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# Effect of Age and Sex on the Response of Walking *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to its Male-Produced Aggregation Pheromone

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**Abstract**—Studies were conducted on the effect of age and sex on the response of *Prostephanus truncatus* (Horn) to its male-produced pheromone using a laboratory walking bioassay. *P. truncatus* adults responded to pheromone from two days of age, but the level of response significantly decreased gradually from 2–10 days of age: newly-emerged, younger beetles seemed to respond more quickly and stay longer in the vicinity of pheromone source than older beetles. There was no significant effect of sex in the level of response to pheromone. The results are discussed in relation to the function of the pheromone: a feeding-based role. The practical implications of the findings on the pest's monitoring using pheromone-baited traps are also highlighted. Copyright © 1996 Elsevier Science Ltd

**Key words**—larger grain borer, *Prostephanus truncatus*, aggregation pheromone, response, walking bioassay

## INTRODUCTION

Since its introduction and subsequent establishment in Africa in the early 1980s, the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), a pest of stored maize and dried cassava, has been studied extensively (Hodges, 1986). A great deal of work on this pest has centered on its control (reviewed in Hodges, 1986; Markham *et al.*, 1991), and monitoring (e.g. Dendy *et al.*, 1989, 1991; Tigar *et al.*, 1993) using traps baited with synthetic analogs of the two-component aggregation pheromone produced by the male (Cork *et al.*, 1991).

Although the pheromone has greatly assisted in the monitoring and detection of *P. truncatus* infestation, almost no information is available about which factors lead to a positive pheromone response. Little is known about the effect of the aggregation pheromone on flight initiation in *P. truncatus*. Flight activity is evident from 4 days of age, reaching a peak at about 8–12 days (Fadamiro, 1995; Fadamiro *et al.*, 1996), but it is not known whether this is due to a lack of response to aggregation pheromone before this time, or due to developmental limitations such as incomplete muscle development, or incomplete cuticle sclerotization (Johnson, 1969).

The biological role of the pheromone is still not clear: is it primarily a sex pheromone, or does it serve to create large aggregations in which individuals enjoy the benefits of group living? The recent report by Smith *et al.* (in press) of a significant reduction in the rate of pheromone

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production by males in the presence of live, conspecific female *P. truncatus*, has by implication suggested a sexual function for the pheromone. If the male-produced pheromone functions mainly as a sex pheromone, it might be expected that females would show a significantly higher level of response than males. Sensitivity to pheromone might also be expected to appear only after the attainment of sexual maturity, and hence would not be expected to be present from the time of emergence. Conversely, if the pheromone serves mainly to bring about aggregation behaviour, sensitivity might be expected to be present from the time of emergence and with no difference in response between the sexes.

This study was therefore conducted in order to shed light on the above proposals and has three main objectives: (i) to determine at what age beetles start to respond to the male-produced pheromone; (ii) to see if the level of pheromone response changes between emergence and 10 days of age; and (iii) to see if any differences in pheromone response exist between the sexes.

## MATERIALS AND METHODS

### *Beetles*

Beetles were cultured at  $29 \pm 0.5^\circ\text{C}$ , 60% relative humidity, under a L12:D12 photoperiod, with no dusk or dawn. Beetles of known age were obtained from cultures at least 30 min before the tests as described in Fadamiro (1995), cleaned, placed in a 9 cm glass Petri dish without food and kept in an incubator at the standard conditions. Sexing of beetles was after Shires and McCarthy (1976).

### *Pheromone*

Pheromone was supplied in polythene vial dispensers ( $20 \times 9 \times 1.5$  mm thick) by the Natural Resources Institute, Chatham, U.K., and upon receipt were stored in a freezer at  $-50^\circ\text{C}$  until needed. The dose used for the experiments was 4 mg based on preliminary tests. Solvent blank vials (control) were also supplied and stored separately. The vials were removed from the freezer at least 15 min before use.

### *Bioassays*

Since flight activity is very rarely displayed by *P. truncatus* before four days of age (Fadamiro, 1995; Fadamiro *et al.*, 1996), a walking bioassay was considered best for this study. Consequently, walking bioassays adapted from White and Birch (1987), were used to assess the response to pheromone.

The test arena consisted of a clean sheet of paper and a plastic ring of diameter 210 mm. The internal surfaces of the ring were coated in flouon to prevent the beetles from climbing out of the arena. In the centre of the paper was a pencil circle of diameter 20 mm, surrounded by another pencil circle of diameter 40 mm (Fig. 1). The bioassay experiments were performed in a fume cupboard at  $28 \pm 2^\circ\text{C}$ , with the extractor fan running continually. Humidity was not controlled, but was in the range of 30–35%.

Twelve beetles of known age were removed from the incubator and placed in the central circle of the arena, and left for 10 min (all timing was by a digital stopwatch, accurate to 0.01 s). At the end of this time, the two beetles nearest to the centre of the arena were removed. If more than two beetles were still in the central circle at the end of this time, the apparatus was left for a further 5 min. At the end of this time, the two beetles nearest the centre were removed. If more than two beetles still remained in the centre, all the beetles were returned to the incubator and a different batch of twelve beetles, of the same age were selected, and the process repeated. This complex selection procedure served two functions. Firstly, to ensure that only active beetles were examined, and secondly, to give the beetles a chance to adjust to the apparatus and disperse from the central circle, before any observations were made.

Having removed two beetles, the pheromone source was introduced into the arena, using a pair of forceps, and stood upright in the central circle. A sheet of glass was placed over the arena, and the behaviour of the beetles observed and recorded for the next 10 min. The outer circle was

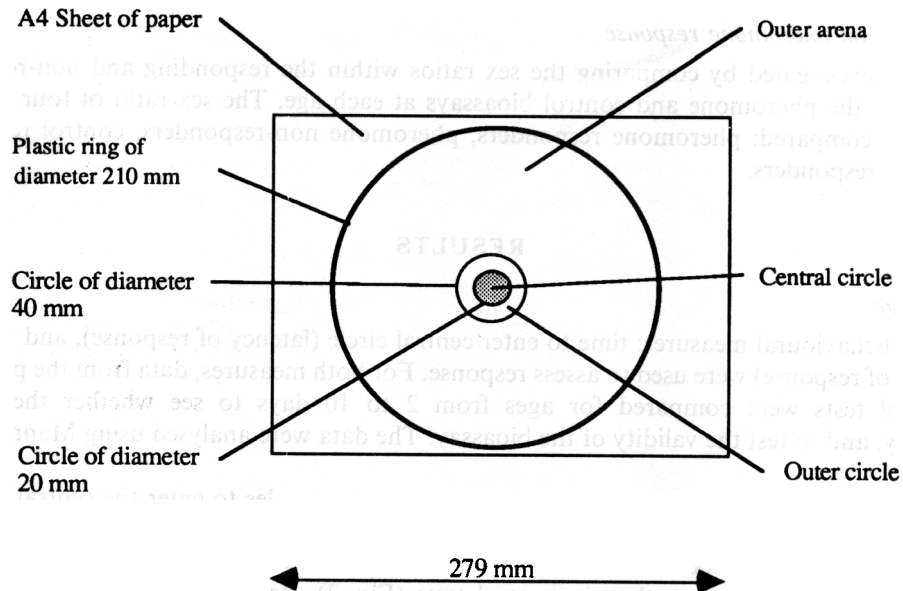


Fig. Diagrammatic representation of the bioassay arena used to test pheromone response in *P. truncatus*.

necessary because preliminary observations had revealed that some beetles exhibited an 'excited' response following exposure to the aggregation pheromone, and would sometimes leave the central circle while still spending most of the time in this section. The outer circle was able to take account of this 'excited' behaviour.

During the 10-min experimental period, observations were made on each of the 10 beetles in the arena. The times when beetles entered and left the outer circle were noted. If a beetle entered the central circle, the time was noted. Beetles within the central circle were timed either until the end of the 10-min period, or until they left the outer circle. Beetles that had entered the central circle and subsequently left the outer circle, were removed and classified as pheromone responders. This practice avoided beetles being counted more than once. Beetles that had entered the central circle and were still within the confines of the outer circle at the end of the 10-min period were also removed and classified as pheromone responders. Times taken to enter the central circle and times spent within the central circle were calculated accordingly. Meanwhile, beetles that had not entered the central circle by the end of the 10-min period, were removed and grouped separately as pheromone non-responders. This group of beetles was given a default time of 600 s before entering the central circle, and 0 s spent within the central circle. The same procedure was used in control tests, except that a blank vial was used in place of a pheromone vial. In the control tests, beetles that entered the central circle and then left the outer circle, or that still remained within the outer circle at the end of the 10-min period, were removed, and classified as control responders. Beetles that had not entered the central circle at the end of the observation period, were removed and grouped together as control non-responders. As before, this group of non-responding beetles was given a default time of 600 s before entering the central circle and 0 s spent in the central circle.

#### *Effect of age on the latency and level of response to pheromone*

This study was based upon observations of 40 experimental beetles in the presence of pheromone, and 10 control beetles, at each age between 2 and 10 days. One-day old beetles were not used in the trials, since owing to a lack of cuticle sclerotization, they were unable to walk. For each set of observations on 10 new beetles, the underlying sheet of paper was changed and the plastic ring, glass sheet, work top and all apparatus were cleaned with alcohol. Separate sets of forceps were used for the pheromone and control tests. During the study, beetles were used in only one set of observations. Observed behavioural measures were: (i) time taken to enter the central circle (latency of response); and (ii) time spent in the central circle (level of response). Short times to enter the central circle and long times spent in the central circle were regarded as a strong response by the beetles to pheromone.

*Effect of sex on pheromone response*

This was investigated by comparing the sex ratios within the responding and non-responding groups, from the pheromone and control bioassays at each age. The sex ratio of four groups of beetles were compared: pheromone responders, pheromone non-responders, control responders, control non-responders.

**RESULTS***Effect of age*

The two behavioural measures: time to enter central circle (latency of response), and time spent there (level of response) were used to assess response. For both measures, data from the pheromone and control tests were compared for ages from 2 to 10 days to see whether they differed significantly, and to test the validity of the bioassay. The data were analysed using Mann-Whitney tests.

At all ages studied, excluding six days, the time taken for beetles to enter the central circle was significantly ( $P < 0.05$ ) less in the pheromone tests, than in the control test (Fig. 2). Similarly, at all ages studied, the time spent by beetles in the central circle, was significantly ( $P < 0.05$ ) longer when pheromone was present, than in control tests (Fig. 3). Both results showing a differential response to pheromone and control sources suggest that the bioassay gave a reliable estimate of response to pheromone.

Using the two measures as above, the data on the effect of age on response to pheromone were square-root transformed and analysed using the General Linear Model (GLM) procedure (SAS Institute, 1985). Analysis of variance showed a just significant effect of age on the time taken to enter the pheromone source in the central circle ( $F_{8,351} = 1.97$ ,  $P = 0.049$ ). A linear regression of age against time taken to enter the center was, however, not significant ( $F_{1,358} = 0.36$ ,  $P = 0.55$ ). The raw data nevertheless suggest that time taken to reach the pheromone source increases with age, and the non-significant regression may have been due to the high mean value recorded for two-day old beetles (Fig. 2).

Similarly, age had a significant effect on the time spent in the central circle in the presence of

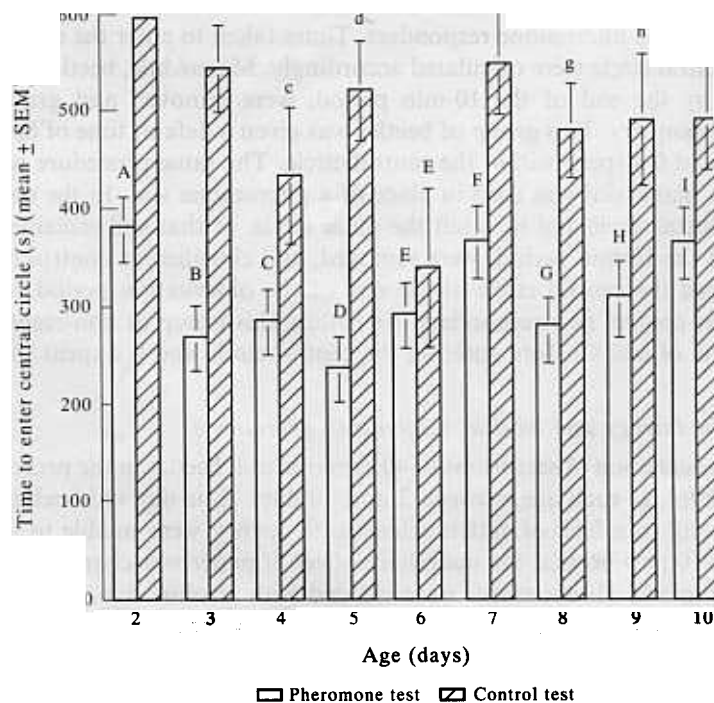


Fig. 2. Behavioural response of *P. truncatus* to pheromone and control sources: latency of response. Columns with the same letter, but in different cases differ significantly ( $P < 0.05$ , Mann-Whitney test).

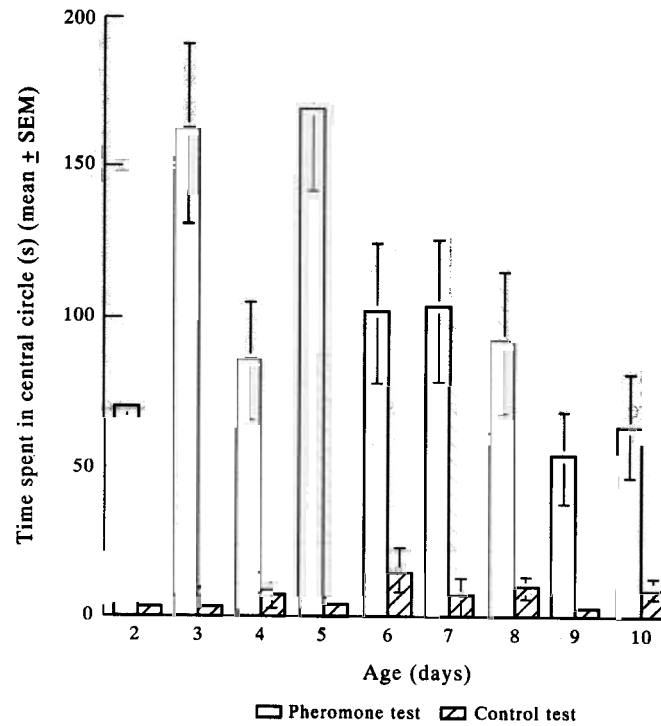


Fig. 3. Behavioural response of *P. truncatus* to pheromone and control sources: time spent in the vicinity of source. Significant differences were recorded between pheromone and control tests at all ages ( $P < 0.05$ , Mann-Whitney test).

pheromone ( $F_{8,351} = 2.62$ ,  $P = 0.009$ ). Time spent by beetles in the central circle decreased with increasing age. On regression, a significant, negative linear relationship was recorded between age and time spent in the central circle ( $F_{1,358} = 5.39$ ,  $P = 0.02$ ) (Fig. 4). The data again demonstrated the particularly low mean value recorded for two-day old beetles.

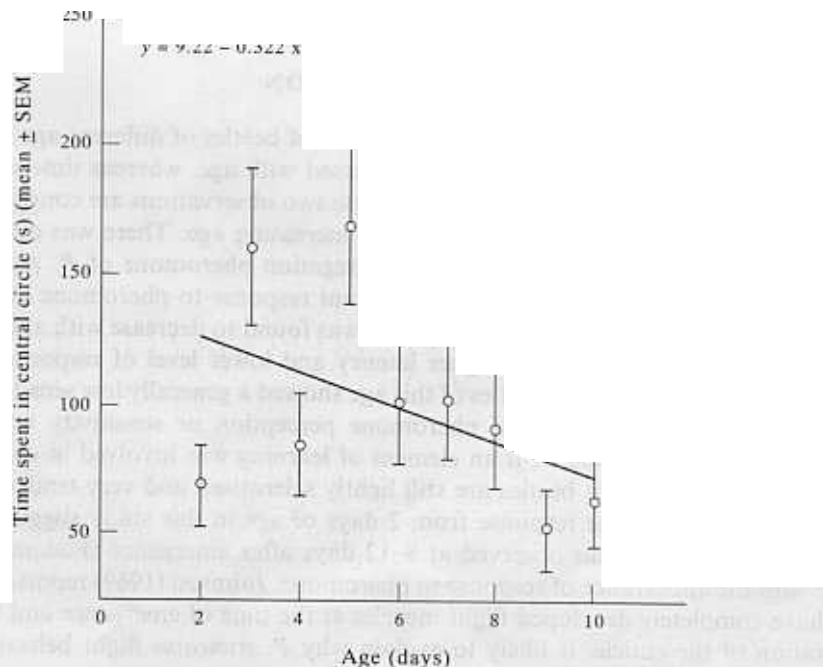


Fig. 4. Effect of age on time spent by *P. truncatus* in the vicinity of pheromone. Significant linear regression ( $P < 0.05$ ).

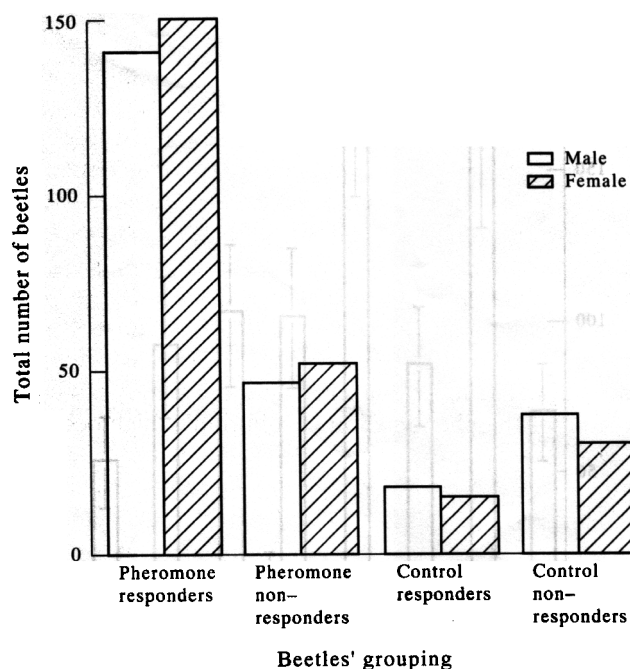


Fig. 5. Effect of sex on the response behaviour of *P. truncatus*. The ratio of male and female in the four groups of beetles did not deviate from 1:1 ( $P > 0.05$ ).

#### Effect of sex

Shires (1979) recorded a 1:1 sex ratio (male:female) for adult *P. truncatus*. However, the sex ratio recorded in the present study was 1.16:1 (male:female). A correction was therefore made for this sex ratio bias, and the data for the four groups of beetles were examined using a chi-squared goodness-of-fit test, testing the null hypothesis (Parker, 1979).

No significant effect of sex was recorded among the four groups of beetles. The sex ratio of beetles from the four groups did not deviate from unity (1:1):  $\chi^2 = 0.22$ ,  $P > 0.5$ , for pheromone responders,  $\chi^2 = 0.16$ ,  $P > 0.5$ , for pheromone non-responders,  $\chi^2 = 0.13$ ,  $P > 0.5$ , for control responders, and  $\chi^2 = 0.97$ ,  $P > 0.1$ , for control non-responders (Fig. 5).

## DISCUSSION

The results demonstrate the differential responses of beetles of different age to pheromone. The time taken to reach the pheromone source increased with age, whereas time spent in the vicinity of the pheromone source decreased with age. These two observations are consistent with an overall reduction in beetle response to pheromone with increasing age. There was no difference between the sexes in response to the male-produced aggregation pheromone of *P. truncatus*.

Although adult *P. truncatus* showed a significant response to pheromone at all ages between 2 and 10 days, the level of response to pheromone was found to decrease with age during this period. The observations of a particularly higher latency and lower level of response to pheromone by two-day old beetles suggest that beetles of this age showed a generally low sensitivity to pheromone. This low sensitivity might occur if pheromone perception or sensitivity was dependent upon complete cuticle sclerotisation or if an element of learning was involved in orientation behaviour to pheromone. Two-day old beetles are still lightly sclerotised and very tender (Fadamiro, 1995). The presence of pheromone response from 2 days of age in this study suggests that the peak of flight activity in *P. truncatus* observed at 8–12 days after emergence (Fadamiro, 1995) does not coincide with the appearance of response to pheromone. Johnson (1969) reported that many insects do not have completely developed flight muscles at the time of emergence and this, or incomplete sclerotization of the cuticle, is likely to explain why *P. truncatus* flight behaviour peaks at 8–12 days, rather than earlier.

The decrease in sensitivity to aggregation pheromone with increasing age has also been recorded

for many other beetle species. Walgenbach and Burkholder (1986) found that adult maize weevils, *Sitophilus zeamais* Motschulsky, up to one-week old showed a significant response to pheromone, weevils from 2 to 6 weeks old showed no response to pheromone, and weevils from 8 to 10 weeks were repelled significantly by pheromone. Similar observations regarding pheromone response were also made for the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Pierce *et al.*, 1983).

The presence of pheromone sensitivity from the time of emergence, together with the absence of differences between the sexes, suggests that the aggregation pheromone of *P. truncatus* is associated with feeding behaviour. The existence of a feeding-based aggregation pheromone has been reported for many other stored product beetles: e.g. *Carpophilus hemipterus* (L.) (Bartelt *et al.*, 1990), *Sitophilus* spp (Walgenbach and Burkholder, 1986) and *Rhyzopertha dominica* (Fabricius) (Obeng-Ofori and Coaker, 1990). Aggregation pheromones associated with feeding can be beneficial in a number of ways. The individuals involved may enjoy a reduced likelihood of falling victim to predation, because of the phenomenon of 'dilution' which occurs in large groups (Krebs and Davies, 1993). In some cases, aggregation may also confer a specific feeding advantage. In the case of *P. truncatus*, a high level of response to pheromone by the newly-emerged beetles may be very beneficial in reducing the cost of initiating attack on a new food resource.

The decrease in pheromone response observed in *P. truncatus* and these other beetle species could be explained by changes in the importance of particular behaviours with increasing age. For example, although some beetle species such as those of the family Anobiidae do not feed as adults prior to reproduction, members of the families Bostrichidae and Curculionidae do (Burkholder, 1982; Burkholder and Ma, 1985). Thus, in the case of *P. truncatus* feeding is of paramount importance after emergence in order to prepare for reproduction. A high sensitivity to feeding-based aggregation pheromone would be expected at this time. Subsequently though, as reproduction becomes the behaviour of primary importance, a relative decrease in pheromone sensitivity might be expected. An additional benefit of aggregation may however be the increased mating chances of individuals.

Walgenbach and Burkholder (1986) recorded an increase in sensitivity of adult *S. zeamais* to pheromone under conditions of food shortage. Pike (1993) demonstrated that *P. truncatus* in suitable food are not attracted to pheromone and Fadamiro (1995) proposed that dispersing *P. truncatus* are mostly young pre-reproductive and reproductively active males and females seeking new resources to colonise. Since the aggregation pheromone appears to be concerned with dispersal to new food resources (Fadamiro, 1995), and in view of the increased flight activity of *P. truncatus* under conditions of crowding and poor food quality (Fadamiro *et al.*, 1996), we might also expect to see this increase in pheromone sensitivity in *P. truncatus* at times of food shortage. More evidence for the feeding-based role of aggregation pheromone in *P. truncatus* could be gained by studying the effects of food volatiles, starvation and crowding on pheromone response at different ages.

These findings may have important implications for the use of pheromone in field monitoring of populations of *P. truncatus*. Given the reduction in pheromone sensitivity with age indicated in this study, we can predict that beetles caught in pheromone traps in maize stores, or in the field, are likely to be comparatively young. Males and females are likely to be captured in similar proportions. These results also indicate however that pheromone trapping alone may not give a true estimate of the population densities of *P. truncatus* present in maize stores, but only of actively dispersing beetles (Fadamiro, 1995), since older beetles may tend to go undetected.

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