Fate of *O, O*-Dimethyl *O*-(3-methyl-4-methylmercaptophenyl) Thiophosphate Sprayed on Tea and Cabbage Leaves

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In the previous report (FUKUDA *et al.*, 1962), the metabolism of *O, O*-dimethyl *O*-(3-methyl-4-methylmercaptophenyl) thiophosphate (Baycid) on and in rice plants was examined, and much difference of the over-all metabolism in rice plants was found between Baycid and methyl parathion. The present experiments were undertaken to examine the behavior of Baycid on and in tea leaves and during the manufacturing process of green tea from tea leaves sprayed with radioactive Baycid. As a comparison, the metabolism of Baycid in cabbage leaves was also examined.

MATERIALS AND METHODS

Test insecticide: The emulsifiable concentrate consisted of ^{32}P -labeled Baycid 55 parts, xylene 20 parts, and emulsifier¹⁾ 25 parts. Radioactive Baycid was supplied through the courtesy of Nihon Tokushu Nôyaku Seizô Company from Dr. G. SCHRADER of Farbenfabriken-Bayer. An initial specific activity of ^{32}P -labeled Baycid was 3.4 mc per gram.

Application of radioactive Baycid to tea and cabbage: Tea and cabbage were cultured in pots which were kept in the tent for spraying the radioactive Baycid. Ten pots were used for tea and cabbage, respectively.

As shown in the previous report (FUKUDA *et al.*, 1962), the water emulsion of the insecticide was sprayed uniformly by means of a nozzle-boom. The top and side of the vinyl tent were remained to cover closely with vinyl sheet for about 1 hour after the appli-

cation of Baycid until the mist of the sprayed emulsion in the tent was completely fallen. The conditions of the application were as follows; test area, 5.4×1.8 meters, concentration of Baycid in water emulsion, 0.07%, sprayed volume, 12.5 liters per area, and the pressure of compressed air given to the nozzle-boom for spraying was 3.5 kg per cm^2 .

Sampling: At various intervals, younger and older leaves of tea were sampled separately, and cabbage leaves were also sampled at the same time. Samples were sliced finely by scissors, and the sliced samples were mixed uniformly in order to avoid the localization of radioactive materials in samples.

Manufacturing process of green tea: According to the usual manufacturing process of green tea, tea leaves were treated as follows, and the examination of the insecticide metabolites was made at each treatment.

i) Just after steaming (at 95°C) tea leaves for 25 seconds.

ii) Just after rubbing with hands at 55°C for 60 minutes, and drying at 70°C for 50 minutes.

Radioassay: An aliquot of sliced sample was wet ashed with a mixture of concentrated sulfuric acid and nitric acid (5:1). After combustion, a precipitate of ammonium phosphomolybdate containing ^{32}P was prepared by a conventional method, and radioactivity per gram fresh weight of sample was determined. On the other hand, an aliquot of sample was macerated with equal volume of chloroform

1) Sorpol, manufactured by Tôhō Chemical Co., Ltd.

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and 10 per cent trichloroacetic acid aqueous solution (included moisture of sample) in a waring blender, and then centrifuged. The radioactivity of chloroform and aqueous phases was determined per unit volume, and partition ratio of radioactive metabolites between chloroform and aqueous phases was obtained.

Separation of radioactive metabolites in tea and cabbage leaves: The chloroform solution after the partition between chloroform and trichloroacetic acid aqueous phases was concentrated in vacuo after drying over anhydrous sodium sulfate, and the metabolites in the concentrate were separated by the paper chromatography with the following solvent systems.

a) Stationary phase, filter paper impregnated with 50 per cent propylene glycol in ethanol; mobile phase, toluene 7 parts and hexane 3 parts by volume (BENJAMINI *et al.*, 1959).

b) Stationary phase, untreated filter paper; mobile phase, isopropanol 75 parts and concentrated ammonium hydroxide solution 25 parts by volume (PLAPP and CASIDA, 1958).

c) Stationary phase, filter paper impregnated with 5 per cent Silicone 550 in hexane; mobile phase, the upper phase from a mixture of ethanol 10 parts, chloroform 10 parts, and water 6 parts by volume (METCALF and MARCH, 1953).

System a) was mainly used, because of the better separation of Baycid metabolites on chromatograms. Two chromatograms were

made for each sample, and they were used respectively for making radioautogram of the paper chromatogram and for examination of the distribution of ^{32}P on paper chromatogram by radioassay.

RESULTS AND DISCUSSION

Change of radioactive materials on and in tea and cabbage leaves: The time of the disappearance of the radioactive materials deposited on tea and cabbage leaves was shown in Fig. 1. The result was expressed as percentage to the initial radioactivity after the application of ^{32}P -labeled Baycid. The initial deposit which was expressed as Baycid, was as follows; tea leaves, younger, 43.2 p.p.m., older, 90.1 p.p.m., and cabbage leaves, 46.4 p.p.m.

In Fig. 1, the decrease of the radioactive materials within 3 days after the application of Baycid seemed to be due to the translocation of the radioactive materials from the applied leaves to the other parts. Although the disappearance of the radioactive materials just after the application was not examined, the decrease of the radioactive materials by evaporation from leaf surface just after the application could not be neglected. It was found in the experiment with rice plants that the evaporation of Baycid from the leaf surface just after the application is rather rapid (FUKUDA *et al.*, 1962). Moreover, the shower of 11.2 mm. which came 3 hours after the application of Baycid might affect the disappearance of the radioactive materials. The difference in the behavior of Baycid which was observed between tea leaves and cabbage leaves, seemed to be dependent on the translocation rate of the insecticide metabolites in plants and on the property of leaf surface where the insecticide deposited.

The partition ratio of the radioactive metabolites in plants between chloroform and 10 per cent trichloroacetic acid aqueous solution was examined at each sampling time. The partition ratio, $(c/c+w) \times 100$, in which w shows radioactivity in aqueous phase and c in chloroform phase, was shown in Fig. 2. A little difference of the partition ratio was found between younger and older tea leaves.

Fig. 1. Disappearance of radioactive materials deposited on tea and cabbage leaves.

Fig. 2. Partition ratio of Baycid metabolites between chloroform and trichloroacetic acid aqueous solution.

The partition ratio in cabbage leaves lower and earlier than that in tea leaves. It seems that this fact tells the rapid penetration of Baycid into leaf tissues of cabbage.

Separation of chloroform extractable metabolites in plants: After the partition between chloroform and trichloroacetic acid aqueous solution, chloroform extractable metabolites were separated by the paper chromatography. Examination of ^{32}P -labeled Baycid by the paper chromatography with propylene glycol impregnated paper showed that 94.4 per cent of radioactive portion was PS-sulfide. The R_f values of Baycid (PS-sulfide) and its oxidation products were shown in the previous report (FUKUDA *et al.*, 1962). The change of the metabolites within 3 days after the application was not examined, but it was probable

that PS-sulfide disappeared more rapidly in cabbage leaves than in tea leaves (Fig. 3). Like the case of rice plants, the major part of the metabolites in the tea leaves consisted mainly of PS-sulfoxide and PS-sulfone, and the radioactivity in the spot located at R_f value of 0, was relatively low. As shown in the previous report (FUKUDA *et al.*, 1962), the derivatives of phosphate ester (PO-sulfide, PO-sulfoxide, and PO-sulfone) were located at value of 0 on chromatogram. The metabolic rate in tea leaves seemed to be more rapid in younger leaves than that in older ones. On the other hand, the feature of the metabolism in cabbage leaves was a little different from that in rice plants or tea leaves. In the cabbage leaves, the radioactivity in the spot located at R_f value of 0, was high in a similar level to those of PS-sulfoxide and PS-sulfone, and the metabolite which was regarded as S-methyl isomer, also appeared in a significant level. Although all of the hydrolysis products of Baycid were located at R_f value of 0, the radioactivity of the metabolites in cabbage leaves located at R_f value of 0 was too high to account for the amount of the hydrolysis products which partitioned in chloroform phase. This fact would show that the oxidation of thiophosphate to phosphate occurred more in cabbage leaves than in tea leaves.

Change of metabolites during the manufacturing process of green tea: The change of the metabolites during the manufacturing process of green tea from the tea leaves which were collected in 3 hours and 7 days after the

Fig. 3. Metabolism of Baycid on and in tea and cabbage leaves.

application was examined. The results were shown in Table 1. The decrease of the radioactive materials was found in the sample after 3 hours of the application, but scarce in the sample after 7 days. Probably the decrease of the radioactive materials in the sample after 3

Table 1. Change of the radioactive materials during the manufacturing process of green tea from tea leaves applied ^{32}P -labeled Baycid

Sample	3 hours after application			7 days after application		
	Residual ratio	$\frac{w}{c+w} \times 100$		Residual ratio	$\frac{w}{c+w} \times 100$	
Fresh leaves	100%	99.8%		100%	34.8%	
Steamed leaves	66.4	93.2		100	37.5	
Green tea	58.9	88.3		87.1	33.8	

Table 2. Paper chromatographic separation of chloroform extractable metabolites during the manufacturing process of green tea from tea leaves applied with ^{32}P -labeled Baycid.

Probable metabolites	Per cent ^{32}P 3			
	3 hours after application		7 days after application	
	(Fresh leaves)	Green tea	Fresh leaves	Green tea
PS-sulfide	(94.4)	10.0	—	—
PS-sulfoxide	—	33.7	56.5	53.0
PS-sulfone	—	56.3	30.3	23.5
S-methyl isomer ?	(3.6)	—	trace	—
Rf O	(2.0)	—	10.2	23.5

hours was due to the loss by the evaporation of the radioactive materials remained on the leaf surface. In the sample after 7 days, the radioactive materials which penetrated into the leaf tissues hardly decreased during the manufacturing process. The partition ratio of the metabolites between chloroform and 10 per cent trichloroacetic acid aqueous solution, showed small change during the manufacturing process. Chloroform extractable metabolites from green tea were separated by the paper chromatography, and the results were shown in Table 2. In Table 2, the percentage of the metabolites in ^{32}P -labeled Baycid was shown instead of the distribution of the metabolites in the fresh leaves after 3 hours of the application, because the sample 3 hours after the application was missed for analysis. In the sample 7 days after the application, the percentage of each metabolite was a little different between fresh leaves and green tea. During the manufacturing process, the level of PS-sulfoxide and PS-sulfone decreased, but the metabolites which were located at Rf value of 0, increased inversely. It was probable that the metabolites in the tea leaves hardly degraded after steaming process, because the hydrolytic enzymes which participate in the degradation of Baycid in leaf tissues were

inactivated by heating.

SUMMARY

The behavior of ^{32}P -labeled Baycid was compared between tea leaves and cabbage leaves, and the residue of Baycid or its metabolites in tea leaves before and after the manufacturing process of green tea was also examined.

1. It was probable that the penetration rate of Baycid into leaf tissues was more rapid in cabbage leaves than in tea leaves, and that the metabolic rate of Baycid was also rapid in cabbage leaves.

2. In tea leaves, PS-sulfoxide and PS-sulfone was detected as the main metabolites of Baycid and the appearance of the other chloroform extractable metabolites was a little, while in cabbage leaves, the distribution of the metabolites was rather different from that of tea leaves, and the appearance of the metabolite which suggested the oxidation of thiophosphate to phosphate was clear.

3. The absolute amount and the distribution of the metabolites in green tea were hardly changed before and after the manufacturing process of green tea.

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摘 要

茶ならびにカンランに散布した *O,O*-dimethyl *O*-(3-methyl-4-methylmercaptophenyl) thiophosphate の消長

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ポットに栽培した茶ならびにカンランに ^{32}P -Baycid 乳剤を散布し、その変化を追跡した。なお茶の場合には製茶過程における変化もあわせて調べた。

茶葉における新葉と古葉では、その変化はあまり大きな差が認められない。カンランでは茶葉に比較して葉面からの浸透速度も加水分解速度も早い傾向が認められた。茶葉における Baycid の酸化は PS-sulfide → PS-sulfoxide → PS-sulfone の方向へ大部分進み、thiophosphate

(PS-型) の phosphate (PO-型) への酸化は少なかったが、カンランにおいては PO 型酸化物が PS-sulfoxide, PS-sulfone と同程度検出され、更に Baycid の S-methyl 異性体と考えられる物質もかなり検出された。緑茶の製茶過程においては Baycid およびその代謝物質の絶対量も、また各代謝物質の割合もあまり変化が認められない。散布直後の茶葉では製茶過程における加熱により、葉面からの蒸発による減少が認められた。

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オオカバマダラの渡りについて

BROWER, L. P. (1961) Studies on the migration of the monarch butterfly. I. Breeding populations of *Danaus plexippus* and *D. gilippus berenice* in South Central Florida. Ecology 42(1): 76~83.

現在までオオカバマダラの周期的移動は魚に見いだされたものに似て、成虫は秋に南方に旅行し、繁殖することなく越冬し、春に北方に進行しながら産卵し、遂に死ぬと考えられてきた。これに対して著者は北米東南越冬地域におけるオオカバマダラ (*Danaus plexippus*; Monarch butterfly) の越冬状態および渡りについて再考察し、本種の冬季個体群とこれと生態的に近縁の *D. gilippus berenice* (Queen) との関係を知るために、南フロリダ南部の4地点で1956年春と1959年に調査を行なった。

越冬地域で Monarch が繁殖しないとの考えはカリホルニア海岸地域で越冬する多数個体の研究から導きださ

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れたものであるが、著者の研究によると3月下旬~6月中旬に南フロリダの中央部において広く繁殖が行なわれている。このことから Monarch は true migration (同一個体が地点を往復する) をするのではなく、それよりもむしろ remigration (移住した個体自身がもとの場所へ帰るのではなく、移住個体からうまれた個体が帰る) することになる。

そこで Monarch の渡りの根本原因は生態的に類似の Queen との種間競争に関するらしいという仮説をたてた。それには両種の成虫はともにとうわた (*Asclepias* spp.) の花に集まり、吸みつし、産卵し、幼虫がその植物で育つし、また Queen の雌は Monarch がいる時は吸みつ植物をさける傾向があるが、Monarch が北方へ飛び去った後すぐにそれに産卵するなどの明白な事実があるからである。したがって Monarch の消去は Queen の個体群増加と合致する結果となる。

(岐阜大農 福島 正三・金森 伸一)