

Pheromone biology and factors affecting its production in *Tribolium castaneum*

A. Hussain*, T.W. Phillips†, T. J. Mayhew‡ and M.T. AliNiazee§

Abstract

Tribolium castaneum is a cosmopolitan pest of almost all processed as well as bulk commodities. Our objectives were to quantify the amount of pheromone, 4,8-dimethyldecanal, produced by individual males in 48-hour intervals, and to determine the effect of feeding on the production. Pheromone was collected through the aeration of adult males. The male flour beetles were aerated individually for 48 hours and the volatiles collected on the solid phase adsorbent Super Q. In the feeding study 0.5 grams of cracked wheat was provided initially to all beetles and removed after 4 days from half of the replications. GC-MS was used for quantification of the pheromone. Single male *Tribolium castaneum* produced detectable amounts of pheromone in 48 hours. The average production of pheromone was $1.265 \mu\text{g} \pm 0.18$ (SE) in 2 days. The total average production was $19.04 \pm 0.52 \mu\text{g}$. Feeding proved to be a major factor in pheromone production. The amount of pheromone dramatically decreased from $1.10 \mu\text{g}$ with food to 0.12 , 0.082 and $0.10 \mu\text{g}$ in 2, 4, and 6 days of starvation, respectively. When food was reintroduced to the starving beetles the production of pheromone increased to normal and dropped again to 0.06 and $0.04 \mu\text{g}$ in 2 and 4 days of starvation, respectively.

Introduction

The red flour beetle *Tribolium castaneum* is a serious pest of stored foodstuffs. *Tribolium* has been reported from many locations worldwide. Development of strategies alternative to routine chemical control has been a major goal of stored-grain scientists for many years. Pheromone-based monitoring traps for moth and beetle pests of stored products have been used for more than two decades (Burkholder and Ma 1985). Adult male *T. castaneum* secrete an aggregation pheromone, 4,8-dimethyldecanal (DMD), that is attractive to both sexes (Suzuki 1980). The pheromone is purportedly produced from the setiferous glands or patches on the ventral side of the femur (Faustini et al. 1981, 1982). Pheromone extracts of prothoracic femora from unmated male flour beetles elicit higher receptor potentials in the antenna of females than in those of males (Levinson and Mori 1983). Little is known about factors affecting pheromone production in male *T. castaneum*. The objectives of this research project are to quantify the amount of pheromone produced by single male *T. castaneum*, and to determine the effect of food on pheromone production.

* Department of Entomology, University of Agriculture, Faisalabad, Pakistan.

† USDA ARS, Dept. of Entomology, University of Wisconsin, Madison, WI USA 53706.

§ Department of Entomology, Oregon State University, Corvallis, OR USA 97331.

Materials and Methods

Insect cultures

An established laboratory colony of *T. castaneum* was used in all experiments. Flour beetles were reared on a mixture of whole wheat flour and brewer's yeast (95:5) in a growth chamber maintained at $27 \pm 1^\circ\text{C}$, 60% r.h. and 16:8 (light-dark) photo period. Parent beetles were sifted from cultures one week after inoculation. Pupae were sieved out after 3 weeks and were sexed using the pygidial characters described by Ho (1969). Male red flour beetle pupae were maintained separately on flour until used.

Collection of volatiles

For all experiments, insects were placed in $7.5 \text{ cm} \times 2.75 \text{ cm}$ cylindrical glass aeration chambers that were clamped to a stand and oriented vertically. Chambers were composed of a male and female ground glass joint tapered distally at each end to a 1/4 inch glass tube. Top and bottom openings to the chamber were loosely packed with glass wool to prevent insect escape while allowing air flow. House vacuum was used to draw air through charcoal and Tenax prefilters into the aeration chamber, and volatiles were trapped upwind on a glass column packed with the solid phase adsorbent Super-Q (Alltech Assoc., Deerfield, IL). The glass column was a Pasteur pipette from which the tip was removed and the cut end flame polished. Glass wool was used to plug the bottom opening of the column and Super-Q was filled to a level of 2 cm (400–450 mg) and held in place with an additional glass wool plug. Air flow rate on each system was maintained at 200 mL/minute. Following aeration of insects, the volatiles were extracted from the columns by elution with approximately 700 μL of HPLC-grade hexane and 762 ng of N-dodecane was added immediately as an internal standard. All aerations were conducted in a room maintained at $27 \pm 1^\circ\text{C}$ at 60% r.h. with a photoperiod of 16:8 (L:D).

Pheromone production by individual beetles

Five 2-day old adult virgin males were placed individually into glass aeration chambers and each male was provided 0.5 g of cracked wheat as food. Super-Q columns were changed after every 2 days and continuous aeration was carried out for 30 days (i.e. 15 consecutive 2-day collections for each beetle). Analysis of variance (ANOVA) was used to determine if pheromone production differed over time among the 15 2-day collection periods.

Effect of food on pheromone production

Two experiments were conducted to determine if feeding on wheat affected pheromone production and also if exposure to wheat volatiles with no feeding affected pheromone production. A total of ten 4-day-old adult males were aerated individually in glass chambers. Five of these males were des-

ignated as controls and provided with 0.5 g of wheat for the duration of the study. The remaining five beetles were designated experimental and were subjected to successive time periods with and without 0.5 g of cracked wheat as food. Super-Q columns were changed after every 2 days throughout the experiment and the amount of pheromone produced by one beetle was summed for each experimental period. For the first 4 days of the study, the experimental beetles were provided food, and thus were treated the same as the controls. After these 4 days of feeding, the wheat kernels were removed from the experimental arenas and the beetles were aerated without food for 6 days. Food was then reintroduced for 2 days and again was removed for a final 4-day period. Insects in the control group were physically disturbed by shaking the chambers at the time when food was added or removed in the treatment group.

In the food volatiles experiment, eight 4-days old adult males were placed individually in aeration chambers without food. A Pasteur pipette, 7.5 cm in length, was filled with 0.5 grams of cracked wheat kernels. Wheat kernels were suspended in the centre by glass wool on both ends of pipette. Air was passed through charcoal and Tenax prefilters, through the wheat kernels, and then to the aeration chamber. Sixteen adults of the same age were also aerated individually as controls, eight without any food or food volatiles, designated as negative control, and eight with 0.5 grams of wheat in the arena for feeding, designated as positive control. Beetles were aerated for a total of 6 days and Super-Q columns were changed at 3-day intervals; pheromone produced by each beetle was summed for the 6-day period and differences among treatments were determined with ANOVA.

Chemical analyses

Samples from all experiments were concentrated to 20 μ L under a gentle stream of N_2 and were subjected to quantitative analysis by couple gas chromatography–mass spectrometry (GC–MS). Analyses were made using a Shimadzu GC-14A coupled to a Finnigan Model 800 series Ion Trap Detector mass

spectrometer. The injector oven was set at 230°C and the heated transfer line to the spectrometer was set at 265°C. The column used was a 30 m \times 0.252 mm DB-1 (J & W Scientific), temperature-programmed at 40°C for 30 sec, then 20°C/minute to 60°C, held for 1 minute, then increased to 175°C at 10°C/minute, and held at 175°C for 2 minutes, then increased to 280°C at 30°C/minute, and held at 280°C for 3 minutes. Injection was made with the splitter closed initially, but then opened at 30 sec. Initial studies were conducted with the spectrometer in the full scan mode, recording mass fragments from 35 to 350 amu. An authentic sample of 4(R), 8(R,S)-dimethyldecanal was analysed for retention time and mass spectrum, which matched the spectrum published by Suzuki (1981). Preliminary studies with volatiles collected from single male *T. castaneum* confirmed the presence of DMD by matching spectrum and retention time with those of the authentic standard. In order to maximise detection ability for DMD in the experiments described above, the mass spectrometer was operated in the multiple ion detection mode (MID) in which only the characteristic fragment ions $m/z=41$ and $m/z=57$ were detected. These ions are common to both the internal standard, dodecane, and DMD. The quantity of DMD in each sample was determined using the Finnigan ITDS software (Ver. 4.10) by comparison of the peak area of the internal standard (representing 762 ng in the initial solution) and that of DMD from the MID chromatogram.

Results and Discussion

Pheromone was successfully collected and detected from one male in a 2-day period for four of the males in this study; a fifth male died early in the experiment. The mean amounts of pheromone produced per day by each of the four males in this study were 605.6 ng, 612.3 ng, 638.3 ng, and 685.3 ng, which indicates little overall variation among males. However, throughout the 30-day aeration of any given male there was great variation, which can be seen in a plot of the mean pheromone production per 2-day interval (Fig. 1). A fluctuating cycle of two days increase and two days decrease in

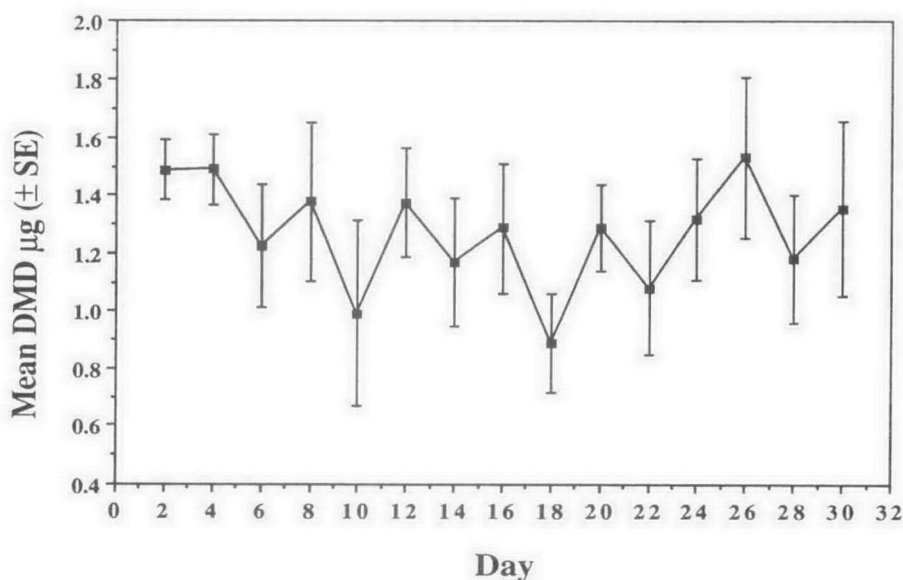


Fig. 1. Amount of pheromone collected from individual *T. castaneum* males beginning 2 days after emergence. Graph shows mean DMD (μ g) ($n=4$) and standard error produced per male every 48 hours up to 30 days of age.

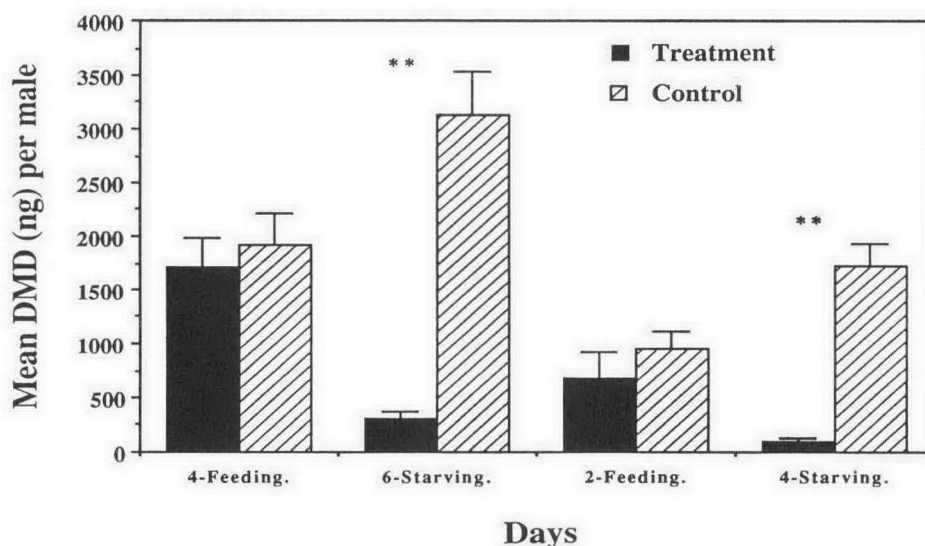


Fig. 2. Effect of feeding on pheromone production by *T. castaneum* males. Histograms show mean DMD (ng) per male and standard error for a given time period and treatment regime. Control is always with food, treatment is either with or without food. Significant difference between treatment and control are indicated as ** ($P < 0.01$, t-test).

pheromone production was observed over a 30-day period. When the mean amounts produced were compared among 2-day intervals, there was no significant difference ($P > 0.05$; ANOVA). The production of DMD remained high up to the last day of the study (1.35 μg). The results on longevity of production show that age does not affect pheromone production, at least during the first month of adulthood. This finding of sustained pheromone production over several weeks is congruent with results by Faustini et al. (1981) who found that globules secreted from the setiferous patches on prothoracic femora of male *T. castaneum* over 100 days were attractive to females and presumably contained pheromone.

The results of the feeding experiment show that there was no significant difference between control and treatment groups when both were provided food, but significant differences occurred when food was removed from the treatment group (Fig. 2). Pheromone production drastically decreased to 310.38 ± 53.67 ng over 6 days when food was removed, compared to 3123.82 ± 410.36 ng for males that had wheat during that period. An increase, equal to control, in DMD production was observed after food was reintroduced to treatment beetles. When wheat was again removed from the treatment chamber, pheromone production was 93.72 ± 35.91 ng in treatment as compared to 1730.00 ± 203.97 ng in control. This reduction was highly significant ($P < 0.001$, t-test). Actual contact and feeding on grain is apparently required for production of pheromone. Male *T. castaneum* exposed to volatiles of cracked wheat produced low levels of pheromone that were not different from those produced by beetles with no food, but were significantly lower than amounts produced by beetles actually feeding on cracked wheat (Fig. 3). These experiments with food provide strong evidence that aggregation pheromone production in *T. castaneum* is dependent on feeding. Starvation has a profound effect on the reduction of pheromone release in male red flour beetles, but pheromone production is easily 'rescued' by providing food.

Feeding has been linked to pheromone production in many other species of beetles, and may be due to one or both of two possibilities (Vanderwel and Oehschlager 1987). Food may provide a direct precursor to the pheromone that is immediately converted upon ingestion or contact. Such is the case in

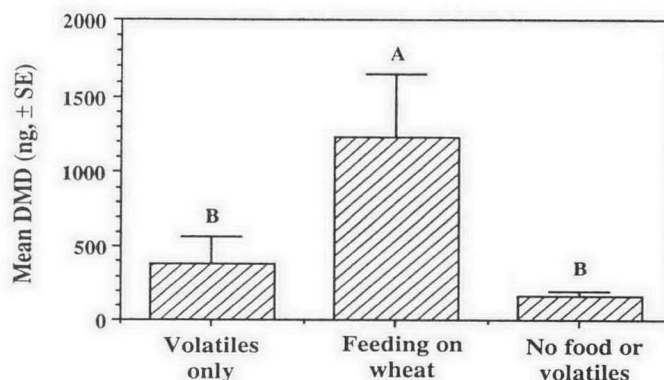


Fig. 3. Effect of wheat volatiles and feeding on the production of DMD by *T. castaneum* males. Histograms show mean DMD (ng) produced per male in 6 days and standard error ($N=8$). Mean amount followed by different letters are significantly different (ANOVA, $P < 0.05$, means comparison by Fisher's LSD).

many scolytid beetles that convert terpenes from pine trees into terpene alcohols that serve as pheromones (e.g. Pierce et al. 1987). In some cases with scolytids, simply the exposure to host plant vapours, with no feeding, can induce pheromone production. This is apparently not the case for *T. castaneum* (Fig. 3). An alternative is that feeding and gut distension induces a neuro-endocrine reaction that triggers pheromone production as a result of hormones (Renwick and Hughes 1977). The outcome of these experiments supports the idea of sanitation in warehouses. Even if beetles contaminate an area briefly, absence of an easily available food source would prevent attraction of additional beetles. Cleaning of residuals of grains should prevent *Tribolium* from aggregation and consequently reduce the chances of population growth.

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