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Multiresidue Analysis of Fungicides in Strawberry

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The overuse of fungicides can cause excessive worker exposure, earlier outbreak of resistant fungus and residues in foodstuffs (Une 1987). In the sales of strawberry in Japan, the variety 'Toyonoka' won popularity rapidly in the late 1980's. But this variety has no resistance to powdery mildew *(Sphaerotheca humuli)* (Honda 1988); farmers were at their wit's end, not knowing how to control this fungus (Ikeda 1991; Kanaiso 1992; Tanigawa 1993). Because strawberry is mainly eaten as raw fruit by children in Japan, the pesticide residue in strawberry is a hygienic problem. No survey has been carried out on the residues of the fungicides for powdery mildew control in Japan. On the other hand, as dicarboximides were often reported residues in strawberry and other foodstuffs, thus it is important to survey the residue levels of dicarboximides in foodstuffs.

In this study, a multiresidue analytical method was established for 7 fungicides to control powdery mildew and 3 dicarboximides in strawberry, and this method was applied to the market survey in Nara, Japan.

MATERIALS AND METHODS

Acetonitrile (MeCN) used as the mobile phase of liquid chromatography (LC) was the grade for LC. The other solvants and anhydrous Na_2SO_4 used in this study were the grade for pesticide residue analysis. Florisil PR™ was supplied by Wako Pure Chemicals Inc. (Ohsaka, Japan), and added 10 v/w% distilled water (H_2O) and then shaken vigorously for 5 min and leaved for a few hours. The buffer solution was made as: 3.58 g of $Na_2HPO_4 12H_20$ and 0.78 g of $NaH_2PO_4 2H_2O$ were diluted in H_2O and made up to 100 mL with H_2O.

The fungicides investigated in this study were tabulated in Table 1, and the corporations from which the analytical standards were supplied were tabulated in Table 2. The analytical standards in first group were dissolved in toluene, and those of the second group in MeCN, and diluted and mixed adequately.

The strawberry samples were bought from the supermarkets in Nara, Japan on Jan.-Mar. 1992, 93. As no variety except for 'Toyonoka'

Table 1. Fungicides investigated in this study

Chemical / Molecular formula	Formula
Bitertanol $C_{20}H_{23}N_3O_2$	*β*-([1,1'-Biphenyl]-4-yloxy)-*α*-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
Chinomethionat $C_{10}H_6N_2OS_2$	6-Methyl-1,3-dithiolo[4,5-b]quinoxalin-2-one
Fenarimol $C_{17}H_{12}Cl_2N_2O$	*α*-(2-Chlorophenyl)-*α*-(4-chlorophenyl)-5-pyrimidinemethanol
Iprodione $C_{13}H_{13}Cl_2N_3O_3$	3-(3,5-Dichlorophenyl)-*N*-(1-methylethyl) 2,4-dioxo-1-imdazolidinecarboxamide
Myclobutanil $C_{15}H_{17}ClN_4$	*α*-Buthyl-*α*-(4-chlorophenyl)-1*H*-1,2,4-triazole-1-propanenitrile
Pyrifenox $C_{14}H_{12}Cl_2N_2O$	1-(2,4-Dichlorophenyl)-2-(3-pyridinyl)-*O*-methyloxime-ethanone
Procymidone $C_{13}H_{11}Cl_2NO_2$	3-(3,5-Dichlorophenyl)-1,5-dimethyl-3-azabicyclo[3,1,0]hexane-2,4-dione
Triadimefon $C_{14}H_{16}ClN_3O_2$	1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone
Triadimenol $C_{14}H_{18}ClN_3O_2$	*β*-(4-Chlorophenoxy)-*α*-(1,1-dimethylethyl)-1*H*-1,2,4-triazole-1-ethanol
Triflumizole $C_{15}H_{15}ClF_3N_3O$	(*E*)-1-[1-[[4-Chloro-2-(trifluoromethyl)phenyl] imino-2-propoxyethyl]-1*H*-imidazole
Vinclozolin $C_{12}H_9Cl_2NO_3$	3-(3,5-Dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione

Table 2. Recovery rates, detection limits, standard concentrations, and supply corporations of the chemicals

Chemical	Recovery rate[a] (%)	Detection limit (μg/g-raw)	Concentration in standard solution(μg/mL)	Standard supply Inc.
Group 1				
Chinomethionat	83±6	0.003	0.050	Wako[b]
Fenarimol	85±5	0.005	0.10	Wako
Iprodione	90±5	0.01	0.20	Wako
Procymidone	86±3	0.005	0.10	Wako
Z-Pyrifenox	76±4	0.005	0.10	Hayashi[c]
E-Pyrifenox	79±3	0.005	0.10	Hayashi
Triadimefon	85±4	0.005	0.10	Wako
Vinclozolin	92±2	0.003	0.050	Wako
Group 2				
α-Bitertanol	76±4	0.005	1.0	Wako
β-Bitertanol	78±4	0.005	-	(Hayashi)[d]
Myclobutanil	78±3	0.01	1.0	Hayashi
α-Triadimenol	77±5	0.01	1.0	GL Sci[e]
β-Triadimenol	79±5	0.01	-	(Wako)[f]
Triflumizole	75±7	0.005	1.0	Wako

a) Figure represents (mean) ± (s. d.) (n = 3)
b) Wako Pure Chemicals Inc. (Ohsaka, Japan)
c) Hayashi Pure Chemicals Inc. (Ohsaka, Japan)
d) Analytical standard of Hayashi Pure Chemicals Inc. contained *α*-,*β*-Bitertanol at the ratio of 80:20
e) GL Science Inc. (Tokyo, Japan)
f) Analytical standard of Wako Pure Chemicals Inc. contained *α*-,*β*-Triadimenol at the ratio of 25:75

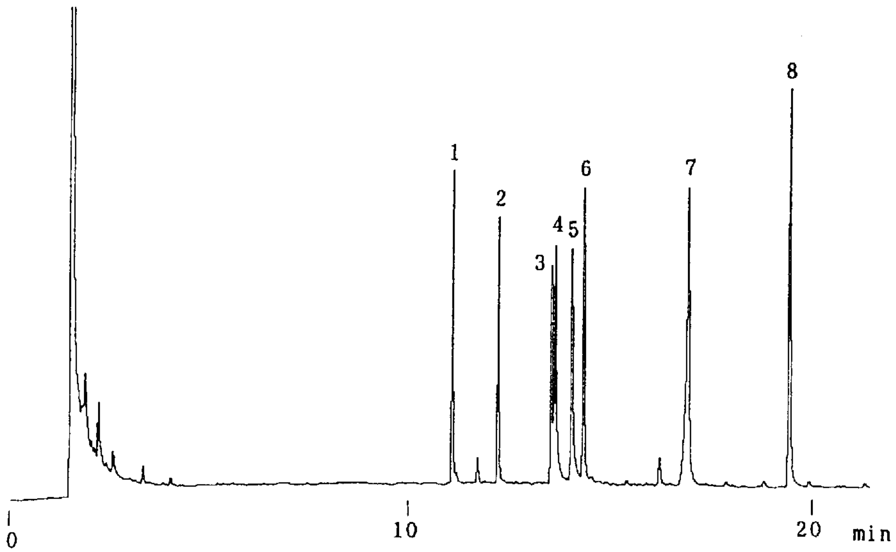

Figure 1. Gaschromatogram of several fungicides by GC-ECD with DB-17
1;Vinclozolin, 2;Triadimefon, 3;Procymidone, 4,5;Z-,E-Pyrifenox, 6;Chinomethionat, 7;Iprodione, 8;Fenarimol

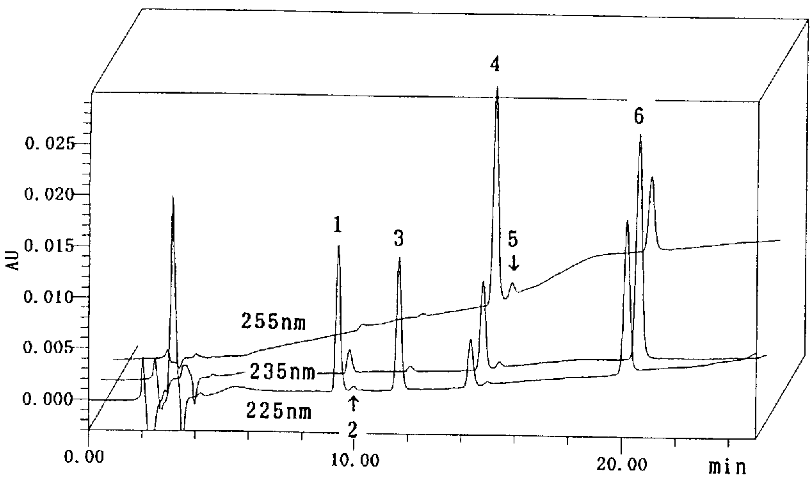

Figure 2. Multiple UV chromatogram of several fungicides by LC-DAD
1,2;α-,β-Triadimenol, 3;Myclobutanil, 4,5;α-,β-Bitertanol, 6;Triflumizole

and 'Nyohou' could be bought, these varieties were analysed 5 samples per year respectively.

The strawberry sample was removed the calyx and cut carefully by kitchen knife not to be crushed pulpy. The cut sample was weighed 20 g-raw, and added 20 mL of the buffer solution and 100 mL of acetone, and then homogenized for 2 min. The suspension was filtered with Toyo 5A filter paper, the residue was washed with 5 mL of acetone three times. The filtrate and the washing were joined and added 100 mL of ethyl acetate and 200 mL of 10% NaClaq, and shaken for 5 min and settled. The lower layer was removed and the upper layer was washed with 100 mL of 10% NaClaq three times. The washed solution was dehydrated by 40 g of anhydrous Na_2SO_4 and evaporated. The residue was dissolved by 2 mL of diethyl ether/ n–hexane (Et/Hx) (1+4), and the solution was loaded into florisil column (10 v/w% hydrated, 5 g, 14 mmø). The column was eluted by 50 mL of Et/Hx (2+3) and acetone/Hx (1+4), and the eluates were evaporated respectively. The residue of the first fraction was dissolved with 5 mL of Hx, and quantified by gas chromatography electron capture detector (GC-ECD). The residue of the second fraction was dissolved in 2mL of MeCN/H_2O (2+3), and analysed with LC-diode array detector (LC-DAD).

The GC and LC analyses were carried out by the following instruments and conditions. GC-ECD: Instrument; Hewlett-Packard (HP) 5890, Column; J&W DB–17 and DB–1 0.25 µm x 0.25 mmø x 30 m, Injector; He, 150 kPa, 230°C, Injection; split (50:1), 3 µL, Oven temp.; 140°C(l min)->(10°C/min)+300°C (5 min), Detector; N, 80 mL/min, 300°C. GC-mass spectrometry (GC-MS): Instrument; HP–5890, 5971, Column; HP Ultra-2 0.11 µm x 0.20 mmø x 25 m, Injector; He, 50 kPa, 200°C, Injection; splitless, 1 µL, Oven temp.; 110°C (1 min)->(10°C/min)+300°C, Ionization; EI. LC-DAD: Instrument; Waters 820, 510 (2 units), 712, 991, Column; Nomura Chemicals Inc. (Seto, Aichi, Japan) Develosil ODS-HG-5 4 mmø x 150 mm, Mobile phase; A;(0.25% Triethylamine (TEA) + 0.1% H_3PO_4)aq : MeCN = 55 : 45, B;(0.25% TEA + 0.1% H_3PO_4)aq : MeCN = 30 : 70, Gradient; A->(l% MeCN/min)->B->A(5 min), 0.6 mL/min, Injection; 50 µL, UV range; 210-350 nm.

RESULTS AND DISCUSSION

As dicarboximides are sensitive to GC-ECD and triflumizole has been usually analysed by LC with UV detector, GC-ECD and LC-DAD were examined for the quantification in this study. In 13 chemicals, α-, β- triadimenol and α-, β- bitertanol were not obtained the sufficient response by GC-ECD but by LC-DAD. According to this result, the first eluant of florisil column chromatography was chosen Et/Hx (2+3) just yet these chemicals eluted. This condition divided 13 chemicals in two groups as in Table 2. The chemicals of the first group were mixed and diluted to be the concentration shown in Table 2. Figure 1 represents the chromatogram of the mixed standard solution by GC-ECD. Figure 2 represents the chromatogram of the mixed solution of the second group by LC-DAD. The second eluant of florisil column chromatography was selected acetone/Hx (1+4) to elute all chemicals quantitatively.

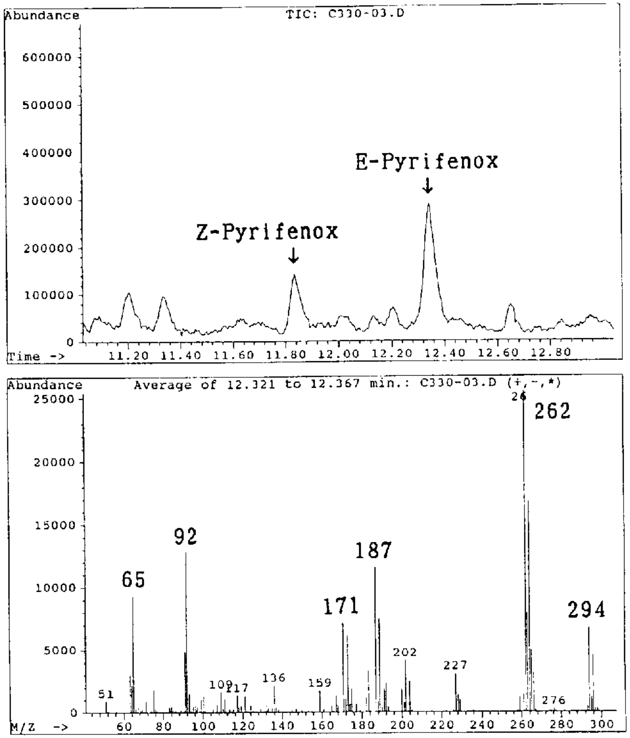

Figure 3. Masschromatogram of the strawberry sample detected the residue of pyrifenox

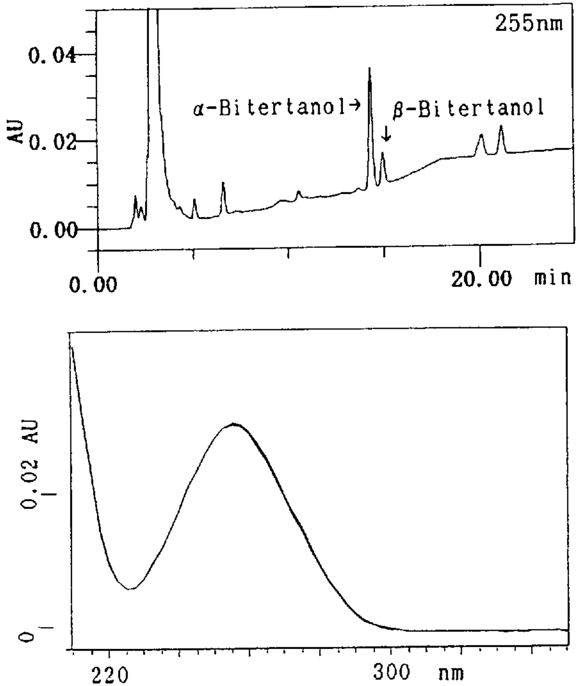

Figure 4. UV chromatogram and spectrum of the strawberry sample detected the residue of bitertanol

Triflumizole degrades both in the acid condition and alkaline one (Goto and Kato 1987). In this method, the homogenization of strawberry was carried out after adding the neutral buffer solution and acetone. This homogenization in the neutral condition increased the recovery rate of trifulumizole from 20% to 76% on analysing strawberry. Table 2 represents the recovery rates of the fungicides. 13 chemicals were recovered in the range of 75-92%.

The residues of 9 fungicides except vinclozolin were detected in the strawberry samples on the market in Nara, Japan, and 12 chemicals detected were identified with GC–MS and LC-DAD. For example, Figure 3 shows the mass-chromatogtram of the strawberry sample detected the residue of pyrifenox. In the upper figure, the total ion chromatogram gave the isolated peaks at the retention times of Z-,E-pyrifenox, the mass-spectra of which agreed well with the standards as the lower figure. Figure 4 shows the result of LC-DAD on the strawberry sample detected the residue of bitertanol. In the upper figure, the UV chromatogram gave isolated peaks at the retention times of α-,β-bitertanol, the UV spectra of which agreed well with the overwritten standard spectrum as the lower figure.

The amounts of the fungicide residues in the strawberry samples on the market in Nara, Japan were tabulated in Table 3, 4. As pyrifenox and myclobutanil were supplemented to the guide for the strawberry production of Nara Prefecture in Apr. 1992, these fungicides were added to this residue survey on Jan. 1993. Triadimenol residues were summed up to triadimefon residue, because the triadimenol product has not been on the market in Japan. All the detected cases were below the maximum residue levels in Japan.

The dicarboximides were detected 60-70% of the strawberry samples both in ’Toyonoka’ and ’Nyohou’. Ambrus et al. (1991) surveyed dicarboximides residues in strawberry on the market in Denmark, Sweden or United Kingdom, and reported that iprodione residues were found in 6 of 236 samples (2.5%), procymidone residues in 2 of 129 samples (1.5%), and vinclozolin residues in 19 of 368 samples (5%) with the detection limit 0.1 µg/g–raw. Luke et al. (1988) surveyed 342 strawberry samples on the market in California, USA, and reported that vinclozolin residues more than 0.1 µg/g-raw were detected in 169 samples (49%). Neidert et al. (1994) surveyed 48 strawberry samples on the market in Canada, and reported that vinclozolin residues more than 0.1 µg/g-raw were detected in 5 samples (10%). In this study, procymidone residues more than 0.1 µg/g-raw were detected in 4 of 20 samples (20%). The incidence of dicarboximides residues by our survey seemed to be in the middle of Ambrus’, Neidert’s and Luke’s.

On the other hand, the fungicides to control powdery mildew were detected often and complexly in ’Toyonoka’ than ’Nyohou’. In these fungicides, Luke et al. (1988) analysed 3 fungicides (fenarimol, myclobutanil, triadimefon), and reported that no residue was detected from 342 strawberry samples in California, USA. Camoni et al. (1993) surveyed fenarimol residue in Italy, and reported that no residue was detected in 215 strawberry samples. Neidert et al.

Table 3. Fungicide residues in the strawberries on the market in Nara, Japan on Jan.-Mar. 1992

Fungicide	Concentration (μg/g-raw)										Maximum residue level
Variety	Toyonoka					Nyohou					
Sample No	1	2	3	4	5	6	7	8	9	10	
Bitertanol[a)]	-[c)]	-	0.27	-	0.02	-	-	-	-	-	1.0[d)]
Chinomethionat	-	-	0.03	-	-	-	-	-	-	-	0.5
Fenarimol	-	-	0.01	-	0.03	-	-	-	-	-	(0.3)[e)]
Triadimefon[b)]	-	-	0.04	-	0.02	-	-	-	-	-	(0.5)
Triflumizole	-	0.08	0.01	-	-	-	-	-	-	-	2.0
Iprodione	0.08	-	-	-	-	-	-	-	-	-	20
Procymidone	-	0.48	-	0.05	-	0.07	-	0.05	0.03	-	(3)
Vinclozolin	-	-	-	-	-	-	-	-	-	-	(20)

a) Figures represents the sum of α-bitertanol and β-bitertanol
b) Figures represents the sum of triadimefon, α-triadimenol and β-triadimenol
c) Not detected
d) Figures are maximum residue level by the Japanese Ministry of Health and Welfare
e) Figures in the parentheses are notified levels for the prohibition against agricultural use by the Environment Agency of Japan

Table 4. Fungicide residues in the strawberries on the market in Nara, Japan on Jan.-Mar. 1993

Fungicide	Concentration (μg/g-raw)										Maximum residue level
Variety	Toyonoka					Nyohou					
Sample No	11	12	13	14	15	16	17	18	19	20	
Bitertanol[a)]	0.13	-	-	-	-	-	-	-	-	-	1.0[d)]
Chinomethionat	-[c)]	-	-	-	-	-	-	-	-	-	0.5
Fenarimol	0.11	0.05	0.01	0.02	-	-	-	0.02	-	-	(0.3)[e)]
Myclobutanil	0.07	-	0.02	-	-	-	-	-	-	0.03	1.0
Pyrifenox[f)]	-	0.03	-	0.02	-	-	0.11	-	-	-	2.0
Triadimefon[b)]	-	0.04	-	-	0.01	-	-	-	-	-	(0.5)
Triflumizole	0.15	0.01	-	-	-	0.03	0.01	0.01	-	-	2.0
Iprodione	-	-	-	-	-	-	-	-	-	-	20
Procymidone	-	0.27	0.15	0.09	-	-	0.02	0.33	0.05	0.11	(3)
Vinclozolin	-	-	-	-	-	-	-	-	-	-	(20)

a)-e) see Table 3
f) Figures represents the sum of Z-pyrifenox and E-pyrifenox

(1994) analysed fenarimol and triadimefon, and reported that no residue was detected from 48 strawberry samples in Canada. In comparison, the residue incidence of these fungicides by our survey seemed remarkably high, but these residues apparently do not control powdery mildew of 'Toyonoka' (Ikeda 1991; Kanaiso 1992; Tanigawa et al. 1993). From the first, this variety was adopted without regard to whether or not it was resistant to powdery mildew (Honda 1988), and won rapidly more than half of the strawberry sales in late 1980's in Japan by the request of the Japanese market system for the advantage of preservation and outside view. In recent years, the resistant fungi to these fungicides have been discovered (Nakano et al. 1992; Ohtsuka and Sou 1990). It seems advisable to consider exchanging 'Toyonoka' variety for one tolerant to control powdery mildew, to retain the effectiveness of these fungicides, and to decrease worker exposure and residues in strawberry fruit.

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