, T2 is typically only 20% of the blend even though it is the major attractant. In a study of flying and walking beetles attracted to different trap types, Hodges et al. (1998) concluded that the presence of T1 was particularly important when the beetle was close to a strong source of T2. In view of this, there could be some potential for improving trapping performance if the standard pheromone lure releases a sub-optimal ratio of blend components. For this reason, the current study set about monitoring the emissions from polythene capsules, loaded with various ratios and weights of T1 and T2, to determine the extent to which artificial lures simulate the quality of typical male release. This led to a field study in Ghana to compare the performance of traps loaded with standard lures and those giving a closer match to the natural pheromone blend. Further improvements in trap performance were sought by an investigation of pheromone 'flash-off' suggested by Compton et al. (1997). The flash-off was believed to occur from lures recently removed from their sealed packaging and to account for high variation in *P. truncatus* catch during the first 2 or 3 days of trapping. This could introduce distortions in trapping performance in research studies where daily or other trapping durations are compared. The phenomenon was investigated in the laboratory by comparing pheromone release between freshly prepared and stored lures and observing the difference in beetle capture profiles between traps holding lures recently removed from packaging and those aired for 24 h prior to use.

## 2. Materials and methods

### 2.1. Pheromone release from polythene capsules

The two pheromone components, T1 and T2, were synthesised at NRI (Cork et al., 1991) and were >97% pure by gas chromatography. Polyethylene capsules (9 mm o.d. × 23 mm with a wall thickness of 1 mm; Just Plastics Ltd.) were impregnated with the pheromone components by adding the pheromone blend and an equal weight of antioxidant (2,6-di-*tert*-butyl-4-methylphenol, BHT) in pentane solvent (100 µl) and allowing the solvent to evaporate before sealing the capsule.

Pheromone was collected from polythene capsules at  $27 \pm 1^\circ\text{C}$  and  $60 \pm 5\%$  r.h. Capsules were placed singly in glass vessels of 30 cm<sup>3</sup> capacity (Fisher Scientific, UK) through which air was drawn at a rate of 1000 cm<sup>3</sup>/min by electrical diaphragm pumps (Capex Mk II; Charles Austin). The intake air was purified by passage through a filter containing activated charcoal (20 cm × 2 cm, 6–18 mesh) and the output air was passed through filters containing Porapak Q (200 mg, 50–80 mesh; Phase Separations, UK) to collect volatiles emitted. A glass round-bottomed flask (1000 cm<sup>3</sup>) was also connected in the system, between the pump and collection filters, to act as a buffer against pressure variations induced by the pump. The air was pumped continuously during the test and the Porapak filters changed after 1 h. Previous studies with two Porapak filters connected in series showed that both T1 and T2 were fully retained on the first filter for at least 3 days. Breakthrough of T1 was apparent after 6 days but not of T2 for at least 13 days.

Volatiles collected on the Porapak were eluted with dichloromethane (750 µl; Fisher Scientific Distol grade) and octyl acetate (5 µg) added as an internal standard. Samples were assayed by gas chromatography, using a fused silica capillary column (30 m × 0.32 mm i.d.) coated with CPWax 52CB (Carbowax equivalent; Chrompack, UK), with helium carrier gas and flame ionisation detection. The temperature was held at 60°C for 2 min, then programmed at 6°C/min to 230°C. The injector temperature was 200°C and the detector temperature was 240°C. The sample (2 µl) was injected splitless and not concentrated to avoid loss of pheromone components. Data were captured and processed with EZChrom V.6 software. Amounts of the two pheromone components were calculated by comparison of peak areas with that of the internal standard and application of correction factors. The latter were derived by calibration of pure synthetic components against the internal standard at the 5 µl level. Peak identity was confirmed at intervals by gas chromatography-mass spectrometry (Finnigan MAT ITD700, Thermoquest) using similar chromatographic conditions.

In a first experiment, capsules were loaded with pheromone, sealed into foil sachets and then stored at  $-18^\circ\text{C}$  for at least 1 week. Sachets were taken from the freezer and allowed to warm to room temperature over 20 min. Then the capsules were removed from the sachets and pheromone collected for 1 h. Two capsules were tested on each of a number of occasions over a period of 15 days (Fig. 1). Initially, a 1:1 mixture of the two components was tested, with total loadings of 2 mg or 4 mg. However, as the 1:1 mixture was found to provide a relatively poor mimic of the natural pheromone blend released by the male beetle, a study was undertaken to observe the component ratios released by 2:1 mixtures; capsules had total loadings of either 3 or 6 mg.

To demonstrate the effects of storing pheromone capsules on the pheromone release characteristics, the pheromone output from freshly prepared capsules was investigated. Capsules

Table 1

Mean ( $\pm$  s.e.) catch of traps baited with lures holding different weights and ratios of the pheromone components T1 and T2 and set to capture beetles 3–8 days after removal from the storage sachet ( $n = 20$ )

Capsule loading (mg)		Mean trap catch
T1	T2	
1	1	10.30 $\pm$ 1.77
2	1	12.00 $\pm$ 2.07
2	2	12.56 $\pm$ 1.55
4	2	16.08 $\pm$ 2.94
		SED 1.52

were loaded with 2 mg of a 1:1 mixture of T1 and T2 and placed in the entrainment apparatus for the first time within 1 h of preparation.

## 2.2. Field trapping trials

Trapping experiments were carried out at Ho, Ghana using pheromone-baited flight traps modified from the Japanese beetle type (Trece Inc., Salinas, CA, USA), as described in Compton et al. (1997). From laboratory studies it had been found that the ratio of the pheromone blends released from the capsules with the two different loadings were not substantially different until about the fourth day. For this reason capsules were left in the open air for 3 days before being loaded into traps, i.e. lures were used to capture beetles from 3 to 8 days after removal from the storage sachet.

In a first experiment, the effect of baiting traps with one of four different pheromone blends (Table 1) was compared with an unbaited control. Traps were hung from trees or bushes at a height of 1.5–2 m, at least 150 m 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. There were 10 trap positions to accommodate two replicates of the five treatments that were allocated to the positions according to two randomised Latin squares. Catches of *P. truncatus* were recorded and trap positions changed each day over a period of 5 days to give a total of 10 replicates for each treatment. The test was then repeated to give a further 10 replicates per treatment.

For statistical analysis, the differences in trap catch according to treatment were investigated by Generalized Linear Modelling (Statistical Package for the Social Sciences, SPSS version 10.0.1, 1999), 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 were considered to be significantly different at the 5% level ( $P \leq 0.05$ ).

In a second experiment, observations were made on the effects of airing capsules for 24 h before using them in traps. Traps were baited with capsules containing a total of 2 mg of the 1:1 mixture of T1 and T2. The capsules were either taken directly from a sachet recently warmed to ambient temperature or had already been aired for 24 h in a laboratory. Two replicates of each of the two treatments were positioned at the opposite corners of a square with sides of about 200 m in a semi-wooded area. Traps were hung at about 2 m above ground and the capsules were put in place at

15.30 h on the first day. Trap catches of both *P. truncatus* and its predator, *T. nigrescens* were recorded daily at 08.30 h for 6 days. This was repeated three more times with the position of aired and non-aired capsules interchanged at each repeat.

### 3. Results

#### 3.1. Pheromone released from polythene capsules

Pheromone release from standard capsules containing 2 mg of the 1:1 mixture of T1 and T2, which had been stored in a freezer, was initially high and declined rapidly over the first 4 h (Fig. 1). After the first day, pheromone release was more uniform, with T1 released faster than T2 for most of the period up to 15 days. The mean daily output per capsule from the end of the first to the 15th day was 29.25 µg/day T1 and 12 µg/day T2. 'Non-standard' capsules containing 4 mg of the 1:1 mixture of T1 and T2 or 3 or 6 mg of a 2:1 mixture, similarly showed a high release of pheromone immediately after removal from storage. The proportions of T1 in the blends released from the standard and non-standard capsules are shown in Fig. 2. For capsules, loaded with a 1:1 mixture of T1 and T2, the proportion of T1 was 50–60% by day 8 and had reached 15–30% by day 15. In contrast, capsules loaded with a 2:1 mixture of T1 and T2, i.e. with relatively more T1, released a blend containing a 75–90% T1 over almost the entire period of 15 days. This was much closer to the mean natural ratio of about 80%.

The pattern of pheromone release from freshly prepared capsules was quite different from those that had been stored in sachets (Fig. 3). Release rates of both components were initially low and increased over the first 2–3 days to levels similar to those from the corresponding stored capsules after 2–3 days, after which release rates from stored and fresh capsules were similar (Figs. 1 and 3).

#### 3.2. Captures of *P. truncatus* in traps baited with lures containing different pheromone blends

The mean catches of *P. truncatus* in traps loaded with different weights and ratios of the two pheromone components are shown in Table 1. Catch differed significantly with treatment ( $F_{(3,79)} = 4.43, P = 0.008$ ). 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, but the difference was significant ( $P < 0.05$ ) in the case of lures with 2 mg of T2.

#### 3.3. Captures in traps loaded with aired and non-aired capsules

During the first trapping period (15.30–08.30 h next day) the *P. truncatus* catch in traps baited with non-aired capsules was very high compared with those baited with aired capsules (Fig. 4). Thereafter, the catch in traps with the two loadings was similar.

For *T. nigrescens* there was a different pattern of catch (Fig. 5). Traps with non-aired capsules showed a higher peak in catch on the second day; otherwise catches between traps were more or less the same.

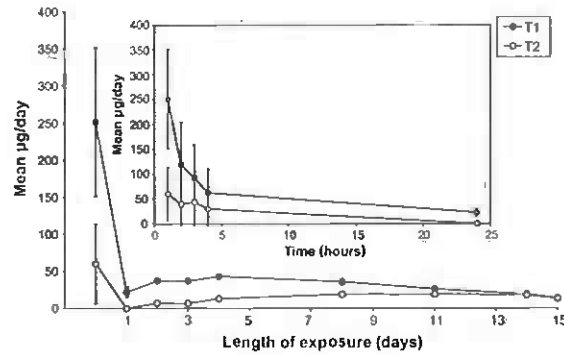


Fig. 1. Mean daily quantities ( $\pm$ s.e.) of *P. truncatus* pheromone released from capsules loaded with 2 mg of the 1:1 pheromone mixture, after storage in a sachet for 30 days with an inset graph to show details of release during the first 24 h (pheromone collected for 1 h and expressed as output in a 24 h period) ( $n = 2$ ).

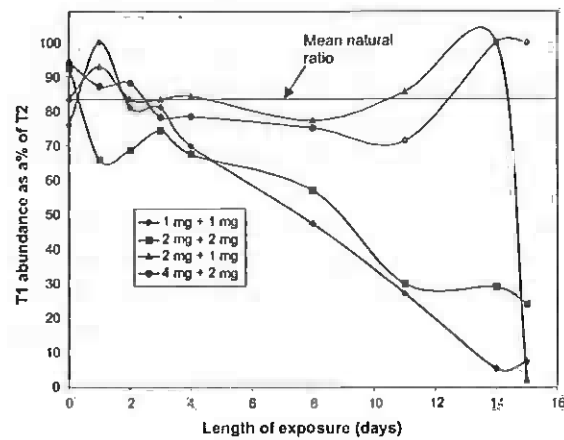


Fig. 2. Mean abundance of pheromone component T2 as a % of T1, released from polythene capsules loaded with different weights of these two compounds and stored in a sachet before exposure ( $n = 2$ ).

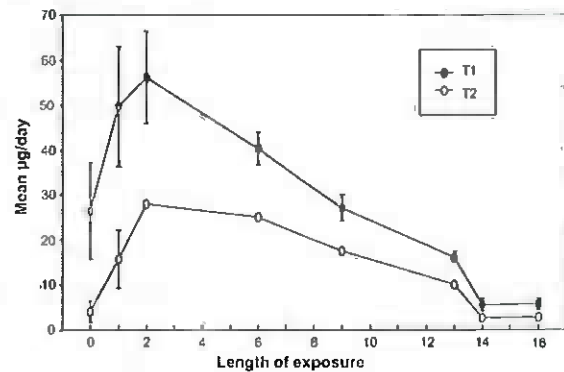


Fig. 3. Mean quantities ( $\pm$ s.e.) of *P. truncatus* pheromone released from freshly prepared capsules loaded with 2 mg of the 1:1 mixture ( $n = 2$ ).

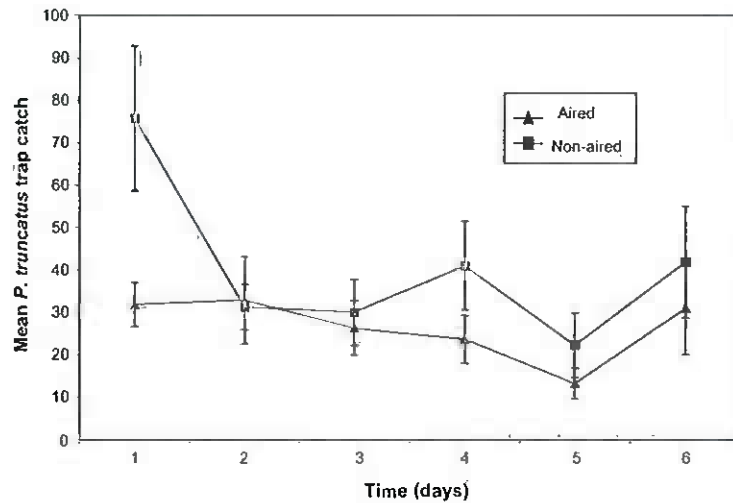


Fig. 4. Mean catch of *P. truncatus* ( $\pm$ s.e.) in traps loaded with capsules (2 mg 1:1 mixture) fresh from a sealed foil sachet or with capsules aired for 24 h before use ( $n = 4$ ).

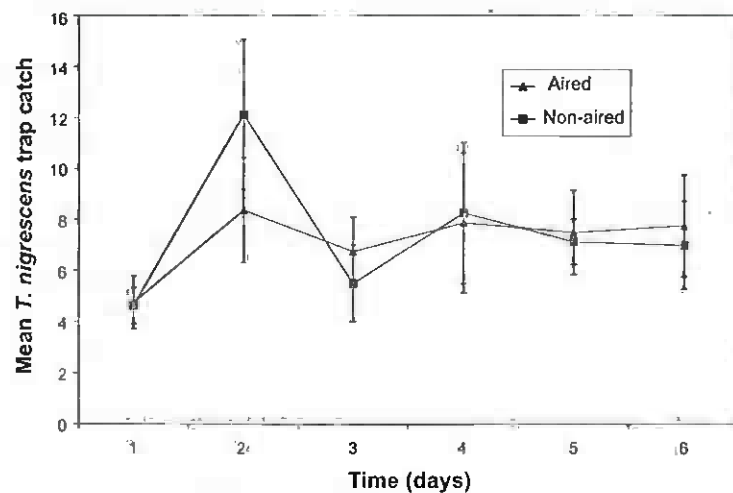


Fig. 5. Mean catch of *T. nigrescens* ( $\pm$ s.e.) in traps loaded with a capsule fresh from a sealed foil sachet or with capsules aired for 24 h before use ( $n = 4$ ).

## 4. Discussion

### 4.1. Pheromone release from polythene capsules

The polyethylene capsules released much greater daily quantities of pheromone than the individual beetles observed by Hodges et al. (2002). The mean daily release from standard capsules loaded with 2 mg of the 1:1 mixture of T1 and T2 was 15 and 24 male equivalents, respectively. Data collected by Hodges et al. (2002) on individual male pheromone release are daily means calculated without a knowledge of the actual release period in any one day; thus it is

not clear to what extent the actual release rates of the capsules mimicked those of the beetles. However, the release ratios of the two components are better understood. During the second week, the blend released from capsules loaded with 2 or 4 mg of the 1:1 mixture declined to only 10–60% T1, while in the same period capsules loaded with 3 or 6 mg of the 2:1 mixture released a blend that contained 60–90% T1. The 2:1 mixture mimicked the mean blend released by male beetles (on average about 80% T1) more closely than the standard lures.

Field testing of different blends of pheromone components provided support for this hypothesis. Traps baited with lures containing a 2:1 blend of T1 and T2 caught more *P. truncatus* than traps baited with lures containing the 1:1 blend with the same loading of T2. Earlier studies on the functions of T1 and T2 in isolation and together have shown that, in flight traps, T2 is the major attractant while T1, at least by itself, attracts few beetles (Leos-Martinez et al., 1995; Hodges et al., 1998). In the one comparison possible in the tests reported here, doubling the amount of T2 while holding that of T1 constant had little effect on catch, raising it from a mean of 12.00 to only 12.56 (Table 1). This might be explained as follows: increasing T2 potentially raises the catch but the associated change from a 2:1 to 1:1 ratio lowers the proportion of T1 which may cancel out any such benefit. Furthermore, Hodges et al. (1998) suggested that the role of T1 could be important where there is extensive exposure to T2 and may modify the response to T2 to facilitate close-range attraction. In the tests here, a blend ratio higher in T1 resulted in greater trap catches, and there was an indication that this advantage was more clearly demonstrated when the absolute values of T2 were higher with catches 17% greater with 1 mg T2 and 29% greater with 2 mg T2. This supports the suggestion that T1 has greater effect at higher T2 concentration. This could be confirmed by observing the electro-physiological responses to different pheromone blends.

Although capsules holding the 2:1 mixture were a superior lure in the flight traps, the actual increase in catch, 17–29%, is unlikely to be very significant when monitoring for the pest in a situation where its presence is already well established. Greater trapping sensitivity might be more of an advantage when the chances of actually catching any *P. truncatus* are low. For example, in localities where the pest has not yet established itself and the population is still small or where the pest is suspected but not yet detected. In these situations, an improved lure may substantially increase the chances of detecting the pest, which may lead to more timely pest management action. It would be helpful to test loadings of the 2:1 mixture above 6 mg to determine whether further increases in trap sensitivity are achievable. The improved lure is likely to be more expensive, the cost of adopting a lure loaded with 3 mg of a 2:1 mixture may be 10% more expensive than the standard lure while a lure with 6 mg could increase costs by up to 40%. However, the use of improved, and hence more costly, lures should not be over emphasised since improvements in trap performance can also be achieved by adopting better trap designs, in particular those that provide easier access for the pest (Compton et al., 1997). It may therefore prove more cost effective to invest in more accessible traps.

#### 4.2. Investigation of the "flash-off" effect

Measurement of release rates from freshly made capsules showed a very low initial release that increased to a maximum over 2–3 days and thereafter declined with an effective lifetime of approximately 15 days at 27°C. These results presumably show that the pheromone components



take 2–3 days to permeate through the polyethylene walls of the capsules. Similar measurements from capsules that had been stored in sealed aluminium foil bags for at least 1 week showed very rapid initial release of both pheromone components. These dropped over the first few hours of exposure, by more than five-fold, to rates similar to those reached by the freshly made capsules after 2–3 days. Under field conditions, lures are typically stored before use, and the early high rates of release confirm the existence of the ‘flash-off’ effect predicted by Compton et al. (1997). This is presumably due to the pheromone permeating through the capsule wall during storage. In the sealed bags used, this will proceed until there is equilibrium, a thin film of pheromone forming on the outer wall of the capsule. This pheromone will volatilise rapidly when the capsule is removed from the bag until a new equilibrium is established after a few hours when release of pheromone is controlled by diffusion through the capsule wall.

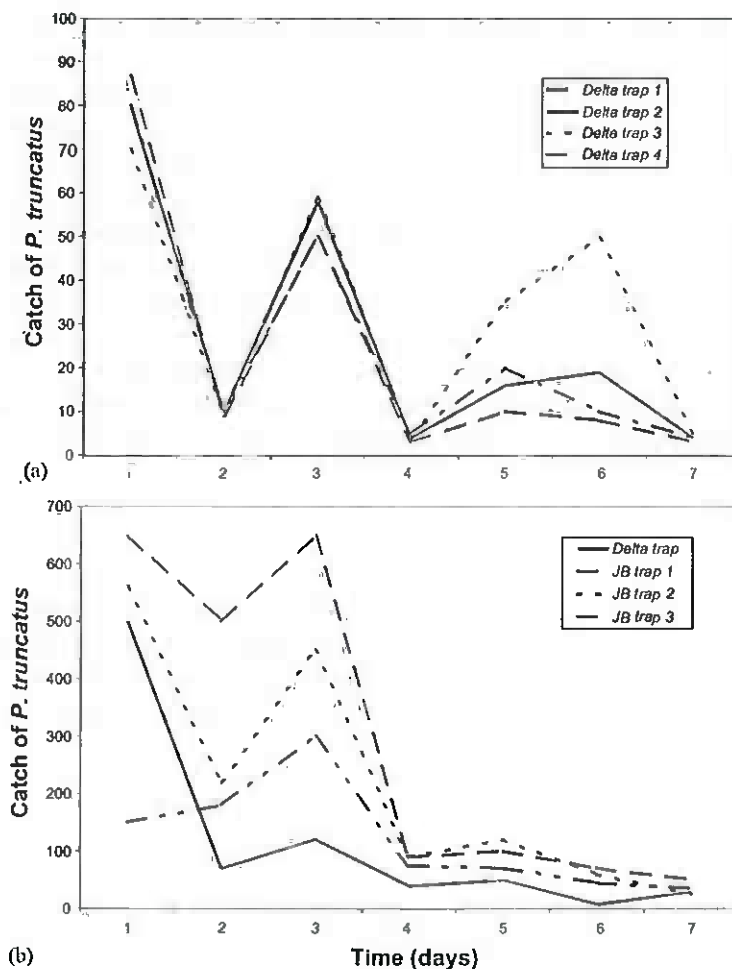


Fig. 6. Numbers of *P. truncatus* captured in two separate trapping studies, with various trap types, showing regular oscillations in catch during the first 7 days of trapping. (a) In Mexico, in delta traps (data from Tigar et al., 1993) and (b) in Ghana, in delta or Japanese Beetle (JB) flight traps (data from Compton et al., 1997).

In the field, during the first trapping period (15.30–08.30 h the following day) the catch of *P. truncatus* in traps baited with lures that had been taken straight from storage was significantly higher than in those baited with capsules that had been aired for 24 h. Thereafter, the catches in traps with either treatment were similar. For *T. nigrescens*, catches during the first trapping period with aired and non-aired lures were the same. Catches of *T. nigrescens* with the non-aired capsules were higher on the second day; thereafter catches with the two types of lures were otherwise similar throughout. These different patterns of catch for *T. nigrescens* and *P. truncatus* could be explained by their different flight behaviours. The traps were put in place for the first time at 15.30 h, and, as *P. truncatus* has a flight peak at dusk (Tigar et al., 1993; Birkinshaw et al., in press), this would have coincided with the initial flash-off of pheromone from the non-aired lures. However, *T. nigrescens* is a diurnal flyer (Birkinshaw et al., in press) and so the traps were in place too late in the day for there to have been a particularly large catch. Instead a high catch was observed in the following period, presumably of beetles that were moving towards the trap but which earlier failed to reach it as lighting conditions/temperatures became unfavourable.

Pheromone measurement in the laboratory and the trapping results with *P. truncatus* indicate that the flash-off is over within a few hours of exposure of the lure. Compton et al. (1997) suggested that the effect of flash-off lasted over at least the first 3 days since they observed a rapid decline in catch during the first 3 days followed by a much longer period of relative stability. However, the results reported by both Compton et al. (1997) and Tigar et al. (1993) show a consistent oscillation in catch for several days after the start of trapping (Fig. 6), and we have also observed this in more recent trapping studies in Ghana. The results reported here suggest that this phenomenon is not associated with flash-off and warrants further investigation.

For many trapping programmes, pheromone flash-off presents no practical drawbacks. 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 1 day before being placed in traps.

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