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Behavior on and in Rice Plants of Diazinon Applied onto the Surface of Paddy Soil.

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19. 水田面に処理されたダイアジノンの水稲への移行と分解.

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水稲を栽培したポットの土壌面に ^{32}P-ダイアジノン粒剤または乳剤を処理し，薬剤の水稲地上部への移行と分解をトレーサー法により試験した．田面水中のクロロホルム可溶 ^{32}P 物質の消失は，乳剤区より粒剤区でゆるやかであった．葉身のクロロホルム可溶 ^{32}P 物質は粒剤区では処理後12, 18日間連続的に増加したが，乳剤区では処理1日後までに最高近くに達し，その後ほぼ同じ水準を保った．クロロホルム可溶 ^{32}P 物質は葉鞘より葉身で大きい傾向を示したが，これはクロロホルム可溶 ^{32}P 物質の葉鞘から葉身への移行が，葉鞘への補給より速いことによるとみられた．田面水中のクロロホルム可溶 ^{32}P 物質は実験期間中その大部分がダイアジノンであった．葉身では処理後1日でダイアジノンは約50%となり，その後もほぼこの水準を保ち，葉鞘ではこれよりやや低かった．葉身の水可溶 ^{32}P 代謝物はジエチルりん酸＞ジエチルチオりん酸＞りん酸・チオりん酸の順で，穂ではジエチルりん酸が主であった．

Diazinon (*O,O*-diethyl (-*O*-2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), is an excellent chemical for the control of various insect pests of crops. This insecticide is now used in Japan for the control of rice insects by "the paddy water application method". Granules and emulsion of Diazinon give simultaneous control of the rice stem borer and certain virus-transmitting leaf-hoppers when applied onto the standing water in rice fields. The method is very simple and has become widespread as a labor-saving practice. When applied by the above-mentioned method, an insecticide should reach into plant portions where insects live or into insects directly from applied zone to kill those insects. Two routes are postulated as pathways in penetration into rice plants of an insecticide applied onto paddy water or soil; one is through the root system which have been proved quantitatively by Tsukano and Suzuki (1962)[1] and Fukuda and Masuda (1965)[2] on γ-BHC and carbaryl, respectively, and the other is through the leaf sheath where an insecticide creeps up by capillarity and/or penetrates into tissues as pointed out by Ishii and Hirano (1962)[3], Tomizawa[4] and Hirano and Yushima (1969)[5] on γ-BHC, Carbaryl, and Diazinon, respectively.

Recent papers have shown that Diazinon was absorbed and translocated into plants from treated soil or irrigation water (Gunner *et al.*, 1967,[6] Onsager and Rusk, 1967,[7] Hirano and Yushima, 1969[5]) or sand (Lichtenstein *et al.*, 1967)[8] and from treated planting water (Miles *et al.*, 1967)[9] or culture solution though it is rapidly hydrolyzed in the foliage (Kansough and Hopkins, 1968)[10].

Present studies have been carried out in order

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to confirm which route is predominant in the penetration of Diazinon into rice plants and to estimate the rate of decomposition in the plants after application of granules and emulsion of Diazinon onto the surface of paddy water.

Materials and Methods

Insecticide: Twenty percent emulsifiable concentrate and 3.2 percent granules of ^{32}P-labelled Diazinon were kindly supplied by Nihon Kayaku Company. The specific activities were 6.3 mc per gram for the former and 4.1 mc per gram for the latter, respectively. Radioactive Diazinon was confirmed to be more than 99% in purity by thin-layer chromatography.

Plants: Rice plants grown in potted soil at different stages were used, *i.e.*, those at the seedling stage (at one week after transplantation) and those at the pre-heading stage (at a few days before heading). The seedlings were tested both in the conditions of flooded and non-flooded soils, and the plants at pre-heading stage were tested only under flooded conditions.

Application of radioactive Diazinon: The labelled Diazinon-containing granules were placed onto the surface of potted soil at a rate of 2.5 mg of active ingredient per pot, equivalent to 1.25 kg per ha.

The emulsifiable concentrate, diluted to 1 : 50 with water, was introduced onto the paddy water at a rate of 4.0 mg of active ingredient per pot, equivalent to 2.00 kg per ha. The pot was 0.02 m^2 in area. Water depth in pots was maintained about 2 cm during the experiment under flooded conditions and the water was drained in order to examine the movement of Diazinon under non-flooded conditions.

Sampling and radioassay: Aerial parts of the plants were cut at 1 cm above the paddy water to avoid the contamination from radioactivity in applied zone or at 1 cm above the soil surface in the experiment under non-flooded conditions. The plants were divided into leaf blades and leaf sheaths. Each portion was chopped finely with scissors. The sliced sample was macerated with an equal volume of chloroform and 10% aqueous trichloroacetic acid (including moisture of the sample) in a Waring blender, and centrifuged to separate into the organic and aqueous layers and the solid matter. An aliquot of each layer was wet-ashed with a mixture of concentrated sulfuric acid and nitric acid (5 : 1). Radioactive phosphoric acid was precipitated as ammonium phosphomolybdate, and its radioactivity was determined by a GM counter. The amounts of radioactive substances which were partitioned into chloroform and aqueous layers, were calculated. The solid matter in the centrifuge bottle was washed with chloroform and 10% aqueous trichloroacetic acid, and the radioactive substances unextractable with these solvents were also determined as a part of the experiments.

Separation of chloroform-extractable metabolites: After partition between chloroform and aqueous trichloroacetic acid, the chloroform layer was dried over anhydrous sodium sulfate, and concentrated at room temperature under reduced pressure. Metabolites in the concentrates were separated by paper or silica gel thin-layer chromatography. In paper chromatography, upper layer of a mixture of ethanol, chloroform and water in a ratio of 2 : 2 : 1 by volume was used as mobile phase, and a filter paper impregnated with 5% Silicone 550 in hexane was used as stationary phase. In thin-layer chromatography, a mixture of hexane 4 parts and acetone 1 part by volume was used as mobile phase. Two chromatograms were made for each concentrate, and one of them was used for the preparation of radioautogram. The other was cut into 20 pieces of an equal length from origin to solvent front, and the radioactivity of each piece was determined in turn by a GM counter. From the results of both radioautogram and radioassay, the percentage of radioactivity corresponding to each metabolite was calculated.

Separation of water-extractable metabolites: The ear (dough stage) and the leaf blade (harvesting stage) were sampled at 38 days and 50 days after application, respectively. After partition between chloroform and aqueous trichloroacetic acid, the aqueous layer was subjected to ion exchange chromatography using Dowex 1-X8 by the method of Plapp and Casida (1958)[11]. Major metabolites were identified by co-chromatography with the authentic compounds.

Results and Discussion

Fig. 1 shows the change of chloroform- or water-soluble radioactive substances in paddy water treated with Diazinon. Chloroform-soluble radioactive substances disappeared rapidly during the first 6 days, and slowly afterwards. In all water samples, measurable amounts of radioactive substances were still found at the end of every experiments. Concentration of Diazinon in water seemed to reach a maximum in one day after application with granules and emulsion. Diazinon applied as granules was persistent as compared with emulsion.

Fig. 2 shows the change of chloroform- and water-soluble radioactive substances in the rice plants applied with Diazinon. In the leaf blades, the amount of the chloroform-soluble substances increased gradually and continuously during the experiment when granules were used, while that in the leaf sheaths increased during the first 6 days, and maintained the similar concentration afterwards. Difference in depth of paddy water had no influence on the systemic activity of the insecticide. Growth stages of the plants had some influence on the activity; the younger plants, increasing in size more rapidly than the older ones, had higher activity per fresh weight. On the other hand, the amount of chloroform-soluble radioactive substances in leaf blades reached near to a maximum during the day after the emulsion application, and then almost unchanged afterwards, while that in leaf sheaths increased rapidly during the first 24 hours and then decreased gradually with days. The radioactivity in the plants was higher in leaf blades than in leaf sheaths throughout the experimental periods except early samples of emulsion application. The ratio of the unextractable activity to total ones was in the range from 0.2 to 0.3 throughout the experiments.

Table 1 shows the ratio of the insecticide into water-soluble conversion products by the formula of $c/c+w+r$ or $c/c+w$, at different intervals, where c and w are the radioactivities partitioned into chloroform and 10% aqueous trichloroacetic acid, respectively, and r is the unextractable radioactivity. In leaf blades, it took 3 days for this ratio became 0.5, and in leaf sheaths, it took 1 to 4 days. It was noticeable that the ratio in leaf sheaths was smaller than in leaf blades in most times. Although this tendency seemingly shows that degradation of Diazinon takes place more rapidly in leaf sheaths than in leaf blades, the fact that chloroform-soluble radioactive substances have increased in leaf blades during the experiments, suggests that translocation of

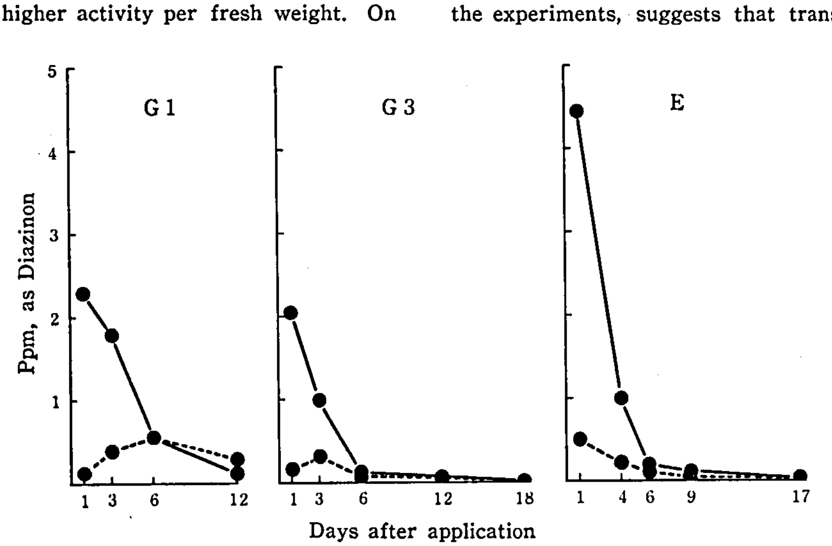

Fig. 1. Changes of chloroform- and aqueous trichloroacetic acid-soluble radioactive substances in paddy water after Diazinon application. Solid lines; chloroform soluble portion, and broken lines; aqueous trichloroacetic acid-soluble portion. G1; granule, 1.25 kg/ha, seedling, water depth 2 cm, G3; granule, 1.25 kg/ha, heading, water depth 2cm, and E; emulsion, 2.00 kg/ha, seedling, water depth 2 cm.

chloroform-soluble radioactive substances from leaf sheaths to leaf blades occured more readily than the supply of diazinon from roots and basal leaf sheaths. This trend is in agreement with the result obtained by Gunner *et al.*, (1966)[6], who found that Diazinon translocated rapidly in bean plants. Hirano and Yushima (1969)[5] have reported that hydrolysis of Diazinon in rice plants was

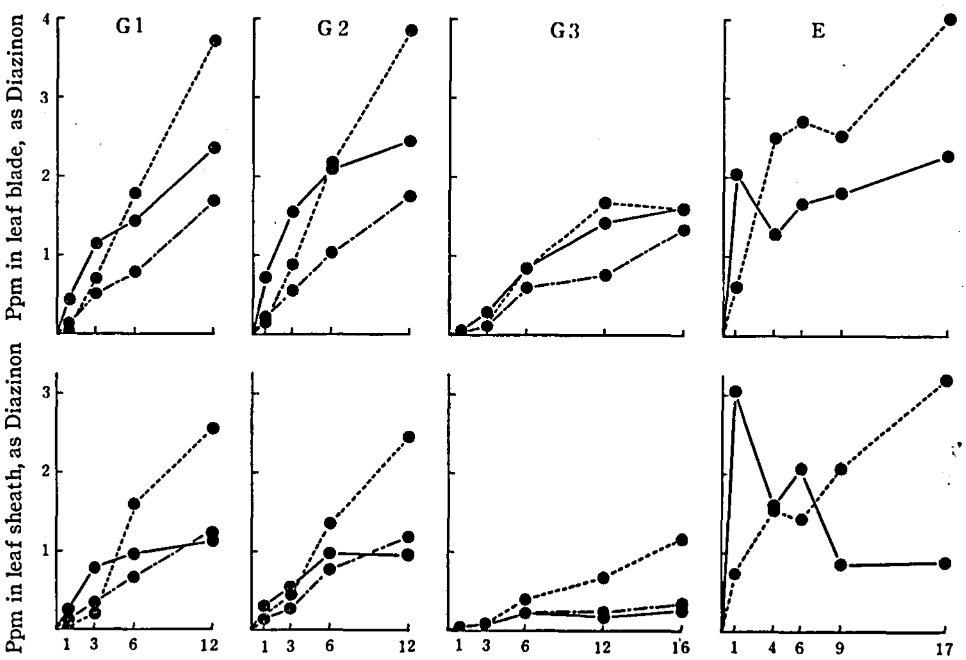

Fig. 2. Changes of chloroform- and aqueous trichloroacetic acid-soluble radioactive substances in plants after Diazinon application. Upper half; leaf blades, lower half; leaf sheaths, solid lines; chloroform-soluble portion, broken lines; aqueous trichloroacetic acid-soluble portion, and chain lines; unextractable portion. G2; granule, 1.25 kg/ha, seedling, water depth 0 cm, and the others are the same in Fig. 1.

Table 1. The ratio of radioactivity partitioned into chloroform to total activity (c/c+w+r* or c/c+w**) in rice plants applied with diazinon.

Diazinon formulation	Depth of paddy water (cm)	Growth stage of plant	Portion	Days after application				
				1	3(4)	6	9(12)	17(18)
Granule*	2	Seedling	Leaf blade	0.69	0.49	0.36	0.30	—
			Leaf sheath	0.67	0.60	0.30	0.23	—
Granule*	0	Seedling	Leaf blade	0.66	0.52	0.40	0.31	—
			Leaf sheath	0.51	0.43	0.31	0.21	—
Granule*	2	Pre-heading	Leaf blade	0.60	0.47	0.36	0.37	0.35
			Leaf sheath	0.60	0.35	0.26	0.16	0.14
Emulsion**	2	Seedling	Leaf blade	0.77	(0.34)	0.38	(0.42)	(0.37)
			Leaf sheath	0.81	(0.51)	0.59	(0.29)	(0.21)

C and w are radioactivities partitioned into chloroform and 10% aqueous trichloroacetic acid, respectively, and r is the unextractable activity.

more rapid in leaf sheaths than leaf blades. The result of present experiment was in disagreement with them. Absorption of Diazinon continued as far as the insecticide remains in paddy water or soil, and this trend differed from the result of Miles *et al.*, (1967)[9] who presumed that absorption of Diazinon by cabbage occures only during restoration of turgidity by the transplanted seedlings.

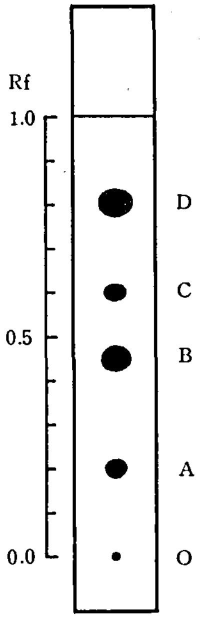

Fig. 3. Tracing of radioautogram of chloroform-soluble substances on TLC plate. Hexane: acetone (4:1) A; unidentified I, B; diazinon oxygen analog, C; unidentified II, and D; Diazinon.

Separation of chloroform-soluble radioactive metabolites in paddy waters and rice plants was carried out by paper or thin-layer chromatography. Fig. 3 shows the positions of the compounds on plates resolved by thin-layer chromatography. Percentage of each compound was calculated from the result of scanning of radioactivity in the radioautogram. Results are shown in Fig. 4. The major portion of chloroform-soluble radioactive substances consisted of Diazinon in paddy water during the experiments, and even at the end of experiments diazinon was more than 60% in both granular and emulsion applications. The remainder was Diazinon oxygen analog and an unidentified metabolite. In leaf blades of plants treated with Diazinon emulsion, chloroform-soluble radioactive substances consisted of diazinon, 30 to 50%, Diazinon oxygen analog, about 30%, and two unidentified metabolites at every sampling time. The constitution of metabolites in leaf sheaths was similar to that in leaf blades. Presence of

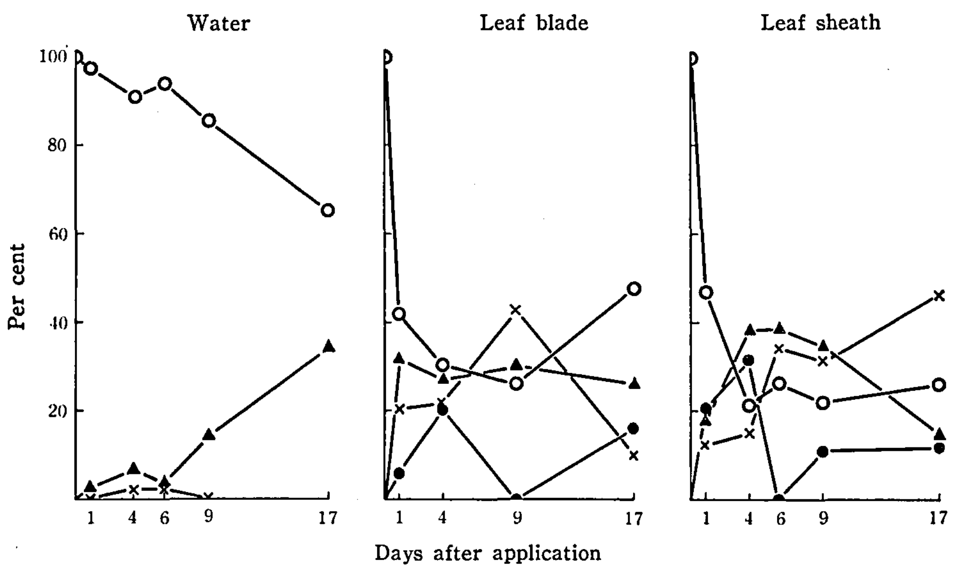

Fig. 4. Separation of chloroform-soluble radioactive substances in paddy water and plants after Diazinon application by TLC. Hollow circles; diazinon, solid circles; unidentified I, triangles; diazinon oxygen analog, and crosses; unidentified II.

Table 2. Separation of water-soluble radioactive substances of diazinon in plants applied with Diazinon granules by Dowex 1-X8.

Plant portion	Per cent of metabolites			
	$(HO)_3PO$ or $(HO)_3PS$	$(C_2H_5O)_2PO(OH)$	$(C_2H_5O)_2PS(OH)$	Unidentified
Leaf blade	7.2	50.4	31.1	11.4
Ear	2.0	85.9	9.8	2.3

a minor amount of Diazinon oxygen analog in field plants applied with Diazinon have been reported by Rall *et al.*, (1966)[12], and Eberle and Novak (1969)[13], but in the present experiment thin layer chromatograms show the presence of this metabolite at relatively high levels. Paper chromatographic separation conducted to the extracts from plants without cleanup was interfered intensively with plant pigments. From these results it is likely that Diazinon was less persistent in rice plants, though more persistent in the paddy water.

As the rice plants had begun to head one week after granular application, the ears in dough stage and leaf blades were sampled. After partition between the organic and apueous solutions, water-soluble metabolites were separated by ion exchange chromatography. As shown in Table 2, diethylphosphoric acid, diethylphosphorothioic acid, and an unidentified metabolite were detected in decreasing order in leaf blades, while diethylphosphoric acid was the major metabolite in the ears. Presence of diethylphosphorothioic acid in leaf blades at a fairly high level indicates that diazinon is decomposed primarily through hydrolysis to diethylphosphorothioic acid, followed by conversion to diethylphosphoric acid and inorganic phosphates.

Summary

^{32}P-labelled Diazinon granules and emulsion were applied onto pots in which rice plants were grown, and translocation and metabolism of the insecticide in aerial portion of the plants were examined by tracer techniques. Chloroform-soluble radioactive substances in paddy water disappeared more slowly in the pots applied with granules than those applied with emulsion. The amount of the chloroform-soluble portion in leaf blades of the plants increased continuously until the end of the experiments in the granular application, while that reached to a maximum within one day and remained unchanged during the experiments in the emulsion application. The chloroform-soluble portion was more concentrated in leaf blades than in leaf sheaths. The ratio, c/c+w or c/c+w+r was generally larger in leaf blades than in leaf sheaths, and it seemed that translocation of the chloroform-soluble portion from leaf sheaths to leaf blades was faster than that from paddy water to leaf sheaths. A major portion of chloroform-soluble radioactive substances was unchanged Diazinon in paddy water during the experiments. In the plants, Diazinon was decreased to about 50% of total chloroform-soluble substances in the first day, and maintained that level in leaf blades but decreased in leaf sheaths. Diazinon oxygen analog and two unidentified metabolites were detected in the plants. Water-soluble radioactive metabolites in leaf blades consisted of diethylphosphoric acid, diethylphosphorothioic acid, and phosphoric or phosphorothioic acid in decreasing order, while only diethylphosphoric acid was predominant in the ears.

Acknowledgement The authors are indebted to Dr. H. Suenaga, the former head of the First Environment Section of this Station, for the encouragement and to Nihon Kayaku Co., for suppling ^{32}P-labelled diazinon. Thanks are also due to Mr. T. Takehisa for his help in the experimental work.

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Entude sur l'Action Insecticide de l'Isobornyl Thiocyanoacétate I. Efficacité de l'Isobornyl Thiocyanoacétate contre la Mouche Domestique (*Musca domestica vicina* Macqu.). A. Hayashi et H. Yamaguchi Dépaltement d'Entomologie Appliquée Laboratoire Pharmaceutique Taisho (Toshimaku, Tokio, Japon) Reçu le 2 Octobre, 1970 *Botyu-Kagaku* 35, 140, 1970 (avec résumé Français)

20. Isobornyl thiocyanoacetateの殺虫作用に関する研究（第1報） Isobornyl thiocyanoacetate のイエバエ成虫に対する殺虫効果について.

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IBTA は低毒性で pyrethroids 系殺虫剤と同様に安全性が高く，高濃度で使用した場合はノックダウン剤として有効で，これに共力剤の混用により効力は増進した．ことに IBTA と S-421 の混用剤は優れた効力をしめし，衛生害虫を対象とした場合，実用的価値のある殺虫剤であることがわかった．

最近，いくつかの殺虫剤について多量の使用が人畜に悪影響をおよぼすことが明かにされつつある．このことは人畜に対し，より安全で残留性の少ない殺虫剤の開発の必要性を示唆するものである．しかし．にわかに新らしい殺虫剤の開発は困難で，その過程として，従来からあるものの再検討から始めるのが容易である．この目的にそうものとして Pyrethroids 系殺虫剤や Isobornyl thiocyanoacetate 剤が考えられる．本実験では本邦において，あまり検討されていなかった Isobornyl thiocyanoacetate の殺虫作用について実験を行い，知見を得たので報告する．

本文に入るに際し，種々御助言を賜った大阪府公衆衛生研究所の武衛和雄博士ならびに供試薬剤を提供いただいた日本樟脳株式会社の浅田四郎氏に謝意を表する．

実験材料および方法

供試薬剤；この実験にもちいた Isobornyl thiocyanoacetate（純度97.0%，以下IBTAとする）日本樟脳株式会社において製造されたもので，殺虫試験用サンプルとして提供をうけたものである。すでに，IBTA は1940年に Herculex Powder Co. ではじめて製造販売され，Thanite の名称で市販されている．本邦においても一時は使用されたが，広く普及するまでにいたらず，殺虫剤指針（厚生省，製薬課編，1965）[6]には共力剤として記載されている．

IBTA と効力比較のために用いた pyrethroids 系殺虫剤は pyrethrins（25% エキス），allethrin, phthalthrin, および chrythron の3種類で，いずれも工業用原体である．

pyrethroids の共力剤は S-421, Synepirin-500, MGK-264 および p. butoxide の4種類を用い，これらの pyrethroids との混用割合は1：1，1：5の組合せとした．供試薬剤は局所施用法の場合以外はエアゾール剤として用いた．なお，エアゾールの製剤条件は次にしめした如くである．

バルブ……………………………プレシジョン型
ノズル……………………………ストレート型
ノズル孔…………………………0.3mm
組成比（原液ケロシン/噴射剤フレオン）…20/80 V
内圧（30°C）………………………4.6kg/cm²
噴射量（25°C）……………………13.7g/30秒

供試昆虫：実験に用いたイエバエ *Musca domestica vicina* Macqu., 高槻系は当研究室において，幼虫期を豆腐粕培基で，成虫期を2%砂糖液で累代飼育中の