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Abstract

Tempranillo grapes were immersed in solutions of pyrimethanil, metalaxyl, dichlofluanid and penconazol fungicides at different concentrations for several different times. Determinations of the fungicide residues was carried out by GC-NPD, with an additional confirmation by GC-MS. The percentage absorption was determined as well as the distribution between surface, skin and pulp of the grapes. The percentage absorption ranged from 7.2 to 85.5 %. These values depended on the time which grapes were in contact with the fungicide solutions. Residues were mainly found in skins, with percentages in skins ranged from 66.3 to 90.3 % of total residues. In pulp, these values were ranged from 3.5 to 31.0 %. The overall methodology was applied to the determination of pyrimethanil in Tempranillo grapes treated with the recommended doses, respecting the safety period. Pyrimethanil residues found in treated grapes were higher in the skin. In grapes collected within the safety period (21 days), the levels found for fungicide residues were below Maximum Residues Levels.

Keywords*:* Pesticides;; grapes; absorption; distribution; penetration

Introduction

Fungicides are widely used in the treatment of grape diseases for winemaking such us grey mold (*Botrytis cinerea)*, powdery mildew (*Uncinula necator)* and downy mildew (*Plasmopara vitícola)*. Although the correct use of these phytosanitary products does not have adverse effects for public or environmental health, indiscriminate treatment applied without respect for safety periods or recommended doses can entail a risk the of residues remaining in grapes used for winemaking. These fungicides may be transmitted to the must and then to the wine during fermentation (Cabras et al. 2000; Fernández González et al. 2003; Nozal et al. 2005) and affect not only the fermentation process but also the final quality of the product, giving rise to off-flavors (Cuinier 1996). Moreover, there may also be a risk for consumer health (Stavropoulos et al. 2001; Navarro et al. 2005). Therefore, rapid and reliable analytical techniques must be developed to control residue levels in grapes, in order to verify their compliance with the Maximum Residue Levels (MRLs) (Teixeira et al. 2004).

Numerous analytical methods have been described for determining the presence of fungicides in winemaking grapes. Determinations are generally carried out by GC with different detectors (NPD, ECD, MS) (Sherma 1999; Fernández et al. 2002; Tadeo et al. 2004; Angioni et al. 2005) or HPLC with UV-VIS or MS detection (Melo et al. 2004; Nozal et al. 2005; de Melo et al. 2006; Vaquero-Fernández et al. 2008). The MS detector is commonly used as confirmatory method (de Melo et al. 2006; Likas et al. 2007)..

In GC analysis, the response of the system to certain pesticides may be affected by the presence of co-extractives from the matrix. Recent related papers in different fields of pesticide analyses report the matrix effect in calibration, and therefore carry out preparation of the calibration solution using extracts from blank samples (matrix-matched calibration) (Likas et al. 2007; Zhu et al. 2007).

Numerous studies on the determination of certain pesticides in grapes at residual levels have been published (Navarro et al. 2000; Rial Otero et al. 2003). The method commonly used is solid-liquid extraction, as a prior isolation step, with solvents such as acetonitrile, hexane, acetone, dichloromethane, ethyl acetate or solvent mixtures as dichloromethane/acetone (Sala et al. 1997; Cabras et al. 2000b; Angioni et al. 2005; de Melo et al. 2006; Likas et al. 2007). Some studies also consider the use of solid phase extraction (SPE) as a further purification process (Fernández González et al. 2003; Rial Otero et al. 2003; Teixeira et al. 2004).

The different effects of the application of fungicides to vines may explain the different locations and concentrations of fungicide residues in grapes. While systemic products penetrate the pulp, those in contact may be found in the skin. The adjuvants used in commercial formulations are important in the penetration of fungicides in tissues (Cabras et al. 2000a; Teixeira et al. 2004). In previous studies, different penetration of pesticides into the pulp has been observed in fruits like apples (Pérez-Clavijo et al. 1996; Sanz-Asensio et al 1999).

This study was designed to determine the residue levels of four fungicides widely used in grapevines of DOCa Rioja (pyrimethanil, metalaxyl, dichlofluanid and penconazol) in different fractions of grape. In this paper, results of a study of distribution of the target fungicides with simulated uptake from solution and in field studies are reported. Different concentrations were tested, equal or higher than the MRLs fixed by European Union.

Experimental

Materials and chemicals

Pesticide analytical standards of pyrimethanil, metalaxyl, dichlofluanid and penconazol were purchased from Riedel-de-Haën (Seelze, Germany). Standards were certified and at least at >99% pure (Pestanal grade).. Metribuzin (a herbicide not used for vineyards) from Riedel-de-Haën (Seelze, Germany) with a purity of 99.8% was used as Internal Standard (IS). Scala® (37.4 % pyrimethanil formulated as suspension concentrate) was supplied by BASF Española S.A. (Barcelona, Spain) for vines treatment. The standards were stored at – 20ºC. HPLC grade ethyl acetate, sodium sulfate anhydrous for GC residue analysis and washed glass wool were obtained from Scharlau (Barcelona, Spain), Ultrapure water was obtained in a Milli–Ro plus system together with a Milli–Q system from Millipore (Bedford, MA, USA).

A Heidolph SilentCruher M (Scwachbach, Germany) and a Centrifuge Eppendorf 5804 (Hamburg, Germany) were used to process samples in the developed method. A Zymark TurboVap® II (Hopkinton, USA) was used to evaporate extracts of ethyl acetate under nitrogen stream and a Büchi rotavapor R-200 to evaporate washing extracts.

The extraction of the skin with water and ethyl acetate was carried out with a Branson 5510 Ultrasonics (Barcelona, Spain).

For solid phase extraction, octadecylsilane 200 mg (LiChrolut RP-18) cartridges were supplied by Merck (Darmstadt, Germany). A Visiprep SPE vacuum manifold from Supelco (Bellefonte, PA, USA) was used to simultaneously process 12 tubes. The system was equipped with a pressure controller (20 mm Hg) to force the sample through the cartridges.

Standard solutions

Stock standards solution (ca. 1000 mg L^{-1}) of each fungicide was prepared in methanol by weighing approximately 0.0250 g of the analyte into a 25 mL volumetric flask and diluting to volume. An intermediary mixed standard solution was prepared by dilution in methanol of the stock standard solution to give a concentration of ca. 100 mg L^{-1} for each compound. Stock and intermediary standard solutions of IS, metribuzin, were prepared in the same way. All standard solutions were stored at -20ºC.

Sampling and processing

In 2005 and 2006, grapes of Tempranillo variety were harvested in the winemaking area of La Rioja. These vines had not been treated with phytosanitary products and collected grapes were considered as control grapes in order to carry out the study of the compounds. The selected treated and control grapes from each bunch (top, middle and bottom) were frozen at –20ºC in 150 mL flasks containing 75 grapes each.

Before using the grapes, they were defrosted in a refrigerator at 4ºC for 24 hours. They were kept at room temperature for one hour before handling. The grapes were weighed , spiked and processed as follows:

a) *Washing procedure*: tap water (50 ml) was added to 25 g of grapes in a 250 mL glass until they were completed covered to dissolve the pesticide residue remaining on the exterior of the fruit. The glass was placed in an ultrasound bath and shaken for 30 minutes to dissolve the residues located on the surface of the grapes. The removed water was concentrated in rotavapor to 5 mL for subsequent SPE analysis. SPE cartridges containing 200 mg of C18 sorbent were used. The C18 cartridge was activated with 5 mL methanol and then 3 mL ultrapure water. Volume sample was 5 mL. A washing step with 2 mL of water/methanol (9/1) was carried out. The extract retained in the solid phase was eluted with 2+2 mL of ethyl acetate. (Method 1).

b) Solvent extraction of fungicides in grape skin: Once the grapes had been washed with water and the washing water had been removed, they were left to dry on filter paper at room temperature for 10 min. The washed and dried grapes were placed in 50 mL of ethyl acetate in a 250 mL glass in order to extract the fungicide residues absorbed on the grape skins. The glass was placed in the ultrasonic bath for 30 min. To ensure full extraction of the skin, four consecutive extractions of 30 min were carried out in the ultrasonic bath for spiked grapes. No signal of fungicides was observed in the third and fourth extracts. The method proposed for grape skin includes two extractions with 50 ml ethyl acetate for 30 min in the ultrasonic bath. The two extracts were combined, dried

with sodium sulfate anhydrous and filtered through glass wool and concentrated in Turbo Vap® to 5 mL (Method 2).

c) *Solvent extraction of fungicides in the whole grape:* Grapes were crushed after removal of ethyl acetate for skin extraction. Ethyl acetate 25 mL was added. The grapes were homogenized with the crusher for 2 minutes and then centrifuged at 6000 rpm for 15 min. The liquid phase was dried with sodium sulfate anhydrous and filtered through glass wool. The extract was concentrated at 5 mL in the Turbo Vap® (Method 3).

The quantification for GC analysis was performed using metribuzin as internal standard at a concentration of 1 mg L^{-1} . Confirmation was performed by GC-MS.

Instrumentation and chromatographic conditions

An Agilent Technologies GC 6890 N equipped with an NPD Nitrogen-Phosphorus Detector system, an autosampler 7683 (Agilent) and a split-splitless injector connected to a HP ChemStation (Hewlett-Packard) were used for determining the fungicides dichlofluanid, metalaxyl, penconazole and pyrimethanil. The capillary column was a HP-5MS (30m x 0.25 mm i.d. with 0.25 µm of cross-linked 5% phenylmethyl siloxane). Injector and detector temperatures were 200ºC and 325ºC, respectively. Oven temperature was programmed as follows: 80ºC for 0.9 min, increased to 225ºC (70ºC min^{-1}) for 5 min, to 275ºC (70ºC min^{-1}) for 3 min. Helium was the carrier and make-up gas at 1.5 mL min^{-1} and 3 mL min^{-1}, respectively. Flow ratios for the NPD detector were 3 mL min^{-1} for hydrogen and 70 mL min^{-1} for air. Injection was performed in splitless mode with a purge time of 0.9 min and the injection volume was 2 µL.

The confirmation of the studied compounds was carried out on an Agilent 7890A GC with MS 5975C detector system. Column, carrier flow and oven temperature program were the conditions above mentioned for GC-NPD system. The volume of sample was 1 µL, injected in splitless mode with splitless time of 1 min. The mass spectrometer was operated in electron impact (70 eV of ion energy), with 3.50 min solvent delay, the interface temperature was kept at 285ºC and the ion source temperature was kept at 230 ºC and the dwell time was 100 ms per ion. Selected monitoring ion (SIM) mode was used (198, 199, and 200 *m/z* for pyrimethanil; 103, 144, and 198 *m/z* for metribuzin; 132, 160, and 206 *m/z* for metalaxyl; 123, 167, and 224 *m/z* for dichlofluanid; 159, and 248 *m/z* for penconazol).

Study of absorption and distribution in grapes

The absorption of the fungicides was studied by soaking 25 g of untreated grapes in 50 mL of aqueous solution spiked with three fungicide concentration levels (1.00, 5.00 and 10.00 mg L^{-1}, corresponding to 2, 10 and 20 µg g^{-1}, respectively), in a 250 ml beaker.The samples were kept in the dark at 4 ºC for 1, 3, 7, 13 and 21 days (the longest safety period for all compounds). The spiking concentrations were similar or higher than MRLs fixed by European Union (10 µg g^{-1} for dichlofluanid, 1 µg g^{-1} for metalaxyl, 0.2 µg g^{-1} for penconazol and 5 µg g^{-1} for pyrimethanil).Three replicates were carried out. The fungicide absorption in the whole grape was calculated as the sum of the three parts: surface, skin and pulp.

Study in field

Tempranillo grapevines in Rioja Baja were treated with Scala® (37.4 % Pyrimethanil) at the recommended doses (2 L ha^{-1}). Scala® is a very widely-used product against *Botrytis cinerea*. Samples were collected seven and twenty-one days after treatment.

Results and discussion

Analytical Method Efficiency

There were no interferences in the extracts obtained from red grapes in GC-NPD chromatogram. The proposed conditions generated narrow and reproducible chromatographic peaks. Clean chromatograms were obtained with only peaks of interest in fortified blank matrix. The GC-MS method allowed confirmation of the fungicides with good resolution and did not show interfering peaks.

Matrix standard calibration were performed for washing water and ethyl acetate from skins, and a matrix matched calibration were carried out for pulp. Linear range, limits of detection (LODs), limits of quantification and repeatabilitiy were determined for the 3 methods and 4 fungicides. Linear range were from 10 to 1400 µg kg^{-1}. Good linearity was obtained for all fungicides and for the entire range of studied concentrations with correlation coefficients better than 0.997. LODs were ranging from 0.04 to 1.67 µg kg^{-1} in surface (washing water); from 0.01 to 1.05 µg kg^{-1} in skin (ethyl acetate); and from 2.46 to 5.64 µg kg^{-1} in pulp. Repeatabilities expressed as RSD were below 11.4 %. The methods were adequate for determining these fungicides.

A matrix matched calibration and in solvent was also carried out for the GC-MS method. In the range from 2.0 to 2000 µg kg^{-1} in grape and water extracts, and in ethyl acetate, excellent linearity was also obtained (correlation coefficients > 0.9995). The GC-MS confirmation method showed LODs and LOQs in grapes below 0.41 and 1.36 µg kg^{-1} for all compounds, values considerably below the fixed MRLs.

Recovery and accuracy were determined with spiked grapes at the three concentration levels and were analyzed in triplicate. The analytical methods presented had recoveries > 90.1 % and precisions (repeatability) < 7.6 % and they were within the acceptable range: 70-110% with relative standard deviation (RSD) <10% (n=3). (Document Nº SANCO/10232/2006).

An analysis of variance performed with the data revealed the absence of significant differences (p<0.05) between the assayed levels of spiking.

Study of fungicides absorption

Grapes in contact with solutions containing the fungicides at three concentration levels were analyzed. The amount of residues present in whole grapes was determined by the proposed methods and the percentage absorption of fungicide relative to its concentration in the solution was calculated. Table 1 shows the results for each compound in all the concentration and time situations.

The amount of absorbed fungicide increased with increasing initial concentration of solutions for any time period. This increase was higher, the higher the contact time.

However, the percentage absorption percentage which was found did not depend on the initial fungicide concentration in contact with grapes for the same contact time. Metalaxyl was the most absorbed fungicide, followed by pyrimethanil, penconazol and dichlofluanid..

To ascertain the fungicide absorption rate at each studied concentration, the experimental data have been fitted to the following mathematical model (Oliva et al. 2005):

$$C_t = C_0 e^{-Kt} \quad (a)$$

$$\text{Ln } C_t = \text{Ln}C_0 - Kt \quad (b)$$

In eq a C_t is the residue concentration at time t ($\mu g\ g^{-1}$), C_0 is the theoretical initial residue concentration at t = 0 ($\mu g\ g^{-1}$), K is the fungicide absorption constant, and t is the time that grapes have been in contact with solutions. This type of analysis, a first order kinetics, allows the behavior of fungicide residues during the time to be known, by showing the correlation that exists between the absorbed residues and the time. It is also possible to study if the correlation existing between variables is more o 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 (Miller and Miller 2002):

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

In eq c, r represents the correlation coefficient and (n - 2) the degrees of freedom. From this equation one obtains a value of distribution of the Student-*t* that can be compared to *t* tabulated values. The fitting results are presented in Table 2.

As can be seen from these data, the linear correlation between Ln C_t and time was good for pyrimethanil, metalaxyl and dichlofluanid for all assays, with correlation coefficients $r > 0.906$. Penconazol did not show a linear correlation, with low correlation coefficients $r < 0.853$. However, this compound presented a linear fit from 1 to 7 days. The Student-*t* test performed to compare the slopes (K values) revealed that there were no significant differences at 95 % confidence level for the three studied concentrations. For the constant values, we can establish an absorption rate for the fungicides as follows: metalaxyl > pyrimethanil > dichlofluanid.

Study of fungicides distribution

The amount of residues in the three parts of the grapes (surface, skin and pulp) was determined using the proposed methods and the percentage distribution relative to the total absorbed residues was calculated. Figure 1 shows this distribution of the fungicide residues in the different parts of the grape as a function of time for the three studied concentrations The highest content was found in the skin for all the compounds, regardless of the fungicide concentration in contact with grapes, with > 66.3 % of the residues for all the fungicides In pulp, the residue amounts ranged from 3.5 to 31.0 %.

A study of fungicide penetration in pulp was performed by calculating the ratio between pulp and skin concentrations (Q) for all the assays. There was no significant difference at 95 % confidence level between concentrations at the same time. The penetration did not depend on the initial solution concentration. The average values for all concentrations are shown in Table 3. Penconazol and dichlofluanid had the highest Q values. Metalaxyl and pyrimethanil had a similar behavior, but different from the other

compounds. The statistical analysis performed for all times showed that metalaxyl and pyrimethanil Q values did not differ at 95 % confidence level from 7 to 21 days. Dichlofluanid presented no significant differences in Q values for 1 to 7 days. Penconazol had higher Q values for the highest contact time.

Analysis of red grapes from La Rioja

The overall methodology was applied to determine pyrimethanil in Tempranillo grapevines. The selected treated grapes from different clusters from the vineyard (top, middle and bottom of the cluster) were frozen at –20ºC in 150 mL flasks containing 75 grapes each during one week for further analysis. The results are shown in Table 4.

The amount of pyrimethanil determined in the three parts of the fruit was higher in the grapes collected after seven days of treatment. A high reduction in pyrimethanil residues were observed over time. This decrease was ca. 99 % from 7 to 21 days. The reduction was due to dissipation and/or a degradation by environmental conditions. Pyrimethanil residues were mainly found in the skin (78.2% and 64.5% in grapes collected after 7 days and 21 days, respectively). Penetration in pulp was higher for grapes collected after 21 days, 15.5% of the residues vs. 10.1% in grapes collected after 7 days. Pyrimethanil penetration observed in field studies was comparable to that observed in laboratory studies.

Conclusions

A simple, rapid, liquid extraction method was developed for the determination of pyrimethanil, dichlofluanid, metalaxyl and penconazol in red grapes, Overall the

methodology involved three procedures for each different part of the berry: surface, skin and pulp. The method gave good recoveries, linearity, precision and accuracy and was highly sensitive. An additional GC-MS confirmatory method was proposed for treated grapes samples.

The study of fungicides mobility in the laboratory showed that percentage absorption did not depend on the initial spiked concentration and the percentage increased with increasing contact time. Pyrimethanil, metalaxyl and dichlofluanid showed a first order kinetics for the absorption during time.

The highest concentrations of residues were found in the skin Penetration into pulp was observed for all the fungicides. The ratios between pulp and skin residues (Q) did not depend on the initial spiked concentration. Penconazol had a higher penetration than the other compounds.

Grapes treated with pyrimethanil and collected after 7 days had higher amounts of residues than those harvested when respecting the safety period. Pyrimethanil residues in field samples was affected by chemical characteristics of the product as well as by other factors, such as environmental factors...

Residues were found mainly in skin. Samples treated in field had similar penetration of pyrimethanil in pulp to samples spiked in the laboratory. Assays performed in the laboratory with grapes are suitable for the simulation of fungicide absorption and

distribution. Grapes collected in the safety period, the levels found for fungicide residues were below Maximum Residues Levels.

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434

Table 1: Fungicide residues and absorption percentage (%) in grapes samples which have been in contact with three different solutions during different times.

Days	Pyrimethanil					
	2 µg g^{-1}		10 µg g^{-1}		20 µg g^{-1}	
	µg g^{-1} (RSD,%)	%	µg g^{-1} (RSD,%)	%	µg g^{-1} (RSD,%)	%
1	0.43 (6.2)	21.6	1.82 (2.2)	18.2	3.36 (2.8)	15.8
3	0.56 (5.3)	28.1	2.46 (7.6)	24.6	4.47 (6.4)	22.9
7	0.96 (2.5)	48.1	4.49 (7.0)	44.9	8.48 (2.6)	42.4
13	1.14 (4.9)	57.1	5.47 (3.8)	54.8	10.7 (5.2)	53.6
21	1.44 (6.2)	72.3	7.14 (7.7)	71.4	14.7 (2.8)	73.5

Days	Metalaxyl					
	2 µg g^{-1}		10 µg g^{-1}		20 µg g^{-1}	
	µg g^{-1} (RSD,%)	%	µg g^{-1} (RSD,%)	%	µg g^{-1} (RSD,%)	%
1	0.23 (9.9)	11.4	0.92 (2.4)	9.2	1.44 (1.8)	7.2
3	0.34 (8.2)	16.8	1.57 (4.6)	15.7	3.10 (3.4)	15.5
7	0.55 (10.0)	27.8	2.89 (4.6)	28.9	6.43 (1.8)	32.2
13	1.08 (5.2)	53.8	5.29 (9.3)	52.9	11.3 (1.7)	56.7
21	1.78 (3.9)	89.0	8.50 (5.8)	85.0	17.9 (5.8)	89.5

Days	Dichlofluanid					
	2 µg g^{-1}		10 µg g^{-1}		20 µg g^{-1}	
	µg g^{-1} (RSD,%)	%	µg g^{-1} (RSD,%)	%	µg g^{-1} (RSD,%)	%
1	0.18 (1.9)	9.2	1.55 (1.1)	15.5	2.96 (2.1)	14.8
3	0.22 (8.4)	11.2	1.72 (3.9)	28.9	3.14 (6.0)	15.7
7	0.31 (2.3)	15.5	2.07 (5.0)	20.7	3.50 (3.6)	17.5
13	0.41 (7.5)	20.4	2.33 (5.4)	23.3	4.17 (4.5)	20.8
21	0.54 (10.3)	27.1	2.69 (9.6)	26.9	5.07 (4.1)	25.3

Days	Penconazol					
	2 µg g^{-1}		10 µg g^{-1}		20 µg g^{-1}	
	µg g^{-1} (RSD,%)	%	µg g^{-1} (RSD,%)	%	µg g^{-1} (RSD,%)	%
1	0.54 (5.0)	27.1	2.00 (3.8)	20.0	3.32 (4.3)	17.5
3	0.73 (4.8)	36.7	2.97 (6.6)	29.7	6.21 (4.8)	31.1
7	1.12 (5.1)	56.5	4.91 (6.9)	23.3	12.0 (6.2)	60.1
13	1.16 (3.3)	58.1	5.66 (8.3)	56.5	12.7 (7.3)	63.4
21	1.22 (3.0)	61.0	6.65 (8.8)	66.5	13.6 (3.0)	68.1

Table 2: Statistical parameters derived from the linear fit of the data for the fungicides absorption during time.

Concentration ($\mu g\ g^{-1}$)	Pyrimethanil			Metalaxyl			Dichlofluanid			Penconazol		
	r	k	*t*	r	k	*t*	r	k	*t*	r	k	*t*
2	0.906	0.058	3.696*	0.988	0.101	11.31*	0.969	0.033	6.807*	0.757	0.046	2.009
10	0.908	0.065	3.747*	0.966	0.108	6.526*	0.962	0.027	6.105*	0.852	0.054	2.827
20	0.923	0.070	4.158*	0.938	0.121	4.685*	0.999	0.027	39.69*	0.731	0.060	1.854

[a] r = correlation coefficient ; k = constant rate ; *t* = calculated value of Student-*t* distribution for $P < 0.05$; significance of the correlation between variables * $P < 0.05$

Table 3 : Average ratios (Q ± standard deviation*) between pulp and skin concentrations for fungicides during time

Days	Pyrimethanil	Metalaxyl	Dichlofluanid	Penconazol
1	0.056 ± 0.006^{a}	0.057 ± 0.002^{a}	0.175 ± 0.025^{a}	0.157 ± 0.015^{a}
3	0.123 ± 0.011^{b}	0.078 ± 0.003^{b}	0.202 ± 0.021^{a}	0.169 ± 0.010^{b}
7	0.162 ± 0.006^{c}	0.117 ± 0.010^{c}	0.226 ± 0.012^{ab}	0.215 ± 0.011^{b}
13	0.184 ± 0.031^{c}	0.119 ± 0.014^{c}	0.254 ± 0.021^{b}	0.273 ± 0.022^{c}
21	0.220 ± 0.028^{c}	0.142 ± 0.006^{c}	0.312 ± 0.023^{c}	0.410 ± 0.045^{d}

* n = 9

a b c d Different letters in the same column means significant differences (P<0.05)

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Table 4: Pyrimethanil residues in grapes from La Rioja

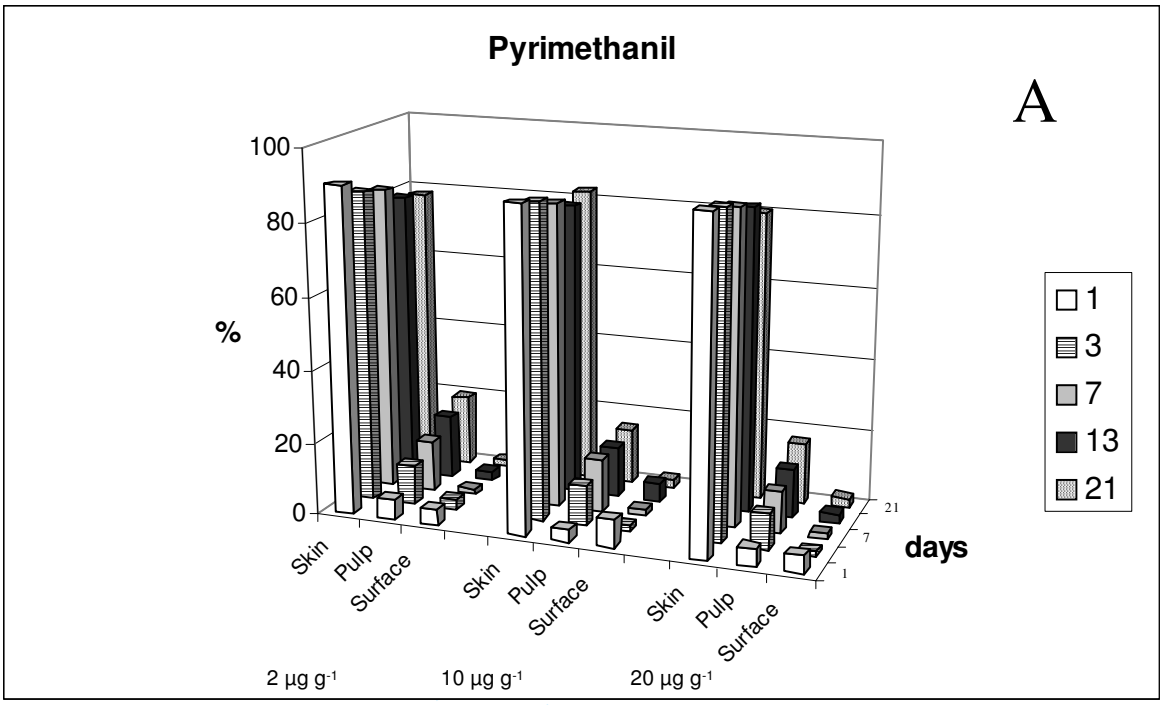
Sample	Pyrimethanil µg kg^{-1} (RSD, %[a])		
	Surface	Skin	Pulp
2006 (7 days)	443 (6.9)	2955 (11.3)	382 (8.6)
2006 (21 days)	4.55 (6.3)	14.7 (13.0)	3.54 (6.5)

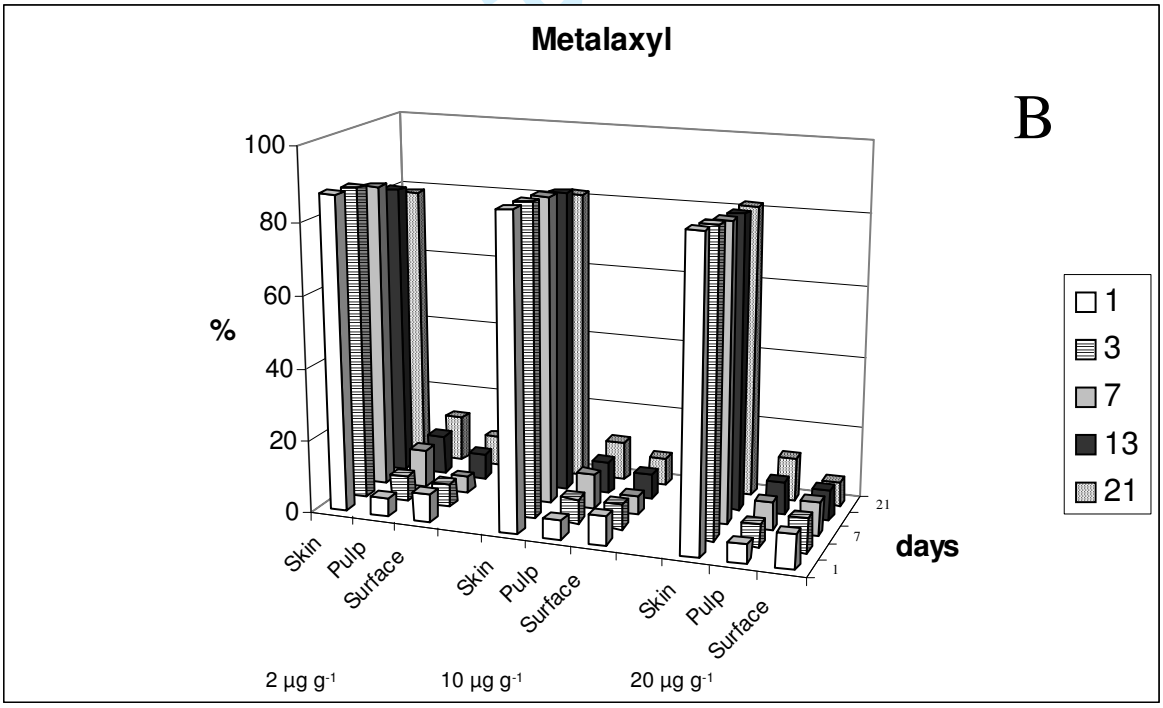
[a] n = 3

FIGURE CAPTIONS

Figure 1. Distribution of fungicides in surface, skin and pulp in grapes which have been in contact with three spiked solutions (2 µg g^{-1}, 10 µg g^{-1}, 20 µg g^{-1}) during 1, 3, 7, 13 and 21 days A) Pyrimethanil B) Metalaxyl C) Dichlofluanid D) Penconazol

Figure 1.


Pyrimethanil
A
100
80
60
40
20
0
%
Skin
Pulp
Surface
Skin
Pulp
Surface
Skin
Pulp
Surface
2 µg g-1
10 µg g-1
20 µg g-1
21
7
1
days
1
3
7
13
21


Metalaxyl
B
100
80
60
40
20
0
%
Skin
Pulp
Surface
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Pulp
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Surface
2 µg g-1
10 µg g-1
20 µg g-1
21
7
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days
1
3
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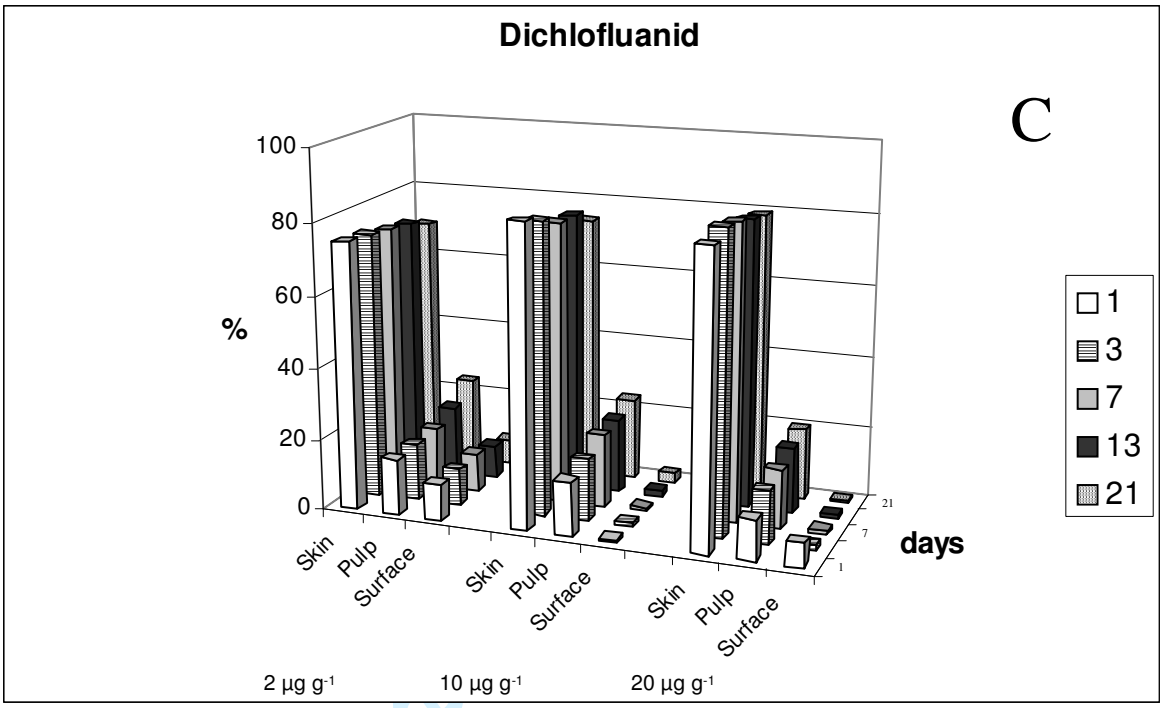
13
21


Dichlofluanid
C
100
80
60
40
20
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%
Skin
Pulp
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Pulp
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days
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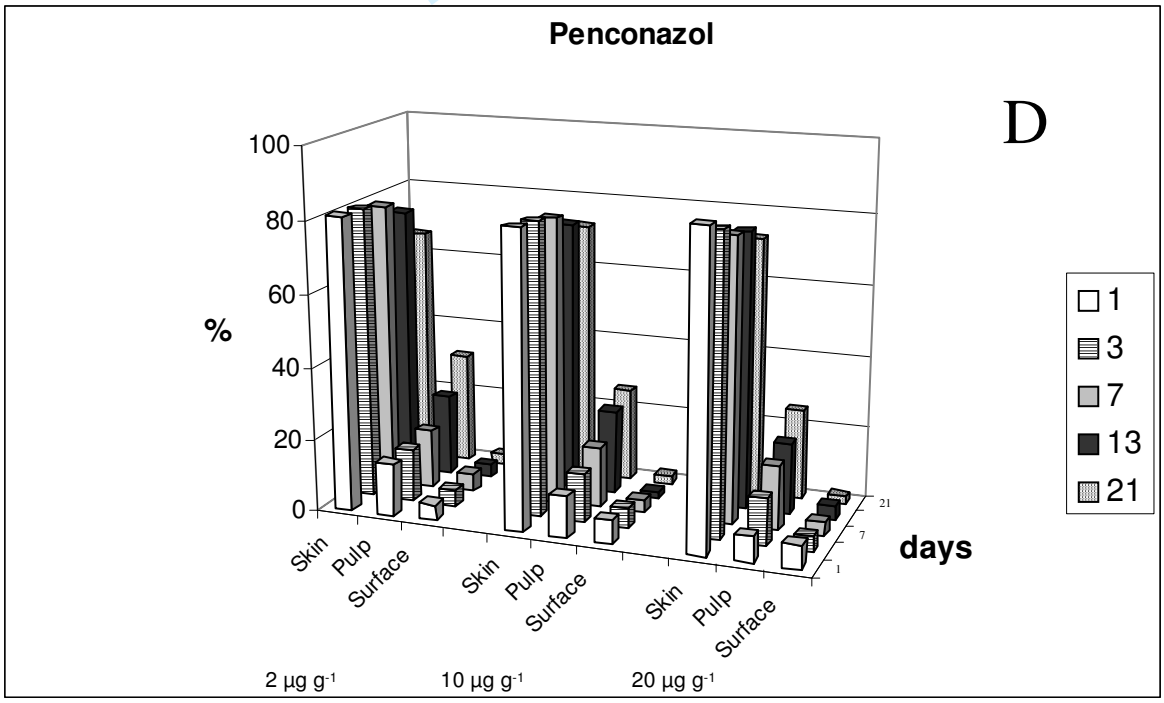
13
21
2 µg g-1
10 µg g-1
20 µg g-1


Penconazol
D
100
80
60
40
20
0
%
Skin
Pulp
Surface
Skin
Pulp
Surface
Skin
Pulp
Surface
21
7
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days
1
3
7
13
21
2 µg g-1
10 µg g-1
20 µg g-1