fatigans (Stone and Brown, 1969), it is probable that a phosphatase was identical to the α- and β-naphthyl acetate hydrolyzing enzyme. This was suggested by the presence of an electrophoretic esterase band which hydrolyzes fenoxon, along with the fact that the largest interstrain difference in degradation was in the production of dimethyl phosphoric acid.

In conclusion, however, the definite identity of the nonspecific esterases with the detoxication enzymes in the organophosphorus resistant strains requires further proof.

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Third Generation Pesticides: the Potential for the Development of Resistance by Insects

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Certain strains of insects that show resistance to insecticides also show cross-resistance to insect juvenile hormone mimics or analogs. In some insects, juvenile hormone tolerance appears to be correlated with high levels of microsomal mixed-function oxidase activity. Genetic tests are described in which the inheritance of juvenile hormone cross-resistance in the housefly was measured. Cross-resistance was controlled by genetic factor(s) on chromosome II, the chromosome which controls high levels of oxidase activity. Experience with present insecticides suggests that through selective pressures from the use of the third generation insecticides high levels of resistance also may develop.

Insect juvenile hormones and compounds which mimic their effects have received a great deal of attention in the last several years as possible insect control agents. Insect hormone studies had their early beginning with Kopec (1922), who first suggested that insect molting was controlled by hormones. Later Wigglesworth (1934) showed that the molting process required a factor which appeared to come from the head of the insect. In 1936, Wigglesworth demonstrated that metamorphosis was inhibited by a factor from the corpora allata. During the intervening years, through the work of a great number of researchers, these early workers' findings have been confirmed.

In insects the processes of growth and development are controlled by three primary hormones, as shown diagrammatically in Figure 1. The brain hormone (BH) is produced by the neurosecretory cells of the brain, transported *via* axoms to the corpus cardiacum, and released from there. The brain hormone stimulates the release of a sec-

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ond hormone known as the molting hormone (MH) or ecdysone. The molting hormone sets in motion the development of a new cuticle and the maturation of the tissues in the insect prior to ecdysis (the casting off of the old skin). In immature insects a third hormone (juvenile hormone) is also released into the blood along with the molting hormone and suppresses or inhibits the full maturation of adult tissues. An ecdysis in the presence of juvenile hormone results in a juvenile stage.

Juvenile hormone (JH) is released from a pair of glands (corpora allata) located behind the brain. In maturing insects JH is no longer secreted, thus allowing for the development and maturation of tissue prior to imaginal ecdysis. Juvenile hormone is found again in the adult and is involved in egg maturation (Figure 1).

The application of juvenile hormone to an insect during tissue maturation when endogenous juvenile hormone is low results in lethal deformities. William (1956) obtained the first juvenile hormone extracts from the abdomens of adult male cecropia moths. He found that treatment of the pupal stage of an insect with hormone extracts disrupted morphogenesis during the pupal-adult molt and produced an intermediate form of insect incapable of survival. These results led to Williams' (1956) suggestion of a hormone-based insecticide. Although other lethal effects of juvenile hormone have been discovered, the disruption of adult development is still one of the primary ways its use has been envisioned in control.

The chemical structure of the cecropia juvenile hormone was elucidated by Roller *et al.* (1967) and this, along with the earlier identification of Schmialek (1961), and demonstration of the juvenile hormone activity of farnesol and a number of terpenoids (Wigglesworth, 1961, 1963) led to the rapid expansion of interest in juvenile hormone research. Williams (1967, 1968) heralded juvenile hormone and its mimics as third generation insecticides. He listed their possible attributes as including specificity, high activity, safety, and possible circumvention of resistance. On the last aspect, he stated that it would be difficult for an insect to develop resistance to its own hormones. Berkoff (1971), Bowers (1971), and Cox (1972) have further elucidated some of the potential of insect hormones as insecticides, as well as some of the problems.

During the last several years, a number of chemical structures with juvenile hormone activity have been synthesized and investigated. A review of insect juvenile hormone analogs and their structure–activity relationships has been published by Slama (1971). High host specificity is associated with these chemicals. Suchy *et al.* (1968) reported almost no change in response to juvenile hormone mimetic compounds at the species level but were able to show slight variations at the genus level and large differences at the family or higher taxonomic levels.

Dyte (1972) was the first to report differences in response by a species of insect to a natural juvenile hormone, cecropia JH (Roller *et al.*, 1967). He determined the juvenile hormone's activities on an insecticide-susceptible strain of *Tribolium casteneum* and compared the results with a strain known to be resistant to DDT as well as 22 organophosphate and four carbamate insecticides. Insects were reared in flour containing various levels of the juvenile hormone, and the number of larvae, pupae, and adults were determined at various time intervals. A summary of his results is shown in Table I. The results clearly show that the juvenile hormone has greater activity on the insecticide-susceptible strain.

In research conducted at Texas A&M (Benskin and Vinson, 1973), evidence was obtained for a difference in response to juvenile hormone preparations between insecticide-resistant and -susceptible strains of the tobacco budworm, *Heliothis virescens* (F.). This insect is resistant to a variety of insecticides, including the organophosphates (Harris *et al.*, 1972; Plapp, 1971). In the *Heliothis*

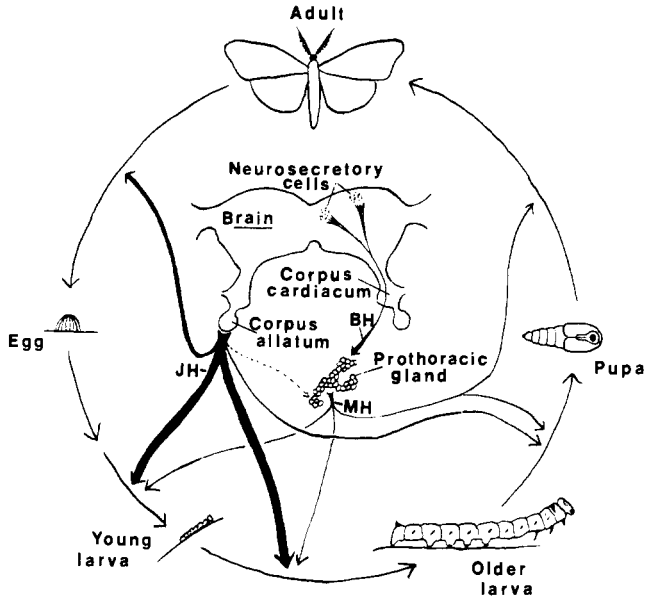


Figure 1. Diagrammatic representation of the endocrine system controlling growth and development in insects. BH = brain hormone; MH = molting hormone; JH = juvenile hormone.

Table I. Stage Reached and Mortality in Susceptible (S) and Resistant (R) Strains of *Tribolium castaneum* after 12 Weeks in Flour Treated with Juvenile Hormone[a]

Juvenile hormone, ppm	Strain	Live larvae, %	Dead larvae, %	Dead pupae, %	Dead adults, %
10	S	0	2	98	0
	R	0	0	82	18
30	S	14	48	38	0
	R	6	12	82	0
100	S	2	98	0	0
	R	4	82	14	0

[a] Data from Dyte (1972).

Table II. Comparison of the Response of Two Strains of Tobacco Budworm Larvae to JH Analogs[a]

Compound	Strain[b]	ED_{50}, μg/larvae	Slope	Resistance value[c]
	Posttreatment temperature, 27°			
ENT 70033	South Florence	4.4	0.5	
	College Station	3.2	0.3	0.7×
JH II	South Florence	1.2	0.6	
	College Station	2.0	0.6	1.7×
	Posttreatment temperature, 17°			
ENT 70033	South Florence	10.7	0.5	
	College Station	20.8	0.4	2.0×
JH II	South Florence	3.5	1.0	
	College Station	18.0	0.4	5.4×

[a] Data from Benskin and Vinson (1973). [b] South Florence susceptible strain. College Station an insecticide-resistant strain. [c] Based on a comparison of the CS strain to the SF strain at the same temperature and with the same JH analog.

study, the response of resistant and susceptible strains to a naturally occurring juvenile hormone, JH II (Meyer *et al.*, 1968), and a synthetic methylenedioxyphenyl derivative, ENT 70033, was measured. Last-instar larvae were treated topically with peanut oil solutions of the test chemicals and held at 17 or 27°. The number of adult moths emerging was used as a criterion of effect. The results (Table II) show a difference in response to both hor-

Table III. Response of Various Strains of the Housefly to ENT-70460 at 0.025 μg/Pupa[a]

Strain	Number tested	Percentage mortality[b]
Susceptible		
NAIDM	60	100
Resistant		
DDT/lindane	18	100[a]
Parathion	66	78.0 ± 8.5[b]
Chlorthion	30	70.7 ± 13.5[b]
Fenthion	77	63.9 ± 6.9[b]
OMS-12[c]	30	30.0 ± 5.8[c]
OMS-15[d]	60	18.9 ± 6.7[c]
Dimethoate	100	14.1 ± 5.0[c]

[a] Data from Cerf and Georghiou (1972). [b] Mean mortality ± standard deviation. Any two means having the same superscript letter are not significantly different at the 5% level as determined by Duncan's multiple range test. [c] *O*-Ethyl *O*-(2,4-dichlorophenyl)phosphormidothioate. [d] *m*-Isopropylphenyl methylcarbamate.

Table IV. Toxicity Data on the Response of Last Instar Larvae of Several Housefly Strains to a Juvenile Hormone Mimic (ENT-70460)[a]

Housefly strains	LC_{50},[b] μg/vial	Resistance[c] value
Orlando Regular	0.02	1
Dld-R;cyw	0.01	0.5
R-Parathion;clw	0.01	0.5
Orlando DDT	0.04	2
DDT-R;dov	0.08	4
kdr-O;ocra;stw	0.09	4.5
stw;bwb;ocra	0.11	5.5
R-Fc	0.15	7.5
R-Baygon;bwb;ocra	0.63	31

[a] Data from Plapp and Vinson (1973). [b] LC_{50} values based on emergence of flies as adults. [c] Resistance are the ratios of the LC_{50} response of the strain to that of the Orlando Regular flies.

mones by the two strains. A greater difference in response occurred when the insects were held at the lower temperature.

Cerf and Georghiou (1972) reported evidence of cross-resistance to a juvenile hormone mimic in several strains of insecticide-resistant houseflies. These authors used ENT-70460 (Zoecon-0515) applied as an acetone solution to unsclerotized pupae. Adult flies which failed to emerge from the puparium were scored as dead. The results of their studies are shown in Table III. The results show a wide difference in response to the juvenile hormone mimic between the various strains tested. The dimethoate-resistant strain showed the greatest cross-resistance. These authors further reported that the resistant strains showed a greater degree of heterogeneity than the susceptible NAIDM-S strain in their response to hormone mimics.

Results similar to Cerf and Georghiou (1972) were reported by Plapp and Vinson (1973). In the latter study, housefly strains were used in which the mechanisms and genes responsible for insecticide resistance are known. It was hoped that the mechanism for juvenile hormone cross-resistance could be determined by comparing the response of the different insecticide-resistant strains. The type of resistance and the chromosomes containing the resistance gene(s) have been described (Bigley and Plapp, 1960; Hoyer and Plapp, 1966; Hoyer *et al.*, 1965; Plapp, 1970b; Plapp and Casida, 1969). In our study, nonfeeding (third-instar) larvae of the housefly were exposed to films of the test chemical. The emergence of adults was used as the criterion of activity.

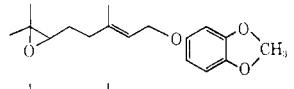
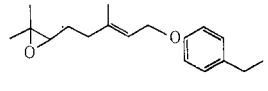
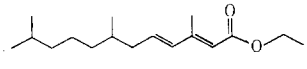
The data (Table IV) show the results of exposure of several housefly strains to ENT-70460. Several insecticide-resistant strains were found to possess slight cross-resistance to this juvenile hormone mimic. Cross-resistance is highest in the R-Baygon;bwb;ocra strain, which has chromosome 2 genes for high microsomal oxidase activity and DDT dehydrochlorianase, as well as a chromosome 4 gene for cyclodiene resistance. The cross-resistance in the Orlando DDT, DDT-T;dov, the kdr-O;ocra;stw and Dld-R;cyw strains is much less than that observed with the R-Baygon;bwb;ocra strain. The only factor in which R-Baygon;bwb;ocra differs from other strains is in having high microsomal oxidase activity. Therefore, it seemed likely that the cross-resistance might be associated with this factor.

Organophosphate resistance itself does not appear to be a prerequisite for juvenile hormone mimic tolerance, since the R-parathion;clw parathion strain in which resistance is due to altered aliesterase activity is very susceptible to ENT-70460. The primary importance of the high oxidase activity is further indicated by the lesser but still measurable tolerance of the R-Fc strain. This strain has a chromosome 5 gene for high oxidase activity which confers an increased ability to metabolize DDT and aldrin (Plapp and Casida, 1969).

In Table V data are shown for the response of a low oxidase (Orlando Regular) and a high oxidase (R-Baygon;bwb;ocra) housefly strains to three additional juvenile hormone analogs. Cross-resistance to all juvenile hormone mimics was found with the R-Baygon;bwb;ocra strain. Cross-resistance was greatest with ENT-70033, a methylenedioxyphenyl juvenile hormone derivative.

Based on these results, it seemed likely that high levels of microsomal oxidase might be involved in juvenile hormone cross-resistance in houseflies. To test this hypothesis and to determine the nature of the genetic factors in-

Table V. The Response of Two Housefly Strains to Several Juvenile Hormone Mimics[a]

JH mimic structure	ENT no.	LC_{50}'s (μg/vial)[b] and resistance values (RV)[c] for each strain tested			
		Orlando Regular		R-Baygon; bwb;ocra	
		LC_{50}	RV	LC_{50}	RV
	70033	17.5	1	422.5	24
	70221	35.3	1	186.2	5.3
	70459	5.1	1	34.7	6.8

[a] Data from Plapp and Vinson (1973). [b] LC_{50} values based on emergence of adult flies. [c] Resistance values are based on a ratio of response of the test strain to that of the Orlando Regular flies.

Table VI. Survival in the Backcross Generation from the Cross F_1 ♂♂ (Rutgers Diazinon-R ♀♀ × stw;bwb;ocra ♂♂) × stw;bwb;ocra ♀♀ following Exposure to a Juvenile Hormone Mimic, ENT 70460

Phenotype	% survival[a]		
	3 μg/vial[b]	0.3 μg/vial[c]	Control[d]
+;+;+	44	48	100
stw;+;+	12	56	32
+;bwb;+	36	56	100
+;+;ocra	44	56	80
stw;bwb;+	12	40	80
stw;+;ocra	8	64	100
+;bwb;ocra	60	72	100
sta;bwb;ocra	12	40	100

[a] Based on estimated numbers of flies expected to emerge (12.5% of total in each phenotype). [b] Total of 200 fly larvae exposed to juvenile hormone; 20 fly larvae/vial. [c] Total of 100 fly larvae exposed to juvenile hormone; 20 fly larvae/vial. [d] Total of 50 fly larvae; 10 fly larvae/vial.

volved, a study of the inheritance of cross-resistance was initiated. For this work a high oxidase-resistant strain, Rutgers Diazinon-R (Philpot and Hodgson, 1971), was crossed with a susceptible strain carrying visible recessive mutants on the second, third, and fifth chromosomes. Males of the F_1 progeny were backcrossed to female of the mutant parent strain to yield a backcross. The inheritance of dominant resistance factors was studied by exposing backcross generation larvae to ENT-70460 and determining the phenotypes which survived the treatment. The methods used were those of Plapp (1970a).

Mature third (last-instar) housefly larvae of the backcross generation were collected from the rearing media and placed in vials treated with several doses of ENT-70460 or an acetone control. In the backcross generation, eight phenotypes in equal numbers would be expected to occur. As seen in the results (Table VI), about equal numbers of the phenotypes did occur in the control and after exposure to a low dose of ENT-70460. The low dose of ENT-70460 appeared to be too low (40% mortality) to give any meaningful results. At the higher dose, 71.5% mortality was obtained. Reduction in emergence was greatest for stw phenotypes.

Pooled results for each chromosome from each test are presented in Table VII, where the four phenotypes homozygous for each mutant are compared with the four phenotypes heterozygous for each mutant. The greater the difference (Δ) in response between the wild-type and mutant flies for each mutant type, the greater the contribution of the chromosome carrying the mutant to the total resistance present. Table VII shows that chromosome 2 supplies the major gene(s) responsible for the cross-resistance to the juvenile hormone mimics investigated. Chromosome 2 has been shown to contain a high oxidase gene for resistance (Philpot and Hodgson, 1971; Tate *et al.*, 1973).

Our results suggest that several juvenile hormone mimics are susceptible to microsomal oxidation. The pathways for the breakdown of racemic juvenile hormone were reported by Slade and Zibitt (1972) for several insect species and are shown in Figure 2. Slade and Zibitt (1972) reported that *Sarcophaga* degraded the racemic juvenile hormone by hydrolyzing the epoxide ring almost exclusively. They also reported in preliminary experiments with microsomal preparations that ester diol formation was indicated as the major metabolic pathway *in vitro* in the housefly. Their results, along with the information provided by our backcross studies, suggest that microsomal oxidation may be a primary factor responsible for the cross-resistance observed.

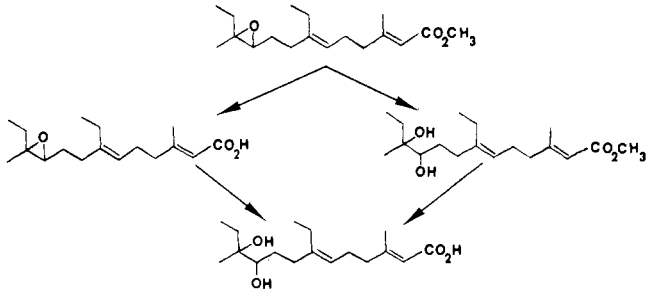


Figure 2. Pathways for the degradation of racemic juvenile hormone identified in *Hyalophora cecropia* and in part in *Manduca sexta, Schistocerca vaga,* and *Sarcophaga bullata* (after Slade and Zibitt, 1972).

The demonstration of high levels of cross-resistance to different juvenile hormone mimics was expected. Several workers have already demonstrated a rapid turnover of juvenile hormone in several insect species (Ajami and Riddiford, 1973; Slade and Zibitt, 1972). A system capable of rapidly disposing of native juvenile hormone is essential if insect metamorphosis is to occur. Further, a mechanism for the rapid degradation and elimination of residual juvenile hormone just prior to pupation would be expected. Perry and Buckner (1970) reported the presence of cytochrome P-450 in larval houseflies. As pointed out by Cerf and Georghiou (1972), it is perhaps unfortunate that the highest sensitivity to juvenile hormone in an insect coincides with the presence of a greater capacity for metabolism and an elimination of xenobiotics.

It is unfortunate that high levels of cross-resistance occur in strains containing high microsomal oxidase activity. Brown (1968) reported over 230 insect species that have developed resistance to various insecticides. Microsomal mixed-function oxidases play a major role in this resistance in insects (Hodgson, 1968). As more chemicals become useless against certain insect species, the problems of the insect control rapidly increase. When juvenile hormone was first proposed as a third generation insecticide by Williams (1967, 1968), it was hoped that these chemicals might offer a means of controlling many of the pests which have developed resistance to the presently used insecticidal compounds.

The demonstration of cross-resistance based on inherently higher microsomal enzyme activities indicates that many insects may have already circumvented the third generation insecticides and may have a degree of cross-resistance to these compounds. Consequently these species, for which juvenile hormone was to offer hope for

Table VII. Contribution of Each Chromosome to Juvenile Hormone Resistance to ENT 70460 (Average % Survival of Wild-Type Phenotypes *vs.* Average % Survival of Mutant Phenotypes)

Chromosome	Marker	% survival								
		3 μg/vial			0.3 μg/vial			Control		
		Wild type	Mutant	Δ	Wild type	Mutant	Δ	Wild type	Mutant	Δ
2	stw	46	14	32	58	50	8	78	95	−17
3	bwb	30	27	3	54	52	2	95	78	17
5	ocra	32	26	6	58	50	8	95	78	17

control, may already have an inherent capacity for resistance to them. It is worth noting, however, that even though cross-resistance has been shown with several insect species, the most active juvenile hormones appear to be more toxic in these strains than many of the presently used pesticides. High juvenile hormone activity coupled with specificity may still offer considerable hope for the future.

The work with juvenile hormone mimetic compounds to date indicates that these compounds may well be degraded by insects in much the same way as our present insecticides. The results further suggest that juvenile hormone mimetic compounds will be subject to all the problems associated with other xenobiotics used for the control of insects, namely the development of resistance. These juvenile hormone mimics will also apply pressure in favor of the development of cross-resistance by interacting with the microsomal enzyme system through induction.

Although insect juvenile hormone mimetic compounds still appear to offer promise as third generation insecticides, they are not the panacea originally predicted. We must continue our efforts to find new ways to control insect pests. We must not be deluded into the hope that juvenile hormone and its mimics will reach the marketplace devoid of the same problems besetting our present day insecticides.

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Microsomal Cytochrome P-450: Characterization and Possible Role in Insecticide Resistance in *Musca domestica*

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Cytochrome P-450 plays a central role in oxidative metabolism of xenobiotics. Recent studies of insecticide-resistant housefly strains have shown that cytochrome P-450 is qualitatively and quantitatively different from that in susceptible strains. Cytochrome P-450 was characterized through the following difference spectra: carbon monoxide; type I substrate; type II (including *n*-octylamine); type III; and ethyl isocyanide. It is probable that more than one cytochrome P-450 occurs in the housefly resembling, in some respects, the cytochromes P-450 from normal and induced mammals. The type I spectrum characteristic of mammalian microsomes cannot be demonstrated in the insecticide-susceptible CSMA housefly strain, although it is apparent in some resistant strains. Current work concerns the correlation of cytochrome P-450 types with the genetics of resistance and on structure–function relationships in difference spectra. Genes on chromosomes II and V appear to control qualitative differences between cytochrome P-450 from different strains, the same chromosomes associated with resistance involving high oxidase activity.

The microsomal mixed function system, which includes cytochrome P-450, plays a major role in the metabolism of xenobiotics in mammals (Gillette *et al.*, 1969) and insects (Hodgson, 1968; Hodgson and Plapp, 1970). An excellent review by Wilkinson and Brattsen (1972) has recently summarized the information on insect microsomal oxidations available through early 1972. The present article deals particularly with two areas, methodology and interpretation of optical difference spectra as applied to cyto-

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