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Dissipation of Foliar Residues of Diafenthiuron and Its Metabolites

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Diafenthiuron, 1-*tert*-butyl-3-(2,6-diisopropyl-4-phenoxyphenyl)-thiourea, is an insecticide and acaricide (Kadir and Knowles 1991), and can be converted into the corresponding carbodiimide [*tert*-butyl-(2,6-diisopropyl-4-phenoxyphenyl)-carbodiimide, designated as CGA-140408] probably responsible for the bioactivity (Ruder and Kayser 1993). The insecticidal activity against two-spotted spider mites was strongly correlated to the residue level of the carbodiimide on cotton leaves (Drabek et al. 1992). Pesticide foliar residues are routinely monitored because of safety and health concerns for farm workers and consumers. They can remain in a dislodgable state, be absorbed into the cuticular layer or translocated into the inner plant tissues. Dislodgable foliar residues (DFRs) are the loosely bound chemicals on leaf surfaces and vary significantly depending on the environmental conditions, formulation type, crop species, and the habits of workers (Davis et al. 1981; Sears et al. 1987), which are the main portion for worker exposure. For example, Popendorf and Leffingwell (1978) reported on the oxidative transformation of parathion into a more toxic paraoxon on orange leaves. Therefore, the fate of those residues in various leaf compartments needs to be investigated for assessing their exposure and health risk. Several studies have been carried out on the predictive modeling of foliar residues (Estesen and Buck 1990) and residue levels in different leaf compartments (Thompson et al. 1984; Sears et al. 1987).

This study was designed to obtain information of (a) transformation of diafenthiuron in/on the leaf surface of Chinese cabbage, (b) dissipation patterns of foliar residues of diafenthiuron and its metabolites, and (c) localization of diafenthiuron and its metabolites in different leaf compartments of leaves. The present study was carried out in a greenhouse to exclude rainfall, which significantly affects the residual patterns of test compounds.

MATERIALS AND METHODS

Diafenthiuron, CGA-140408, and 1-*tert*-butyl-3-(2,6-diisopropyl-4-phenoxyphenyl)-urea (CGA-177960) were kindly provided by Syngenta Korea Ltd. HPLC grade solvents were purchased from Duksan Co. (Ansan, Korea). All other

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chemicals and reagents were of analytical grade and commercially available.

A test was carried out with Chinese cabbages in a greenhouse at Seoul National University, Suwon, Korea. Diafenthiuron EC (25% a.i.) was sprayed on the leaves with a personal sprayer at rates of 1 (plot A) and 10 g a.i/1000 m^2 (plot B). Each plot consisted of twenty plants. The mean, lowest, and highest temperatures were 20, 14 and 29°C, respectively, during the experimental period.

Five leaves were sampled at 0, 2, 7, 9, 12, and 17 days after the application. Residual diafenthiuron and its metabolites were sequentially washed with 0.1% Tween 80 aqueous solution (100 mL), dichloromethane (80 mL), and methanol (100 mL). For each washing, whole leaves were immersed in the washing solution, shaken for 1 min, and washed with an additional amount of washing solution (20mL). The aqueous washing solutions were extracted with dichloromethane (2 × 100 mL), and the extracts were combined and concentrated to dryness *in vacuuo.* The residues were dissolved in 2 mL of methanol and analyzed by HPLC for the concentrations of diafenthiuron and metabolites as dislodgable residues. Organic solvents were directly concentrated, and adjusted to an appropriate volume prior to HPLC measurement for cuticular residues. Remaining leaves were macerated with acetonitrile (100 mL) for 1 min with a homogenizer (Samsung, Korea). After filtration, an aliquot (20 mL) of the extracts was partitioned with hexane (3 × 20 mL) and dried over anhydrous sodium sulfate. After the hexane layer was concentrated to dryness, the residues were dissolved in acetonitrile (2 mL) and analyzed by HPLC for diafenthiuron in inner tissues. For the purification of CGA-140408 and CGA-177960 in the same tissues, N,N'-dicyclohexylcarbodiimide (50 μL, 5 g/L) was added into the hexane extracts and concentrated *in vacuo*. The residues were dissolved in 2 mL of mixed hexane and chloroform (4/1, v/v) and cleaned up with aminopropyl Sep-Pak cartridges, which had been pre-conditioned with 8 mL of mixed hexane and chloroform (4/1, v/v). CGA-140408 and CGA-177960 were eluted with 4 mL of mixed hexane and chloroform (7/3, v/v) and 4 mL of mixed hexane and chloroform (3/2, v/v), respectively. Each eluent was dried under a gentle stream of nitrogen gas. The residues were dissolved in 1 mL of methanol and analyzed by HPLC. Recovery tests were carried out at concentrations of 1 and 10 mg/kg fresh weight on the whole leaf tissues. The same procedures of extraction and cleanup were followed for the remaining leaves. For the quantitation of residues, an HP-1100 series HPLC equipped with UV-VIS detector was used. The HPLC column was Symmetry C18 (Waters, 150 × 3.9 mm, 5 μm). Isocratic elution was performed with mixtures of methanol and water at a ratio of 4/1 (v/v) for diafenthiuron and CGA-177960, and at a ratio of 9/1 (v/v) for CGA-140408. The flow rate and the detection wavelength were 0.8 mL/min and 250 nm, respectively.

RESULTS AND DISCUSSION

Initial concentrations of diafenthiuron in whole leaves immediately after application were 4.61 and 27.03 mg/kg in plots A and B, respectively, and

decreased rapidly at similar rates (Figure 1). Half-life ($t_{1/2}$) of diafenthiuron on whole leaves from the two plots was 4.0 days. CGA-140408 and CGA-177960 were found in all compartments, but the levels varied in different leaf compartments.

Total residues including diafenthiuron, CGA-140408 and CGA-177960 gave similar results which the half-lives of total residues being 4.4 and 4.2 days in plots A and B, respectively. Residue levels of CGA-140408 and CGA-177960 rapidly reached maximum 7-9 days after treatment, then gradually decreased in both test plots (Table 1).

Table 1. Concentration ratio of metabolites relative to the concentration of diafenthiuron on whole leaves*

Day	Plot A		Plot B	
	CGA-140408	CGA-177960	CGA-140408	CGA-177960
0	1.05	0.33	2.43	1.02
2	1.36	1.62	0.97	3.19
7	3.11	3.09	0.70	3.89
9	1.94	4.41	0.36	3.34
12	0.85	2.67	0.16	2.48
17	0.14	1.71	0.08	0.84

* Ratio = (metabolite molar concentration / diafenthiuron concentration) × 100.

Dislodgable diafenthiuron residues decreased more rapidly ($t_{1/2}$, 3.1 days in both plots) than those of whole leaf residues ($t_{1/2}$, 4.2 to 4.4 days). Because the dislodgable residues are more labile against degradation by sunlight, heat, and other environmental factors, half-lives of dislodgable residues are generally short, typically in a range of 1 to 10 days (Davis et al. 1981; Nigg et al. 1992; Sears et al. 1987). The experimental results on leaf surface residues also followed the general tendency. Volatilization of diafenthiuron could not play a key role on the decline of the dislodgable residues because of its low vapor pressure (Tomlin 1997). Drabek et al. (1992) reported on the rapid photochemical conversion of diafenthiuron on a Teflon plate and cotton leaf. Photodegradation of diafenthiuron on Chinese cabbage leaves also may occur. Maximum concentrations of dislodgable CGA-177960 were 4.9 and 6.0% of the initial concentrations of dislodgable diafenthiuron in plots A and B, respectively, while that of CGA-140408 was below 1.5%. However, the concentrations of dislodgable residues were 20- to 30-fold lower than those in cuticles. Highest concentrations of residues were observed in chloroform and methanol extracts (leaf cuticular layers). The results suggest the rapid translocation of the chemicals into cuticular layer.

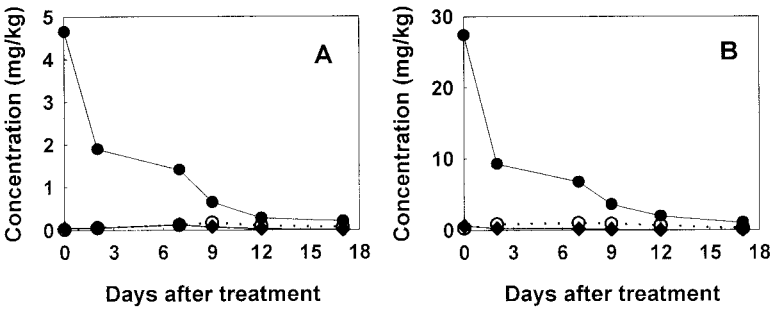

Figure 1. Residues of diafenthiuron, CGA-140408 and CGA-177960 on whole leaf tissues in plot A (**A**) and plot B (**B**). Data were averages of two replicates. -●-, diafenthiuron; -♦-, CGA-140408; and -o-, CGA-177960.

The levels of diafenthiuron in cuticles were 485-fold higher than those of inner tissues, 111-fold for CGA-140408, and 37-fold for CGA-177960 (Table 2). The dissipation pattern coincided with that of dislodgable residues showing rather extended half-lives (3.8 and 4.0 days). Concentrations of the metabolites stayed below 4% of cuticular diafenthiuron during the experimental period. In comparison with other tissue compartments, residue accumulation time lag was observed in the inner tissue samples (Figure 2). Maximum concentrations of diafenthiuron and its metabolites in the inner tissues were reached 2-7 days after treatment and gradually decreased therefore (Figures 2 and 3). Residue levels of the inner tissues were very low in comparison with those in other leaf compartments. Maximum concentration of CGA-140408 was 0.06 ppm at 2 days after treatment.

The time-lagged accumulation of diafenthiuron and metabolites in the inner tissue could be explained by the fact of a slow diffusion process of chemicals from cuticle into other leaf compartments (Bukovac and Petracek 1993; Davis et al. 1979; Schonherr and Baur 1994; Schreiber and Schonherr 1993). Sears et al. (1987) and Thompson et al. (1984) also reported the delayed accumulation in a penetration study.

In comparison with other compartments, the residues in the inner tissues dissipated slowly. Accumulation profiles of the metabolites differed significantly among different tissues (Figures 2 and 3).

The concentrations of the metabolites in inner tissues were 60-150% of diafenthiuron in the same tissues while the levels in other layers were maintained below 4 to 5%. These discrepancies could be explained by two factors: different degradation rates of diafenthiuron and its metabolites as observed in cotton leaves (Drabek et al. 1992) and faster translocation of the metabolites into the inner tissues. Different translocation rates may be the main cause of higher level of

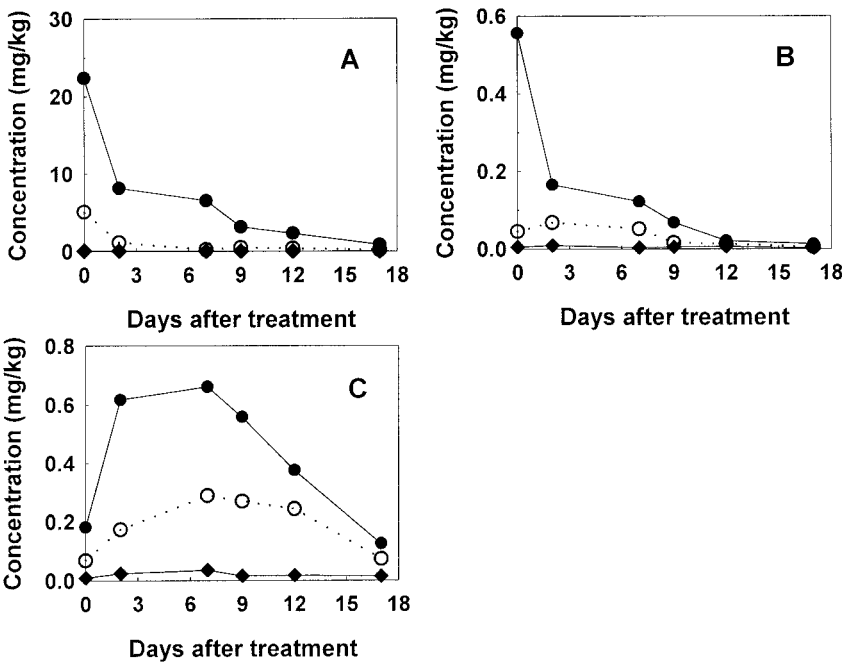

Figure 2. Distribution of residues of diafenthiuron (A), CGA-140408 (B) and CGA-177960 (C) in different leaf compartments in plot B. -o-, DFRs; -●-, cuticle, -♦-; tissue

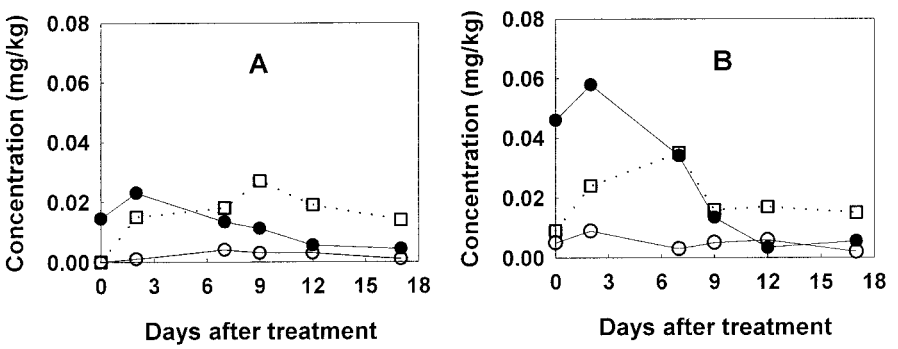

Figure 3. Concentration of diafenthiuron, CGA-140408 and CGA-177960 in inner tissues in plot A (A) and plot B (B): -●-; diafenthiuron, -o-; CGA-140408 and -□-; CGA-177960

metabolites in the inner tissues.

Table 2. Concentration ratio of diafenthiuron and its metabolites in different leaf compartments

Chemical	Days after Application	Ratio*			
		Plot A		Plot B	
		Dislodgable	Cuticle	Dislodgable	Cuticle
Diafenthiuron	0	35.0	286.4	110.2	484.7
	2	4.7	77.6	19.6	140.8
	7	3.4	102.2	6.3	191.7
	9	2.9	55.6	34.0	232.5
	12	2.1	47.7	108.0	680.9
	17	2.0	46.0	26.8	152.5
CGA-140408	0	0.0	0.0	9.0	111.4
	2	3.0	53.9	7.6	18.3
	7	1.3	31.0	17.0	40.6
	9	0.5	26.2	3.2	13.6
	12	0.4	10.7	2.2	3.5
	17	0.0	5.2	2.5	6.5
CGA-177960	0	0.0	0.0	7.6	21.3
	2	0.7	3.2	7.3	26.7
	7	1.3	5.4	8.3	20.0
	9	0.3	6.0	16.9	37.1
	12	0.1	5.2	14.4	23.0
	17	0.0	4.5	4.9	8.9

* Concentration of each compartment was divided by the residual level of the inner tissue.

In conclusion, diafenthiuron, sprayed on Chinese cabbage, was rapidly distributed into the cuticular layer with only a small portion persisted on the surface as DFRs or penetrated into deeper tissues. The residues in cuticles and leaf surfaces dissipated rapidly, which fitted with the first-order kinetics. The exception was inner tissue samples, where a time lag of the residue accumulation was observed. Diafenthiuron on a leaf surfaces was transformed into CGA-140408 and CGA-177960 in a successive manner, and similar results were observed in the inner tissues. Application rates did not affect the dissipation rate constants.

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