原　著

Synergistic Attractancy of Cheese Components for Cheese Mites, *Tyrophagus putrescentiae*.

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1. チーズ成分の相乗作用によるケナガコナダニ誘引性. 芳沢宅実, 山本 出, 山本 亮 (東京農業大学農学部農芸化学科, 東京都世田谷区), 45. 11. 6. 受理.

チェダー・チーズ中のケナガコナダニ誘引因子として, heptan-2-one, octan-2-one, nonan-2-one および 8-nonen-2-one からなる主誘引因子の他に, 新たに 3-methylbutanol を同定した. 3-methylbutanol は単独でも活性を有するが, 上記のメチルケトン類と組合せると明瞭な相乗作用を示した. これらの誘引成分は, 乳製品のケナガコナダニ誘引性を決定する重要因子と考えられた.

The attractancy of cheddar cheese for the cheese mites, *Tyrophagus putrescentiae*, was not due to one component in the cheese, but to multicomponents. In the previous paper,[1] four methyl ketones, heptan-2-one, octan-2-one, nonan-2-one and 8-nonen-2-one, were reported as the cheese mite attractants in the main attractive fraction separated from the cheese. These ketones were individually inactive or less active, but their mixture showed a synergistic attractancy for the mites. We noticed also that their attractancy was increased by combining other components in the cheese. This paper describes the identification of 3-methylbutanol as another attractive factor in the cheese and its synergism with the above methyl ketones.

Experimental

Bioassay with an Odor Trap Tube Olfactometer (Fig. 1).

The cheese mites were reared on a dried yeast and starved for one day prior to the test. Two gathered pieces of filter paper (5×30mm) loaded with an amount of testing material were placed in two s-tubes (1 dia. ×6cm long), and two papers without any material were in the other two c-tubes. All tubes were arranged in a cruciform pattern in a petri dish (15cm dia.), with their open ends pointing toward the outside. A few thousand of the mites of all stages and both sexes were introduced in the center of the dish, which was then placed on a basin filled with water and held in the dark at 25±5°C for one hour. The mites which migrated to each tube were killed by adding hot water and then counted under a microscope after pouring the water on a filter paper. The degree of attractancy was expressed as follows:

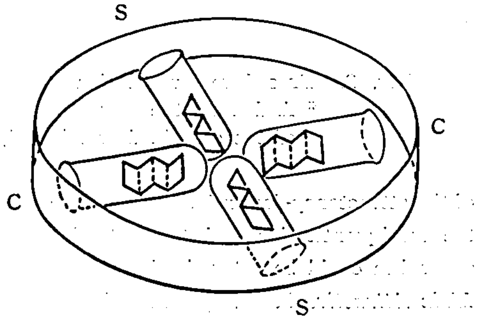

Fig. 1. Odor trap tube olfactometer for the cheese mite.
Each tube contains a filter paper loaded with or without a test material. The cheese mites were introduced in the center of the dish.

$$\text{Attractancy}(\%) = \frac{S}{S+C} \times 100$$

where S and C were numbers of trapped mites in s and c-tubes, respectively. Statistically, the attractancy over 65% was significantly associated with the presence of the attractant, but 70% or more was usually adopted as an indication of attractancy.

In order to establish the semiquantitation of a test material and the detection of its synergism, the above method was applied to a *cross test*, in which a standard material was placed into c-tubes, and the attractancy of a sample in s-tubes was expressed as indicated above. By an analysis

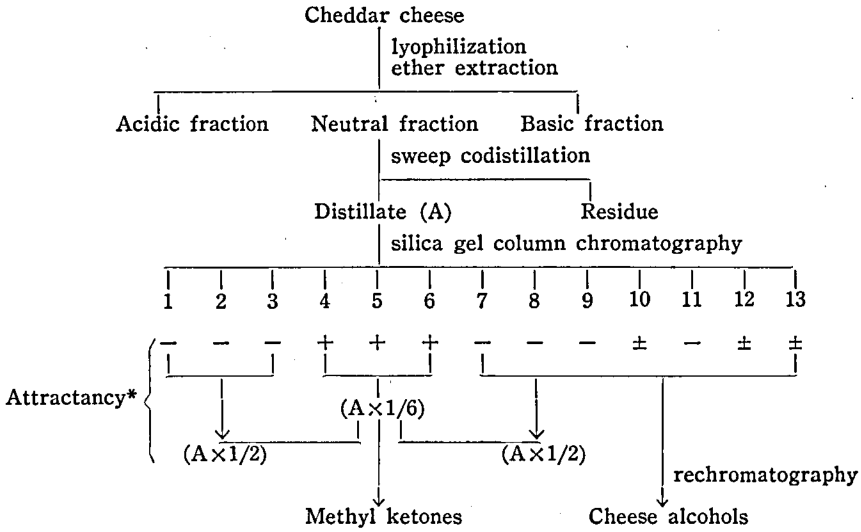

Fig. 2. Procedure for isolation of cheese mite attractants from cheese.
* A denotes the attractancy of distillate and A×1/6 denotes that the attractancy of the combined fractions 4, 5 and 6 was the one sixth of A.

of the attractancy-dose relationship on the sample, the semiquantitation and the detection of its synergism could be determined.

Main Attractive Factors for the Cheese Mites.

From the main attractive fraction in cheddar cheese (Figs. 2 and 3), heptan-2-one, octan-2-one, nonan-2-one and 8-nonen-2-one were identified as cheese mite attractants.[1] Among them, 8-nonen-2-one was found not only as a new component in cheese volatile but also the first case as a natural product, which was shown to be identical with the synthetic one by combined gaschromatography and mass-spectroscopy (Fig. 4). These methyl ketones were individually inactive or less

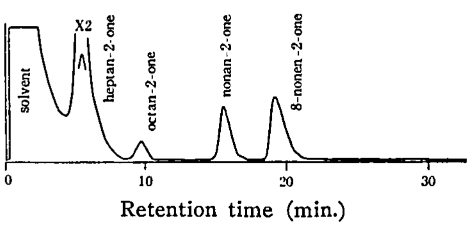

Fig. 3. Gas chromatogram of Fr. 6 in the main attractive fractions.
Other methyl ketones such as undecan-2-one and tridecan-2-one were also identified from the main attractive fractions, but they were not involved in the attractancy.

active, but their mixture was considerably more attractive (Table 1). Fig. 5 indicates that the methyl ketones showed a synergistic attractancy for the mites and that their mixture was almost comparable to the attractancy of 0.1g of cheese at the level of 1.0μg per tube.

Table 1. Attractancy of methyl ketones in cheese for cheese mites.

Methyl ketones	Attractancy (%/μg/tube)		
	0.1	1.0	10
heptan-2-one	44.2±10.1	54.2±7.9	48.0±13.2
octan-2-one	64.7± 4.3	66.3±6.9	66.2± 3.6
nonan-2-one	68.8± 5.1	60.8±5.8	65.3± 5.3
8-nonen-2-one	62.5±14.0	81.0±8.9	71.5±13.2
mixture*	71.1± 9.7	78.3±9.1	71.7±10.0

* Mixture of heptan-2-one, octan-2-one, nonan-2-one and 8-nonen-2-one in the ratio of 5:1:1:1.

Identification of Cheese Alcohols.

It was indicated that attractive factors other than the methyl ketones were included in alcoholic fractions (Frs. 7-13) and esteric fractions (Frs. 1-3) as shown in Fig. 2.

The eluates obtained with the polar solvent system of more than 20% ether in *n*-pentane from various runs were recombined to yield 365mg

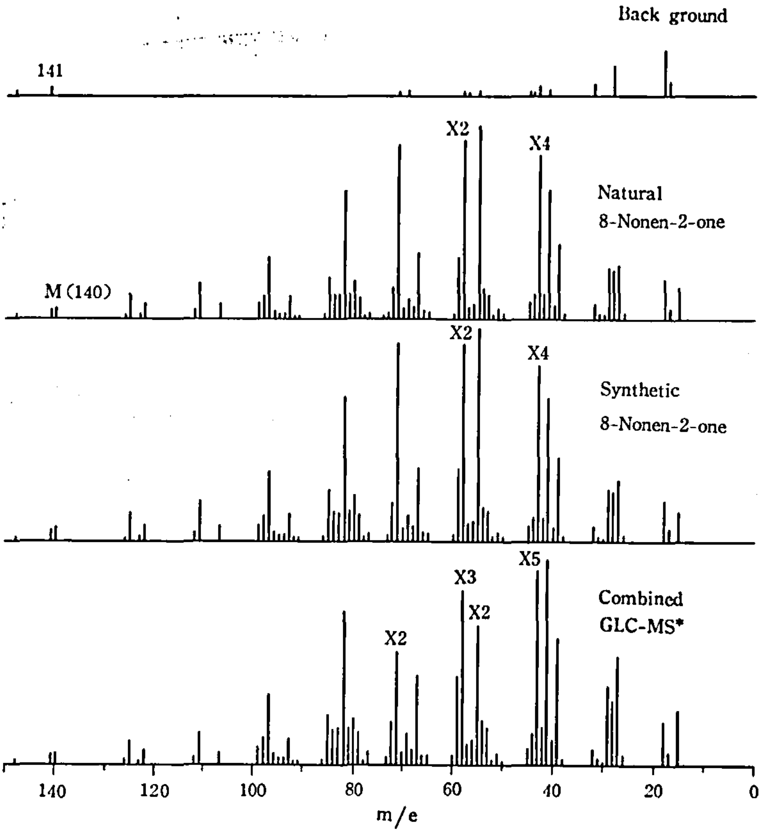

Fig. 4. Combined gas chromatography-mass spectrometry of 8-nonen-2-one as a new cheese volatile.

* A mixture of isolated and synthetic 8-nonen-2-ones gave a single peak of the retention time of 21.61 minutes.

GLC condition: Hitachi K-53 gas chromatograph, 0.5mm i.d. ×45m column packed with MBM, column temp.; isothermal at 270°C, carrier gas; He 1.0kg/cm^2 (inlet press.),

MS condition: Hitachi RMU mass spectrometer, ionizing volt; 70eV.

which was estimated to be from approximately 200kg of cheese (1.8×10^{-4}% of cheese). The eluate (330 mg) were subjected to a silica gel chromatography (60g in 3×23cm column), eluted successively with *n*-pentane (100m*l*), 30% ether in *n*-pentane (200m*l*), 50% ether in *n*-pentane (100m*l*), ether (200m*l*), acetone (100m*l*) and methanol (100m*l*), and separated into ten fractions. Almost all the materials were concentrated in fractions 6 and 7 which were the eluates with ether, and they showed slight attractancies.

Co-gas Chromatography: Fractions 6 and 7 were gas-chromatographed as shown Fig. 6. By the co-gas chromatography with authentic samples, the folloiwng alcohols were shown in each fraction (Table2); 2-pentanol, 3-methylbutanol, 2-heptanol and 2-nonanol in Fr. 6; 2-butanol, *n*-butanol, 2-pentanol, 3-methylbutanol, *n*-pentanol, 2-heptanol and n-hexanol in Fr. 7.

3, 5-Dinitrobenzoate Derivatives: The eluate in fraction 6 or 7 in one m*l* of dry pyridine was treated with approximately 100mg of 3,5-dinitrobenzoyl chloride at room temperature overnight and diluted with ether. From the ethereal solution, the 3,5-dinitrobenzoates were obtained by an ordinary method and gas-chromatographed (3mm i.d. ×2m column packed with 10% SE-30 on celite 545, isothermal at 250°C, flow rate of He gas;

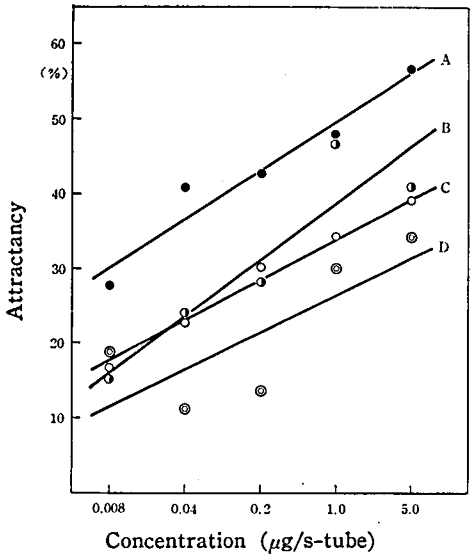

Fig. 5. Relative attractancy of methyl ketones to neutral volatiles of cheese.

A(●)*... Y=9.6 logA+49.7, a mixture of heptan-2-one, octan-2-one, nonan-2-one and 8-nonen-2-one in the ratio of 5:1:1:1.

B(◑)*... Y=10.9 logA+38.6, a mixture of heptan-2-one, octan-2-one and nonan-2-one in the ratio of 5:1:1.

C(○)*... Y=7.7 logA+33.8, a mixture of heptan-2-one, nonan-2-one and 8-nonen-2-one in the ratio of 5:1:1.

D(◎)*... Y=7.0 logA+26.3, a mixture of octan-2-one and 8-nonen-2-one in the ratio of 1:1.

* Each mark indicates an average of six repetitions. Cheese neutral volatiles at the concentration of 0.1g equivalent of cheese was placed in c-tube as a standard material.

37.5m*l*/min.). Components listed in Table 2 were detected by comparing the retention data with the authentic derivatives. Furthermore, individual peaks on the gas chromatogram were collected and cochromatographed on thin layer plates (Table 2). 3-Methylbutyl 3,5-dinitrobenzoate was identical with the authentic one on an infrared spectrum, and its mass spectrum showed diagnostic fragments of 3-methylbutyl group at m/e 282 (M^+), 267 ($M-CH_3$), 252 ($M-2CH_3$) and 239 ($M-CH(CH_3)_2$).

Attractancy of Identified Alcohols for the Cheese Mites.

Individual alcohol identified from cheese was diluted with 4-folds of liquid paraffin, and subjected to the attractivity test at concentrations of 0.1, 1.0 and 10μg per tube (Table 2).

3-Methylbutanol was the most attractive, and *n*-hexanol and 2-nonanol were slightly attractive. The mixture of the primary alcohols such as *n*-butanol, 3-methylbutanol, *n*-pentanol and *n*-hexanol in the ratio of 5:2:1:1 was prominently active, whereas the mixture of the secondary alcohols such as 2-pentanol, 2-heptanol and 2-nonanol in the ratio of 6:5:1 was only slightly attractive. The effect of 3-methylbutanol to other primary alcohols (the mixture of *n*-butanol, *n*-pentanol and *n*-hexanol in the ratio of 5:1:1) was examined by the cross test with the mixture of four primary alcohols in c-tubes as a standard material (Fig. 7). It was shown that the attractancy of 3-methylbutanol was not increased by combining it with other primary alcohols, indicating that the attractancy of alcoholic fractions could be represented with that of 3-methylbutanol.

Synergistic Attractancy between the Methyl Ketones and 3-Methylbutanol.

The synergistic increase of attractancy was observed by recombining the ketonic and the alcoholic fractions separated from cheese (Fig. 2), from which four methyl ketones and 3-methylbutanol, respectively, were identified as attractive factors. For further demonstration of the synergism between the methyl ketones and 3-methylbutanol, three samples: the mixture of heptan-2-one, octan-2-one, nonan-2-one and 8-nonen-2-one in the ratio of 5:1:1:1; 3-methylbutanol; and the mixture of the ketones and the alcohol in an equal ratio, were compared. Five μg of 3-methylbutanol was used as a standard material in c-tubes. Fig. 8 shows that the attractancy of the mixed methyl ketones was superior to that of 3-methylbutanol, and that the whole combination synergistically increased the attractancy.

Discussion

Though aliphatic ketones have been known to be alarm substances for some ants,[2-6] they have never been reported as a food attractant. The four methyl ketones identified as main attractive

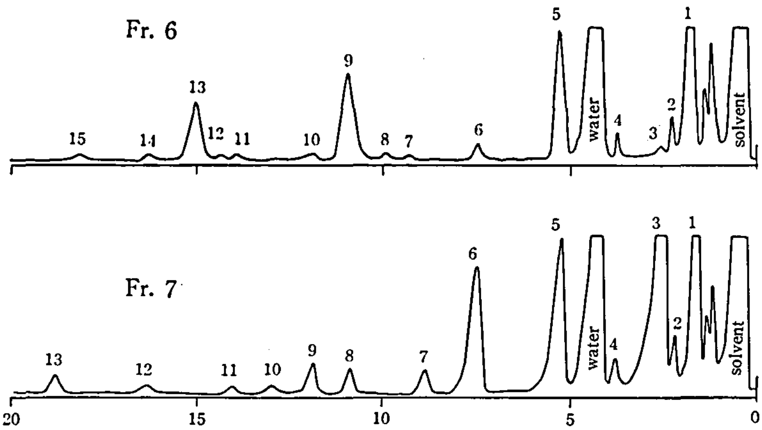

Fig. 6. Gas chromatograms of alcoholic fractions separated from cheese.
GLC condition: JGC-750 gas chromatograph (TCD detector), 3mm i. d. ×2m column packed with 20% PEG-20M on 60-80 mesh celite 545, column temp.; isothermal at 60°C for 5min., then temperature programmed 8°C/min. to 190°C, carrier gas; He 35ml/min., bridge current; 100mA.

Table 2. Identified cheese alcohols and their attractancies for cheese mites.

GLC Peak No. Fr. 6	GLC Peak No. Fr. 7	Identity	Retention time tR/tR(a)	3, 5-DNB GLC tR/tR(b)	3, 5-DNB TLC Rf(c)	Attractancy (%/μg/tube) 0.1	Attractancy (%/μg/tube) 1.0	Attractancy (%/μg/tube) 10
1	1	ethanol	0.230	0.562	0.689	51.2± 3.5	62.5±10.8	57.0± 5.6
—	3	2-butanol	0.338	0.730	1.111	55.2± 4.3	53.0± 7.3	56.7± 3.2
—	5	*n*-butanol	0.689	0.873	1.000	52.6± 5.9	54.5± 7.2	51.8± 6.7
5	5	2-pentanol	0.689	0.873	1.244	46.8± 6.3	53.8±12.0	57.3± 2.3
6	6	3-methylbutanol	1.000	1.000	1.132	61.4± 9.9	86.0± 8.2	95.7± 0.4
—	7	*n*-pentanol	1.210	1.120	1.122	68.7± 6.0	59.8± 6.3	64.2± 5.2
9	8	2-heptanol	1.487	1.389	1.400	64.3± 6.1	63.5± 9.2	61.8±10.3
—	9	*n*-hexanol	1.615	1.448	1.290	55.0± 1.4	55.7± 5.7	69.2± 8.4
13	—	2-nonanol	2.025	2.190	1.495	58.8± 7.2	63.5± 9.2	70.7± 4.6
—	—	mixture of 1-ols(d)	—	—	—	74.5± 5.8	88.2± 1.4	93.7± 3.6
—	—	mixture of 2-ols(e)	—	—	—	48.3±10.1	50.8±18.3	73.0± 4.6

(a): Relative retention time caluculated on basis tR/tR of 3-methylbutanol=1.000.
(b): Relative retention time caluculated on basis tR/tR of 3-methylbutyl 3,5-dinitrobenzoate=1.000 at 250°C of column temperature.
(c): Relative Rf-value caluculated on basis Rf/Rf of *n*-butyl 3,5-dinitrobenzoate=1.000, developer; *n*-hexane-AcOEt (9:1).
(d): Mixture of *n*-butanol, 3-methylbutanol, *n*-pentanol and *n*-hexanol in the ratio of 5:2:1:1.
(e): Mixture of 2-pentanol, 2-heptanol and 2-nonanol in the ratio of 6:5:1.

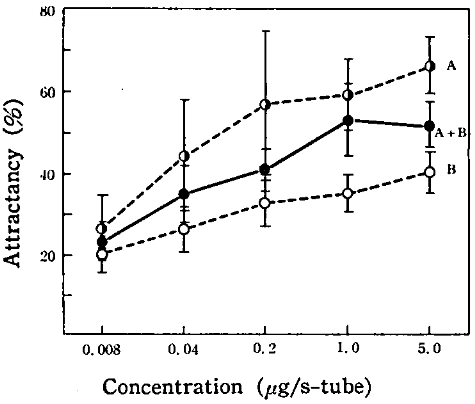

Fig. 7. Additive effect on attractancy of cheese alcohols.
A: 3-methylbutanol.
B: a mixture of *n*-butanol, *n*-pentanol and *n*-hexanol in the ratio of 5:1:1.
A+B: a mixture of *n*-butanol, 3-methylbutanol, *n*-pentanol and *n*-hexanol in the ratio of 5:2:1:1.
Five μg of the mixture of four alcohols (A+B) was used as a standard material in c-tube. Data from six repetitions.

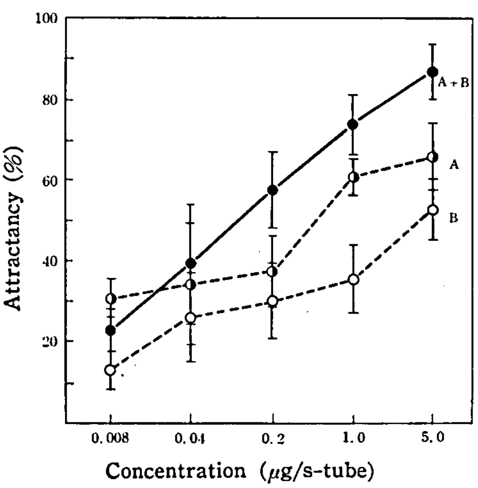

Fig. 8. Synergistic increase in attractancy from methyl ketones by 3-methylbutanol.
A: a mixture of heptan-2-one, octan-2-one, nonan-2-one and 8-nonen-2-one in the ratio of 5:1:1:1.
B: 3-methylbutanol.
A+B: a mixture of A and B in an equal ratio.
Five μg of 3-methylbutanol was used as a standard material in c-tube. Data from six repetitions.

principles did not individually show any significant attractancy except 8-nonen-2-one. Among synthetic isomers of 8-nonen-2-one (***trans*** and ***cis*-Δ3**, ***trans*-Δ5**, ***trans*-Δ6**, ***trans*** and ***cis*-Δ7**), only *cis*-3-nonen-2-one showed slight attractancy, but it was inferior to 8-nonen-2-one.[10]

Reactions of the neutral volatiles from the cheese with several chemical reagents resulted in a considerable decrease of attractancy, particularly by 3,5-dinitrobenzoylation.[7] This fact meant that the 3-methylbutanol had been removed from the attractive mixture by the reaction. The prominent attractancy of 3-methylbutanol was not observed in 2-methylbutanol, 2-pentanol and *n*-pentanol, but 4-methylpentanol was comparable to 3-methylbutanol. From these results, it is suggested that terminal hydroxyl and terminal branched methyl groups are required for the partial structure of alcohols in order to show their attractancies to the mites.

The definite quantitation of the synergism for these attractants was not obtained since the numbers of mites introduced to the test varied; however, the synergistic effect observed in cheese was apparently simulated in the synthetic mixture of heptan-2-one, octan-2-one, nonan-2-one, 8-nonen-2-one and 3-methylbutanol. In general, the methyl ketones and 3-methylbutanol in dairy products were fermentatively biosynthesized from fatty acids of milkfats and L-leucine, respectively.[8,9] This leads to a speculation that the level of these precursors in dairy products and the extent of their fermentative decompositions may be related to their attractancies for the mites.

The preliminary study on the relative attractancy of some dairy products indicated that the attractive substances in skim milk, dry whole milk, cheddar cheese and blue cheese were found to be concentrated in neutral volatiles, and that the relative attractancies were 15:43:100:156.[10] The slight activity of skim milk may be due largely to the lack of milkfats, and the existence of milkfats in dry whole milk reflected on its attractancy. A fermentative process promoted the production of 3-methylbutanol as well as the methyl ketones in case of cheese, particularly, blue cheese. These relationship suggests that the difference in the ratio of the methyl ketones and 3-methylbutanol in dairy products may be the determining factors of their attractancy for the mites, and that these attractants also may be one of the factors on the host selection of cheese mites.

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Summary

Besides methyl ketones, such as heptan-2-one, octan-2-one, nonan-2-one and 8-nonen-2-one, another attractive principle in cheddar cheese was identified. This was 3-methylbutanol, which showed the synergistic attractancy for the cheese mite, *Tyrophagus putrescentiae*, by mixing with the above methyl ketones. These components were considered to be important factors for the attractancy of dairy products to the cheese mite.

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Fate of Organophosphorus Insecticides in Soils. Part I. The Retention of ^{32}P-Labeled Disulfoton and Dimethoate in the three Soils. Ikuro Kawamori, Tetsuo Saito and Kisabu Iyatomi (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, Japan) Received December 19, 1970. *Botyu-Kagaku* 36, 7, 1971.

2. 有機燐殺虫剤の土壌施用に関する研究. 第1報 ^{32}P 標識 Disulfoton 及び Dimethoate の土壌における保持. 川森郁郎, 斎藤哲夫, 弥富喜三 (名古屋大学農学部害虫学教室, 名古屋市千種区不老町) 45. 12. 9. 受理.

^{32}P 標識 Disulfoton 及び Dimethoate の土壌による保持を沖積層土壌 (未耕地, 愛知県岡崎市), 頁岩風化土壌 (水田, 愛知県知多郡) 及び火山灰土壌 (畑地, 栃木県栃木農試) を用いて調べた.

土壌に施用した薬量と保持量との間には直線関係が見られ, Disulfoton は Dimethoate より多く土壌に保持された. また, 両薬剤に対する各土壌の保持量は火山灰土壌において最大であり, 頁岩風化土壌, 沖積層土壌の順であった. 保持薬量の多くが有機物の抽出方法で得られる画分に見い出され, 保持量は土壌の粘土含量よりむしろ有機物含量に密接な関連があるものと推論された.

薬剤と土壌構成物間の作用について薬剤の水溶性の相違から検討した.

Introduction

The application of systemic organophosphorus insecticides to soils offers an advantageous method of pest control because of their ecologically selective toxicities to the phytophagous insect pests and labour saving for their application.

Most studies on the fate of pesticides in soils have been confined to chlorinated hydrocarbon insecticides and organic herbicides, and there are few reports on organophosphorus insecticides, especially systemic organophosphates. Since it is