Table 3. Content of Sumithion applied immediately after reaping. [1,2]

Days[3]	1	3	5
Peel			
Packed after dried	1.81	1.59	1.48
Packed immediately after dipping	1.65	1.62	1.39
Flesh			
Packed after dried	0.042	0.037	0.015
Packed immediately after dipping	0.053	0.035	0.042

1) Means of three replications. Expressed as μg of Sumithion per gram of tissue.
2) Faint color was also observed in the control runs, probably due to the contaminant pigments from banana tissues. This value was subtracted.
3) Days after bananas were brought out from the cellars.

harvest when bananas are actually placed on the counter of a grocer.

Thus, in light of the permissible limit in food being 1 ppm and 8 ppm for parathion and for malathion respectively (OFDA, 1957), it can be concluded that the content of Sumithion in bananas indicated above is quite negligible and completely harmless to human body from the view point of public health.

This work was carried out under the guidance of Prof. Sei-ichi Okui, Institute of Pharmacy, School of Medicine, Tohoku University, to whom we are much obliged.

Sammary

The amount of Sumithion residue in banana tissue was analyzed after the fruit was dipped in the emulsion of Sumithion. One and one half μg of Sumithion per gram of banana was adherred to the surface and it was penetrated into the flesh with the lapse of time. But the transfer seems very gradual. On around the tenth to fifteenth day after application of Sumithion when bananas are actually placed on the counter of a grocer, the content of Sumithion remaining was at most 1.8μg and 0.05μg per gram of peel and flesh respectively. It can be concluded that this content is quite negligible and completely harmless to human body from the view point of public health.

A Genetic Study on Sevin-Resistance and Joint Toxic Action of Sevin with γ-BHC against House Flies. Tsutomu KASAI and Zen-ichi OGITA (Department of Genetics, Medical School, Osaka University, Osaka, Japan) Received Dec. 7, 1964. *Botyu-Kagaku*, 30, 12, 1965.

3. イエバエにおけるセビン抵抗性の遺伝的解析ならびにセビンと BHC 混合による Joint toxic action. 笠井 勉・荻田善一（大阪大学医学部遺伝学教室） 39. 12. 7 受理

最近 DDT 抵抗性のシラミがカーバメートに感受性であるとか或はカーバメートで淘汰されたカが，ディルドリンや DDT に感受性になることが報告され，塩素系殺虫剤とカーバメート系殺虫剤が逆相関交差抵抗性 (negatively correlated cross-resistance) を示す関係にあるかも知れないことが暗示されている．しかしながら，キイロショウジョウバエではセビン抵抗性は，DDT, BHC やパラチオン抵抗性と同一の遺伝子によつて支配されていることが明らかにされたので，イエバエにおけるカーバメート抵抗性について遺伝的解析をおこなつた．イエバエにおいてはセビン抵抗性は主として第5染色体上の遺伝子によつて支配されており，γ-BHC 抵抗性は主として，第2染色体上の遺伝子によつて支配されている．それ故にこれらの間には逆相関交差抵抗性は認められない．しかしながら γ-BHC とセビン又は他のカーバメート系殺虫剤を混合する時いくつかの系統のイエバエに対して顕著な殺虫効力の増加が認められた．これは γ-BHC とカーバメート系殺虫剤が相互に殺虫作用機構が異なるためにもたらされた dissimilar joint action であると結論した．

In controlling resistant insects, it is very promising to use negatively correlated substances which were clearly shown by Ogita in *Drosophila melanogaster*. It was reported that phenylthiourea (PTU) and its halogen-substituted derivatives were negatively correlated substances

to DDT[1~6]. Other negatively correlated substances were found in DDT-resistant house flies for a crude preparation of diisopropyl tetrachloroethylphosphate by Mitlin et al.[7], and for cetyl bromoacetate by Ascher[8], although no similar result was obtained with new strains by another worker[9]. These authors did not confirm their respective results from a genetic basis.

Recent works suggest the possibility of a negatively correlated cross-resistance between carbamate insecticides and chlorinated hydrocarbons. Cole and Clark[10] reported that two DDT-resistant strains of the body lice were more susceptible to Sevin and *m*-isopropylphenyl N-methylcarbamate than a DDT susceptible strain. Georghiou and Metcalf[11] selected *Anopheles albimanus* with *m*-isopropylphenyl N-methylcarbamate and found that *m*-isopropylphenyl N-methylcarbamate-resistant strain was more susceptible to dieldrin and DDT than non-selected strains. On the other hand, Kikkawa[12] reported that the resistance to Sevin in *Drosophila melanogaster* is controlled by a gene which was also responsible for the resistance to DDT and parathion, and that negatively correlated corss-resistance was absent between DDT and Sevin. The above results indicate the need for further study of Sevin-resistance and relations between Sevin and chlorinated hydrocarbon insecticides in the house fly.

In the present study, a genetic analysis was made of resistance to Sevin in comparison with resistance to chlorinated hydrocarbon insecticides in house flies. It was noteworthy that a combined use of γ-BHC with carbamates resulted in an increased insecticidal action despite a finding that no negatively correlated cross-resistance was observed between Sevin and γ-BHC or DDT.

Materials and Methods

Insecticide resistant and susceptible strains of house flies reared at Osaka University were used. They included : (1) *NAIDM*, a susceptible strain obtained from Yamasaki of Tokyo University, (2) *Lab*, a susceptible strain obtained from Tsukamoto of Osaka University, (3) *ro* ; *ct* ; *cm*, a susceptible and multichromosomal mutant strain (2 ; 4 ; 5=*rough eyed* ; *cut wings* ; *carmine eyed* obtained from Hiroyoshi of Osaka University, (4) *RP*, a diazinon-resistant strain obtained from Yasutomi of National Institute of Health in Tokyo, and (5) 203 *d*, a diazinon-resistant strain obtained from Tsukamoto.

The carbamate insecticides used were Sevin (1-naphthyl N-methylcarbamate), UC-10854 (*m*-isopropylphenyl N-methylcarbamate) and C-3 (*m*-methylphenyl N-methylcarbamate). *p, p'*-DDT {1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane} and γ-BHC (1, 2, 3, 4, 5, 6-hexachlorocyclohexane) were used as chlorinated hydrocarbon insecticides. They were obtained from Japan Agricultural Chemicals and Insecticides Co., as technical pure samples.

Insecticidal action was tested by topical application. Female flies about 2 days old were treated with acetone solution of insecticides and their mortality was counted routinely 24 hours later. To determine which chromosome was responsible for insecticide-resistance, males of the F_1-hybrid offspring of a cross (*ro* ; *ct* ; *cm* ♀ × 203*d* ♂) were backcrossed to females of the *ro* ; *ct* ; *cm* strain. Adult flies of both sexes of the resultant backcross progeny were then tested for resistance by topical application. Mortality was counted in 24 hours, and each phenotypic marker was compared.

Joint action of a carbamate insecticide and a chlorinated hydrocarbon insecticide was tested by topical application of acetone solution containing two insecticides at various concentrations which was prepared by mixing with chemicals for LD-50, at the rates of 10 : 0, 8 : 2, 6 : 4, 4 : 6, 2 : 8, and 0 : 10 in volume. Then house flies treated with the mixture were comparatively studied as to mortality with those sujected to an insecticide alone.

Results

A susceptible and multichromosomal mutant strain, *ro*; *ct*; *cm*, and a diazinon-resistant strain, 203 *d*, the latter being over a hundred times resistant as the former[13] were used for a genetic analysis of insecticide-resistance. The mortality-dosage regression lines of the *ro* ; *ct* ; *cm* and the 203 *d* strains and their F_1-progeny on topical application of Sevin or γ-BHC, are shown in Figs. 1

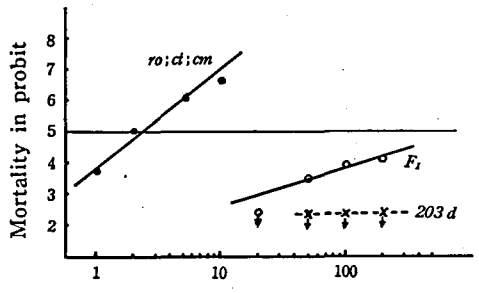

Fig. 1. Levels of resistance to Sevin in resistant and susceptible strains and their hybrids of house flies. One hundred female flies were used for each determination.

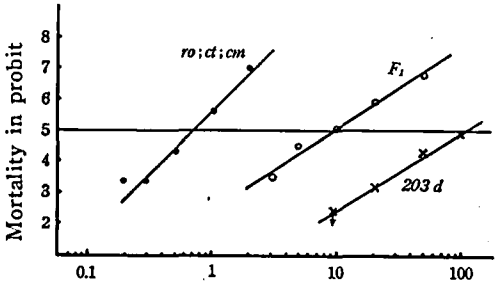

Fig. 2. Levels of resistance to γ-BHC in resistant and susceptible strains and their hybrids of house flies. One hundred female flies were used for each determination.

and 2. The 203*d* strain proved to be highly resistant to Sevin making it impossible to measure the LD-50 of Sevin which was higher than 200μg per female fly. The LD-50 for the *ro* ; *ct* ; *cm* strain is about 2μg Sevin per female fly. The F_1-hybrid showed an intermediate resistance of their parent flies. The 203*d* strain was also resistant to γ-BHC, showing its LD-50 over a hundred times resistant than the *ro*;*ct*;*cm* strain. It was revealed that F_1-hybrid flies showed an intermediate resistance against γ-BHC as well as against Sevin. Thus 203*d* strain proved to be multiple resistant against diazinon[13,14], DDT[14], Sevin and γ-BHC.

A question arose to study whether the multiple resistance shown in the 203*d* strain was due to only one factor or not, that is, a single resistant gene was responsible for multiple resistance or many genes were related to multiple resistance. Thus a genetic analysis was conducted with Sevin and γ-BHC. F_2-progeny flies obtained from a backcross {*ro* ; *ct* ; *cm* ♀ × F_1(*ro* ; *ct* ; *cm* ♀ × 203*d*♂)♂} were separated into 8 different phenotypes and were tested for insecticide-resistance following a topical application of Sevin or γ-BHC.

Resistance of each phenotypic F_2-progeny obtained from the backcross against Sevin showed

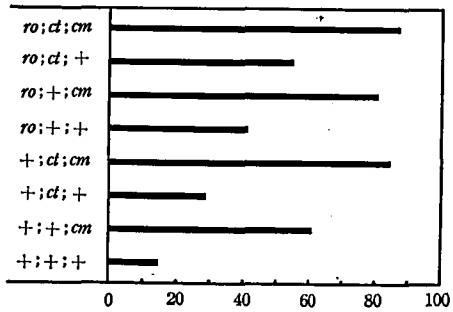

Fig. 3. Mortality of each phenotypic F_2-progeny obtained from a backcross {*ro* ; *ct* ; *cm* ♀ × F_1(*ro* ; *ct* ; *cm* ♀ × 203*d*♂)♂} by topical application of 10μg Sevin in acetone per fly. Eight hundred F_2-progenies of both sexes were used.

a clear segregation (Fig. 3). Namely, mortality rates of *ro* ; *ct* ; +, *ro* ; + ; +, + ; *ct* ; +and+ ; + ; + (wild type) progenies which had the 5th chromosome carrying a factor of resistance derived from the 203*d* in heterozygous condition, were lower than those of *ro* ; *ct* ; *cm*, *ro* ; + ; *cm*, + ; *ct* ; *cm* and + ; + ; *cm* progenies, in which both the 5th chromosomes were derived from the susceptible strain. These data evidently indicate that the most important effect on Sevin-resistance may be due to the 5th chromosome. The effect of 2nd or 4th chromosome on the Sevin-resistance should not be entirely neglected. From the results in Fig. 3, it was found that mortality rates of phenotypes of the 2nd chromosomal and 4th chromosomal mutants were slightly higher than those of the 2nd or 4th chromosomal heterozygotes. However, such finding may not be of so important in pursuing resistance, although it affords certain modifying factors.

Resistance of phenotypic F_2-flies obtained from the backcross against γ-BHC are shown in Fig. 4.

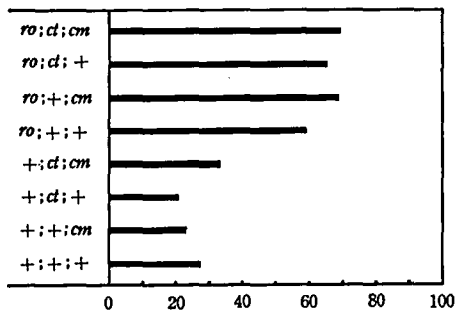

Fig. 4. Mortality of each phenotypic F_2-progeny obtained from a backcross {*ro* ; *ct* ; *cm* ♀ × F_1(*ro* ; *ct* ; *cm* ♀ × 203*d* ♂) ♂} by topical application of 2μg γ-BHC in acetone per fly. Eight hundred F_2-progenies of both sexes were used.

The mortality rates of *ro* ; *ct* ; *cm*, *ro* ; *ct* ; +, *ro* ; + ; *cm* and *ro* ; + ; + progenies which had the 2nd chromosomes derived from the susceptible strain in homozygous condition, were higher than those of + ; *ct* ; *cm*, + ; *ct* ; +, + ; + ; *cm* and + ; + ; + progenies which were heterozygous for the 2nd chromosomes. These data evidently indicate that a semi-dominant gene or genes responsible for the γ-BHC-resistance are linked with the *ro* character, i.e., with the 2nd chromosome.

The genetic factors responsible for Sevin-resistance and γ-BHC-resistance are quite different, as one is located on the 5th chromosome and the other on the 2nd chromosome. This suggests that the mechanism of insecticidal action is different between Sevin and γ-BHC. Therefore a joint action of the insecticides was tested as an example of a *dissimilar joint action* mentioned by Metcalf[16].

Table 1. Insecticide susceptibility of several strains of house flies by topical application.

Strain	LD-50 (μg/female fly)				
	Sevin	UC-10854	C-3	γ-BHC	DDT
NAIDM	10	0.7	—	0.5	0.2
Lab	—	0.7	5	0.5	0.2
ro ; *ct* ; *cm*	1.5~2	0.7	4	0.4~0.5	0.1~0.3
RP	30	—	—	0.04	4.4
203*d*	100<	—	—	100<	100<

Sevin=1-naphthy N-methylcarbamate
UC-10854=*m*-isopropylphenyl N-methylcarbamate
C-3=*m*-methylphenyl N-methylcarbamate

The LD-50 of Sevin, its derivatives, γ-BHC and DDT are shown in Table 1. The acetone solutions giving LD-50 of two insecticides to be tested were mixed at various concentrations before they were topically applied to house flies. Fig. 5A shows a joint action of Sevin and γ-BHC against mixed population consisting of the *ro* ; *ct* ; *cm* (which is susceptible to Sevin but moderately resistant to γ-BHC) and the *RP* (which is moderately resistant to Sevin but susceptible to γ-BHC) strain in equal numbers. In this case, Sevin alone killed almost all the *ro* ; *ct* ; *cm* flies, but not the *RP* flies, and γ-BHC alone acted reciprocally, whereas higher mortality rates were caused by mixture solutions. This joint action may result from the heterogeneous constitution of insects. Joint action of several carbamates and γ-BHC against some strains of house flies is shown in Fig. 5B~5I. It is evident that a combined use of carbamates with γ-BHC shows a higher mortality than that of these two agents

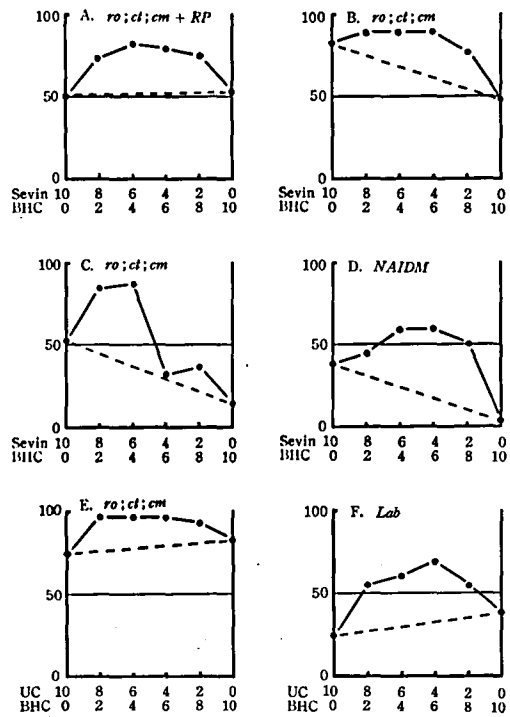

Fig. 5. A—F

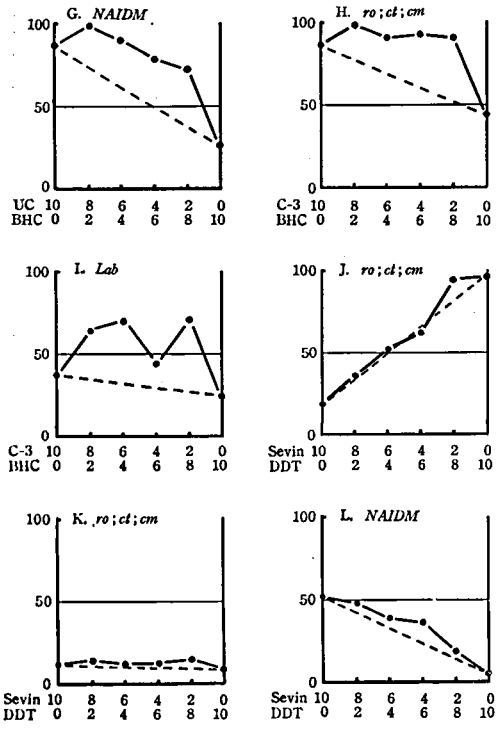

Fig. 5A~5L. Joint action of carbamates and chlorinated hydrocarbon insecticides against several strains of house flies. The abscissa represents the proportion of insecticides which were prepared for LD-50 as shown in Table 1, except Fig. 5A in which Sevin and γ-BHC were prepared to give 10μg and 0.1μg per female fly respectively. The ordinate shows mortality in 24 hrs. Fifty female flies were used for each determination.

separately employed. However, no combinations with other chlorinated hydrocarbon insecticide, DDT, and Sevin, resulted in an increase in mortality over a respective single use, in some preliminary experiments (Fig. 5J~5L).

Discussion

As mentioned above, the most important factor responsible for Sevin-resistance is located on the 5th chromosome as well as diazinon-resistance and DDT-resistance genes[13], and many genes responsible for esterases and acid phosphomonoesterase activities[14,15]. The locus of the main Sevin-resistace gene is left to be defined. It is understood that Sevin has no negatively correlated activity to DDT, for Sevin-resistant strain 203*d* is also resistant to DDT.

A question arises if there may be a negatively correlated cross-resistance between Sevin and γ-BHC, when the *ro* ; *ct* ; *cm* and the *RP* strains are compared, since the *ro* ; *ct* ; *cm* strain is more resistant to γ-BHC than the *RP* strain, whereas the former is more susceptible to Sevin than the latter. However, such a result is not due to a negatively correlated cross-resistance, but to the variation found in strains, for the main factor responsible for resistance to γ-BHC is located on the 2nd chromosome. The factor responsible for γ-BHC-resistance is quite different from that for Sevin-resistance. Thus it is suggested that the mechanism of insecticidal action of Sevin is different from that of γ-BHC.

A combined use of two insecticides having different physiological action may be useful for the control of an insect population with varying resistant levels, for one insecticide may be influential in affecting part of the population, leaving the rest of the population under the influence of the other. Joint action of Sevin and γ-BHC was tested against a mixed population consisting of the *ro* ; *ct* ; *cm* and the *RP* strains which have different resistant levels to the insecticides. Obviously the majority of the *ro* ; *ct* ; *cm* flies were killed by Sevin, whereas the *RP* flies were mostly killed by γ-BHC, so that a combined use of the two agents resulted in higher mortality of insects.

Further, joint action of Sevin with γ-BHC was tested against several strains with almost the same level of resistance. The experimental results showed higher mortality than when these agents were separately employed. Metcalf[16] summerized joint action of insecticides into several types on the basis of response and physiological action. The joint action of Sevin and γ-BHC may be classified as *dissimilar joint action* according to his designation, where toxicants A and B applied separately produce a common response yet when applied jointly produce the same response by action on separate and distinct physiological systems (A) and (B). Thus, it was concluded that two factors might be res-

ponsible for an increase in insecticidal action following a combined use of Sevin and γ-BHC, i. e., a joint action for the genetic heterogneity of insect population viewed on an insecticide-resistance basis, and a *dissimilar joint action.*

Increase in insecticidal action was also evident in the combinations of γ-BHC with other carbamates such as *m*-isopropylphenyl N-methylcarbamate and *m*-methylphenyl N-methylcarbamate. This increase suggests that carbamates such as Sevin and *m*-isopropylphenyl N-methylcarbamate have the same mode of action when jointly used with γ-BHC. It may be assumed that the mode of action of DDT is different from that of γ-BHC, for a combined use of DDT with carbamates failed to show any increase in mortality. This assumption coincided with the results obtained of a genetic analysis made by Tsukamoto[13,17] that the most important genetic factor responsible for DDT-resistance is located on the 5th chromosome where γ-BHC-resistance gene absent.

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Summary

A genetic study was made of resistance to Sevin and γ-BHC against house flies. A genetic factor or factors controlling Sevin-resistance are located on the 5th chromosome, whereas those of γ-BHC-resistance are on the 2nd chromosome. A combined use of γ-BHC with carbamates resulted in an increased insecticidal action, despite a finding that no negatively correlated cross-resistance was observed between Sevin and γ-BHC or DDT. It was concluded that two factors might be responsible for the increased insecticidal action following a combined use of Sevin and γ-BHC, i. e., a joint action for the genetic heterogeneity of insect population viewed on an insecticide-resistance basis, and a *dissimilar joint action* reported by Metcalf.

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