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INFLUENCE OF DIET QUALITY AND PHYSIOLOGICAL CONDITION ON BOLL WEEVIL (*ANTHONOMUS GRANDIS*) PHEROMONE PRODUCTION

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Abstract

Recent research examining individual male boll weevils has indicated higher rates of pheromone production than were previously reported. However, influences on pheromone production of food type and size have not been examined using these techniques. We examined the influences of square size (5-6 mm versus 8-9 mm diameter) and diet switching (maintained on 6-7 mm diameter squares versus switching from squares to 15-mm diameter bolls) on pheromone production by individual weevils. Weevils were fed squares of the assigned size class for 4 d (square size) or 5 d (switching) before daily pheromone collections were initiated on days 5-7 (square size) or 6-9 (switching). No influence of square size on pheromone production was demonstrated (5-6 mm squares, 52.9 ± 6.6 µg/weevil/day; 8-9 mm squares, 85.5 ± 14.7 µg/weevil/day). However, pheromone production did increase from the 5th day (60.3 ± 10.3 µg/weevil/day) to the 7th day (83.4 ± 8.7 µg/weevil/day). In the second experiment, weevils switched from squares to bolls produced more pheromone (76.2 ± 9.7 µg/weevil/day) than weevils remaining on squares (50.2 ± 4.9 µg/weevil/day). Also, the increase in pheromone production from the 6th to 9th day of adulthood was greater for weevils switched to bolls than for weevils remaining on squares. In both studies, pheromone production was positively associated with the presence of well-developed accessory glands. These results provide a basis for additional studies of the dynamics of boll weevil pheromone production.

Introduction

Recent work done in this lab (Spurgeon and Marshall 2000) demonstrated that male boll weevils produce substantially more pheromone than was originally indicated in the seminal studies on the subject (Hedin et al. 1974, McGovern et al. 1976, McKibben et al. 1976, Dickens et al. 1988). Estimates of pheromone production from these earlier studies were based mainly on extracts of collected feces or whole animals. In contrast, analysis of volatiles released by individual males has shown that the bulk of the pheromone occurs in the air as volatiles, with only a few percent remaining in the feces (Spurgeon and Marshall 2000, Spurgeon 2001). Other recent studies have indicated that boll weevil diet has a profound influence on reproductive development (Spurgeon and Raulston 1997, 1998, Spurgeon and Esquivel 2000), but corresponding influences on pheromone production have not been examined. Our objectives were to examine the influences of square size, and of diet switching (square to bolls) on pheromone production of individual male weevils.

Materials and Methods

Collection of Weevils of Known Age

Adult boll weevils were reared from field-collected infested squares. Oviposition-punctured squares were collected from cotton plants and held in screened cages in an environmental chamber at 29.4 ± 1°C and under a 13:11 [L:D] h photoperiod. Squares were examined for the presence of pupae 5 or 6 d after collection. Pupae were removed from the squares and held in groups of 35–50 on a thin layer of moistened vermiculite in disposable petri plates. They were examined at least once daily and newly eclosed weevils were sexed using the method of Sappington and Spurgeon (2000), weighed, and assigned to experimental treatments. Weevils used in these experiments ranged in weight from about 8 – 20 mg.

Food Collection

Because pheromone production rates are known to vary considerably on the bases of food type and quality (Hardee, 1972), we exercised considerable care to provide the highest quality food possible. Squares and bolls were obtained from greenhouse-raised cotton plants and fruit exhibiting any damage, browning, or discoloration was discarded. Bracteoles were left intact on both squares and bolls. Fresh fruit was usually collected daily, and was fed to weevils 2 to 4 hours after lights-on (0600h) so the weevils would have access to fresh food in good condition for the bulk of the photophase.

Pheromone Collection Apparatus and Analysis

Volatile compounds were collected from the headspace of 120-ml Qorpak neckless wide-mouth bottles with teflon-lined lids (Qorpak, Bridgeville, PA). The lid of each vessel was penetrated by two holes, each fitted with a teflon reducing union, which attached volatile collection and trap columns to the collection vessel. Both collection and trap columns were 8 × ¼ inch (length × o.d.) glass tubes (Envirochem, Lancaster, PA) packed with a 5-cm bed of Super Q resin (Alltech Associates, Deerfield, IL) held in place by plugs of glass wool. The trap column removed volatiles from incoming air and the collection

column adsorbed pheromone from air drawn through the vessel. Eight vessels were attached to a manifold through which air was drawn by a diaphragm vacuum pump set at 51 cm of mercury. Airflow rates were maintained at 1 - 1.5 liter/min by individual flow meters between the respective collection columns and the manifold. The collection apparatus was placed in a vacant fume hood maintained at 29 ± 2°C by a thermostatically controlled oscillating heater. Temperature conditions were recorded with a HOBO data logger. Experiments were conducted under a 13:11 [L:D] h photoperiod supplied by a fluorescent light controlled by an electric timer.

Each sample represented a 24-h collection interval. At the end of each collection period, pheromone was eluted from each collection column directly into GC sample vials with a volume of dichloromethane (GC grade) sufficient to result in 1.0 ml eluant volumes. Each vial was mixed by agitation and duplicate 1-µl injections were analyzed by GC/FID. Pheromone content of each sample was calculated from the average of the two injections. In addition, frass was brushed from the food source (square or boll) into the collection vessel and extracted within the vessel by gentle swirling with 1.0 ml of GC-grade dichloromethane. Pheromone content of the frass was estimated based on GC/FID analysis of duplicate 1-µl injections of the extract.

Samples were analyzed on a Hewlett-Packard 5890 series II GC (Hewlett-Packard, Palo Alto, CA) using a DB-5 column (30 m × 0.25 mm i.d., J. & W. Scientific, Folsom, CA) and a flame-ionization detector. The temperature program for analysis was as follows: injector temperature 180°C; detector temperature 300°C; flow rate 2.0 ml/min; initial column temperature 60°C maintained for 5 min, increased to 110°C at 30°C/min, held at that temperature for 1 minute, increased to 150°C at 3°C/min and maintained for 3 min, and finally increased to 300°C at 70°C/min and maintained for 5 min. Total GC run time was 31.98 min. Concentrations of pheromone components were calculated by comparing the areas under peaks from the samples to corresponding areas for the four components of a synthetic Grandlure standard (ISP Fine Chemicals, Columbus, OH) of 20.5mg/100 ml.

Dissection Criteria for Rating Accessory Glands

Because a strong correlation between pheromone production and accessory gland condition was previously reported (Spurgeon 2001) the condition of the accessory glands was determined by dissection of each weevil immediately following the end of the last 24h pheromone collection period. Based on the results of Spurgeon (2001) accessory glands were classified as: 1) undeveloped (small, transparent) or 2) developed (enlarged, grayish in coloration, contents semi-solid).

Comparison of Large and Small Squares

Ten newly eclosed males were placed singly in 100x15 mm petri dishes with a 1 cm section of cotton wick soaked with water and a fresh cotton square. Weevils were randomly assigned to one of two square size treatments (large 8 – 9 mm diameter; or small, 5 – 6mm diameter). Square diameter was measured at the widest portion of the bud. Fresh squares were provided daily for 4 d post-eclosion. The morning of the fifth day four weevils from each treatment were placed individually into Qorpak collection jars with a plastic scintillation vial full of distilled water and a cellulose wick. Each weevil was provided a square of the assigned size class daily for 3 d. In order to compensate for any temperature gradient in the fume hood where the apparatus was housed, jars with weevils assigned to each treatment were alternated along the row of eight stations. After each 24-h collection period the weevils and water vials were transferred to a fresh jar and the square was replaced with fresh one of the same size. The collection and trap columns were removed and replaced, and the collection traps were eluted as above. The inlet trap was eluted into a waste beaker and all the traps were blown to dryness with pure dry nitrogen. The entire changeover of the 8 samplers took about 15 min.

Diet Switching from Squares to Small Bolls

Ten newly eclosed weevils, obtained and sexed as above, were placed singly in 100x15 mm petri dishes with a 1 cm cotton wick soaked with water and a fresh 6 –7 mm diameter square. At this time they were randomly assigned to one of the two treatments, switching or non-switching. Squares were replaced daily for 5 d. The morning of the sixth day four individuals of each treatment were individually placed into Qorpak collection jars with a plastic scintillation vial full of distilled water and a cellulose wick. Four of the weevils were again given a 6 – 7 mm square daily, and the remaining 4 received a small, fresh cotton boll, 15 ± 1 mm in diameter, each day. Other than the differences in food provided the procedure was the same as for the square size experiment.

Statistical Analysis

Single-factor ANOVAs were run to test the significance of accessory gland condition in relation to the pheromone production on the last 24 h of collection using StatView 5.0 (SAS Institute, 2001, Cary, NC). Because accessory glands can change considerably over a 24 h period (DWS, unpublished data), it was appropriate to only consider the relationship between accessory gland condition and pheromone production from the most recent sampling period. Repeated measures ANOVAs using the REPEATED statement of PROC GLM (SAS Institute, 2001, Cary, NC) were used to test the significance of

treatment (food type), replicate, day of sampling, and their interactions. Based on sphericity tests, significance of model terms containing the repeated factor (day) were assessed using the Greenhouse-Geisser adjusted *P*.

Results

Comparison of Large and Small Squares

Almost all male boll weevils that had been fed on either small (5-6 mm diameter) or large (8-9 mm) squares were producing pheromone by the time they were dissected at 7 d post-eclosion. The mean daily rates of pheromone production did not differ statistically between square size classes (small squares, 52.9 ± 6.6 μg; large squares, 85.5 ± 14.7 μg; $F = 3.53$; df = 1,23; $P = 0.07$). Daily pheromone production increased over the three-day collection period ($F = 6.28$; df = 2,46; *G-G adjusted* $P<0.01$) from 60.3 ± 10.3 μg per weevil on the first collection day to 83.4 ± 8.7 μg per weevil on the third day. No other main effects or interactions were significant.

Two individuals with undeveloped accessory glands produced less than 0.5 μg of pheromone in the 24 h preceding dissection, while males with developed accessory glands produced an average of 89.1 ± 8.3 μg. Thus, accessory gland condition was highly significant in relation to pheromone production ($F = 7.64$, df =1,29; $P <0.01$) although these results should be interpreted with caution because of the small number of weevils with undeveloped accessory glands.

Diet Switching from Squares to Small Bolls

Male weevils that had been fed for five days on squares generally continued to produce pheromone whether switched to bolls or continued on squares. The mean daily pheromone production by weevils switched to bolls (76.2 ± 9.7 μg) was greater than that for weevils maintained on squares (50.2 ± 5.9 μg) ($F= 5.06$; df = 1,40; $P = 0.03$). Also, mean daily pheromone production generally increased throughout the 4-d collection period (day 1, 47.0 ± 5.8 μg; d 4, 75.3 ± 6.7 μg; $F = 10.12$; df = 3,120; *G-G adjusted* $P < 0.01$). However, examination of the diet by day interaction ($F = 4.68$; df = 3,120; *G-G adjusted* $P <0.01$) indicated that the amount of increase in pheromone production with increasing age differed between the diets. Examination of the daily pheromone production patterns (Fig. 1) indicated that pheromone production by weevils in the respective diet treatments were roughly similar on the first day of pheromone collection but that pheromone production of weevils switched to bolls increased over the subsequent 3 days while pheromone production of weevils maintained on squares remained relatively static.

There was a strong association between the physiological state of the accessory glands and the production of pheromone. Most weevils produced pheromone on either diet treatment. There was, however, little or no pheromone production by seven individuals that were evenly distributed between the two diets. These individuals all had accessory glands rated as undeveloped. The weevils with developed accessory glands produced significantly more pheromone on the day of dissection (86.3 ± 6.3 μg) than those with small glands (1.6 ± 1.4 μg) ($F = 36.17$, df = 1,60, $P < 0.01$). Interestingly, one of the seven weevils rated with undeveloped glands did produce about 10 μg of pheromone the last day.

Discussion

The relationship between accessory gland condition and pheromone production that we observed was consistent with the observations by Spurgeon (2001). Thus, this relationship appears to have some general application. Dissection of trap-captured or field-collected weevils and scoring of the accessory glands should provide a meaningfull assessment of the pheromone production activities of these males.

An unexpected result of our research was that males switched to small bolls continued to produce pheromone at levels even higher than those of males maintained on squares. That these males spent the first five days of adult life, feeding on 6-7 mm squares may prove to be critical in understanding the physiology underlying their ability to continue pheromone production on what is commonly considered a less than ideal food source. In this regard, the relative suitability of squares and young bolls for male reproductive development in various time frames needs to be further addressed.

Disclaimer

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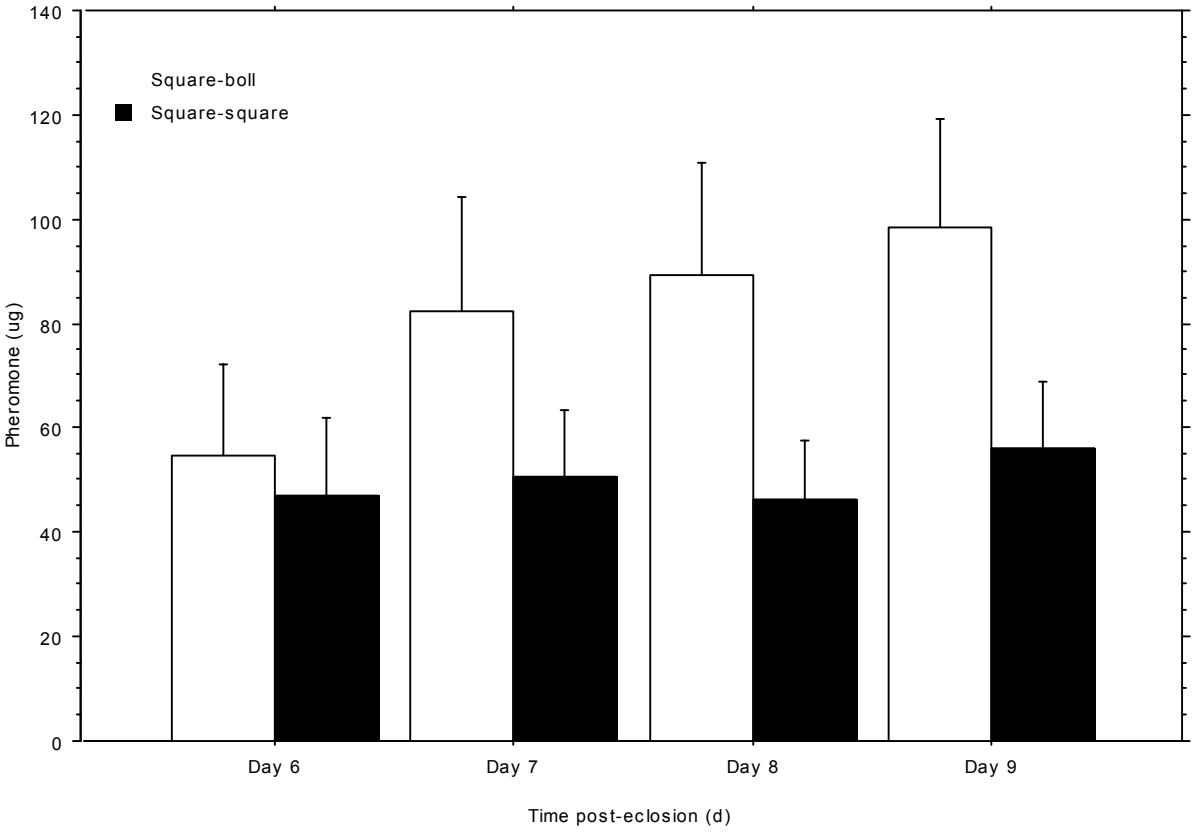

Figure 1: Mean daily pheromone production over a 4-d sampling period of weevils reared 5 d post-eclosion on 6-7 mm squares, then either switched to 15 mm bolls (Square-boll) or left on squares (Square-square). Error bars represent the 95% confidence interval.