CORRELATION BETWEEN ACCESSORY GLAND CONDITION AND PHEROMONE PRODUCTION BY MALE BOLL WEEVILS

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Abstract

Improved understanding of the dynamics of boll weevil pheromone production would facilitate ecological interpretation of weevil dispersal patterns and development of improved trapping systems. Methods for measuring pheromone production by individual male weevils were recently developed in this laboratory. Initial observations suggested a correlation between accessory gland condition and pheromone production. A system of classifying accessory glands was devised and used in a new study to further examine this relationship. Accessory glands were divided among four classes. Class 1 and 2 glands were transparent, and class 3 and 4 glands were more translucent. Class 1 glands were smaller and less apparent than class 2 glands. Similarly, class 3 glands were smaller than class 4 glands, which dominated the volume of the abdomen. Relationships between pheromone production and accessory gland classes were examined by analysis of variance and simple linear regression. Pheromone production varied considerably within accessory gland class, but weevils with class 3 or 4 glands produced substantial amounts of pheromone while weevils with class 1 or 2 glands produced little or no pheromone. Regression analyses also indicated a relationship between accessory gland condition and pheromone production. Observed relationships between gland condition and pheromone production are sufficient to permit the identification of pheromone-producing weevils from field or trap collections. This ability should be useful in field studies of the role of pheromone in the ecology and distribution of boll weevil populations.

Introduction

The boll weevil pheromone trap is an indispensable tool for detecting and monitoring populations in organized boll weevil management programs. However, our knowledge of the dynamics of pheromone production by male weevils is incomplete. A more thorough understanding of this phenomenon would improve our ability to interpret seasonal trap capture and dispersal patterns, and may lead to development of improved trapping systems.

Traditional methods of estimating boll weevil pheromone production rely on extraction of pheromone from frass produced by large numbers of weevils (Hedin et al. 1974, McGovern et al. 1976, McKibben et al. 1976, Dickens et al. 1988). Use of such methods precludes examination of the dynamics of pheromone production by individual weevils. More recently, simple and effective methods for measuring pheromone production of individual male weevils were developed (Spurgeon and Marshall 2000). Use of these new methods yielded two particularly notable results; a greater capacity for pheromone production by the male weevil than was previously recognized, and that a relatively small proportion of the total pheromone produced was contained in the frass. During development of the new pheromone measurement methods a correlation between accessory gland condition and pheromone production was noted (unpublished observations). Existence of such a relationship provides a valuable tool for trapping studies when morphology of captured weevils is examined. The objective of the present study was to further examine this relationship.

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Materials and Methods

Pheromone Collection Apparatus and Analysis

Pheromone was collected and analyzed by methods similar to those described by Spurgeon and Marshall (2000). Qorpak neckless wide-mouth bottles (120-ml, Qorpak, Bridgeville, PA) with teflon-lined lids were used as headspace collection vessels. The lid of each vessel was penetrated by two 29/64-inch holes which were fitted with 3/8-inch to 1/4-inch Swagelock teflon reducing unions (Swagelock, Solon, OH). The 3/8-inch side of the union was placed on the underside of the lid to provide an air-tight seal. The unions were used to attach volatile collection and trap columns to the collection vessel. Each collection vessel was equipped with two 8 X 1/4 inch (length X o.d.) columns (Envirochem, Lancaster, PA); a trap column to remove volatiles from incoming air, and a collection column to collect pheromone from air drawn through the vessel. Columns were packed with a 2-inch bed of Super Q resin (Alltech Associates, Deerfield, IL) held in place by glass wool. The collection columns of 8 vessels were connected to a manifold through which air was drawn by a diaphragm vacuum pump set at 20 inches of mercury. Air flow rates through the vessels was regulated at about 1 liter/min by individual flow meters between the collection columns and the manifold. The collection apparatus was housed in a fume hood which was maintained at about 27.5"1°C by a thermostatically controlled oscillating heater. The hood was lighted by two 40-W flourescent bulbs controlled by an electric timer to provide a photoperiod of 13:11 [L:D] h.

Following a 24-h collection period, pheromone was eluted from each collection column directly into gas chromatography sample vials with a volume of methylene chloride (GC grade) sufficient to result in 1.0 ml eluant volumes. Each vial was mixed by agitation and duplicate injections of a 1-:l aliquot were analyzed by GC. Pheromone content of each sample was calculated from the average of the two injections. In addition, frass was brushed from the food source (cotton square) into the collection vessel and extracted within the vessel by gentle swirling with 1.0 ml of GC-grade hexane. Pheromone content of the frass was estimated by GC 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 X 0.25 mm i.d., J&W Scientific, Folsum, CA) and a flame-ionization detector. The temperature program for analysis was as follows: Injector temperature 200ºC; detector temperature 300ºC; flow rate 2.0 ml/min; initial column temperature 60ºC maintained for 7 min, increased to 110ºC at 30ºC/min, decreased to 100ºC at 2ºC/min and maintained for 5 min, and finally increased to 300ºC at 10ºC/min and maintained for 10 min. Total GC run time was 48.67 min. Concentrations of pheromone components were calculated by comparing the areas under peaks from the samples to corresponding areas for standards of known concentration (ISP Fine Chemicals, Columbus, OH). Total quantity of pheromone eluted from a column or extracted from a frass sample was calculated by adding the quantities of the 4 individual components.

Collection of Pheromone from Boll Weevils

Adult boll weevils were reared from field-collected infested squares. Oviposition-punctured squares were collected from cotton plants and held in screened cages within an environmental chamber at 29.4°C and with a 13:11 [L:D] h photoperiod. Beginning 5 or 6 d after square collection, squares were examined for the presence of pupae. Pupae were removed from the squares, placed in groups of 35 to 50 on a thin layer of moistened vermiculite within 15 X 100 mm petri plates, and returned to the environmental chamber. The plates were examined daily for adult eclosion. Newly eclosed adults were sexed using the criteria of Agee (1964), as described by Sappington and Spurgeon (2000). When males were sufficiently sclerotized to walk, they were weighed and placed in separate collection vessels.

Each day each male weevil was supplied a fresh, uninfested square with bracts intact and measuring 6 to 7 mm in diameter. Also, water was supplied in a 7.5-ml plastic vial closed with a cotton wick. Trap columns, collection columns, and collection vessels were replaced after 5 days, and pheromone was collected for 24 h. At the end of the collection period, pheromone was eluted from the collection columns and extracted from the frass, and duplicate aliquots were analyzed as previously described. Four repetitions of the experiment, each involving 8 male weevils, were conducted between 12 July and 11 August 2000. One weevil died before the end of the pheromone collection period, and was not included in the analyses.

Accessory Gland Ratings

Accessory glands were rated by dissection under a stereomicroscope at 15-20X immediately following the pheromone collection period. Based on previous observations, accessory glands were assigned to one of four classes according to their color and relative size. Class 1 glands are very small and transparent. These glands are inconspicuous, and considerable skill and effort is required to locate them in weevils containing substantial fat bodies. Class 2 glands are larger than class 1 glands but are also transparent (Fig 1). These glands are usually large enough to be readily observable in all but the fattest weevils. Class 3 glands are slightly to considerably larger than class 2 glands and translucent because of a gray, semi-solid, almost granular material in part or most of their length (Fig 2). Class 4 glands are very large and their presence appears to dominate the volume of the abdomen. This class also contains the gray material, typically throughout the length of the glands.

Statistical Analyses

For each statistical test, duplicate analyses were conducted for pheromone collected from the headspace, pheromone extracted from frass, and total pheromone. Initial analyses used simple linear regression (PROC REG; SAS Institute 1988) to examine potential relationships between adult weight and pheromone production of weevils within accessory gland classes. Results of these analyses were used to determine whether pheromone production should be corrected for adult weight in subsequent analyses. The influence of accessory gland condition on pheromone production was examined by two methods. Because the gland classification system was categorical rather than quantitative, differences in pheromone production among gland classes were examined by analysis of variance using the SAS procedure PROC GLM (SAS Institute 1988). The analysis of variance model included repetition of the experiment and gland class as main effects, and the repetition by gland class interaction. When the interaction term was not significant and its omission reduced the error mean square, it was removed from the model. Means of the main effects were separated using the REGWQ option of PROC GLM. The relationship between gland condition and pheromone production was further explored by simple linear regression using pheromone production as the response variable and accessory gland class as the independent variable (PROC REG; SAS Institute 1988). Data of all experimental repetitions were pooled for the regressions.

Results and Discussion

Pheromone production over the 24-h collection period varied among the 31 weevils from 0–245.8 µg, and averaged 82.0 µg. Only about 2% of the total pheromone was obtained from frass extractions. These results were generally consistent with the report of Spurgeon and Marshall (2000), who reported that pheromone from the frass constituted <6% of the total pheromone collected.

Regressions of pheromone production on weevil weight indicated a significant relationship only for pheromone extracted from the frass of weevils with class 2 glands (F=924.38; df=1, 2; P=0.021; r^2=0.999). Because only 3 weevils were included in this analysis, and results of other analyses failed to indicate similar relationships, pheromone production estimates were not corrected for adult weight in subsequent analyses.

Pheromone from headspace collections (F=7.44; df=3, 22; P<0.01) and total pheromone production (F=7.06; df=3, 22; P<0.01) differed significantly among repetitions of the experiment (Table 1). Such differences were not detected for pheromone extracted from the frass (F=0.51; df=3, 24; P=0.68; Table 1). Spurgeon and Marshall (2000) observed similar variation among experimental repetitions. In fact, square size was more stringently controlled in the present study in hopes of minimizing this source of variation. Genetic differences among individual weevils is a likely source of at least part of the variability among repetitions, but further studies will be required to distinguish the effects of differences among weevils from the potential effects of subtle variations in food quality.

All measures of pheromone production indicated differences associated with accessory gland condition (Table 2; headspace, F=12.76; df=3, 22; P<0.01; frass, F=3.89; df=3, 24; P=0.02; total pheromone, F=12.76; df=3, 22; P<0.01). In addition, the repetition by gland class interactions were significant for headspace collections (F=4.64; df=2, 22; P=0.02) and total pheromone (F=4.36; df=2, 22; P=0.03), indicating some degree of difference in the relationship between gland class and pheromone production among experimental repetitions. In general, class 3 and 4 accessory glands (containing the gray material) were associated with high levels of pheromone production while classes 1 and 2 were associated with little or no production of pheromone. Although the analyses did not indicate differences in pheromone production between gland classes 3 and 4, these classes are easily distinguished from classes 1 and 2, and this distinction may provide a useful diagnostic tool in ecological studies where field or trap collected weevils are dissected.

Despite the discrete nature of the accessory gland classes, regression analyses indicated a significant relationship between gland class and pheromone production for headspace collections (headspace =53.97[gland class]-78.17; F=21.44; df=1, 29; P<0.01; r^2=0.425), frass extractions (frass=1.25[gland class]-1.99; F=10.21; df=1, 29; P<0.01; r^2=0.260), and total pheromone (total pheromone=55.23[gland class]-80.16; F=22.02; df=1, 29; P<0.01; r^2=0.432). Fit of the respective models was generally not sufficient to provide a useful level of predictive power. This lack of fit was expected given the subjective nature of the gland classes, but considerable variability in the relationship was also contributed by the wide ranges of pheromone production rates among weevils within gland classes 3 and 4.

Pheromone production in the present study was generally higher than that observed by Spurgeon and Marshall (2000). Some of this increase may have resulted from increased proficiency in executing the methods, and from the different photoperiod used in the present study. Several recent reports have emphasized the prominent influence of diet on boll weevil reproductive development (Spurgeon and Raulston 1996, 1997, 1998), and Spurgeon and Esquivel (2000) found square size to be an influential factor. It seems likely that these same factors may influence pheromone production. If so, differences in the sizes of squares fed in the respective studies may also provide an explanation for observed differences in pheromone production. Further examination of this potential relationship could yield valuable insight into the ecology of the boll weevil.

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Table 1. Mean daily pheromone production by 6-day old male boll weevils in 4 experimental repetitions.

Experiment Repitition	*n*	Pheromone in Headspace (µg)[a]	Pheromone in Frass (µg)[a]	Total Phermone (µg)[a]
12 July	8	92.29 a	1.53 a	93.83 a
21 July	7	88.89 a	1.76 a	90.66 a
31 July	8	40.37 b	1.80 a	42.17 b
11 Aug	8	100.55 a	1.70 a	102.25 a

[a]Means within a column followed by the same letter are not significantly different ("=0.05; REGWQ multiple range test)

Table 2. Influence of accessory gland condition on mean daily pheromone production by 6-day old male boll weevils .

Accessory Gland Class[a]	*n*	Pheromone in Headspace (µg)[b]	Pheromone in Frass (µg)[b]	Total Phermone (µg)[b]
1	2	0.00 b	0.00 b	0.00 b
2	3	1.81 b	0.23 b	2.04 b
3	21	84.81 a	1.65 ab	86.46 a
4	5	140.30 a	3.44 a	143.74 a

[a]Gland classes are defined as: 1, very small, colorless, and inconspicuous; 2, small and colorless but apparent; 3, moderate in size and translucent; 4, large, dominating the abdomen, and translucent.
[b]Means within a column followed by the same letter are not significantly different ("=0.05; REGWQ multiple range test)

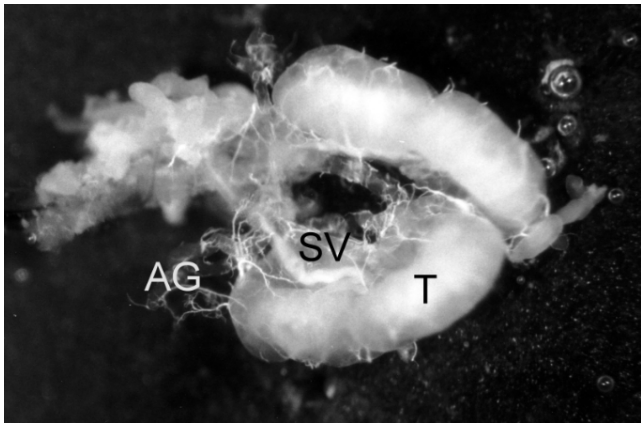

Figure 1. Reproductive system of the male boll weevil illustrating class 2 accessory glands; T, testis; SV, seminal vesicle; AG, accessory gland.

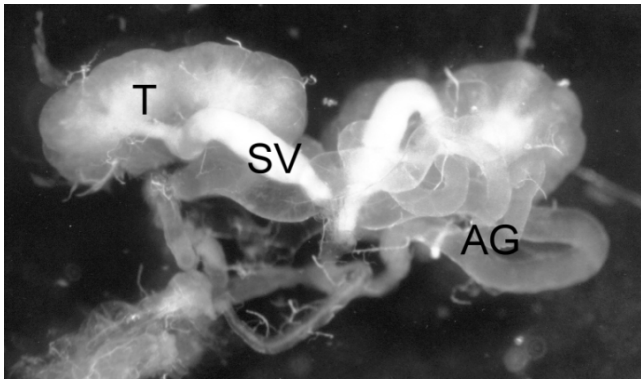

Figure 2. Reproductive system of the male boll weevil illustrating class 3 accessory glands; T, testis; SV, seminal vesicle; AG, accessory gland.