

Monitoring Degradation of Dicarboximidic Fungicide Residues in Soils

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Iprodione, procymidone, and vinclozolin are dicarboximidic fungicides commonly used in preharvest viticulture treatments to prevent gray mold (*Botrytis*) and other fungal diseases. The aim of this study is to determine the degradation of these fungicides in five vineyard soils of Penedès after three treatments at 100-day intervals with commercial formulations under laboratory conditions. The analyses were conducted over a period beginning the same day of each treatment and lasting for up to 77 days afterward. In these conditions, the degradation rates of all three fungicides increased with the number of applications. Times elapsed to 90% degradation of iprodione, procymidone, and vinclozolin following first, second, and third treatments were about 35, 21, and 7 days, respectively. No residues were found at 77 days. These results showed that the residual levels of these three fungicides in soils decreased with time.

Keywords: *Iprodione; procymidone; vinclozolin; dicarboximidic fungicides; residues; soils*

INTRODUCTION

Iprodione, procymidone, and vinclozolin dicarboximidic fungicides used in preharvest viticulture treatments to prevent *Botrytis* were introduced into the market due to the resistance of some *Botrytis cinerea* strains to systemic benzimidazole fungicides (Bolay and Rochaix, 1976).

Iprodione and vinclozolin are contact fungicides, whereas procymidone has moderate systemic activity (*The Pesticide Manual*, 1995).

In a previous paper we studied the persistence of dicarboximidic fungicides in grapes, must, and wine (Garcia-Cazorla and Xirau-Vayreda, 1994). In this work we examine the persistence or degradation of these fungicides in five vineyard soils of Penedès (Catalonia).

There is little information about the determination and degradation of these fungicides in soils. Walker (1987) examined the effects of soil pH on degradation rates of iprodione and vinclozolin in a silty clay loam soil. Mitchell and Cain (1996) indicated the enhanced degradation of iprodione and vinclozolin in soils with no previous history of any pesticide input by only one application of the fungicides. Slade et al. (1992) stated that enhanced degradation of iprodione and vinclozolin in a clay loam soil was induced in the laboratory by using three successive applications of fungicides but could not be induced in the case of procymidone. Cain and Mitchell (1996) described the isolation and characterization of the organism responsible for the enhanced degradation of vinclozolin.

The objective of this study was to determine the degradation of these dicarboximidic fungicides in vine-

Table 1. Physicochemical Properties of the Soils in Study

property	soil				
	1	2	3	4	5
humidity (%)	22.8	16.7	19.1	12.7	18.9
active lime (% CaCO ₃)	6.0	5.5	16.0	0.5	3.0
salinity (mS/cm)	0.523	1.875	0.498	0.410	0.448
soluble salts (%)	0.03	0.12	0.03	0.03	0.03
pH	8.05	7.98	8.35	8.15	8.35
organic carbon (%)	0.96	0.63	0.61	0.65	0.38
organic matter (%)	1.66	1.09	1.06	1.12	0.66
texture	clay-loam	silt-loam	loam	sandy-loam	sandy-clay-loam
total N (%)	0.17	0.13	0.11	0.16	0.11
assimilable P (mg/kg)	12.4	21.8	8.5	17.1	8.1
cation-exchange capacity (meq/100 g)	32.0	20.8	20.0	27.1	28.2
exchangeable K ⁺ (meq/100 g)	1.20	1.28	0.69	0.97	0.55
exchangeable Mg ²⁺ (meq/100 g)	1.45	1.98	0.39	3.56	3.96
exchangeable Na ⁺ (meq/100 g)	1.00	1.53	0.20	1.07	1.39
exchangeable Ca ²⁺ (meq/100 g)	27.01	15.03	18.36	19.45	21.39

yard soils of Penedès under laboratory conditions, after three treatments at 100-day intervals, like those used by viticulturists to prevent vine diseases. These treatments may yield deposits in soils of different quantities of these products that could provide information on the degree of persistence in the soil environment.

MATERIALS AND METHODS

Standards and Formulations. Iprodione and procymidone at 99.0% purity and vinclozolin at 99.6% purity were obtained from Dr. Ehrenstorfer at Promochem (Germany).

Wettable powder formulations of Rovral (registered name for iprodione), 50% active ingredient; Sumisclax (registered name for procymidone), 50% active ingredient; and Ronilan

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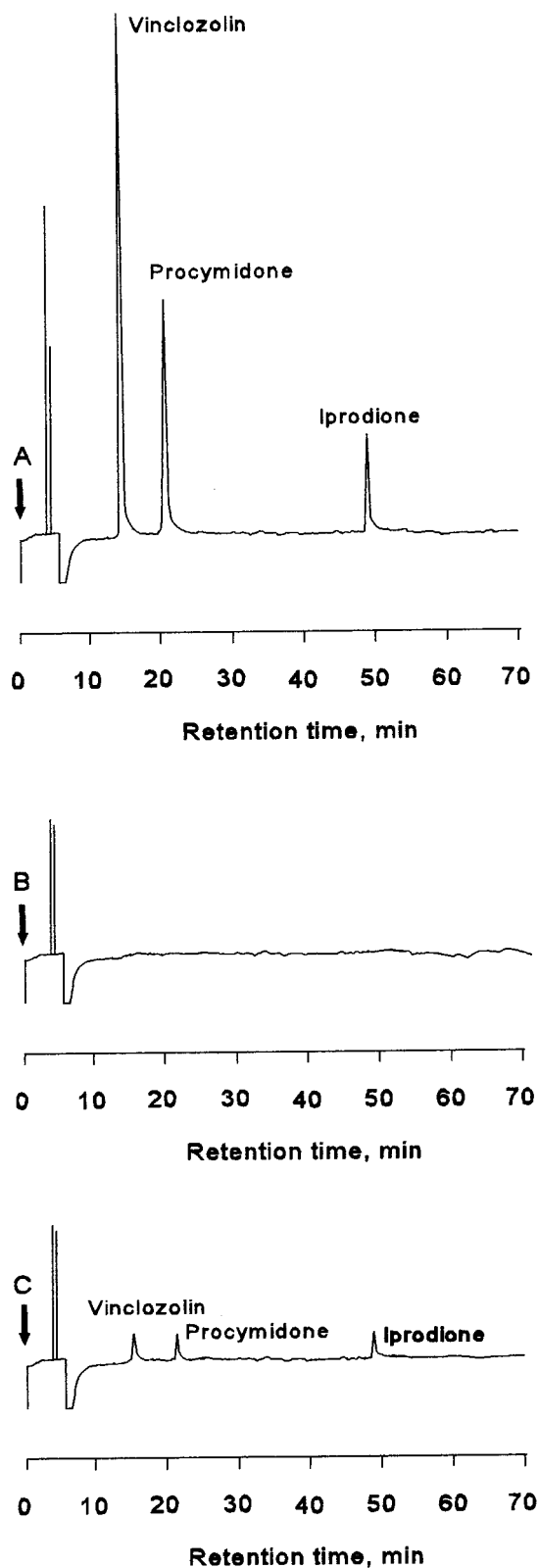


Figure 1. Chromatograms of a mixed standard working solution (A), a blank soil sample (B), and a soil sample fortified with standard dicarboximidic fungicides at a level close to their detection limit: iprodione, 11 $\mu\text{g}/\text{kg}$; procymidone, 5 $\mu\text{g}/\text{kg}$; vinclozolin, 3 $\mu\text{g}/\text{kg}$ (C). Chromatographic conditions: SPB-5 fused silica capillary column, 30 m \times 0.25 mm i.d., 0.25 μm film; column temperature, 165–200 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/\text{min}$ and hold 200 $^{\circ}\text{C}$ for 55 min; flow rate, 1 mL/min, He; detection, ECD; sample, 1 μL ; split ratio, 100/1.

Table 2. Recoveries of Dicarboximidic Fungicides

fungicide	fortification level (mg/kg)	% recovery \pm SD ^a
iprodione	0.500	87.3 \pm 3.7
	1.500	87.1 \pm 4.0
	3.000	86.1 \pm 5.4
	4.500	89.3 \pm 2.5
	\bar{x}	87.4 \pm 3.6
procymidone	0.500	83.3 \pm 2.9
	1.500	84.5 \pm 6.6
	3.000	86.3 \pm 5.1
	4.500	86.1 \pm 0.7
	\bar{x}	85.0 \pm 4.0
vinclozolin	0.500	84.7 \pm 6.9
	1.500	83.6 \pm 4.0
	3.000	81.8 \pm 3.3
	4.500	81.1 \pm 3.5
	\bar{x}	82.9 \pm 4.2

^a Each value was the mean of three separate determination.

(registered name for vinclozolin), 50% active ingredient, were used. The dose of each fungicide used was 4 mg of active ingredient/kg of soil, which was administered by spraying 50 mL of aqueous suspension (160 mg/L of commercial formulation).

Soil Sampling Plan. For this study we selected five fields of 3 ha with different types of soil to obtain reliable information on different vineyard zones of the Penedès with a known fungal treatment history.

To choose the sampling points, we used the systematic or grid sampling method (Martin, 1993), which attempts to guarantee complete coverage of soil composition because sampling points are located at regular intervals on a grid.

Sampling and Sample Preparation. From each field, 12 samples of 2.5 kg were taken from the first 20-cm depth of the soil. These samples were mixed and taken as representative of the quality and composition of the soil. Labeled polyethylene bags were used to store samples before reduction.

At the laboratory soils were air-dried at 20 $^{\circ}\text{C}$ with a relative humidity of 20% (Jackson, 1962). After air-drying, the soils were ground and sieved through a 2-mm mesh. The materials obtained constitute the fine earth fraction, which was stored in labeled polyethylene bags at room temperature.

In separate samples of each soil, physical and chemical analyses were performed to obtain a correct pedologic characterization. The soils were of the following textures: clay loam, silt loam, loam, sandy loam, and sandy clay loam (Day, 1965) with 0.96, 0.63, 0.61, 0.65, and 0.38% organic carbon, respectively (Jackson, 1962). Soil pH values (Walker, 1987), measured using a glass electrode in a 1:2.5 suspension of the soil in distilled water, were 8.05, 7.98, 8.35, 8.15, and 8.35, respectively, in the different samples. The main properties of the soils studied are given in Table 1.

The degradation of the fungicides was studied on 18 separate subsamples of 1 kg of each soil. Each subsample was placed in a 30 \times 25 \times 8 cm polypropylene container at 20 $^{\circ}\text{C}$ and adjusted to 18% relative humidity (mean value of humidity of soils studied) by periodic additions of water. The soils were mixed thoroughly.

A suspension of the commercial formulation of each pesticide in water was then applied to the soil subsamples to give an initial concentration of 4.0 mg/kg.

Six subsamples were treated with iprodione, six with procymidone, and six with vinclozolin. These treatments were performed three times at 100-day intervals.

Triplicate samples of soil (10 g) from each container were taken immediately after each application and then 7, 14, 21,

Table 3. Degradation of Iprodione in Soil

applications	days elapsed	field soil 1		field soil 2		field soil 3		field soil 4		field soil 5	
		\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)
first	0	3.851	0.0	4.167	0.0	3.942	0.0	4.025	0.0	3.990	0.0
	7	2.862	25.7	3.153	24.3	3.140	20.4	3.007	25.3	2.523	36.8
	14	1.626	57.8	2.137	48.7	1.139	71.1	1.685	58.1	1.601	59.9
	21	0.818	78.8	1.112	73.3	0.685	82.6	0.754	81.3	0.478	88.0
	35	0.323	91.6	0.605	85.5	0.221	94.4	0.335	91.7	0.216	94.6
	56	0.061	98.4	0.091	97.8	0.036	99.1	0.070	98.3	0.030	99.3
	77	NQ ^b	100.0	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0
second	0	3.963	0.0	3.892	0.0	3.982	0.0	4.029	0.0	3.974	0.0
	7	1.830	53.8	1.871	51.9	1.779	55.3	1.986	50.7	1.844	53.6
	14	0.495	87.5	0.398	89.8	0.434	89.1	0.407	89.9	0.527	86.7
	21	0.279	93.0	0.338	91.3	0.313	92.1	0.311	92.3	0.407	89.8
	35	0.081	98.0	0.100	97.4	0.085	97.9	0.132	96.7	0.154	96.1
	56	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0
	77										
third	0	3.982	0.0	3.978	0.0	4.051	0.0	4.046	0.0	4.098	0.0
	7	0.344	91.4	0.679	82.9	0.423	89.6	0.426	89.5	0.363	91.1
	14	0.218	94.5	0.067	98.3	0.058	98.6	0.192	92.8	0.111	97.3
	21	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0
	35										
	56										
	77										

^a Mean of 18 determinations. ^b NQ, not quantifiable.

Table 4. Degradation of Procymidone in Soil

applications	days elapsed	field soil 1		field soil 2		field soil 3		field soil 4		field soil 5	
		\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)
first	0	4.013	0.0	4.047	0.0	4.027	0.0	4.083	0.0	3.968	0.0
	7	3.324	17.2	3.320	18.0	3.216	20.1	3.345	18.1	3.212	19.0
	14	2.071	48.4	2.183	46.1	1.979	50.9	2.036	50.1	1.968	50.4
	21	1.410	64.9	1.501	62.9	1.221	69.7	1.456	64.3	1.241	68.7
	35	0.550	86.3	0.611	84.9	0.389	90.3	0.561	86.3	0.422	89.4
	56	0.107	97.3	0.194	95.2	0.070	98.3	0.152	96.3	0.062	98.4
	77	NQ ^b	100.0	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0
second	0	4.019	0.0	3.992	0.0	4.148	0.0	4.015	0.0	4.036	0.0
	7	1.777	55.8	1.860	53.4	1.869	54.9	1.818	54.7	1.871	53.6
	14	0.554	86.2	0.558	86.0	0.532	87.2	0.548	86.4	0.563	86.0
	21	0.359	91.1	0.406	89.8	0.403	90.3	0.462	88.5	0.427	89.4
	35	0.086	97.9	0.137	96.6	0.095	97.7	0.142	96.5	0.100	97.5
	56	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0
	77										
third	0	3.994	0.0	4.085	0.0	4.046	0.0	4.087	0.0	4.030	0.0
	7	0.425	89.4	0.447	89.1	0.438	89.2	0.445	89.1	0.429	89.4
	14	0.076	98.1	0.102	97.5	0.065	98.4	0.065	98.4	0.070	98.3
	21	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0
	35										
	56										
	77										

^a Mean of 18 determinations. ^b NQ, not quantifiable.

35, 56, and 77 days later for residual pesticide analysis. Samples that could not be analyzed immediately were stored at $-15\text{ }^{\circ}\text{C}$.

Extraction Procedure. Ten grams of homogeneous soil sample was placed into a 1×13 cm centrifuge tube, and 10 mL of cyclohexane was added. The tube was shaken for 15 min, placed in an ultrasound bath for 10 min, and then centrifuged for a further 15 min at 4500 rpm.

Method. Gas chromatography was performed as described by Alonso (1981) with modifications to improve the method and adapt it to soil samples.

One microliter of the organic phase was injected into a Hewlett-Packard chromatograph (model 5890) equipped with a Ni electron capture detector (ECD), under the following conditions: fused silica open tubular column, 0.25 mm i.d. \times 30 m in length, coated with SPB-5, 0.25- μm film; oven temperature programmed to rise from 165 to 200 $^{\circ}\text{C}$ at 2 $^{\circ}\text{C}/$

min and hold at 200 $^{\circ}\text{C}$ for 55 min; injector temperature, 250 $^{\circ}\text{C}$; detector temperature, 300 $^{\circ}\text{C}$; carrier gas, helium, and auxiliary gas, argon/methane (95:5), with a flow rate of ~ 1 mL/min; split ratio, 100/1. The chromatograms shown in Figure 1 were obtained under these conditions.

RESULTS AND DISCUSSION

The retention times were 50.1 min for iprodione, 21.4 min for procymidone, and 14.2 min for vinclozolin. The detection limits were 11 $\mu\text{g}/\text{kg}$ for iprodione, 5 $\mu\text{g}/\text{kg}$ for procymidone, and 3 $\mu\text{g}/\text{kg}$ for vinclozolin. The precision of the modified method was verified on the basis of the results obtained from 10 determinations of a soil sample. The average contents for iprodione, procymidone, and vinclozolin were, respectively, 2.119 ± 0.193 , $1.943 \pm$

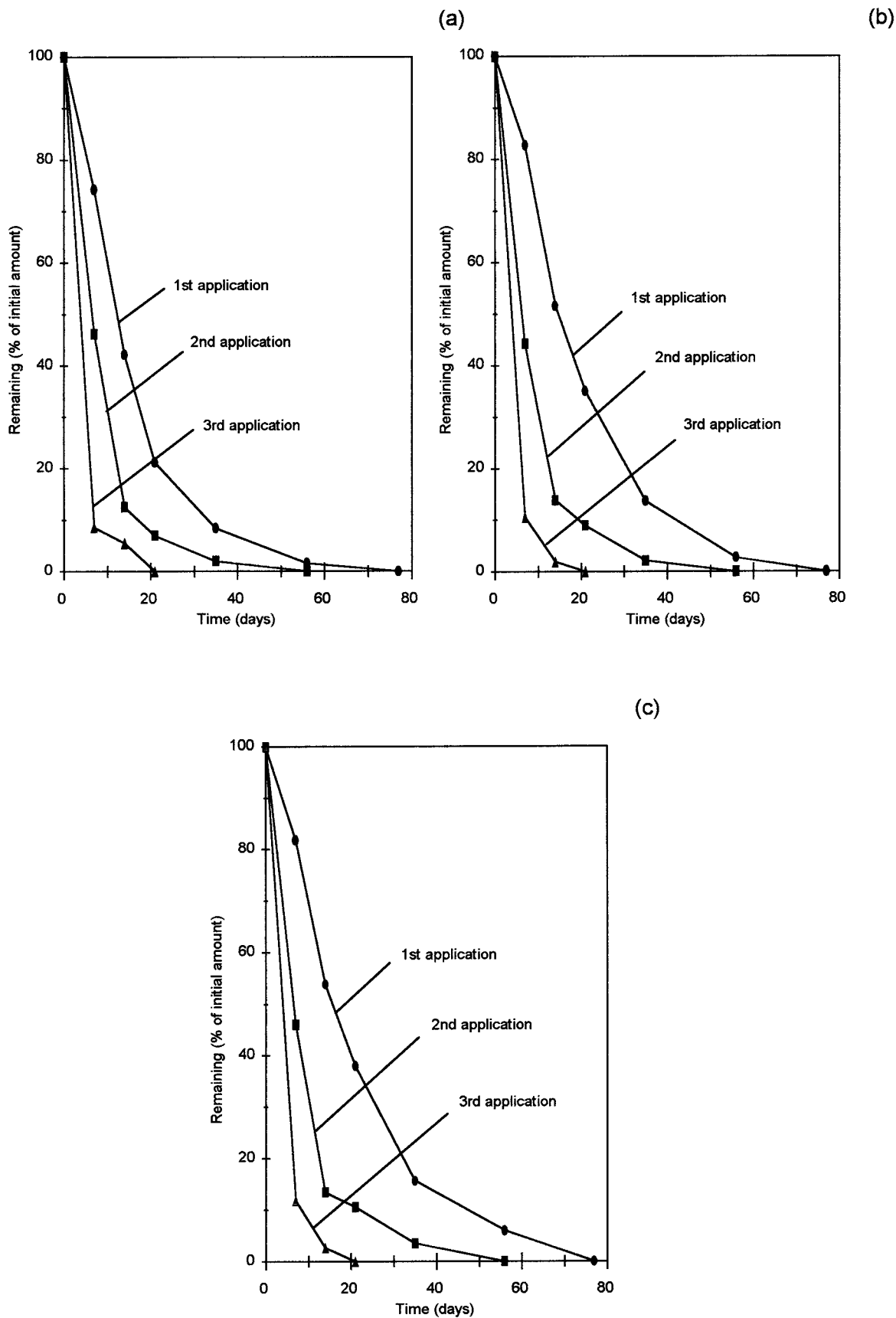


Figure 2. Degradation of (a) iprodione, (b) procymidone, and (c) vinclozolin in field soil 1.

0.136, and 1.969 ± 0.119 mg/kg. The relative standard deviations (RSD) were 9.1, 7.0, and 6.1%, respectively, which is acceptable according to Horwitz (1983). Recoveries were determined with untreated samples forti-

fied at four addition levels: 0.5, 1.5, 3.0, and 4.5 mg/kg (Table 2). For iprodione the mean recovery in soils was $87.4 \pm 3.6\%$, for procymidone, $85.0 \pm 4.0\%$, and for vinclozolin, $82.9 \pm 4.2\%$.

Table 5. Degradation of Vinclozolin in Soil

applications	days elapsed	field soil 1		field soil 2		field soil 3		field soil 4		field soil 5	
		\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)	\bar{x}^a (mg/kg)	degradation (%)
first	0	4.070	0.0	4.006	0.0	4.022	0.0	4.056	0.0	3.986	0.0
	7	3.332	18.1	3.288	17.9	3.301	17.9	3.329	17.9	3.279	17.7
	14	2.195	46.1	2.243	44.0	2.053	48.9	2.149	47.0	2.046	48.7
	21	1.546	62.0	1.569	60.8	1.409	65.0	1.496	63.1	1.405	64.8
	35	0.639	84.3	0.674	83.2	0.528	86.9	0.602	85.2	0.508	87.3
	56	0.246	94.0	0.233	94.2	0.151	96.2	0.203	95.0	0.141	96.5
	77	NQ ^b	100.0	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0
second	0	3.999	0.0	4.084	0.0	4.115	0.0	3.983	0.0	4.028	0.0
	7	1.839	54.0	1.805	55.8	1.921	53.3	1.802	54.8	1.880	53.3
	14	0.539	86.5	0.504	87.7	0.575	86.0	0.526	86.8	0.561	86.1
	21	0.424	89.4	0.427	89.5	0.442	89.3	0.421	89.4	0.412	89.8
	35	0.138	96.5	0.130	96.8	0.123	97.0	0.117	97.1	0.152	96.2
	56	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0
	77										
third	0	4.006	0.0	4.060	0.0	4.008	0.0	4.042	0.0	4.016	0.0
	7	0.478	88.1	0.433	89.3	0.403	89.9	0.426	89.5	0.442	89.0
	14	0.109	97.3	0.077	98.1	0.089	97.8	0.091	97.8	0.122	97.0
	21	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0	NQ	100.0
	35										
	56										
	77										

^a Mean of 18 determinations. ^b NQ, not quantifiable.

Table 6. Summary of Fungicide Degradation Data According to the Student–Newman–Keul's Test (SNK)

fungicide	treatment		
	first	second	third
iprodione	C ^a	F	G
procymidone	B	E	H
vinclozolin	A	D	I

^a Treatments indicated by a different letter are significantly different ($p < 5\%$, $p =$ probability).

The residual levels of iprodione, procymidone, and vinclozolin obtained in the analysis of six subsamples of soils from fields 1–5, after the first, second, and third applications, are shown respectively in Tables 3, 4, and 5 and in Figure 2. Each value is the mean of three determinations.

The results showed that the degradation rate for all three fungicides increased with the number of applications: the time elapsed for 90% of loss was approximately 35 days in the first treatment, 21 days in the second treatment, and 7 days in the third treatment. No residual pesticide levels were detected at 77 days. Therefore, the degradation rate for all three fungicides in soils was stimulated by previous treatments.

The SYSTAT program (v. 7.0) was used to compare the overall results obtained for the degradation of fungicide residues and the soils of each field, the behavior of each fungicide, and the number of treatments.

Analysis of variance (ANOVA) indicates clear differences between kinetics of degradation and type of soil ($p = 0.0\%$, $p =$ probability) and also between kinetics of degradation and order of application ($p = 0.0\%$).

According to the Student–Newman–Keul's test (SNK), after each treatment there were significant differences among the means of iprodione, procymidone, and vinclozolin degradation residues ($p < 5\%$). To provide a better understanding, we have used a separation code, in which treatments indicated by different letters are significantly different (Table 6).

To summarize, the residues of iprodione, procymidone, and vinclozolin in field soils decrease with time.

The degradation rate is also influenced by the number of applications: it is faster on the second application and more so after the third.

In the study of the possible correlation between the degradation rate and physicochemical properties of the soils, we can conclude that, in general, an increase in the pH or Ca/K ratio in the soil is associated with an acceleration of the fungicide degradation. On the other hand, the fungicide degradation is delayed when the concentrations of K and P in the soil increase. Other influencing factors could be the soil microorganisms or enzymatic systems that need some adaptation time to the pesticides as substrate before rapid degradation can proceed (Cain and Mitchell, 1996).

We believe that the data offered here can be considered as a further contribution to the study of the degree of fungicide persistence in vineyard soils and perhaps the start of more detailed further studies.

LITERATURE CITED

- Alonso, A. Análisis de residuos de pesticidas en vino (Analysis of pesticide residues in wine.). *Quim. Ind.* **1981**, 27 (2), 103–105.
- Bolay, A.; Rochaix, M. Fight against gray mold *Botrytis cinerea*. Effects of new fungicides. *Bull. O.I.V.* **1976**, 49, 106–129.
- Cain, B.; Mitchell, A. Enhanced degradation of the fungicide vinclozolin: isolation and characterization of a responsible organism. *Pestic. Sci.* **1996**, 48 (1), 13–23.
- Day, P. R. *Particle Fractionation and Particle-Size Analysis*; Agronomy 9, Part I; American Society of Agronomy: Madison, WI, 1965.
- Garcia-Cazorla, J.; Xirau-Vayreda, M. Persistence of Dicarboximide Fungicide Residues in Grapes, Must, and Wine. *Am. J. Enol. Vitic.* **1994**, 45 (3), 338–340.
- Horwitz, W. Report of the Committee on Laboratory Studies. *J. Assoc. Off. Anal. Chem.* **1983**, 66 (2), 455–466.
- Jackson, M. L. *Soil Chemical Analysis*; Constable: London, 1962.
- Martin, R. *Soil Sampling and Methods of Analysis*; Canadian Society of Soil Science; Lewis Publishers: Chelsea, MI, 1993.

Mitchell, A.; Cain B. Rapid onset of the accelerated degradation of dicarboximide fungicides in a UK soil with a long history of agrochemical exclusion. *Pestic. Sci.* **1996**, *48* (1), 1–11.

Slade, A.; Fullerton, R.; Stewart, A.; Young, H. Degradation of the dicarboximide fungicides iprodione, vinclozolin and procymidone in Patumahoe clay loam soil, New Zealand. *Pestic. Sci.* **1992**, *35* (1), 95–100.

Tomlin, C., Ed. *The Pesticide Manual*, 10th ed.; British Crop Protection Council (BCPC): Faenham, U.K., 1995.

Walker, A. Further Observations on the Enhanced Degradation of Iprodione and Vinclozolin in Soil. *Pestic. Sci.* **1987**, *21*, 219–231.

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