EFFECT OF JUVENILE HORMONE III AND BETA-BISABOLOL ON PHEROMONE PRODUCTION IN FAT BODIES FROM MALE BOLL WEEVILS, ANTHONOMUS GRANDIS BOHEMAN (COLEOPTERA: CURCULIONIDAE)

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Abstract—1. Fat bodies from 1 to 10-day-old male boll weevils were treated with juvenile hormone III, beta-bisabolol, and juvenile hormone + beta-bisabolol.
2. We measured the amount of sex pheromone produced after 2 hr of incubation with the above compounds.
3. More pheromone was produced by the juvenile hormone III + beta-bisabolol treatment than by the others.

INTRODUCTION

The boll weevil (Anthonomus grandis Boheman) sex pheromone was first isolated and identified by Tumlinson et al. (1969) and shown to consist of four components. The four compounds are: (+)-cis-2-isopropenyl-1-methylcyclobutaneethanol (cpd 1), cis-3,3-dimethyl-α,β-cyclohexanecethanol (cpd 2), cis-3,3-dimethyl-α,γ-cyclohexanecetaldehyde (cpd 3) and trans-3,3-dimethyl-α,γ-cyclohexanecetaldehyde (cpd 4).

In earlier articles Wiygul et al. (1982) localized the sex pheromone in the fat bodies of adult males, and determined the effect of glucose and ATP on sex pheromone production in fat bodies (Wiygul and Sikorowski, 1985).

Juvenile hormone (JH) has been implicated in pheromone production in other coleoptera (Hughes and Renwick, 1977). In our laboratory Hedlin et al. (1982) found that boll weevil pheromone production was increased when JH III was fed to adult males. Dickens et al. (1988) reported that pheromone production by this insect could be stimulated by both antennectomy and topical application of JH analog (JHA).

Beta-bisabolol is a sesquiterpene alcohol that has been found in the cotton plant and other closely related Malvaceae. Its structure was elucidated by Minyard et al. (1968). Minyard et al. (1969) reported that it is the most abundant polar compound in cotton bud essential oil and is attractive to boll weevils in bioassays in the laboratory. McKibben et al. (1974) found that beta-bisabolol plus caryophyllene oxide added to the synthetic boll weevil pheromone grandlure attracted 25% more boll weevils in the field than did grandlure alone.

In this present study we wished to determine the effect of beta-bisabolol and JH III on the production of pheromone from adult male boll weevil fat bodies. From this we hope to gain further insight into the mechanisms of production of the compounds that make up the pheromone.

MATERIALS AND METHODS

Source and treatment of insects

The insects, received as imagos from the Robert T. Gast Rearing Facility (USDA-ARS), were reared by the methods of Roberson and Wright (1984). Males were selected and placed in 2 x 10 x 19cm cages that were kept at 24°C and 50% r.h. with a 16:8 light:dark cycle. The weevils were held 100 to the cage and were fed fresh cotton squares (flower buds) daily.

Pheromone production

Pheromone production was determined for fat bodies of adult male boll weevils incubated in: (1) 5 ml saline solution + 10 mg ATP; (2) 5 ml saline solution + 10 mg ATP + 5 μg JH III; (3) 5 ml saline solution + 10 mg ATP + 25 μg beta-bisabolol; and (4) 5 ml saline solution + 10 mg ATP + 5 μg JH III + 25 μg beta-bisabolol. The saline solution consisted of 9 g NaCl + 0.2 g KCl + 0.299 g CaCl2 + 0.1 g NaHCO3 + 0.01 g NaH2PO4 + distilled water to make 1 liter. Fat bodies from 20 insects were incubated in 5 ml of the experimental saline solutions, and incubated for 2 hr at 23°C. This was considered to be one replicate and each treatment was replicated four times for each day of the 1–10 day-of-age test period. A total of 800 insects were used for each treatment during the study.

At the end of the 2 hr incubation period, the saline solution and fat bodies were extracted for pheromone according to the method of McKibben et al. (1976), except that the samples were shaken in separatory funnels with pentane to extract the pheromone rather than extracted in microsoxhlet units. After extraction, the samples were analyzed by gas-liquid chromatography using a 30 m x 0.315 mm fused silica capillary column with a DB-5 liquid phase. Alpha-terpenol was used as an internal standard to calculate the quantity of pheromone on a per insect basis. Each of the four compounds that comprise the boll weevil aggregation pheromone (Tumlinson et al., 1969) was reported as ng compound I, II, III, or IV per weevil. Also, total quantities of components were added together and

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RESULTS AND DISCUSSION

Total pheromone production (components summed) by the four experimental groups from day 1 to day 10 is shown in Fig. 1. Fat bodies incubated with both JH III, and JH III + beta-bisabolol produced more pheromone than those incubated with ATP alone. In fact, those incubated with JH III + beta-bisabolol produced more pheromone than any other group. Incubation of excised fat bodies in saline with ATP + JH III + beta-bisabolol led to a significant increase, $P < 0.05$ by Duncan's New Multiple Range Test, in total pheromone production on day 6 when compared to the other treatments. A second peak in pheromone production occurred on day 10; however, differences observed on this day were not significant due to considerable variability among the means. Similar peaks in pheromone production on days 6 and 10 occur in male boll weevils (Gueldner and Wiygul, 1978), and a peak in pheromone production by fat bodies of male boll weevils incubated with glucose on day 6 is also known (Wiygul and Sikorowski, 1985).

Quantities of the four individual pheromone components produced by each experimental group were similar except for compound 1 on day 6 (Fig. 2). On this day fat bodies incubated with JH III and beta-bisabolol produced significantly more compound 1 than the other groups ($P < 0.05$ by Duncan's New Multiple Range Test). Production of each pheromone component by boll weevil fat bodies is known to be enhanced by energy sources such as glucose and ATP (Wiygul and Sikorowski, 1985). Both JH III (Hedin et al., 1982) and a JH analog (Dickens et al., 1988) also increase pheromone production in intact boll weevils. Although the mechanism by which addition of JH III and beta-bisabolol enhance pheromone production by boll weevil fat bodies incubated with ATP might not be determined by our current experiments, it is interesting to note that JH III must exert its effect by directly affecting fat body metabolism. JH has previously been shown...
to directly affect fat body metabolism in female insects during vitellogenesis (Pan and Wyatt, 1971; Raabe, 1982). The effects of beta-bisabolol on pheromone production are even less clear. However, since addition of beta-bisabolol to the saline + ATP and saline + ATP + JH III fat body incubates led to increases in production of compound, it might be speculated that beta-bisabolol serves as a precursor for the pheromone component. This would be fortuitous since beta-bisabolol is the major volatile of cotton (Thompson et al., 1971), and has been reported only in cotton and closely related Malvaceae.

REFERENCES


