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Residues of Spiroxamine in Grapes Following Field Application and Their Fate from Vine to Wine

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Dissipation of the fungicide spiroxamine in grapes of two vine varieties, Roditis and Cabernet Sauvignon, exposed to field treatments was evaluated. Vines of a grape vineyard located in central Greece were sprayed once or twice with a commercial formulation of the fungicide at 30 g a.i./hL. Residues in grapes, must, and wine were determined by gas chromatography/IT-MS after extraction with cyclohexane−dichloromethane (9:1), with a limit of quantitation 0.02 mg/kg in grapes and 0.012 mg/kg in wine. Under field conditions, spiroxamine dissipation on grapes was faster during the first 2 weeks and then slower to the sixth week. About 7 days after application, half of the initial spiroxamine concentration remained on the grapes; the respective proportion at 42 days was about 10%. At 14 and 35 days, residues were lower than 0.44 and 0.22 mg/kg, respectively, values below the maximum residue levels set by the European Union (1 mg/kg). Spiroxamine residues transferred from grapes into the must and through the vinification process into the wine were also studied. Mean transfer factors of 0.26 and 0.55 were found from grapes into wine for the wines obtained without maceration and with maceration, respectively. Residues in wine, prepared from grapes with a spiroxamine content of 0.11−0.20 mg/kg, varied from <0.026 to 0.09 mg/kg. Spiroxamine diastereomer B was found to dissipate slower than diastereomer A in the field as well as during the vinification process.

KEYWORDS: Spiroxamine; pesticides; residues; grapes; wine; vinification

INTRODUCTION

The main distribution area of grapevines is the Mediterranean countries, where about three-fourths of the total world distribution is grown. To obtain good quality table grapes and wine, the vine must be protected from pathogen attacks until it ripens. Among the principal pathogen of vines is powdery mildew, and fungicides have to be used to control this fungus. Most studies on fungicide residues deal with their fate in vine and from vine to wine in regard with the old classes of fungicides and recently with a number of new chemical classes, such as the strobilurines (*1*).

Spiroxamine, with IUPAC name 8-*tert*-butyl-1,4 dioxaspiro-[4.5]decan-2-ylmethyl(ethyl)(propyl)amine, is a new fungicidal active substance belonging to the spiroketalamine class of substances with protective, curative, and eradicative effects against mildews (*2*). Spiroxamine is primarily used in cereal and recently in grape cultivations either as a single agent product or in combination with other fungicidal substances. It is comprised of cis and trans diastereoisomers: the A and the B forms, respectively. All isomers have partly different biological activities interacting with different steps in sterol biosynthesis, as δ-14 reductase and/or δ-8-7 isomerase inhibitor (*3*). Spiroxamine was recently registered in Greece and other European countries for use in vine production with a maximum residue level (MRL) provisionally established by the European Union at 1 mg/kg and a preharvest interval of 14 days for table grapes and 35 days for wine grapes. As grape and wine have an important contribution to the human diet, data on the fate of spiroxamine residues in grapes after application are essential, for both the establishment of MRLs and the calculation of the theoretical maximum daily intake. Furthermore, some countries require data on the residues fate during the wine-making process, to assess the real consumer exposure for the establishment of MRL (*4*).

In recent years, an important number of research works dealt with the behavior of pesticides in grapes (*5*−*8*), their fate from vine to wine, and the influence of various technological processes on their residues found in the produced wine (*1*, *4*, *5*, *8*−*13*). However, to the best of our knowledge, no data have been published on the fate of spiroxamine residues in grapes after treatments on vine or during the winemaking process. This paper reports the results of (i) spiroxamine residue dissipation

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in grapes of two vine varieties (Roditis and Cabernet Sauvignon) after field treatments for two consecutive years and (ii) spiroxamine residue fates from vine to wine.

MATERIALS AND METHODS

Field Trials. The experimental trials were carried out in a vineyard, located at Nea Aghialos, Magnesia, in central Greece. The experiments were performed on two grape varieties, one white grape (cv. Roditis) and one red grape (cv Cabernet Sauvignon); both plants were 10 years old. Both varieties were grafted on 1103P rootstock and with a double cordon as a training system. The vines were spaced 1.20 m from each other within a row and 2.70 m between the rows, and during the experimental period, they received routine horticultural practices (potassium and magnesium fertilization in December, bush pruning in late January, nitrogen fertilization early in February, and KNO_3 fertilization in mid-June). There were four experiments in the above vineyard: a white grape variety sprayed twice on August 13 and 27, 2002; a white grape variety sprayed twice on August 19 and 26, 2003; a white grape variety sprayed once on August 26, 2003; and a red grape variety sprayed once on August 16, 2003. Each experiment was divided into four randomized plots of 12 vines each. Three of them, used as replicates, were treated with the fungicide spiroxamine, and the other one was left untreated to be used as a control. A 50% spiroxamine commercial formulation was used (Prosper 50 EC, Bayer Hellas, Greece) at the recommended dose (30 g of active ingredient/hL of water), using a pressurized hand-gun applicator at high volume until run off. In both years, care was taken to ensure that vines were well-covered with the spraying mixture. Meteorological data were collected by an agrometeorological station, located near the vineyard. During the experiments and for the years 2002/2003, respectively, the average daily air temperatures were 24.8/25.6 °C for August and 20.2/20.6 °C for September; the average relative humidities were 67.0/61.5% in August and 83.5/63.7% in September; and the rainfalls were 5/15 mm in August and 24.6/6.2 mm in September.

Sampling. For studying the dissipation of spiroxamine on grapes, samples were collected at time 0 days (3 h postapplication, when the spraying mixture had dried) and 2, 4, 7, 10, 14, 21, 28, 35, 42, and 50 days after the last application (DAA). The samples consisted of randomly collected parts of at least 12 bunches of separate vines, and the overall sample weight was 1.5−2 kg with 1 kg of the minimum weight recommended in the FAO/WHO guidelines (*14*). Grape samples were forwarded to the laboratory, blended after removal of the stems, subdivided into 50 g aliquots as analytical replicates, and stored in individual bags at −18 °C until extraction. For studying the fate of spiroxamine residues from vine to wine, samples (∼18 kg) of grapes were processed to produce must and wine. Wine was also produced from grapes collected from the control plot to be used as a control sample.

Vinification Processing. The vinification procedure was performed at laboratory scale. Six vinification experiments were performed, four for grapes of the Roditis variety, collected at 21 and 35 (three vinifications) DAA, respectively, and two for grapes of the Cabernet variety, collected at 14 and 35 DAA, respectively. The grape samples were divided into two equal parts, pressed, and stemmed, and 100 mg of sodium metabisulfite and 200 mg of dry yeast were added per kilogram of grapes. One part was allowed to ferment with the skins (vinification with maceration); the other was dripped, and the resulting must was allowed to ferment (vinification without maceration) at room temperature. A 2 × 50 g aliquot of cloudy must was taken and centrifuged at 4000 rpm for 5 min in order to evaluate the amount of lees and the residue concentration in the clear must. Alcoholic fermentation had a regular course in all samples, and after 21 days, the obtained wine was racked, filtered, and analyzed for spiroxamine residues. The samples of grapes, must, and clear must were conserved at −18 °C until analysis.

Chemicals. An analytical standard of spiroxamine (97.2% purity) was supplied by Bayer. The composition of diastereomers A and B in the standard was 54.3 and 45.7%, respectively. Stock (1000 mg/L) and working (2, 20, and 100 mg/L) standard solutions of spiroxamine were prepared in methanol. Spiroxamine calibration solutions were prepared in matrix (grape and wine) extract in the range of 0.10−6.0 μg/mL, by drying control sample extracts under a stream of nitrogen and reconstituting with the appropriate standard solution in cyclohexane. Cyclohexane and dichloromethane were of pesticide residue grade (Labscan). Anhydrous sodium carbonate and bisulfate were of proanalysis grade (Panreac).

Table 1. Retention Time (t_R), Target and Qualifier Ions with Their Abundances, and LOQs for Spiroxamine Diastereomers A and B in Grapes and Wine Analyzed by the GC-IT-MS Method

	t_R (min)	target ion *m/z*	qualifier ions *m/z* (abundances)	LOQ (mg/kg) grapes	LOQ (mg/kg) wine
spiroxamine				0.02	0.012
A	13.4	100	72 (67), 126 (20), 58 (14)	0.01	0.006
B	14.7	100	72 (67), 126 (20), 58 (14)	0.01	0.006

Analytical Procedures. All grape, must, and wine samples were analyzed for spiroxamine residues by gas chromatography (GC), with an IT-MS detector according to a method for spiroxamine determination in grapes and wine (*15*) with some modifications in the sample preparation step in order to decrease the determination limit of spiroxamine in wine samples. Sample preparation and extraction are briefly described below.

Grape, Must, and Wine Extraction Procedure. Ten grams of sample was placed with an amount of Na_2CO_3 (0.20 g for grape samples and 0.10 g for must or wine samples) into a screw-capped centrifuge tube and homogenized in a vortex. Ten milliliters of cyclohexane−dichloromethane (9:1 v/v) was added, and the mixture was homogenized with an Ultra Turrax at low speed for 40 s. The tubes were centrifuged for 10 min at 4000 rpm, and 5 mL (6 mL for wine samples) of the organic layer was transferred into a pear-shaped flask and evaporated carefully to dryness on a rotary evaporator. The residue was redissolved in 1 mL (750 μL for wine extracts) of cyclohexane and transferred to GC vials for injection into the GC/MS system. On the basis of this extraction procedure, the concentration factor of the sample in the final solution corresponds to 5 g of grape and must matrix/mL and 8 g of wine matrix/mL. The concentration factors were different, to decrease the determination limit of spiroxamine in wine samples.

GC Determination. Chromatographic analyses were performed with a Thermo Quest TRACE 2000 gas chromatograph equipped with a splitless injector, an Rtx-5 ms capillary column, 30 m × 0.25 mm i.d., 0.25 μm film thickness (Restek, Bellefonte, United States), coupled to a GCQ plus (Thermo Quest, Austin, TX) ion trap mass spectrometer with Xcalibur version 1.1 software. The GC operating conditions were as follows: oven temperature program started from 70 °C (1 min), increased to 180 °C (30 °C/min), to 260 °C (1.5 °C/min), and then to 300 °C (30 °C/min), and held for 5 min; carrier gas flow, helium at 1 mL/min; injector, 230 °C; splitless injection, splitless time, 1 min; and injection volume, 1 μL. The ion trap mass spectrometer was operated in the electron ionization (EI) mode at 70 eV. The ion source was set at 200 °C, and the transfer line was set at 275 °C. The acquisition scan mode was set at full scan (*m/z* 45−500).

RESULTS AND DISCUSSION

Spiroxamine Determination−Method Efficiency. The described method of analysis of spiroxamine residues in grape, must, and wine samples by GC-IT-MS is fast and relatively simple. **Table 1** lists the retention times, the target and qualifier ions, the abundance ratios, and the limits of quantification (LOQs) of spiroxamine diastereomers in grapes and wine.

Analysis was performed with reconstructed ion chromatograms (RICs) using the target ion *m/z* 100 (**Figure 1**). All control sample's RICs show no evidence of chromatographic interference in all kinds of samples: grapes, must, and wine. Confirmation of spiroxamine diastereomers in samples was performed by assessing their retention time, the presence of the target and qualifier ions, and their relative abundances, which should be within ±30% of those obtained from the standard material (*16*).

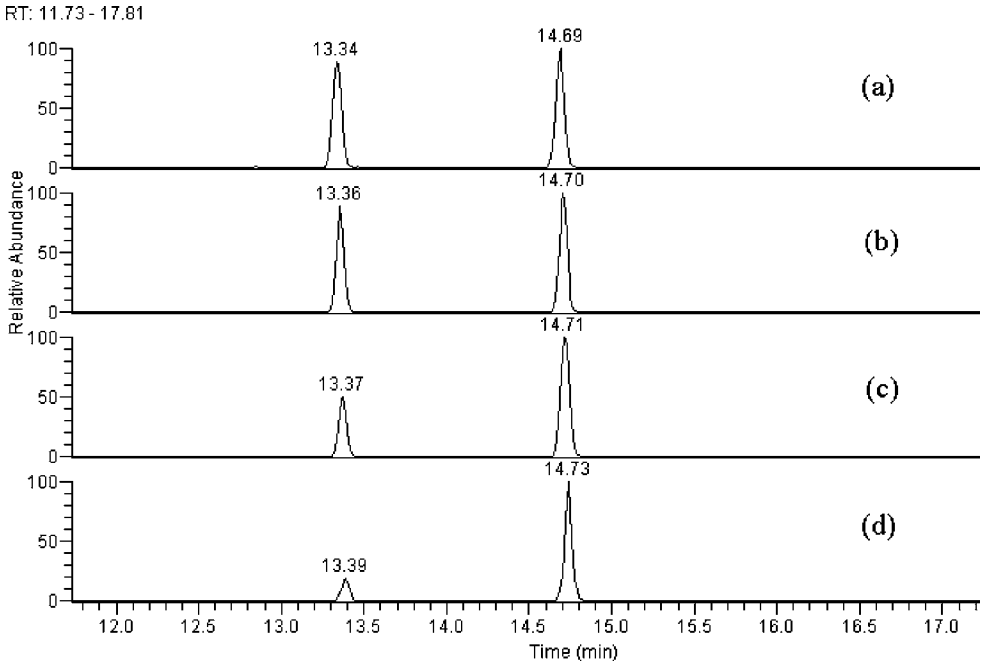

Figure 1. Typical RICs for *m*/*z* 100 of spiroxamine standard at 0.10 mg/L (**a**), of grape sample at 0.86 mg/kg at 0 days postapplication (**b**), of grape sample at 0.30 mg/kg at 14 days postapplication (**c**), and of the obtained wine sample at 0.08 mg/kg (**d**).

The detector responses for A and B diastereomers in the investigated range of concentrations present typical shallow curves, which fit better to a second-order polynomial rather than to a linear relationship. With such a polynomial relationship, the correlation coefficients of the calibration curve ($n = 9$) were >0.999 for both grapes and wine-matched standard solutions. Quantitation was performed with the external standard method, and the spiroxamine concentration in samples was calculated as the sum of the concentrations of the two diastereomers A and B.

The efficiency of the method has been evaluated by spiking control grape, must, and wine samples with spiroxamine working solutions at various levels (0.02−1.2 mg/kg for grapes and must and 0.012−0.30 mg/kg for wine). Recovery values for grapes and must ranged from 87 to 97% for diastereomer A and from 85 to 101% for diastereomer B with a maximum 11% coefficient of variation (CV) ($n = 4$). Recovery values for wines ranged from 93 to 102 for diastereomer A and from 95 to 104 for diastereomer B with a maximum 8% CV ($n = 4$). All of these values of recovery and CV presented good method's accuracy and repeatability as they are within the accepted range for residue determinations (*16*, *17*).

Dissipation of Residues in Grapes. The data relating to the residues in grapes from the field experiments carried out in 2002 and 2003 are reported in **Tables 2** and **3**. It should be noticed that during the experimental periods grapes had attained their final growth state; therefore, any diluting effect was negligible. No residues of spiroxamine were detected in any analyzed control grape sample.

Spiroxamine concentrations in grape samples of Roditis vine after two applications ranged from 0.74 to 1.12 mg/kg at 0 DAA in the 2002 experiment and from 0.88 to 1.32 mg/kg in the respective 2003 experiment, while after a single application in the 2003 experiments, the respective values ranged from 0.77 to 1.02 mg/kg for Cabernet and from 0.73 to 0.82 mg/kg for Roditis vines.

Thereafter, spiroxamine residues in grapes decreased with time and were found 33−40% of the initial concentration at 14 DAA and 11−20% at 35 DAA. Mean concentrations in grapes varied from 0.28 to 0.38 mg/kg at 14 DAA and from 0.11 to 0.20 mg/kg at 35 DAA. In all of the individual analyzed subsamples (one from each plot), the spiroxamine concentration was lower than 0.44 mg/kg at 14 DAA or 0.22 mg/kg at 35 DAA. These time intervals are the legal required preharvest intervals (PHI) for table and wine grapes, respectively, and spiroxamine residue values measured were clearly below the MRL values established in the European Union (1 mg/kg) or in Australia (2 mg/kg).

Spiroxamine dissipation was faster during the first 2 weeks and then slower to the sixth week. Half of the initial spiroxamine concentration was at approximately 7 DAA in all cases, while

Table 2. Residues (Mean ± Standard Deviation)[a] of Spiroxamine and Its A and B Diastereomers in Grapes at Various Time Intervals (Days) after the Last of Two Applications (DAA) for Experiments on Roditis Variety Grapes

year and vine variety	DAA (days)	mg/kg		
		spiroxamine	A diastereomer	B diastereomer
2002 Roditis	(–0)[b]	0.24 ± 0.07	0.10 ± 0.05	0.14 ± 0.05
	0	0.92 ± 0.19	0.42 ± 0.10	0.50 ± 0.11
	2	0.73 ± 0.12	0.33 ± 0.09	0.39 ± 0.13
	4	0.52 ± 0.08	0.23 ± 0.03	0.29 ± 0.04
	7	0.51 ± 0.15	0.23 ± 0.07	0.28 ± 0.09
	14	0.37 ± 0.07	0.15 ± 0.02	0.22 ± 0.04
	21	0.31 ± 0.05	0.13 ± 0.02	0.18 ± 0.03
	28	0.29 ± 0.07	0.12 ± 0.02	0.17 ± 0.04
	35	0.20 ± 0.03	0.08 ± 0.01	0.12 ± 0.03
	42	0.16 ± 0.01	0.06 ± 0.01	0.10 ± 0.02
2003 Roditis	(–0)[b]	0.31 ± 0.05	0.13 ± 0.02	0.18 ± 0.03
	0	1.11 ± 0.22	0.52 ± 0.10	0.59 ± 0.13
	2	0.69 ± 0.20	0.32 ± 0.09	0.37 ± 0.11
	4	0.63 ± 0.05	0.29 ± 0.01	0.34 ± 0.04
	7	0.56 ± 0.03	0.28 ± 0.09	0.32 ± 0.02
	14	0.37 ± 0.05	0.15 ± 0.01	0.22 ± 0.03
	21	0.34 ± 0.09	0.14 ± 0.03	0.20 ± 0.06
	28	0.24 ± 0.06	0.09 ± 0.02	0.15 ± 0.04
	35	0.13 ± 0.03	0.05 ± 0.007	0.08 ± 0.02
	42	0.10 ± 0.02	0.04 ± 0.006	0.07 ± 0.02
	50	0.10 ± 0.02	0.03 ± 0.006	0.06 ± 0.02

[a] $n = 3$, three samples, one from each plot replicate. [b] (–0) is sampling before the last application.

Table 3. Residues (Mean ± Standard Deviation)[a] of Spiroxamine, and Its A and B Diastereomers in Grapes at Various Time Intervals after One Single Application (DAA) for Experiments at Roditis and Cabernet Vine Varieties

year and variety	DAA (days)	mg/kg		
		spiroxamine	A diastereomer	B diastereomer
2003 Roditis	0	0.78 ± 0.05	0.37 ± 0.03	0.41 ± 0.03
	2	0.50 ± 0.06	0.23 ± 0.04	0.27 ± 0.06
	7	0.39 ± 0.06	0.18 ± 0.01	0.21 ± 0.03
	14	0.28 ± 0.04	0.12 ± 0.02	0.16 ± 0.05
	21	0.23 ± 0.01	0.09 ± 0.006	0.14 ± 0.02
	28	0.13 ± 0.02	0.05 ± 0.01	0.08 ± 0.03
	35	0.11 ± 0.01	0.04 ± 0.003	0.07 ± 0.01
	42	0.08 ± 0.01	0.03 ± 0.004	0.05 ± 0.01
2003 Cabernet	0	0.86 ± 0.14	0.41 ± 0.056	0.45 ± 0.08
	2	0.57 ± 0.06	0.26 ± 0.03	0.31 ± 0.06
	7	0.40 ± 0.04	0.17 ± 0.03	0.23 ± 0.05
	14	0.30 ± 0.06	0.12 ± 0.03	0.18 ± 0.05
	21	0.23 ± 0.01	0.09 ± 0.01	0.14 ± 0.03
	28	0.20 ± 0.05	0.08 ± 0.01	0.12 ± 0.03
	35	0.15 ± 0.03	0.05 ± 0.01	0.10 ± 0.02
	42	0.14 ± 0.04	0.05 ± 0.01	0.09 ± 0.03
	50	0.08 ± 0.02	0.03 ± 0.006	0.05 ± 0.02

[a] $n = 3$, three samples, one from each plot replicate.

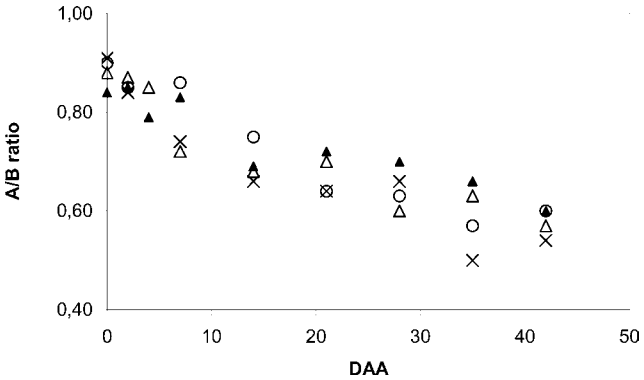

Figure 2. Evolution of spiroxamine diastereomers ratio (A/B) on grapes at various days after the last application (DAA) on Roditis variety grapes for 2002 (solid triangles) and 2003 (triangles) and after one application for 2003 on Roditis (circles) and Cabernet varieties (crosses).

at 42 DAA about 10% of the initial concentration still remained on grapes.

From the data of **Tables 2** and **3**, the concentration ratio of the two spiroxamine diastereomers A:B was evaluated and is shown in **Figure 2**. The ratio was found to decrease with time. Thus, at 0 DAA, the ratio values varied from 0.84 to 0.91 while at 42 DAA, they varied from 0.56 to 0.60. This indicates that diastereomer A dissipated faster than B. At 35 DAA, diastereomer A was 30−40% of the total spiroxamine concentration in grapes and diastereomer B was 60−70%. So, grapes harvested for table consumption or for vinification contained more diastereomer B that A.

Residues in Wine. Spiroxamine residues transferred from grapes into the must and through the vinification process into the wine are presented in **Tables 4** and **5** for Roditis and Cabernet grapes, respectively. Residue concentrations in wine without maceration, prepared from grapes with a spiroxamine content from 0.11 to 0.20 mg/kg, collected at the indicated time for grape wine preharvest intervals (35 days), varied from <0.026 to 0.046 mg/kg. This corresponds to 20−27% of the initial spiroxamine concentration in the grapes. Residues in wine prepared with maceration from the same, as above, grapes varied from 0.06 to 0.09 mg/kg, which was about 45−62% of those found in the grapes. Residue concentrations in wine prepared from grapes collected at 21 DAA were found 0.12 and 0.22 mg/kg for wine without and with maceration, respectively, corresponding to 35 and 65% of the initial spiroxamine concentration in the grapes.

Table 4. Fate of Spiroxamine Residues (mg/kg) and of the Ratio of Diastereomers A and B (A/B) from Grapes to Wine for Four Vinification Experiments on the Roditis Variety[a]

		must		wine	
	grapes	unprocessed	centrifuged	without maceration	with maceration
spiroxamine	0.13[a]	0.07	0.07	0.036	0.08
A	0.05	0.02	0.02	0.006	0.02
B	0.08	0.05	0.05	0.03	0.06
A/B ratio	0.63	0.40	0.40	0.20	0.33
spiroxamine	0.20[b]	0.06	0.07	0.046	0.09
A	0.08	0.02	0.02	0.006	0.02
B	0.12	0.04	0.05	0.04	0.07
A/B ratio	0.66	0.50	0.40	0.15	0.29
spiroxamine	0.34[c]	0.29	0.27	0.12	0.22
A	0.14	0.10	0.09	0.02	0.05
B	0.20	0.19	0.18	0.10	0.17
A/B ratio	0.70	0.52	0.50	0.20	0.29
spiroxamine	0.11[d]	0.04	0.04	<0.026	0.06
A	0.04	0.01	0.01	<0.006	0.01
B	0.07	0.03	0.03	0.02	0.05
A/B ratio	0.59	0.33	0.33	<0.30	0.20

[a] Grapes collected at 35 DAA (two applications, 2003). [b] Grapes collected at 35 DAA (two applications, 2002). [c] Grapes collected at 21 DAA (two applications, 2003). [d] Grapes collected at 35 DAA (one application, 2003).

Table 5. Fate of Spiroxamine Residues (mg/kg) and of the Ratio of Diastereomers A and B (A/B) from Grapes to Wine for Two Vinification Experiments on the Cabernet Variety (2003)

		must		wine	
	grapes	unprocessed	centrifuged	without maceration	with maceration
spiroxamine	0.15[a]	0.09	0.07	<0.031	0.07
A	0.05	0.03	0.02	<0.006	0.02
B	0.10	0.06	0.05	0.025	0.05
A/B ratio	0.50	0.50	0.40	<0.24	0.40
spiroxamine	0.30[b]	0.19	0.17	0.08	0.17
A	0.12	0.07	0.05	0.01	0.04
B	0.18	0.12	0.12	0.07	0.13
A/B ratio	0.66	0.58	0.42	0.14	0.31

[a] Grapes collected at 35 DAA. [b] Grapes collected at 14 DAA.

Mean transfer factors from grapes into must, centrifuged must, and wine from all of the experiments studied are presented in **Figures 3** and **4**, for Roditis and Cabernet grapes, respectively. A similar pattern of spiroxamine transformation during the wine making process was observed for the two grape varieties. Spiroxamine residues in the produced must were clearly lower than in grapes, presenting a mean transfer factor from grapes to must of 0.55, indicating that half of spiroxamine is transferred in the juice product and the rest remains on the solid commodity of the fruit. However, after centrifugation of the must, spiroxamine residues transferred were almost completely in the centrifuged must, indicating that spiroxamine is not adsorbed by the suspended solids in the must.

At the end of the wine-making process, the residue remaining in wine was lower than in grapes, presenting a mean transfer factor from grape to wine of 0.26 for the wines obtained without maceration and 0.55 for the wines obtained with maceration.

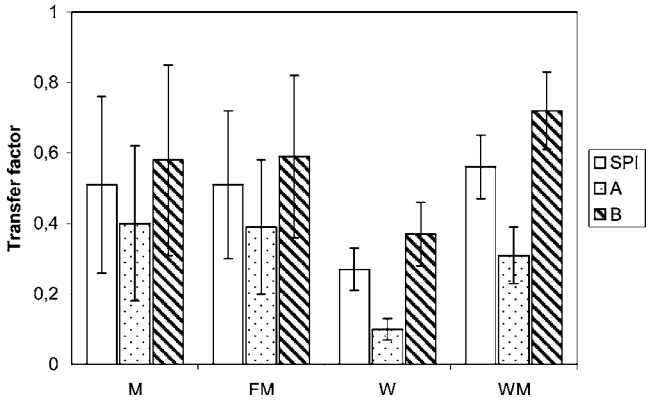

Figure 3. Mean transfer factors of spiroxamine (SPI) and spiroxamine diastereomers A (A) and B (B) from grapes of Roditis variety to must (M), centrifuged must (FM), and wines obtained without (W) or with (WM) maceration. Error bars represent standard deviation of the transfer factor for the four vinifications presented in **Table 4**.

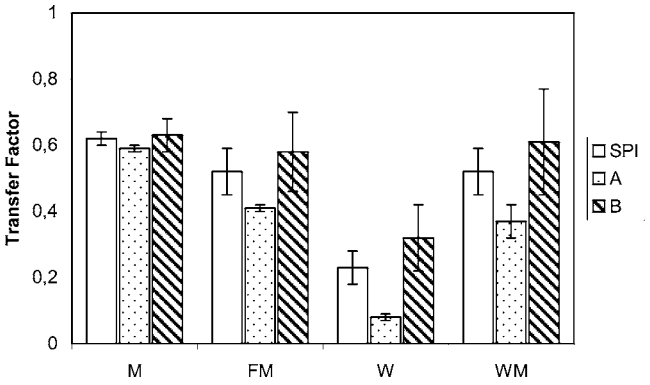

Figure 4. Mean transfer factors of spiroxamine (SPI) and spiroxamine diastereomers A (A) and B (B) from grapes of Cabernet Sauvignon variety to must (M), centrifuged must (FM), and wines obtained without (W) or with (WM) maceration. Error bars represent standard deviation of the transfer factor for the two vinifications presented in **Table 5**.

This indicates that the employed wine-making technique influences the transfer of spiroxamine residues from grape to wine. The higher transfer factors observed for wines obtained with maceration may be related to the high value of spiroxamine solubility in water (>200 g/L at pH 3), which enhanced immigration of spiroxamine from the solid commodity into the hydroalcoholic solution during the fermentation process.

In the wine obtained by vinification without maceration, the diastereomer B was with the major residue level, while levels of A were low, even below the quantitation limit (**Tables 4** and **5** and **Figure 1d**). The residue levels in the obtained wine were about 10 and 36% of that measured in grapes for diastereomers A and B, respectively.

Although, as referred above, grapes harvested for vinification contained more diastereomer B than A and the A/B ratio values ranged from 0.50 to 0.70 (**Tables 4** and **5**), the A/B ratio values in the produced wines ranged from 0.14 to <0.30, indicating a different transformation of A and B isomers during the wine-making process. As diastereomers A and B passed from grapes to wine at almost the same proportion and they are both totally transferred to the centrifuged must (**Figures 3** and **4**), their different fates may be attributed to the fermentation step. Diastereomer B dissipates slowly from must to wine as more than half of the residue found in must remained in the produced wine. On the contrary, diastereomer A dissipates faster, as approximately one-quarter of the residue in must was found in the produced wine. Thus, the behavior of diastereomer A from must to wine seems to be independent of the adsorption on particles (lees) and to be rather related to a selective degradation, chemical hydrolysis in acid solutions, or microbial degradation or to a selective adsorption of A by microbial population of yeast.

The same behavior to a smaller degree was observed in vinification with maceration. The residue levels in the obtained wine were about 33 and 70% of that measured in grapes for diastereomers A and B, respectively.

Conclusions. Spiroxamine showed a relatively fast dissipation in field-sprayed grapes, and half of the initial concentrations were measured about 7 days after the last application. Spiroxamine diastereomers A and B presented different dissipation rates with A dissipating faster relative to the B.

About 23−56% of spiroxamine residues was transferred from grapes to wine, depending on the wine-making technique. Grapes harvested at the legal PHI used for vinification had a spiroxamine concentration clearly lower than the MRL values. Residues in the obtained wines were found higher than 0.01 mg/kg, indicating that the concentration remaining in wine must be taken into account when assessing consumer exposure for the establishment of a MRL. The major diastereomer present in the obtained wines was B, having a higher concentration in the grapes used for vinification and also dissipating slower than A in the must used to produce the wine.

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