EFFECTS OF ANTENNECTOMY AND A JUVENILE HORMONE ANALOG ON PHEROMONE PRODUCTION IN THE BOLL WEEVIL (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Aggregation pheromone production by male boll weevils, *Anthonomus grandis grandis* Boheman can be stimulated by both antennectomy and topical application of a juvenile hormone analog (JHA, methoprene). Since JHA decreases sensitivity of antennal olfactory receptors, its effects on pheromone production may possibly be by either stimulating release of some blood-borne factor or decreasing antennal input.

Key Words: Insect, pheromone, regulation, antenna, hormone, olfaction, Anthonomus grandis grandis.

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INTRODUCTION

Stimulation of pheromone production in insects has been attributed to hormonal factors (Barth, 1961; Borden et al. 1969; Hughes and Renwick 1977a,b; Harring 1978; Renwick and Dickens 1979). For example, in the bark beetle, *Ips paraconfusus* Lanier (Coleoptera: Scolytidae), juvenile hormone (JH) stimulates the brain-corpora cardiaca complex to release a brain hormone (BH) which induces pheromone production (Hughes and Renwick 1977a). A similar mechanism involving BH in pheromone production probably exists for another beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) (Menon 1976) and recently a brain peptide was shown to activate pheromone production in certain Lepidoptera (Raina and Klun 1984). The release of these stimulatory agents into the hemolymph has been attributed to such factors as stretching of the gut (Hughes and Renwick 1977a) and photoperiod (Raina and Klun 1984).

The male boll weevil aggregation pheromone is released from the frass and consists of four components: I [(+)-*cis*-2-isopropenyl-1-methylcyclobutaneethanol]; II (*cis*-3,3-dimethyl-Δ^1,β-cyclohexane-ethanol); III (*cis*-3,3-dimethyl-Δ^1,α-cyclohexaneacetaldehyde); and IV (*trans*-3,3-dimethyl-Δ^1,α-cyclohexaneacetaldehyde) (Tumlinson et al. 1969). Previous research in our laboratory has shown that pheromone production increases through adult life and exhibits not only a circadian rhythm but also longer rhythms with peaks occurring every several days when insects are held under constant temperatuare (24°C) and photoregimen of 16 hours of light and 8 hours of darkness (LD 16:8) (Gueldner and Wiygul 1978). Boll weevils held in constant darkness produce very little pheromone. However, insects held under constant light release pheromone in an arrhythmic manner in which pheromone production increases to a peak level before declining to a low level. Other experiments indicated that when JH III was fed to adult males, production

of the aggregation pheromone increased (Hedin et al. 1982). Thus pheromone production in the boll weevil, as in other insects, appears to be under hormonal control (Barth 1961; Borden et al. 1969; Hughes and Renwick 1977a,b; Harring 1978; Renwick and Dickens 1979) and is influenced by photoperiod (Gueldner and Wiygul 1978; Raina and Klun 1984). However, the mechanism by which the boll weevil and other insects regulate daily pheromonal output is poorly understood. We report the discovery that antennal input may play a role in the regulation of production of the aggregation pheromone in the boll weevil thus providing a possible mechanism for modulation of pheromonal output.

MATERIALS AND METHODS

Experimental Methodology

We used adult male boll weevils from a small laboratory colony annually infused with feral insects. In our first experiment, four groups of twenty insects (80 insects each for untreated control and each treatment; 320 insects total) were used for the untreated control and each treatment. The three treatments consisted of (1) topical application of 10 μg of a JH analog (JHA, methoprene, obtained from Zoecon Corp., Palo Alto, CA) dissolved in 2μl of >99.9% pure acetone on the prothoracic sternum after frass collection on days 3 and 6 following adult emergence, (2) antennectomy following frass collection on day 3, and (3) antennectomy and topical application of JHA (as above) after frass collection on day 3. Insects which were not antennectomized were maintained in separate incubators.

A second experiment was performed in order to determine possible solvent effects and general effects of extirpation of an appendage on pheromone production. In this experiment, four groups of twenty insects were also used (80 insects each for untreated and solvent controls, and 2 treatment groups; 320 insects total). In addition to the untreated control, the three experimental groups were: (1) a solvent control consisting of topical application of 2μl of >99.9% pure acetone on the prothoracic sternum after frass collection on day 3; (2) extirpation of the tarsal and pretarsal segments of the right prothoracic leg following frass collection on day 3, and (3) topical application of 10 μg of JHA dissolved in 2μl of >99.9% pure acetone on the prothoracic sternum after frass collection on day 3.

All insects were held under LD 16:8 at 24°C and fed daily 1 fresh field-collected cotton square (DPL61) for each 5 weevils. Each group of twenty insects was housed in a plastic container (45 cm^3) with screen floor (49 cm^2) and plastic top with a screened hole (3 cm diameter). Pheromone-laden frass was collected daily between 0800 and 1000 hours, 4 to 6 hours after the onset of photophase, from aluminum foil covering the bottom of the screen floor. A standard (α-terpineol) was added to each frass sample which was then extracted in a micro-Soxhlet apparatus with 10 ml of pentane (McKibben et al. 1976). Gas liquid chromotography (GLC) with a 30 m × 0.315 mm fused silica column with a DB-5 liquid phase was used to quantify each of the four pheromone components.

Data Analyses

In the first experiment, treatment means were analyzed by a factorial treatment structure (i.e. antennectomy, with and without; JHA, with and without) and least significant difference (LSD) values (Ostle 1963).

Total pheromone production was calculated by summing amounts of each of the four components. Day means were compared for each treatment by analysis of variance (ANOV) combined over days using each day as a subunit. The LSD values were based on error b from analysis of the log transformed values. Treatment means were compared each day by ANOV for randomized complete block design. All significant differences reported are $P < 0.05$.

RESULTS

In the first experiment, pheromone production by the untreated control group was typical for male boll weevils fed cotton squares and held at LD 16:8 (Gueldner and Wiygul 1978) (Fig. 1A). Total pheromone produced by each insect increased through day 5 to ca. 500 ng. This initial peak was followed by a slight decline with a second slight increase in pheromone production day 10.

In the experimental group treated topically with JHA on days 3 and 6, pheromone production on the day following treatment increased 2 to 3 times that of the preceding day. The increase in pheromone was significantly ($P < 0.05$) greater than the control for both topical treatments (Fig. 1B). JHA clearly incrased the quantity of pheromone produced within 24 hours post-treatment.

The two other experimental groups demonstrated the effects of antennectomy on pheromone production and how this deprivation of antennal input might interact with the effects of JHA. In the first group antennectomized on day 3, pheromone production increased significantly ($P < 0.05$) within 48 hours post-treatment (Fig. 1C.) to a level significantly greater ($P < 0.05$) than both the control and JHA-treated groups.

In the last experimental group, each insect was both antennectomized and treated with JHA on day 3 (Fig. 1D.). Similar to the JHA-treated only group, pheromone production increased within 24 hours to a level significantly greater ($P < 0.05$) than either the control or the group which received antennectomy only. Within 48 hours pheromone production in this group reached a level similar to that observed for the antennectomized group which was significantly greater than the group receiving JHA treatment only on day 3. Factorial analysis of treatment means for groups receiving antennectomy, JHA or both on day 3 revealed no significant interaction ($P < 0.05$) between antennectomy or JHA treatment.

Total pheromone production by the control group and each experimental group for the 10-day period following adult emergence is summarized in Table 1. Total pheromone produced by each experimental group was signficantly greater than the control.

Results of the second experiment showed that pheromone production increased significantly ($P < 0.05$) from days 3 and 4 in the untreated control, the tarsectomized group and the JHA treatment group (Fig. 2A., 2C., and 2D.). However the increase in pheromone production elicited by JHA treatment (4.19 times) from days 3 to 4 was significantly ($P < 0.05$) greater than increases observed for the untreated control (2.01 times) and tarsectomized (1.77 times) groups. While increases in pheromone production in these latter 2 groups were not significantly ($P < 0.05$) different, both were signficantly greater than the solvert control (Fig. 2B). The dramatic increase in pheromone production 48 hours following antennectomy in the first experiment (Fig. 1C. and 1D.). was not observed for the tarsectomized group in the second experiment (Fig. 2C.).

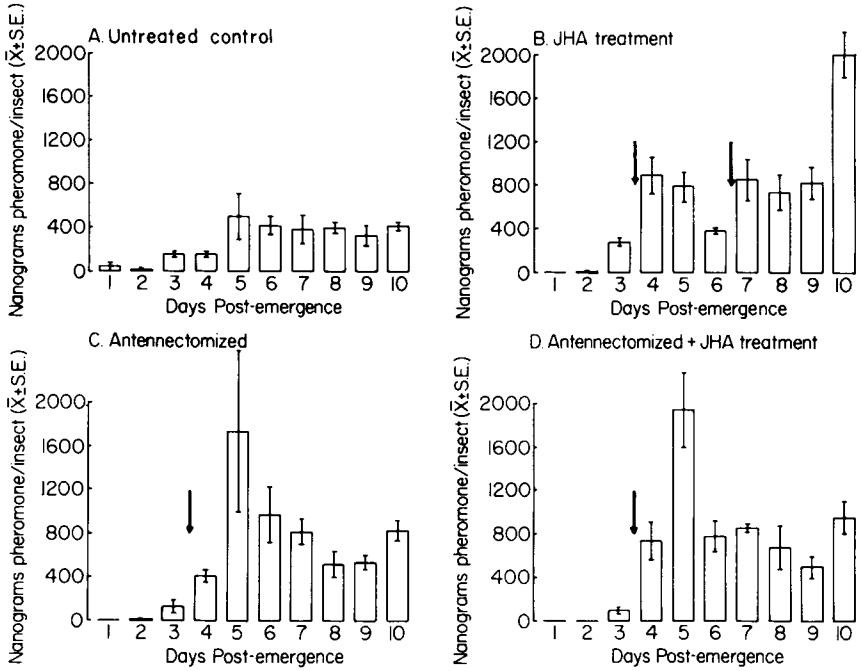

Fig. 1. Daily pheromone production ($\bar{X} \pm$ S.E.) by untreated control males (A.), males treated topically with 10 µg of JHA in 2 µl of acetone on days 3 and 6 (B.), males antennectomized on day 3 (C.), and males treated topically with 10 µg of JHA dissolved in 2 µl of acetone solvent, and antennectomized on day 3 (D.). Vertical arrows indicate treatment dates.

Table 1. Total amount of pheromone produced by control vs. three experimental groups from emergence through day 10 of adult life.

	Total amount of pheromone produced by each insect (ng) ($\bar{X} \pm$ S.E.)
Control	2813 ± 592 a*
JHA	6794 ± 855 b
Antennectomized	5892 ± 1398 b
Antennectomized + JHA	6539 ± 853 b

* Values followed by different letters significantly different ($P < 0.05$).

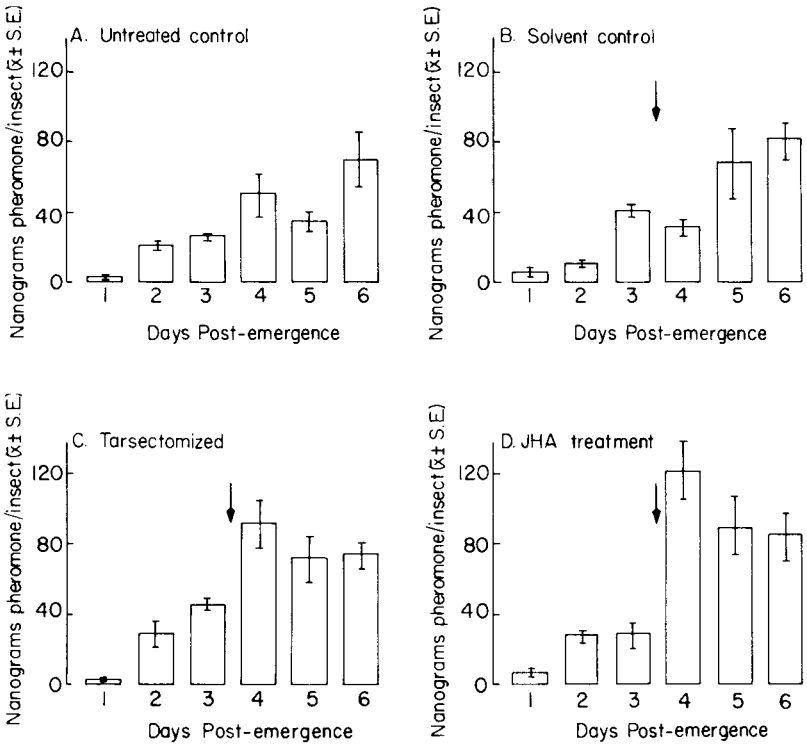

Fig. 2. Daily pheromone production ($\bar{X} \pm$ S.E.) by untreated control males (A.), males treated topically with 2 µl of acetone solvent on day 3 (B.), males foretarsectomized on day 3 (C.), and males treated topically with 10 µg of JHA dissolved in 2 µl of acetone solvent on day 3 (D.). Vertical arrows indicate treatment dates.

DISCUSSION

Our results indicate that both antennectomy and JHA have significant effects on pheromone production in the boll weevil. In other coleopterous insects, JH and JHA increase pheromone production by stimulating release of BH (Menon 1976; Hughes and Renwick 1977a). Since output interneurons connect olfactory glomeruli to other regions of the brain (Ernst et al. 1977; Matsumoto and Hildebrand 1981; Boeckh et al. 1984), e.g. the protocerebrum, information may be fed to the pars intercerebralis, where neurosecretory cells play an important role in the control of corpus allatum activity (Highnam 1964; Girardie 1967). Alternatively, antennectomy could stimulate release of a hormone responsible for stimulation of pheromone release from the brain itself as shown for certain Lepidoptera (Raina and Klun 1984).

The stimulatory effect of JHA on pheromone production is also of interest since recent experiments on both the boll weevil (Dickens 1986) and another insect (Palaniswamy et al. 1979) have shown JHA to decrease sensitivity of antennal receptors for pheromones as well as selected plant ordors in the boll weevil.

Furthermore, it was proposed for locusts that antennectomy might affect nymphal pigmentation, adult growth and morphometrics by changing activity of the corpora allata (Mordue 1977). Since similar changes in pigmentation and morphometrics took place naturally under uncrowded conditions, it was proposed that extirpation of antennae might reduce relevant sensory input which might simulate an uncrowded condition, perhaps by interfering with perception of a gregarization pheromone (Ellis and Gillet 1967; Nolte et al. 1970). In the boll weevil, one might speculate that when pheromone production was high, antennal sensitivity might be low possibly due to regulation by concurrently high JH levels; the high JH levels being responsible for the stimulation of pheromone production. It might be contemplated that this possible decrease in sensitivity during pheromone production could be especially significant for the boll weevil and other coleopterous insects which utilize aggregation pheromones (Tumlinson et al. 1969; Birch 1984). Since both sexes respond to the aggregation pheromone (Dickens 1984, 1986), a decrease in the sensitivity of antennal receptors of the individual producing the pheromone would decrease the likelihood of its responding to its own or nearby pheromone sources.

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