CONTINUATION OF PHEROMONE PRODUCTION BY BOLL WEEVILS FOLLOWING HOST REMOVAL

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Abstract

Comprehensive knowledge of the chemical ecology of the boll weevil, especially with regard to the production of and response to pheromone, is critically important to continued efforts to improve pheromone traps, lures, and the interpretations of trapping data. In light of recent reports demonstrating a strong correlation between weevil accessory gland condition and pheromone production, existing dissection data from trapping studies implied that at least some trap-captured weevils were capable of continued pheromone release. Thus, the possibility of weevils continuing to release pheromone in traps was investigated by monitoring pheromone production by individual weevils following their removal from food. Pheromone production during the 24-h collection period immediately prior to food removal averaged 46 µg. Of the 13 pheromone-producing weevils, 12 weevils continued to release pheromone during first 24-h starvation period, but the average pheromone production level during this period dropped to 10 µg. During the second day of starvation, only 5 weevils continued to release pheromone and average daily pheromone production was reduced to 3 µg. In comparison, daily pheromone production by weevils with continued access to food varied little throughout the study period. These results clearly demonstrate that pheromone-producing weevils can continue to release pheromone in the absence of a host. This previously unrecognized capability could represent an important source of variation in trapping studies, particularly those involving comparisons of traps or lures.

Introduction

The pheromone trap is a key component of management and eradication programs directed against the boll weevil, *Anthonomus grandis* Boheman, but trap data are difficult to interpret because of the day-to-day variability in captures and the inability to identify the source of captured weevils. An extensive trapping study was initiated in 2000 to provide some insight on the source of captured weevils by characterizing and comparing the seasonal physiological condition of trap-captured weevils to those infesting the standing cotton crop (unpublished data). One of the morphological characters examined in the study was male accessory gland condition, which was recently shown to be strongly correlated with pheromone production (Spurgeon and Marshall 2000, Spurgeon 2001, Spurgeon 2003). During dissections of weevils in the trapping study, we observed that a substantial proportion of trap-captured weevils contained accessory glands indicative of those associated with pheromone production. It is generally accepted that boll weevils require access to a suitable food source to produce pheromone. However, the dynamics of cessation of pheromone production, as may occur when a weevil is captured in a trap or otherwise isolated from its host, have not been examined. Herein, we report preliminary observations regarding the influences of host removal on continuation of weevil pheromone production.

Materials and Methods

Weevil Source

Adult boll weevils were reared from oviposition-punctured squares collected from commercial cotton fields. Collected squares were placed in screened cages, which were held in an environmental chamber at 29.4 ± 1°C and a 13:11 (L:D) h photoperiod. Beginning 5 or 6 d after collection, squares were checked daily for pupae. Harvested pupae were held in groups of 35 – 50 on a thin layer of moistened vermiculite contained in a Petri Plate. Pupae were held under the same environmental conditions as the infested squares, and were examined at least once daily for the presence of adults. Newly-eclosed adults were sexed using the method of Sappington and Spurgeon (2000), and the males were weighed after they were sclerotized sufficiently to permit walking. Only males weighing > 10 mg were used in the study.

Pheromone Collection Apparatus

The headspace collection vessels were 120-ml wide-mouth glass bottles (Qorpak, Bridgeville, PA) capped with Teflon-lined lids. Two holes (1.15-cm diam.), separated by 2.5 cm, were drilled in the middle portion of the lids to accommodate 1.1-cm to 0.6-cm Teflon-lined stainless steel reducing unions (Swagelock, Solon, OH). One of the unions was used to fit a collection column (collect pheromone) and the other was used to fit a trap column (remove volatiles from incoming air) to the vessel lid. Both columns (Supelco, Bellefonte, PA) were 17.8 cm long with an outside diameter of 0.6 cm, and were packed with a 5-cm bed of Super Q resin (Alltech Associates, Deerfield, IL) held in place by plugs of fused silica wool. The ends of the collection column were offset 1.5 – 2.0 cm above the ends of the trap column in each collection vessel to enhance sampling of the entire vessel volume.

The collection columns of eight vessels were connected to a manifold. Air was drawn through the manifold by a diaphragm pump, and flow rates through each vessel were regulated to about 1 liter/min. The entire apparatus was housed in a fume hood lined with foam insulation, which was maintained at 27 ± 2°C with a thermostatically-controlled electric heater. A 13:11 (L:D) h photoperiod was provided by two 40-W fluorescent bulbs suspended from the ceiling of the fume hood and controlled by an electric timer.

At the end of the each pheromone collection period, pheromone was eluted from the collection columns using GC grade methylene chloride. The first 1 ml of eluant from each collection column was captured directly into a GC sample vial, and each vial was immediately capped with a Teflon-lined lid. Sample vials were mixed by agitation prior to analysis on a Shimadzu 17A GC (Shimadzu Scientific Instruments, Columbia, MD) equipped with 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 from each sample vial 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 maintained at 60°C for 7 min, increased to 110°C at 25°C/min and maintained for 4 min, increased to 150°C at 20°C/min and maintained for 5 min, and finally increased to 300°C at 30°C/min and maintained for 8 min, for a total program time of 33 min. Pheromone contents of samples were calculated using GC Real Time Analysis Software, and were based on areas under component peaks in reference to corresponding areas generated by external standards of known concentrations of Grandlure (ISP Fine Chemicals, Columbus, OH). The four pheromone components were totaled for each column, and estimates from the two injections were averaged before statistical analysis. Spurgeon and Marshall (2000) and Spurgeon (2003) found that only about 5% of the total pheromone produced was present in the frass. Therefore, we made no effort to recover pheromone from the frass in this study.

Experimental Procedure

In each replication of the experiment, 10 to 12 newly-eclosed male weevils were transferred individually to 100- by 15-ml Petri plates. Because Spurgeon (2003) reported daily pheromone production continued to increase through the ninth day of adulthood, a 9-d preconditioning period was used to promote a high level of pheromone production. During this period, each weevil was provided a freshly-picked square (6 – 7 mm diameter with intact bracteoles) each day and a 1-cm length of cotton dental wick saturated with water. Squares were replaced before 1000 h (CDT), and the cotton wicks were replenished with water as needed. Weevils were held at 29.4 ± 1°C with a 13:11 (L:D) h photoperiod throughout the feeding period.

Beginning on the morning of the 10th day of adulthood (when weevils were 9-d-old), eight weevils were randomly selected and transferred individually to the vessels for pheromone collection. Pheromone was collected over three consecutive 24-h periods after introduction to the vessels (hereafter referred to as Day 0, 1, and 2). At the beginning of the first collection period (Day 0), each weevil was provided a fresh square (6 – 7 mm diameter) and water in a 4-ml glass vial closed with a cotton wick. Pheromone production was estimated during this initial period to detect any differences between the respective groups of weevils that existed before experimental treatments of "continued feeding" or "food removed" were assigned. At the beginning of the second 24-h collection period, weevils were randomly assigned to the feeding treatments. Those designated as "continued feeding" were provided a fresh square each day for the remaining collection periods, while those assigned to the "food removed" treatment were denied access to food. Weevils assigned to the "food removed" treatment were, however, provided a 4-cm by 4-cm section of crumpled paper toweling as a resting site to prevent continuous walking. At the end of each collection period, weevils were transferred to new vessels equipped with new water vials, paper refuges, and collection and trap columns.

Statistical Analysis

Weevils that died during the experiment or did not produce pheromone during the initial 24-h collection period (Day 0) were excluded from analysis. Daily pheromone production was analyzed by repeated-measures mixed-model analysis of variance using the SAS procedure PROC MIXED (SAS, ver. 8.02, SAS Institute, Cary, NC). Fixed effects included feeding treatment, collection period, and their interaction. Random effects included replication and the replication-by-feeding treatment interaction. Collection period was the repeated factor, and corrected degrees of freedom were obtained using the DDFM=KR option in the MODEL statement. Based on preliminary analyses a heterogeneous autoregressive covariance structure (TYPE=arh(1) option of the REPEATED statement) was used in the analysis. Least-square means corresponding to levels of fixed effects were compared using the ADJUST=TUKEY option of the LSMEANS statement, and the effects of feeding treatment within each collection period were examined using the SLICE option.

Results and Discussion

Only 5 of the 32 weevils introduced into the study died or did not produce pheromone during the initial (Day 0) baseline pheromone production period. Thus, daily pheromone production estimates for 27 weevils were available for analysis. On average, weevils provided continued access to squares produced greater quantities of pheromone than did weevils denied access to squares after the initial day of pheromone collection (49 ± 3.2 and 20 ± 2.7 µg, respectively) (F = 47.69; df = 1, 47; P <0.001). Differences were also detected among the three days of pheromone collection (F = 10.79; df = 2, 26.9; P <0.001),

but temporal changes in pheromone production differed between the two feeding treatments (treatment-by-day interaction, $F = 21.20$; df = 2, 26.9; $P < 0.001$). Further examination of the treatment-by-day interaction with the SLICE option indicated pheromone production between weevils assigned to the "continued feeding" and "food removed" treatments were similar on the initial day (Day 0) of pheromone collection ($F = 0.08$, df = 1, 41.1; $P = 0.777$) (Table 1). However, differences between the two treatments were observed on the subsequent days (Day 1, $F = 70.73$; df = 1, 21; $P < 0.001$ and Day 2, $F = 125.49$; df = 1, 16.8; $P < 0.001$). Results provided by the SLICE option also indicated that the amounts of pheromone produced by weevils in the "continued feeding" treatment varied little among days of collection ($F = 2.04$; df = 2, 18.4; $P = 0.159$), while levels of pheromone production by weevils denied continued access to food generally declined with increasing time of starvation ($F = 24.85$; df = 2, 7.86; $P < 0.001$). Consequently, differences in pheromone production observed between the two feeding treatments were primarily caused by the sharp decline in pheromone production associated with isolation of weevils from their food. In addition to differences in the amounts of pheromone produced, differences were also observed between feeding treatments in the proportions of weevils producing measurable quantities of pheromone. All weevils that produced pheromone during the initial collection period and that were provided continual access to squares, continued to produce pheromone for the duration of the experiment. In contrast, of the 13 pheromone producing weevils observed in the "food removed" treatment, 12 weevils continued to produce pheromone on the first day of starvation, but only 5 weevils produced pheromone during the second day of starvation.

A sound understanding of the chemical ecology of the boll weevil, especially with regard to the production of and response to pheromone, is important to continued efforts to improve pheromone traps, lures, and the interpretations of trapping data. Existing dissection data from trapping studies implied that at least some captured weevils were capable of continued pheromone release (unpublished data). Capture of such weevils in evaluations of trap or lure designs could effectively mask the true differences between experimental treatments. The full extent of this potentially confounding factor may depend on the numbers of pheromone-producing weevils captured, and the amount and duration of continued pheromone release following capture. Nonetheless, our results illustrate a previously unrecognized source of variation that should be considered in pheromone trapping studies, particularly those involving evaluation or comparison of new traps or lures. Additional research will be necessary to determine maximum duration over which weevils may produce pheromone in the absence of food. However, in the absence of such data, our results are sufficient to accentuate the need to conduct trap or lure evaluations during periods when the opportunity to capture pheromone-producing weevils is minimized, such as before the initiation of square production in cotton or after harvest.

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Table 1. Changes in daily pheromone production (mean ± SE) of 9-d-old boll weevils initially fed (Day 0 baseline) and subsequently denied access to squares (food removed) for 2 days compared with weevils having continued access to squares (continued feed).

		Daily pheromone production (μg)		
Feeding treatment	***n***	**Day 0 (baseline)**	**Day 1**	**Day 2**
Continued feed	14	43 ± 4.4	54 ± 5.0	48 ± 3.9
Food removed	13	46 ± 6.1	10 ± 1.6	3 ± 1.0