Original Article

Translocation and Metabolism of Procymidone in Cucumber and Bean Plants

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Translocation and metabolism of a systemic fungicide procymidone [Sumilex®, *N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide] radiolabeled at the carbonyl carbon were examined in cucumber and bean plants under laboratory conditions. The fungicide disappeared from the plants with a half-life of 20 days, after foliar application at a rate of 250 µg per leaf. Procymidone was metabolized in plants via hydroxylation at the methyl group or the 4-position of phenyl group, or hydrolysis at the cyclic imide linkage. Very little of radioactivity translocated to other parts of the plants including edible portions. When the fungicide was applied to the surface of a cucumber fruit, approximately 24% of the applied radioactivity penetrated into the fruit in a day. However, there was no significant increase of radioactivity in the fruit thereafter. When bean plant seedlings were cultivated for 42 days in Kodaira light clay and Takarazuka loamy sand soils treated with 10 ppm of ¹⁴C-procymidone, shoots, and pods and seeds contained 12.3–15.3 ppm and 0.42–0.66 ppm of ¹⁴C, respectively. Preincubation of procymidone treated soils for up to 5 months significantly lowered ¹⁴C residues taken up in plants, especially in shoots and pods and seeds.

INTRODUCTION

Procymidone [Sumilex®, *N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide] is a systemic fungicide for the control of plant diseases such as gray mould of grape and vegetable crops, and Sclerotinia rot of kidney beans and vegetable crops.^{1–3)} From the point of environmental safety, study on metabolism of procymidone in rats had already been performed and it was shown that the fungicide was biodegradable and not persistent in animal bodies even after repeated oral administration.⁴⁾

This report is concerned with the translocation and metabolism of procymidone in cucumber and kidney bean plants after leaf, fruit or root treatment of ¹⁴C preparation labeled at the carbonyl group.

MATERIALS AND METHODS

1. Chemicals

Procymidone labeled with ¹⁴C at the carbonyl group was synthesized by Yoshitake *et al.*⁵⁾ in Takarazuka Research Center of Sumitomo Chemical Co., Ltd. The specific activity was 4.67 mCi/mmol with more than 99.9% of radiochemical purity as determined by thin layer chromatography (TLC), radioautography and liquid scintillation counting. Unlabeled procymidone and the following compounds were prepared for the reference⁶⁾: *N*-(4-hydroxy-3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide [referred to P-4-OH], 2-(3,5-dichlorophenylcarbonyl)-1,2-dimethylcyclopropane-1-carboxylic acid [P-NH-COOH], *N*-(3,5-dichlorophenyl)-1-hydroxymethyl-2-methylcyclopropane-1,2-dicarboximide [P-CH₂OH], *N*-(3-chlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide [P-3-Cl] and 1,2-dimethyl-

Table 1 Thin-layer chromatographic properties of procymidone and its metabolites.

Compound	R _f values with indicated solvent systems ^{a)}		
	A	B	C
Procymidone	0.58	0.41	0.55
P-3-Cl	0.54	0.36	0.34
P-4-OH	0.42	0.22	0.26
P-NH-COOH	0.08	0.14	0.17
P-CH ₂ OH	0.17	0.18	0.14
CP-(COOH) ₂	0.01	0.04	0.12

^{a)} A: chloroform/ethyl acetate (4/1), B: *n*-hexane/acetone (5/2), C: toluene/acetic acid (7/1).

cyclopropane-1,2-dicarboxylic acid [CP-(COOH)₂].

2. Radioassay

Liquid scintillation counting (LSC), combustion analysis and radioautography were carried out according to the methods reported previously.⁷⁾

3. Thin Layer Chromatography

Precoated silica gel 60F-254 chromatoplates (20×20 cm, 0.25 mm layer thickness, E. Merck) were used for an analytical purpose. The R_f values for procymidone and its metabolites are listed in Table 1. The radioactive compounds were detected by autoradiography and unlabeled compounds were detected by UV light or by exposure to iodine vapor.

4. Foliar Treatment of Cucumber and Bean Plants

A six-week old cucumber plant (*Cucumis sativus* L. cv Kurumeochiai H-type), bearing two to three fruits per plant, in Wagner pots (26.5 cm i.d. ×30 cm height) with soil, and five-week old kidney bean plants (*Phaseolus vulgaris* L.) in the stage of flowering were selected in the experiment. These plants were held in a greenhouse at 25±2°C with a relative humidity of 40 to 80% before or after treatment. The weight of cucumber fruits was ca. 5 g at the time of treatment and ca. 20 g at harvest. ¹⁴C-Procymidone was diluted with

four parts of a 1 : 9 mixture (w/v) of emulsifying agent (Hymal PS-10AS, Matsumoto Yushi Seiyaku) and isophorone, and then mixed with water to form an aqueous suspension with a concentration of 500 ppm active ingredient. This suspension (0.5 ml) was evenly applied to the upper surface of the 6th cucumber leaf in the vicinity of a fruit bearing area, or of the 4th leaf of bean plants at a rate of 250 µg per leaf by a micropipette. This treatment was calculated from a practical application rate of 1.5 kg/ha in the field.

At specified intervals, two of the cucumber and bean plants were harvested, and divided into a treated leaf, other untreated leaves and stems (designated as shoots) and into edible and root portions. The treated leaf was rinsed three times with 50 ml of acetone (surface wash) and then homogenized in methanol/chloroform (2/1) with a Waring blender to extract internal radiocarbon (extract). The homogenate was filtered and the residue was reextracted twice with the same solvent system. Both shoots and edible portions were extracted in the same way as described above. The root portions were thoroughly washed with tap water, frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. The homogenate was extracted twice with 50 ml of methanol for ¹⁴C analysis.

5. Cucumber Fruit Treatment

Potted 8-week old cucumber plants were used in the experiment. ¹⁴C-Procymidone in ether (1.5 ml) was applied to the surface of the cucumber fruits (ca. 30–40 g) by a brush at a rate of 300 µg per fruit, and held in a greenhouse at 25±2°C for up to 12 days. At specified intervals, two treated fruits were removed and washed three times with 50 ml of acetone (surface wash). Then, the fruits were cut into small pieces and homogenized in a mixer for 10 min with 5 ml of methanol/chloroform (2/1) per gram plant material. The mixture was filtered and the residue on the filter paper was rinsed with more solvent (extract).

6. Uptake of Soil Residues by Bean Plants

Characterization of soil used for this study is as follows. Kodaira light clay soil with sand, silt, clay and organic matter contents of 31, 40, 29%

and 15.3%, respectively, pH (H₂O) 5.5; Takarazuka loamy sand soil with sand, silt, clay and organic matter contents of 95, 3, 2% and 2.7%, respectively, pH (H₂O) 7.0. Detailed characteristics of the soils were reported previously.⁸⁾ ¹⁴C-Procymidone was applied at a rate of 10 ppm on dry weight basis, and the soils were mixed well, and then each 300 g soil sample was placed into a plastic pot (11 cm i.d. × 7 cm height). The treated soil sample was moistened to 40% of the maximum water-holding capacity and incubated for 2 weeks or 5 months at 25 ± 2°C in the dark. Then, 14-day old seedlings of kidney bean plants were transplanted into the pots and held in a greenhouse at 25 ± 2°C. About 30–50 ml of water was applied to each pot every day. After 42 days, the plants were carefully pulled out of the soils and the roots were thoroughly washed with 200 ml of water. The harvested plants were sectioned into roots, shoots and edible portions, and each part of the plant was analyzed as mentioned above. The soil was extracted three times with 900 ml of ethyl acetate/1 N HCl (2/1) by shaking for 10 min followed by centrifugation at 500 rpm for 15 min. The soil residue was reextracted twice in the same solvent system. The combined ethyl acetate extract and aqueous layer were radiocounted, and the organic layer was

concentrated and examined by TLC. The unextracted soil residue was combusted to ¹⁴CO₂ prior to LSC.

RESULTS AND DISCUSSION

1. Foliar Treatment

After the foliar application of ¹⁴C-procymidone to cucumber and bean plants at a rate of 250 µg per leaf, recovery of the total radiocarbon gradually decreased. From the cucumber plants 63.6% of the applied ¹⁴C was recovered after 23 days, and 38.7% from bean plants after 30 days (Table 2). Most of the recovered ¹⁴C was found in the treated leaves, with less than 3% of the applied ¹⁴C in other parts of the plants.

At harvest, 25 ppb and 17 ppb procymidone equivalent to the radiocarbon were present in the edible portions of cucumber and bean plants, respectively (Table 3). Shoots contained 38 ppb to 19 ppb procymidone equivalent to the radiocarbon, whereas no ¹⁴C was detected in roots. The evidence indicates that very little ¹⁴C translocated from the treated leaves to other parts of the plants including the edible portions. However, the findings were not consistent with those of the previous studies on cucumber young seedlings,²⁾ which showed that approximately 15% of the applied

Table 2 Metabolism of procymidone in cucumber and kidney bean plants after foliar or fruit treatment with ¹⁴C preparation.

Days after application	% of the applied ¹⁴ C					
	Cucumber ^{a)}		Bean ^{a)}		Cucumber fruits ^{b)}	
	8	23	12	30	5	12
Treated leaves or fruits						
Surface wash	73.2	45.5	52.4	29.9	44.7	38.9
Procymidone	73.2	45.5	51.2	28.4	44.7	38.9
P-NH-COOH	<0.1	<0.1	0.8	1.3	<0.1	<0.1
Others	<0.1	<0.1	0.4	0.2	<0.1	<0.1
Extract	9.5	18.0	5.2	8.3	22.2	20.4
Procymidone	9.1	17.3	4.9	7.7	21.4	19.0
P-NH-COOH	0.2	0.1	0.1	0.1	0.4	0.2
P-4-OH	<0.1	0.1	<0.1	0.1	0.1	0.2
P-CH ₂ OH	<0.1	0.1	<0.1	0.1	<0.1	0.1
Others	0.2	0.4	0.2	0.3	0.3	0.9
Pulps	<0.1	0.1	0.1	0.5	3.5	4.9
Total	82.7	63.6	57.7	38.7	70.4	64.2

^{a)} foliar treatment, ^{b)} fruit treatment.

Table 3 Residue levels of radiocarbon in cucumber and bean plants after foliar application of ^{14}C -procymidone at a rate of 250 μg per leaf.

	^{14}C , ppm procymidone equivalent	
	Cucumber ^{a)}	Bean ^{b)}
Treated leaves (0 day)	40	120
At harvest		
Treated leaves	18	38
Shoots	0.038	0.019
Edible portions	0.025	0.017
Roots	<0.001	<0.001

^{a)} after 23 days, ^{b)} after 30 days.

^{14}C translocated from the treated leaves to the untreated upward and downward portions. This difference might be due at least in part to the difference in the developmental stage of plants to which procymidone had been applied.

As shown in Fig. 1, the fungicide disappeared from the treated leaves of cucumber and bean plants with a half-life of approximately 20 days, and at harvest declined to 62.8% and 36.1% of the applied dose. Together with a large amount of unchanged procymidone, four minor metabolites were detected by silica gel TLC in either solvent system A or B, and three metabolites were identified by TLC cochromatography with the authentic standards. All of the products were procymidone derivatives cleaved at the cyclic imide linkage (P-NH-COOH), and hydroxylated at the methyl group (P-CH₂OH) and at the 4-position of phenyl group (P-4-OH), which amounted in total to less than 2% of the applied ^{14}C in cucumber and bean plants. The shoots, roots and edible portions were not analyzed for ^{14}C because of lower radiocarbon levels.

2. Fruit Treatment

When cucumber fruits were treated with ^{14}C -procymidone at a rate of 7.5–10 ppm, approximately 24% of the applied ^{14}C penetrated into the fruits one day after treatment and located mainly in the peeled fraction. Nevertheless, there was no significant increase in the amount of radiocarbon thereafter. After 12 days, approximately 64% of the applied

Fig. 1 Residue levels of procymidone in the treated leaves of bean and cucumber plants after foliar application.

^{14}C was recovered from the cucumber fruits under the greenhouse conditions (Table 2). However, procymidone disappeared much more rapidly from the cucumber fruits with half-lives of 3–7 days under practical field conditions.⁹⁾

On the treated fruits only unchanged procymidone was detected and no degradation product was observed with a sufficient quantity to permit the detection on TLC, whereas trace amounts of P-NH-COOH, P-4-OH and P-CH₂OH occurred in the fruits.

3. Uptake of Soil Residues by Bean Plants

Kidney bean seedlings were grown for up to 42 days in the soil treated with 10 ppm of ^{14}C -procymidone, and the radiocarbon in shoots, roots and edible portions was determined (Fig. 2). Although the shoots contained 12.3 ppm procymidone equivalent to ^{14}C with Kodaira soil and 15.3 ppm with Takarazuka soil, the concentration in the edible portions was 0.42 ppm and 0.66 ppm with Kodaira and Takarazuka soils, respectively. Approximately 5–6 ppm of ^{14}C residue was found in roots with both soils. When bean seedlings were transplanted in the soil preincubated with 10 ppm of ^{14}C -procymidone for 5 months, the ^{14}C residues in plants were lower than in those without preincubation. After 42 days, shoots, pods and seeds contained 5.2–6.1 ppm and 0.33–0.38 ppm procymidone equivalent to ^{14}C ,

Fig. 2 Uptake and distribution of ¹⁴C in bean plants 42 days after transplantation to Kodaira and Takarazuka soils treated with ¹⁴C-procymidone at the rate of 10 ppm.

respectively.

Procymidone amounted to approximately 80–90% of the radiocarbon in shoots and roots, and 30–65% in pods and seeds. The metabolites found in the leaves treated with ¹⁴C-procymidone such as P-NH-COOH, P-4-OH and P-CH₂OH were also detected in shoots and roots, but none in the edible portions. Most of the metabolites in pods and seeds were polar and located at the origin of silica gel TLC in solvent system A. Although further characterization is yet to be carried out, they may be conjugated metabolites. Thus, procymidone residue in soil is readily taken up by bean plants, and translocated to shoots except for the edible portions. The lower concentration of procymidone in pods and seeds may be caused by lower transpiration capacity of the edible portions. Such a distribution pattern has been observed in tomato when benomyl was used as a systemic fungicide.¹⁰⁾

A slightly large amount of radiocarbon was found in bean plants grown in Takarazuka soil with a lower organic matter content than in Kodaira soil with a higher organic matter content. This appears to relate to more extensive adsorption of procymidone to Kodaira soil¹¹⁾ which results in reduction of an amount of a fungicide available for plant uptake as in the case of other pesticides.^{12–14)}

In the treated Kodaira and Takarazuka soils, at least seven degradation products were detected on silica gel TLC in solvent system A or B, and P-NH-COOH, P-4-OH, P-3-Cl and CP-(COOH)₂ were included. Evidence for their

Fig. 3 Metabolic pathways for procymidone in plants.

identity was obtained by TLC cochromatography with the authentic standards.

Thus, although uptake and translocation of procymidone varied to some extents depending upon the routes of application, growth stage of the plants, and soil properties, translocation to the edible portion proceeded only to a limited extent. The identified procymidone metabolites in cucumber and bean plants were analogous to those in mammals⁴⁾ (Fig. 3).

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要 約

殺菌剤プロシミドンのキュウリおよびインゲンマメにおける移行と代謝

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浸透移行性殺菌剤プロシミドン(スミレックス®)のキ

ュウリおよびインゲンマメにおける代謝について検討した。 ^{14}C 標識体を $250\text{ }\mu\text{g/葉}$ の割合で葉面処理して温室内で栽培すると、プロシミドンは可食部を含む他の無処理部にほとんど移行することなく、両植物ともに半減期が約20日の速度で減少した。 ^{14}C 標識体をキュウリ果実表面に $7.5\sim 10\text{ ppm}$ の割合で処理すると、処理1日後に添加 ^{14}C の約24%が果実内部に浸透したものの、その後増加する傾向は認められなかった。本化合物は、可食部を含む植物体内において、メチル基の水酸化、フェニル基4位の環水酸化、環状イミドの加水分解を経て徐々に代謝された。一方、 ^{14}C 標識体を 10 ppm の割合で処理した小平軽植土や宝塚壤質砂土にインゲンマメを移植して42日間栽培すると、地上部には $12.3\sim 15.3\text{ ppm}$ の ^{14}C が存在したが、可食部における ^{14}C 残留量は $0.42\sim 0.66\text{ ppm}$ であった。プロシミドン処理土壌を好氣的畑地条件で5カ月間前もってインキュベートすると、土壌からインゲンマメに取りこまれる ^{14}C が著しく低下した。