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PRELIMINARY OBSERVATIONS ON THE DAILY PATTERN OF PHEROMONE PRODUCTION BY INDIVIDUAL BOLL WEEVILS

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Abstract

A sound understanding of the chemical ecology of the boll weevil, *Anthonomus grandis* Boheman, on cotton (*Gossypium hirsutum* L.) is important to efforts to improve pheromone trapping technologies. Recent reports of new methods of examining pheromone production by individual male weevils have indicated that most pheromone is not in the frass as was previously assumed. We used these new methods to evaluate the daily pattern of pheromone production of individual weevils, and the influence of time of food replacement on those patterns. Weevils fed daily at 0730 h (CDT) produced more pheromone (2.62 ± 0.30 µg/h) than weevils fed at 1530 h (1.66 ± 0.30 µg/h). Hourly pheromone production was highest during the intervals of 0730 – 1130 h (2.98 ± 0.37 µg) and 1130 – 1530 h (3.30 ± 0.37 µg), intermediate between 1530 – 1930 h (1.67 ± 0.31 µg), and lowest between 1930 – 0730 h (0.58 ± 0.16 µg). Weevils fed at 0730 h produced more pheromone during the 1130 – 1530 h period than weevils fed at 1530 h, but pheromone production by the two groups was more similar for other periods. Our results indicated less distinct daily patterns, and greater amounts of pheromone released during the scotophase, than were previously reported. Our results likely differ from the daily patterns of pheromone production occurring in the field because we did not simulate field temperatures. Still, they provide insights previously unavailable from the literature. These results also suggest additional study could further improve our understanding of the dynamics of boll weevil pheromone production.

Introduction

A sound understanding of the chemical ecology of the boll weevil, *Anthonomus grandis* Boheman, on cotton (*Gossypium hirsutum* L.) is important to continued efforts to improve pheromone traps, lures, and the interpretations of trapping data. As a result, this aspect of the boll weevil's biology has been extensively studied (Hardee 1972, Hardee and Mitchell 1997). However, newer techniques permit quantification of the pheromone produced by individual male weevils and incorporate recently obtained knowledge regarding the effects of adult diet on physiological condition (Spurgeon and Marshall 2000, Spurgeon 2003). Because these newer techniques sample pheromone actually released into the atmosphere surrounding the weevils, they avoid the assumptions inherent to earlier methods that sampled pheromone contained in the feces or frass. In contrast with earlier investigations of pheromone production (Hedin et al. 1974, McGovern et al. 1976, McKibben et al. 1976, Dickens et al. 1988), these more recent studies indicated that only a small proportion of the total pheromone produced is contained in the frass, and that the weevil's pheromone production capability is much greater than was previously suspected. Therefore, it would seem expedient to use these newer techniques to reevaluate some of the more fundamental aspects of boll weevil pheromone production.

The boll weevil exhibits daily patterns in trap response (Guerra 1983) and pheromone production (Gueldner and Wiygul 1978), and these patterns appear to be roughly similar. However, the patterns of pheromone production reported by Gueldner and Wiygul (1978) were based on pheromone extracted from frass. Our objective was to reevaluate the daily pattern of pheromone production using methods similar to those of Spurgeon (2003). In addition, because the time of food replacement could result in experimental artifacts that influence the daily pattern of pheromone production observed in the laboratory, we incorporated two daily feeding times to investigate the potential influence of this factor. Because our methods could not distinguish between pheromone production and release *per se*, we use the term 'pheromone production' in this report as it has been previously used in the literature so as to encompass both of these processes.

Materials and Methods

Experimental Insects

Adult boll weevils of known age were reared from field-collected, oviposition-punctured squares. The squares were collected from plants before abscission, and transported to the laboratory where they were held in screened cages within an environmental chamber maintained at 29.4±1°C with a 13:11 [L:D] h photoperiod. The contents of the squares were examined daily beginning five or six days after collection. Pupae removed from the squares were held in groups of 35 – 50 on a thin layer of moistened vermiculite in disposable Petri plates. Plates were held in the same chamber as the infested squares, and were examined at least once daily for the presence of newly eclosed adults. Adults were sorted by sex on the day of eclosion using the method of Sappington and Spurgeon (2000). Males were weighed to the nearest 0.1 mg after they were sufficiently sclerotized to walk. Because squares collected from the field deteriorate fairly rapidly in the laboratory, weevils present as eggs or early instar larvae at the time of collection may produce unusually small adults. This is particularly likely if the collected squares are also small. To avoid this potential source of bias we used only males weighing >10 mg in the study.

Pheromone Collection Apparatus and Procedure

The headspace collection vessels were 120-ml neckless wide-mouth bottles (Qorpak, Bridgeville, PA) with Teflon-lined lids. Two 1.15-cm diameter holes in the lid of each vessel were each fitted with a 1.1-cm to 0.6-cm Teflon-lined stainless steel reducing union (Swagelock, Solon, OH). The reducing unions were used to fit a volatile collection column (to collect pheromone) and a trap column (to remove volatiles from incoming air) to each vessel. Both columns (Supelco, Bellefonte, PA) were 17.8 cm long by 0.6 cm outside diameter, and were packed with a 5-cm long bed of Super Q resin (Alltech Associates, Deerfield, IL) held in place by plugs of fused silica wool. When installed in the vessels, the bottom of the collection column was 1.5 – 2 cm higher than the bottom of the trap column to ensure that the volume of the vessel was thoroughly sampled.

During periods of pheromone collection, the collection columns of eight vessels were attached to a manifold through which air was drawn by a diaphragm pump. Pump vacuum was adjusted to about 68 kPa, and air flow through each vessel was regulated at about 1 liter/min by individual flow meters. The entire apparatus was housed in a fume hood with a sealed exhaust. Walls of the hood were sheathed with foam insulation, and the hood was maintained at about 27±2°C by a thermostatically controlled electric heater. A 13:11 [L:D] photoperiod was maintained by two 40-W fluorescent bulbs controlled by an electric timer.

At the end of each pheromone collection period, pheromone was eluted from each collection column directly into respective GC sample vials, using a volume of GC grade methylene chloride sufficient to result in 1.0-ml eluant volumes. Each vial was mixed by agitation before analysis on a Shimadzu 17A GC (Shimadzu Scientific Instruments, Columbia, MD) using dual DB-5 columns (60 m by 0.32 mm i.d.; J&W Scientific, Folsum, CA) and flame-ionization detectors. A separate 2-µl injection of each sample was analyzed on each column. The GC operating conditions and temperature program were as follows: injector temperature 200°C; detector temperature 300°C; flow rate 2.0 ml/min; initial column temperature 60°C maintained for 7 min, increased to 110°C at 25°/min and maintained for 4 min, increased to 150°C at 20°/min and maintained for 5 min, and finally increased to 300°C at 30°/min and maintained for 8 min. The total program time was 33 min. The boll weevil pheromone is composed of four components. Pheromone contents of samples, which were the sums of the four components, were calculated based on areas under component peaks, in reference to corresponding areas for external standards of known concentrations of Grandlure (ISP Fine Chemicals, Columbus, OH) using GC Real Time Analysis software (LabSolutions GCsolution Analysis, ver. 2.10, Shimadzu, Kyoto, Japan). Estimates from the two injections were averaged before statistical analysis. Because Spurgeon and Marshall (2000) and Spurgeon (2003) found that only about 5% of the total pheromone produced is present in the frass, we made no effort to recover pheromone from the frass.

Experimental Procedure

In each replication of the experiment, 10 to 12 males were transferred individually to 100 by 15-mm Petri plates. Each weevil was provided a fresh 6 – 7-mm diameter square with intact bracteoles each day. A short (~1 cm) section of saturated cotton dental wick was provided as a water source. Weevils were held in an environmental chamber under the same conditions as the infested squares and were fed daily before 1000 h (CDT). Beginning on the morning of the 9th day of adulthood (when weevils were 8-d-old), weevils were assigned to feeding treatments of either "morning" (fed at 0730 h) or "afternoon" (fed at 1530 h). These feeding treatments were then maintained until the termination of the replication.

At the beginning of the 10th day of adulthood (when weevils were 9-d-old), one weevil from each feeding treatment was randomly assigned to each of four pheromone collection vessels (a total of eight weevils per replication). All weevils were introduced into the collection vessels at 0730 h, and those assigned to the morning feeding treatment received fresh squares at that time. Weevils from the afternoon feeding treatment were accompanied by the squares from their respective Petri plates, after frass was wiped from the surfaces of the squares. In addition, each collection vessel was equipped with a 4-ml glass vial filled with water and fitted with a cotton wick stopper. Pheromone was eluted from the columns four times during the 24-h volatile collection period; at 1130, 1530, 1930, and 0730 h on the following morning. This provided estimates of pheromone production corresponding to three 4-h periods during the photophase and one 12-h period encompassing the first and last 30 min of the photophase and the entire scotophase. At the end of each volatile collection period, weevils and their respective feeding squares and water vials were transferred to new collection jars, except at 1530 h, when squares of weevils assigned to the afternoon feeding treatment were replaced. The experiment was replicated four times, with weevils of the respective replicates originating from Hidalgo, Hill, and Limestone (two replicates) Counties, Texas. Thus, a total of 32 weevils (16 weevils per feeding treatment) were used in the study.

Statistical Analysis

The estimated amounts of pheromone produced during each period were divided by the duration of each period to provide estimates of hourly pheromone production (µg/h). Weevils that produced no pheromone during the 24-h collection period or that died were excluded from analysis. Estimates of hourly pheromone production were analyzed by repeated-measures mixed-models analysis of variance using the SAS procedure PROC MIXED (SAS, ver. 8.02, SAS Institute, Cary, NC). Fixed effects included feeding treatment, daily period, and their interaction. Random effects included replication and the replication-by-feeding treatment interaction. Corrected degrees of freedom were obtained using the DDFM=KR option of the MODEL statement. Daily period was the repeated factor, and based on the results of preliminary analyses an unstructured

covariance structure (TYPE=UN option in the REPEATED statement) was selected for the analysis. Least-squares means corresponding to levels of the fixed effects were compared using the ADJUST=TUKEY option of the LSMEANS statement. In addition, the effects of feeding treatment within each daily period were examined using the SLICE option of the LSMEANS statement.

Results and Discussion

Of 32 weevils in the experiment, one weevil failed to produce pheromone (morning feeding treatment) while one in the afternoon feeding treatment died after pheromone collection began. Thus, data for 30 weevils were available for analysis. The 15 weevils assigned to the "morning" feeding treatment ranged in weight from 10.30 to 18.37 mg (mean ± SE, 14.37 ± 0.73 mg), while those assigned to the "afternoon" treatment ranged from 10.34 to 17.77 mg (mean ± SE, 14.34 ± 0.56 mg). Of those weevils producing pheromone, the total 24-h pheromone production ranged from 1.98 µg (afternoon feeding treatment) to 86.22 µg (morning feeding period).

Analyses indicated that weevils fed at 0730 h produced more pheromone (2.62 ± 0.30 µg/h) than weevils fed at 1530 h (1.66 ± 0.30 µg/h; $F = 6.13$; df = 1, 23.7; $P = 0.02$). Also, pheromone production varied among collection periods ($F = 36.55$; df = 3, 26; $P < 0.01$). Hourly pheromone production was similar during the 1st (0730 – 1130 h; 2.98 ± 0.37 µg) and 2nd (1130 – 1530 h; 3.30 ± 0.37 µg) collection periods, but hourly production during those periods was greater than during the 3rd period (1530 – 1930 h; 1.67 ± 0.31 µg). Hourly pheromone production was least during the 12-h period spanning the scotophase (1930 – 0730 h; 0.58 ± 0.16 µg). Although the feeding treatment-by-collection period interaction failed to detect differences between feeding treatments in the daily pattern of pheromone production ($F = 2.76$; df = 3, 26; $P = 0.06$), results provided by the SLICE option indicated that weevils fed at 0730 h produced more pheromone than weevils fed at 1530 h during the 2nd collection period (1130 – 1530 h; $F = 5.22$; df = 1, 26.7; $P = 0.03$). Analyses of other slices did not indicate differences between the feeding treatments during other collection periods (Fig. 1; period 1, $F = 2.68$; df = 1, 27.6; $P = 0.11$; period 3, $F = 2.73$; df = 1, 26.5; $P = 0.11$; period 4, $F = 1.04$; df = 1, 18.9; $P = 0.32$). Therefore, the daily pattern of pheromone production was somewhat more pronounced for weevils fed at 0730 h than for weevils fed at 1530 h (Fig. 1).

Gueldner and Wiygul (1978) reported that peak pheromone production occurred between 0700 – 1000 or 1000 – 1300 h, and that 100-fold differences were observed between daily highs and lows of pheromone release. Although the timing of the daily peak in pheromone production that we observed was generally consistent with their report, other characteristics of the daily pattern differed between studies. Specifically, the daily patterns we observed were much less marked than those reported by Gueldner and Wiygul (1978), and our results showed that the time of day when weevils were fed influenced the amount of pheromone produced as well as the pattern of production. We could likely obtain further information regarding the effects of time of feeding by examining pheromone production at one or more additional ages more distant from the time that the feeding treatments were established. Finally, Gueldner and Wiygul (1978) reported that light stimulates pheromone release while we observed that substantial quantities of pheromone continued to be released during the scotophase. Therefore, it appears that the daily pattern of pheromone production is more likely a product of an entrained rhythm than a consequence of light-stimulated pheromone production or release.

It was expected that our results would differ, at least somewhat, from those reported earlier because of the uniqueness of the methodology we used, and because of our care in regulating the weevils' diets. Most studies of pheromone production provide little information regarding diet other than its type (squares, bolls, artificial diet). For example, Gueldner and Wiygul (1978) provided one square per five weevils daily, but did not specify the sizes of squares or whether they were intact or debracted. In contrast, we closely controlled diet quality because of previous reports relating pheromone production to the condition of male accessory glands (Spurgeon 2001, 2003), and because our experience regarding the role of diet in reproductive commitment (Spurgeon and Raulston 1998, Spurgeon and Esquivel 2000) suggests the dietary factors controlling reproductive commitment also influence male accessory gland condition (unpublished data, illustrated in Spurgeon et al. 2003).

The daily pheromone production patterns we observed likely differ from those that occur in the field because we made no effort to simulate natural daily temperature profiles. Still, our results provide some insights beyond those that were previously available in the literature, and suggest considerable opportunity to continue to expand our knowledge of the dynamics of boll weevil pheromone production.

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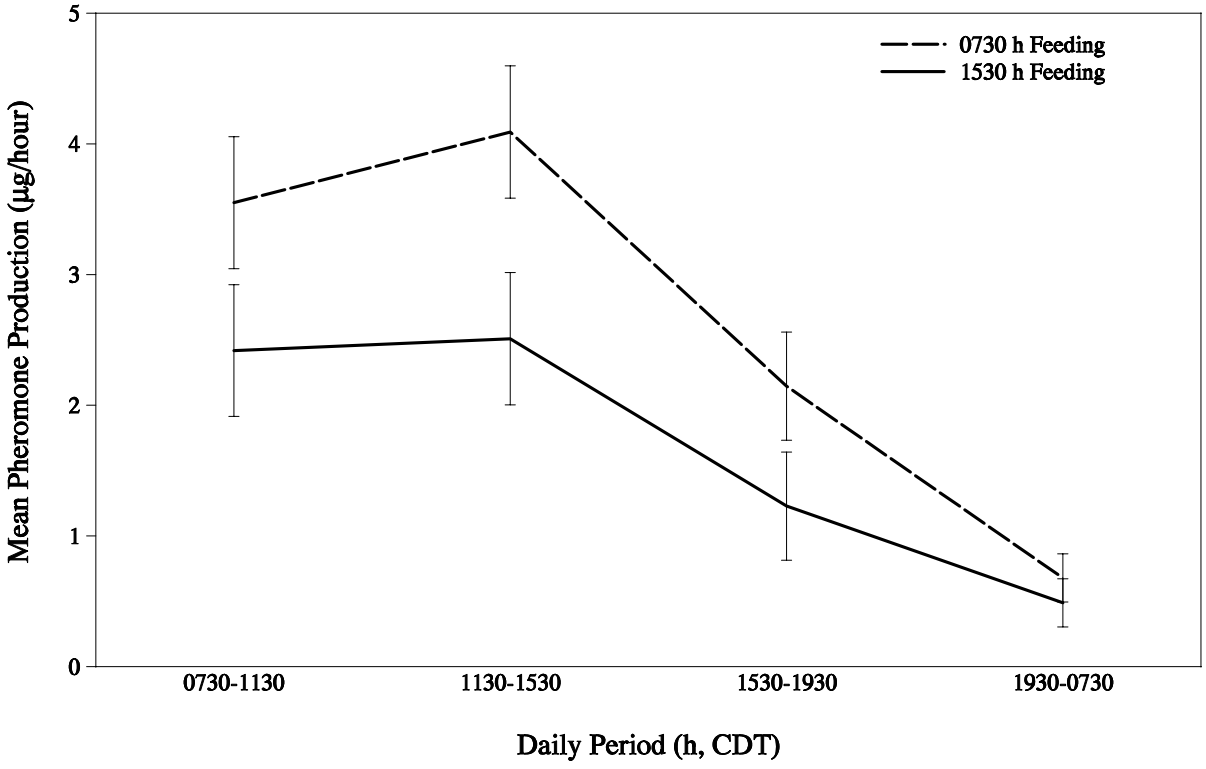

Figure 1. Mean hourly pheromone production (± SE) during four daily collection periods for male boll weevils fed at either 0730 or 1530 h (CDT).