JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fungicide Dissipation Curves in Winemaking Processes with and without Maceration Step

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The evolution of residual levels of four fungicides (cyprodinil, fludioxonil, pyrimethanil, and quinoxyfen) during the elaboration of three types of wine with maceration (traditional red wine, carbonic maceration red wine, and red wine of long maceration and prefermentation at low temperature) and two types of wine without maceration (rosé and white) has been studied. The disappearance curves of each fungicide have been analyzed during the period of each winemaking process (21 days) and during the different enological steps involved in the elaborations. The residual levels of fludioxonil reduce most quickly during the winemaking processes without maceration, whereas the decrease in levels of pyrimethanil was the slowest in practically all cases (with and without maceration). During carbonic maceration winemaking, the decay constant of cyprodinil was greater than that of the other pesticides in all assays (time and steps).

KEYWORDS: Fungicide residues; dissipation curves; winemaking; maceration; constant rate

INTRODUCTION

The main distribution area of the grapevine is in European countries. Among these, France, Italy, and Spain control the sector on an international scale. Spain dedicates the largest surface in the world to the cultivation of the grapevine (1230 $\times$ 10^3 ha, in 2001). The major part of this surface is inscribed in 56 Apellations d'Origine Controlles (AOC). Three of these belong to Murcia (southeastern Spain), an area of peculiar climatic characteristics that favor the development of pests and diseases. The principal parasites of the vine are the grape moth (*Lobesia botrana*), downy mildew (*Plasmopora viticola*), powdery mildew (*Uncinula necator*), and, on some occasions, gray mold (*Botrytis cinerea*) (*1*−*4*). To control these parasites, vine growers use insecticides and fungicides. This is important in maintaining grape productivity and wine quality. However, in many cases, when the dose and/or the established preharvest time for each product is not respected, hazardous residues are left, and these become a permanent danger to the quality of the wine, the environment, and consumer health (*5*−*14*). In this sense, the elaboration method, the correct winemaking processes, and the correct use of phytosanitary products are influential in the dissipation and elimination of the current residues in grapes and must.

Most studies on pesticide residues deal with the transformation from vine to wine, and the results reported show, on the one hand, their fate during vinification and the influence of each technological process on the residue amount and, on the other hand, that is almost impossible not to find residues in wine, albeit at very low or nondetectable levels (*15*−*26*).

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With this aim, this work was designed to study the evolution of four fungicides during the elaboration of wines obtained with and without maceration. The dissipation curves of each fungicide in each winemaking process were elaborated. These fungicides, two anilinopyrimidines (cyprodinil and pyrimethanil), a phenylpyrrole (fludioxonil), and a phenoxyquinoline (quinoxyfen), are the most frequently used to control diseases in the Jumilla area production.

MATERIALS AND METHODS

Phytosanitary Treatments and Sampling. Prior to the phytosanitary treatments, Monastrell and Airén grapes were collected from vineyards situated in Jumilla, Murcia (southeastern Spain) and delivered in plastic containers (15 kg per container). These grapes were sprayed with fungicide formulations at the recommended dose using a hand-gun applicator (**Table 1**). Two hours later, winemaking process were performed at laboratory scale following the usual wine production method applied in Jumilla. All assays were made in triplicate. In addition, a control of each vinification was made. Samples were taken in each step of the winemaking process to study the dissipation of fungicides during the elaborations.

Winemaking Processes. Among the different winemaking methods, conventional schemes of elaboration adopted in AOC Jumilla (Murcia, Spain) were used (**Figures 1**−**4**). For winemaking with maceration, 9 kg of grapes (Monastrell variety) was crushed and 80 mg/kg of sulfite was added. The crushed harvest was allowed to ferment with skins for 4 days in the case of the traditional winemaking method, for 4 days at 5 °C and 6 at room temperature in the case of long maceration winemaking, and for 10 days in an atmosphere saturated with carbon dioxide in the carbonic maceration process. The mixtures were then pressed to separate the skins. At the end of fermentation, the wine was separated from the lees and clarified with bentonite plus gelatin (40 g/hL and 8 mL/hL, respectively). Clarified wine was filtered by nylon filters.

10.1021/jf040299v CCC: $30.25
Published on Web 01/07/2005

Table 1. Phytosanitary Products and Treatment Dose

active ingredient	formulation	dose	preharvest time (days)	MRL[a] (mg/kg)
cyprodinil	Switch WG	100 g/hL	21	2
fludioxonil	Switch WG	100 g/hL	21	1
pyrimethanil	Scala 40% SC	200 cm^3/hL	21	5
quinoxyfen	Arius 25% SC	30 cm^3/hL	30	1

[a] Established for wine grapes by Spanish legislation.

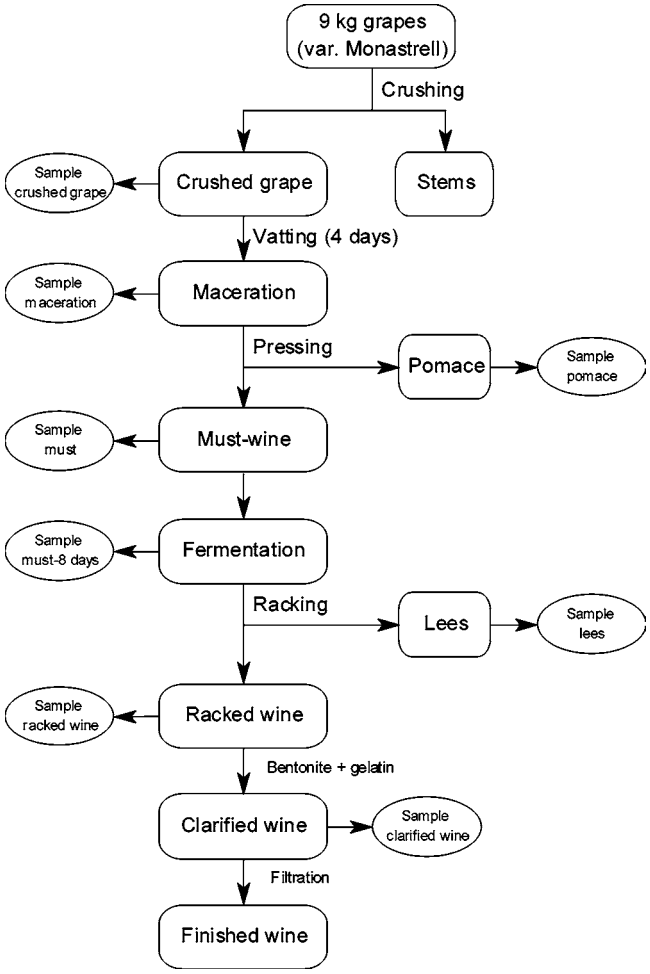

Figure 1. Scheme for traditional winemaking used in this study and sampling points.

For rosé winemaking, the same weight of Monastrell grapes was used. Grapes were crushed and pressed to obtain the must. The must was clarified before beginning fermentation, at 5 °C during 36 h. After alcoholic fermentation of the clarified must, the wine was racked to separate it from lees and clarified with bentonite plus potassium caseinate (40 g/hL and 6 g/hL, respectively). A nylon filter was used for filtration.

White wines were obtained with the same method as employed for rosé wines but with 9 kg of Airén variety grapes.

Pesticides and Reagents. Cyprodinil [*N*-(4-cyclopropyl-6-methylpyrimidim-2-yl)aniline], fludioxonil [4-(2,2-difluoro-1,3-benzodioxol-4-yl)pyrrole-3-carbonitrile], pyrimethanil [*N*-(4,6-dimethylpyrimidin-2-yl)aniline], and quinoxyfen (5,7-dichloro-4-quinolyl-4-fluorophenyl ether) analytical standards were purchased from Novartis Agro (cyprodinil and fludioxonil), Dr. Ehrenstorfer (pyrimethanil), and Dow Agro Sciences (quinoxyfen). These chemicals were at least 98.5% pure. Acetone, dichloromethane, hexane, isooctane, and toluene were used for pesticide residues (SDS, France), and sodium chloride was used for analytical grade (Panreac). Stock standard solutions containing all pesticides (≈100 mg/L each) were prepared in isooctane plus toluene

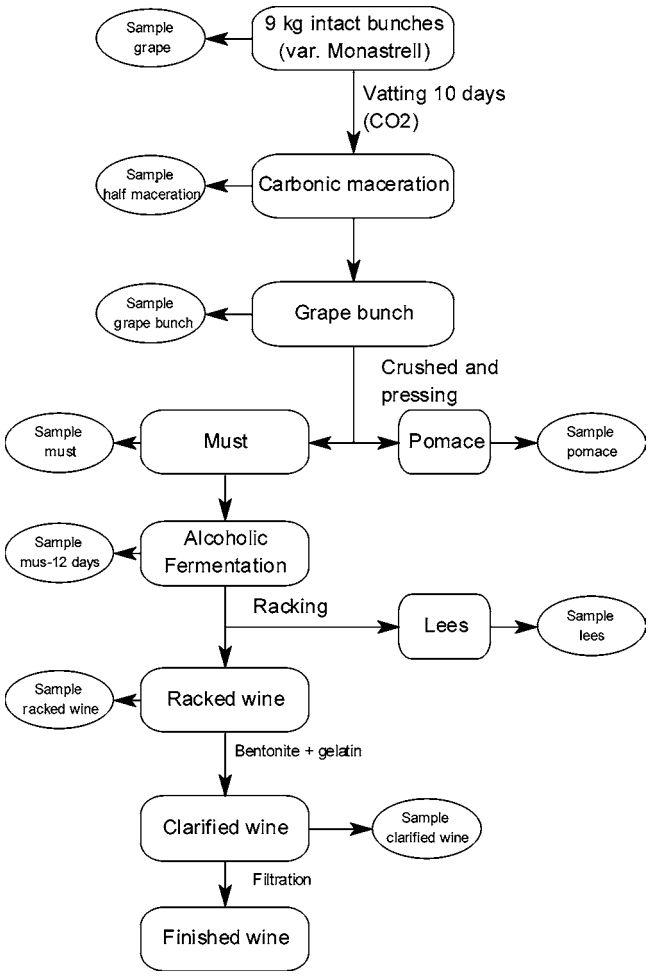

Figure 2. Scheme for carbonic maceration winemaking used in this study and sampling points.

(1+1, by volume). Several dilutions were prepared to check the linearity of response of detectors and to obtain the detection limits in each case by dilution in the same solvent.

Extraction Process. For the extraction of cyprodinil, fludioxonil, pyrimethanil, and quinoxyfen, an on-line microextraction process was used. The plant material is extracted with an acetone/dichloromethane mixture (cyprodinil, fludioxonil, and pyrimethanil) and hexane (quinoxyfen), followed by filtering and concentration of the obtained extract.

(a) Extraction of Cyprodinil, Pyrimethanil, and Fludioxonil from Grapes and Pomace. Five gram samples were homogenized at 8000 rpm during 3 min in a high-speed electric mixer (Polytron-Aggregate, Kinematica, Germany) with 30 mL of acetone/dichloromethane (1:1, v/v) and 2 g of NaCl resulting. The homogenized mixture was filtered through 1 PS Phase Separator paper (Whatman 2100150 1 PS). The filter was washed with 10 mL of the mixture solvent. All of the fractions were picked up in a concentration flask and concentrated to dryness by rotary vacuum evaporation. The dry extract was dissolved in 5 mL of isooctane/toluene (1:1, v/v).

(b) Extraction of Cyprodinil, Pyrimethanil, and Fludioxonil in Must, Wine, and Lees. Five milliliters of must or wine, 20 mL of acetone/dichloromethane (1:1, v/v), and 2 g of NaCl were placed in a glass flask of 30 mL with hermetic closing. The mixture was homogenized for 10 min in an ultrasonic bath (Ultrasons 613, Selecta) and passed though 1 PS Phase Separator paper (Whatman 2100150 1 PS). Flasks and filters were washed with 10 mL of mixture solvent. Organic fractions were evaporated through rotary vacuum evaporation, and the residue was dissolved in 5 mL of isooctane/toluene (1:1, v/v).

(c) Extraction of Quinoxyfen in Grapes, Pomace, Must, Wine, and Lees. Ten grams or milliliters of plant material and 20 mL of hexane were homogenized for 30 min in a rotary shaker (Unite-Mixer Lab Line 1306, Biomedical Products, Inc.). A 10 mL aliquot of the organic

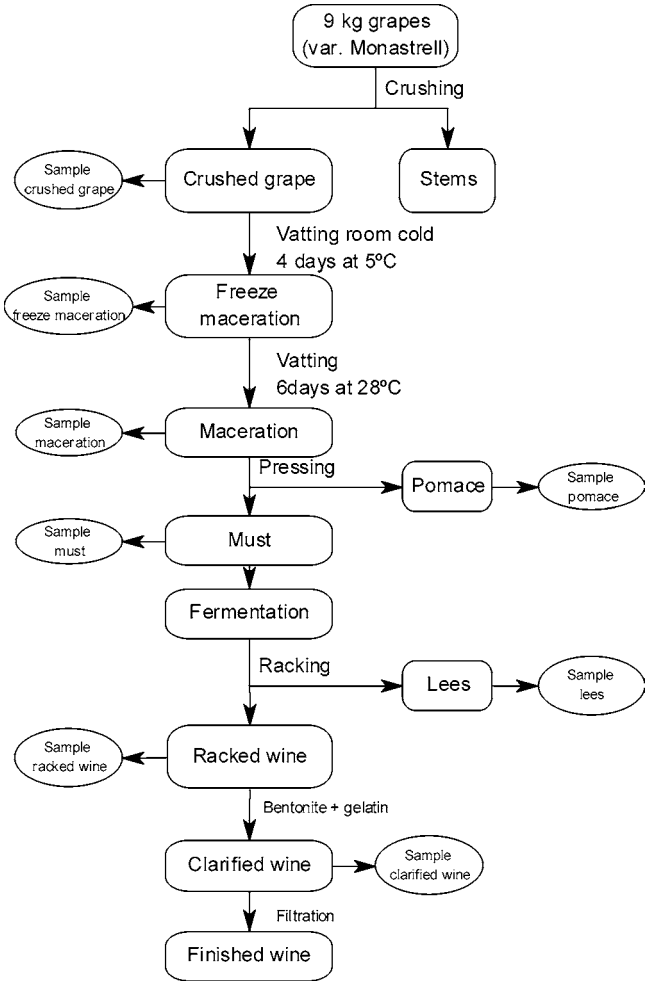

Figure 3. Scheme for long maceration and prefermentative at low-temperature winemaking used in this study and sampling points.

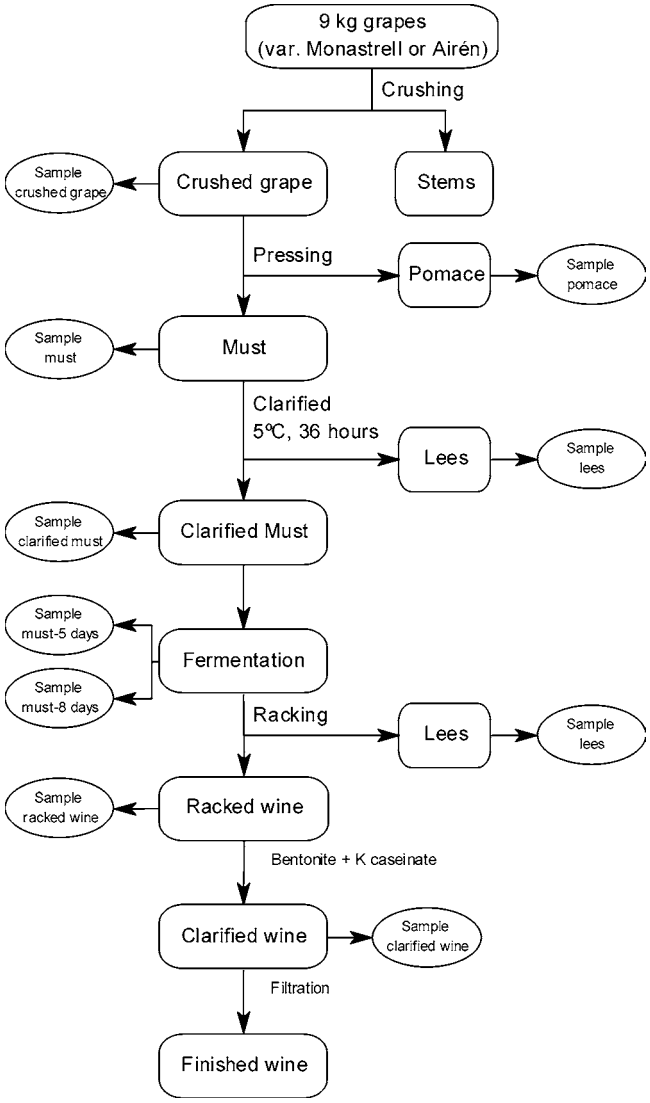

Figure 4. Scheme for rosé (var. Monastrell) and white (var. Airén) winemakings used in this study and sampling points.

phase was concentrated to dryness by rotary vacuum evaporation and dissolved in 5 mL of isooctane/toluene (1:1, v/v).

Recovery Assays. To determine percentage recoveries of cyprodinil, fludioxonil, pyrimethanil, and quinoxyfen, untreated grapes, must, and wine were spiked with the fungicides tested and processed according to the process described above. Recovery assays were carried out at 0.01−0.5 mg/kg. The results of recovery analysis were compared with standard dilutions used in the fortification. At each fortification level five replicates were analyzed. To verify the absence of matrix effect, a blank of each sample (grape, must, and wine) was made.

Pesticide Analysis. Cyprodinil, fludioxonil, and pyrimethanil were determined by GC with a nitrogen−phosphorus detector (NPD), using a Hewlett-Packard 6890 gas chromatograph equipped with a NPD, a split−splitless injector, an autosampler HP-6890 (Hewlett-Packard), and an HP Chemstation system (Hewlett-Packard). The capillary column was an HP-5 fused-silica (Hewlett-Packard) packed with 5% diphenyl and 95% methyl siloxane (30 m × 0.32 mm i.d.; film thickness, 0.25 μm). The injector and detector were operated at 250 and 300 °C, respectively. The oven operating temperatures were as follows: 90 °C for 1 min, programming rate at 10 °C/min (from 90 to 180 °C), held for 1 min, 1 °C/min (from 180 to 205 °C), and 30 °C/min from 205 to 250 °C. N_2 was the carrier and makeup gas at 1 and 9 mL/min, respectively. The plasma of the detector was obtained with H_2 (3 mL/min) and air (60 mL/min). The sample (2 μL) was injected in the splitless mode. The total run time was 37.50 min (*27*).

Quinoxyfen was determined by GC with a ^{63}Ni electron-capture detector (ECD). An HP Vectra VL integrator was used in combination with the gas chromatograph (Autosystem Perkin-Elmer). The column used was an HP-5 fused-silica (Hewlett-Packard) with the same characteristics as the one previously described. The injector and detector temperatures were set at 200 and 350 °C, respectively. The sample (2 μL) was injected in the splitless mode, and the oven temperature was programmed as follows: 90 °C for 1 min, raised to 280 °C (20 °C/min), and held for 9 min. Both carrier and makeup gases were N_2, at 3 and 60 mL/min, respectively. The total run time was 19.50 min.

Statistics. The informatics pack SPSS version 11.0 for Windows was used for descriptive statistics and linear fit of the data for the dissipation of fungicides studies.

RESULTS AND DISCUSSION

Analytical Method Efficiency. The described methods of analysis of cyprodinil, fludioxonil, pyrimethanil, and quinoxyfen residues in grape, must, and wine are relatively simple, and no cleanup of the extracts is necessary. The detector response was linear in the range of the concentrations researched (0.01−2 mg/kg for all active ingredients except for fludioxonil, which was 0.05−6 mg/kg), with correlation coefficients $r = 0.999$ ($n = 7$) for all pesticides. The precision of the detectors was also acceptable, with coefficients of variability of the repeatability and reproducibility ($n = 5$) oscillating between 0.8 and 8.6 for pyrimethanil and fludioxonil, respectively (repeatability), and ranging from 5.27 to 13.50 for pyrimethanil and quinoxyfen, respectively (reproducibility). The detection limits obtained were 0.02 ng for cyprodinil, pyrimethanil, and quinoxyfen and 0.10 ng for fludioxonil (**Table 2**).

Table 2. Linear Correlation Coefficients, Coefficients of Variability of Repeatability and Reproducibility ($n = 5$), and Detection Limits for the Tested Fungicides

active ingredient	linearity (r)	repeatability	reproducibility	detection limit (ng)
cyprodinil	0.999	1.1	6.59	0.02
fludioxonil	0.999	8.6	10.56	0.10
pyrimethanil	0.999	0.8	5.27	0.02
quinoxyfen	0.999	5.3	13.50	0.02

Table 3. Mean Recovery Percents ($n = 5$) and Variability Coefficients (% ± CV) for the Four Fungicides in Grape, Must, and Wine at Two Fortification Levels

fungicide	spike level (mg/L)	% ± CV grape	% ± CV must	% ± CV wine
cyprodinil	0.01	93.5 ± 17.5	103.2 ± 6.4	102.0 ± 3.0
	0.1	94.7 ± 8.7	101.7 ± 6.7	95.0 ± 6.4
fludioxonil	0.05	98.6 ± 17.2	100.8 ± 3.2	100.0 ± 5.1
	0.5	78.8 ± 6.2	107.0 ± 4.1	102.0 ± 1.9
pyrimethanil	0.01	92.6 ± 14.3	103.4 ± 5.7	99.0 ± 9.9
	0.1	89.3 ± 7.0	101.0 ± 1.0	96.7 ± 6.2
quinoxyfen	0.05	87.5 ± 6.3	96.3 ± 2.8	110.5 ± 13.7
	0.5	92.1 ± 4.9	91.6 ± 2.3	105.3 ± 7.6

All recovery values ($n = 5$) in grapes were >87.5% in all cases, with the exception of fludioxonil at the highest spiked level, for which the median value was 78.8%. In must and wine, the recoveries from fortified samples were in the range of 91.6−110.5%. The coefficients of variability in grapes, must, and wine were <10% in all cases except for cyprodinil and fludioxonil in grapes and for quinoxyfen in wine, for which these coefficients were >10% (**Table 3**).

The theoretical limits of determination calculated were 0.01 mg/kg for cyprodinil and pyrimethanil, 0.05 mg/kg for fludioxonil, and 0.005 mg/kg for quinoxyfen. All were far below the maximum residue limit (MRL) established by the different legislations for these compounds.

Dissipation of Residues. The residual concentrations found during the different elaborations are shown in **Tables 4−7**. To ascertain the dissipation rate of residues in each winemaking process, the experimental data have been fitted to the following mathematical model (*28*, *29*):

$$R_t = R_0 \, e^{-kt} \quad \text{(a)}$$

$$\text{Ln } R_t = \text{Ln } R_0 - Kt \quad \text{(b)}$$

In eq a, R_t is the residue concentration at time t (mg/kg), R_0 is the theoretical initial residue concentration at $t = 0$ (mg/kg), K is the fungicide decay constant, and t is the time elapsed since the phytosanitary treatment. In eq b, Ln R_0 and K are constants, and Ln R_t and t are variables; the second one depends on the first.

This type of analysis allows the behavior of fungicide residues during the winemaking process to be known, by showing the correlation that exists between the residual levels and the time and also the fungicide decay constants. These values of K represent the tendency of the residues of each fungicide to be reduced to a greater or lesser degree during the overall winemaking process depending on factors such as degradation, adsorption on skins and lees or on clarifying agents, variety of grape, and winemaking method, among others. In addition, it is also possible to study if the correlation existing between variables is more or less significant from the statistical point of view, through a statistical demonstration with the number of pairs of values used in the coefficient calculus. The following equation is then used (*30*):

$$t = |r| \sqrt{(n-2)} / \sqrt{(1-r^2)} \quad \text{(c)}$$

Equation c enables us to obtain a value of distribution of the Student t that can be compared to t tabulated values. If t calculated is superior to t tabulated, the correlation between both variables is statistically significant. In eq c, r represents the correlation coefficient and n the degrees of freedom.

Taking into consideration the arguments presented, two studies were carried out for each vinification. The first was made using the evolution of residual levels during the time of the vinification process and the second with the evolution of residual concentrations during the steps involved in the different winemaking methods.

The study based on the time employed in the transformation from grape to wine began with the crushing of grapes (first day) and concluded 21 days later (clarified wine). The results of the fit are presented in **Table 8**.

As can be seen from these data, the linear correlation between Ln R_t and the time was not good in all assays, with correlation coefficients <0.8 for cyprodinil in both winemakings without maceration (rosé and white) and for fludioxonil in the winemaking of long maceration. The most unfavorable coefficient was calculated for pyrimethanil in the elaboration of white wine ($r = 0.688$). The other values oscillated between 0.808 and 0.975, corresponding, respectively, to quinoxyfen in the elaboration of long maceration and fludioxonil in the elaboration by carbonic maceration. However, it is more important to determine whether the correlation between both variables is statistically significant than to ascertain the absolute value of the correlation coefficient (r). Thus, the data in **Table 8** indicate that in the winemakings without maceration (rosé and white), and with the exception of the pyrimethanil assay in the rosé winemaking, there is no statistically significant correlation between the quantity and the time, even though we have values for r that are in some cases >0.9.

For winemakings with maceration it is observed that independent of the type the only significant correlation between concentration and time is for cyprodinil and pyrimethanil.

In relation to fungicide decay constant values, we can establish a dissipation rate for the four fungicides. For each winemaking process it was as follows:

traditional (maceration of 4 days): F > Q > C > P

long maceration
(4 days at 5 °C and 6 at room temperature):
F > C > P > Q

carbonic maceration
(maceration of 10 days in CO_2 atmosphere):
C > F > Q > P

rosé and white wine: F > Q > C > P

For all winemaking methods, except carbonic maceration, fludioxonil was the fungicide that showed the highest constant value (K). For maceration carbonic winemaking the highest value of K corresponded to cyprodinil. Pyrimethanil decay constants were the lowest in all vinifications with the exception of the long maceration winemaking process, for which the lowest was presented for quinoxyfen.

Table 4. Residual Concentrations of Cyprodinil ($n = 3$) Found in the Different Control Stages of Each Vinification

	winemaking method				
stage	traditional	carbonic maceration	long maceration	rosé	white
grape (mg/kg)		3.12 ± 0.15			
half maceration (mg/kg)		2.63 ± 0.16			
grape bunch (mg/kg)		2.14 ± 0.21			
crushed grape (mg/kg)	3.67 ± 0.23		7.47 ± 4.61	2.67 ± 0.12	4.24 ± 0.16
freeze maceration (mg/kg)			5.82 ±1.08		
maceration (mg/kg)	3.21 ± 0.36		4.75 ± 4.53		
pomace (mg/kg)	7.41 ± 0.58	4.43 ± 0.28	14.63 ± 4.30	1.65 ± 0.09	2.47 ± 0.09
must (mg/L)	0.76 ± 0.08	0.33 ± 0.04	0.37 ± 0.22	4.78 ± 0.19	3.62 ± 0.21
lees (mg/kg)				7.88 ± 0.26	8.91 ± 0.35
clarified must (mg/L)				1.23 ± 0.07	0,97 ± 0,08
must 5 (mg/L)				0.71 ± 0.03	0,77 ± 0,06
must 8 (mg/L)	0.47 ± 0.03			0.43 ± 0.02	0,74 ± 0,03
must 12 (mg/l)		0.30 ± 0.02			
lees (mg/kg)	2.07 ± 0.12	0.27 ± 0.02	1.61 ± 0.05	6.08 ± 0.31	4,73 ± 0,25
racked wine (mg/L)	0.23 ± 0.02	0.26 ± 0.01	0.17 ± 0.003	0.34 ± 0.02	0,60 ± 0,07
not clarified wine (mg/L)	0.11 ± 0.01	0.20 ± 0.01	0.17 ± 0.01	0.36 ± 0.04	0,57 ± 0,02
clarified wine (mg/L)	0.11 ± 0.02	0.12 ± 0.02	0.16 ± 0.02	0.32 ± 0.03	0,40 ± 0,03

Table 5. Residual Concentrations of Fludioxonil ($n = 3$) Found in the Different Control Stages of Each Vinification

	winemaking method				
stage	traditional	carbonic maceration	long maceration	rosé	white
grape (mg/kg)		9.74 ± 1.23			
half maceration (mg/kg)		6.35 ± 0.76			
grape bunch (mg/kg)		5.88 ± 0.92			
crushed grape (mg/kg)	11.68 ± 1.20		4.67 ± 1.62	8.31 ± 0.89	12.50 ± 1.26
freeze maceration (mg/kg)			3.93 ± 0.76		
maceration (mg/kg)	10.03 ± 1.36		3.24 ± 2.91		
pomace (mg/kg)	21.06 ± 2.38	12.86 ± 1.29	9.60 ± 0.81	3.77 ± 0.31	4.55 ± 0.23
must (mg/L)	1.46 ± 0.32	4.02 ± 0.62	0.26 ± 0.00	17.90 ± 1.23	21.20 ± 1.35
lees (mg/kg)				44.20 ± 2.25	61.30 ± 3.12
clarified must (mg/L)				2.52 ± 0.32	1.32 ± 0.15
must 5 (mg/L)				<DL[a]	1.00 ± 0.13
must 8 (mg/L)	<DL			<DL	0.69 ± 0.16
must 12 (mg/L)		1.61 ± 0.15			
lees (mg/kg)	10.26 ± 0.98	2.23 ± 0.34	1.79 ± 0.23	20.10 ± 1.75	14.10 ± 0.56
racked wine (mg/L)	<DL	0.97 ± 0.15	<DL	<DL	<DL
not clarified wine (mg/L)	<DL	0.96 ± 0.13	<DL	<DL	<DL
clarified wine (mg/L)	<DL	0.64 ± 0.08	<DL	<DL	<DL

[a] DL, detection limit = 0.05 mg/kg.

Table 6. Residual Concentrations of Pyrimethanil ($n = 3$) Found in the Different Control Stages of Each Vinification

	winemaking method				
stage	traditional	carbonic maceration	long maceration	rosé	white
grape (mg/kg)		4.47 ± 0.49			
half maceration (mg/kg)		4.48 ± 0.56			
grape bunch (mg/kg)		4.15 ± 0.48			
crushed grape (mg/kg)	5.40 ± 0.36		5.35 ± 2.25	3.52 ± 0.36	5.18 ± 0.69
freeze maceration (mg/kg)			3.72 ± 1.05		
maceration (mg/kg)	5.22 ± 0.48		3.61 ± 0.88		
pomace (mg/kg)	16.41 ± 1.36	7.57 ± 0.56	12.02 ± 0.33	3.25 ± 0.21	5.32 ± 1.01
must (mg/L)	1.67 ± 0.15	1.76 ± 0.16	0.712 ± 0.11	6.69 ± 0.65	7.01 ± 0.85
lees (mg/kg)				11.10 ± 0.80	13.60 ± 1.58
clarified must (mg/L)				4.77 ± 0.46	3.30 ± 0.27
must 5 (mg/L)				3.09 ± 0.24	2.99 ± 0.36
must 8 (mg/L)	1.39 ± 0.20			2.77 ± 0.21	2.86 ± 0.41
must 12 (mg/L)		1.76 ± 0.19			
lees (mg/kg)	6.72 ± 1.05	1.49 ± 0.09	3.56 ± 1.00	14.60 ± 1.05	8.93 ± 1.23
racked wine (mg/L)	0.91 ± 0.09	1.79 ± 0.25	0.46 ± 0.05	1.95 ± 0.08	3.02 ± 0.15
not clarified wine (mg/L)	0.73 ± 0.12	1.39 ± 0.09	0.46 ± 0.10	1.80 ± 0.11	2.59 ± 0.26
clarified wine (mg/L)	0.66 ± 0.08	1.26 ± 0.08	0.43 ± 0.16	1.70 ± 0.09	2.58 ± 0.28

From the above classification, it can be deduced that the maceration process does not affect the order of the dissipation of the fungicides studied. However, on observing the *K* values we see that these are greater for fludioxonil when there is no maceration and greater for pyrimethanil when there is maceration. This is a consequence of the different values of dissolubility in water of the two products (1.8 mg/L for fludioxonil and 121 mg/L for pyrimethanil).

Table 7. Residual Concentrations of Quinoxyfen ($n = 3$) Found in the Different Control Stages of Each Vinification

	winemaking method				
stage	traditional	carbonic maceration	long maceration	rosé	white
grape (mg/kg)		0.240 ± 0.237			
half maceration (mg/kg)		0.216 ± 0.155			
grape bunch (mg/kg)		0.207 ± 0.142			
crushed grape (mg/kg)	0.838 ± 0.110		0.353 ± 0.001	0.421 ± 0.113	0.375 ± 0.038
freeze maceration (mg/kg)			0.280 ± 0.099		
maceration (mg/kg)	0.683 ± 0.591		0.272 ± 0.004		
pomace (mg/kg)	1.465 ± 0.566	0.044 ± 0.017	0.701 ± 0.102	0.227 ± 0.108	0.067 ± 0.028
must (mg/L)	0.157 ± 0.072	0.216 ± 0.085	0.172 ± 0.067	0.495 ± 0.097	0.341 ± 0.254
lees (mg/kg)				0.849 ± 0.290	1.536 ± 0.080
clarified must (mg/L)				0.179 ± 0.150	0.169 ± 0.097
must 5 (mg/L)				0.124 ± 0.093	0.113 ± 0.013
must 8 (mg/L)	0.103 ± 0.068			0.124 ± 0.000	0.099 ± 0.059
must 12 (mg/L)		0.083 ± 0.036			
lees (mg/kg)	0.801 ± 0.652	0.339 ± 0.304	0.108 ± 0.010	0.792 ± 0.673	1.217 ± 0.211
racked wine (mg/L)	<DL[a]	0.031 ± 0.000	<DL	<DL	<DL
not clarified wine (mg/L)	<DL	<DL	<DL	<DL	<DL
clarified wine (mg/L)	<DL	<DL	<DL	<DL	<DL

[a] DL, detection limit = 0.005 mg/kg.

Table 8. Statistical Parameters Derived from the Linear Fit of the Data during the Time Employed in Each Vinification (21 Days)

	traditional method			carbonic maceration method			long maceration			rosé method			white method		
fungicide	*r*	|*k*|	*t*	*r*	|*k*|	*t*	*r*	|*k*|	*t*	*r*	|*k*|	*t*	*r*	|*k*|	*t*
cyprodinil	0.879	0.211	3.684*	0.940	0.238	4.751*	0.869	0.242	3.505*	0.786	0.146	2.546	0.748	0.139	2.252
fludioxonil	0.890	0.688	1.947	0.975	0.210	7.605**	0.746	0.252	1.586	0.883	1.199	1.881	0.900	1.765	2.063
pyrimethanil	0.829	0.142	2.965*	0.919	0.136	4.020*	0.910	0.160	4.390*	0.854	0.090	3.280*	0.688	0.076	1.896
quinoxyfen	0.906	0.533	2.145	0.896	0.181	2.850	0.808	0.096	1.942	0.871	0.543	2.509	0.922	0.342	3.362

[a] r = correlation coefficient; $|k|$ = absolute value of constant rate; t = calculated value of t distribution for $P < 0.05$; significance of the correlation between variables: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 9. Statistical Parameters Derived from the Linear Fit of the Data during the Steps Envolved in Each Vinification

	traditional method			carbonic maceration method			long maceration method			rosé method			white method		
fungicide	*r*	|*k*|	*t*	*r*	|*k*|	*t*	*r*	|*k*|	*t*	*r*	|*k*|	*t*	*r*	|*k*|	*t*
cyprodinil	0.974	1.236	7.509**	0.947	1.254	5.511*	0.936	1.021	5.533**	0.962	0.990	6.066**	0.971	0.993	7.045**
fludioxonil	0.890	1.374	1.947	0.979	1.104	8.404**	0.834	0.968	2.135	0.868	1.198	1.746	0.900	1.770	2.061
pyrimethanil	0.957	0.858	5.679*	0.926	0.711	4.235*	0.942	0.683	5.596**	0.973	0.567	7.300**	0.946	0.565	5.072*
quinoxyfen	0.906	1.064	2.144	0.895	0.907	2.843	0.883	0.361	2.661	0.908	1.117	2.164	0.991	0.683	7.521

[a] r = correlation coefficient; $|k|$ = absolute value of constant rate; t = calculated value of t distribution for $P < 0.05$; significance of the correlation between variables: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The differences in the order of dissipation between traditional winemaking and that of long maceration with 4 days of prefermentative maceration at low temperature (5 °C) may be due to the low temperature during the first 4 days, temperatures which modify the levaduriforme population and affect the yeast metabolism and the fermentative kinetics of both the endogenous compounds of the must and the exogenous ones (pesticides) (*31*, *32*). Indeed, studies related to fermentation microflora show the capacity of some yeasts to degrade or adsorb residues of certain active materials and thus decrease their concentrations during the fermentative phase (*33*−*36*).

The second study was made by taking into account the steps of the winemaking process. For this purpose four to five winemaking steps for each elaboration were established as follows: *traditional winemaking process to obtain red wines*, crushing grapes (phase 0), maceration period (phase 1), must (phase 2), racked wine (phase 3), and clarified wine (phase 4); *winemaking process to obtain red wines of long maceration and prefermentary process at low temperature*, crushing grapes (phase 0), freeze maceration (phase 1), room-temperature maceration (phase 2), must (phase 3), racked wine (phase 4) and clarified wine (phase 5); *carbonic maceration winemaking to obtain red wine*, grape (phase 0), half maceration period (phase 1), must (phase 2), racked wine (phase 3), and clarified wine (phase 4); and *winemaking without maceration to obtain rosé and white wines*, crushing grapes (phase 0), must (phase 1), clarified must (phase 2), racked wine (phase 3) and clarified wine (phase 4).

In this case, the linear fit of results was performed by taking into consideration the enological steps of each winemaking process; that is, the relationship between each phase and the preceding phase was based not on the time elapsed but on the residual values linked to the stage in question. Thus, the time (t) was substituted for the steps in eqs a and b. The results of the study are shown in **Table 9**.

In **Table 9**, correlation coefficients superior to that calculated for time were observed in all cases, except for fludioxonil in the rosé winemaking process ($r = 0.868$ vs $r = 0.883$). All values varied between a minimum of 0.868 (fludioxonil in rosé winemaking) and a maximun of 0.991 (quinoxyfen in white winemaking). The correlation between the residues of cyprodinil and pyrimethanil and the different steps of all winemaking

process was statistically very significant. On the contrary, with the exception of winemaking with carbonic maceration, the disappearance of quinoxyfen residues during the steps of the winemaking process was not significant in any of the cases.

With regard to the calculated values of the constant rate, the following dissipation rate was found for each vinification:

traditional (maceration of 4 days): F > C > Q > P

long maceration
(4 days at 5 °C and 6 at room temperature):
C > F > P > Q

carbonic maceration
(maceration of 10 days in CO_2 atmosphere):
C > F > Q > P

rosé: F > Q > C > P

white wine: F > C > Q > P

In general, the results obtained were very similar to those observed in the other study (for time). Fludioxonil showed the highest decay constant values in the winemaking process without maceration and in the traditional winemaking, and pyrimethanil presented the lowest in all cases with the exception of the long maceration winemaking process.

In this study differences existed between the winemaking processes with and without maceration for cyprodinil and fludioxonil. In vinifications with maceration cyprodinil showed higher constant rates than in vinifications without this maceration phase. However, decay constant values of fludioxonil were superior in rosé and white winemaking process than in the process with maceration phase. The tendency of pyrimethanil and quinoxyfen to decay in the transition from one step to the next in the context of overall vinification methods (with or without maceration) was very similar. The constant rates calculated for these fungicides ranged from 0.565 to 0.858 for pyrimethanil in white winemaking and traditional processes, respectively, and from 0.683 to 1.064 for quinoxyfen in the same vinifications as the previous case. The only important difference for quinoxyfen was appreciated between long maceration winemaking process and rosé vinification.

At the end of both studies we can affirm that in the winemaking process with maceration the relationships between the residue levels and the steps were more significant than those calculated between residue levels and time. Correlation coefficients were superior in the study performed by steps for all fungicides. The major difference between studies, time, or step appeared in the winemaking without maceration process.

In conclusion, the present study suggests that the elaboration of fungicide dissipation curves is a valuable tool to ascertain the evolution and behavior of the different active ingredients during the conversion of grapes to must and that of must to wine.

The results obtained from this mathematical model may also serve to discuss or evaluate which MRL should be established by law for wine, because at present, the MRLs applied are those established by different legislations on the harvested grape. We would also know the initial residues in the grape from levels found in the wine and according to the type of winemaking employed. In recent years, several countries have been planning studies to ascertain factors pertaining to the concentration or elimination of pesticide residues according to the type of winemaking and, thus, establish the MRL in wine.

The winemaker may also benefit when choosing which winemaking process to follow when grapes with pesticide residues arrive at the winery. For example, if the grapes present fludioxonil residues, they should not undergo carbonic maceration because this process leads to a slower elimination and does not remove all of the residues in the wine.

Knowing the *K* value of a fungicide may be of help in predicting the behavior of a compound of the same family with a similar chemical structure during the winemaking.

ACKNOWLEDGMENT

We are grateful to BSI (Jumilla, Murcia, Spain) for supplying wine grapes and clarifying agents. Thanks for the help given.

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Received for review June 23, 2004. Acepted November 1, 2004.

JF040299V