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Residue-free Wines: Fate of Some Quinone outside Inhibitor (QoI) Fungicides in the Winemaking Process

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The fate of three fungicide residues (fenamidone, pyraclostrobin, and trifloxystrobin) from vine to wine was studied to evaluate the decay ratio and the influence of the technological process. The aim of this work was to identify pesticides that can degrade rapidly or be eliminated together with byproduct (lees and cake) of the winemaking process to obtain wine free of residues. The disappearance rate on grapes was calculated as pseudo-first-order kinetics, and the half-life ($t_{1/2}$) was in the range from 5.4 ± 1.9 to 12.2 ± 1.2 days. The mechanism of dissipation of the three quinone outside inhibitor (QoI) fungicides was studied using different model systems. It was observed that the main mechanism responsible for disappearance was photodegradation. For active ingredients (ai) the half-lives of fenamidone, pyraclostrobin, and trifloxystrobin were 10.2 ± 0.8, 20.1 ± 0.1, and 8.6 ± 1.0 h, respectively, whereas for formulation higher half-lives were observed when epicuticular waxes were present (from 13.8 ± 0.2 to 26.6 ± 0.1 h). After winemaking, fenamidone, pyraclostrobin, and trifloxystrobin residues were not detected in the wine, but they were present in the cake and lees. This was due to the adsorption of pesticide residues to the solid parts, which are always eliminated at the end of the alcoholic fermentation. The data obtained in these experiments suggest that these three active ingredients could be used in a planning process to obtain residue-free wines.

KEYWORDS: QoI fungicides; model systems; residues; wine; grapes

INTRODUCTION

The main pests of vine are downy mildew (*Plasmopora viticola*), powdery mildew (*Uncinula necator*), and gray mold (*Botrytis cinerea*). The pesticides mainly employed against these adversities belong to the chemical classes of acylaniline, triazol, and dicarboximides and have been commercially used since the 1970s. It was only in the 1990s that new fungicides, belonging to new chemical classes, became available. These compounds have a different mode of action compared to traditional fungicides. Trifloxystrobin (methyl (*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α-trifluoro-*m*-tolyl)ethylideneaminooxy]-*o*-tolyl} acetate) and pyraclostrobin (methyl *N*-(2-{[1-(4-chlorophenyl)-1*H*-pyrazol-3-yl]oxymethyl}phenyl) *N*-methoxy carbamate) are strobilurines (**Figure 1**) having activities, similar to that of natural strobilurines, that are quite significant against downy and powdery mildew. They act on the respiration process by blocking the transport of electrons within the mitochondria from cytochrome *b* to cytochrome *c*1 by binding to a specific site (*1*). Fenamidone ((*S*)-1-anilino-4-methyl-2-methylthio-4-phenylimidazolin-5-one) (**Figure 1**) is a pesticide active against downy mildew, belonging to the imidazolinone class, having

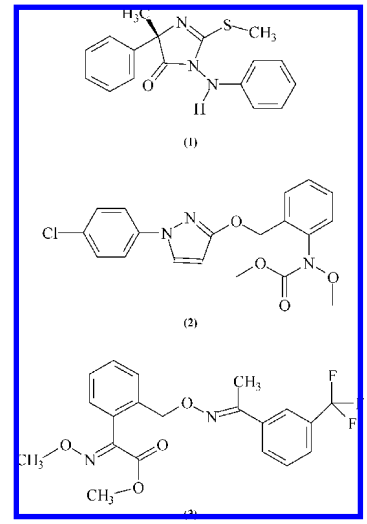

Figure 1. Structures of fenamidone (**1**), pyraclostrobin (**2**), and trifloxystrobin (**3**).

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an activity that is similar to that of strobilurines class (*2*). Strobilurine compounds are important tools for creating new strategies against resistance. Recently, the presence of xenobiotic residues in food has generated a great alarm among consumers. Many studies on the evolution of residues from grapes to wine have shown that it is possible not to transfer certain pesticides from grapes to wine during the process of winemaking (*3*−*12*). Therefore, it would be possible to eliminate grape parasites and to obtain residue-free wines.

EXPERIMENTAL PROCEDURES

Field Trials. The trial was carried out in a grape vineyard (cv. Cabernet Franc) located at Ussana, near Cagliari, Italy. A random block scheme was used with four replications for each experiment, and each block contained 80 plants. Flint (50% trifloxystrobin), Cabrio Top (5% pyraclostrobin), and Elicio (4.44% fenamidone) were the commercial formulations applied, together, at the doses recommended by the manufacturer (15, 150, and 40 g/hL, respectively). The fungicides were sprayed with an AM-190 portable motor sprayer (Oleo-Mac, Reggio Emilia, Italy). The fungicide treatments were carried out on September 10, 2007. From each block 3 kg grape samples were collected randomly, before and about 1 h after the treatment (when the canopy was dry) and subsequently at the 3rd, 7th, 14th, 21st, and 28th days. Meteorological data were collected by an agrometeorological station AD-2 (Silimet, Modena, Italy) located near the vineyard. During the experiments the maximum, minimum, and average temperatures were 32.9, 16.9, and 23.9 °C, respectively; total rainfall was 0.5 mm in one rainy day, and the average daily solar radiation was 278 W/m^2.

Winemaking. Each of the four samples was divided into three parts. One part was used for determination of the pesticide residues in the grapes; the other two (ca. 1 kg each) were processed with and without skins as described by Cabras et al. (*13*). Fermentation was regular in all samples, and after 15 days, the wines were centrifuged and analyzed for fungicide residues.

Chemicals. The active ingredient (ai) standards (purity ≥ 99%) were kindly provided by the manufacturer. Ethyl acetate, hexane, acetonitrile, and methanol were of HPLC grade (Carlo Erba, Milan, Italy). Water was distilled and filtered through a Milli-Q apparatus (Millipore, Bedford, MA). Standard stock solutions (≈1000 mg/L) were prepared in acetonitrile. Working standard solutions were obtained by dilution with the extract from untreated (control) grapes, must, and wine.

Apparatus and Chromatography. A liquid chromatograph model 2010 Shimadzu (Milan, Italy), equipped with a SIL-10ADvp autosampler, two pumps LC-10ADvp, a diode array detector DAD SPD-M10Avp, and a detector MS 2010, and connected to LC solution version 1.2 software was used. The analytical column was a Synergi Hydro RP-C_{18} end-capped (150 mm × 4.6 mm i.d. × 4 μm particle size) (Phenomenex, Castel Maggiore (BO), Italy) with a precolumn LiChrospher 100 RP-C_{18} (4 mm × 4 mm × 5 μm) (Merck, Darmstadt, Germany). The mobile phase was methanol/water (80:20 v/v) with 0.1% of trifluoroacetic acid, delivered at a flow of 0.40 mL/min in isocratic mode. The injected volume was 20 μL. The mass spectrometer detector was operated in the positive electron spray ionization (ESI). The ion source was set at 280 °C and 4.5 V, and the curved dissolvation line (CDL) temperature was 280 °C and 200 V. Nitrogen, produced with a gas generator UHPLCMS 12W (Dominick Hunter, Charlotte, NC), was used as desolvation gas at a temperature of 350 °C and with a flow of 4.5 L/h; the capillary was set at 4.5 kV. The SIM mode was used to monitor the $[M + H]^+$ adducts *m*/*z* 312 for fenamidone, *m*/*z* 388 for pyraclostrobin, and *m*/*z* 409 for trifloxystrobin. Matrix-matched standards were prepared at the same concentrations as that of the calibration solutions by adding the appropriate amounts of standards to the control matrix extracts. Quantitative determination of the three fungicides was realized by integrating the peak area of the LC-MS chromatograms. The analysis time was 20 min.

Extraction Procedures. A 5 g aliquot of grape or a 5 mL aliquot of must and wine samples was weighed or measured in a 30 mL screw-capped tube; after the addition of 10 mL of a mixture of ethyl acetate/hexane (50:50, v/v), the tubes were agitated for 15 min in a rotary shaker (Falc Instuments, Bergamo, Italy). The phases were allowed to separate, and 1 mL of organic phase was evaporated to dryness under a gentle nitrogen stream. The dry extract was dissolved in 1 mL of mobile phase before injection in the HPLC-MS system.

Statistical Assays. This method was validated under EURACHEM Guide (1998) (*14*) and EURACHEM/CITAC Guide (2000) (*15*) recommendations. Analysis of variance (ANOVA) was carried out with the STATISTICA program, using Turkey's test at $p \leq 0.05$.

Model Systems and Cuticular Wax Extraction. To establish the fungicide disappearance mechanisms (thermodegradation, evaporation, codistillation, and photodegradation) in grape, model systems were used according to the method of De Melo Abreu (*10*). Grape wax extraction was performed as described by McDonald (*16*).

Test A (Effect of Evaporation and Thermodegradation on Pesticides). The fungicide was dissolved in acetone and poured on a regenerated cellulose membrane. After the solvent was evaporated, the membrane was placed in a 10 mL screw-capped vial. The control vial was stored in the dark at room temperature. The sample vials were placed in a thermostatic stove at 50 °C for 24 h, then removed and stored at −20 °C for 5 h to allow the ai in the gaseous state to condense on the inside of the vial. The membrane was then transferred to another vial and treated following the method described under Extraction Procedures to determine the pesticide residue in the membrane (*m*). Five milliliters of extraction mixture was added into the vial (v), shaken, and analyzed following the method described under Extraction Procedures to determine the amount of pesticides evaporated from the membrane. The amount of thermodegraded pesticides was measured by subtracting the sum of pesticide residues detected in the membrane and on the inside of the sample vials from the amount detected in membrane of the control vial $[c - (v + m)]$.

Test B (Effect of Codistillation on Pesticides). The fungicide was dissolved in acetone and poured on membranes of regenerated cellulose. When the solvent evaporated, the membrane was placed onto the vial and then closed with a screw-holed cap. The 10 mL vial was filled with 5 mL of water, weighed, and placed in a thermostatic stove at 50 °C. A control vial, treated in the same manner, was stored in the dark at room temperature. After 24 h, the sample vial was returned to room temperature and weighed to determine the amount of water evaporated. During the evaporation, the water passing through the membrane could entrain the pesticide residues from the membrane (codistillation process). After measuring, if the amount of pesticides left in the sample membrane (*m*) was lower than that in the control membrane (*c*), the loss ($c - m$) in ai could be due to the codistillation process. A correct estimation of the codistillation is obtained by considering the losses due to evaporation and thermodegradation determined with test A.

RESULTS AND DISCUSSION

Analytical Method. The proposed chromatographic method allowed a good separation of the three fungicides (fenamidone t_r 9.5 min; pyraclostrobin t_r 11.3 min; trifloxystrobin t_r 14.7 min) (**Figure 2**). For the three fungicides calibration standards ranged from 0.01 to 1.00 mg/kg. In this range a good linearity for the mass detector was achieved with R^2 ranging from 0.996 to 0.999. Due to the extraction method, no cleanup was necessary. The recovery for the three fungicides ranged from 82 to 113% for grapes, from 80 to 105% for must, and from 85 to 109% for wines. Precision and accuracy were valued with $n = 6$ for standards, grape, must, and wine samples. The maximum variation coefficients were 9.9% in repeatability and 11.3% in reproducibility (**Table 1**). The limits of quantitation (LOQs), calculated as 10-fold increases of the signal-to-noise ratio, for the three fungicides are reported in **Table 2**.

Residues in Grapes and Wine. *Trifloxystrobin. (a) Grapes.* After treatment, trifloxystrobin residues on grapes were 0.56 ± 0.16 mg/kg (**Table 3**), and they decreased according to a pseudo-first-order kinetics ($R^2 = 0.911$) with a half-life of 6.4 ± 1.0 days. After 3 days from treatment, the residues of this fungicide were 0.32 ± 0.1 mg/kg, and after 7 days they were 0.25 ±

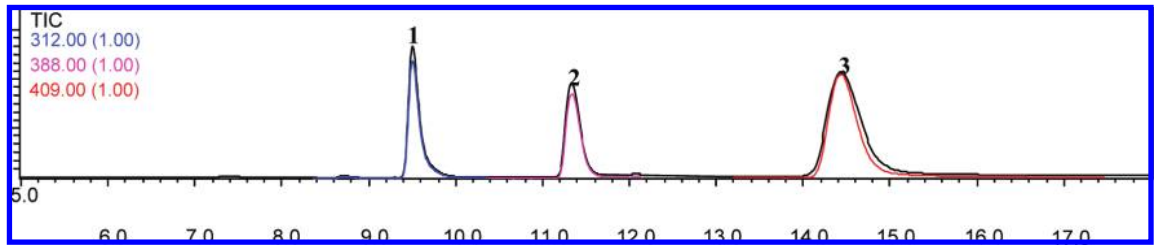

Figure 2. Ion chromatogram of fenamidone (peak 1), pyraclostrobin (peak 2), and trifloxystrobin (peak 3). Standard mix at 0.1 mg/kg.

Table 1. Validation Parameters for QoI Fungicides in Grapes, Must, and Wine at Three Different Levels of Fortification

	trifloxystrobin	pyraclostrobin	fenamidone
Repeatability in Grapes ($n = 6$), CV (%)			
0.1 mg/kg	8.6	4.4	9.9
0.5 mg/kg	5.1	4.8	8.4
1.0 mg/kg	8.1	6.9	8.4
Intermediate Precision in Grapes ($n = 6$), CV (%), mg/kg			
0.1 mg/kg	4.5	5.4	3.6
0.5 mg/kg	4.2	11.3	5.6
1.0 mg/kg	6.9	7.5	5.9
Recovery in Grapes ($n = 6$) ± SD, R (%)			
0.1 mg/kg	82 ± 10	99 ± 5	87 ± 4
0.5 mg/kg	94 ± 12	96 ± 4	96 ± 8
1.0 mg/kg	90 ± 9	113 ± 7	95 ± 9
Recovery in Wines ($n = 6$) ± SD, R (%)			
0.1 mg/L	93 ± 6	109 ± 3	98 ± 5
0.5 mg/L	85 ± 4	98 ± 8	93 ± 4
1.0 mg/L	103 ± 5	104 ± 7	94 ± 5
Recovery in Musts ($n = 6$) ± SD, R (%)			
0.1 mg/L	80 ± 4	96 ± 8	91 ± 7
0.5 mg/L	89 ± 6	105 ± 4	101 ± 9
1.0 mg/L	85 ± 8	100 ± 4	96 ± 8

Table 2. Limits of Quantitation (LOQ) of QoI Fungicides in Grapes, Must, and Wine

compound	LOQ in grapes (mg/kg)	LOQ in must (mg/L)	LOQ in wines (mg/L)
trifloxystrobin	0.02	0.02	0.04
pyraclostrobin	0.03	0.02	0.06
fenamidone	0.02	0.02	0.05

Table 3. Residues of the Three QoI Fungicides and Dissipation Half-Lives in Grapes after Field Treatment ($n = 4$)

days after treatment	trifloxystrobin (mg/kg ± SD)	pyraclostrobin (mg/kg ± SD)	fenamidone (mg/kg ± SD)
0	0.56 ± 0.16	0.38 ± 0.15	0.93 ± 0.22
3	0.32 ± 0.10	0.25 ± 0.07	0.58 ± 0.25
7	0.25 ± 0.05	0.15 ± 0.09	0.35 ± 0.07
14	<0.02	0.13 ± 0.03	0.26 ± 0.02
21	<0.02	0.10 ± 0.02	0.18 ± 0.07
28	<0.02	0.09 ± 0.06	0.18 ± 0.02
$t_{1/2}$ (days)	6.4 ± 1.0	5.4 ± 1.9	12.2 ± 1.2
R^2	0.911	0.998	0.870
MRL	5.0	2.0	0.5

0.05 mg/kg; from the 14th day until harvest time, the residues of trifloxystrobin were no longer detectable (<0.02 mg/kg). In Europe and in Italy the maximum residue level (MRL) of this fungicide is 5 mg/kg with a preharvest interval (PHI) of 35 days (*17*). Considering our data, it is not clear why the legislature set a MRL of 5 mg/kg and such a long PHI. In addition, with a high disappearance rate ($t_{1/2} = 6.4 \pm 1.0$ days), even after repeated treatments, it is unlikely to have residues of 5 mg/kg at harvest time. The model systems showed that the ai did not thermodegrade (**Table 4**), evaporate (**Table 4**), or codistillate (**Table 5**). The only mechanism that affected the disappearance was photodegradation. The active ingredient in the formulation was more stable in the presence of epicuticular waxes ($t_{1/2} = 6.9 \pm 0.3$ versus 13.8 ± 0.2 h) (**Table 6**). The solubilization of ai in epicuticular waxes significantly decreases the photodegradation rate, probably due to the lipophilic features of trifloxystrobin (log $K_{ow} = 4.5$).

(b) Winemaking. The grape samples used in the winemaking process were harvested 3 days after the treatment (0.32 ± 0.10 mg/kg). In the winemaking without maceration 30% of cakes and 70% of must were obtained. The residues in the must were 0.39 ± 0.06 mg/L (**Table 7**). Trifloxystrobin residues in both samples, must and grapes, did not show a statistically significant difference. The cleanup of must by centrifugation allowed the complete removal of the residue. The amount of lees was 4% w/v, calculated as wet weight, with a residue of 12.27 ± 0.67 mg/kg. The residue in the cake was 0.03 ± 0.01 mg/kg. This indicated that the residues were mainly adsorbed on the solid parts and particularly on the lees. The wine obtained without maceration was residue-free. In the wine processed with maceration, the three fractions were analyzed: wine (67%), cake (29%), and lees (4%) (**Table 8**). Residues in wine were not detectable (<0.04 mg/L), whereas in cake and in lees they were 0.23 ± 0.04 and 7.13 ± 0.21 mg/kg, respectively. This result confirms that the ai was preferably adsorbed in lees. The Italian MRL for wine is 0.3 mg/L (*18*). Our experiments showed that in wine trifloxystrobin residues are below the LOQ. At 14 days from treatment and at harvest time, residues of trifloxystrobin were not detectable. Residues were selectively adsorbed in cakes and lees. On the basis of our experimental data it is difficult to understand such a high MRL on wine; a more appropriate MRL value for wine would correspond to the LOQ.

Pyraclostrobin. (a) Grapes. After treatment, the pyraclostrobin showed a residue of 0.38 ± 0.15 mg/kg (**Table 3**) comparable with trifloxystrobin; this result is consistent with the same doses used in the treatment (7.5 g of ai/hL). Pyraclostrobin residues decreased with time: 3 days after treatment they were 0.25 ± 0.07 mg/kg, and after 7 days, they were 0.15 ± 0.09 mg/kg. The half-life, calculated as pseudo-first-order kinetics ($R^2 = 0.998$), was 5.4 ± 1.9 days. This indicates that pyraclostrobin decreased quickly, similarly to trifloxystrobin, and it suggests that the residues at harvest time might be very low even after several treatments. The disappearance study with model systems indicates that, like trifloxystrobin, pyraclostrobin does not thermodegrade (**Table 4**), evaporate (**Table 4**), or codistillate (**Table 5**); the only mechanism that affects disappearance from grapes is photodegradation. With regard to photodegradation in the presence of epicuticular wakes, the behavior of the formulation containing pyraclostrobin was similar to that of the formulation containing

Table 4. Results of Evaporation and Thermodegradation of Active Ingredients and Formulations with and without Epicuticular Waxes (mg/kg ± SD) (Test A)[a]

sample	waxes	control (*c*) (mg/kg)	vial (*v*) (mg/kg)	membrane (*m*) (mg/kg)	difference [*c* − (*v* + *m*)] (mg/kg)
trifloxystrobin	without	2.22 ± 0.22	nd	2.22 ± 0.16	ns
	with	2.40 ± 0.24	nd	2.33 ± 0.08	ns
Flint (formulation)	without	1.49 ± 0.06	nd	1.44 ± 0.10	ns
	with	1.45 ± 0.11	nd	1.45 ± 0.07	ns
pyraclostrobin	without	3.49 ± 0.16	nd	3.52 ± 0.14	ns
	with	3.69 ± 0.27	nd	3.61 ± 0.18	ns
Cabrio Top (formulation)	without	1.38 ± 0.10	nd	1.56 ± 0.15	ns
	with	1.42 ± 0.16	nd	1.47 ± 0.12	ns
fenamidone	without	1.11 ± 0.07	nd	0.83 ± 0.04	0.28 ± 0.02*
	with	1.04 ± 0.05	nd	0.96 ± 0.03	0.08 ± 0.02*
Elicio (formulation)	without	0.44 ± 0.06	nd	0.44 ± 0.05	ns
	with	0.42 ± 0.03	nd	0.41 ± 0.03	ns

[a] nd, not detectable; ns, not significant; *, significant $p < 0.05$).

Table 5. Results of Codistillation of Active Ingredients and Formulations with and without Epicuticular Waxes (mg/kg ± SD) (Test B)[a]

sample	waxes	control (*c*) (mg/kg)	membrane (*m*) (mg/kg)	difference (c − m) (mg/kg)
trifloxystrobin	without	2.20 ± 0.18	2.00 ± 0.20	ns
	with	2.40 ± 0.20	2.14 ± 0.13	ns
Flint (formulation)	without	3.61 ± 0.41	2.91 ± 0.78	ns
	with	3.74 ± 0.34	3.73 ± 0.30	ns
pyraclostrobin	without	2.06 ± 0.08	2.09 ± 0.18	ns
	with	2.31 ± 0.09	2.31 ± 0.11	ns
Cabrio Top (formulation)	without	2.15 ± 0.07	1.97 ± 0.17	ns
	with	2.18 ± 0.09	2.29 ± 0.15	ns
fenamidone	without	2.10 ± 0.20	1.90 ± 0.33	ns
	with	1.70 ± 0.15	1.80 ± 0.10	ns
Elicio (formulation)	without	1.43 ± 0.05	1.44 ± 0.05	ns
	with	1.63 ± 0.06	1.61 ± 0.05	ns

[a] nd, not detectable; ns, not significant; *, significant $p < 0.05$.

Table 6. Results of Sunlight Photodegradation of Active Ingredients and Formulations: Half-Lives ($t_{1/2}$) Calculated in Hours with and without Epicuticular Waxes ($n = 4$)

sample	$t_{1/2}$ (h) without waxes	$t_{1/2}$ (h) with waxes
frifloxystrobin	8.6 ± 1.0	11.1 ± 0.6
Flint (formulation)	6.9 ± 0.3	13.8 ± 0.2
pyraclostrobin	20.1 ± 0.1	13.3 ± 0.2
Cabrio Top (formulation)	6.4 ± 0.2	18.5 ± 0.2
fenamidone	10.2 ± 0.8	3.3 ± 0.2
Elicio (formulation)	8.6 ± 0.6	26.6 ± 0.1

trifloxystrobin (**Table 6**). These data indicate that, like trifloxystrobin, the MRL for grapes (2 mg/kg with a PHI of 35 days) (*17*) is not consistent with field results.

(b) Winemaking. As for trifloxystrobin, the grape samples used in the winemaking process were harvested 3 days after treatment, and the residues of pyraclostrobin were 0.25 ± 0.07 mg/kg. The must showed a residue of 0.30 ± 0.07 mg/L (**Table 7**). Residues in the two samples, must and grapes, did not show any statistical difference. As for trifloxystrobin the cleanup of must by centrifugation allowed the complete removal of this ai. The amount of lees was 4% w/v, with a residue of 7.44 ± 0.59 mg/kg. The residue in the cake was 0.06 ± 0.02 mg/kg. This indicated that, as for trifloxystrobin, the residues of pyraclostrobin were preferably adsorbed by the lees. The wine obtained without maceration showed a residue below the LOQ (<0.06 mg/L). Residues on the three fractions obtained from winemaking without maceration (wine, 67%; cake, 29%; and lees, 4%) were not detectable in the wine fraction (<0.06 mg/L), whereas they were present in cake (0.40 ± 0.04 mg/kg) and in lees (3.95 ± 0.33 mg/kg) (**Table 8**). This confirms that also this ai was preferably adsorbed in lees. The Italian MRL for wine is 0.05 mg/L (*19*). Our experiments confirmed that this MRL value is appropriate and consistent with our field results.

Table 7. Residues (mg/kg or mg/L ± SD) of the Three QoI Fungicides during Winemaking without Maceration (Grapes Harvested at the Third Day)

	trifloxystrobin	pyraclostrobin	fenamidone
grapes	0.32 ± 0.10	0.25 ± 0.07	0.58 ± 0.25
must	0.39 ± 0.06	0.30 ± 0.07	0.64 ± 0.08
centrifuged must (66%)	<0.02	<0.02	0.22 ± 0.08
cakes (30%)	0.03 ± 0.01	0.06 ± 0.02	0.28 ± 0.33
lees (4%)	12.27 ± 0.67	7.44 ± 0.59	10.25 ± 1.09
centrifuged wine	<0.04	<0.06	<0.05.

Table 8. Residues (mg/kg or mg/L ± S.D.) of the Three QoI Fungicides during Winemaking with Maceration (Grapes Harvested at the Third Day)

	trifloxystrobin	pyraclostrobin	fenamidone
grapes	0.32 ± 0.10	0.25 ± 0.07	0.58 ± 0.25
centrifuged wines (67%)	<0.04	<0.06	<0.05.
cakes (29%)	0.23 ± 0.04	0.40 ± 0.04	0.15 ± 0.03
lees (4%)	7.13 ± 0.21	3.95 ± 0.33	6.70 ± 1.14

Fenamidone. (a) Grapes. After treatment, fenamidone showed a residue of 0.93 ± 0.22 mg/kg (**Table 3**), which is about 2-fold higher compared to the residues of trifloxystrobin and pyraclostrobin. This result can be explained because the quantity of ai used was more than double (17.8 g of ai/hL) that used in the experiments for trifloxystrobin and pyraclostrobin. Fenamidone

decreased with time up to the 21st day. From the 21st day to harvest time the residues detected were constant (0.18 mg/kg). The half-life, calculated as pseudo-first-order kinetic (R^2 = 0.870), was 12.2 ± 1.2 days. This indicates that fenamidone decreased much more slowly than trifloxystrobin and pyraclostrobin. At the PHI (30 days) we detected a residue of fenamidone below the MRL (0.18 vs 0.5 mg/kg) (*17*). Analyses with model systems showed that the only mechanism affecting disappearance of fenamidone from grapes was photodegradation. The photodegradation experiments using Elicio formulation (**Table 6**) showed that fenamidone photodegraded with a lower rate ($t_{1/2}$ = 26.6 ± 0.1 h) when the ai was incorporated in epicuticular waxes. These data suggest that coformulants might be incorporated in waxes, lowering the photodegradation of this ai.

(b) Winemaking. As for trifloxystrobin and pyraclostrobin, the grapes used in winemaking were harvested at the third day after treatment, and the residue was 0.58 ± 0.25 mg/kg, comparable to the MRL value (0.5 mg/kg) (*17*). In the winemaking process carried out without maceration, the residue of fenamidone in the must was 0.64 ± 0.08 mg/L (**Table 7**). Residues in the two samples, must and grapes, did not show any statistical differences. The cleanup of must by centrifugation did not completely remove this ai, leaving a residue of 0.22 ± 0.08 mg/L. At the end of fermentation the residues were not detectable in wine (<0.05 mg/L). The sum of residues detected in cakes (30%) and lees (4%) was 0.49 ± 0.11 mg/kg. These data indicate that the ai was adsorbed in cakes and lees. The wine obtained without maceration showed a residue below the LOQ (<0.05 mg/L). However, some residues were found in cake (0.15 ± 0.03 mg/kg) and in lees (6.70 ± 1.14 mg/kg) (**Table 8**). The sum of these quantities is 0.31 ± 0.05 mg/kg, which is 53% of ai detected in grapes. In Italy the MRL for fenamidone in wine is 0.5 mg/L (*20*).

In conclusion, residues of the three fungicides in grapes, at harvest time, were below the MRL values (*17*). Nevertheless, our field results for trifloxystrobin and pyraclostrobin were not consistent with the MRL values. In our opinion, these MRL values are too high if compared with field results. On the basis of the model system experiments the main process responsible for the QOI degradation is photodegradation. In the winemaking process, with and without maceration, we found that QoI residues were adsorbed in the solid phases (cake and lees), whereas in the wine they were below the LOQ. The data obtained in our experiments suggest that these three active ingredients may be used in a planning process to obtain residue-free wines.

LITERATURE CITED

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