えられることが推察された.

摘　　要

マツを加害する穿孔虫類の防除を目的とする薬剤の有効度評価に，適切なる試験方法を確立する目的で，餌木とした7本のマツの樹枝部から脱出する，キイロコキクイムシ成虫の分布を，10cm の長さを調査単位として，3日ごとに最高15回まで調べた．その分布は，おおむね過大分散を示し，負の二項分布によく適合した．とくに異常と認められたものをのぞくことによって，各供試木における調査日間の共通のk_eの値の算定は可能であった．供試木中，1本を除外すれば，供試木全体の共通な k_e の値は求められた．このことは供試木をさらに多くとれば，全体に共通なk_eの値が求められるものと推測された．なお同一の供試木における共通のk_eを Blisss and Owen[4] の簡便法と Bliss and Fisher[2] の最尤法の二方法で求め，その結果を比較した．

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Summary

In July 1967, some branches, having diameters ranging from 2 to 6cm, were cut from 7 living pine trees which were about 50 years old. These branches were placed at the base of the tree from which they were taken for oviposition of *Cryphalus fulvus* Niijima for a period of 20 days. Under a laboratory condition of 25°C and 60% relative humidity, the number of adults that emerged per unit of 10cm of a branch was counted every 3 days. The number of adults counted per unit showed over dispersion. The spatial distributions of 92 out of 94 samples could be well fitted to the negative binominal. Through observation of the spatial distribution data it was possible to fit a common k_e to each of 5 trees. It was also possible to calculate a common k_e in the remaining 2 trees if one or two unusual samples were omitted. Estimation of a common k_e among all the trees was possible if one unusual tree was omitted. The estimated result was 0.296. There was a small difference between the common $\hat{k}_e$'s of the same tree depending upon whether it was calculated by the method of Bliss and Owen[4] or that of Bliss and Fisher[2].

Studies on the Increment of the Efficacy of Insecticides (VIII) Metabolism of ^{3}H-Pyrethroids in the Adult House Fly, *Musca domestica vicina* Macq. Akifumi Hayashi*, Tetsuo Saito and Kisabu Iyatomi (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Nagoya) Received July 17, 1968. *Botyu-Kagaku*, 33, 90, 1968.

14. 殺虫剤の効力増進に関する基礎的研究 (VIII). ^{3}H-アレスリンおよび ^{3}H-フタルスリンのイエバエにおける代謝について. 林　晃史*, 斎藤哲夫, 彌富喜三 (名古屋大学農学部害虫学教室)

^{3}H-アレスリンと ^{3}H-フタルスリンに共力剤 piperonyl butoxide, *n*-propyl isome, safroxan, MGK-264 および sulfoxide を 1:10 の割合で混用し，ピレスロイドの体内への吸収ならびに代謝について調べた．その結果，共力剤を加用することによって，排泄量が少なくなり，体内存在量の多くなることが認められた．また，表皮残存量からみると共力剤の加用は有効成分の表皮透過性を低下せしめるものと考えられる．

なお，代謝物をペーパークロマトグラフィーで調べた結果，両薬剤とも6つの成分が分離され，共力剤を加用することによって分解物の量は少なく，このことより，共力剤の作用機構の一つはピレスロイドの分解を抑制するものではないかと考える．

The penetration and metabolism of pyrethroids in insects have been studied by Winteringham (1955), Hopkins *et al.* (1957), Chang *et al.* (1964),

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and Yamamoto and Casida (1966), with ^{14}C-labeled allethrin, pyrethrin I, and cinerin I. In this paper, the penetration and metabolism of ^{3}H-allethrin and ^{3}H-phthalthrin, applied singly or in combination with synergists to the adult house fly, were studied.

I. Materials and Methods

(A) *Materials*

^{3}H-Labeled allethrin and ^{3}H-labeled phthalthrin were prepared according to the ^{3}H-exchange method, and purified chromatographically by courtesy of Sumitomo Atomic Energy Industrial Company. Both compounds were identical with authentic materials in thin-layer and gas-liquid chromatographic analyses. Their specific activities were 43.3 mCi/g and 1.78 mCi/g, respectively. The following synergists were used in combination with pyrethroids in a 10:1 ratio by weight; piperonyl butoxide, S-421, *n*-propyl isome, sulfoxide, safroxan, and MGK-264 (technical grade, commercially available).

(B) *Test insects*

The house flies, *Musca domestica vicina* Macq., used in these experiments were of an insecticide-susceptible strain ("Takatsuki"). Flies, 4 to 5 days-old, were sexed under a light CO_2 anesthetization and the female flies were used throughout all experiments. They have been routinely reared in this laboratory.

Pyrethroid dissolved in acetone with or without one of the synergists was topically applied to the dorsal surface of the thorax of female flies in volume of 0.5 μl per fly by means of a micrometer syringe. The treated flies were reared in a room kept at 25°C, supplied with 2.0% sugar solution. Mortality counts were made 24 hrs after the treatment. Results are shown in Table 1.

Table 1. Toxicities of synergized pyrethroids topically applied to house flies.

Synergist	LD_{50} μg/Fly (24 hrs)	
	Allethrin	Phthalthrin
—	0.398	0.646
S-421	0.080	0.149
P. butoxide	0.166	0.109
Safroxan	0.131	0.178
Sulfoxide	0.167	0.214
MGK-264	0.168	0.178
n-propyl isome	0.171	0.164

(C) *Extraction of allethrin and phthalthrin from house flies*

One-half μl of acetone solution of ^{3}H-allethrin or 1 μl of acetone solution of ^{3}H-phthalthrin was topically applied to each of the anesthetized house flies. The flies were held in a room kept at 30°C. After 1, 3, 5, and 24 hrs of the applications, ten flies were rinsed with 4.0 m*l* of 90% aqueous acetone to remove the remaining insecticide on the surface of the insects. Aliquots of the washings were taken into vials for counting their radioactivities. The washed flies were homogenized with 1.5 m*l* of aqueous acetone in a glass homogenizer. The homogenate was centrifuged at 2,000 r.p.m. for 15 min, and the resulting supernatant was separated. The precipitate was washed with aqueous acetone, and recentrifuged in order to obtain the supernatant. The washing was further carried out twice, and the supernatants thus obtained were combined, and made up to 5.0 m*l* adding aqueous acetone. Each one-half m*l* of an aliquot was taken into a vial for the determination of the radioactivity of the absorbed pyrethroid in the house flies. The excretion of ^{3}H-labeled pyrethroid was investigated by means of washing the inside of glass jar with 20 m*l* of aqueous acetone in which the flies had been kept. Two m*l* of the washing was taken into a vial for the determination of radioactivity. Radioactivity was counted by a liquid scintillation spectrometer, Packard Tri-Carb 314 EX type, in the following way. Acetone was allowed to evaporate under a dry-air stream, and the resulting residue was dissolved in 10 m*l* of dioxane scintillator solution (Bray 1960) for the counting. Internal standard corrections were applied for quenching. These results are shown in Tables 2, 3, 4, and 5.

(D) *Measurement of metabolites of ^{3}H-labeled allethrin and phthalthrin*

The test chemicals were applied to each 100 house flies as described above. After 3 and 24 hrs, the house flies were homogenized in aqueous acetone. The homogenate was centrifuged at 2,000 r.p.m. for 15 min. The procedures of

Table 2. Absorption and excretion of ^{3}H-Allethrin topically applied to house flies.

Material	1 hr. (dpm)			3 hrs. (dpm)			5 hrs. (dpm)			24 hrs. (dpm)		
	outer	internal	excreta	outer	internal	excreta	outer	internal	excreta	outer	internal	excreta
Allethrin	1793	2787	242	865	3552	611	724	2499	466	194	1402	1654
All.+S-421	3113	6965	123	1518	3388	229	1446	3369	342	358	6165	201
All.+P. butoxide	3482	3693	148	2186	2856	116	3019	4108	128	906	4361	388
All.+Safroxan	—	—	—	—	—	—	—	—	—	532	5750	372
All.+Sulfoxide	—	—	—	—	—	—	—	—	—	840	4583	444
All.+MGK-264	—	—	—	—	—	—	—	—	—	231	1545	1277
All.+*n*-Propyl isome	—	—	—	—	—	—	—	—	—	755	4578	586

Table 3. Absorption and excretion of ^{3}H-Phthalthrin topically applied to house flies.

Material	1 hr. (dpm)			3 hrs. (dpm)			5 hrs. (dpm)			24 hrs. (dpm)		
	outer	internal	excreta	outer	internal	excreta	outer	internal	excreta	outer	internal	excreta
Phthalthrin	256	325	45	415	471	99	274	211	71	21	353	269
Pht.+S-421	294	163	27	247	389	31	371	332	31	127	486	85
Pht.+P.butoxide	474	335	19	336	315	71	406	305	43	291	421	109
Pht.+Safroxan	—	—	—	—	—	—	—	—	—	59	387	87
Pht.+Sulfoxide	—	—	—	—	—	—	—	—	—	129	424	87
Pht.+MGK-264	—	—	—	—	—	—	—	—	—	108	298	91
Pht.+*n*-Propyl isome	—	—	—	—	—	—	—	—	—	218	378	71

Table 4. Penetration of ^{3}H-Allethrin topically applied onto dorsal surface of house flies.

Time	Material	Percentages of allthrin (%)		
		outer	internal	excreta
1 (hr.)	Allethrin	37.2	57.8	5.0
	+S-421	30.5	68.3	1.2
	+P. butoxide	47.5	50.4	2.0
3	Allethrin	17.2	70.6	12.2
	+S-421	29.6	66.0	4.5
	+P. butoxide	42.4	55.4	2.2
5	Allethrin	19.6	67.7	12.6
	+S-421	28.0	65.3	6.6
	+P. butoxide	41.6	56.6	1.8
24	Allethrin	6.0	43.1	50.9
	+S-421	5.3	91.7	3.0
	+P. butoxide	16.0	77.1	6.9
	+Safroxan	8.0	86.4	5.6
	+Sulfoxide	14.3	78.1	7.6
	+MGK-264	7.6	50.6	41.8
	+*n*-P-isome	12.8	77.3	9.9

Table 5. Penetration of ^{3}H-Phthalthrin topically applied surface of house flies.

Time	Material	Percentages of Phthalthrin (%)		
		outer	internal	excreta
1 (hr.)	Phthalthrin	40.9	51.9	7.2
	+S-421	60.7	33.7	5.6
	+P. butoxide	57.2	40.5	2.3
3	Phthalthrin	42.1	47.8	10.1
	+S-421	37.0	58.3	4.6
	+P. butoxide	46.5	43.6	9.8
5	Phthalthrin	49.3	37.9	12.8
	+S-421	50.5	45.2	4.2
	+P. butoxide	53.8	40.5	5.7
24	Phthalthrin	3.3	54.9	41.8
	+S-421	18.2	69.6	12.2
	+P. butoxide	35.4	51.3	13.3
	+Safroxan	11.1	72.6	16.3
	+Sulfoxide	20.2	66.3	13.6
	+MGK-264	21.7	60.0	18.3
	+*n*-P-isome	32.7	56.7	10.6

Table 6. Paper chromatography of ^{3}H-Allethrin and its degradates.

Material	After 3 hrs						After 24 hrs					
	Rf 0.02	0.16	0.40	0.52	0.68	0.93	Rf 0.02	0.16	0.40	0.52	0.68	0.93
Allethrin hydrolyzed with *N*/2 NaOH, 78°C, 1.5 hr	(48.9)	(%)	(51.1)									
Allethrin	22.9	6.0	0.5	0.2	0.5	69.9	63.3	4.2	0.4	0.8	1.3	30.0
All.+S-421	7.7	1.9	0.3	1.2	5.7	83.2	39.6	11.6	0.7	0.4	1.4	46.3
All.+P. butoxide	10.7	0.6	0.1	0.3	1.1	87.2	39.9	12.6	0.3	0.3	2.6	44.3
All.+Safroxan	—	—	—	—	—	—	32.6	7.7	0.3	0.4	0.3	58.7
All.+Sulfoxide	—	—	—	—	—	—	41.0	0.5	0.6	0.1	0.1	57.7
All.+MGK-264	—	—	—	—	—	—	47.8	6.1	10.2	0.4	0.7	34.8
All.+*n*-Propyl isome	—	—	—	—	—	—	31.0	7.0	0.2	0.4	3.3	58.1

* Rf: 0.02=Allethrolone, 0.40=chrysanthemic acid, 0.93=Allethrin

Table 7. Paper chromatography of ^{3}H-Phthalthrin and its degradates.

Material	After 3 hrs.						After 24 hrs.					
	Rf 0.02	0.29	0.40	0.58	0.83	0.90	Rf 0.02	0.29	0.40	0.58	0.83	0.90
Phthalthrin hydrolyzed with *N*/2 NaOH, 78°C, 1.5 hr	(11.9)	(%)	(88.1)									
Phthalthrin	37.5	4.0	6.4	5.3	6.2	40.6	56.6	4.1	1.7	1.4	4.2	32.0
Pht.+S-421	28.9	1.5	2.4	2.9	11.2	63.1	33.4	3.0	2.6	1.7	0.7	58.6
Pht.+P. butoxide	4.2	3.5	0.8	0.2	0.9	90.4	29.8	5.9	0.3	1.2	3.7	59.1
Pht.+Safroxan	—	—	—	—	—	—	18.7	5.8	5.1	5.0	5.6	59.8
Pht.+Sulfoxide	—	—	—	—	—	—	44.3	0.1	1.9	1.8	0.1	57.8
Pht.+MGK-264	—	—	—	—	—	—	25.0	6.5	10.4	8.8	7.2	42.1
Pht.+*n*-Propyl isome	—	—	—	—	—	—	26.9	2.2	5.3	11.5	7.3	46.8

Rf: 0.02=tetrahydrophthalimide-*N*-methylol 0.40=chrysanthemic acid
0.04=tetrahydrophthalimide 0.90=Phthalthrin

washing and centrifuging were repeated three times as mentioned above. The combined supernatants were concentrated to a small volume at 40°C by means of a vacuum rotary evaporator. The concentrate was spotted on Toyo No. 51A filter paper treated with 50% *N*, *N*-dimethylformamide in acetone and then developed with ligroin saturated with *N*, *N*-dimethylformamide. After drying, the filter paper was cut into pieces of 1cm section. Each section was put into a vial in which 10m*l* of the dioxane scintillator solution was added, and then radioactivity was counted. These results are listed in Tables 6 and 7.

II. Results and Discussion

As shown in Table 1, allethrin was proved to be more toxic than phthalthrin in topical application test against house flies. S-421 was an effective synergist for allethrin, and piperonyl butoxide for phthalthrin. These results are in accordance with the observations by Yasutomi (1960), Hayashi (1962), and Incho *et al.* (1962). As shown in Tables 4 and 5, the penetration of allethrin through the integument of house flies occurred rapidly. One hour after the treatment, 57.8% of the applied dose was absorbed. When allethrin was applied in combination with either S-421 or piperonyl butoxide, the rate of absorption after one hour was 68.3 or 50.4%, respectively. Twenty-four hours after the treatment, 6.0% of the total allethrin was found on the exterior surface of the house flies, 43.1% in the body,

and 50.9% in the excreta. When allethrin was jointly applied with piperonyl butoxide, amounts of both remaining allethrin on the cuticular surface and the absorbed one into the body were increased, but the excretion rate was decreased. This is the same result as that by Hopkins *et al.* (1957) who have used ^{14}C-labeled allethrin in combination with piperonyl butoxide. The amount of unabsorbed allethrin on the body surface increased when applied in combination with piperonyl butoxide, and the amount remaining allethrin in the body increased when applied with S-421. The remaining period of phthalthrin on the cuticular surface of the house flies was longer than that observed with allethrin. Five hours after the treatments, 42.9% of phthalthrin was recovered from the outer surface, whereas only 19.6% of allethrin was recovered. However, there were small differences between phthalthrin and allethrin at 24 hrs after the treatments, in the amounts remained either outside or inside of the body, and excreted. An increase of the unabsorbed pyrethroid, when treated with one of the synergists, seems to be due to the dilution of the pyrethroid by the synergist on the insect integument, as suggested by Winteringham (1955) who studied using allethrin and piperonyl cyclonene.

The present experiments revealed that the amounts of both pyrethroids remaining on the cuticular surface and those retained in the inside of body were increased, and that the rate of excretion was reduced, when these pyrethroids were topically applied to house flies in combination with a synergist. This may provide an important clue to elucidate the mechanism of synergistic action.

Metabolites of allethrin and phthalthrin in the house flies were investigated by paper chromatography. Paper chromatograms showed six spots including five metabolites in the extract of house flies treated with allethrin and phthalthrin. Winteringham (1955), Bridges (1957), Hopkins *et al.* (1957), and Chang *et al.* (1964) have already reported that there were chrysanthemic acid, an alcoholic substance, and unidentified substances in the metabolites of pyrethroids.

On the chromatograph, allethrolone, chrysanthemic acid, and allethrin were detected with Rf values of 0.02, 0.40 and 0.93, respectively, and three metabolites with Rf values of 0.16, 0.52 and 0.68 were not yet identified. Very weak radioactivity on the spot corresponding to chrysanthemic acid was observed. When allethrin was applied alone to house flies, 63% of it was metabolized to allethrolone, 30% unchanged, and the remaining 7% was unknown compounds after 24 hrs from the treatment. When allethrin was applied with either S-421 or piperonyl butoxide, radioactivities found in the spots with Rf values of 0.02 and 0.16 were higher than those of allethrin alone. The radioactivity at Rf value of 0.93, corresponding to allethrin, was increased when combined with any one of the synergists. There was no relationship between the radioactivity of allethrin spot on the chromatogram and synergistic effectiveness of the synergists used in the present study, except for MGK-246.

When phthalthrin was applied, tetrahydrophthalimide-*N*-methylol, chrysanthemic acid, and phthalthrin were detected with Rf values of 0.02, 0.40 and 0.90, respectively, and other three spots with Rf values of 0.29, 0.58 and 0.83 were unidentified. When phthalthrin was applied in combination with any one of the synergists, the radioactivity of the spot with Rf 0.02 corresponding to tetrahydrophthalimide-*N*-methylol was smaller than that phthalthrin applied without the synergists. The radioactivity of phthalthrin recovered in the house fly bodies was higher when it was applied with the synergists. No qualitative difference was found in the metabolites of pyrethroids when applied with or without synergists. The present experimental results coincided with that of Hopkins *et al.* (1957).

In view of the fact that the decomposition of allethrin and phthalthrin was depressed in insect body when those were applied with synergists, the mechanism of synergists may be explained by the depression of the decomposition of the pyrethroids in insects.

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Summary

1. Allethrin and phthalthrin were applied topically to the adult house flies in combination with one of the following synergists at a ratio of 1 : 10 ; piperonyl butoxide, S-421, *n*-propyl isome, sulfoxide, safroxan, and MGK-264. The LD_{50} values evaluated at 24 hrs after treatment showed that S-421 was the most effective synergist for allethrin, whereas piperonyl butoxide for phthalthrin.

2. The radioactivities of ^{3}H-labeled allethrin and phthalthrin on the cuticular surface, in the body, and in the excreta were measured at regular intervals after the treatment. In all cases the radioactivity recovered in the excreta decreased, whereas that in the body and on the cuticular surface increased when the pyrethroids were applied with synergists. Synergists probably caused the depression of the permeability of pyrethroids through the integument of the house flies.

3. Five compounds were detected as metabolites of both pyrethroids by paper chromatography. The decomposition of the pyrethroids was depressed by the addition of synergists. It may be suggested that the inhibition of the decomposition of the pyrethroids in insects is one of synergistic mechanisms of synergists.

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綜　　説

Central Nervous System Control of Circadian Rhythmicity in Insect. Junko Nishiitsutsuji-Uwo (Department of Agricultural Chemicals, Shionogi & Co., Ltd., Doshomachi, Osaka)

昆虫の体内時計. 宇尾淳子（塩野義製薬株式会社・植物薬品部）

はじめに

生体を取りかこむ環境は，その変化においてめざましい程周期的である．周期性は主として地球の自転や公転，又月の公転に起因している．そしてこれらの日々の，月々の，年間の“時”というものが，生体に対して何等かの影響を与えている．

遠く1729年に，フランスの地質学者 De Mairan は或種の植物の葉が，昼と夜とで上ったり，下ったりする運動を日々繰返すことに興味をもった．そこで彼はオジギソウ *Mimosa* を洞くつの中に入れて，日々の光や温度のサイクルから遮断してみたところ，おどろくべきことに，やはりこの葉は日々の上下運動を繰り返すことを発見した．この現象は19世紀の植物学者によって詳しく追究せられ，同じ世紀の後半においてすでに，日々の光の周期は植物のもっているどの様な種類の周期性をもひき起こす原因ではなくて，単に他の原因によって内部に生じでる周期性の“timing”をcotrolするに過ぎないことが明らかにされた．すなわち De Mairan の現象は完全に内因的であって，外因的な原因によって生ずるのではないことが，現在迄に数多くの研究者によって証明されている．近年 Franz Halberg は De Mairan の oscillation を“circadian”—ラテン語 circa, dies 約1日の意—という用語で現わすことを提案した．この用語は生物のもつ oscillation の周期と，地球の自転—正確に24時間—との間の区別を理論的に強調すると共に，昼行性，diurnal (又は夜行性，nocturnal) という用語—各生物にある固有の遺伝的性質としてのニュアンスをもつ—のもつ不明瞭さと矛盾とを補っている[24]．

現在 De Mairan の現象はおどろく程多種類の生物で観察されている．すなわち，単細胞生物，多細胞生物，もちろん人間も含めて，この地球という遊星の上に住むあらゆる生きとし生けるものの生理機構の一般