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WISCONSIN UNIV-MADISON DEPT OF ENTOMOLOGY  
A NEW APPROACH FOR THE CONTROL OF COCKROACHES UTILIZING THE ENT--ETC(U)  
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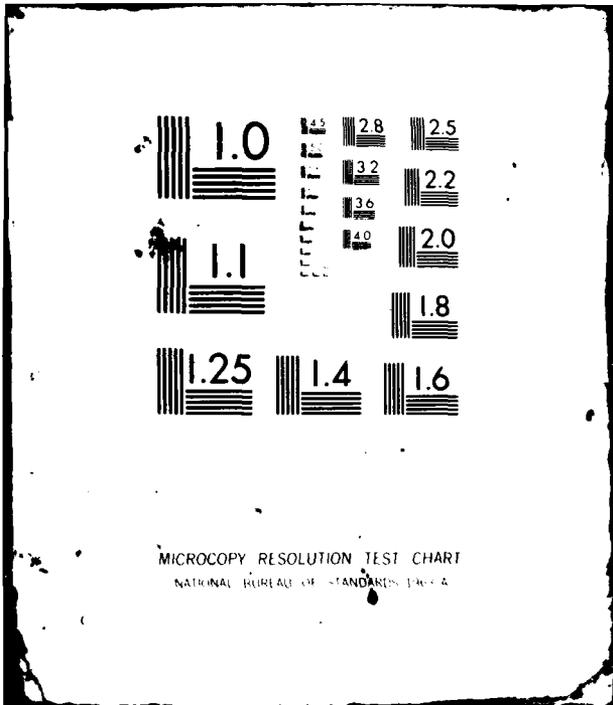
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) <b>The entomophilic nematode, <u>Neoaplectana carpocapsae</u>=DD-136, and associated bacterium, <u>Xenorhabdus nematophilus</u> have been tested in the laboratory as far as potential and capacity to infect and kill the German cockroach, <u>Blattella germanica</u>. A concentration of 2 X 10<sup>5</sup> infective stage nematodes in 2 ml of 0.1% formalin in water produced approximately 85% mortality. Another pathogenic nematode, <u>N. glaserii</u> at a concentration of 5 X 10<sup>4</sup> gave approximately 95% mortality in three days. <u>Neoaplectana</u></b>			

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carpocapsae did not effectively reproduce in its host, whereas N glaserii developed about three fold.

A total of 23 food related substances were evaluated as potential cockroach attractants using olfactometry and choice boxes methodology. Only three substances drew 60% or more response: oleyl alcohol, palmitic acid and an ethanol extract of finigreek seed. Of interest was the difference in response to oleyl alcohol and oleic acid, which are quite similar in structure--the alcohol form being first in attraction in this test, whereas the acid form ranked next to last.

Tests were conducted to determine the effects on cockroach reproduction of the juvenile hormone analogs ZR-512 and ZR-515. Both were tested as topical treatments at concentration of 0.01 and 0.001% (w/w) on filter paper. Exposure to the filter paper ranged from 1 minute to 24 hours in duration. ZR-512 resulted in 95% reduction in offspring and ZR-515 resulted in 91% reduction in fecundity. Follow-up cooperative studies with these two compounds, and two additional JH analogs, ZR-619 and ZR-777 are underway at the Navy Disease Vector Ecology and Control Center (DVECC), Naval Air Station, Alameda, California; Zoecon Corporation, Palo Alto, California; and the University of Wisconsin, Madison, Wisconsin.

OFFICE OF NAVAL RESEARCH

Contract #N00014-80C-0080

Task No. NR 139-002

ANNUAL REPORT NO. 1

A New Approach for the Control of Cockroaches Utilizing the Entomophilic  
Nematode DD-136 in Conjunction with Attractants

by

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October 1981

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The broad overall objective of this project is to develop a successful pest management scheme to control cockroaches on board naval ships and on shore facilities, based on the use of promising entomophilic nematodes in conjunction with baits, attractants and harborage-inoculation devices. In addition, ~~we are evaluating~~ four juvenile hormone analogs<sup>USDA 1073A</sup> for possible use in controlling cockroaches on naval ships and shore facilities.

For presentation, the results of our research are divided into three parts: I.- Nematode Studies, II.- Food Attractant Study, and III.- Juvenile Hormone Analog Study. Progress under each of these subdivisions is summarized below.

#### I. - Nematode Studies

##### Introduction

The potential of entomogenous nematodes for the biological control of insect pests has been recognized for some time. Of the hundreds of insect pests tested to date, nearly all have proved susceptible to and are killed by Neoplectana species (Dutky 1974). The best known of the Neoplectanid species is N. carpocapsae Weiser, which was first described by Weiser from diseased codling moth larvae in Czechoslovakia in 1954. Dutky and Hough (1955) later isolated a similar nematode which came to be known, after some disagreement as to its species status, as the DD-136 strain of Neoplectana carpocapsae.

In 1965, Poinar and Thomas published a description of an entomopathogenic bacterium, Achromobacter nematophilus, which was found in association with this particular nematode. The bacterial symbiont was found to be carried in the intestinal lumen of the infective stage juvenile nematodes. When the infective juveniles invade the haemocoel of a suitable host, the bacterium is

voided thru the anus and proliferates, causing a fatal septicemia. Further studies by Poinar (1966) demonstrated the importance of A. nematophilus in the reproduction and development of the entomogenous nematode inside the host cadaver. Bedding (1976) showed that the bacteria of Neoaplectana were capable of converting a wide range of substrates into media suitable for the reproduction of their associated nematodes.

More recently, entomogenous nematodes in the newly described genus Heterorhabditis have shown similar symbiotic associations with bacteria. This has prompted Thomas and Poinar (1979) to create a new genus, Xenorhabdus, to accommodate the large, gram negative, rod shaped, facultatively anaerobic, entomopathogenic bacteria that are found intimately associated with entomogenous nematodes. To date, nine strains of Xenorhabdus have been described, all of which are mutualistically associated with members of either the Heterorhabditis or Neoaplectana.

The success of entomogenous nematodes and its associated bacterium against such a wide range of insect hosts has prompted investigations of whether this complex could not also be used in a biological control program aimed at suppression of the German cockroach, Blattella germanica. Initial investigations focused on the potential and capacity of the DD-136 strain of Neoaplectana carpocapsae and Neoaplectana glaserii to infect and kill German cockroaches under laboratory conditions.

#### Materials and Methods

Unanesthetized adult and nymph roaches were exposed to varying concentrations of nematodes (in 0.15% formalin) placed on filter paper in a petri dish (15 x 100 mm, 20 per plate). The roaches were then examined at 24, 48, and 72 hr. intervals to determine the extent of infection and/or

mortality. For those roaches infected with the DD-136 strain of Neoplectana carpocapsae, isolations of its associated entomopathogenic bacterium, Xenorhabdus nematophilus, were attempted from the cadavers.

Isolates of bacterial symbionts for stock cultures were obtained by:

- 1) Macerating surface sterilized infective stages in a tissue homogenizer and streaking to appropriate media, or
- 2) by selecting the symbiont from the bacteria obtained from streaking the hemolymph aseptically obtained from Galleria melonella larvae recently infected by nematodes.

A pure culture of X. nematophilus was also obtained from G. O. Poinar Jr. and G. M. Thomas at the University of California, Berkeley.

#### Observations and Results

a) DD-136 strain of N. carpocapsae

Of the approximately 1000 roaches tested to date, about 85% died within 3 days of constant exposure to the nematodes ( $2 \times 10^5$  nematodes in 2 ml 0.15% formalin/plate) under the conditions described. Infective stage juveniles were readily observed in the body cavity of the dead roaches, especially in the abdomen, genitalia, head capsule and legs in about 60% of the cases. Attempted isolations of the symbiotic bacterium from the dead roaches was generally unsuccessful. Reproduction and propagation of the nematodes in the roach cadavers was very limited.

b) N. glaserii

Of the approximately 500 roaches tested to date, about 95% died within 3 days of constant exposure to the nematodes ( $5 \times 10^4$  nematodes in 2 ml. 15% formalin/plate). Reproduction and propagation of the nematodes in

roach cadavers was more successful, yielding several times the quantity of infective juvenile nematodes used in the inoculation.

#### Discussion

These preliminary investigations indicate that entomogenous nematodes, including N. glaserii and the DD-136 strain of N. carpocapsae, have the capacity to infect and kill German cockroaches under laboratory conditions. N. glaserii has demonstrated the desirable attribute of also being able to reproduce in the roach cadavers, so that a new generation of infective juveniles emerge seeking a new host. This may be due to the fact that this particular nematode is one that has been shown to be able to live and breed independent of its gut symbionts (Poinar, personal communication). If, as Poinar pointed out, (1966), X. nematophiles is needed for the reproduction and development of the DD-136 strain of N. carpocapsae inside a host cadaver, then the apparent inability of this nematode to reproduce within the roaches may be related to this phenomena. Future studies will investigate the role of the bacterial symbiote in the reproduction and development of the DD-136 strain inside the host cadavers. Other studies will focus on the susceptibility of the German cockroach to the nematode Heterorhabditis heliothidis. The entomopathogenicity of various Xenorhabdus strains for cockroaches will also be investigated.

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## II. - Food Attractant Study

### Introduction

Control agents for insect pests often cannot be universally distributed to reach pests wherever they may be. Insects may hide where no aerosol may reach. Biological agents such as nematodes, may require moisture that only exists in specific microhabitats. Whenever dispersal may only be local, a control agent relies on the pest being drawn to it (or at least not repelled by it). Several sorts of substances may be used to attract insects. Among them are 1) pheromones, hormones that produce a response in an individual other than the producer, and of which there are several sorts, and 2) food substances.

Two types of pheromones have been isolated from the German cockroach Blatella germanica. They are the sex pheromone and an aggregation pheromone. Unlike pheromones of many other insects, they are perceived only locally, generally by touch (Persoons and Ritter 1979). This renders them relatively useless in drawing roaches to bait stations, though the aggregation pheromone has been shown to mask the repellancy of insecticides (Rust and Reiersen 1977).

Since neither of the major pheromones could attract German roaches at a distance, a study on attraction to food substances was begun. The literature was searched for suggested food components; any substance that could not be chemically quantified was discarded. The final list included several fatty acids and their esters, sugars, alcohols, and flavor extracts.

### Methods

The attractiveness of a substance is most easily measured by presenting two equal areas to the test animals - One laced with the 'test substance' while the other is not. The degree to which the 'attractant' side is preferred is its measure of attraction. Data were gathered on the degree to which German cockroaches were attracted to an area so laced, relative to a similar untreated area, for the various test substances suggested by the literature.

Two such methods for measuring attraction are found in the literature: olfactometry (Burkholder 1970) and the use of choice boxes (Ebellig, et al. 1966; and Rust and Reiersen 1977). Olfactometry has an advantage in that it excludes tactile sensation of a suspected attractant, but it is rather time consuming. This method will be discussed later as it was used for a final evaluation of the better attractants. Time is not bettered much by the choice box, since numbers of roaches must, as in olfactometry, be counted after the run and placed back into the population before they can respond again. To circumvent this problem during the first evaluation of the many substances to be tested, the foraging behavior of a colony of roaches was observed on videotape at 3X normal speed. Members of the colony could choose between two feeding stations, one labelled with a test substance, while the other was not.

The colony was housed in a large arena. A stainless steel circular arena 1.0 meters across and 0.15 meters deep was painted with a flat grey enamel that contrasted with the roaches, while not presenting a glare to the video camera. The inside of the wall was greased with a 1:1 (volume to volume) mixture of petroleum jelly and parafin oil to discourage climbing. A final barrier to escape was a coating of Tanglefoot® at the lip of the arena.

The colony was established at the center of the arena. A harborage was

made from 1/8 inch masonite using ten square pieces 10 cm on a side separated by square pieces 2 cm x 2 cm. Watering stations were made from 8 oz. medicine bottles with a wick and plug made from 6 in. dental rolls (by Johnson & Johnson). Feeding stations consisted of 1/8 in. thick circular glass plates 88 mm in diameter, with one nugget of Purina® Dog Chow at the center. Two each of watering and feeding stations were placed in the arena, all equidistant from the harborage and wall, with watering stations at 0° and 180° and feeding stations at 90° and 270°. The colony was growing, and was culled whenever it was too large to be contained within the harborage. At any time, the colony contained between 300 and 700 individuals.

Feeding stations were laced with an 'attractant' using a 2 cm segment cut from 6 in. dental rolls (Johnson & Johnson) impregnated one half hour before filming, with a solution of the test substance for the test feeding station or the solvent only for a control. Clean (washed with sodium hydroxide) plates were used at the beginning of each run.

All experiments were carried out under diffuse light in a secluded room at off hours to remove the influence of lab activity. Recording began at 5:00 - 6:00 P.M., with the test substance on one side of the arena. After six hours of recording, a second run was started using the same substance at the opposite side of the arena. Results were monitored at 3X normal speed on videotape. A response was tallied for a feeding station when an individual stepped up on to the glass plate. Individuals leaving a plate were tallied again only if they disappeared from view or returned to the harborage.

Independence of feeding between the two feeding stations was established using two unlabelled plates. The chi-squared ( $\chi^2$ ) statistic was used to test for results significantly different from independence. Substances were ranked for attractiveness by the proportion of responses (in their favor).

The highest ranked test substances underwent further testing to determine if they would be attractive where tactile senses could not be used, using a two choice olfactometer (Burkholder 1970). This device consists of two glass plates with a brass spacer between them which collectively enclose a 'Y' shaped cavity. A vacuum line draws air at 0.6 litres per minute from the bottom leg of the 'Y'. Air enters the chamber at the ends of the other two legs. Incoming air was dessicated and charcoal filtered. For one of the upper legs, air was laced with the test substance by passing over a 2 cm segment of dental roll that had been impregnated with the chemical. Roaches numbering 150 to 200 are rendered unconscious with CO<sub>2</sub> gas and introduced to the bottom leg of the 'Y'. The apparatus is covered with black felt to prevent disturbance by lab activity. After 25 minutes, the chamber is flooded with CO<sub>2</sub> to anesthetize the roaches. Similar limits are drawn on each of the upper legs of the 'Y', and counts are made for each side within these limits. The apparatus was thoroughly cleaned between runs with acetone to remove any aggregation pheromones.

The two sides of the olfactometer were tested for independence five times initially, and once at the beginning of each operating day. Five tests of each 'attractant' were performed on each side of the olfactometer, yielding proportions of responses in favor of the chemical. The t distribution was used to test for departures from independence.

#### Results and Discussion

A review of the literature provided a list of 23 substances that had been implicated as roach attractants. Three more were added because of their similarity to members of the original list. This list of these substances containing descriptions of their preparation is displayed as Table 1.

Results of the arena experiment are analyzed in Table 2. Rank was determined by the proportion of responses in favor of the 'attractant'. The chi-squared ( $\chi^2$ ) value was not used in ranking since  $\chi^2$  for a given proportion will increase with sample size and the sample size varied considerably.

Confidence limits may be estimated for proportions taken from single samples (Dixon & Massey 1969) but the method is graphical and thus crude. A simple way to determine sufficiency of smaller samples is to observe variation in data as it accumulates to determine a point where adding further observations will make little change in the estimate (often referred to as a 'running mean'). Table 3 displays such a running mean for the proportion of responses as sample increases. In most cases 200 responses was a sufficient sample. Aberrant results were lost to sample size after 300 responses. Very few of the samples are smaller than this.

Several of the test substances appeared to draw roaches in significant numbers. Only three drew 60% or more of the responses: oleyl alcohol, palmitic acid, and the extract of finigreek seed. Rather surprising was the difference in response to oleyl alcohol and oleic acid, which are quite similar in structure. It is possible that one or the other of these results is in error, but this is unlikely considering the size of the samples and their high degree of consistency. It is also possible that the alcohol group may increase reception on the part of the roach.

Tests of the three best attractants in the olfactometer were rather disappointing (see Table 4). Normality was assumed for the distribution of proportions around the mean, since all values were between 0.30 and 0.70 (see Sokal & Rohlf 1981, section 13.10), and a t-test was used to examine for departures from independence. Only one set of results approached significance: that for palmitic acid. A possible explanation is that roaches

cannot detect odors at any great distance (Trospen, personal communication), and thus leading one to suspect that providing a good harborage is a better way to attract roaches than with food attractants.

Table 1. A list of test substances and descriptions of their preparation.

Group	Test Substance	Solvent	%	Dose (ml)
Saturated Fatty Acids	Capric Acid	Ethanol	2	0.5
	Caproic Acid	Ethanol	2	0.5
	Caprylic Acid	Ethanol	2	0.5
	Myristic Acid	Ethanol	2	0.5
	Lauric Acid	Ethanol	2	0.5
	Palmitic Acid	Ethanol	2	0.5
	Stearic Acid	Ethanol	< 1	1.0
Unsaturated Fatty Acid	Oleic Acid	Ethanol	2	0.5
Pure Saturated Fats	Tricaprin	Ethanol	2	0.5
	Tripalmitin	Ethanol	< 1	1.0
Fatty Acid Esters	Methyl Palmitate	Ethanol	2	0.5
	Ethyl Palmitate	Ethanol	2	0.5
	Methyl Myristate	Ethanol	2	0.5
Sugars	Sucrose (refined)	H <sub>2</sub> O	2	0.5
	Sucrose (Brown sugar)	H <sub>2</sub> O	5	0.5
	Galactose	H <sub>2</sub> O	2	0.5
	Maltose	H <sub>2</sub> O	2	0.5
	Arabinose	H <sub>2</sub> O	2	0.5
Alcohols	Glycerol	Ethanol	2	0.5
	Nonadecanol	Acetone	2	0.5
	Cetyl Alcohol	Ethanol	2	0.5
	Sorbitol	Ethanol	2	0.5
	Oleyl Alcohol	Ethanol	2	0.5
Unknowns	Finigreek seed (crude extract)	Ethanol	*	0.5
	Artificial Maple Flavor	H <sub>2</sub> O	*	0.5
	Artificial Banana Flavor	Ethanol	*	0.5

\* Concentrations are unknown. Artificial flavors by French's were diluted to 10% original volume. Finigreek extract was prepared by lightly grinding 100g of Finigreek seed in a mortar and leaching in 250 ml Ethanol for 2 weeks.

Table 2. Summary of observations in the arena. Under 'Total' proportion (R) represents an average (weighed by sample size) from both sides of the arena. N is Sample size, R is fraction of responses on favor of substance tested,  $\chi^2$  is chi squared for 1 degree of freedom, and P is the likelihood by chance of a  $\chi^2$  greater than or equal to the observed.

Rank	Test Substance	Run 1			Run 2			Total			P
		N	R	$\chi^2$	N	R	$\chi^2$	N	R	$\chi^2$	
1	Oleyl Alcohol	400	.718	75.69	400	.615	21.16	800	.666	88.45	0.0001
2	Palmitic Acid	212	.623	12.75	222	.622	13.13	434	.622	25.89	0.0001
3	Finigreek Seed Alcohol Extract	313	.601	12.68	411	.594	14.42	724	.597	26.30	0.0001
4	Nonadecanol	400	.593	13.69	400	.570	7.84	800	.581	21.13	0.0001
5	Tricaprin	400	.558	5.29	400	.583	10.89	800	.570	15.68	0.0001
6	Galactose	262	.561	3.91	236	.576	6.48	498	.568	9.29	0.005
7	Brown Sugar	900	.566	15.47	886	.544	7.59	1786	.556	22.39	0.0001
8	Maltose	304	.549	2.96	261	.559	3.68	565	.554	6.58	0.015
9	Cetyl Alcohol	330	.561	4.84	446	.536	2.29	776	.546	6.68	0.010
10	Arabinose	115	.626	7.31	107	.449	1.13	222	.541	1.46	--
11	Glycerol	669	.529	2.27	816	.526	2.16	1485	.527	4.42	0.025
12	Sucrose (refined)	618	.534	2.85	738	.515	0.66	1356	.524	3.0	0.10
13	Banana flavor	556	.520	0.87	719	.527	1.12	1507	.523	1.94	--
14	Sorbitol	788	.513	0.51	395	.530	2.57	951	.521	2.63	--
15	Methyl Palmitate	164	.470	0.61	364	.525	0.89	528	.508	0.12	--
16	Lauric Acid	437	.508	0.11	349	.499	<0.01	786	.504	0.05	--
17	Stearic Acid	774	.481	1.16	511	.505	0.05	1285	.490	0.49	--
18	Tripalmitin	400	.525	1.00	400	.445	4.84	800	.485	0.72	--
19	Caproic Acid	287	.505	0.03	395	.458	2.75	682	.477	1.32	--
20	Myristic Acid	302	.487	0.21	286	.455	2.36	588	.471	1.97	--
21	Artificial Maple flavor	172	.477	0.37	358	.458	2.51	530	.464	2.72	--
22	Ethyl Palmitate	400	.443	5.29	400	.480	0.64	800	.461	4.81	0.015
23	Caprylic Acid	367	.458	2.62	471	.461	2.91	838	.459	5.52	0.02
24	Methyl myristate	470	.438	7.16	492	.455	3.93	962	.447	10.81	0.005
25	Oleic Acid	342	.427	7.30	520	.454	4.43	862	.443	11.14	0.001
26	Capric Acid	683	.399	27.48	503	.459	3.34	1186	.425	26.71	0.0001

Table 3. Running mean for the proportion of responses in favor of an 'attractant'. No real changes in magnitude or direction (from 1:1) after 300 responses.

Responses used in estimating mean	1-100	1-200	1-300	1-400
Substance				
Galactose #1	.5600	.5700	.5554	
Galactose #2	.5900	.6100	.5363	
Tripalmitin #1	.5000	.4950	.5033	.5250
Tripalmitin #2	.4300	.4450	.4633	.4450
Nonadecanol #1	.7000	.6300	.6233	.5925
Nonadecanol #2	.5000	.5350	.5667	.5700
Ethyl Palmitate #1	.3600	.5000	.4666	.4425
Ethyl Palmitate #2	.4800	.4700	.4700	.4800
Tricaprin #1	.6100	.5800	.5833	.5825
Tricaprin #2	.6100	.5600	.5600	.5575
Oleyl Alcohol #1	.6700	.6950	.6967	.7175
Oleyl Alcohol #2	.6600	.6300	.6033	.6150

Table 4. Results of Olfactometry. Significance of deviations from independence (mean = 0.5) were determined using a t-test. 'Treatment' indicates the test substance used, 'side' indicates left (L) or right (R) of the olfactometer, and 'N' indicates the number of replicates. Solutions are the same as those used in the arena. Dosage in each case is 1 ml.

Treatment	Side	N	Mean	SD	t	% ile (t distribution)
CONTROL	L	21	0.50804	0.0653	0.564	0.7106
Oleyl Alcohol	L	5	0.47746	0.1030	-0.490	0.3248
Oleyl Alcohol	R	5	0.50788	0.0462	0.382	0.6390
Palmitic Acid	L	5	0.44569	0.0583	-2.083	0.0529
Palmitic Acid	R	5	0.50789	0.0256	0.690	0.7358
Finigreek Extract	L	5	0.48754	0.0822	-0.339	0.3758
Finigreek Extract	R	5	0.48676	0.0600	-0.493	0.3238

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### III. - Juvenile Hormone Analog Study

Data were collected to 1) determine the effects of exposure to juvenile hormone analogues on the reproductive value of the German cockroach, 2) determine whether or not these hormone analogues are repellent to German roaches, and 3) if they are repellent, the degree to which food attractants might mask repellancy.

Four juvenile hormone analogues were obtained from the Zoecon corporation. ZR512 and ZR515 hydroprene and methoprene and respectively were used in preliminary studies. Two new hormone analogues, ZR619 and ZR777, are being tested along with those previously mentioned, in further studies. Acetone solutions of the hormone analogues were prepared at concentrations of 0.001% and 0.01% (100 x volume/volume), no more than two weeks before exposing roaches. Solutions and hormone stocks were kept below 0°C to slow deterioration.

Preliminary studies on the effects of ZR512 and ZR515 varied the concentration, exposure time, and instar exposed. Whatman® number 5 filter paper (5.5 cm diameter) was saturated with 2 ml hormone solution and allowed to dry. Densities were  $8.42 \times 10^{-5}$  ml/cm<sup>2</sup> for 0.001% solutions, and  $8.42 \times 10^{-4}$  ml/cm<sup>2</sup> for 0.01% solutions. Exposures to filter paper were 1 minute, 1, 4, 12, and 24 hours in duration. Sixty individuals from each instar were exposed to each treatment, 10 individuals per filter paper. Each group began with a 1:1 sex ratio. The exceptions were instars one and two, in which individuals could not easily be sexed. A 1:1 sex ratio was assumed. Upon reaching adulthood, treated individuals were allowed to mate with untreated individuals. The number of ootheca and offspring production were recorded. The Duncan multiple range test was used to separate treatments into groups with similar distributions and means.

Tests for repellancy and masking utilized the two-choice olfactometer described earlier. Only one hormone analogue, ZR515, has been tested to date, along with two attractants: oleyl alcohol and finigreek seed extract. Two cm segments of dental rolls (by Johnson and Johnson) were impregnated with test substances, and allowed to dry, not more than 1.0 hour before a test. Air was passed over these segments before entering the test side of olfactometer. Separate segments were used for each substance. Tests for the olfactometer's independence were performed once before each run.

Current research on the effects of juvenile hormone analogues on the German cockroach includes 4 aspects: the specification of reproductive value for treated individuals, carryover of developmental damage to the second generation, heritability of resistance to the hormone analogues, and the consequences of long term exposure for a population.

Six each of the previously mentioned treatments, varying exposure time, concentration, and age with 10 individuals each are being performed with all of the hormone analogues. Treated individuals are mated with treated and untreated individuals. In addition to measuring fecundity of the parents, offspring are being observed for developmental abnormalities, and fecundity. Separating offspring of treated parents on the basis of clutch size and measurement of their clutch size in response to treatment will yield data on the heritability of resistance.

For the long term exposure experiment, 20 populations were established in glass cages 16" x 8" x 4", with 2 harborages at one end and food and water at the other. Feeding stations were 60 mm petri dish halves containing Purina® Dog Chow. Watering stations were 4 oz. Nalgene® bottles wicked and plugged with the dental rolls previously mentioned. Harborages were made from 1/8 in. masonite, 3 squares 80 mm per side separated by 2 squares 20 mm per side. A

strip of filter paper 8" x 3" was laid across the floor of each cage, after it had been impregnated with 2 ml of one of the 0.01% solutions of a juvenile hormone analogue, and allowed to dry. The strip separated harborages from food and water, with 6 inches free space on the harborage side and 7 inches on the food side. Populations are cleaned of dead individuals and other debris with an aspirator, rendered unconscious with CO<sub>2</sub> gas, and photographed for records of size, sex, and age structure.

Remaining analogues will be tested for repellancy in the manner already described.

#### Results and Discussion

In the preliminary studies 0.01% ZR515 was the most effective treatment. Apart from the large number of morphological aberrations produced, it at best resulted in a 95% reduction of offspring production. ZR512 at 0.01% did not do as well, producing only a 91% reduction in fecundity.

In the follow up experiment, the results of the initial exposure shows that ZR-515 at a concentration of .01% was again the most effective treatment, followed by ZR-512 at the same concentration. ZR-619 and ZR-777 were virtually ineffective in reducing cockroach ootheca production and nymph emergence. Preliminary results of the progeny experiment indicate that although a large percentage of the progeny of all experiments exhibit banded bodies and the wing distortions, the fecundity of the second generation was as high as the controls. There also appears to be no difference in the JHAs tested or the concentrations that were used in their effects on the second generation.

Table 1 presents the results of tests for repellancy. For this series, an expected value for right and left sides of the olfactometer is generated that is significantly different from 1:1. These values will be used for

independence. The lower concentration of hydroprene (0.001%) did not show significant repellancy. The higher concentration (0.01%) showed significant repellancy on either side. Masking with either 2% oleyl alcohol or finigreek seed extract brought the results back to independence.

No results are available yet from the long term exposure experiment, as populations were established and exposed only 3 weeks ago. The second series of photographs is not in yet, though observations reveal substantial growth for the controls, while the age structure was highly skewed toward adults in the populations exposed do hydroprene.

**DATA  
FILM**