Physiological Entomology (1996) **21**, 179–187

Factors influencing response of flying *Prostephanus truncatus* to its male-produced aggregation pheromone

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Abstract. The effects of age, sex, mating status, pre-exposure to pheromone, resource (diet) quality, and starvation on the response of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to its male-produced aggregation pheromone were investigated. Age was an important factor determining the level of response to pheromone: young adults were more responsive than older adults. The suitability of the resource in which *P.truncatus* is present affected its dispersal tendency, but not its sensitivity to pheromone *per se*. Sex, mating status, brief prior exposure to pheromone, and starvation did not influence the response of the larger grain borer to pheromone. The orientation behaviour of the beetle is described, and it is proposed that the pheromone primarily functions as a 'suitable resource location and colonization signal'.

Key words. *Prostephanus truncatus*, larger grain borer, Coleoptera, Bostrichidae, pheromone, flight, response, wind tunnel.

Introduction

The identification and chemical synthesis of pheromones has revolutionized the monitoring of insect pest populations and many stored products pest species are now being monitored by commercially available pheromone trapping systems (Burkholder & Ma, 1985). However, for a pheromone to be used successfully, and with optimum efficiency, in monitoring a species, the intrinsic and extrinsic factors that can influence the response of the species to its pheromone need to be investigated, because differential response will lead to variable catch data (Walgenbach & Burkholder, 1986). This information is also crucial to confident interpretation of laboratory bioassays (Pierce *et al.*, 1983).

Factors that influence the physiological state of insects, such as age, mating status, diet quality, and feeding status, are known to affect production of (for example, Mayhew & Phillips, 1994), as well as response to (e.g. Borden, 1977; Pierce *et al.*, 1983; Walgenbach & Burkholder, 1986; White, 1989; Obeng-Ofori & Coaker, 1990) pheromone in insects.

The male-produced aggregation pheromone of *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) is currently employed in the monitoring of this storage pest, particularly in Africa, where it has continued to spread since its introduction from Central America in the late 1970s (Hodges, 1994). However, it is not known which beetles in a population are attracted to the pheromone and what factors influence their response, making the variable trap catches recorded in the field difficult to interpret. The absence of information concerning the factors influencing the response of the beetle to pheromone is probably because, to date, no detailed laboratory flight bioassays have been conducted on its response behaviour: a walking bioassay was used to assess the beetle's attraction to synthetic pheromone (Cork *et al.*, 1991). Furthermore, the biological function(s) of the 'aggregation' pheromone is/are still not clear: the report that male *P.truncatus* stops pheromone production in the presence of a female, or chemical residues produced by the female (Smith *et al.*, 1996), has by implication suggested a sexual function for the pheromone.

Pike (1993) observed in Mexico that once present in a suitable food source, even at relatively high population density, *P.truncatus* are not attracted from the food into nearby pheromone-baited traps. However, it is not clear whether this change in behavioural response is due to their reduced tendency to disperse from a suitable resource (Fadamiro *et al.*, 1996), or rather due to other changes in these beetles, such as changes in the responsiveness to pheromone at peripheral or CNS levels.

This paper reports on the investigations carried out on the influence of age, sex, mating, pre-exposure to pheromone, resource quality and starvation state on the response of *P.truncatus* to pheromone.

Materials and Methods

Insects. A Tanzanian strain of *P.truncatus* reared on whole, clean maize at 30 ± 1°C and 65 ± 5% r.h. under a LD 12:12 h photocycle with no dusk or dawn was used in this study. Beetles of known age were collected from cultures established on milled grain that passed through an Endecotts sieve of mesh size

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3.35 mm to ease removal of beetles from culture (Fadamiro, 1995), and were checked for all parts before use in the bioassays. Sexing of beetles was as described by Shires & McCarthy (1976).

Wind tunnel. The wind tunnel was a clear, rectangular, glass, flight tunnel 160 cm long and 75 cm high and wide with moveable visual floor patterns (Fadamiro, 1995). Experiments were carried out at 29 ± 1°C, 30 ± 5% r.h., 20 ± 1 cm/s wind speed, at 10–12 h of the photophase under a light intensity of 3700 lux except where otherwise stated.

Pheromone. The male-produced aggregation pheromone of *P.truncatus* consists of two components together known as 'Trunc-call'. The major and minor components were identified as 1-methylethyl(2*E*)-2-methyl-2-pentenoate (Trunc-call 1 or T1), and 1-methylethyl(2*E*,4*E*)-2,4-dimethyl-2,4-heptadienoate (Trunc-call 2 or T2) respectively (Cork *et al.*, 1991; D. Hall, personal communication). The pheromone (T1:T2 in the ratio of 1:1) and solvent blank control used in this study were supplied by the Natural Resources Institute, Chatham, U.K. (NRI) in polythene vial dispensers (20 × 9 × 1.5 mm thick). Both were stored separately at –50°C before use. For the tests, a 0.2 mg dose of the pheromone was used, being the dose that gave the best attraction in the wind tunnel (Fadamiro, 1995).

Effects of age and pre-exposure. In this experiment, the effect of beetle age from 5 to 40 days old on their response to pheromone was tested at 5-day intervals. Three-day-old beetles were collected *en masse* from cultures started on kibbled maize grains as described in Fadamiro (1995), and were randomly separated into eight groups each containing about 200 beetles. Each group of beetles was placed in a 9 cm glass petri-dish with food. In this and subsequent experiments, the food consisted of a mixture of whole maize, kibbled maize, and maize flour approximately in ratio 1:1:1, except otherwise stated. At the appropriate age, twenty beetles from each age group were randomly selected and released in the presence of 0.2 mg of pheromone in the wind tunnel. The numbers of beetles taking flight, orienting to (defined as a directional upwind flight at the level of the pheromone plume and to within 10 cm distance of the source), and landing on, the pheromone source were recorded for 10 min in this and the subsequent experiments (except where otherwise stated). After this 10 min observation period all twenty beetles were removed from the wind tunnel and held in another 9 cm petri-dish without food. 15 min later, these same beetles were again released in the wind tunnel in the presence of a fresh 0.2 mg pheromone vial. The flight response parameters were again observed for 10 min, recorded, and compared with those of the 'first exposure' to give an indication of the possible effect of prior exposure of beetles to pheromone on their subsequent response. In a control experiment carried out on the same day, twenty beetles were selected from the petri-dish and tested for flight in the presence of a solvent blank vial in the wind tunnel. The same parameters were observed and recorded for 10 min. On the appropriate days, 5-, 10-, 15-, 20-, 25-, 30-, 35- and 40-day-old beetles were bioassayed in this way. Both the pheromone and control experiments were replicated four times for each age class.

Effect of sex. This experiment was designed to test for any difference between the proportions of male and female beetles attracted to pheromone, and also to check for any difference in the latency (time taken) of response between the sexes.

Beetles aged 7–15 days old were first pre-selected in the wind tunnel for flight activity as descibed in Fadamiro (1995) to reduce the labour of sexing larger numbers of beetles many of which would not fly or orient, and then sexed. Five pairs of males and females were then randomly chosen and released in the wind tunnel in the presence of pheromone. An exposure period of 10 min was used. After the first 5 min had elapsed, the beetles that landed on the pheromone source were removed, placed in a marked petri-dish and sexed later at the end of the test. At the end of the 10 min exposure period, the beetles landing on the pheromone source, those landing elsewhere, and those remaining on the release platform (i.e. the beetles that did not take flight) were collected and sexed. The total number of beetles of each sex that landed on pheromone source was calculated as the sum of beetles of each sex that landed on pheromone source after 5 min, and those that landed at the end of the experiment (10 min). The experiment was replicated ten times using each beetle only once.

Effect of mating. To investigate the effect of mating on flight activity and response to pheromone, unmated newly-emerged *P.truncatus* (aged 2 days old) were carefully sieved from cultures set up for this purpose. Observations had shown that the beetles do not display courtship behaviour during the first 3 days after emergence (Fadamiro, 1995). Isolating the beetles at the pupal stage could impair on their flight activity as adults since the removal of freshly-emerged day-old beetles from culture sometimes reduced their flight activity at a later stage (Fadamiro, 1995). There were four treatment levels in this experiment: unmated females, unmated males, mated females, and mated males.

The 2-day-old beetles were sexed and about fifty members of each sex were placed separately in a 9 cm petri-dish with food. For the mated treatments, twenty-five pairs of beetles (male and female) were placed together in a 9 cm petri-dish. The petri-dishes were marked accordingly and kept at the standard culturing conditions for 10 days. When the beetles were at least 12 days old, twenty beetles from each group were selected and released together in the wind tunnel first without pheromone, and at least 10 min later in the presence of pheromone (for the mated beetles, twenty beetles of each sex were released in the wind tunnel). There were four replicates for each of the four groups of beetles and the order of observations was randomized.

Effect of resource quality. In this experiment, the effect of the quality of the food medium in which *P.truncatus* is present was investigated in relation to its readiness to respond to pheromone. Based on a previous study on flight behaviour (Fadamiro *et al.*, 1996), the hypothesis tested here was that beetles in a food medium of lower quality (food quality is defined in terms of reproductive success) would more readily respond to pheromone than those in a suitable food resource.

The resource quality treatments in this experiment were whole maize, kibbled maize, maize flour, and no-food. The major quality difference in these food types is that whole maize was superior to maize flour as rearing media for the beetle (Fadamiro, 1995). Twenty beetles aged 7–15 days were randomly chosen and placed in a 9 cm petri-dish with lid containing about 50 ml of each food treatment (except for the no-food treatment). Each treatment was observed separately in the wind tunnel in presence of pheromone. The numbers of beetles taking flight, and responding (orienting) to pheromone were compared for the treatments during a 10 min observation period. There were four replicates per treatment and the order of observation was randomized.

Effect of starvation. The effect of three levels of starvation on response of *P.truncatus* to pheromone was studied: the groups of beetles compared were unstarved beetles (i.e. not more than 2 h of being starved), beetles starved for 1 day, and those starved for 2 days.

Eleven-day-old beetles were removed from a culture and randomly allocated to three groups each containing about 150 beetles. Beetles in each of two randomly chosen groups were placed in a 9 cm petri-dish containing about 100 ml of food. The beetles in the third group were placed in a similar petri-dish but without food. The petri-dishes were then marked accordingly one for each of the three groups and placed in an incubator at the standard culturing conditions for 2 days. 24 h later (i.e. on the second day), the beetles in the petri-dish marked for 1-day starvation level were removed, and placed in a similar petri-dish but without food. 48 h after start of the experiment the beetles in the three petri-dishes were removed and held separately in petri-dishes without food. After 2 h of removal from culture, twenty beetles (ten pairs) from each group were randomly selected and released in turn in the presence of pheromone in the wind tunnel. The parameters observed during a 10 min observation period were as for the first experiment above (effects of age and pre-exposure). There were six replicates per group and all observations were randomized.

Statistical analysis. Data from all the experiments (except those from the effect of sex) were analysed using the GLM procedure in SAS Institute (1985). Data on take-off were expressed as a proportion of the numbers of beetles released, whereas those on orienting to, and landing on, a pheromone source were expressed as a proportion of the 'fliers' (fliers were the beetles that took flight), or as a proportion of the number of released beetles (as appropriate), arcsine-square root transformed (arcsine-square root transformation was chosen based on the results of the descriptive test) and subjected to weighted (by *n*) analysis of variance. This approach took into account the effect of unequal number of observations (*n*) (i.e. unequal number of 'fliers' within the treatments). The Tukey test was used to compare means.

Chi-square (χ^2) was used to analyse the data on the effect of sex on response to pheromone. Goodness of fit tests were used for the raw data on the sex ratios of the beetles that landed on pheromone source, both after the first 5 min, and also the total after the 10 min observation period (Parker, 1979).

Results

Description of oriented flight behaviour

When placed on the release platform in the wind tunnel in the presence of pheromone, a responding *P.truncatus* is generally characterized by a very short period (<1 min) of immobility (similar to being feign-dead) especially if on its back. This is followed by a short period of activation or excitation on the release platform in which the beetle raises its head and thorax, vibrating its antennae, fanning its wings and sometimes making short flights from one part of the platform to another. No directional response, however, occurs at this stage. This stage is usually followed by initiation of directed locomotory activity in form of take-off.

Beetles taking-off from the release platform, in the presence of pheromone plume, consistently displayed three major kinds of behaviour: (1) A small proportion (varying with pheromone concentration) exhibited phototaxis (flew upward towards the overhead lights), either throughout the observation period or for some part of it: some beetles did fly toward, and alighted on, the downwind screen having first flown upward towards light. (2) A very small proportion (about 5%) of 'fliers' took-off straight downwind, landing on the downwind screen. A few of the beetles that landed on the downwind screen in this group, and those from class 1 above, later resumed flight upwind, oriented towards the odour source, and eventually landed on it. These beetles were classed as 'responders' (see class 3 below). (3) A greater proportion (about 80%) of 'fliers' took-off directly upwind from the release platform, flying at the level of pheromone plume and were classed as 'responders'.

After taking-off, a responder usually headed slowly upwind at the level of the pheromone plume turning periodically as it flew along. Nearer to the source (*c.* ≤10 cm from the pheromone source), the beetle reduced its flight speed, hovered for some seconds (characterized by side-to-side movements with zero net displacement upwind), and eventually made an upwind 'surge' that took it to the source, landing either on the platform holding the pheromone dispenser or in most cases, directly on the pheromone vial. On the pheromone source, the beetle walked round with the antennae raised. In very few cases courtship behaviour was observed among beetles on the pheromone source. Most beetles remained on the source till observation was completed.

In the absence of pheromone, flight was erratic and preponderantly phototactic. No oriented upwind flight was recorded to the solvent blank control.

Effect of pre-exposure of beetles to pheromone

Data on the effect of pre-exposure of beetles to pheromone were pooled for all age groups since no interactions were recorded between age and exposure to pheromone.

There was no significant effect of pre-exposure of beetles to pheromone on their behaviour when subsequently re-exposed. First, the proportions of beetles taking flight were not different for both first and second exposures ($F_{1,\,24} = 3.29$, $P = 0.08$) (Fig. 1). Similarly, no differences were recorded between data collected from the two exposures on proportions orienting to ($F_{1,\,18} = 0.18$, $P = 0.68$), and landing on ($F_{1,\,18} = 1.03$, $P = 0.32$), the pheromone source (Fig. 1).

Effect of age

A significant effect of age was recorded on the flight activity of beetles with or without exposure to pheromone. The proportions taking flight were significantly higher for beetles aged 10 and 15 days old than for those aged 5, 35 or 40 days old ($F_{7,\,24} = 5.19$, $P < 0.001$, data collected when beetles were not

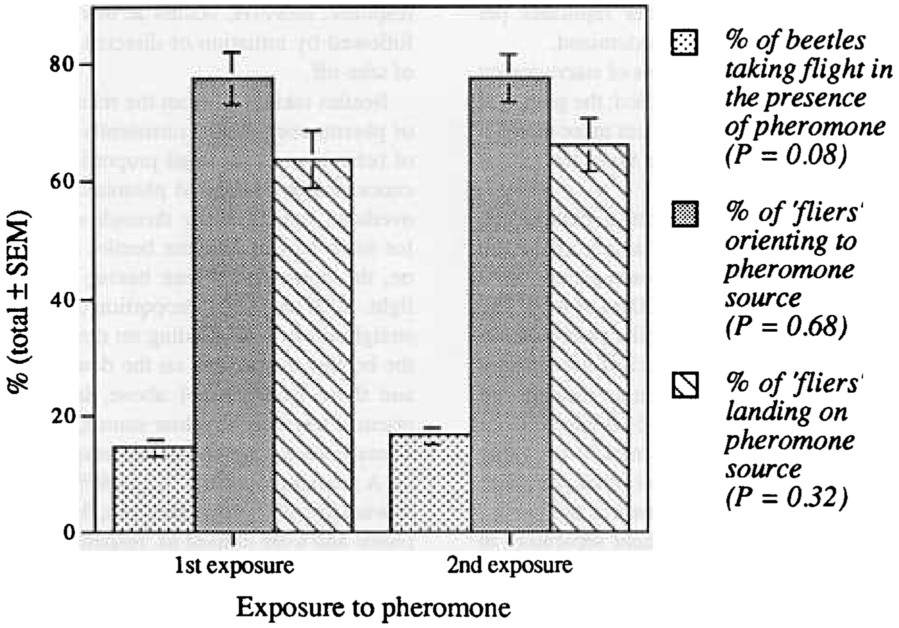

Fig. 1. Effect of pre-exposure of *P.truncatus* to pheromone on its response during subsequent re-exposure. Figure shows mean flight activity and response to pheromone during first and second exposures to 0.2 mg of pheromone. The period between the first and second exposure was 15 min. None of the observed parameters was significant (at $P < 0.05$).

exposed to pheromone). Since prior exposure of the beetles to pheromone did not significantly influence their probability to take-off when re-exposed (Fig. 1), data obtained from first and second exposures to pheromone were pooled. The result also showed a significant effect of age on flight initiation in the presence of pheromone ($F_{7,24} = 7.49$, $P < 0.0001$) (Fig. 2). No significant interaction on flight initiation was recorded between age and the effect of pre-exposure of beetles to pheromone ($F_{7,24} = 0.89$, $P = 0.53$).

Because beetles previously exposed to pheromone did not show any difference in their response behaviour when re-exposed (Fig. 1), data from both exposure times (first and second exposure) on the proportions of beetles orienting to, and landing on, the pheromone source were pooled. A significant difference was

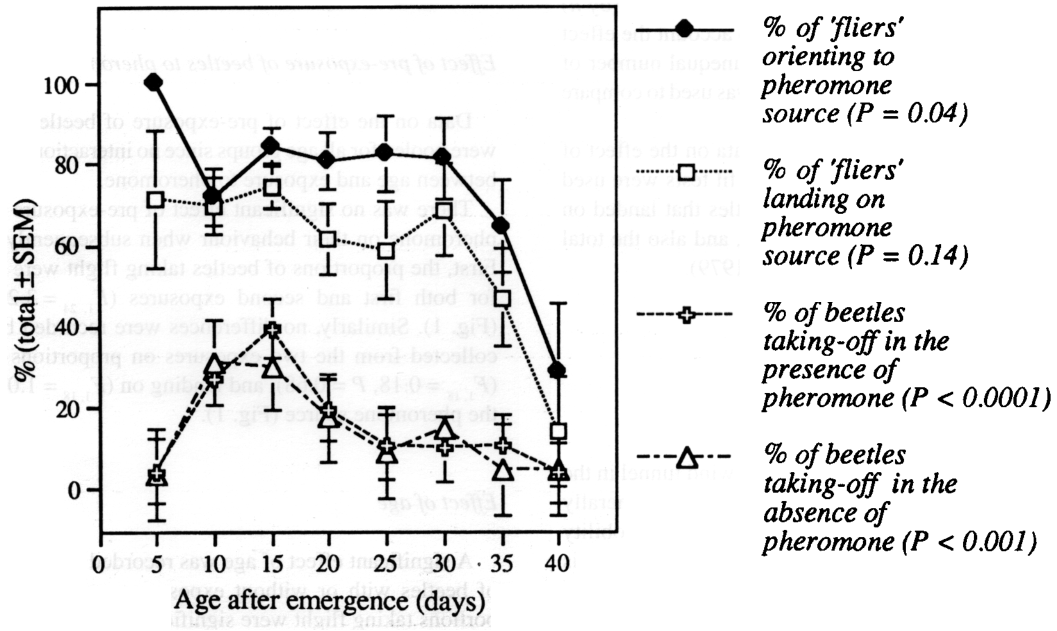

Fig. 2. Effect of age on the flight activity and response of *P.truncatus* to pheromone. The difference in response to pheromone (i.e. per cent of fliers orienting to pheromone) was only significant between 5- and 35–40-day-old beetles (at $P < 0.05$).

recorded for the proportion of fliers orienting to pheromone: a significantly higher proportion (100%) of 5-day-old 'fliers' oriented to the pheromone source than older 35- or 40-day-old 'fliers' (65% and 29% respectively, $F_{7,18} = 2.71$, $P = 0.04$, see Fig. 2). Although there was a trend of decrease in the proportion of beetles landing on the pheromone source with increase in age, this was not significant ($F_{7,18} = 1.86$, $P = 0.14$; see Fig. 2). No interactions were recorded between age and whether or not beetles were pre-exposed to pheromone ($F_{7,18} = 0.85$, $P = 0.56$, data on proportion orienting; and $F_{7,18} = 1.72$, $P = 0.17$, data on proportion landing on pheromone).

Effect of sex

Approximately equal numbers of each sex landed on the pheromone source both after the first 5 min ($\chi^2 = 0.69$, $P > 0.1$), and at the end of the 10 min observation period ($\chi^2 = 0.15$, $P > 0.5$). Similarly, no association was recorded between sex and response behaviour of the beetles ($\chi^2 = 1.63$, $P > 0.1$): there were no sex differences in the flight behaviour of beetles (Table 1).

Effect of mating

The data obtained from the mating experiment were compared between the four treatments: unmated females, unmated males, mated females, and mated males. No significant differences were recorded for flight activity without pheromone ($F_{3,12} = 2.35$, $P = 0.12$), or in the presence of pheromone ($F_{3,12} = 0.10$, $P = 0.96$) between beetles from the four groups. Similarly, there were no differences between the response of fliers in the four groups, to pheromone: the proportions of fliers orienting to ($F_{3,12} = 0.93$, $P = 0.46$), and landing on ($F_{3,12} = 0.25$, $P = 0.86$), pheromone were equal in the four groups (Table 2).

Effect of resource quality

Resource quality exerted a significant effect on flight activity of *P.truncatus* ($F_{3,20} = 78.41$, $P < 0.0001$) (Fig. 3) There was also a significant difference ($F_{3,20} = 32.93$, $P < 0.0001$) in the proportion of beetles placed in each resource type, which responded to pheromone (i.e. number of beetles responding to pheromone/n, where n = total number of beetles released per resource type). Fig. 3 shows that both this proportion and that of flight activity were lowest in the whole maize treatment. The apparent difference in the proportion of beetles responding to pheromone is explained by difference in numbers of take-off. In fact the proportion of those taking-off which responded to pheromone was constant across treatments ($F_{3,20} = 0.15$, $P = 0.93$, see Fig. 3).

The overall result shows that the recorded difference in response to pheromone was linked to differences in the flight activity of beetles present in the four resource types.

Effect of starvation

There was a significant effect of starvation on flight activity of beetles in the absence of pheromone ($F_{2,9} = 9.17$, $P = 0.007$) with the greatest flight activity recorded for unstarved beetles (Fig. 4). However, this was not significant in the presence of

Table 1. Response of *P.truncatus* to pheromone in relation to sex.

	Sex		
Observed parameters	Female	Male	*P* value
No. of beetles landing on pheromone source after first 5 min	21	15	>0.1
Total number of beetles landing on pheromone source after 10 min	32	28	>0.5
No. of beetles landing elsewhere in the tunnel after 10 min	7	12	>0.1
No. of beetles remaining on release platform after 10 min	11	10	>0.1

Table shows the sex distribution of beetles exhibiting the various observed parameters.

Table 2. Effect of mating on the flight activity and response of *P.truncatus* to pheromone.

	Sexual grouping of beetles				
Observed parameters	Unmated females	Unmated males	Mated females	Mated males	*P* value
Per cent take-off in the presence of solvent vial	38.8 ± 4.3	35.0 ± 2.9	28.8 ± 3.1	26.3 ± 4.3	0.12
Per cent take-off in the presence of pheromone	40.0 ± 5.4	36.3 ± 3.7	38.8 ± 4.3	37.5 ± 7.2	0.96
Per cent of fliers orienting to pheromone	78.9 ± 8.4	75.7 ± 3.5	74.9 ± 3.0	67.5 ± 9.5	0.46
Per cent of fliers landing on pheromone source	65.8 ± 7.5	62.8 ± 2.6	62.0 ± 9.8	60.0 ± 8.2	0.86

Table shows mean (± SEM) per cent flight activity and response of fliers to pheromone. None of the parameters was significant between the four groups (at $P < 0.05$).

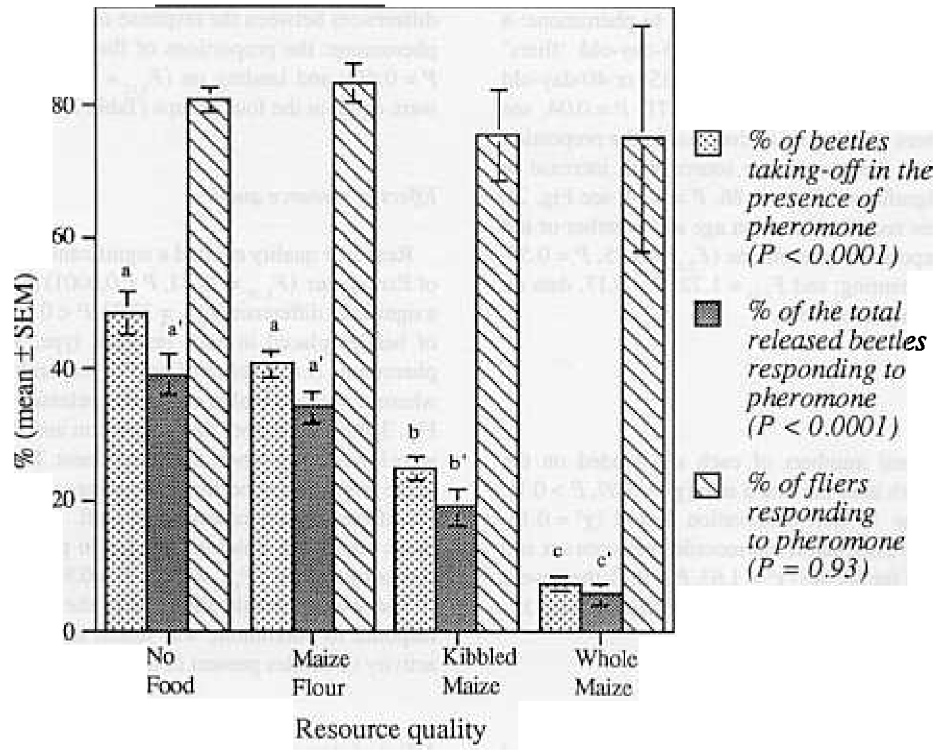

Fig. 3. Effect of resource quality on the flight activity and response of *P.truncatus* to pheromone. Figure shows mean percentage of beetles flying or responding and those of fliers responding to pheromone. No significant difference was recorded between the response of fliers in the different resource treatments to pheromone. The significant difference ($P < 0.05$) between the proportion of the total released beetles in the different treatments, responding to pheromone is related to differences in their flight activity.

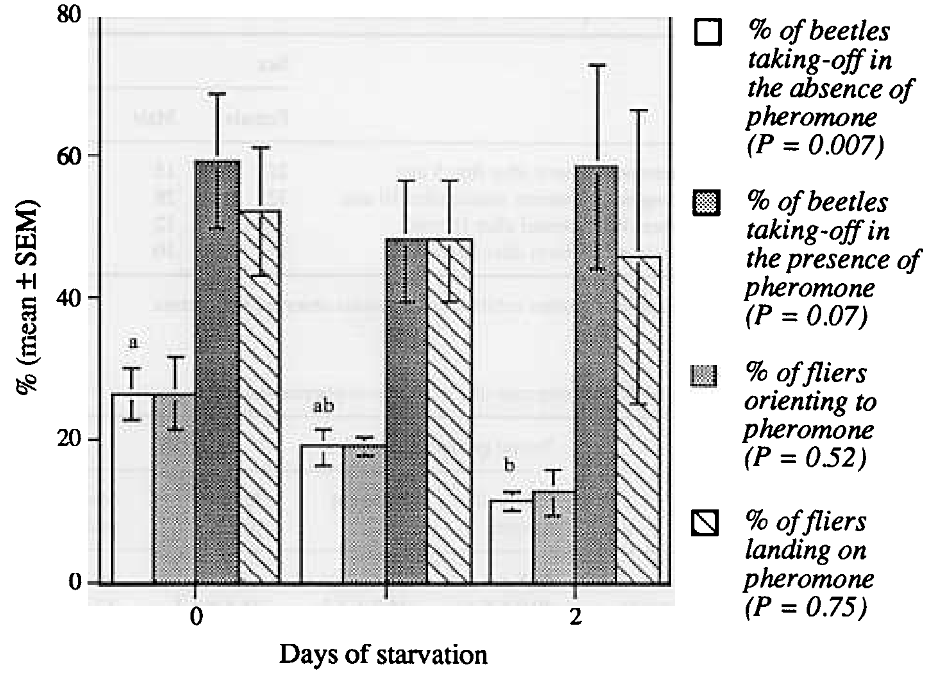

Fig. 4. Effect of starvation on response of *P.truncatus* to pheromone. Starvation did not affect response to pheromone but did affect flight take-off ($P < 0.05$).

pheromone ($F_{2,9} = 3.74$, $P = 0.07$). No significant differences were recorded for proportions of the released beetles orienting to ($F_{2,9} = 3.46$, $P = 0.08$), and landing on ($F_{2,9} = 3.06$, $P = 0.1$), pheromone. Also, comparing the fliers in each treatment, no significant differences were recorded for proportions orienting to ($F_{2,9} = 0.70$, $P = 0.52$), and landing on ($F_{2,9} = 0.29$, $P = 0.75$), the pheromone source (Fig. 4).

Overall, there was a significant effect of starvation on flight activity but not on the response of fliers to pheromone.

Discussion

The results of these studies show that age is a major factor that determines the level of response of *P.truncatus* adults to their aggregation pheromone. The suitability of the resource in which the larger grain borer is present affects its tendency to disperse, but not its behavioural sensitivity to pheromone, *per se*. The sex, mating status, brief pre-exposure to pheromone, and starvation states of individual beetles do not influence their response to pheromone. The aggregation pheromone is proposed to function primarily as a 'suitable resource location and colonization signal'.

Pre-exposure to pheromone

In this study, prior exposure of beetles to pheromone was found to have no effect on their response behaviour when subsequently re-exposed to the same dose of pheromone. This suggests that the beetles were neither sensitized nor habituated at the behavioural level, by a brief exposure to pheromone at the dose tested in this experiment.

Many authors have reported decreases in the response of insects to chemical odours following pre-exposure (e.g. Trayner, 1970; Bartelt & Lawrence, 1973). However, direct comparisons cannot be made between these studies and the present because different pre-exposure durations, as well as pheromone doses, were used. Habituation is an all-or-nothing phenomenon, which will only occur when the concentration of the stimulus evoking it is above the threshold level for response (Trayner, 1970). Although the pheromone dose used in the current study was above the threshold for response, it is possible that it was below the threshold level for initiation of habituation. Further studies on pre-exposure to different doses of the pheromone will shed light on the above speculation.

Age

Significant effects of age were recorded both for flight activity, and response to pheromone. Fadamiro *et al.* (1996) report on the effect of age on flight activity of *P.truncatus*. In the current study, the proportion of 5-day-old fliers responding to pheromone was higher than of older, 35–40-day-old fliers. 5-day-old beetles were used as the starting point in the current bioassay since newly-emerged (<4 days old) beetles do not normally fly (Fadamiro *et al.*, 1996). However, this does not indicate their lack of tendency (or inability) to respond to pheromone. A laboratory walking bioassay has shown that both sexes of *P.truncatus* commence response to pheromone as early as when 2 days old, and in fact, in a comparative study between 2- and 10-day-old beetles, the level of response to pheromone was found to gradually decrease slightly with age (Boughton & Fadamiro, 1996). This is in broad agreement with the results of the current flight studies in which a gradual general decrease in response to pheromone was recorded with age, becoming significant in 35-day-old beetles.

The decrease in the response of *P.truncatus* to pheromone with increasing age, recorded in this study, has also been reported for other insects especially long-lived beetles with male-produced aggregation pheromone (see for example: Pierce *et al.*, 1983; Walgenbach & Burkholder, 1986; White, 1989; Obeng-Ofori & Coaker, 1990), although most of these studies used walking bioassays. Generally, the age at which pheromone production and perception starts seems to be a function of the reproductive biology of a species, and therefore varies from species to species. For instance, newly emerged adults of *Oryzaephilus* spp. (Coleoptera: Silvanidae) are unresponsive to aggregation pheromone. Sensitivity to pheromone reaches a maximum after 4 weeks and remains high throughout the adult life (Pierce *et al.*, 1983). This is broadly in agreement with White's (1989) observation on *Oryzaephilus surinamensis*. Faustini *et al.* (1982) reported that *Tribolium brevicornis* did not respond to the male-produced pheromone until 2 days old, reaching the peak of response after 6 days. In their study on *Sitophilus zeamais* (Coleoptera: Curculionidae), Walgenbach & Burkholder (1986) recorded a more dramatic relationship between age and response to pheromone: weevils less than 1 week old were attracted to pheromone, those from 2 to 6 weeks old showed almost no response, whereas older 8–10-week-old beetles were significantly repelled by pheromone.

The age at which pheromone production begins in *P.truncatus* is not clear, but in the closely related species *Rhyzopertha dominica*, pheromone was produced 3–5 days after feeding began and production continued over the course of 1 month, ceasing only, when food was not present (Mayhew & Phillips, 1994). In *P.truncatus*, pheromone is similarly produced only when present in a suitable resource (D. Hall, personal communication). The need to feed of long-lived beetles such as *P.truncatus* after emerging as adults in order to reproduce has been highlighted by Burkholder (1982). In the larger grain borer, feeding by adults starts soon after emergence, coinciding with the start of pheromone response. The newly emerged adults seemed to prefer kibbled maize or maize flour, since the mouthparts are probably not sclerotized enough to bore into hard whole maize grains (H. Fadamiro, unpublished observation). Because newly-emerged adult beetles could suffer mortality by boring into hard, whole grains, it is therefore very desirable for them to be most responsive to an aggregation pheromone associated with location of suitable resources. This will reduce the 'energy costs' of singly boring into hard grains, since the 'first arrivals' to the source (especially the older beetles) may have started boring activities. Levels of pheromone response matched the period in the life history of reproductive activity.

Sex and mating

There was no sex difference in the response of *P.truncatus* to pheromone, and mating did not change the response of the beetle to pheromone, suggesting that the male-produced aggregation pheromone does not primarily play a sexual role. The mating result is in agreement with the olfactometer studies of Obeng-Ofori & Coaker (1990) on some long-lived stored product beetles (including *P.truncatus*) whose males produce aggregation pheromones. They recorded, for all the beetles, a lack of post-mating reduction in pheromone response.

Resource quality

Data from this experiment show that whereas the beetles readily disperse from an unsuitable food, there was no significant effect of resource quality on the proportion of fliers responding to pheromone. This suggests that the mechanism underlying Pike's (1993) observation of lack of response to pheromone by *P.truncatus* infesting a suitable food is due to the reduced dispersal tendency of such beetles, and possibly not due to internal changes in their sensitivity to pheromone. Fadamiro *et al.* (1996) discuss the observed effect of resource quality on the tendency of beetles to disperse and proposed a hypothesis involving the dispersal of reproductively active male and female beetles from habitats of low resource quality to those that better support reproduction.

Starvation

In the present experiment, unstarved beetles (i.e. ≤2 h of starvation) were found to display more sustained-flight activity than those starved for 1 or more days. Starvation, however, did not affect the response of flying *P.truncatus* to pheromone, suggesting that starvation does not alter sensitivity of beetles to pheromone, but reduces their dispersal tendency. Once a starved beetle has taken flight, it is indeed beneficial for it to respond to the feeding-related aggregation pheromone. In the maize weevil, *Sitophilus zeamais*, starvation of up to 12 h has been reported to increase the response of 2-day-old beetles (but not of older 10-week-old beetles) to aggregation pheromone in a pitfall bioassay, declining afterwards (Walgenbach & Burkholder, 1986).

The biological function of the pheromone

The results, particularly of the age and resource quality experiments, and the absence of sex and sexual differences in response, strongly suggest a primary 'suitable resource location and colonization' function for the male-produced aggregation pheromone of *P.truncatus*. Related functions have been reported for the aggregation pheromones produced by the males of many other beetles including bark beetles (Borden, 1985), *Carpophilus* spp. (Bartelt *et al.*, 1992), *Sitophilus* spp. (Walgenbach & Burkholder, 1986), *Tribolium* spp. (Obeng-Ofori & Coaker, 1990) and *Rhizopertha dominica* (Khorramshahi & Burkholder, 1981; Obeng-Ofori & Coaker, 1990). In these long-lived beetles the aggregation pheromone has been suggested to function primarily to signal the presence of exploitable food sources, followed by the aggregation of both sexes by the sources. Shorey (1973) suggested that aggregation pheromones in the beetles arose primarily as a mechanism to cause aggregation at a suitable food source, implying that a sexual function arose secondarily.

In the case of *P.truncatus*, the aggregation pheromone can be advantageous to individuals in an 'aggregate' in many ways. First, in terms of costs, initial attack by the larger grain borer of a suitable resource such as maize grains or cobs is difficult. Thus, the aggregation of many individuals on a suitable resource would reduce the costs of initiating attacks by individual beetles; it only requires an individual to initiate attack successfully by boring into a resource for later beetles to benefit from a significant reduction in boring effort, since they may easily make their own tunnels in kernels that had already been attacked. Indeed, Fadamiro (1995) observed that *P.truncatus* prefers to bore into already attacked maize kernels, and in a culture jar, it is common observation to find up to four or more beetles within a maize kernel, although many other kernels would go unattacked.

The attraction of both sexes to suitable food resources will undoubtedly result in increased mating chances. However, an alternative hypothesis that the male pheromone currently functions primarily as a sex pheromone, but is exploited by other males, is not supported by the present study, although this may be the evolutionary origin of the behaviours.

Acknowledgments

The authors are grateful to Dr Martin Birch for his comments. We thank Drs Rick Hodges and David Hall of the Natural Resources Institute, Chatham Maritime, U.K., for supplying the parent beetle culture, and pheromones respectively. *P.truncatus* was reared under a MAFF licence number PHF 1231A/286/122 and funding was provided by the Rhodes Trust, Oxford (H.Y.F.).

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Accepted 29 May 1996