

tion of pigeons, and Hart² did not report any seasonal difference in the shivering of pigeons. Although a progressive decrease was found in shivering as Ta approached the critical point, so conflicting with the results of West¹⁶, we did not find a linear correlation between the intensity of shivering and M. Although shivering disappeared, M remained elevated.

The relatively high critical temperature of the pigeon can be understood, when it is pointed out that unlike mammals, shivering is perhaps the only mechanism by which birds produce extra heat when Ta decreases below thermoneutrality. Thus shivering is elicited just at birds' critical temperature. Furthermore, the shivering threshold is correlated to bird's size and insulation, being highest in small tropical birds and lowest in 'insulative specialists', like arctic gallinaceous birds.

Pigeons acclimated to long photophase (groups 3 and 4) regulated their Tb's and Tf's on a lower level (about 2 °C) than the controls or 'winter' pigeons, especially below their critical temperature (see fig.). The result is in agreement with previous observations that winter birds have a higher Tb and a greater difference between skin and Ta compared with summer birds⁶. The increased skin temperature of the body and extremities has also been noticed in cold-acclimated pigeons⁹.

In conclusion it can be stated that photoperiod is important in birds' thermoregulatory adaptation; it provokes changes in insulation, in the lower critical temperature, in the shivering threshold and in the relation between skin temperature and Ta. These photoperiod-induced changes in pigeons' heat production, Tb and Tf may result from the

influence of the photoperiod on catecholamine and lipid metabolism (as found just recently¹³), and they show that the pineal gland has an important role to play in the temperature regulation of birds. It is thought that the thermoregulatory influence may be mediated through an inhibitory action of pineal melatonin on the thyroid gland (discussed previously by Ralph et al.¹⁷ and Saarela¹⁸).

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Enhancement of boll weevil *Anthonomus grandis* Boh. (Coleoptera: Curculionidae) pheromone biosynthesis with JH III¹

P. A. Hedin, O. H. Lindig and G. Wiygul

Boll Weevil Research Laboratory, USDA-SEA-AR, Mississippi State (Mississippi 39762, USA), 31 March 1981

Summary. The juvenile hormone JH III, when incorporated at 1.0 ppm in the diet of adult male boll weevils (*Anthonomus grandis* Boh.), increased the biosynthesis of its 4 pheromone compounds by 3 times. The biosynthesis at lower and higher levels of JH III was less. JH I was not active at any of the concentrations tested.

The 4 monoterpene compounds that comprise the male boll weevil (*Anthonomus grandis* Boh.) pheromone [I, (+)-cis-2-isopropenyl-1-methylcyclobutaneethanol; II, (Z)-3,3-dimethyl- $\Delta^{1\beta}$ -cyclohexaneethanol; III, (Z)-3,3-dimethyl- $\Delta^{1\alpha}$ -cyclohexaneacetaldehyde; and IV, (E)-3,3-dimethyl- $\Delta^{1\alpha}$ -cyclohexaneacetaldehyde] were identified and synthesized in 1969². The biosynthesis of these compounds has since been reported to be a function of a number of factors. It increases with age³, it is higher in the summer than in winter with insects reared in the laboratory⁴, it decreases when males are fed a laboratory diet rather than cotton (*Gossypium hirsutum* L.) buds⁵, it further decreases if chemosterilants are incorporated in the diet⁵, and it decreases in insects with a high gut bacterial load⁶. The pheromones may be synthesized in the fat bodies of the abdomen shell or in the gut, because the greatest concentrations are found in these tissues⁴. If synthesized in the fat bodies, they may be transported to the gut via the malpighian tubules for excretion with fecal pellets. The most concentrated source of the pheromones is the fecal pellets⁵, and the release is rhythmic with the peak from 07.00 to 13.00 h⁷. The peak periods for feeding and fecal release also occur during these hours.

For physiological studies and field release programs, it is

very important that laboratory reared insects possess high vigor, and that males be sexually competitive with wild males. Improved pheromone biosynthesis would contribute to these objectives. Amerasinghe⁸ reported that the pheromone biosynthesis of allatectomized *Schistocerca gregaria* males injected with juvenile hormone JH I (3, 11-dimethyl-10 epoxy-7-ethyl-trans, trans-2, 6-tridecadienoic acid, methyl ester) and JH III (10-epoxy-3,7,11-trimethyl-2,6-trans, trans-dodecadienoic acid, methyl ester) was restored. Therefore, this study was initiated to determine whether JH I or III would promote pheromone biosynthesis in the male boll weevil. Because administration of these compounds by topical application would be of little practical significance

Table 1. Pheromone biosynthesis of male boll weevils fed diets containing JH I, $\mu\text{g}/\delta/\text{day}$

Days	JH I, ppm				Cotton buds
	0.0	0.1	1.0	10.0	
1-4	0.15	0.18	0.15	0.14	0.16
5-8	0.20	0.16	0.13	0.21	0.30
9-12	0.26	0.25	0.20	0.26	0.48

Table 2. Pheromone biosynthesis of male boll weevils fed diets containing JH III, $\mu\text{g}/\delta/\text{day}$

Days	JH III, ppm							Cotton buds
	0.0	0.1	0.3	1.0	3.0	10.0	100.0	
1-4	0.10	0.20	0.06	0.14	0.08	0.08	0.16	0.16
5-8	0.13	0.26	0.19	0.45 ^a	0.56 ^b	0.34	0.31	0.30
9-12	0.35	0.34	0.26	1.11 ^c	0.78 ^d	0.30	0.47	0.48

^a Significant at the 5% level in comparison to 0.13 $\mu\text{g}/\delta/\text{day}$. ^b Significant at the 10% level in comparison to 0.13 $\mu\text{g}/\delta/\text{day}$. ^c Significant at the 5% level in comparison to 0.35 $\mu\text{g}/\delta/\text{day}$, and significantly different from 0.0 ppm and cotton buds by Duncan's New Multiple Range Test. ^d Not significant at the 5% level.

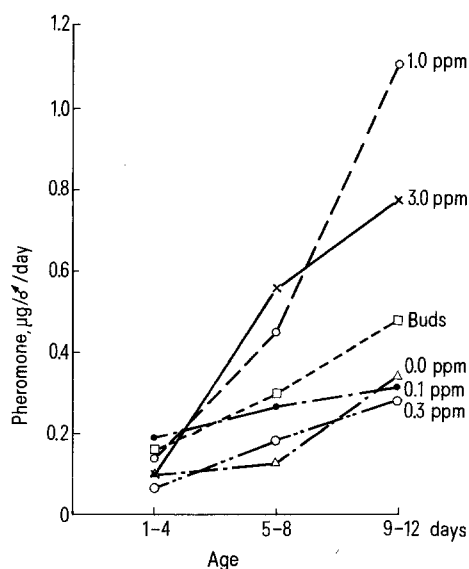
even though successful, they were evaluated by incorporation in the laboratory diet. Because JH I, II and III have not been found in the boll weevil, even though we have attempted their isolation by established methods, there is no direct precedent for this approach. However, the isolation of several juvenile hormone mimics⁹ and 20-hydroxyecdysone^{10,11} from the boll weevil has been accomplished, so it can be assumed that this insect is under hormonal controls common to other insects.

Materials and methods. Newly emerged males were placed in screened bottom plastic cages in groups of 100 and fed for 12 days on the standard adult diet^{6,12} to which 0.1, 0.3, 1.0, 3.0, 10.0 and 100.0 ppm of JH I and JH III (Calbiochem Corp, La Jolla, CA) was added by dilution in 100% ethanol, mixing, and heating to evaporate the ethanol. Diets containing no JH I or III, and fresh cotton buds fed daily were included for comparison. Agar was included with the diet so that cylindrical plugs were formed on cooling in tubes, and 100 g quantities were sufficient for 100 insects for about 6 days. Refrigerated diet was provided daily. Frass that fell through the screened cages was collected daily and refrigerated. Pooled samples from days 1 to 4, 5 to 8, and 9 to 12 were steam distilled into hexane. After concentration, analysis was performed on an aliquot with a 76 m \times 0.8 mm stainless steel capillary gas chromatographic column coated with OV-17[®] at 130 °C⁵. Carrier gas flow was 8.0 ml/min N₂. An internal standard of α -terpineol was added to each sample for quantitation. The analytical data were processed to obtain the total pheromone content in $\mu\text{g}/\delta/\text{day}$. The approximate ratio of individual components regardless of diet was 6:6:2:1:I:II:III:IV. The data were statistically

analyzed by Duncan's New Multiple Range Test and the 2 tailed t-test.

Results and discussion. Pheromone biosynthesis totals from insects fed the JH I diets are summarized in table 1, and those from insects fed JH III diets are summarized in table 2 and in the figure. The results were averages of 4 tests, each containing 3 replicates, conducted at intervals from January 1979 to September 1980.

JH I did not promote pheromone biosynthesis at any tested level. JH III significantly increased the pheromone biosynthesis of males fed 1.0 ppm at 5-8 days and 9-12 days. The increase in pheromone biosynthesis of males fed 3.0 ppm was significant only at the 10% level at days 5-8, because of greater data variability, and noticeably higher than all of the remaining treatments at days 9-12, but not significant because of the aforementioned variability. Insects fed at 0.1, 0.3, 10.0 and 100.0 ppm did not synthesize a greater amount of pheromone than the control. Staal¹³ has attributed the failure of increasing amounts of JH to elicit increasingly greater effects on insects to a cellular sensitivity which limits the capacity of the cell to respond to a given stimulus. The lower doses (0.1 and 0.3 ppm) did not stimulate pheromone synthesis, presumably because of a threshold effect. In 2 previous tests, 1.27³ and 1.19⁵ $\mu\text{g}/\delta/\text{day}$ of pheromone was biosynthesized by insects fed cotton buds, and 0.46 μg ⁷ by insects fed the laboratory diet. These present analyses (buds: 0.48 μg , diet: 0.35 μg) are somewhat lower. The failure of JH levels below 1.0 ppm to elicit a significant increase in pheromone biosynthesis, and the decrease in biosynthesis with 10 and 100 ppm suggests that there is a relatively precise optimal level. The average daily consumption of diet of adult insects has been found to be 3 mg¹⁴; thus the apparent average daily intake of JH III at 1.0 ppm is 3 ng. Then, a cumulative dose of 15-20 ng over a 5-8-day period appears to be sufficient to elicit an improved rate of biosynthesis.



Boll weevil pheromone biosynthesis of males fed the laboratory diet incorporated with JH III.

- 1 In cooperation with the Miss. Agr. and For. Exp. Sta., Miss. State, MS 39762. Mention of a proprietary product does not necessarily imply endorsement of this product by the USDA.
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