MONITORING PHEROMONE PRODUCTION OF INDIVIDUAL BOLL WEEVILS

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Abstract

The boll weevil pheromone trap is a critically important tool in boll weevil population monitoring and management, but much remains to be learned regarding the factors influencing pheromone production by male weevils and their repercussions to trapping efforts. Standard methods of measuring boll weevil pheromone production rely on relatively large groups of weevils and assume that most or all of the pheromone produced is present in the frass. We devised and evaluated a technique to monitor pheromone production by individual weevils, using an adsorbent to collect pheromone from the air around the weevil (headspace). Pheromone recovery efficiency of the method was about 95%. Pheromone production indicated by frass extractions was similar to that in previous reports. However, measurements of pheromone from the headspace indicated that pheromone in the frass represents a small fraction of the total pheromone produced, and that the boll weevil can produce a much larger amount of pheromone than was previously recognized. In addition, use of our technique allowed detection of pheromone production at an earlier weevil age than is typically reported. Our techniques appear uniquely suitable for monitoring boll weevil pheromone production and should prove invaluable in further investigations of the ecology and management implications of boll weevil pheromone production.

Introduction

The boll weevil pheromone trap is widely and routinely used for population detection and monitoring, and has become an indispensable component of organized boll weevil management programs. However, much remains to be learned regarding the factors controlling pheromone production by male weevils, and the implications of these factors to trapping efforts. Early efforts to measure pheromone production relied on weevil response to fed males in laboratory bioassays or traps in the field (Hardee 1970, Klassen and Earle 1970, Earle and Leopold 1975), but these methods were decidedly qualitative. Subsequently, pheromone production was estimated by more direct, gas chromatographic (GC) methods (Hedin et al. 1974, McGovern et al. 1976, McKibben et al. 1976, Dickens et al. 1988). Although these techniques allowed quantitative estimation of pheromone production, the procedures used introduced at least 3 major sources of potential error: 1) they involved collection of frass from relatively large numbers of weevils, and thus did not provide estimates of variation in pheromone production among weevils within a treatment, 2) they assumed that most or all of the pheromone produced was present in the frass, and 3) they failed to account for pheromone that volatilized between frass collection periods. In addition, frass produced over several days was pooled to facilitate analysis in some studies (McGovern et al. 1976, Earle et al. 1978, Villavaso et al. 1983), therefore daily patterns in pheromone production could not be reliably examined.

Collection of pheromone from the air surrounding the weevil (headspace) seems a promising means of directly examining pheromone production. Chang et al. (1988) used this approach to measure pheromone produced by weevils on different cotton genotypes. However, they estimated the pheromone produced by replicates of 60 or 100 weevils. Thus, variation in response of individual weevils was not examined. The development of techniques to estimate pheromone produced by single male weevils would provide a valuable tool for further investigations of the impacts of age, physiological condition, food source, and mating status on pheromone production. Improved understanding of these factors could lead to development of improved pheromone lures and better understanding of the role of boll weevil pheromonal communication in the agroecosystem. Our objective was to devise and evaluate a method for monitoring boll weevil pheromone production of individual male weevils.

Materials and Methods

Pheromone Collection Apparatus

Final configuration of the pheromone collection apparatus was determined in preliminary experiments. 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. Each column was packed with a 2-inch bed of Super Q resin (Alltech Associates, Deerfield, IL) held in place by glass wool. The collection columns of up to 8 vessels were connected to a manifold through which air was

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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 under continuous incandescent light in a vacant fume hood which was maintained at about 29±2˚C by a thermostatically controlled oscillating heater.

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.

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. Injector temperature was 200˚C, detector temperature was 300˚C, and flow rate was 2.0 ml/min. The initial column temperature of 65˚C was maintained for 7 min after injection, increased to 110˚C at 70˚C/min, decreased to 100˚C at 4˚C/min and held at that temperature for 5 min, and finally increased to 300˚C at 70˚C/min and maintained at that temperature for 5 min, giving a total elapsed time of 23 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.

Determination of Collection Efficiency

Collection efficiency of the apparatus was determined by recovery of pheromone volatilized from sections of commercial boll weevil pheromone lure (Plato Industries, Houston, TX). Two plugs were cut from each of 4 lures using a #3 cork borer. One of each pair of plugs was used to determine pheromone recovery while the other was used as a control for determination of the original pheromone content of the lure. The control plug was placed in a GC sample vial and stored at 4˚C until analysis.

Sample plugs were placed in separate collection vessels and headspace volatiles were collected for 24 h. At the end of the collection period, pheromone was eluted from the collection columns and analyzed by GC as previously described except that triplicate injections of the 1-µl aliquot were used. Also, both sample and control plugs were extracted with 1.5 ml hexane for 24 h at room temperature. Residual pheromone content of the plugs was calculated by averaging the results of GC analysis of duplicate injections of a 1-µl aliquot of each extract. Total pheromone content of each sample plug was calculated by adding the pheromone recovered from the headspace to the residual pheromone extracted from the lure plug. Percent recovery of the pheromone was calculated by dividing the total pheromone recovered from each sample plug (headspace collection plus extraction; corrected for plug weight) by the pheromone extracted from the respective control plug (corrected for plug weight) and multiplying by 100.

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 (1999), and males were immediately placed in separate collection vessels.

Each male weevil was supplied a fresh, uninfested square with bracts intact and measuring 6 to 9 mm in diameter. Also, water was supplied in a 7.5-ml plastic vial closed with a cotton wick. Squares, water vials, trap and collection columns, and collection vessels were replaced daily for 6 d. Pheromone was eluted from the collection columns and duplicate aliquots were analyzed as previously described. In addition, frass was collected from the collection vessel and the surface of the square and extracted with 1.0 ml of GC-grade hexane. Pheromone content of the frass was estimated based on GC analysis of duplicate injections of a 1-µl aliquot of the extract. Five separate experiments, each involving 8 male weevils, were conducted between 20 July and 16 September 1999. However, among days within experiments sample size varied from 6 to 8 because 1 or 2 weevils died before the end of the 6-d measurement period in all but one of the experiments.

Daily pheromone production was compared among repetitions of the experiment and among weevil ages using the SAS procedure PROC GLM (SAS Institute 1988). The ANOVA model included a term for the experiment by age interaction in addition to the main effects. Means for the main effects were separated using the REGWQ option of PROC GLM (SAS Institute 1988).

Results

Collection Efficiency

Sample and control plugs cut from the pheromone lures averaged 28.66±0.51 mg (0±SE), and the average difference in weight between paired sample and control plugs was <3%. An average of 2.04±0.28 µg of pheromone was eluted from the collection columns, and pheromone retained by the sample plugs averaged 6.84±0.29 µg. Total pheromone recovered from the sample plugs averaged 8.89±0.09 µg compared with 9.37±0.17 µg for the control plugs. Thus, efficiency of recovery averaged 94.98±2.51%. Based on these results, we concluded it was unnecessary to correct estimates of weevil pheromone production to account for efficiency of recovery.

Collection of Pheromone from Boll Weevils

The average daily production of pheromone per male across the entire study was 16.50 µg, with 15.54 µg recovered from the headspace and 0.96 µg extracted from frass. Thus, >94% of the total pheromone recovered was obtained from the headspace. However, average daily pheromone production varied significantly among repetitions of the experiment for headspace collections (F=18.08; df=4,189; P<0.01), frass extractions (F=28.32; df=4,190; P<0.01), and total pheromone (F=19.46; df=4,189; P<0.01) (Table 1).

Pheromone production tended to increase with male age (Table 2). This age effect was reflected in both headspace collections (F=30.24; df=5,189; P<0.01) and frass extractions (F=14.37; df=5,190; P<0.01). Because the majority of pheromone was recovered from the headspace, temporal patterns of total pheromone production were similar to those observed for headspace collections (F=30.12; df=5,189; P<0.01) (Table 2). However, temporal trends in pheromone production were not identical among repetitions of the experiments because the experiment by age interactions were significant for headspace collections (F=4.59; df=20,189; P<0.01), frass extractions (F=5.17; df=20,190; P<0.01), and total pheromone (F=4.74; df=20,189; P<0.01). The significant experiment by age interaction appeared to result from differences among repetitions in the age at which weevils first produced pheromone and the maximal observed level of pheromone production. One group of weevils produced sufficient pheromone for detection in the headspace during the 1st 24 h after eclosion, 1 group produced pheromone by the 2nd day, and remaining groups did not produce pheromone until the 3rd or 4th day. Pheromone was first detected in frass extractions on the 3rd or 4th day.

Discussion

Our estimates of pheromone content of the frass were within the range depicted in earlier reports. Hedin (1976) indicated the average daily production of pheromone in the frass was about 1.3 µg. Villavaso et al. (1983) reported an average daily production of 0.443 µg per male over a 7-d period. Dickens et al. (1988) reported that average daily pheromone production increased through day 5 to about 0.5 µg. In our study, observations over a 6-d feeding period indicated weevils produced an average of 0.96 µg of pheromone per day.

More remarkable than our estimates of pheromone in frass were our estimates of pheromone released into the headspace. Most studies of boll weevil pheromone production assume that most or all of the pheromone is present in the frass. However, Chang et al. (1988) collected pheromone from the headspace and concluded that pheromone production indicated by this method was of similar magnitude to that obtained by extraction of frass in other studies. In contrast, our experiment indicated >94% of the total pheromone collected was obtained from the headspace, and our estimates of total pheromone production were more than an order of magnitude greater than those previously reported. These results clearly indicate that pheromone in the frass reflects only a minor portion of total pheromone production.

By monitoring pheromone in the headspace we were also able to detect pheromone production at weevil ages younger than those previously reported. Using frass extraction techniques, McKibben et al. (1976) found that pheromone production by groups of 50 to 150 weevils was too low for quantitation during the 1st 6 days of age. Hedin (1976) stated that pheromone production was very limited for the 1st 5 days after adult emergence. Also, Hedin et al. (1974) suggested that newly eclosed males were not capable of synthesizing the 2 aldehyde components of the pheromone, and that the aldehydes are not found in the males for the 1st 6 days after emergence. Our techniques permitted the detection of measurable amounts of pheromone as early as the day of eclosion, and we generally detected pheromone in the headspace a day earlier than in the frass.

Another noticeable attribute of our results was the marked variation in pheromone production among repetitions of the experiment, and among weevils within repetitions. At least a portion of this variation may be explained by variations in food quality; several recent reports indicate a prominent influence of diet on boll weevil reproductive characters (Spurgeon and Raulston 1996, 1997, 1998) and pheromone production may be similarily influenced. Alternatively, observed variations in pheromone production may have resulted from genetic or physiological differences among weevils. Because we now have a suitable means of monitoring pheromone production by individual weevils, the contributions of these and similar factors can be easily and unambiguously investigated.

In summary, the pheromone collection apparatus and procedure we devised appear uniquely suitable for monitoring pheromone production of individual male boll weevils. These techniques provide an improved capability to study the various environmental, physiological, and genetic factors influencing pheromone production, and should prove invaluable to further study of the ecology and management implications of boll weevil pheromone production.

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Table 1. Variation among 5 experiments in daily mean pheromone production by single male boll weevils over 6 d as indicated by headspace collections and frass extractions.

Experiment	Pheromone in Headspace (μg)[a]	Pheromone in Frass (μg)[a]	Total Pheromone (μg)[a]
20 July	35.38 a	3.08 a	38.48 a
27 July	8.83 bc	0.69 b	9.52 bc
19 Aug	17.74 b	0.39 b	18.13 b
25 Aug	10.66 bc	0.38 b	11.04 bc
16 Sep	4.01 c	0.21 b	4.22 c

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

Table 2. Variation among weevil ages in daily mean pheromone production by single male boll weevils as indicated by headspace collections and frass extractions.

Weevil Age (d)	Pheromone in Headspace (μg)[a]	Pheromone in Frass (μg)[a]	Total Pheromone (μg)[a]
1	0.54 c	0 b	0.54 c
2	0.14 c	0 b	0.14 c
3	1.81 c	0.30 b	2.12 c
4	15.31 b	1.51 a	16.81 b
5	36.15 a	1.92 a	38.06 a
6	42.11 a	2.21 a	44.32 a

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