

Mechanism of Dicofol Resistance in Spider Mites II: Thin Layer Chromatographic Identification of Dicofol Metabolites in Citrus Red Mite, *Panonychus citri* McGREGOR. Katsuhiko TABATA* and Tetsuo SAITO (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Chikusaku, Nagoya, Japan) Received May 17, 1973. *Botyunkagaku*, 38, 151, 1973.

23. ハダニの Dicofol 抵抗性の作用機構 (第2報), ミカンハダニ雌成虫における Dicofol 代謝物の TLC による同定 田畑勝洋*, 斎藤哲夫 (名古屋大学農学部害虫学教室, 名古屋市千種区不老町) 48. 5. 17 受理

^3H -dicofol (0.02 $\mu\text{g}/2\text{ ml}/1\text{頭}$) 処理の dicofol 感受性 (WSS) および抵抗性 (WRS) ミカンハダニ雌成虫を処理32時間後, クロロホルムと水 (1:1) を加えて, ホモジェネエートにし, これより得たクロロホルム抽出物および水溶性物について薄層クロマトグラフによってその同定を試みた. Dicofol 感受性および抵抗性ミカンハダニのクロロホルム抽出物は DDE, DBP およびその他の DDT 誘導物質ではなく, dicofol そのものであった. 一方, dicofol 抵抗性ミカンハダニにおいて, 体内へ浸透した ^3H -dicofol の約20%は水溶性物質として代謝されることはすでに述べたが, この水溶性物質はシリカゲル薄層クロマトグラフ D (Fig. 3 参照) において R_f 値 0.6~0.7 をもつ物質であり, DBH やその他の DDT 誘導物質の同溶媒系を用いたときの値とことなる R_f 値をもつ化合物であった.

本実験結果より dicofol 抵抗性ミカンハダニは dicofol を分解し, 水溶性物質に代謝することが主な解毒過程であると考えられる.

Introduction

Dicofol (Kelthane®), a hydroxy analog of DDT, is one of the most effective compound against phytophagous mites but not insect pests. While the use of dicofol has rapidly increased to control not only citrus red mite (*Panonychus citri* McGREGOR) but also other injurious mites, the dicofol resistance in *P. citri* McGREGOR presently has become a major problem on control in citrus orchards in Japan. However, little attention has been paid to studies on dicofol metabolism in mites, although these would be valuable in relation to investigations of the mite resistance.

Chlorinated hydrocarbon insecticides have been used extensively during the past two decades in controlling various insect pests. In relation to DDT and its related compounds, there have been a number of detailed studies of their metabolisms in insects. In many insects DDT-dehydrochlorinase, a glutathione dependent enzyme,

catalyzed the degradation of *p,p'*-DDT to *p,p'*-DDE^{1,2)}. An alternate biotransformation in *Drosophila melanogaster* gave rise to the hydroxy analog of DDT^{3,4,5)}. Similarly, when third-instar larvae of *Triatoma infestans* were topically applied with DDT-¹⁴C, two metabolites were detected, dicofol and a compound that behaved like 4,4'-dichlorobenzohydrol. The latter also appeared after treatment with dicofol⁶⁾. Furthermore, investigation showed that a kind of soil microorganism was capable of degrading DDT to a dicofol-like compound⁷⁾.

The previous study, therefore, sought to determine the fate of dicofol after topical application to both dicofol susceptible and resistant strains of *P. citri* McGREGOR in vivo⁸⁾. As a result, it was found that resistant strains have higher ability than susceptible strains to metabolize dicofol to the water soluble metabolites and that no significant difference in cuticle permeability between susceptible and resistant strains was found. From these results, the authors concluded that difference in dicofol metabolism is one of factors responsible for dicofol resistance.

Basing on the facts described above, tentative identifications of the chloroform extractable

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metabolites and the water soluble metabolites from dicofol susceptible and resistant mites applied topically with ^3H -dicofol were carried out by thin-layer chromatography technique.

Materials and Methods

Radio-labeled compound: ^3H -ring labeled dicofol was supplied by Sanyo trading Co., Ltd. via Loam and Haas Co., U.S.A. with a specific activity 0.96 mCi per mg and had a radiochemical purity of ca. 94% by thin-layer chromatography (tlc) using system B as shown in Table 1.

Table 1. Solvent components of thin-layer chromatography on silica gel F₂₅₄ precoated chromatoplates.

System	Development Solvent
A	<i>n</i> -heptane
B	<i>n</i> -heptane : acetone (98 : 2)
C	petroleum ether : ethyl ether : acetic acid (80 : 30 : 1)
D	<i>n</i> -butanol : acetic acid : water (4 : 1 : 2)

Mites: Dicofol susceptible strain (WSS) and resistant strain (WRS) of *P. citri* McGREGOR came from Wakayama citrus experiment station in 1969 were reared on citrus plant in green house at the Nagoya University for 3 years and adult female mites were used throughout this experiment.

WSS: A dicofol susceptible strain obtained from citrus orchards where dicofol has not been sprayed. The LD-50 by topical application was approximately 0.001 $\mu\text{g}/\text{mite}^{\text{a}}$.

WRS: Highly resistant to dicofol, originally collected from citrus orchards being sprayed twice per annum in 1965-1969. The topical LD-50 was approximately 0.366 $\mu\text{g}/\text{mite}^{\text{a}}$.

Treatment: Twenty $\text{m}\mu\text{g}$ of ^3H -dicofol in 2 $\text{m}\mu\text{l}$ of furfuryl alcohol solution applied on the idosoma of adult female of *P. citri* McGREGOR (WSS and WRS) and subsequently held on cover glass at 25°C for 32 hours in plastic cage with ca. 74% of relative humidity without food.

Radioassay: Tritium labeled compounds in *P. citri* McGREGOR topically applied with ^3H -dicofol were assayed by a liquid scintillation spectrometer (Aloka LSC-502). Two counting

formulations were used; solution A for counting the water soluble metabolites, consisting of 6.0 grams of PPO (2,5-diphenyloxazole), 112 grams of naphthalene, 270 mg of POPOP (1,4-bis-2-(5-diphenyloxazolyl)-benzene), and enough reagent grade dioxane to bring the volume to 1 liter and solution B for counting the chloroform extractable metabolites, consisting of 4 grams of PPO and 5mg of POPOP per liter of reagent grade toluene. All counts were corrected for quenching (external standard) and background.

Extraction of radioactive materials from treated mites: Thirty two hours after topical application with ^3H -dicofol (0.02 $\mu\text{g}/2 \text{ m}\mu\text{l}/\text{mite}$), 300 mites were washed on their cuticle with *n*-hexane twice. The mites were transferred to 10 ml of centrifuge tube and 2 ml of extraction solvent, chloroform-water (1 to 1), was added and homogenized by the grass bar. The homogenate was partitioned three times with equal volume (1 ml) of chloroform, which yielded the chloroform extractable metabolites and water soluble metabolites (Figure 1). The radioactivities of the chloroform extractable metabolites from

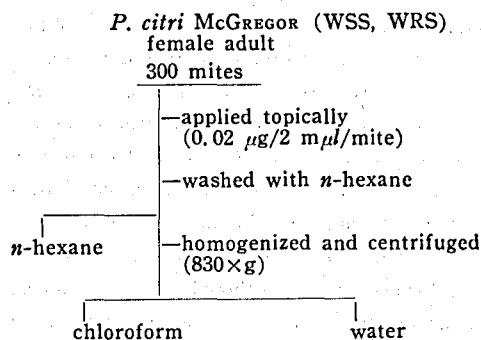


Fig. 1. Flow diagram for the standard extraction procedure on the female adult of citrus red mite applied topically with ^3H -dicofol.

dicofol susceptible and resistant mites and the water soluble metabolites from dicofol resistant mites were ca. 18,500, 17,000 and 1,630 cpm per ml respectively.

Chromatography: The chloroform extractable metabolites and water soluble metabolites isolated as described above were chromatographed in solvent system A, B, C and D given in Table 1.

The chromatographic techniques were carried out on the thickness of 0.25 mm plates (20×20 cm) of the silica gel with a fluorescent indicator (Silica gel F₂₅₄, Merck) using the solvent systems described in Table 1. The plates were activated at 110°C for one hour and then cooled to the room temperature prior to use. Tentative identifications were based on comparisons of the chromatographic behaviour with that authentic reference compounds. The positions of the ra-

dioactivity were compared with the positions of the reference spots detected by ultra-violet light. The areas containing the radioactivity were scraped and counted by the scintillation spectrometer.

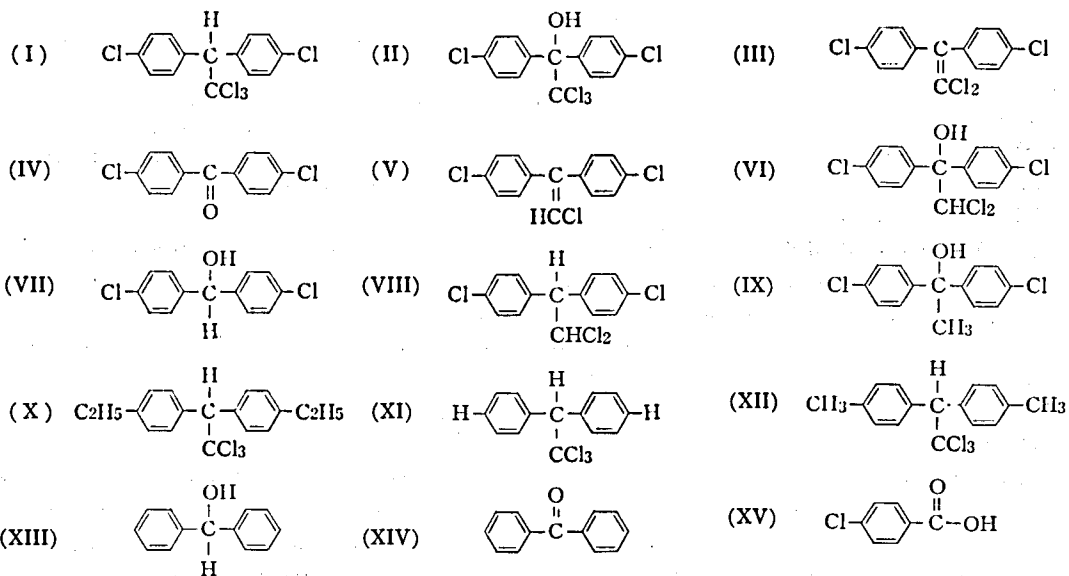
Results and Discussion

In previous experiments with the fate of topically applied ³H-dicofol in citrus red mite, dicofol was absorbed rapidly and was not

Table 2. R_F-Values of dicofol and its related compounds.

Compound		Solvent system			
		system A	system B	system C	system D
<i>p,p'</i> -DDT	(I)	0.35-0.42	0.72-0.74	0.82-0.87	0.91-0.95
Dicofol (DCF)	(II)	0.03-0.09	0.47-0.52	0.81-0.86	0.91-0.95
DDE	(III)	0.57-0.63	0.84-0.88	0.80-0.92	0.93-0.95
DBP	(IV)	0.01-0.05	0.62-0.68	0.82-0.87	0.90-0.94
DDMV	(V)	0.51-0.56	0.82-0.87	0.82-0.87	0.93-0.95
FW-152	(VI)	0.01-0.03	0.29-0.33	0.73-0.78	0.91-0.95
DBH	(VII)	0.00-0.01	0.08-0.12	0.48-0.54	0.92-0.95
DDD	(VIII)	0.21-0.25	0.64-0.68	0.80-0.85	0.90-0.94
DMC	(IX)	0.03-0.07	0.55-0.63	0.85-0.90	0.87-0.95
C ₂ H ₅ -DDT	(X)	0.15-0.20	0.79-0.83	0.83-0.92	0.92-0.95
H-DDT	(XI)	0.28-0.32	0.74-0.80	0.83-0.88	0.92-0.95
CH ₃ -DDT	(XII)	0.33-0.40	0.75-0.80	0.85-0.90	0.91-0.95
Bh	(XIII)	0.00-0.02	0.03-0.07	0.53-0.57	0.83-0.91
Bp	(XIV)	0.00-0.04	0.30-0.36	0.70-0.73	0.85-0.95
CBA	(XV)	0.00-0.00	0.00-0.02	0.39-0.43	0.83-0.93

Formula



extensively degraded, that is, about 40 to 50% of treated dicofol was penetrated within 32 hours after topical application in WSS and WRS strains. Approximately 20% of absorbed ^3H -dicofol was found as water soluble metabolites in WRS but only small amount in WSS, which was considered as one of the factors responsible for resistance⁹⁾.

The thin-layer chromatographic identifications of dicofol metabolites from the resistant and susceptible of *P. citri* McGREGOR topically applied with ^3H -dicofol (0.02 $\mu\text{g}/2\text{ m}\mu\text{l}/\text{mite}$) were investigated by means of the extraction procedure as shown in Figure 1.

The chromatographic behaviours of dicofol and its related compounds in several solvent systems are shown in Table 2. The *n*-heptane-acetone system (system B) was useful for separating dicofol and 4,4'-dichlorobenzophenone (DBP), and *n*-butanol-acetic acid-water system (system D) was used principally to separate the water soluble metabolites.

Only one internal metabolite was detected in the chloroform extract of the mites intoxicated with ^3H -dicofol by the thin-layer chromatography. This compound has the same chromatographic mobility as authentic dicofol having R_f 0.03-0.06 on tlc system A, R_f 0.47-0.52 on tlc system B, R_f 0.81-0.86 on tlc system C, and R_f 0.91-0.95 on tlc system D. Therefore, it should be noticed that more than ca. 85% of ^3H -dicofol absorbed in WSS and ca. 30% in WRS were found as internal radioactivity after 32 hours and were not metabolized. It is well known that DDT is dehydrochlorinated by DDT-dehydrochlorinase to yield DDE in the insect body^{1,2)}. DDT is also converted to dicofol, an alcoholic type compound, and DBP, a ketonic compound of DDT derivatives, were discovered in several species of insects^{3,4,5,10)}. Dicofol has been known to decompose to DBP and chloroform under alkaline condition. However, the formation of DBP and/or DDE from WSS and WRS applied topically with ^3H -dicofol may not occur at least in vivo. The small amounts of activity found at R_f 0.57-0.63 on tlc system A, R_f 0.84-0.88 on tlc system B, R_f 0.90-0.92 on tlc system C and R_f 0.93-0.95 on tlc system D could be accounted

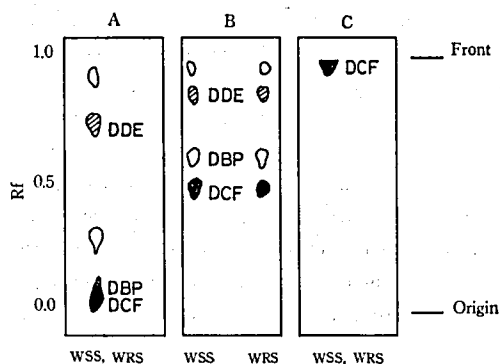


Fig. 2. Cochromatograms of the chloroform extractable metabolites in *P. citri* McGREGOR.

WSS : dicofol susceptible strain.

WRS : dicofol resistant strain.

Black spot show the strongest radioactivity, shaded spot weak radioactivity. Detector : UV light.

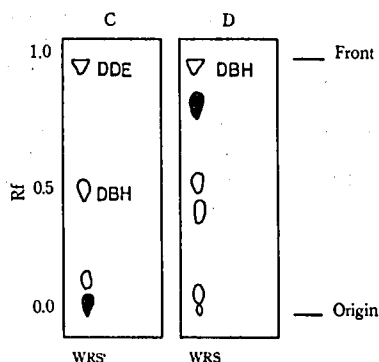


Fig. 3. Cochromatograms of the water soluble metabolites in *P. citri* McGREGOR.

WRS : dicofol resistant strain.

Black spot show the strongest radioactivity, shaded spot weak radioactivity. Detector : UV light.

for in terms of trace quantities of radioactive impurities present in the original ^3H -dicofol, although this compound has the same R_f value of DDE. Further proof was obtained by comparing the R_f values of the chloroform extractable metabolites from WSS and WRS, with ^3H -dicofol, nonlabeled dicofol and DDE.

The large amount of ^3H -dicofol is metabolized to form the water soluble metabolites in WRS. This compound was cochromatographed identically with the authentic compounds in solvent

system C and D as shown in Figure 3. The thin-layer chromatography on silica gel F₂₅₄ plates gave a compound with R_f value of 0.00-0.07 on tlc system C and also 0.6-0.7 on tlc system D. This R_f value does not correspond to that obtained with 4,4'-dichlorobenzohydrol (DBH) having R_f 0.48-0.54 on tlc system C and R_f 0.92-0.95 on tlc system D and/or other authentic compounds using in this study, although DBH appeared after topical application with dicofol-¹⁴C to third-instar larvae of *Triatoma infestans*⁶⁾. Therefore, the results of this study indicated hydrolytic detoxication of dicofol in WRS was a major pathway, although the chemical nature of this compound is not known.

Summary

- 1) Tentative identifications of the chloroform extractable metabolites and the water soluble metabolites from dicofol susceptible (WSS) and resistant (WRS) mites applied topically with ³H-dicofol (0.02 µg/2 mµl/mite) were carried out by the thin-layer chromatography using several solvent systems.
- 2) As indicated by the thin-layer chromatography of the chloroform extractable metabolites from both dicofol susceptible and resistant mites, they were not DDE, DBP and/or other related authentic compounds but dicofol itself.
- 3) Approximately 20% of ³H-dicofol absorbed are metabolized to form the water soluble metabolite(s) in the dicofol resistant mites. This compound has 0.6-0.7 of R_f value on tlc system

D but this R_f value does not correspond to DBH or other authentic compounds used in this experiment.

- 4) The results of this study indicated hydrolytic detoxication of dicofol in resistant mites was a major pathway, although the chemical nature of the water soluble metabolite is not known.

Acknowledgments: The authors deeply indebted to Dr. K. Iyatomi and Dr. T. Miyata for their valuable advice and encouragement. The authors are also grateful to Mr. H. Honda for rearing the mites and to Sanyo trading Co., Ltd. for supplying the authentic dicofol used in this study. Thanks are due to Roam and Haas Co., U. S. A. for supplying ³H-dicofol.

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The Resistant Level of the Housefly to Several Insecticides in Sapporo City and Isolated Islands in Hokkaido. Akifumi HAYASHI and Megumi HASEGAWA* (Laboratory of Applied Entomology, Taisho Pharmaceutical Co., Ltd. Hokkaido Institute of Public Health.*) Received April 9, 1973. *Botyu-Kagaku*, 38, 155, 1973. (with English Summary 157)

24. 札幌市内と北海道離島におけるイエバエの殺虫剤感受性について 林 晃史, 長谷川 恩* (大正製薬株式会社防虫科学研究室, 北海道立衛生研究所*) 48. 4. 9 受理

札幌市内のイエバエの殺虫剤感受性を詳細に検討した結果, malathion に対して強い抵抗性を持つことが確認された。また, 北海道内周辺の島々についても同様なことが観察され, 今後, イエバエの駆除には DDVP が適切ではないかと考えられる。

林ら (1971)^{1,2)} は北海道内におけるイエバエの各種殺虫剤に対する感受性について調査し, 札幌系が malathion に対して非常に強い抵抗性を持つことを

明らかにした。今回はこのような現象が市内全般にみられるか否かについて知る目的で, 市内を区分して採集し, 実験を行ない知見を得たので報告する。また,