Pheromone biology of the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae)

T.J. Mayhew* and T.W. Phillips*†

Abstract

The objectives of this study were to document pheromone production over time by male *Rhyzopertha dominica*, and then to investigate the effects of feeding, food nutritional value, mating, and population density, on pheromone production. The male-produced pheromones DL-1 and DL-2 were collected through aeration using the solid-phase adsorbent, Super-Q. Sexed *R. dominica* adults, 24 hours post-emergence, were placed individually in 7.5 cm × 2.75 cm cylindrical aeration chambers containing cracked wheat. Volatiles were collected for 24-hour periods and quantified using GC-MS. Pheromone was produced 3-5 days after feeding began and, once started, production did not cease over the course of one month. The ratio of the two pheromone components continually changed over the test period. The onset of pheromone production following feeding was on average 4.71 days ± 1.06 (SE). The maximum amount of DL-1 produced in a 24-hour period was 1114.756 ng ± 109.9 (SE), occurring 18 days after feeding began. Similarly, the maximum amount of DL-2 produced in 24 hours was 960.377 ng ± 78.0 (SE), occurring 14 days after feeding began. Pheromone production ceased when food was not present. Pheromone production increased proportionally as the content of wheat flour increased relative to non-nutritive cellulose in the food substrate. Pheromone production levels between mated and unmated males of the same age were not significantly different. Pheromone production was negatively correlated with population density levels.

Introduction

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), is a destructive internal feeding pest of stored grains throughout the world. Both the larvae and adults can easily attack whole, sound grain. *R. dominica* males produce an aggregation pheromone that was first reported by Khorramshahi and Burkholder (1981), and later isolated and identified by Williams et al. (1981). The pheromone was found to be made up of two unsaturated esters given the trivial names of dominicalure 1 and dominicalure 2 (Williams et al. 1981). Synthetic pheromone is available commercially and is used in flight traps for survey and detection (e.g. Cogburn et al. 1984; Leos-Martinez et al. 1986, 1987; Fields et al. 1993). However, there has been limited work on the environmental or physiological factors affecting pheromone production in *R. dominica*. The objectives of this study are to quantify pheromone production by individual beetles over time and to then investigate the effects of feeding, food nutritional value, mating, and population density on pheromone production.

* Department of Entomology, University of Wisconsin, Madison, WI USA 53706.
† USDA ARS Stored-Product Insects Research Unit,. Madison, WI USA 53706.

Methods and Materials

General

R. dominica were reared on a diet of sifted (#40 U.S. standard sieve) whole wheat flour and brewer's yeast (95:5). Pupae were sexed according to the pupal sexual dimorphism first reported by Potter (1935) and isolated. One-day-old adults were placed individually in a 7.5 cm × 2.75 cm (OD) cylindrical aeration arena containing several kernels of soft pastry wheat. Air was drawn by house vacuum through charcoal and Tenax™ (283 mg) prefilters, through the aeration arena, then through a glass column (106 mm × 6 mm ID) containing 209 mg Super-Q™ adsorbent at a rate of 0.5 L/min. Columns were extracted with 530 µL of hexane following an aeration and 716 ng of tetradecane was added as an internal standard. Samples were concentrated to 20 µL under a stream of pure N_2 at room temperature and analysed by coupled gas chromatography–mass spectrometry (GC–MS). The GC used was a Shimadzu GC-14A with a DB-1 fused silica capillary column (25 m by 0.25 mm ID.) and operated under the following conditions: injector temperature 130°C, heated transfer line 265°C, oven temperature 40°C for 30 seconds, 20°C per minute to 60°C, hold one minute, then 10°C per minute to 175°C, hold 30 seconds, then 30°C per minute to 280°C with a final hold time of 2 minutes; injection was made splitless, and the splitter was opened at 30 seconds. The mass spectrometer was a Finnigan-Mat 800 series ion trap detector operated in the multiple ion detection (MID) mode to scan for m/z = 85 for the internal standard and m/z = 111–115 for the two pheromone components. A linear regression equation was developed to quantify each pheromone relative to the internal standard.

Daily pheromone production was determined by placing single adult males in 10 aeration devices with 12 kernels of cracked wheat (ca. 0.4857 g) and collecting volatiles daily for 30 days. The insects were observed each day to determine when feeding began. The presence of frass was used as an indication of feeding. Mean amounts of DL-1 and DL-2 produced per day were determined.

The effect of mating on pheromone production was investigated by aerating individual virgin adult males and females, 10 each, with 12 kernels of cracked wheat. Volatiles were collected on days 8–10, and on day 10 five females were marked with a permanent marker and paired individually with five males. The remaining five females and five males were left unpaired and volatiles were collected from all preparations on days 10–12. On day 12, the mating pairs were separated and beetles were again aerated individually with fresh wheat. Volatiles were collected on days 12–14. Mated females were held in the aeration system for an additional 14 days to check for progeny development to confirm mating.

The role of feeding on pheromone production in *R. dominica* was studied by comparing volatiles from fed and unfed beetles. Two groups of 10 single-beetle aerations were established with 12 kernels of cracked wheat each. Volatiles were collected daily from day 8 to day 14 from all beetles. One group of beetles, referred to as controls, were allowed to feed for the duration of the experiment, while treatment beetles had food removed for 2 days and then it was returned. Quantities of pheromone produced were compared (t-test) between fed and unfed beetles within each sample period.

The effects of male density on individual pheromone production was examined by analysing volatiles from seven replicates of the following treatments: single male, 5 males, and 15 males in each chamber. All chambers were provided with seven kernels (ca. 0.2906 g) of cracked wheat. The quantity of pheromones produced per individual male on days 8–10 was calculated by dividing the total amount of pheromone produced in each treatment group by number of insects in each group. Data were analysed with the general linear models procedure (Proc GLM, SAS Institute 1985), and means were separated by the protected least significant difference (LSD) test.

Results and Discussion

In all but one case beetles produced pheromone after they began feeding, which averaged 4.71 d ± 1.06 (SE) after frass was observed. The maximum mean amount of DL 1 produced in a 24 hour period was 1114.756 ng ± 109.9 (SE) occurring 18 days after feeding began (Fig. 1). Similarly the maximum mean amount of DL 2 produced in 24 hours was 960.377 ng ± 78.0 (SE) occurring 14 days after feeding began. Williams et al. (1981) reported that 2000 mixed sex beetles produced a total of 660 µg of DL-1 and DL-2 combined in a 20-day aeration period, which is equivalent to approximately 30 ng/d if a 1:1 sex ratio is assumed. Dowdy et al. (1993) reported that one male infesting grain for one day produced 10 µg of pheromones. The maximum total amount of the two pheromones produced by one male in a 24-hour period in our study was 2.075 µg. Variation among studies in amounts of pheromone produced by *R. dominica* probably reflects differences in collection methods. Dowdy et al. (1993) measure headspace volatiles that had adsorbed on wheat kernels after infestation, and did not collect volatile directly from beetles. Williams et al. collected pheromone from large groups of beetles of unknown age and sex, thus rendering estimates of production per beetle inaccurate. In the present study we collected pheromone directly from individual beetles of known age, and we sampled pheromone throughout a defined time course.

There was no effect of mating on pheromone production. No significant differences were observed in the level of pheromone production between mated and unmated males during any of the collection periods, whether females were present or not. Pheromone production by males joined with females was 403.5 (±127.4) ng/d of DL-1 compared to 510.4 (±91.8) ng/d of DL-1 for unmated males during the same period. Pheromone production trends were similar for DL-2 form males of different mating status. Pheromones were not detected in any of the female aerations, confirming that DL-1 and DL-2 are male-produced pheromones. All male-paired females produced progeny following the experiment, thus confirming they were mated. Many insect species are known to cease pheromone production following mating (e.g. in Lepidoptera, Raina and Menn 1987). Males of the bark beetle *Ips paraconfusus* reduce production of their aggregation pheromone as they are joined by females, and pheromone production ceases when the harem (3–5 females) is complete (Borden 1967). Our experiment with *R. dominica* paired one female with one male. Since the mating system and host use patterns of *R. dominica* are not understood relative to a nonstorage habitat, we cannot be certain that we are testing the effect of mating on pheromone production adequately.

Pheromone production was very dependent on feeding. We found that pheromone production nearly ceased within 24 hours after food was removed, but then resumed within 24 hours when food was returned to the beetles (Fig. 2 for DL-1). In another study (unpublished) there was no pheromone production when beetles were deprived of direct contact with wheat but exposed to wheat volatiles, thus feeding or direct contact with food is essential for pheromone production. Feeding is a prerequisite in several other species of stored-product beetles that use aggregation pheromones (e.g. Phillips et al. 1985; Pierce et al. 1984). Wheat may contain direct precursors or nutrients that contribute to pheromone production by male *R. dominica*. The association of pheromone production with a food resource would ensure a responding female that there is a suitable habitat for her progeny. Conversely, other males that respond to pheromones from feeding males

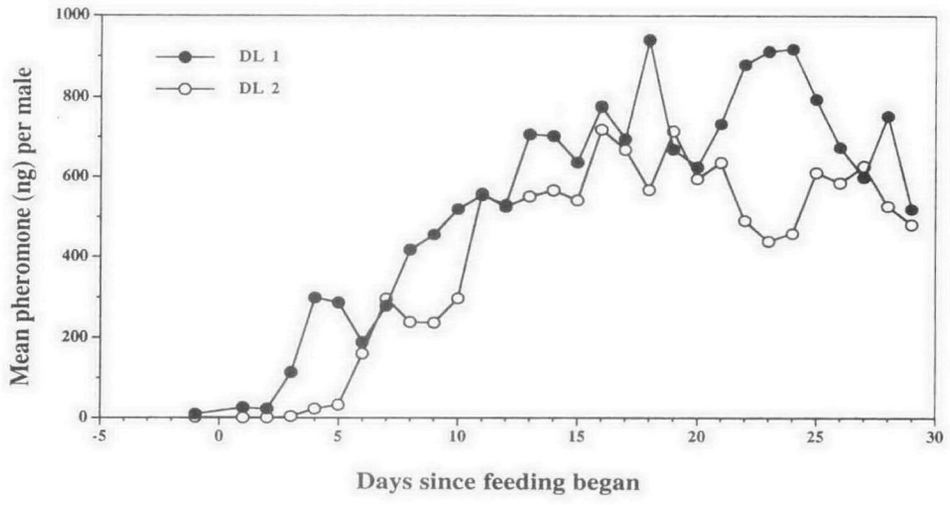

Fig. 1. Mean pheromone production by individual male *R. dominica* (n=6–8 males) feeding on wheat kernels in glass aeration chambers over a 30-day period.

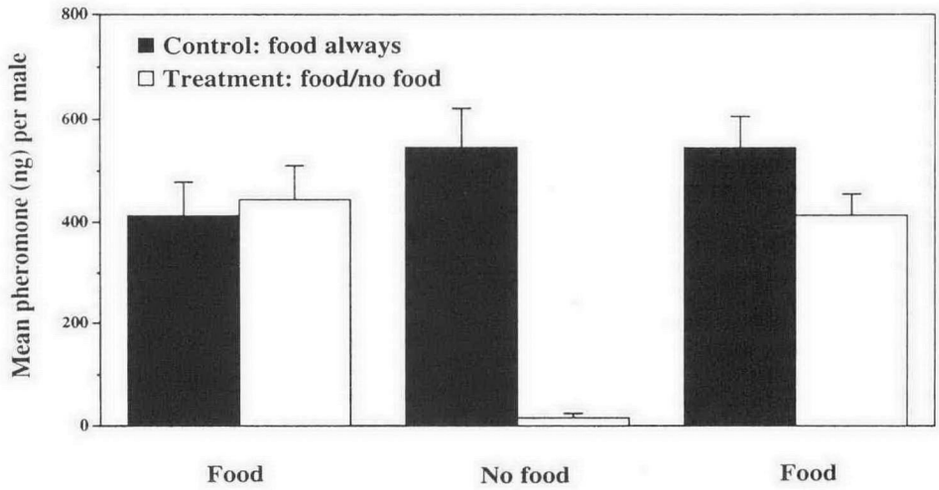

Fig. 2. Mean (± SE) production of DL-1 by male *R. dominica* feeding on wheat kernels during consecutive 24-hour periods. Treatment beetles were provided with wheat at first, then had the wheat removed, and then had the wheat returned; control beetles were provided with wheat for the duration of the experiment. Trends for DL-2 were similar to these for DL-1.

may exploit this signal to locate females at these attractive sites.

Pheromone production by males in groups of 5 or 15 males was significantly lower than pheromone production by single males (Fig. 3). Food may have been limiting in this experiment, since seven wheat kernels were provided in all cases, and caused reduction in pheromone production. Reduction of pheromone production by males in a group may also be a strategy by individual males to conserve energy from pheromone biosynthesis.

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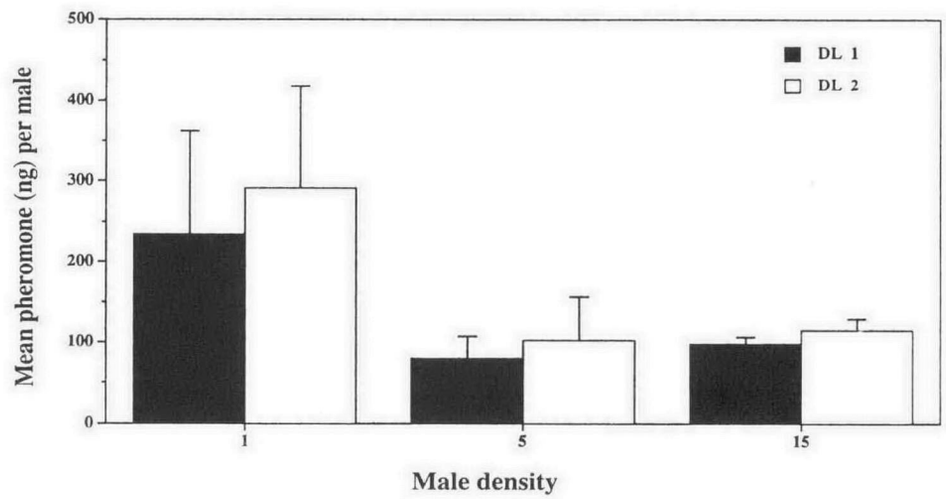

Fig. 3. Mean production of DL-1 and DL-2 in 24 hours by male *R. dominica* held singly or in groups of 5 or 15 males with seven wheat kernels.

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