



# JH III production, titers and degradation in relation to reproduction in male and female *Anthonomus grandis*

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## Abstract

Juvenile hormone (JH) is necessary for the production of vitellogenin (Vg) in the boll weevil, *Anthonomus grandis*. Occurrence of Vg in this species is typically restricted to reproductively competent females, and is not detected in untreated males. However, the JH analog, methoprene stimulates Vg production in intact males and in the isolated abdomens of both male and female boll weevils (where in each case no Vg is detected without treatment), suggesting that males are competent to produce Vg but are normally not stimulated to do so. Preliminary work indicating that male boll weevil corpora allata (CA) produced little or no JH in vitro suggested that failure of males to produce Vg might be due to very low JH levels compared to females. This study re-examines the question of JH in male boll weevils by determining in vitro production of JH III by male CA during the first 10 days after adult emergence, determining hemolymph JH esterase activity during this same time period and hemolymph JH III titers in adults of both sexes. We also re-examine the ability of isolated male abdomens to produce Vg in response to hormonal stimulation, analyzing the effect of a wide range of methoprene and JH III dosages. Results indicate that male *A. grandis* have circulating JH titers and JH production similar to females. JH esterase activity is slightly but significantly higher in males than females. Vg production by isolated abdomens of both sexes after stimulation with methoprene or JH III was confirmed. Dose response studies indicated that high levels of methoprene were less effective than intermediate doses in stimulating Vg synthesis in both sexes. We conclude that the sexually dimorphic effect of JH on Vg synthesis is not due to differences in JH production or differences in JH titer between the sexes.

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## 1. Introduction

Juvenile hormones (JH) have many roles in insect development and behavior, including control of metamorphosis, control of reproductive development and behavior, and control of migratory and/or social behavior. In boll weevils we have shown that JH is necessary for vitellogenin (Vg) synthesis in females while

a brain factor is necessary for Vg uptake and completion of oogenesis (Taub-Montemayor and Rankin, 1997). Very low to undetectable JH titer is characteristic of reproductive diapause in females. Our early results analyzing in vitro production of JH by corpora allata (CA) cultures seemed to indicate that adult male boll weevils might have very low levels of the hormone at any time. High JH esterase activity was correlated with overwintering success in both sexes in lab and field populations (Taub-Montemayor et al., 1997b), suggesting that high JHE activity may be necessary during diapause to keep JH levels low enough to ensure that no

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reproductive development begins prematurely. Why this should be necessary in males if JH levels were constantly low was an unanswered question. There are also various reports of JH effects in male *Anthonomus grandis* that are difficult to reconcile with a finding of negligible JH production in male boll weevils. For example, Hedin et al. (1982) and Dickens et al. (1988) showed that JH III and JH analog treatment enhanced aggregation pheromone production in intact male boll weevils. And Wiygul et al. (1990) further demonstrated an increase in pheromone production in fat bodies incubated in saline with ATP, JH III, and beta-bisabolol.

The production of Vg in boll weevils is typically restricted to reproductively competent females, and has not been detected in males except in response to JH analog treatment (Taub-Montemayor and Rankin, 1997). The JH analog, methoprene, stimulates Vg production in isolated abdomens of both sexes (where it does not occur without juvenoid treatment) and in intact males (Taub-Montemayor and Rankin, 1997), suggesting that males are competent to produce Vg but are normally not stimulated to do so. Based on our preliminary results indicating negligible JH production by male CA, we suspected that the sexual dimorphism with respect to Vg production in boll weevils might be due to normal differences in levels of JH between males and females. There are reports of such differences in other species. For example, sexually dimorphic production of JH has been carefully documented in *Diptera punctata* using a series of implantation and allatectomy studies to determine the role of JH in sexual behavior of both sexes (Tobe and Stay, 1977; Tobe et al., 1979; Szibbo and Tobe, 1982). In *D. punctata*, males have both smaller glands and less synthetic ability (Szibbo and Tobe, 1982). Production of JH by female CA closely tracks the gonotrophic cycle (ranging from 5 to 10 pmol JH/h after ecdysis and oviposition increasing to > 50 pmol JH/h during oocyte growth) (Tobe and Stay, 1977; Tobe, 1980) while synthesis rates in males remain at a constant, relatively low level (< 8 pmol JH/h) (Tobe et al., 1979).

In an interesting variation on the traditional model of production of JH by the CA, the male in a number of species of moths lack the enzyme JH acid methyltransferase in their CA and thus produce JH acid rather than JH. JHA is converted in the male accessory glands to JH and may be passed to the female via the spermatophore (Bhaskaran et al., 1988). Similarly, the male accessory glands of *Aedes aegypti* synthesize JH III from L-[Methyl-<sup>3</sup>H] methionine, indicating a complete JH III biosynthetic pathway in these glands although in this case the male CA also synthesize JH III. Cusson et al. (1993) found that products of in vitro incubation of CA from armyworm moths, *Pseudaletia unipuncta* differ by sex both qualitatively and quantitatively and also vary with age and rearing conditions. Male CA produce and release JH acids I–III as well as homo-(and/or) di-homo-farnesoic acid.

Female CA produce and release JH I–III and homo/di-homo-methyl farnesoate. The patterns of release of CA products are correlated with pheromone communication in both sexes. In contrast, male CA in both *Choristoneura fumiferana* and *C. rosaceana* synthesize JH in vitro in quantities and homolog proportions similar to females and cannot synthesize JH in the male accessory glands (Cusson et al., 1999). Thus, it would not have been out of question to find that even if male boll weevil CA did not produce JH III, the hormone was still important in the life cycle of the male and produced by another tissue.

The present study re-examines in detail the capacity of male boll weevil CA to synthesize JH III in vitro and also determines circulating JH III titers in boll weevils of both sexes. Hemolymph JH esterase activity in adult male *A. grandis* was also monitored and the ability of males to produce Vg with and without hormonal stimulation was re-examined. We have not, as yet, examined the male accessory glands for JH biosynthetic activity, but the results of the present study do not suggest the need for that experiment.

## 2. Materials and methods

### 2.1. Weevils

Laboratory-strain insects were purchased and shipped as eggs from the Gast Rearing Facility at the USDA/ARS Boll Weevil Research Laboratory, Mississippi State, MS. This facility and the mass rearing procedures are described by Griffin et al. (1983). During the course of this study, the rearing facility was relocated twice, first to the USDA/ARS facility in Mission, TX, and then to Benzon Research, Inc. The initial JH titer analyses (2–10 days) were performed on animals from Mission, while the extension of the JH titers to 14 days and the determination of a possible diel cycle of JH was done using animals from Benzon Research, Inc. Adult and larval media were prepared according to Vanderzandt and Davich (1958, 1961). Dishes with pupae were checked daily for newly emerged adults, and adults were removed to form cohorts according to date of emergence (defined as day 1). Cohorts were provided with water and pellets of medium and maintained in environmental chambers in a 13:11 h, L:D photcycle, [31:26 °C] and in a 12:12 h, L:D photcycle, [26:21 °C] to delay reproductive development (for the hormone replacement study). The JH titer determination from 8 to 14 days and the diel study were performed on weevils reared in 11:13 h, L:D photcycle, [31:26 °C].

### 2.2. Sample preparation

Hemolymph was collected from adult weevils by puncturing the metathoracic coxa while applying light

pressure to the abdomen to produce a bead of hemolymph that was then drawn into a microcapillary tube and either used immediately or sealed in the collecting tube and stored at  $-80^{\circ}\text{C}$ . For Vg analysis by polyacrylamide gel electrophoresis followed by Western blotting, samples were placed into denaturing sample buffer containing sodium dodecylsulfate and  $\beta$ -mercaptoethanol, and incubated at  $90^{\circ}\text{C}$  for 5 min prior to loading the gel. For JHE activity analysis, hemolymph was diluted into 0.1 M phosphate buffer (pH 7.5) just prior to analysis. For JH titer determinations, hemolymph samples were placed into 0.5 ml acetonitrile immediately following collection and stored at  $-80^{\circ}\text{C}$  until analyzed.

### 2.3. Vitellogenin assay

Hemolymph samples were analyzed for the presence or absence of Vg with the Western blot procedure of Towbin et al. (1979) as modified by Taub-Montemayor and Rankin (1997).

### 2.4. Radiochemical assay for JH production

The rates of JH release by CA *in vitro* were determined according to Pratt and Tobe (1974) and Tobe and Pratt (1974) as modified by Taub-Montemayor et al. (1997a). Glands were examined for linear release of JH in a time course study reported previously (Taub-Montemayor et al., 1997a). Due to the small size of the CA, brain–corpora cardiaca (CC)–CA complexes were cultured rather than isolated CA to minimize damage to the CA during dissection. Removal of brain–CC–CA complexes required 1.5 h for 12 individuals. The pooling of 12 individuals per culture vial was necessary due to the low levels of JH production. Some retention of JH in the glands was found; therefore, cultured tissue was homogenized within the culture media following termination of incubation and prior to extraction of JH from the media.

### 2.5. JH-esterase activity assay

JH esterase activity of hemolymph samples was assayed using the phase partition assay of Hammock and Sparks (1977) as described in detail by Hammock and Roe (1985). Hemolymph samples were diluted 1:1000 in 0.1 M phosphate buffer (pH 7.5) with phenylthiourea and held on ice. 100  $\mu\text{l}$  aliquots were transferred to small test tubes pre-coated with Prosil-28 (PCR Inc., Gainesville, Florida) and were allowed to come to room temperature before addition of 1  $\mu\text{l}$  of the substrate [ $^3\text{H}$ ] JH III (NEN Research Products, sp. act. 606.8 GBq/mmol) in ethanol to a final concentration of  $5 \times 10^{-6}\text{M}$ . Following extraction with iso-octane (Sigma), the aqueous portion was removed and analyzed by

scintillation counting. Calculation of activity was performed according to Hammock and Roe (1985).

### 2.6. JH titer determinations

Hemolymph samples (volume of 0.5  $\mu\text{l}$ ) were immediately added to 0.5 ml acetonitrile. 1.0 ml of 0.9% NaCl was added, and the sample was extracted twice with 1.0 ml hexane. The pooled hexane fractions were stored at  $-80^{\circ}\text{C}$  until analyzed by radioimmunoassay (Hunnicutt et al., 1989; Huang et al., 1994) as modified by Min et al. (2004). Hemolymph samples were collected between 12 and 2 p.m. on day 2 through day 14 after eclosion for at least 4 individuals at each age and analyzed by RIA for JH titer. Titer determinations for the assessment of daily rhythms were performed at 7:30 am, 12:30 pm, 5:30 p.m. and 12:30 a.m. (0.5, 5.5, 10.5 after lights on and 6.5 h after lights off) in 8, 9, 10-day males and females with lights on at 7 a.m. and off at 6 p.m.

### 2.7. Treatments

Decapitation was performed on day 1 using a sharp, single-edge razor blade. The decapitation and incision sites were sealed using low melting point, NU-BASE PLATE<sup>®</sup> wax (Electron Microscopy Sciences, Fort Washington, PA) or the same wax containing 10  $\mu\text{g}$  JH III or methoprene [Isopropyl (2E,4E,7S)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate]/1  $\mu\text{l}$  of wax. JH III and methoprene were applied to the decapitation site in melted wax to avoid the damaging effects of acetone and other solvents on the isolated abdomens (Taub-Montemayor and Rankin, 1997). The methoprene-RS and -S forms were a gift from Thomas P. Bowman, Zoecon Co., Dallas, TX. Sample collection of 0.5  $\mu\text{l}$  of hemolymph for Vg analysis was done on 9 and 10 days following decapitation and treatment.

### 2.8. Statistical methods

Data were analyzed using Minitab<sup>™</sup> statistical software version 14. Non-parametric tests were used to compare JH production: Kruskal–Wallis for analysis within the sexes and Mann–Whitney for between-sex comparisons. JHE activity between males and females were compared using Mann–Whitney. JH titer profiles between the two sexes, different ages, and time of day sampled were compared using one-way analysis of variance. Correlations between JH production and JH titers were examined with Spearman's correlation. Differences in response to hormone treatments were examined for significance by Fisher's exact probability test. A critical alpha level of 0.05 was applied in all cases.

### 3. Results

#### 3.1. JH production

JH production was examined daily from day 2 following eclosion to day 10. This time frame was chosen because under the rearing regime used in these experiments, all females produce Vg by day 5 and have oviposited at least once by day 7. Each determination involved pooling brain-CA-CC complexes from 12 adults of the same age, according to Taub-Montemayor et al. (1997a). JH production in males was low on day 2 and rose to a peak on day 4 and again on day 9 (Fig. 1) These results are similar in character to those obtained by Taub-Montemayor et al. (1997a) in females reared in the same conditions, though the timing of the putative peaks in activity is slightly different, occurring 1 day later in females than in males: day 4 and 9 in males as compared to 5 and 10 in females (Fig. 1). Although the day-4 peak in males was significant (Kruskal-Wallis  $H = 17.03, p < 0.05$ ) relative to previous and subsequent values, apparent differences between the sexes were not significant nor were the other putative peaks in either sex. The quantity of JH produced and the degree of variability in JH production were similar in males and females.

#### 3.2. JHE activity

Previous results indicated that JH acid is the primary product of JH degradation in *A. grandis* hemolymph (Taub-Montemayor et al., 1997b). In our examination of males from day 2 to day 10 after adult emergence, we found an elevation of JHE activity at day 2 that declined to low levels by day 7 and remained low through day 10.

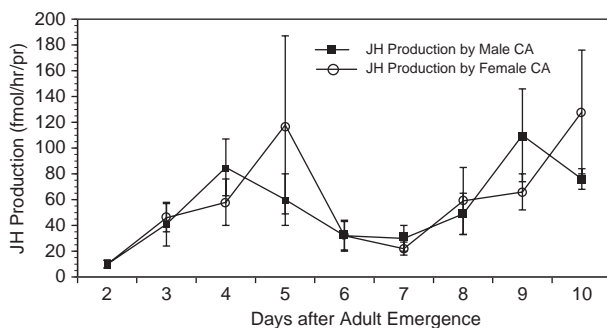


Fig. 1. Relationship of JH synthesis for male and female boll weevils reared in conditions to encourage reproductive development. The error bars are SEM. Each in vitro CA assay required 12 individuals per assay and each assay was replicated for females as follows ( $n$  in parenthesis): 2 days (4), 3 days (4), 4 days (2), 5 days (2), 6 days (2), 7 days (2), 8 days (4), 9 days (2), and 10 days (4), data from Taub-Montemayor et al., 1997a. Replicates for males are as follows ( $n$  in parenthesis): 2 days (4), 3 days (4), 4 days (8), 5 days (6), 6 days (4), 7 days (5), 8 days (5), 9 days (8), and 10 days (5). No significant differences were found between males and females (one-way ANOVA).

This is a similar pattern to that found in females except that JHE activity is higher in males and remains high longer than in females (Fig. 2). Significant differences in JHE activity between males and females were found at 4, 5, and 10 days (Mann-Whitney,  $p < 0.05$ ).

#### 3.3. JH titers

Hemolymph levels of JH III were determined from day 2 to day 10 following eclosion for both sexes (Fig. 3). Titer determinations for males and females were virtually identical throughout except on day 10 when male titers seemed to drop somewhat relative to females, though the difference was not significant. There were no significant increases in JH titer corresponding to the putative peaks in JH production even on day 4 in males. (Note that on days 4, 5, and 10, JH esterase levels were significantly higher in males than in females.) In a different titer study (Fig. 4), using boll weevils aged 8–14 (reared under a short photoperiod regime) there again appeared to be no significant differences in JH titer between the sexes. In all cases, these comparisons were made using a one-way analysis of variance.

#### 3.4. Diel JH titers

Although hemolymph collection was routinely limited to a 2-h period each day (12–2 pm) to reduce any possible effects of diel periodicity of JH production, we also checked for diel periodicity of JH III titers by collecting hemolymph 4 times daily in different individual males and females on days 8, 9 and 10 after emergence (Fig. 5). There were no significant differences in JH titer with time of day, during the photophase (0.5, 5.5, and 10.5 h after lights on) (one-way ANOVA).

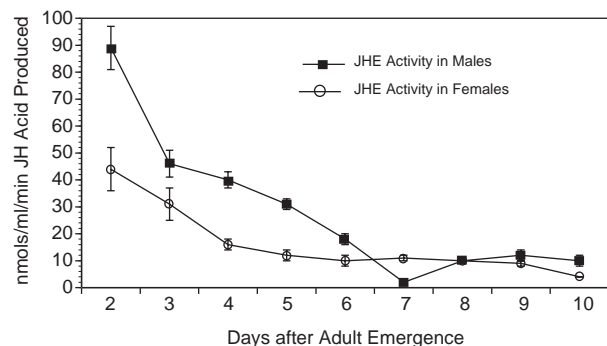


Fig. 2. Relationship of JH degradation for male and female boll weevils reared in conditions to encourage reproductive development. The error bars are SEM. JHE activity in hemolymph was determined for samples from individual males as follows ( $n$  in parenthesis): 2 days (48), 3 days (24), 4 days (59), 5 days (59), 6 days (48), 7 days (57), 8 days (58), 9 days (60), and 10 days (35). Females were replicated with five individuals for each age, data from Taub-Montemayor et al. (1997a). Significant differences between males and females at 4, 5, and 10 days (one-way ANOVA,  $p < 0.05$ ).

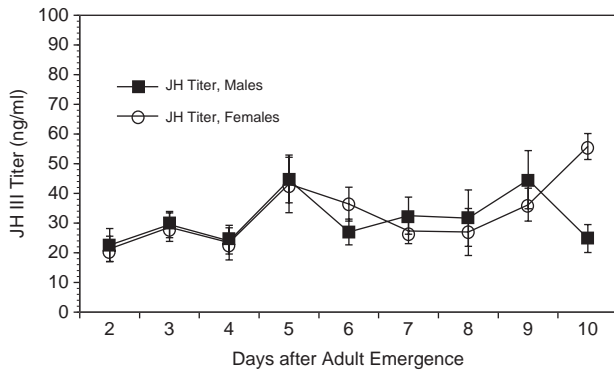


Fig. 3. Relationship of JH titers for male and female boll weevils reared in conditions to encourage reproductive development. JH hemolymph titers in males and females were determined from day 2 to day 10 following eclosion. The error bars are SEM. Sample sizes for each sex are as follows ( $n$  in parenthesis): males day 2 (5), day 3 (8), day 4 (5), day 6 (5), day 7 (7), day 8 (6), day 9 (7), day 10 (5) and females day 2 (5), day 3 (6), day 4 (4), day 5 (5), day 6 (4), day 7 (5), day 8 (4), day 9 (7), and day 10 (4). Vg detected on day 5 in all females tested ( $N = 35$ ).

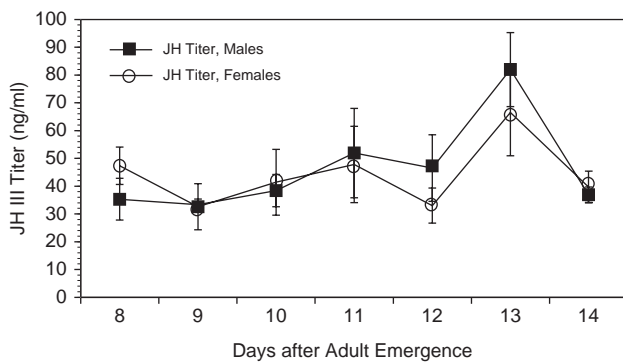


Fig. 4. JH titers in male and female boll weevils reared in short day conditions. JH hemolymph titers in males and females were determined from day 8 to day 14 following eclosion. The error bars are SEM. Sample sizes for each sex was 5 each day.

However, significant differences in JH titers were observed within but not between sexes during the scotophase (i.e., 6.5 h after lights off-half-way through the scotophase) (one-way ANOVA,  $p < 0.05$ ).

### 3.5. Hormone treatments

Decapitated females responded to treatments of JH III and methoprene by producing Vg as expected. A low number of decapitated males also produced Vg in response to hormone treatment but not nearly as many as the previous study. Females responded to methoprene more strongly than to JH III but this difference was not evident in decapitated males. Indeed, in these experiments males were fairly insensitive to both compounds relative to previous work (Fig. 6). The response to

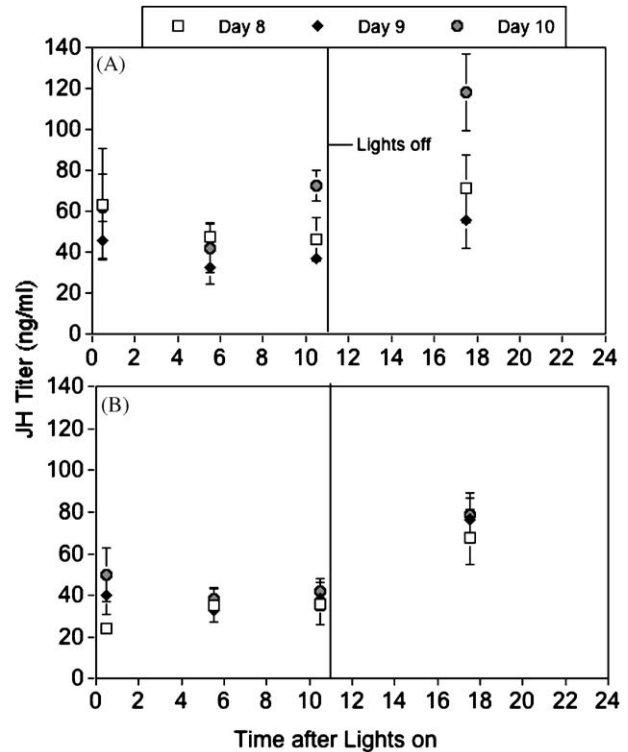


Fig. 5. Diel JH titers for males (A) and females (B). Titer determinations for the assessment of daily rhythms were performed at 7:30 a.m., 12:30 p.m., 5:30 p.m. and 12:30 a.m. (0.5, 5.5, 10.5, 16.5 h after lights on, respectively) in 8, 9, and 10 day insects with lights on at 7 a.m. and off at 6 p.m.  $N = 5$  for each point.

increasing doses of JH and methoprene varied. In females methoprene showed a clear positive dose-response effect except at the highest dose (200  $\mu\text{g}$ ), which was significantly less effective than the 100  $\mu\text{g}$  dose (Fisher's Exact,  $p = 0.002$ ). In contrast, while there was an increased response between 0.1 and 10  $\mu\text{g}$  JH III, further increases did not cause any further significant increase. In males, there was also some indication that intermediate levels of stimulation (10  $\mu\text{g}$ ) were most effective with both treatments, but the differences were not significant.

## 4. Discussion

The results reported here differ in two respects from previous studies. Taub-Montemayor et al. (1997a) indicated that male CA produced little or no JH III in vitro. The male JH production determinations were done as a minor part of a larger study that was primarily focused on understanding the endocrinology of oogenesis in *A. grandis* and were some of the first boll weevil CA determinations done in this lab or elsewhere. Since

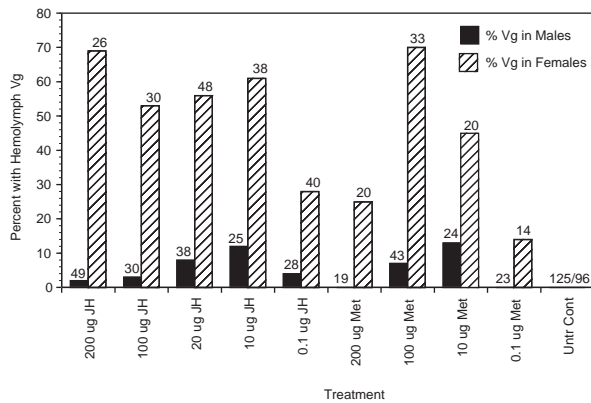


Fig. 6. Vg production in response to hormone replacement in decapitated weevils. Each treatment was performed on 1-day, decapitated weevils reared in short-day, low-temperature to delay reproductive development, and results reflect analysis of hemolymph collected on 9–10 days following decapitation. Numbers for each treatment group appear above each bar.

the present more detailed determinations have clearly shown the male CA capable of JH production, we must conclude that inexperience with the dissection and assay was the cause of our earlier negative result. Secondly, the JH titer studies reported here confirm the reliability of the CA assays done in both sexes. Clearly, male boll weevils produce quantities of JH similar to females (between 10 and 110 fmol/CA pr/h) at least for the first 9 days of adult life, well after first oviposition has occurred in females. JH titer determinations also confirm that circulating levels of the hormone are similar in males and females, although timing of JH peaks may differ slightly, if indeed the peaks are real. Since the JH production determinations were done with pooled samples and the peaks in synthesis are not reflected in the JH titer determinations even in day 4 males where the JH production peak was statistically significant, they may simply reflect periods of greater variability in development within the population. On the other hand, there is a corresponding difference in JHE activity on day 4 and 5 in males that might serve to bring down circulating JH titers in spite of a peak in JH production. If these subtle differences are real, they may indicate some interesting differences in life history between the sexes at this time. A strong correlation ( $\rho = 0.831, p < 0.005$ ) between *in vitro* JH production and JH titers was found in females. This correlation was less strong in males ( $\rho = 0.446, p > 0.05$ ) possibly because of the relationships suggested above.

JH III titer determinations in these experiments reflect values in line with those obtained from the CA culture experiments and for titers recorded in some Coleoptera, but they differ substantially from others. For example, JH titers in the boll weevil range from 20 to 80 ng/ml, comparable to levels found in the Colorado potato beetle (de Kort et al., 1982). However, those reported

for burying beetles range from 0 to almost 8000 ng/ml depending on the phase of the reproductive cycle and sex (Scott et al., 2001; Panaitof et al., 2004). *In vitro* assay of boll weevil CA results in JH release in fmol range rather than pmol amounts as in the burying beetle (Scott et al., 2001), and JHE levels are considerably higher in the boll weevil as well. Whole-body JH titer determinations were examined in both sexes of *Tenebrio molitor* in response to infection by *Hymenolepis diminuta* (Cole et al., 2002). These authors found whole-body JH titers ranging from 100 pg/g wet weight to 539 pg/g, but comparisons are difficult when techniques differ substantially.

The two sets of determinations of CA biosynthesis and JHE activity in boll weevils (i.e., males vs. females) were done several years apart and included intervening changes in the source colony location. Yet the timing of Vg production and the JH titer determinations indicate that reproductive development was similar in all populations. Under the rearing conditions used in the CA culture experiments, 100% of the females displayed Vg in the hemolymph by day 5 and had oviposited by day 7 (Taub-Montemayor et al., 1997a). In this study again, all females displayed hemolymph Vg by day 5.

The JH titer data in males indicated that there might be a decline in JH levels in males around day 10 that was not seen in females. However, in a different study in which JH titer determinations were extended until day 14, there were no differences between the sexes in older individuals and JH titers continued to rise slightly from day 10 to day 14. Unfortunately, the second JH titer study is not strictly comparable to the first since it was done on animals reared under short photoperiod. Yet titers were virtually identical between the two studies for days 8, 9, and 10, and it thus seems likely that the apparent drop in JH titers in males on day 10 is not real.

A large-magnitude rise (>15–20 fold increase in mean titer; >100 fold increase in some individuals) in JH titer has been reported in the cricket, *Gryllus firmus*, near the end of the photophase-beginning of the scotophase (Zhou and Zera, 2004). In a preliminary study of diel JH titer changes in boll weevils we found that JH titers approximately doubled during the scotophase, but did not see the extraordinary increase seen in crickets. Boll weevil JH titers are quite stable during the photophase. Samples collected at 0.5, 5.5, and 10.5 h after lights on were not significantly different between or within gender or time period groups. In contrast, at 6.5 h after lights off (half-way through the 13 h scotophase), the levels of JH had risen significantly. The scotophase differences may be important and are worth pursuing further. We plan to extend this preliminary study to include more daily sample times and groups of animals reared under both long or short-day photoperiod regimes. For the purposes of the present experiments, however, it seems clear that our bleeding times minimized any possible diel

effects on the results. Hemolymph samples were always taken from 5 to 7 h after lights on when there were no diel differences in JH titers between the sexes.

JH and methoprene treatment studies confirmed the ability of boll weevils of both sexes to respond by producing hemolymph Vg although only the female normally does so, at least at levels detectable with our antibody (22.5 ng of purified protein/well (Taub-Montemayor and Rankin, 1997)). This result is not unique to boll weevils. Although Vg is generally a female specific protein, it has been reported in the hemolymph of male *Bombyx mori* (Lamy, 1984; Lamy and Julien-Laferrière, 1974), *Rhodnius prolixus* (Engelmann, 1979; Harnish and White, 1982) and *Galleria mellonella* (Lamy, 1979), and trace amounts have been reported in male hemolymph of several other insects (see Mundall et al., 1983 for review).

Differences in response of boll weevils to the naturally occurring JH III vs. methoprene are presumably due to differences in degradation between the two compounds, as has been observed elsewhere (Henrick et al., 1973, 1976; Chen et al., 1976), JH III being degraded quickly by JH esterase and other pathways, methoprene being quite stable. The dose–response results for JH III and methoprene suggest that even though the male fat body is competent to respond, it is much less responsive than female tissue, and this in itself may be enough to account for the difference between the sexes in Vg production. Similar differences in sensitivity of female and male tissues to JH treatment have been observed in *D. punctata* (Mundall et al., 1983), *Leucophaea maderae* (Don-Wheeler and Engelmann, 1991), and *Locusta migratoria* (Dhadialla and Wyatt, 1983).

The work reported here indicates that the gender difference in Vg production in boll weevils is not due to sex-specific differences in JH production or JH titers. It does not address what other factors might cause this difference. In addition to JH production, JH degradation and the presence of JH binding proteins can all affect measurable or effective JH titers (de Kort and Granger, 1996; Gilbert et al., 2000). There are no published reports describing JH binding proteins for the boll weevil, and we did not investigate them in this study. In other insects, however, these proteins seem to function in transport of JH, prevention of adsorption/degradation and possibly as a JH reservoir. Differences in JH binding proteins between the sexes might, therefore, be responsible for the difference in apparent sensitivity of the fat body to identical JH titers in males and females. Alternatively, the sexes may differ in number or type of JH receptors or some other essential intracellular factor (Edwards et al., 1993) or differences in gene dosage. Vg transcripts have been shown to differ between males and females in *Locusta* (Dhadialla et al., 1987). Clearly, the present study can do no more than set the stage for the additional work necessary to

understand differential tissue sensitivities to JH between male and female boll weevils.

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