

- mol. Soc. Am. 46, 63 (1953)
- 13) O'Brien, R.D. : Can. J. Biochem. Physiol. 37, 1113 (1959)
- 14) Roan, C.C., Fernando, H.E. and Kearns C. W. : J. Econ. Entomol. 43, 319 (1950)
- 15) Robbins, W.E. and Dahm, P.A. : J. Agr. Food Chem. 3, 500 (1955)
- 16) Sato, T. and Tomizawa, C. : *Botyu-kagaku* 25, 85 (1960)
- 17) Shishido, T. and Suwanai, M. : J. Agr. Chem. Soc. Japan 32, 956 (1958)
- 18) Yushima, T. and Chino, H. : *Öyô-Kontyu* 9, 52 (1953)
- 19) Zeid, M.I., Dahm, P.A., Hein R.E. and McFarland, R.H. : J. Econ. Entomol. 46, 324 (1953)

Fate of Organophosphorus Insecticides Sprayed on Rice Plant. Chojiro TOMIZAWA, Toshiro SATO (National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo). Hiroo YAMASHINA*, Hiroshi KUBO** (Kyushu Agricultural Experiment Station, Chikugo, Fukuoka). Received June 11, 1960. *Botyu-Kagaku*, 25, 99, 1960 (in English)

18. 水稻に散布した有機燐殺虫剤の動態 富沢長次郎, 佐藤敏郎(農林省 農業技術研究所), 山科裕郎, 久保博司(農林省 九州農業試験場) 35. 6. 11. 受理

水稻に散布した ^{32}P ラベル付 malathion および ^{35}S ラベル付 methyl parathion の行動を追跡した。 ^{32}P ラベル付 malathion を動力噴霧機で散布し、散布後の空気中および水中の経時変化を測定した。水稻葉に附着した ^{32}P ラベル付 malathion の ^{32}P は葉面から直接蒸発するほか、葉組織内へ滲透した後、葉鞘、根を経て他の茎へ移行してゆく。一枚の葉では葉鞘から葉身へ向う傾向が認められる。 ^{35}S ラベル付 methyl parathion の水稻葉における行動は ^{32}P ラベル付 malathion に類似した傾向を示す。水稻葉組織内へ滲透した malathion および methyl parathion は極めて短時間に分散される。

Organophosphorus insecticides have been extensively used for the control of rice plant pests. Parathion and malathion are representative of organophosphorus insecticides against rice stem borer (*Chilo suppressalis* Walker) and green rice leafhopper (*Nephotettix bipunctatus cincticeps* Uhler), respectively. Many works on the application of organophosphorus insecticides in paddy fields have been carried out, but the results of these works do not always agree, because of the difficulty in examining the behavior of insecticides in minute amounts^{3,7,9}. For this purpose, the use of a radioactive insecticide will give more reliable results for the fate of insecticides than chemical analysis or bioassay. The present experiments were undertaken to examine the fate of radioactive malathion and methyl parathion under rice plant environment with particular reference to the insecticide in dynamic state.

Materials and Methods

Radioactive Insecticides : ^{32}P -labeled malathion was prepared according to the description of March

et al.,²⁾ starting from ^{32}P -labeled phosphoric acid (100mc). Radioactive red phosphorus was prepared by heating ^{32}P -labeled phosphoric acid with charcoal at 1000°⁴⁾. Chromatographic analysis showed that the radioactive portion was 96.5% malathion, with an initial relative activity, 3928 cpm.

The preparation of ^{35}S -labeled methyl parathion has been described previously⁶⁾. Chromatographic analysis showed that the radioactive portion was more than 99% methyl parathion, with an initial relative activity, 1385 cpm. ^{35}S -labeled methyl parathion was used on a small scale as compared with ^{32}P -labeled malathion. The methods employed for the investigation of radio-tracer preparations of the insecticides have been described in detail.⁶⁾

Paper Chromatography : The following solvent systems of paper chromatography were used for

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separation of malathion and degradation products: (1) filter paper uniformly deposited with silicic acid; mobile solvent, the upper phase from a mixture of hexane 10 parts, acetic acid 2 parts and water 1 part¹⁾; (2) filter paper impregnated with 2% silicone 550 in hexane; mobile solvent, isopropyl alcohol 10 parts, acetic acid 2 parts and water 10 parts. The system (2) was a modification of that used by March et al⁶⁾. The results were shown largely with chromatographs carried out with solvent system (1).

In the case of methyl parathion, filter paper impregnated with 5% silicone 550 in hexane was used with a solvent system of the upper phase from a mixture of ethyl alcohol 10 parts, chloroform 10 parts and water 6 parts³⁾. The ascending technique of paper chromatography was employed, using strips of Toyo filter paper No. 51, 300 mm in length and 2 mm in width. Radioautographs of paper chromatograms were prepared by exposing filter paper to X-ray film for an enough time to sensitize, and density scans of radioautographs were prepared by means of a densitometer.

Discussion of Results

Spraying with ^{32}P -labeled Malathion in Tent. For examining the behavior of malathion after spraying under the conditions which imitated those of a paddy field, the following experiment was carried out. The rice plants which were planted in pots and concrete containers, were kept in a tent which was covered with "vinyl-sheets". The vinyl-sheet which covered the top of the tent was removed after spraying. The tent had an area of 2.73 m $\times$ 1.94 m. Concentrated emulsion of malathion consisted of ^{32}P -labeled malathion 50 parts, emulsifier (Sorpul 213)** 25 parts and xylene 25 parts. A water emulsion which was prepared from diluting the concentrated emulsion of malathion and two thousand times of its weight of water was sprayed in the tent by means of a power sprayer with four nozzles, under a pressure of 30 pounds (Fig. 1). The diameter of each nozzle was 0.8 mm. Sprayed volume was 0.66 l and the amount of ^{32}P -labeled malathion was 165 mg. The dosage used in this experiment corresponded to about 108 l per 1/4 acre. The



Fig. 1. Spraying with ^{32}P -labeled malathion.

varieties of the rice plant used in the experiment were as follows: Norin No. 17 at ear-forming stage, Norin No. 18 at tillering stage, and Tomoemasari at heading stage. On spraying with ^{32}P -labeled malathion, the heads of Tomoemasari were covered with bags made of polyethylene film in order to avoid the deposition of ^{32}P -labeled malathion on the heads. The time required for spraying was 14 sec.

Time Course of Malathion Concentration in Air after Spraying. Immediately after spraying, the air within the tent was collected by means of a syringe which had a capacity of 50 cc. The air which was taken into the syringe was absorbed by 5 ml of methyl alcohol, and wet ashed with a mixture of concentrated sulfuric acid and nitric acid (5:1). Sampling was made at various intervals until 120 min. after spraying (Fig. 2). Malathion concentration in air decreased rapidly within 10 min. After 10 min. the concentration

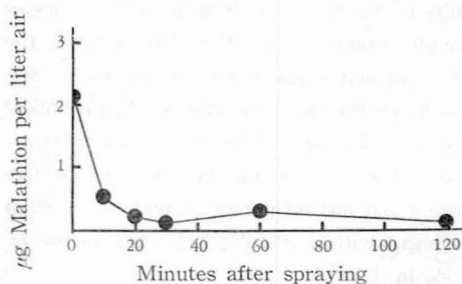


Fig. 2. Change of malathion concentration in air after spraying.

* Polyvinyl chloride plastics sheets.

** Manufactured by Toho Chemical Co., Ltd.

did not change so much. The residual activity in air may not be due to the mist of the malathion water emulsion, but to the vapor of malathion.

Time Course of Malathion Concentration in Water: It is reasonable to suppose that a greater part of malathion sprayed in a paddy field drops on the surface of irrigated water after spraying. It was reported by Yamashina⁹⁾ that the percentage of ethyl parathion deposited on rice plants in spraying ethyl parathion in a paddy field was very low. In the present experiment, the water of the concrete container in which the rice plants were planted, was collected at various intervals after spraying. Each container had an area of 45cm×45cm, and water depth was 5cm. In the similar manner to that of the preceding experiment, a portion of water was pipetted from the surface of water. As shown in Fig. 3, more than 95%

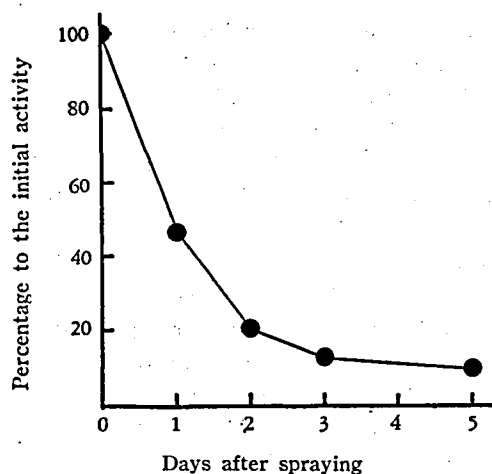


Fig. 3. Change of radioactivity in water after spraying.

of initial activity disappeared within two days. The malathion concentration just after spraying, was 4.3 μg per ml of water. As shown in Table 1, total amount of malathion which dropped on the surface of water was 1139 mg, but the amount of malathion used in the experiment was actually 165 mg. The discrepancy between both figures may be due to the fact that malathion was more concentrated near the surface of the water just after spraying. With the elapse of time, however, malathion may diffuse into deeper layers of water, and some parts of malathion may be absorbed

Table 1. Distribution of malathion in water just after spraying.

Total amount of malathion dropped on the surface of the water which had an area of 2.73m×1.94m and water depth 5cm, on the assumption that malathion distributed uniformly in water (calculated on the basis of 4.3 μg malathion per ml of water)	1139mg
Actual amount of malathion sprayed	165mg

also by soil particles under the water layer¹⁰⁾. As shown in Fig. 5, the result of paper chromatography carried out with water which was sampled just after spraying, showed that the radioactive portion consisted largely of malathion (δ).

Persistence of Radioactivity of Malathion and Methyl Parathion on and in Rice Plant: After spraying with ^{32}P -labeled malathion, a rice plant was pulled out from the pot, and the root was washed in running water. Whole plant was wet ashed with a mixture of concentrated sulfuric acid and nitric acid (5:1), and a precipitate of ammonium phosphomolybdate containing ^{32}P was obtained. The variety of the rice plant used in this experiment, was Norin No. 18 which had an average top height 41cm, average number of tillers 10 and average weight 10.8 g per plant. The changes of radioactivity on and in rice plants are shown in Fig. 4. The initial radioactivity corresponded to 47.5 μg malathion per g fresh weight.

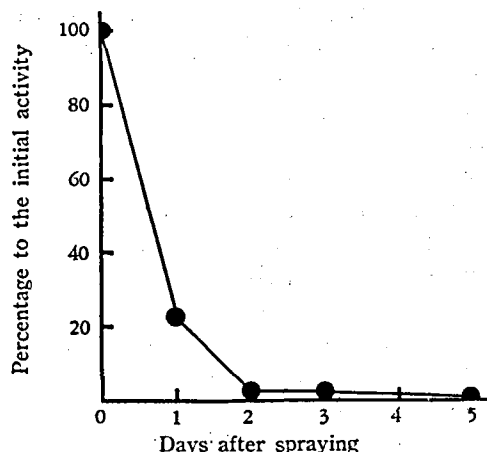


Fig. 4. Change of radioactivity on and in rice plant after spraying.

The decrease in percentage of radioactivity would be due to the translocation of radioactive material to the other parts of the plant, that is, roots and leaves unfolded after spraying, in addition to the evaporation of radioactive material from the leaf surface. The evaporation of methyl parathion and malathion which were left as residual film was very rapid⁶⁾. The rate of the translocation in the plant body was examined by using the variety Tomoemasari at heading stage. Two samplings were made in 2 days and 7 days after spraying. The sampled plants were divided into three portions: leaf blade, leaf sheath and heads. It was found that the radioactive material was translocated from leaf sheath to leaf blade and heads, as the data shown in Table 2. It seemed

Table 2. Translocation of radioactive material in the rice plant sprayed with ³²P-labeled malathion.*

After spraying	Leaf sheath	Leaf blade	Heads
2 days	0.426	0.026	0.029
7 days	0.143	0.344	0.458

* Figures show ppm of radioactive material expressed as malathion per tissue.

that in one leaf, radioactive material moved from the base to the top.

The experiment with ³⁵S-labeled methyl parathion was carried out on a small scale. The rice plant variety, Norin No. 1 at heading stage, which was planted in a pot, was sprayed with a water emulsion of ³⁵S-labeled methyl parathion. As in the case of the experiment with ³²P-labeled malathion, the heads of the plants were covered with bags made of polyethylene in order to avoid the deposition of the insecticide on heads. Concentrated emulsion of methyl parathion consisted of ³⁵S-labeled methyl parathion 45 parts, emulsifier (Sorpul 2020)* 30 parts and xylene 25 parts, and diluted with one thousand times of water just before spraying. 15 ml of the water emulsion was sprayed on three pots by means of a small glass sprayer. Each pot contained two hills of rice. After spraying, the pots were kept in a greenhouse at a temperature of about 30° and the samples for radioassay

Table 3. Translocation of radioactive material in the rice plant sprayed with ³⁵S-labeled methyl parathion.

After spraying	Leaf sheath	Leaf blade	Heads
1 hour		16.755	
2 hours		12.553	
1 day	5.616	3.787	
2 days	6.020	3.306	
3 days	2.024	5.850	
6 days	1.849	3.543	4.870
9 days	2.459	3.959	4.464
12 days		4.207	6.201

Figures show ppm of radioactive material expressed as methyl parathion per tissue.

were taken at various intervals. As shown in Table 3, the tendency of the rise and fall of radioactivity among leaf blade, leaf sheath, and heads was not so different from that of malathion, but a fluctuation in data which might be due to irregularity of the spraying condition was found. The fact that the amount of insecticide deposited on the rice plant was different between ³²P-labeled malathion and ³⁵S-labeled methyl parathion, would be depend on the difference in the spraying method and the dosage of insecticide.

Metabolism of Malathion and Methyl Parathion in Rice Plant: The degradation in rice plants was examined by paper chromatography with three varieties of the rice plant sprayed with ³²P-labeled malathion in the tent. Six hours after spraying, the surfaces of the leaves of Norin No. 17 were rinsed with a mixture of water 100 parts, benzol 0.5 part and Triton x-100 0.5 part, for eliminating the radioactive material deposited on the surface of the leaves. Rinsed leaves were macerated in a mortar, and methyl alcohol extract of the leaves was prepared. The result of paper chromatography carried out with the extract is shown in Fig. 5. Quite a small part of the radioactive material in the extract remained as malathion (δ), and the greater part of radioactive material was located at the origin of the paper chromatograph (α). A peak (γ) which appeared to correspond to dimethyl hydrogen phosphorodithioate, was also detected. One day after spraying, when the

* Manufactured by Toho Chemical Co., Ltd.

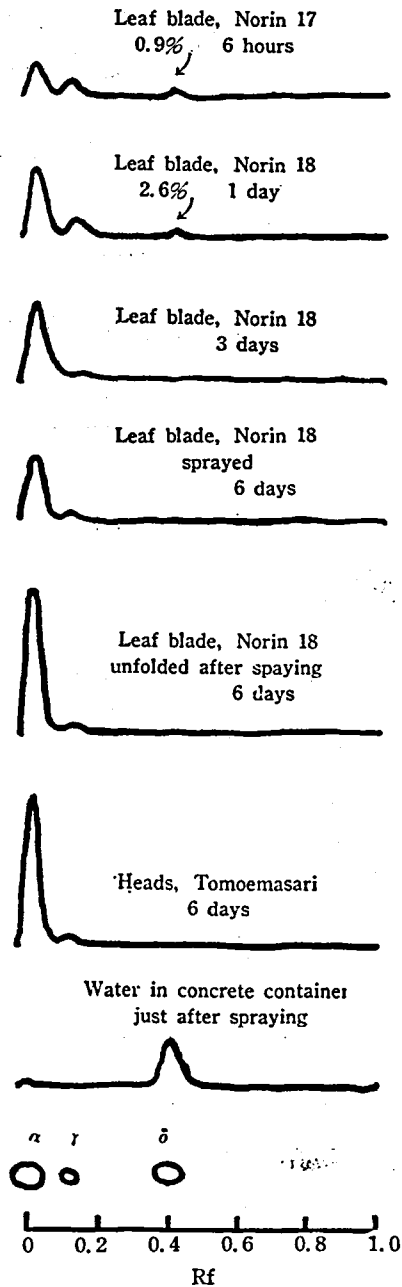


Fig. 5. Metabolism of malathion in the rice plant, based on density scans of radioautographs prepared from chromatograms of alcohol extracts of the tissues (Silicone paper).

surface of the leaves was not rinsed with a mixture of water, benzol and Triton x-100, the percentage of radioactivity which remained as malathion was high as compared with that of the

extract six hours after spraying. It was probably due to the malathion on the surface of the leaves which did not yet penetrate into the leaf tissues. No malathion was detected on and in the leaves three days after spraying. It was also found that the greater part of the radioactive material which was contained in the leaves unfolded after spraying was located at the origin of the paper chromatograph (α). The radioactive material which was located at the origin of the paper chromatograph was probably due to ionic metabolites. The distribution of the radioactive material contained in the heads was similar to that of the leaves. On the other hand, when 5 μ g of malathion were spotted on filter paper, and exposed to sunshine, the degradation of malathion was not so rapid, and even three days after the initiation of the experiment more than half of the radioactive material remained as malathion (Fig. 6). The peak (β) appeared to correspond to mala-oxon. Although malathion penetrated into the leaf tissues, it seemed that malathion rapidly degraded in the plant system.

The metabolism of methyl parathion was examined with the rice plant variety, Norin No. 1 which was used for estimating the persistence of radioactivity of methyl parathion on and in rice plants. Sampling was made at various intervals after spraying with ^{35}S -labeled methyl parathion. After dividing the rice plant into three portions of leaf blade, leaf sheath and heads, leaf sheath and heads macerated in a mortar, and macerates were extracted with acetone. Only in the case of leaf blade, radioactive material on the surface of the leaf blade was rinsed with a water emulsion consisting of water 100 parts, xylene 0.5 part and emulsifier (Sorpul 2020) 0.5 part, and then macerated. The result of paper chromatography was shown in Fig. 7. In leaf blade, the penetration of radioactive material into the leaf tissues was remarkably rapid. No methyl parathion was detected in the radioactive material which penetrated into the leaf tissues at any sampling time. On the other hand, the penetration of ethyl parathion into the leaf tissues was found by chemical analysis of parathion⁹⁾. The difference of degradation in rice plant between ethyl para-

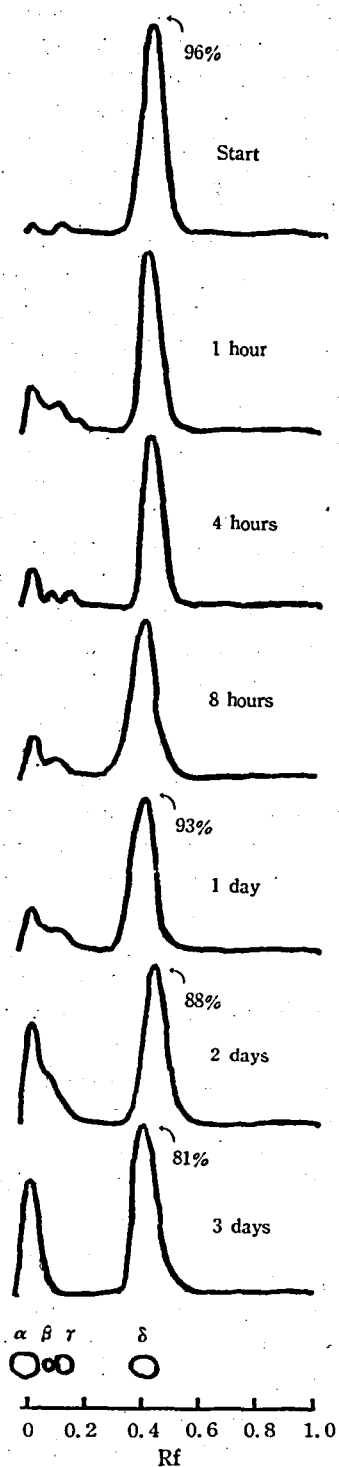


Fig. 6. Change of malathion exposed to sunshine, based on density scans of radioautographs of chromatograms (silica paper).

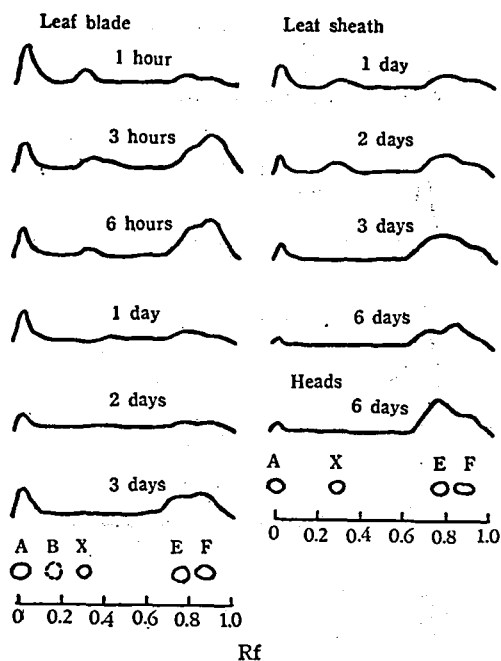


Fig. 7. Metabolism of methyl parathion in the rice plant based on density scans of radioautographs prepared from chromatograms of alcohol extract of the tissues (silicone paper).

thion and methyl parathion would depend on the susceptibility of the insecticides to hydrolysis. The chromatographic pattern of radioactive material in leaf sheath was similar to that of leaf blade. In both the cases of the blade and leaf sheath, spots E and F increased with time. Spot E appeared to correspond to dimethyl hydrogen phosphorothioate. The position of spot X on radioautograph corresponded to the Rf value of some material on paper chromatograph which is supposed to be degradation product(s) of chlorophyll. It was not, therefore, probable that spot X was a real degradation product of methyl parathion. It could be a material which was produced by a reaction between radioactive material and plant constituent before or after the preparation of the extract. Spot X disappeared with time. The chromatographic pattern of radioactive material in heads was similar to that of leaf sheath, but the ratio of each degradation product was different between leaf sheath and heads.

Summary

1. The behavior of ^{32}P -labeled malathion under

an environment surrounding rice plant was examined. Malathion concentration in air after spraying, decreased rapidly within ten minutes, and a small radioactivity which could be attributed largely to the vapor of malathion remained until two hours after spraying, when sampling ceased.

2. After spraying, a greater part of radioactivity of ^{32}P -labeled malathion could be located on the surface of the water in the concrete container in which the rice plants were planted. $4.3/\mu\text{g}$ per ml of water were attained just after spraying, which was the maximum concentration throughout the experiment. The malathion then diffused gradually into deeper layers of the water, and some parts of radioactive material appeared to be absorbed by soil particles under the water layer. More than 95% of radioactivity disappeared from the water within two days.

3. When ^{32}P -labeled malathion was sprayed on rice plant, the disappearance of radioactive material from rice plants was due to the translocation to the roots and leaves which unfolded spraying, in addition to the evaporation from leaf surface. After penetrating into leaf tissues, radioactive material was translocated from the leaf sheath to the leaf blade and heads. In spraying with ^{35}S -labeled methyl parathion, the tendency of translocation of radioactive material in rice plants was similar to that in ^{32}P -labeled malathion.

4. Although both ^{32}P -labeled malathion and ^{35}S -labeled methyl parathion penetrated into leaf tissues of the rice plant, they appeared to have a very short life in the plant body. A small amount of malathion was detected in the leaf tissues, six hours after spraying, while methyl parathion was not detected at any sampling time. The radioactive material which was translocated to the

unsprayed parts of the rice plant consisted only of degradation products both in the cases of malathion and methyl parathion.

Acknowledgement

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References

- 1) Asakawa, M. and Suwanai, M. : Japanese Appl. Entomol. Zool., 1, 67 (1957)
- 2) March, R.B., Metcalf, R.L., Fukuto, T.R. and Maxon, M.G. : J. Econ. Entomol., 48, 355 (1955)
- 3) Metcalf, R.L. and March, R.B. : Science, 117, 527 (1953)
- 4) Ooeterbaan, R.A. and Van Rotterdam, J. : J. Amer. Chem. Soc., 78, 5641 (1956)
- 5) Ozaki, K. : Bull. Natl. Inst. Agr. Sci., Series C, 4, 177 (1954)
- 6) Sato, T. and Tomizawa, C. : *Botyu-Kagaku*, 25, 85 (1960)
- 7) Suenaga, H. and Hashizume, B. : Bull. Kyushu Agr. Exptl. Sta., 1, 297 (1953)
- 8) Uezima, T., Hashizume, B. and Yamashina, H. : *Öyô-Kontyu* 9, 157 (1954)
- 9) Yamashina, H. : Summary on the studies of spraying agricultural chemicals, 8, Assoc. Plant Protection (1955)
- 10) Yamashina, H. and Uezima, T. : *Öyô-Kontyu* 12, 138 (1956)