EFFECT OF A JUVENILE HORMONE ANALOGUE, FENOXYCARB ON PHEROMONE PRODUCTION AND REPRODUCTION IN THE CALIFORNIA FIVESPINED IPS, *IPS PARACONFUSUS* LANIER

by

Ningmei Chen

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Examining Committee:

Chairman: Dr. M. Mackauer

Dr. J. Borden, Senior Supervisor

Dr. K. Nair, Professor

Dr. B. McKeown, Professor, External Examiner

Date approved 4 Dec., 1986

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Author: [redacted]
(signature)

Ningmei Chen
(name)

Dec. 4, 1986
(date)

ABSTRACT

The effects of fenoxycarb, (ethyl[2-(p-phenoxyphenoxy)-ethyl]carbamate), an insect growth regulator with juvenile hormone activity, on pheromone production and reproduction in *Ips paraconfusus* Lanier were studied. Topical application of fenoxycarb in acetone induced newly-emerged male beetles to become attractive to females, as measured by positive responses to male abdominal extracts in a laboratory bioassay. Two pheromones, ipsdienol and ipsenol, were detected by gas chromatography in the abdominal extracts of fenoxycarb-treated males. Pheromone production was minimal at a dose of 0.1 μg/insect of fenoxycarb, maximal at 10 μg, and was reduced to trace amounts at a dose of 100 μg. In comparison, peak production of pheromones was induced at a dose of 0.1 μg/insect of natural juvenile hormone (JH III). Treatment with 10 μg of fenoxycarb resulted in the occurrence of pheromones 12 h after exposure, maximal pheromone content between 16 to 20 h, and undetectable amounts after 36 h. Topical treatment of either female or both parent beetles with 50 μg/insect of fenoxycarb resulted in a significant reduction in egg hatchability and number of progeny produced by beetles allowed to infest logs of ponderosa pine, *Pinus ponderosa* Laws. When pairs of beetles were allowed to attack logs that were surface-treated with fenoxycarb, the effects on reproduction at a dose of 100 μg/cm^2 of bark surface were comparable to those of a topical treatment at a dose of 50 μg/insect. However, at doses of 1000 or 10,000

$\mu g/cm^2$ the adverse effects included reduction in the length of egg galleries, fecundity (number of egg niches), and number of freshly-laid eggs. Percent reduction in progeny was found to be 35.8, 82.5, and 95.7 for doses of 100, 1,000, and 10,000 $\mu g/cm^2$, respectively, if broods were allowed to emerge. This study indicates that fenoxycarb exhibits strong juvenile hormone activity and acts as an effective chemosterilant on *I. paraconfusus*.

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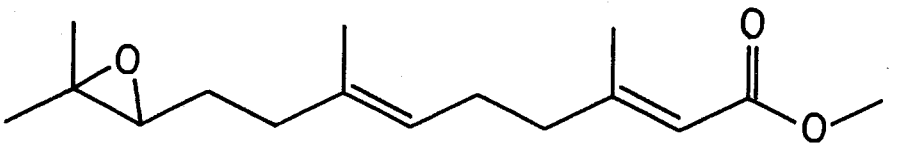
CHAPTER I

INTRODUCTION

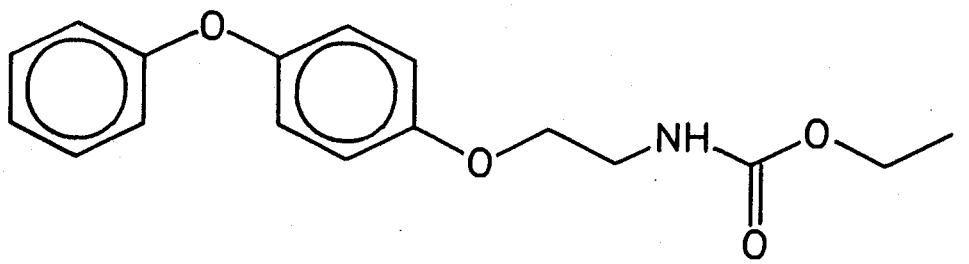
Insect growth regulators (IGRs) with juvenile hormone activity, may severely disrupt insect development and reproduction. They have been regarded as promising potential tools for use in insect pest management (Staal 1975). Such potential has been realized with the discovery and commercial development of methoprene for control of various insects, primarily mosquitoes (Schaefer and Wilder 1972, 1973), fleas (Chamberlain 1979), certain livestock pests (Harris *et al.* 1973), greenhouse and ornamental pests (Staal *et al.* 1973; Hamlen 1975) and storage pests (Loschiavo 1976; McGregor and Kramer 1975). However, certain limitations, such as lack of adequate efficacy, insufficient environmental stability, or high cost of manufacture, have been associated with most of the juvenile hormone analogues (JHAs) synthesized so far (Staal 1977).

Encouraging new evidence of practical potential has arisen from the recent tests of a non-terpenoid compound, fenoxycarb, (ethyl[2-(p-phenoxyphenoxy)ethyl]carbamate) (Fig. 1), a non-neurotoxic insecticide that exhibits strong juvenile hormone activity against numerous insect pests in various orders (Anonymous 1983; Dorn *et al.* 1981; Masner *et al.* 1981). This compound achieved satisfactory control of a broad spectrum of lepidopterous and coleopterous pests in stored products (Kramer

Fig. 1. Structural formulae of JH III and fenoxycarb.



JH III



Fenoxycarb

et al. 1981). It was also efficacious against leafrollers in orchards (Reede *et al.* 1984, 1985). Fenoxycarb is highly persistent in various environmental conditions, with residual activity up to 1 year in stored wheat (Edwards and Short 1984) and at least 4 weeks on apple leaves (Dorn *et al.* 1981; Reede *et al.* 1984). On the other hand, fenoxycarb demonstrates a very low mammalian toxicity (acute oral LD_{50}, rat>10,000 mg/Kg, Anonymous 1983). Trials under laboratory and various field conditions have shown that fenoxycarb appears to be innocuous against beneficial arthropods (Dorn *et al.* 1981; Parrella *et al.* 1983; Reede *et al.* 1984). Furthermore, it is effective against insects which have developed resistance to conventional insecticides (Edwards and Short 1984). Finally, due to its chemical structure, the compound is easy to synthesize and is therefore relatively inexpensive (Karrer and Farooq 1981).

Bark beetles are major forest insect pests throughout much of the Northern Hemisphere. Their biology is different from that of many other insects in that they spend most of their life cycle in the protected subcortical area of their hosts, and that adult beetles, which appear only briefly during their dispersal and attacking phases, produce aggregation pheromones which play a vital role in concentrating the dispersing beetle population on susceptible new hosts (Borden 1974, 1984).

Control of bark beetles with JHAs would be most feasible if the compound were applied to the outer bark of unattacked hosts, so that attacking beetles would be exposed to it as they

attempted to bore into the outer bark. To be most effective the JHA should be capable of affecting fecundity, but should not inhibit pheromone production by the attacking beetles. Such bark treatments have not been rigorously evaluated; however, prolonged emergence of brood *Ips typographus* L. was observed following treatment of logs prior to attack with a JHA (Novák *et al.* 1976). Research with topical applications indicates that JHAs may exert the desired detrimental effect on reproduction (Van Sambeek and Bridges 1981), while at the same time stimulating pheromone production (Borden *et al.* 1969; Hughes and Renwick 1977a, 1977b; Harring 1978; Conn 1981; and Bridges 1982).

Other phenomena associated with reproduction, including flight muscle degeneration (Borden and Slater 1968; Unnithan and Nair 1977); ovary development (Sahota *et al.* 1970; Fockler and Borden 1973), and mating behavior (Fockler and Borden 1973) may, depending on the dose given, also be stimulated or repressed by JHAs. In addition, JHAs may disrupt embryogenesis, if eggs are treated (Ibaraki and Sahota 1976; Novák *et al.* 1976) and metamorphosis, if last-instar larvae or pupae are treated (Novák *et al.* 1976; Van Sambeek and Bridges 1980).

The California fivespined ips, *Ips paraconfusus* Lanier, is an aggressive secondary bark beetle of moderate economic importance in pine forests of the Pacific Southwest of North America (Furniss and Carolin 1977). It is an excellent subject for research on reproductive biology and the effect of JHAs

because its aggregation pheromones are known (Silverstein *et al.* 1966; Wood *et al.* 1966, 1968), and it is sensitive to exogenous applications of JH or JHAs (Borden *et al.* 1969; Hughes and Renwick 1977a; Borden and Slater 1968; Unnithan and Nair 1977). Moreover, it is easily cultured in the laboratory, and readily responds to its pheromones in laboratory olfactometers (Wood and Bushing 1963; Borden 1967).

My objectives were: 1) to determine whether fenoxycarb can induce pheromone production by *I. paraconfusus*; and 2) to determine by topical and bark treatments if fenoxycarb can adversely affect the reprodction of *I. paraconfusus*.

CHAPTER II

MATERIALS AND METHODS

General Methods

Insect Rearing

I. paraconfusus were obtained from a continuously-reared, laboratory culture maintained on logs of ponderosa pine, *Pinus ponderosa* Laws., in laboratory cages at 25±1°C and variable R.H. All logs were cut from green trees near Princeton, B.C. Emergent brood beetles were collected daily. Their sex was determined by the presence of the pars stridens on the posterior dorsal area of the head of females, but not males (Wood 1961). Beetles were stored on moistened paper towels in glass jars at 4 °C until used for experiments.

Maintenance and Handling of Test Materials

Two forms of fenoxycarb, were supplied by the Elanco Division, Eli Lilly Canada. Inc., Winnipeg, Manitoba: a granular technical grade material (96.6% purity) for topical treatment of insects and and a liquid formulation (125 g/L) for treatment of logs. Technical fenoxycarb was kept in a glass vial at room temperature; 104 mg of the material was dissolved in acetone to make a main stock of 100 µg/µL. The main stock was subseqently diluted in acetone to give 0.1, 1, 10, and 50 µg/µL. All

extracts were stored at 4 °C in 2 mL vials with teflon-lined lids. The liquid formulation was also refrigerated at 4°C. A stock solution of 50 μg/μL was prepared by diluting 3.5 ml of formulation in 5.25 ml acetone. Further dilutions were made to give 5 and 0.5 μg/μL solutions which were used immediately.

JH III (96.9%, Sigma Chemical Co., St. Louis, U.S.A., 52 mg) was diluted with acetone to give a 100 μg/μL stock solution, which was divided into 5 approximately equal parts and stored individually in 2 ml vials with teflon-lined lids at -15°C. One vial was used whenever solutions of 0.1, 1, 10 μg/μL were needed.

Effect of Fenoxycarb on Pheromone Production

Treatment of Beetles

Adult beetles were immobilized by cooling at 4°C and stuck on scotch tape with their abdominal venter upward. Each beetle received 2 topical applications, made to the abdominal venter, of 0.5 μL of a given solution approximately 1 min apart administrated with a 25 μL Hamilton syringe attached to a 50-stop Hamilton repeating dispenser. Treated beetles were incubated individually for a specific length of time at room temperature in glass cylinders (1.78 cm ID) placed in petri dishes (Conn 1981). All petri dishes contained a saturated cotton wick and were sealed with parafilm to minimize loss of

moisture.

Preparation of Abdominal Extracts

Groups of 12 or 24 newly-emerged male beetles were either: 1) treated topically with acetone extracts of fenoxycarb or JH III at 0.1, 1, 10, 100 μg/insect and incubated at room temperature for 20 h; 2) treated with 1 μL acetone only applied in the same manner and held as above; 3) left untreated and held as above; or 4) introduced into preformed holes in ponderosa pine logs (Miller and Borden 1985) and allowed to bore in the inner bark for 20 h. For analysis by gas chromatography (GC), additional groups of 12 males were topically treated with 10 μg of fenoxycarb and incubated for 12, 16, 20, 28, or 36 h. All insects were taken from petri dishes or excised from the bark, and their abdomens (containing hindgut and Malpighian tubules) were removed and immediately immersed in double distilled pentane in 2 ml glass vials in dry ice.

For bioassays, 24 beetle abdomens from each treatment group were pooled and crushed with a glass rod in 200 μL of pentane. After crushing, samples were allowed to warm up slightly at room temperature and the clear extracts were transferred to clean 2 ml glass vials with one pentane rinse to give a 240 μL extract at a concentration of 1 abdomen/10μL of solution. Vials containing extracts were covered with teflon-lined lids and stored at -44°C until used in bioassays.

For analysis by GC the same extraction procedure was followed except that: 1) single abdomens were extracted with 100 µL of pentane without a rinse; and 2) solutions of 2 internal standards, 3- and 2-octanol, each at a concentration of 2.5 µL (2 mg) in 10 mL of pentane, were added before and after crushing, respectively, at the rate of 2.5 µL/sample (Conn 1981).

Bioassay Procedure

The attractiveness of male abdominal extracts was bioassayed with recently-emerged female *I. paraconfusus* in an open arena olfactometer (Conn 1981). The arena was a 24 cm diam. disposable filter paper. A glass tube (7 cm long and 1 cm ID) containing a rolled-up filter paper (4.25 cm diam.) impregnated with a stimulus was placed to allow an air stream to flow at a rate of 1500 mL/min through it toward the insect release point at the center of the arena. Bioassays were conducted at room temperature under red light.

Three types of stimuli were tested: 1) beetle abdominal extracts; 2) a pentane control; and 3) a pheromone standard consisting of racemic ipsenol, racemic ipsdienol, and cis-verbenol (Borregaard, A.S., Sarpsborg, Norway) at concentrations of 2, 1.5, and 1 ng/µL in pentane, respectively. Each stimulus was assayed at the rate of 20 µL, or 2 male equivelants for abdominal extracts, for each group of test

females. A fresh stimulus tube was used for each test, and the arena was changed for each new stimulus.

Beetles that walked normally and had no missing appendages were held in groups of 20 in 35 mm diam. petri dishes lined with moistened filter paper at 2-4°C. They were brought to room temperature about 2 min prior to use. Each group of insects was released in the center of the arena and given 2 min to respond. An insect was counted as a positive responder if it walked upwind and stopped within 1 cm of the stimulus source or circled around the edge of the airstream more than once. It was classified as non-responder if it walked off the arena or remained in the arena after 2 min without responding. Once its status was classified, an insect was returned to the petri dish, which was placed at the end of a rotation of 13 other petri dishes. No group of insects was reused for at least 60 min. There was no evidence that this procedure resulted in a change in response through time in this or in studies with other scolytids (Conn 1981; Stock and Borden 1983).

The bioassay experiment was completed in 2 sessions of 4 h each in the same day. Assays were conducted before each session with pentane and the pheromone standard to ensure that the insects were responding consistently.

Gas Chromatography

GC Analysis was conducted on a Hewlett-Packard 5880A gas chromatograph fitted with a flame-ionization detector and a glass capillary column (30 m x 0.50 mm ID) coated with SP-1000 (Supelco Inc., Bellafonte, Pa., U.S.A.). The oven temperature was programmed from 120 to 180°C at the rate of 4°C/min, with an injector temperature of 260°C and detector temperature of 275°C.

Samples which were kept in dry ice were warmed to room temperature, and 2 µL of each sample was injected manually into the injection port. A standard sample consisting of the synthetic pheromones, ipsenol, ipsdienol, and cis-verbanol was routinely put through the GC under identical conditions as the beetle extracts. Determination of pheromone content in the samples was based on comparison of retention times with those of the standard sample. Quantification of ipsenol and ipsdienol was achieved by using the area under the 3-octanol peak as a reference. The 2-octanol peak was used to check for possible loss of volatiles during processing of the samples.

Effect of Fenoxycarb on Reproduction

Treatment of Parent Insects

Male beetles were: 1) treated topically with 10 or 50 µg of fenoxycarb in acetone; 2) treated with 1 µL of acetone; or 3)

left untreated. After 1 h at room temperature, males in all 3 groups were introduced individually into predrilled holes in the bark of ponderosa pine logs (about 80 cm long and 20-30 cm in diam.). The holes were drilled at approximately 30 cm centers in vertical rows 12 cm apart. The beetles were confined with geletin capsules taped over the holes (Miller and Borden 1985). Females were treated in the same manner as males, but 24 h later. Each female was introduced into a capsule to pair with a male.

There were 8 treatment combinations randomly assigned to one of 8 logs cut from a single uninfested ponderosa pine. Treatment combinations were: 1) both sexes received 10 μg fenoxycarb; 2) male received 10 μg fenoxycarb, female acetone; 3) female received 10 μg fenoxycarb, male acetone; 4) both sexes received 50 μg fenoxycarb; 5) male received 50 μg fenoxycarb, female acetone; 6) female received 50 μg fenoxycarb, male acetone; 7) both sexes received acetone; and 8) both sexes left untreated.

The experiment was terminated 2 weeks after the female beetles were paired with males. The logs were debarked and the following data recorded: length of egg galleries, and the number of egg niches, larvae, eggs, and larval mines associated with each gallery. Data were not recorded if one or both parents was found dead or missing.

Treatment of Logs

Juvenile Assessment Experiment

Hollow glass cylinders (1.78 cm ID) were attached, at the same intervals as the preformed entrance holes above, on 5 ponderosa pine logs, approximately 85 cm long and 22-23 cm in diameter cut from a single green tree. These logs were then randomly assigned to 5 different treatments. Using 100 μL Hamilton syringes, 500 μL of liquid formulation fenoxycarb in acetone at 0.5, 5, or 50 μg/μL were administrated in 3 applications of 250, 150, and 100 μL each at about 10 min intervals to the bark surface enclosed by the glass cylinders to give approximately 100, 1,000, and 10,000 $\mu g/cm^2$ of bark surface. Treated logs were allowed to air dry for 2 h before introduction of insects. Controls were logs treated with 500 μL of acetone or left untreated. One male and one female beetle were put simultaneously into each glass cylinder and confined therein by covering the open end with parafilm throughout the entire experiment except when inspection and frass removal was conducted. Logs were kept separately in laboratory cages at 25±2°C. The next day, any male or female beetles found dead or apparently moribund were replaced. Frass was removed from the glass cylinder every other day. Two weeks after the beetles were put into logs, the logs were debarked and the galleries examined as above.

Brood Emergence Experiment

The same experimental setup as for juvenile assessment was followed except that: 1) beetle progeny were allowed to develop and emerge; 2)parent beetles were introduced into logs in rows with centers 40 cm apart and 16 cm between rows; and 3) frass was removed weekly until brood emergence. Brood beetles were collected twice a day from the wall and floor of individual cages and their number recorded. The experiment was terminated 30 days after the first appearance of emergent beetles in the cage containing the acetone-treated log. All logs were stripped; those galleries containing egg niches excavated by introduced beetles were considered as successful attacks and were used in calculating average brood size for each treatment group. Beetles that successfully attacked but produced no emergent brood, as indicated by no emergent holes, were regarded as completely sterilized.

Statistical Analysis

Proportional data, including percentages of responding females in bioassays, males containing pheromones in GC analyses, and beetle pairs producing emergent brood were converted using an arcsin-square-root transformation and compared using a Newman-Kuels test modified for testing proportions (Zar 1984).

A maximum likelihood method [1] was used to estimate the mean amounts of pheromone produced by males treated with fenoxycarb or JH III, primarily to recover valuable information regarding the effect of treatments from zero values which were probably resulted from a certain threshold (8.0 ng) of the GC equipment. Differences among estimated means were then assessed by the Newman-Kuels test, and differences from zero by t-tests.

Data on mean cumulative brood emergence were fitted into linear regression equations after log(y+1) transformations, and the slopes of the regression lines were compared by the Bonferroni simultaneous test.

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[1]Based on an exponential model developed by Drs. M. A. Stephens, K. L. Weldon, and T. Swartz, and Mrs. S. Ng, Department of Mathematics and Statistics, Simon Fraser University.

CHAPTER III

RESULTS AND DISCUSSION

Effect of Fenoxycarb on Pheromone Production

Within 20 h after topical treatments of male beetles with fenoxycarb or JH III their abdominal extracts became attractive to females, when compared with the responses of females to pentane or extracts of either untreated or acetone-treated males (Table 1). Response was maximal to extracts of males treated with 10 μg of fenoxycarb, but significantly less than maximal to extracts of males subjected to the 100 μg treatment. Abdominal extracts from males treated with 0.1 μg of JH III were as attractive to females as those from males treated with 10 μg of fenoxycarb (Table 1). However, there was a progressively weaker response as the dose of JH III increased. The pheromone standard extracts from males fed in logs (and thus exposed to the pheromone precursor, myrcene) elicited the strongest responses by females (Table 1).

The bioassay results were supported by gas chromatographic analysis (Table 2). Treatment of male beetles with fenoxycarb or JH III resulted in the occurrence of ipsenol and ipsdienol in their abdominal extracts (as determined by identical retention times as those of synthetic ipsenol and ipsdienol). In agreement with the bioassay results (Table 1), the optimal dosages for fenoxycarb and JH III were 10 and 0.1 μg, respectively.

TABLE 1. **Response by *I. paraconfusus* females in laboratory bioassays to pentane extracts of abdomens from males treated with fenoxycarb or JH III, and extracted 20 h after treatment.**

Stimulus source	Dosage	No. females tested	Response[b] (%)
Pentane	20 µL	120	3.3a
Pheromone standard[a]	20 µL	121	66.1f
Abdominal extracts from			
males in logs 20 h	2 male equiv.	140	90.7g
untreated males	"	121	9.0ab
acetone-treated males	"	140	7.9ab
Abdominal extracts from fenoxycarb-treated males			
0.1 µg fenoxycarb	"	140	14.3bc
1.0 µg fenoxycarb	"	140	22.9c
10.0 µg fenoxycarb	"	121	48.3d
100.0 µg fenoxycarb	"	121	18.2bc
Abdominal extracts from JH III-treated males			
0.1 µg JH III	"	140	41.4d
1.0 µg JH III	"	119	16.8bc
10.0 µg JH III	"	140	15.0bc
100.0 µg JH III	"	120	13.3bc

[a] (±)ipsenol + (±)ipsdienol + cis-verbenol at 40, 30, and 20 ng/20 µL, respectively.

[b] Percentages followed by the same letter are not significantly different, Newman-Kuels test (α=0.05) modified for proportional data (Zar 1984).

TABLE 2. **Effects of topical treatment with fenoxycarb or JH III on pheromone production by male** *I. paraconfusus* **extracted and analyzed 20 h after treatment.**

Treatment (n=12 males/treatment)	Percentages of treated males containing pheromones[a]	Amount (ng) of pheromone/male[b] ($\bar{x}$ ± S.E.)	
		Ipsdienol	Ipsenol
Controls			
In logs 20 h	83.3c	129.9 ± 28.5	816.5 ± 191.0
Untreated	0.0a	0.0	0.0
Acetone-Treated (1 μL)	0.0a	0.0	0.0
Treated with fenoxycarb in 1 μL acetone			
0.1 μg	25.0b	3.3 ± 1.0a	10.6 ± 3.0b
1.0 μg	25.0b	7.3 ± 2.1b	26.0 ± 7.5c
10.0 μg	50.0bc	12.7 ± 3.7c	45.1 ± 13.0d
100.0 μg	0.0a	0.0	0.0
Treated with JH III in 1 μL acetone			
0.1 μg	33.3b	6.4 ± 1.8b	21.0 ± 6.1c
1.0 μg	16.7ab	3.8 ± 1.1a	12.6 ± 3.6b
10.0 μg	8.3ab	0.0	4.5 ± 1.3a
100.0 μg	0.0a	0.0	0.0

[a] Percentages followed by the same letter are not significantly different, Newman-Kuels test (α=0.05) modified for testing proportional data (Zar 1984).

[b] Zero values indicate no detectable amount (<8.0 ng) of pheromone. Where mean amounts >0.0, original data (excluding data from males in logs 20 h) have been replaced by maximum likelihood estimates of means from an exponential model derived from the original data. Exponentiality is assumed because by the Anderson-Darling Statistic, there is no evidence that the data do not follow an exponential distribution. Ajusted means within a column followed by the same letter are not significantly different, Newman-Kuels test (α=0.05). All adusted means are significantly different from zero, *t*-test, P<0.05.

However, there was no measurable pheromone (threshold = 8.0 ng) in males treated with 100 μg of either fenoxycarb or JH III. On the other hand, treatment with fenoxycarb at the dose of 0.1 μg resulted in a significant increase in percent of treated males containing pheromone and in amount of pheromone content (Table 2).

The results of both bioassays (Table 1) and GC analysis (Table 2) corroborate the conclusions of Borden *et al.* (1969) and Hughes and Renwick (1977a) that JH stimulates pheromone biosynthesis in *I. paraconfusus*, even though the potency of JH III was found to be at least 100x higher than in the experiment conducted by Borden *et al.* (1969). The results also clearly indicate that fenoxycarb mimics the function of juvenile hormone in stimulating pheromone biogenesis in *I. paraconfusus*, and suggest that it possesses high biological activity for scolytid beetles.

The diminished pheromone activity (Table 1) or detectable content (Table 2) with treatment dosages higher than optimal has not been observed before in *I. paraconfusus*. However, inhibitory effects on pheromone production with exogenous JHs or JHAs has been observed in a number of insects, and several hypothesis have been proposed to account for this phenomenon. Hedin *et al.* (1982) found that JH III incorporated at 1.0 ppm in the diet of male boll weevils, *Anthonomus grandis* Boheman, caused a 3-fold increase in the biosynthesis of 4 pheromone compounds. However, at dosages of 10 and 100 ppm, there was a decrease in pheromone

synthesis. The results obtained by Hedin *et al.* (1982) are consistent with Staal's (1975) hypothesis that the failure of increasing amounts of JH to elicit increasing greater effects may be due to a cellular sensitivity which limits the capacity of a cell to respond to a given stimulus.

Decreases in pheromone content following application of JHA in the southern pine beetles, *D. frontalis* Zimmermann were observed by Bridges (1982), who suggested that JH might stimulate release of pheromones. Treatment with JHAs resulting in a significant reduction in pheromone activity was also observed in female yellow mealworms, *Tenebrio molitor* L. (Menon and Nair 1972), and in female cockroaches *Byrsotria fumigata* (Guerin) (Bell and Barth 1970). For both insects, the reduced activity was attributed to stimulation of yolk deposition by an excess of JHA, which might in some way have inhibited pheromone production. Fockler and Borden (1973) reported that a high dosage (50 μg) of a JHA inhibited mating activity of the striped ambrosia beetle, *Trypodendron lineatum* (Olivier), whereas a lower dosage (0.05 μg) accelerated the activity. They attributed this phenomenon to a toxic effect of high dose of JHA since there was some mortality in treated insects. However, such a toxic effect was not evident in my experiment; there was no mortality, nor apparent loss of vigor of *I. paraconfusus* treated with any dose of fenoxycarb or JH III. Therefore, the failure to detect pheromone following treatment with either material suggests an inhibitory effect. Such an effect could result from

negative feedback on the release of brain hormone, which, as demonstrated by Hughes and Renwick (1977a), is essential for normal pheromone biogenesis in *I. paraconfusus*. Inhibition of brain hormone release by high JH titer has been reported for certain diapausing larval lepidopterans, such as the cabbage armyworm, *Mamestra brassicae* L. (Hiruma *et al.* 1978), and the tobacco hornworm, *Manduca sexta* (L.) (Nijhout and Williams 1974; Rountree and Bollenbacher 1984). Thus, the optimal (non-inhibitory) effect of 100 μg JH III found by Borden *et al.* (1969) may have been due to the nonvolatile solvent, peanut oil, which might have served as a slow release medium. The same dosage of the same compound carried by highly active solvent acetone may have caused a rapid saturation of JH receptors on the corpora cardiaca (Hughes and Renwick 1977a), or may have activated some other negative feedback mechanism that inhibited pheromone biosynthesis.

When male beetles were treated with 10 μg fenoxycarb and then extracted and analyzed at various post-treatment times, pheromone was first detected 12 h after treatment (Figs. 2, 3). The highest proportions of individuals contained pheromone at 16 and 20 h; pheromone-containing males were fewer after 28 h, and none contained detectable pheromone after 36 h. The proportions of beetles containing pheromone were reflected by the amount of ipsenol, and to a lesser extent ipsdienol, content/male (Fig. 3).

Fig. 2, 3. Relationship between time after treatment of male *Ips paraconfusus* with fenoxycarb and percent of treated males containing ipsenol, ipsdienol or both (Fig. 2), and amount (ng±S.E.) of pheromone content/ male (Fig. 3). Bars in Fig. 2 with same letter above are not significantly different, Newman-Kuels test (α=0.05) modified for proportional data (Zar 1984).

Fig. 2

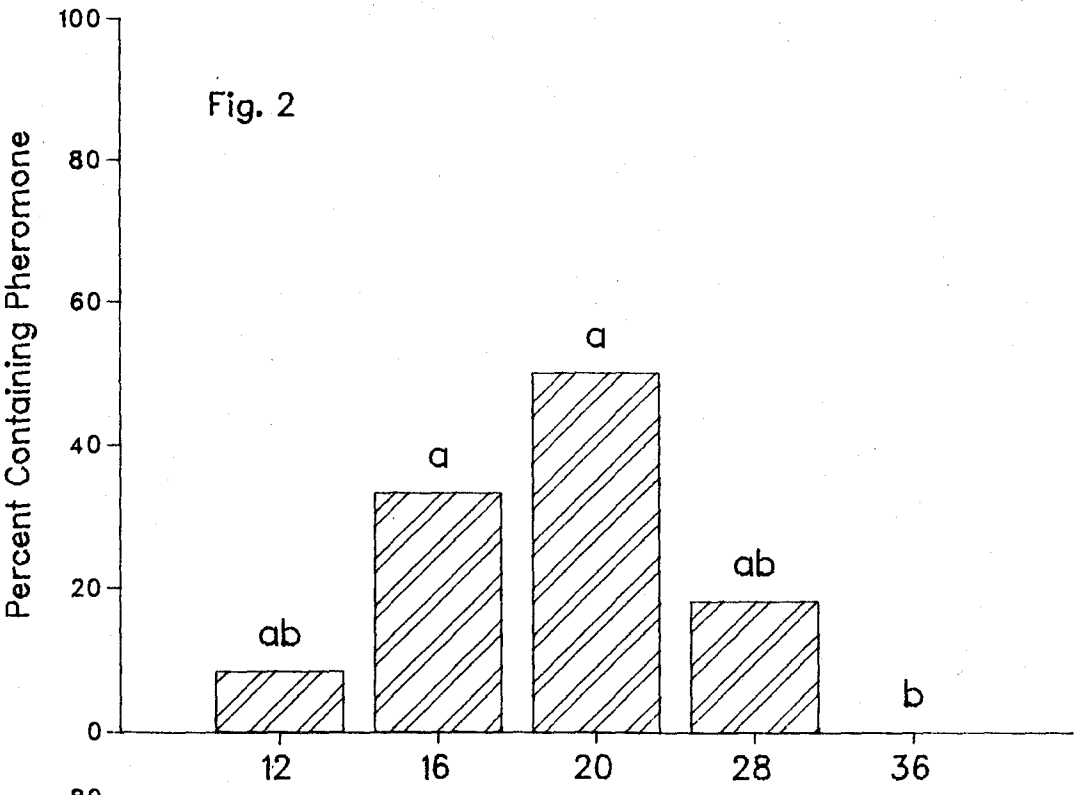
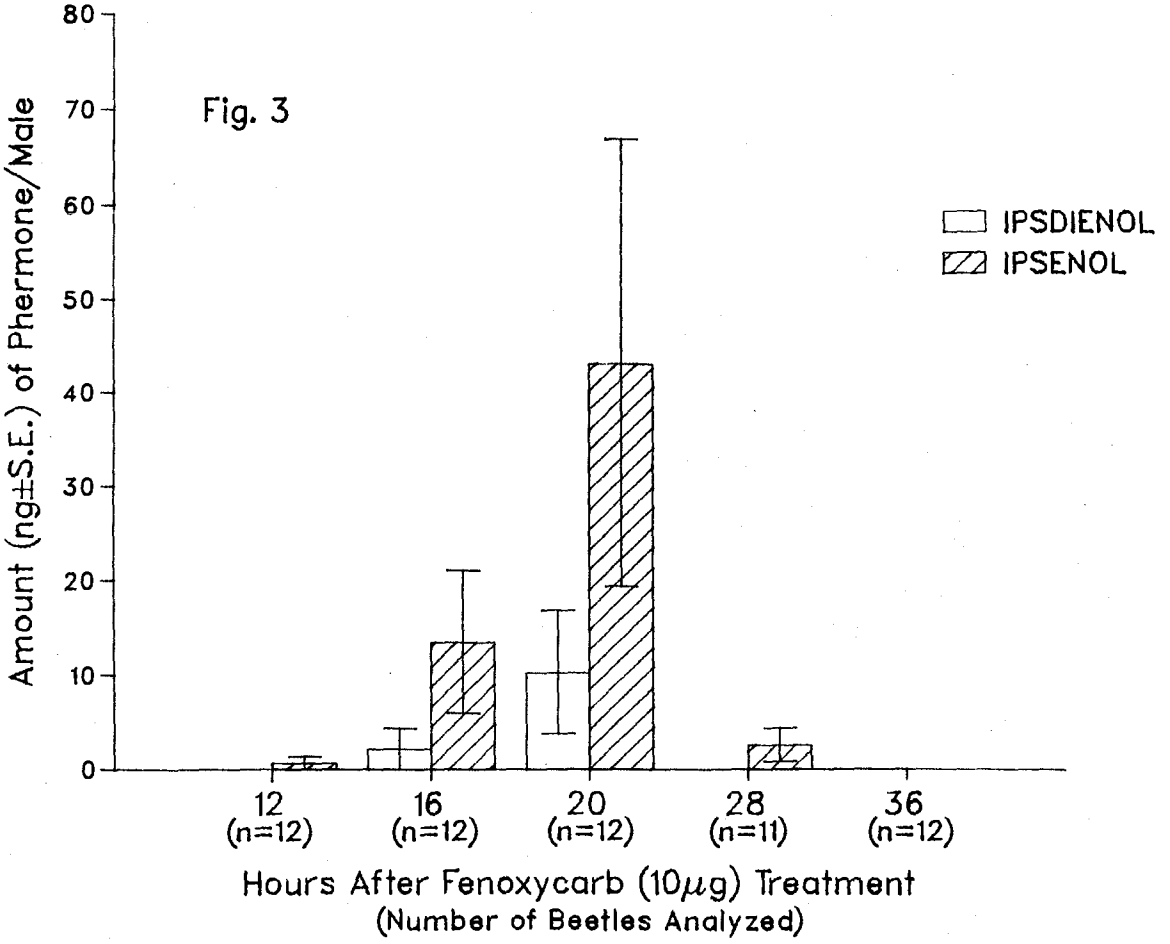

Percent Containing Pheromone
100
80
60
40
20
0
ab
a
a
ab
b
12
16
20
28
36

Fig. 3


Amount (ng±S.E.) of Phermone/Male
80
70
60
50
40
30
20
10
0
IPSDIENOL
IPSENOL
12 (n=12)
16 (n=12)
20 (n=12)
28 (n=11)
36 (n=12)
Hours After Fenoxycarb (10μg) Treatment
(Number of Beetles Analyzed)

These results agree in part with those of Borden *et al.* (1969), who concluded that it required at least 12 h for JH-induced pheromone synthesis to produce sufficient pheromone to elicit a positive response by females, whereas it required only 3 h for beetles boring and feeding on fresh ponderosa pine bark to possess highly attractive guts. The lag between pheromone synthesis induced by stimuli associated with boring and feeding activity and by exogenous JH treatment may provide indirect evidence for Hughes and Renwick's (1977a) hypothesis regarding neural and hormonal control mechanism of pheromone biosynthesis in *I. paraconfusus*. The production of JH and subsequent release of brain hormone in response to the removal of neural inhibition, e.g. as a result of gut distention by feeding (Hughes and Renwick 1977a; Harring 1978), might occur quite rapidly. On the other hand, subtle changes in hemolymph JH titers resulting from slow rates of penetration of topically-applied material through the cuticle and transport to the target tissues could limit the rate of pheromone production. Possibly, however, beetles in fresh host material simply may have abundant supplies of ingested myrcene as a pheromone precursor (Byers 1981), and thus produce detectable levels of ipsdienol and ipsenol in a shorter time than JH- or JHA-treated beetles, which must de-derivatize limited amounts of sequestered precursor (Hughes 1975) prior to converting it to ipsdienol and ipsenol. Borden *et al.* (1969) and Hughes and Renwick (1977a) monitored the pheromone content in treated male *I. paraconfusus* for only 24 and 22 h, respectively. As JH III- or

fenoxycarb-treated beetles were denied food, their pheromone production must be constrained by the amount of endogenous sequestered precursor (Borden 1984). It is likely, therefore, that this supply was consumed within about 20 h after treatment and that the volatile pheromones were lost eventually by emission from the hindgut (Pitman *et al.* 1965; Renwick *et al.* 1966) to the external environment, and/or by diffusion into the haemocoel, resulting in undetectable levels by 36 h (Figs. 2, 3). The observation that pheromone production induced by exogenous JH or JHA varies not only with dosage but also with time may be of concern in future studies.

Effect of Fenoxycarb on Reproduction

Topical treatment of both sexes or females with 50 μg of fenoxycarb caused a significant reduction in mean brood size, as assessed by the number of immature progeny in logs debarked 14 days after they were attacked by treated or control beetles (Tables 3, 4). Treatment of the bark with fenoxycarb also had a significant adverse effect on reproductive success as assessed by the type of gallery constructed (Fig. 4), the number of immature progeny (Tables 5, 6) or emergent brood beetles (Table 7, Fig. 5). Reduction in reproductive success was associated primarily with a significant decrease in egg hatchability (Tables 4, 6), but following pre-attack bark treatment with 1,000 or 10,000 μg/cm^2 of fenoxycarb, there was also a reduction in fecundity (as indicated by mean number of egg niches) (Table 5).

Acetone treatment, either topical or applied to the bark, had no significant effect on the reproductive capacity of *I. paraconfusus*. My results agree with those of Van Sambeek and Bridges (1981) for *D. frontalis* , but differ from those of (Critchley and Almeida 1973) in which the reproductive petential of a cotton stainer, *Dysdercus fasciatus* Signoret was significantly reduced by acetone treatment. Hatchability in all control treatments (ranging from 84.4 to 97.3) (Table 4, 6) was lower than that (98.2%) reported by Wood and Stark (1966) for *I. paraconfusus* reared under similar conditions. However, their

TABLE 3. **Gallery characteristics and brood production in ponderosa pine logs debarked 14 days after attack by fenoxycarb-treated** *I. paraconfusus*.

Treatment		No. beetle pairs	Gallery characteristics[a]			Brood production[a]	
Males	Females		Length of egg gallery (cm)	No. egg niches/ gallery ($\bar{x}$ ± S.E.)	No. larval mines/ gallery ($\bar{x}$ ± S.E.)	No. larvae/ gallery ($\bar{x}$ ± S.E.)	No. eggs/ gallery ($\bar{x}$ ± S.E.)
untreated	untreated	8	15.7 ± 1.0	52.4 ± 6.0	43.6 ± 5.1a	40.1 ± 4.3a	6.3 ± 2.0
acetone	acetone	7	14.1 ± 0.9	48.0 ± 3.0	42.9 ± 3.4a	36.3 ± 3.7a	4.0 ± 1.0
acetone	10 µg fenoxycarb	6	15.2 ± 1.8	50.2 ± 6.0	41.7 ± 5.1ab	33.2 ± 5.2ab	5.0 ± 2.0
10 µg fenoxycarb	acetone	6	13.7 ± 1.5	42.8 ± 5.0	37.5 ± 3.5ab	31.5 ± 2.9abc	3.5 ± 2.0
10 µg fenoxycarb	10 µg fenoxycarb	5	13.7 ± 1.3	41.0 ± 3.0	33.4 ± 1.9ab	22.0 ± 2.2abc	3.2 ± 2.2
acetone	50 µg fenoxycarb	7	12.7 ± 1.6	36.1 ± 4.1	21.1 ± 5.8b	14.9 ± 4.2c	3.7 ± 2.0
50 µg fenoxycarb	acetone	8	13.3 ± 1.1	43.3 ± 5.5	37.8 ± 4.4ab	29.7 ± 3.9abc	2.6 ± 1.0
50 µg fenoxycarb	50 µg fenoxycarb	9	13.3 ± 1.1	38.8 ± 4.1	22.9 ± 5.5b	17.0 ± 4.0bc	1.0 ± 0.8

[a] Values within columns 1, 2, and 5 are not significantly differnt (Anova, $P>0.05$). Values within columns 3 and 4 followed by the same letter are not significantly different, Newman-Kuels test, ($\alpha=0.05$).

TABLE 4. **Reproductive success of** *I. paraconfusus* **in ponderosa pine logs debarked 14 days after attack by parent beetles treated topically with fenoxycarb.**

Treatment		No. beetle pairs	Reproductive success[a]		
Males	Females		Egg hatchability[b] (%)	Brood size[c] ($\bar{x}$ ± S.E.)	Reduction of progeny[d] (%)
untreated	untreated	8	93.5 ± 3.4a	46.4 ± 5.5a	--
acetone	acetone	7	97.3 ± 0.8a	40.3 ± 3.6a	--
acetone	10 µg fenoxycarb	6	91.1 ± 3.2a	38.2 ± 6.9ab	11.9
10 µg fenoxycarb	acetone	6	96.0 ± 2.4a	35.0 ± 4.0ab	19.3
10 µg fenoxycarb	10 µg fenoxycarb	5	88.2 ± 2.8a	25.2 ± 4.2ab	41.9
acetone	50 µg fenoxycarb	7	59.1 ± 15.7b	18.6 ± 5.5b	57.1
50 µg fenoxycarb	acetone	8	93.9 ± 2.7a	32.2 ± 4.6ab	25.7
50 µg fenoxycarb	50 µg fenoxycarb	9	56.1 ± 13.0b	18.0 ± 4.3b	58.5

[a] Data from Table-3 used to calculate egg hatchability and brood size. Values within a column followed by the same letter are not significantly different, Newman-Kuels test (α=0.05).

[b] $$\frac{\text{No. larval mines}}{\text{No. egg niches - No. eggs}} \times 100$$

[c] $$\frac{\text{No. larvae + No. eggs}}{\text{No. beetle pairs}}$$

[d] $$100 - \frac{\text{Mean brood size in treatment}}{\text{(Mean brood size in untreated control + Mean brood size in acetone-treated control)/2}} \times 100$$

TABLE 5. **Gallery characteristics and brood production in ponderosa pine logs debarked 14 days after treatment of the bark with fenoxycarb and subsequent attack by** *I. paraconfusus*.

Treatment	beetle pairs	Gallery characteristics[a]			Brood production[a]	
		Length of egg gallery (cm)	No. egg niches/ gallery ($\bar{x}$ ± S.E.)	No. larval mines/ gallery ($\bar{x}$ ± S.E.)	No. larvae/ gallery ($\bar{x}$ ± S.E.)	No. eggs/ gallery ($\bar{x}$ ± S.E.)
Controls:						
untreated	8	15.0 ± 1.2a	53.3 ± 5.7a	40.0 ± 5.3a	35.1 ± 4.9a	6.5 ± 1.6ab
acetone	8	16.5 ± 0.8a	56.4 ± 3.5a	41.9 ± 3.4a	36.4 ± 2.9a	9.5 ± 1.5a
100 μg fenoxycarb/cm²	7	13.7 ± 1.5a	54.4 ± 6.5a	25.6 ± 4.1b	19.7 ± 2.9b	9.3 ± 1.6a
1,000 μg fenoxycarb/cm²	8	10.3 ± 1.1b	41.8 ± 4.7ab	14.8 ± 5.6bc	11.0 ± 4.6b	4.1 ± 1.0b
10,000 μg fenoxycarb/cm²	9	9.4 ± 0.9b	28.8 ± 6.0b	7.2 ± 2.9c	4.9 ± 2.4c	2.4 ± 1.1b

[a] Values within a column followed by the same letter are not significantly different, Newman-Kuels test, (α=0.05).

TABLE 6. **Reproductive success of** *I. paraconfusus* **in ponderosa pine logs debarked 14 days after attack by parent beetles boring through fenoxycarb-coated bark.**

treatment	No. beetle pairs	Reproductive success[a] Egg hatchability[b] (%)	Brood size[c] ($\bar{x}$ ± S.E.)	Reduction of progeny[d] (%)
Controls:				
untreated	8	84.4 ± 5.3a	41.6 ± 6.1ab	-
acetone	8	89.5 ± 2.1a	45.9 ± 2.3a	--
100 μg fenoxycarb/cm²	7	57.5 ± 7.2b	29.0 ± 3.7b	33.7
1,000 μg fenoxycarb/cm²	8	31.6 ± 11.0c	15.1 ± 5.1c	65.5
10,000 μg fenoxycarb/cm²	9	21.6 ± 8.1c	7.3 ± 3.6c	83.3

[a] Data from Table-3 used to calculate egg hatchability and brood size. Values within a column followed by the same letter are not significantly different, Newman-Kuels test (α=0.05).

[b] $$\frac{\text{No. larval mines}}{\text{No. egg niches - No. eggs}} \times 100$$

[c] $$\frac{\text{No. larvae + No. eggs}}{\text{No. beetle pairs}}$$

[d] $$100 - \frac{\text{Mean brood size in treatment}}{(\text{Mean brood size in untreated control + Mean brood sizein acetone-treated control})/2} \times 100$$

FIG. 4. Comparison between *I. paraconfusus* galleries from untreated log (left) and from log treated prior to attack with fenoxycarb at a dose of 1,000 $\mu g/cm^2$ (right).

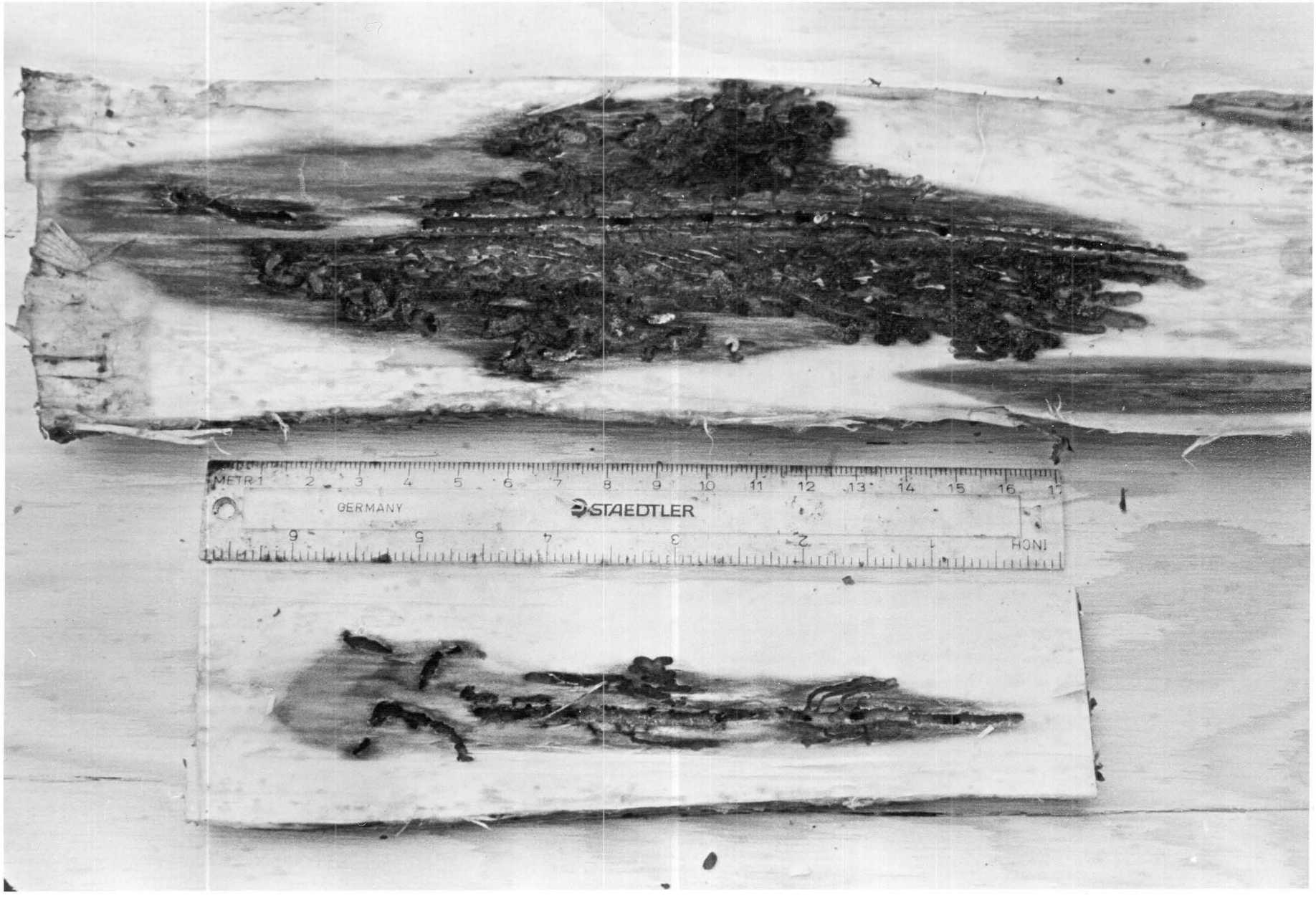


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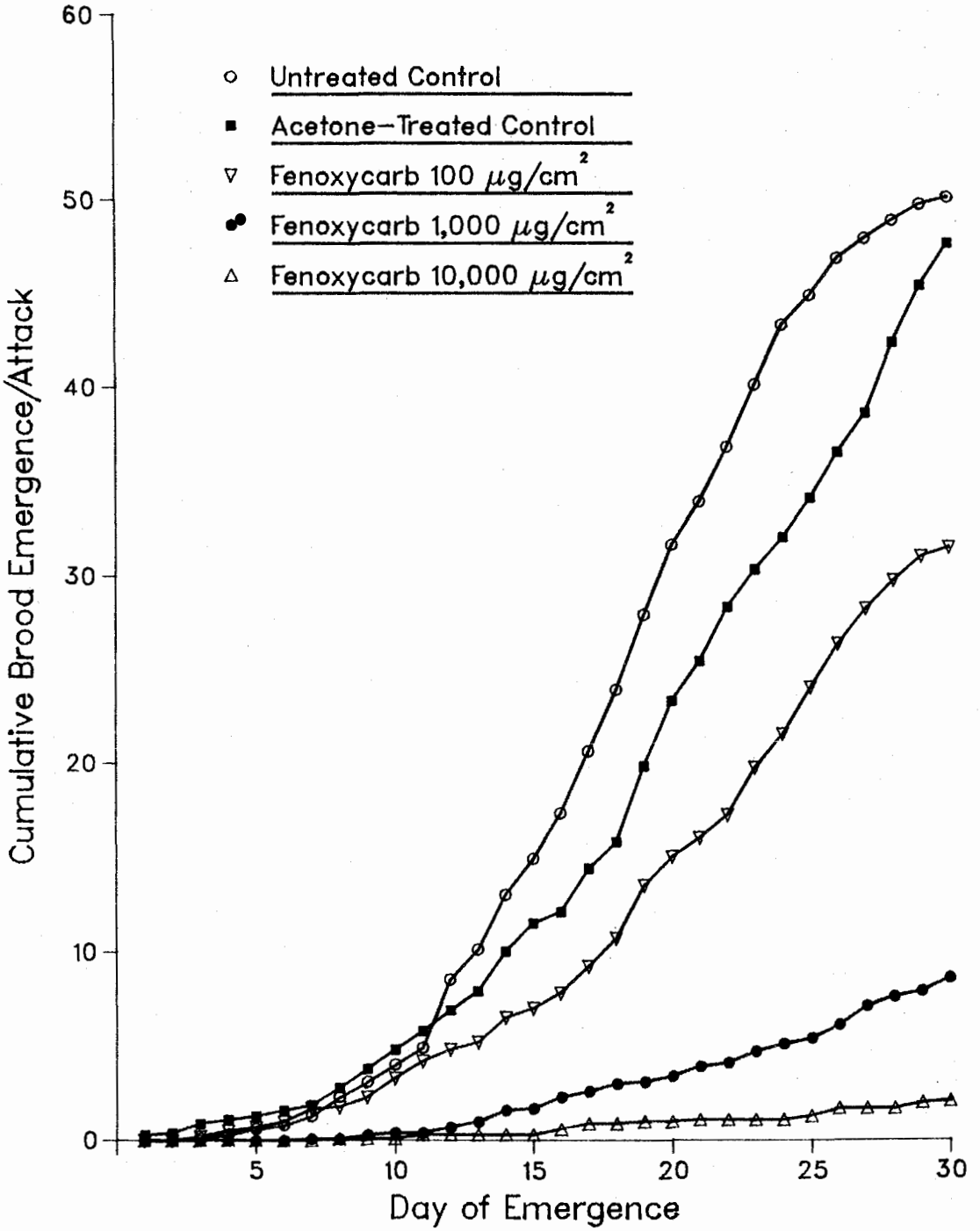
Table 7. **Effect of pre-attack application of fenoxycarb to the bark of ponderosa pine logs on subsequent, 30-day production of emergent brood** *I. paraconfusus*.

Treatment	No. beetle pairs attacking successfully	Percent of successful attack producing emergent brood[a]	Average No. emergent beetles per successful attack	Reduction of progeny[b] (%)
Controls:				
untreated	8	100.0a	50.3	-
acetone	8	100.0a	47.8	-
100 µg fenoxycarb/cm²	6	83.3ab	31.5	35.8
1,000 µg fenoxycarb/cm²	7	57.1bc	8.6	82.5
10,000 µg fenoxycarb/cm²	7	42.9b	2.1	95.7

[a] percentages followed by the same letter are not significantly different, Newman-Kuels test (α=0.05) modified for proportional data (Zar 1984).

[b] $\frac{\text{Mean no. emergent beetles in pooled controls - No. emergent beetles in treatment}}{\text{Mean no. emergent beetles in pooled controls}} \times 100$

Fig. 5. Cumulative emergence of *I. paraconfusus* from logs treated with 100, 1,000, or 10,000 µg of fenoxycarb as compared with emergence from untreated and aectone-treated control logs.


Untreated Control
Acetone–Treated Control
Fenoxycarb 100 μg/cm²
Fenoxycarb 1,000 μg/cm²
Fenoxycarb 10,000 μg/cm²
Cumulative Brood Emergence/Attack
Day of Emergence
0
10
20
30
40
50
60
5
10
15
20
25
30

formula for estimating egg hatchability was derived from the ratio of the number of larval mines to the sum of the number of collapsed eggs plus the number of larval mines because of the variation in number of empty egg niches in their study. However, in my experiment, very few empty egg niches were observed and there was no obvious variation among different treatments.

Neither length of the egg gallery nor the number of egg niches were significantly affected by topical treatment with fenoxycarb (Table 3), suggesting that unlike neuro-toxic chemicals, it does not exert a profound direct action on the behavior of *I. paraconfusus*. A similar phenomenon was observed by Van Sambeek and Bridges (1981) in *D. frontalis* treated topically with methoprene.

The significant reduction in mean brood size, but not fecundity following topical treatment of both sexes, or of females alone, with 50 μg of fenoxycarb (Table 3) can be mainly attributed to diminished numbers of larvae, which, in turn, reflect a significant reduction in hatchability (Table 4). On the other hand, the absence of any significant effect after males were treated with 50 μg of fenoxycarb suggests that fenoxycarb has little influence on the fertility of male beetles. Moreover, if fenoxycarb is transfered to untreated females by treated males, the dose rate was too low to affect the reproductive potential of females. The sexually selective impact of fenoxycarb on reproduction by females could result from formation of defective eggs and/or from an effect on

embryogenesis in JHA-contaminated eggs (Sehnal 1976; Staal 1975).

Fenoxycarb applied to outer bark surface even at a dosage as low as 100 $\mu g/cm^2$ caused a similar detrimental effect on reproduction of *I. paraconfusus* (Table 5, 6) as 50 μg topical treatments (Table 3, 4). Since the male beetles initiate attack in *I. paraconfusus*, and injest host material while doing so, they could possibly have taken in enough fenoxycarb to affect their reproductive success. However, females that join males do not begin to feed until they construct egg galleries deep in the phloem tissue. Thus they most likely absorbed fenoxycarb primarily through the cuticle while they were on the outer bark or in the entrance hole awaiting completion of the nuptial chamber by the males.

At the dosages of 1,000 or 10,000 $\mu g/cm^2$ of bark surface even the mean length of gallery , fecundity, and the mean number freshly-laid eggs were significantly reduced (Table 5; Fig. 4). The drastic impact of these larger dosages may be partially attributed to a toxic effect of fenoxycarb, since many beetles in these 2 treatments had to be replaced the day after the initial introduction. However, once egg gallery construction began there was no abnormal mortality in any treatment group. Another possible reason for this drastic impact could be that fenoxycarb might have caused resorption of mature oöcytes and deformation of ovarioles, as observed by Rohdendorf and Sehnal (1973) in reproducing female firebrats, *Thermobia domestica*

(Parkard). Lack of availability of oöcytes, or mature eggs, might in some manner have signalled a premature termination of the reproductive cycle, resulting in a cessation of gallery construction by female beetles. In *I. paraconfusus*, egg laying proceeds only as the egg gallery is lenthened, a common oviposition behavior among *Ips* spp. (Schmitz 1972; Miller and Borden 1985).

The ultimate test of the impact of any pesticide on bark beetle reproduction is in the F_1 brood beetles that emerge from an infested host. In part, this impact can be seen in a comparison of the slopes of regression lines [2] derived from the cumulative emergence data in Fig. 5. These comparisons (Bonferroni simultaneous test, α=0.05) yielded the following ranking from hightest to lowest: untreated = acetone-treated > 100 µg fenoxycarb/cm^2 > 1,000 µg fenoxycarb/cm^2 = 10,000 µg fenoxycarb/cm^2.

Complete sterilization of 1, 4 and 3 beetle pairs as indicated by no emergence holes, was observed in the 100, 1,000 and 10,000 µg/cm^2 treatments, respectively (Table 7). At the treatment doses of 1,000 or 10,000 µg/cm^2 this decrease in reproducing pairs was significant. For the 10,000 µg/cm^2

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[2] Regression equations, after log(y+1) transformation, are:
y = 0.0011 + 0.0675x, p<0.0001, r^2 = 0.9387;
y = 0.1440 + 0.0569x, p<0.0001, r^2 = 0.9753;
y = 0.0139 + 0.0555x, p<0.0001, r^2 = 0.9786;
y = -0.1741 + 0.0396x, p<0.0001, r^2 = 0.9732;
y = -0.0927 + 0.0188x, p<0.0001, r^2 = 0.9552;
respectively, for untreated control, acetone-treated control, 100 µg/cm^2 fenoxycarb treatment, 1,000 µg/cm^2 fenoxycarb treatment, and 10,000 µg/cm^2 fenoxycarb treatment.

treatment there were only of 2.1 emergent beetles/attack, a net increase in population size of virturally zero. There was also a considerable delay in the onset of brood emergence from logs treated with 1,000 and 10,000 $\mu g/cm^2$ of fenoxycarb, as compared to that from the control logs (Fig. 5). In *I. typographus* there was a 12-day emergence period by brood beetles from log treated prior to attack with JHA, whereas there was only a 7-day emergence period from control log (Novák *et al.* 1976). Retarded developmental and growth rates of progeny produced by adults treated with JHA or exposure to JHA-contaminated host foliages has been reported for the white pine weevil, *Pissodes strobi* (Peck) by McMullen and Sahota (1974). However, JHAs could either cause a prolonged developmental time, or, simply delay the production of the first viable eggs until the beetles begin to recover from the JHA treatment.

The higher percent reductions in progeny of emergent brood beetles (Table 7) than that of the brood exposed at 14 days after attack (Table 6) may suggest a persistent influence of fenoxycarb on the reproducing parents and/or an additional mortality of the progeny after 14 days in the logs.

CHAPTER IV

CONCLUDING DISCUSSION

That fenoxycarb mimics the function of juvenile hormone in stimulating pheromone biosynthesis in male *I. paraconfusus* was clearly demonstrated in this study (Tables 1, 2). Its high biological activity was evidenced by a strong stimulatory effect at a low topical treatment dosage of 0.1 μg. Two interesting phenomena regarding the pattern of JH-induced, pheromone biogenesis by *I. paraconfusus* were observed. Firstly, with a post-treatment incubation period of 20 h, high dosage(s) of exogenous fenoxycarb or JH III inhibited pheromone biogenesis, while lower dosages were strongly stimulatory (Table 2). Such an inhibitory effect may result from a rapid saturation of JH receptors on the corpora cardiaca, or from an activation of some other negative feedback mechanism. This phenomenon could be used in investigation of the neural and hormonal control of pheromone biogenesis in *I. paraconfusus*. Secondly, JHA-induced pheromone production varied with time (Fig. 2, 3). While the occurrence of pheromone content 12 h after treatment may reflect the rate of response of treated insects to the JHA stimulus, the gradual disappearance of pheromone may suggest loss due to external release or internal diffusion over time. Both phenomema are of concern in future studies, as they may affect the observed activity of JHAs.

It is also clear from the results that at competetive dosages with other JHA's fenoxycarb is an effective chemosterilant against reproductive *I. paraconfusus* (Tables 3-7, Figs. 4, 5). Noticeably, this effect results from the profound indirect impact of fenoxycarb on egg hatchability and, to a lesser extent, from the direct impact on fecundity of the treated or exposed beetles. However, fenoxycarb appears to have little influence on the fertility of male beetles, even at dosages sufficient to affect pheromone production. It follows that those species in which the female beetles initiate the attack might be more prone to fenoxycarb-coated hosts than those in which the male initiate the attact. The former species are reasonable targets for further investigation.

This study disclosed the dual impact of fenoxycarb, i.e. stimulation of pheromone production by one sex and inhibition of reprodction by the other, and yielded positive evidence for the feasibility of exposing attacking bark beetles via contact with fenoxycarb-coated bark. Additional research is needed to demonstrate whether or not fenoxycarb affects pheromone-based communication by bark beetles in the field, and to evaluate the efficacy of fenoxycarb in controlling bark beetle infestations.

REFERENCES

Anonymous. 1983. Ro 13-5223. Technical data sheet. Dr. R. Maag Ltd., CH-8157. Maag Technical Bulletin, Dielsdorf, Switzerland.

Bell, W. J. and R. H. Barth Jr. 1970. Quantitative effects of juvenile hormone on reproduction in the cockroach *Byrsotria fumigata*. J. Insect Physiol. 16: 2303-2313.

Borden, J. H. 1967. Factors influencing the response of *Ips confusus* (Coleoptera: Scolytidae) to male attractant. Can. Entomol. 99: 1164-1193.

Borden, J. H. 1974. Aggregation pheromones in the Scolytidae. pp. 135-160. In M. C. Birch (ed.). Pheromones. North-Holland Publ., Amesterdam.

Borden, J. H. 1984. Aggregation pheromones. Vol. IX. pp. 275-285. In G. A. Kerkut and L. I. Gilbert (eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology.Pergamon, Oxford.

Borden, J. H. and C. E. Slater. 1968. Induction of flight muscle degeneration by synthetic juvenile hormone in *Ips confusus*. Z. vergl. Physiol. 61: 366-368.

Borden, J. H., K. K. Nair, and C. E. Slater. 1969. Synthetic juvenile hormone: induction of sex pheromone production in *Ips confusus*. Science 166: 1626-1627.

Bridges, J. R. 1982. Effects of juvenile hormone on pheromone synthesis in *Dendroctonus frontalis*. Environ. Entomol. 11: 417-420.

Byers. J. A. 1981. Pheromone biosynthesis in the bark beetle, *Ips paraconfusus*, during feeding or exposure to vapours of host plant precursors. Insect Biochem. 11: 563-569.

Chamberlain, W. F. 1979. Methoprene and the flea. Pest Control 47: 22-26.

Conn, J. E. 1981. Pheromone production and control mechanisms in *Dendroctonus ponderosae* Hopkins. M.Sc. Thesis. Simon Fraser University, Burnany, B. C. Canada.

Critchley, B. R. and A. A. Almeida. 1973. Side effects of solvents, especially acetone, used for the application of juvenile hormone mimics and chemosterilants. Bull. Entomol. Res. 63: 1-6.

Dorn, S., M. L. Frischkencht, V. Martinez, R. Zurflüh, and U. Fischer. 1981. A novel non-neurotoxic insecticide with a broad activity spectrum. Z. Pflanzenkr. Pflanzenschutz 88: 269-275.

Edwards, J. P. and J. E. Short. 1984. Evaluation of three compounds with insect juvenile hormone activity as grain protectants against insecticide-susceptible and resistant strains of *Sitophilus* species (Coleoptera: Curculionidae). J. Stored Prod. Res. 20: 11-15.

Fockler, C. E. and J. H. Borden. 1973. Mating activity and ovariole development of *Trypodendron lineatum*: effect of a juvenile hormone analogue. Ann. Entomol. Soc. Amer. 66: 509-512.

Furniss, R. L. and V. M. Carolin. 1977. Western Forest Insects. U.S.D.A. Forest Serv. Misc. Publ. No. 1339. Washington, D.C.

Hamlen, R. A. 1975. Insect growth regulator control of longtailed mealybug, hemispherical scale, and *Phenacoccus solani* on ornamental foliage plants. J. Econ. Entomol. 68: 223-226.

Harring, C. M. 1978. Aggregation pheromones of the European fir engraver beetles *Pityokteines curvidens, P. spinidens and P. vorontzovi* and the role of juvenile hormone in pheromone biosynthesis. Z. angew. Entomol. 85: 281-317.

Harris, R. L., E. D. Frazer, and R. L. Younger. 1973. Horn flies, stable flies, and house flies: development in feces of bovines treated orally with juvenile hormone analogues. J. Econ. Entomol. 66: 1099-1102.

Hedin, P. A., O. H. Lindig, and G. Wiygul. 1982. Enhancement of boll weevil *Anthonomus grandis* Boh. (Coleoptera: Curculionidae) pheromone biosynthesis with JH III. Experientia 38: 375-376.

Hiruma, K., S. Yagi, and N, Agui. 1978. Action of juvenile hormone on the cerebral neurosecretory cells of *Mamestra brassicae* in vivo and vitro. Appl. Entomol. Zool. 13: 149-157.

Hughes, P. R. 1975. Pheromone of *Dendroctonus*: origin of α-pinene oxidation products present in emergent adults. J. Insect Physiol. 21: 687-691.

Hughes, P. R. and J. A. A. Renwick. 1977a. Neural and hormonal control of pheromone biosynthesis in the bark beetle, *Ips paraconfusus*. Physiol. Entomol. 2: 117-123.

Hughes, P. R. and J. A. A. Renwick. 1977b. Hormonal and host factors stimulating pheromone synthesis in the female western pine beetles, *Dendroctonus brevicomis*. Physiol. Entomol. 2: 289-292.

Ibaraki, A. and T. S. Sahota. 1976. Effect of insect growth regulators on survival of Douglas-fir beetle progeny. Can. For. Serv. Bi-Monthly Res. Notes 32: 3, 5.

Karrer, F. and S. Farooq. 1981. Some insect growth regulators with aromatic rings: Their synthesis and biological properties. Part I, pp. 289-302. In F. Sehnal, A. Zabzá, J. J. Menn, and B. Cymborowski (eds.), Regulation of insect development and behaviour. Wroclaw Tech. Univ. Press, Wroclaw, Poland.

Kramer, K. J., R. W. Beeman, and L. H. Hendricks. 1981. Activity of Ro 13-5223 and Ro 13-7744 against stored product insects. J. Econ. Entomol. 74: 678-680.

Loschiavo, S. R. 1976. Effects of the synthetic insect growth regulators methoprene and hydroprene on survival, development or reproduction of six species of stored-products insects. J. Econ. Entomol. 69: 395-399.

Masner, P., S. Dorn, W. Vogel, M. Kalin, O. Graf, and E. Günthart. 1981. Types of responses of insects to a new IGR and to proven standards. Part II, pp. 808-818. In F. Sehnal, A. Zabzá, J. J. Menn, and B. Cymborowski (eds.), Regulation of insect development and behaviour. Wroclaw Tech. Univ. Press, Wroclaw, Poland.

Menon, M. and K. K. Nair. 1972. Sex pheromone production and reproductive behaviour in gamma-irradiated *Tenebrio molitor*. J. Insect Physiol. 18: 1323-1331.

McGregor, H. E. and K. J. Kramer. 1975. Activity of insect growth regulators, hydroprene and methoprene, on wheat and and corn against several stored-grain insects. J. Econ. Entomol. 68: 668-670.

Miller, D. R. and J. H. Borden. 1985. Life history and biology of *Ips latidens* (Leconte) (Coleoptera: Scolytidae). Can. Entomol. 117: 859-871.

McMullen, L. H. and T. S. Sahota. 1974. Effect of a juvenile hormone analogue on developmental rate and growth rate of progeny in *Pissodes strobi* (Coleoptera: Curculionidae). Can. Entomol. 106: 1015-1018.

Nijhout, H. F. and C. M. Williams. 1974. Control of moulting and metamorphosis in the tobacco hornworm, *Manduca sexta* (L.): Cessation of juvenile hormone secretion as a trigger for pupation. J. Exp. Biol. 61: 493-501.

Novák, V., F. Sehnal, M. Romaňuk, and L. Streinz. 1976. Responses and sensitivity of *Ips typographus*. L. (Col., Scolytidae) and *Hylobius abietis* L. (COl., Curculionidae) to juvenoids. Z. angew. Entomol. 80: 118-131.

Parrella, M. P., G. D. Christie, and K. L. Robb. 1983. Compatibility of insect growth regulators and *Chrysocharis parksi* (Hymenoptera: Eulophidae) for the control of *Liriomyza trifolii* (Diptera: Agromyzidae). J. Econ. Entomol. 76: 949-951.

Pitman, G. B., R. A. Kliefoth, and J. P. Vité. 1965. Studies on the pheromone of *Ips confusus* (LeConte). II. Further observation. on the site of production. Contrib. Boyce Thompson Inst. 23: 13-18.

Reede, R. H. de, R. F. Groendijk, and A. K. H. Wit. 1984. Field tests with insect growth regulators, epofenonane and fenoxycarb, in apple orchards against leafrollers and side-effects on some leafroller parasites. Entomol. Exp. Appl. 35: 275-281.

Reede, R. H. de, P. Alkema, and L. H. M. Blommers. 1985. The use of the insect growth regulators fenoxycarb and epofenonane against leafrollers in integrated pest management apple orchards. Entomol. Exp. Appl. 39: 265-272.

Renwick, J. A. A., G. B. Pitman, and J. P. Vité. 1966. Detection of a volatile compound in hindguts of male *Ips confusus* (LeConte) (Coleoptera: Scolytidae). Naturwissenschaften 53: 83-84.

Rohdendorf, E. B. and F. Sehnal. 1973. Inhibition of reproduction and embryogenesis in the firebrat, *Thermobia domestica*, by juvenile hormone analogues. J. Insect Physiol. 19: 37-56.

Rountree, D. B. and W. E. Bollenbacher. 1984. Juvenile hormone regulates ecdysone secretion through inhibition of PTTH release. Amer. Zool. 24: 31A.

Sahota, T. S., J. A. Chapman, and W. W. Nijholt. 1970. Ovary development in a scolytid beetle *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae): effect of farnesyl methyl ether. Can. Entomol. 102: 1424-1428.

Schaefer, C. H. and W. H. Wilder. 1972. Insect developmental inhibitors: A practical evaluation as mosquito control agents. J. Econ. Entomol. 65: 1066-1071.

Schaefer, C. H. and W. H. Wilder. 1973. Insect developmental inhibitors: 2. Effects on target mosquito species. J. Econ. Entomol. 66: 913-916.

Schmitz, R. F. 1972. Behavior of *Ips pini* during mating, oviposition, and larval development (Coleoptera: Scolytidae). Can. Entomol. 104: 1723-1728.

Sehnal, F. 1976. Action of juvenoids on different groups of insects. pp. 301-322. In L. I. Gilbert, (ed.), The Juvenile Hormones. Plenum, New York.

Silverstein, R. M., J. O. Rodin, and D. L. Wood. 1966. Sex attractants in frass produced by male *Ips confusus* in ponderosa pine. Science 154: 509-510.

Staal, G. B. 1975. Insect growth regulators with juvenile hormone activity. Ann. Rev. Entomol. 20: 417-460.

Staal, G. B. 1977.Insect control with insect growth regulators based on insect hormones. Ponlif. Accad. Sci. Varia 41: 353-377.

Staal, G. B., S. Nassar, and J. W. Martin. 1973. Control of the citrus mealybug with insect growth regulators with juvenile hormone activity. J. Econ. Entomol. 66: 851-853.

Stock, A. J. and J. H. Borden. 1983. Secondary attraction in the western balsam bark beetle, *Dryocoetes confusus* (Coleoptera: Scolytidae). Can. Entomol. 115: 539-550.

Unnithan, G. C. and K. K. Nair. 1977. Ultrastructure of juvenile hormone-induced degenerating flight muscles in a bark beetle, *Ips paraconfusus* Cell Tiss. Res. 185: 481-490.

Van Sambeek, J. W. and J. R. Bridges. 1980. influence of the juvenile hormone analogue, methoprene, on development of the southern pine beetle, *Dendroctonus frontalis* Simm. (Col., Scolytidae). Z. angew. Entomol. 89: 479-488.

Van Sambeek, J. W. and J. R. Bridges. 1981. influence of the juvenile hormone analogue methoprene on reproduction of the southern pine beetles, *Dendroctonus frontalis* Zimm. J. Georgia Entomol. Soc. 16: 77-83.

Wood, D. L. 1966. Stridulation in the genus *Ips* De Geer. Pan-Pacif. Entomol. 37: 187-188.

Wood, D. L., L. E. Browne, R. M. Silverstein, and J. O. Robin. 1966. Sex pheromones of bark beetles. I. Mass production, bio-assay, source, and isolation of the sex pheromone of *Ips confusus* (LeC.). J. Insect Physiol. 12: 523-536.

Wood, D. L., L. E. Browne, W. D. Bedard, P. E. Tilden, R. M. Silverstein, and J. O. Rodin. 1968. Response of *Ips confusus* to synthetic sex pheromones in nature. Science 159: 1373-1374.

Wood, D. L. and R. W. Bushing. 1963. The olfactory response of *Ips confusus* (LeConte) (Coleoptera: Scolytidae) to the secondary attraction in the laboratory. Can. Entomol. 95: 1066-1078.

Wood, D. L. and R. W. Stark. 1966. The effects of Gamma Radiation on the biology and behavior of adult *Ips confusus* (LeConte) (Coleoptera: Scolytidae). Can. Entomol. 98: 1-10.

Zar, J. H. 1984. Biostatistical Analaysis. 2nd Ed., Prentice-Hall,Englewood Cliffs, New Jersey.