N O T E

Continued Pheromone Release by Boll Weevils (Coleoptera: Curculionidae) Following Host Removal[1]

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Pheromone traps are a key component of management and eradication programs directed against the boll weevil, *Anthonomus grandis grandis* Boheman, but trap data remain difficult to interpret because of the day-to-day variability in captures. 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 interpretations of trapping data. It is widely accepted that male boll weevils require a suitable food source to initiate and maintain high levels of pheromone production. However, the dynamics of cessation of pheromone production, as may occur when a weevil is captured in a trap or otherwise isolated from a food, have not been thoroughly investigated. Hardee (1970, Contrib. Boyce Thompson Inst. 24: 315–322) concluded boll weevils could continue to release relatively small amounts of pheromone up to 24 h after removal from food. However, this conclusion was based on the response of weevils in olfactometer studies—not direct measurements of pheromone.

We conducted an extensive trapping study 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 (*Gossypium hirsutum* L.) crop (C.S., unpubl. data). One of the morphological characters examined in the study was male accessory gland condition, which is strongly correlated with pheromone production (Spurgeon 2001, Proc. Beltwide Cotton Conf., Pp. 1138–1140). During dissections of weevils in the trapping study, we observed a substantial proportion of trap-captured weevils contained accessory

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glands indicative of those associated with pheromone production. Consequently, we speculated that some male weevils captured in traps may continue to release pheromone. Such an occurrence could potentially confound trapping results, particularly those involving evaluations of new lure or trap designs. The objective of our study was to confirm whether boll weevils could continue to release pheromone in the absence of food. Herein, we present information on the duration and quantities of pheromone released by boll weevils following their removal from a food source.

Adult boll weevils used in the study 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. Squares were checked 5 or 6 d after collection and daily thereafter for pupae. Harvested pupae were held in groups of 35 to 50 on a thin layer of moistened vermiculite contained in a petri dish (100 × 15 mm). 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, Ann. Entomol. Soc. Am. 93: 610–615), and the males were weighed after they were sclerotized sufficiently to permit walking. Only males weighing >10 mg were used in the study. Ten to 12 newly eclosed male weevils were transferred individually to petri dishes (100 × 15 mm). Because Spurgeon (2003, Environ. Entomol. 32: 31–38) reported daily pheromone production continued to increase through the ninth day of adulthood, a 9-d feeding period was used to promote a high level of pheromone production. During this period, each weevil was provided a freshly picked square (6- to 7-mm diam prefloral bud with intact bracteoles) each day and a 1-cm length of cotton dental wick saturated with water. Squares were replaced before 10:00 a.m., 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 glass vessels for pheromone collection. Pheromone was collected over five consecutive 24-h periods after introduction to the vessels (hereafter referred to as Day 0, 1, 2, 3, and 4), using materials and procedures described by Spurgeon and Suh (2007, J. Entomol. Sci. 42: 250–260). At the beginning of the first collection period (Day 0), each weevil was provided a fresh square (6- to 7-mm diam) 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 that existed between the respective groups of weevils before experimental treatments of "continued feeding" or "food removed" were assigned. At the beginning of the second 24-h collection period, four weevils were randomly assigned to each feeding treatment. 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 × 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 pheromone collection vessels equipped with new water vials, paper refuges, and collection and trap columns.

Table 1. Changes in daily pheromone production (μg, mean ± SD) of 9-d-old boll weevils initially conditioned to produce high levels of pheromone (Day 0 baseline) and subsequently denied or provided continued access to cotton prefloral buds (squares) for 4 d.

	Feeding Regime	
Days	**Continued Feeding**	**Food Denied**
0 (baseline)	45.3 ± 14.49	50.2 ± 14.71
1	57.4 ± 17.88	13.1 ± 7.72
2	51.3 ± 13.92	1.7 ± 2.70
3	55.2 ± 15.43	0.2 ± 0.41
4	56.8 ± 14.15	<0.1 ± 0.09

The amount of pheromone produced by each weevil during each daily collection period was determined by gas chromatography using the exact equipment and methods previously described (Spurgeon and Suh 2007). The experiment was conducted a total of four times (trials) with eight weevils per trial. Weevils that died during a pheromone collection period or did not produce >20 μg of pheromone during the initial 24-h collection period (Day 0) were excluded from analysis. Because of the relatively small sample of weevils available in each trial, data from all trials were pooled prior to statistical analysis. The amounts of pheromone produced by weevils at Day 0 were compared between treatment groups using the PROC TTEST statement of SAS (SAS Institute 2012, SAS Institute, Cary, NC). The mean ± SD amount of pheromone measured for each treatment group during each pheromone collection period was calculated using the PROC MEANS statement of SAS (SAS Institute 2012, SAS Institute, Cary, NC).

Of the 32 weevils introduced into the study, eight weevils either died during a pheromone collection period or did not produce sufficient amounts of pheromone (>20 μg) during the initial baseline pheromone collection period (Day 0). Thus, daily pheromone production estimates of 13 and 11 weevils were available for the "continued feeding" and "food removed" treatments, respectively. Comparisons of baseline pheromone collections (Day 0) indicated both groups of weevils initially released similar quantities of pheromone ($t = -0.82$; df = 22; $P = 0.419$). All weevils in the "continued feeding" treatment continued to release high levels of pheromone during the subsequent 4 d of pheromone collection (Table 1). In contrast, the pheromone quantities and percentages of weevils releasing pheromone in the "food removed" group declined rapidly with each day of starvation (Table 1). All 11 weevils denied food continued to release small quantities of pheromone during the first day of food removal, but only 7 (64%) and 3 (27%) of these weevils continued to release pheromone on the second and third days of starvation, respectively (Table 1). By the fourth day following food removal, only 1 weevil (9%) released measurable quantities of pheromone. Based on those weevils that released

pheromone on the first, second, third, and fourth days of food removal, pheromone quantities averaged 13.1, 2.6, 0.8, and 0.3 μg/weevil, respectively.

Hardee (1970) estimated pheromone production was reduced by approximately 50% and 90% within 1 and 24 h, respectively, following removal of a food source. However, these estimates were based on the numbers of weevils responding to pheromone-producing weevils in olfactometer studies. Based on our direct measurements of pheromone released by weevils, pheromone production was reduced by approximately 75% and 97% on first and second days following food removal, respectively (Table 1). By the third and fourth days of starvation, pheromone quantities averaged $<1\%$ of the initial baseline pheromone levels. Our results conclusively demonstrate that pheromone-producing boll weevils can continue to release small quantities of pheromone for at least 4 d following their removal from a food source. However, the full extent of this potentially confounding factor in trapping studies will likely depend on the numbers of pheromone-producing weevils captured and the elapsed time when weevils last fed prior to their capture in traps. Nonetheless, our results illustrate a previously unrecognized or disregarded occurrence that could provide a source of variation in pheromone trapping studies. As such, our findings suggest new trap or lure evaluations, when possible, should be conducted 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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