Fate of Azoxystrobin, Fluazinam, Kresoxim-methyl, Mepanipyrim, and Tetraconazole from Vine to Wine

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The fate of five fungicide residues (azoxystrobin, fluazinam, kresoxim-methyl, mepanipyrim, and tetraconazole) from vine to wine was studied, to evaluate the decay ratio and study the influence of the technological process. The disappearance rates on grapes were described as pseudo-first-order kinetics (r between 0.96 and 0.99) and half-life ($t_{1/2}$) in the range of 4.3−15.2 days. After wine-making, fluazinam, mepanipyrim, and tetraconazole had negligible residues in all samples. This was due to fermentation in the case of fluazinam and mepanipyrim and to removal during the formation of must in the case of tetraconazole. The residue level of azoxystrobin was higher in the wine obtained by vinification without maceration than with maceration. Azoxystrobin was the only active ingredient found in both grapes and wine. The clarifying process showed that among the clarifying agents used, only charcoal was efficient in decreasing the residues completely.

Keywords: *Fungicide; residues; wine-making; grape*

The main pests of vine are downy mildew (*Plasmopora viticola*), powdery mildew (*Uncinula necator*), and gray mold (*Botritis cinerea*). The main pesticides traditionally employed belong to the chemical classes of acylaniline, triazol, and dicarboximides and have been marketed 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. Azoxystrobin and kresoxim-methyl are strobilurines that showed significant activity against downy and powdery mildew. The mode of action is similar to that of natural strobilurines. They act on the respiration process by blocking the transport of electrons within the mitochondria from cytochrome b to cytochrome c_1 by binding a specific site (Ammermann et al., 1992). Mepanipyrim is an anilinopyrimidine that acts by inhibiting the biosynthesis of methionine by the pathogen (Masner et al., 1994). Fluazinam is a 2,6-dinitroaniline that causes the uncoupling of oxidative phosphorylation in the mitochondria (Guo et al., 1991). Because the mechanism of action of these new compounds is different from that of the usual fungicide, these compounds are important tools for the creation of new strategies against resistance. No works have been found in the literature on the destiny of the pesticide residues of these new compounds in vine and during the wine-making process. This work is an attempt to give a contribution to the knowledge of the fate of the residues of these new active ingredients (AIs) from vine to wine. In addition to the new compounds we also studied tetraconazole, a fungicide of the last generation of the triazoles, which inhibits the biosynthesis of ergosterol (Garavaglia et al., 1996). This work is the continuation of a study of pesticide residues from vine to wine that has so far dealt with cyprodinil, fludioxonil, pyrimethanil, and tebuconazole (Cabras et al., 1997).

EXPERIMENTAL PROCEDURES

Field Trials. The trial was carried out in a grape vineyard (cv. Chardonnay), located at Ussana, near Cagliari, Italy. An espalier vineyard was planted in 1989 with a planting distance of 2.5 × 1.5 m. The plants were completely wetted with 700 L × ha on July 8 and 29. A random-block scheme was used with four replications for each test, and each block contained 60 plants. Quadris (25% of azoxystrobin), Ohayo (39.5% fluazinam), Stroby WG (50% kresoxim-methyl), Kif 3535 (50% mepanipyrim), and Concorde 4 EC (4% tetraconazole) were the commercial formulations applied at the dose recommended by the manufacturer (130, 550, 53, 523, and 19 g of AI/ha, respectively) with an F-320 portable motor sprayer (Fox Motori, Reggio Emilia, Italy). Samplings on dry plants started ∼1 h after the last treatment; random 5-kg samples of grapes were collected from each plot and immediately analyzed for fungicide residues. The samplings and analyses were repeated weekly.

The environmental conditions were continuously recorded with an AD-2 automatic weather station (Silimet, Modena, Italy). During the experiments, the total rainfall was 1.8 mm on 1 rainy day; the maximum and minimum average temperatures were, respectively, 32.9 and 18.6 °C.

Wine-Making. A portion of each of the four grape samples was analyzed directly; the remaining parts were pressed and stemmed together, 200 mg of sodium metabisulfite and 200 mg of dry yeast per kilogram of grapes were added, and the mixed sample was divided into two equal parts. 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). An

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Table 1. Fungicide Residues (Milligrams per Kilogram ± SD) in Grapes, Must, and Wine

			must		wine	
fungicide	days after treatment	grapes	before centrifugation	after centrifugation	without maceration	with maceration
azoxystrobin	0	0.50 ± 0.09	0.37	0.37	0.20	0.12
	7	0.31 ± 0.11	0.17	0.17	0.19	0.09
	14	0.23 ± 0.03	0.13	0.11	0.10	0.06
	21	0.19 ± 0.06	0.13	0.13	0.13	0.09
fluazinam	0	1.21 ± 0.30	0.30	0.08	nd[a]	nd
	7	0.51 ± 0.05	0.20	0.02	nd	nd
	14	0.15 ± 0.03	0.11	nd	nd	nd
	21	0.04 ± 0.01	nd	nd	nd	nd
kresoxim-methyl	0	0.15 ± 0.05	0.13	0.05	0.18	0.09
	7	0.08 ± 0.01	0.11	nd	0.08	0.04
	14	nd	nd	nd	nd	nd
	21	nd	nd	nd	nd	nd
mepanipyrim	0	1.00 ± 0.30	0.96	0.37	nd	nd
	7	0.55 ± 0.19	0.24	0.11	nd	nd
	14	0.34 ± 0.10	0.22	0.13	nd	nd
	21	0.31 ± 0.06	0.16	0.07	nd	nd
tetraconazole	0	0.14 ± 0.02	nd	nd	nd	nd
	7	0.06 ± 0.01	nd	nd	nd	nd
	14	0.03 ± 0.01	nd	nd	nd	nd
	21	nd	nd	nd	nd	nd

[a] nd, not detectable (<0.01).

aliquot of cloudy must (100 g) was taken and centrifuged (4000 rpm for 5 min) to assess the amount of lees and the residue concentration in the clear must. Fermentation had a regular course in all samples, and after 15 days the obtained wines were filtered and analyzed for fungicide residues.

Wine Clarification. Clarification tests were carried out on 1-L samples of residue-free wine after addition of 0.25 mg/L of the studied fungicides. The clarifying agents and doses were those usually applied in oenological practices. Two days after clarification, the clear wine and the control samples (without clarification) were analyzed for fungicide residues. Each clarification test was performed with four replications.

Chemicals. The AI standards (purity > 99%) were graciously provided by the manufacturer. Acetone and hexane were of HPLC grade (Carlo Erba, Milan, Italy). Sodium chloride and anhydrous sodium sulfate were reagents for analysis (Carlo Erba). Triphenyl phosphate (99%) was used as internal standard (i.s.) and was of analytical grade (Janssen, Geel, Belgium).

Standard stock solutions (~500 mg/L) were prepared in methanol. Working standard solutions were obtained by dilution with the extract containing triphenyl phosphate as i.s. at 0.03 mg/kg from untreated (control) grapes, must, and wine.

The clarifying agents used were bentonite (100 g/hL; Superbenton, Dal Cin, Milan, Italy); charcoal (20 g/hL; Decoran, AEB, Brescia, Italy); potassium caseinate (100 g/hL; Caseoflok, Marescalchi, Alessandria, Italy); gelatin (20 g/hL; Gelasil, AEB, Brescia, Italy); and polyvinylpolypyrrolidone (80 g/hL; Fluka, Milano, Italy).

Apparatus and Chromatography. A gas chromatograph Fison HRGC series Mega 2 (Carlo Erba) equipped with a nitrogen−phosphorus detector (NPD-80), a split−splitless injector, and an AS 800 autosampler (Carlo Erba) and connected to an HP 3396-A reporting integrator (Hewlett-Packard, Avondale, PA) was used. The capillary column was a WCOT fused silica column CP-Sil 8 CB liquid phase (25 m × 0.25 mm i.d., 0.12 μm) (Chrompack International, Middelburg, The Netherlands). The injector and detector were operated at 250 and 300 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s). The oven temperature was programmed as follows: 110 °C for 1 min, raised to 260 °C (10 °C/min), held for 5 min. Helium was the carrier gas at 120 kPa, and N_2 was the makeup gas at 80 kPa. The plasma of the detector was obtained using H_2 (60 kPa) and air (110 kPa), the current was 2.75 A, and the voltage was 3.5 V. Calibration graphs for the active ingredients were constructed with the i.s. method by measuring peak heights versus concentrations. A good linearity was achieved in the 0.01−2 mg/kg range, with correlation coefficients between 0.9987 and 0.9995.

Extraction Procedure. A 5-g aliquot of sample was weighed in a 30-mL screw-capped tube; 4 g of NaCl and 10 mL of acetone/hexane (50:50, v/v) were added, and the tube was agitated for 30 min in a rotary shaker. The phases were allowed to separate, and the organic layer was poured into another tube containing 1 g of anhydrous sodium sulfate.

RESULTS AND DISCUSSION

The determinations of pesticide residues were performed according to a method described by Cabras et al. (1998); this method showed good recovery and repeatability and a sufficient limit of determination. The results obtained during the experiment on grapes and on vinification are reported in Table 1.

Azoxystrobin. This fungicide had an initial residue of 0.50 mg/kg that decreases with pseudo-first-order kinetics ($r = 0.98$) and a half-life of 15.2 days; therefore, at harvest time its residue was 0.19 mg/kg. The residue level in the must was 35% lower than in the grapes. The separation of lees (~6%) from must did not affect the residue level, indicating that the residues were not adsorbed on the suspended matter.

The wines obtained with vinification without maceration had residues similar to those found in the must, whereas those obtained by vinification with maceration had residues one-third lower than those found in the grapes. This indicated that the residues were preferably adsorbed on skins. This is confirmed by the residue level that in the wine obtained by vinification without maceration was higher than in that obtained by vinification with maceration.

Fluazinam. This AI degraded more rapidly than azoxystrobin. It had an initial residue of 1.21 mg/kg that became 0.04 mg/kg at harvest time. The half-life was calculated as a pseudo-first-order kinetics ($r = 0.99$) and was 4.3 days. The residue percentage in the must increased as the grape residue decreased. After the first sampling, the residue in the must was 25% of that in the grapes; after 7 days it was 39% and after 14 days, 73%. Clarification by centrifugation caused a significant loss of pesticide and a total loss when the residue was

<0.20 mg/kg. These data showed that the residues were adsorbed both on lees and on skins. At harvest time the wines obtained with the two vinification processes did not have any residues.

Kresoxim-methyl. After treatment, kresoxim-methyl showed a very low residue (0.15 mg/kg). This was due to the low doses × ha used (55 g of AI). After 2 weeks, no residue was found in the grapes. Kresoxim-methyl is structurally similar to azoxystrobin, but it had a different behavior during vinification. The residue levels in the must were similar to those in the grapes, although they rapidly decreased on centrifugation. The wines obtained with vinification without maceration showed a residue similar to that in the must, whereas the wines obtained by vinification with maceration had residues about half that in the grapes.

Mepanipyrim. The half-life, calculated by a pseudo-first-order kinetics ($r = 0.96$), was 12.8 days in grapes. During 3 weeks the residue level decreased from 1.00 to 0.31 mg/kg at harvest time. Must formation caused a residue decrease of about half compared to the residue found in the grapes. This was also obtained with centrifugation of the must. This behavior is similar to that of fluazinam. No residue was detectable in any of the wines. Mepanipyrim is structurally similar to pyrimethanil but showed a completely different behavior both in its degradation in grapes and during wine-making (Cabras et al., 1997).

Tetraconazole. As with kresoxim-methyl, the low doses employed (28 g of AI × ha) caused a very low (0.14 mg/kg) residue level just after treatment. The disappearance rate was fast, and the half-life calculated as a pseudo-first-order kinetics ($r = 0.99$) was 6.3 days. During wine-making, the residue was completely eliminated, indicating a high affinity of tetraconazole for the solid matter. Clarification showed that only charcoal was effective in reducing the residue completely, whereas the other clarifying agents were ineffective.

Conclusions. The used fungicides showed decay rates with pseudo-first-order kinetics and r between 0.96 and 0.99 and $t_{1/2}$ between 4.3 and 15.2 days. Azoxystrobin and kresoxim-methyl are structurally similar but showed different behaviors during wine-making. The former decreased during wine-making, showed no decrease during centrifugation, and decreased during the fermentation process. The latter showed a decrease during centrifugation and fermentation with maceration, while wine-making and fermentation without maceration did not affect the residue level. Fluazinam, mepanipyrim, and tetraconazole did not present residues in wine, as a result of fermentation in the case of fluazinam and mepanipyrim and as result of wine-making in tetraconazole. Vinification with maceration led to wines with residue levels of azoxystrobin and kresoxim-methyl lower than that obtained without maceration. At harvest time azoxystrobin was the only pesticide with a residue in wine. The clarifying agents bentonite, potassium caseinate, gelatin, and PVPP did not affect the residue levels in wine, whereas charcoal proved very effective and led to complete elimination of the fungicide residue.

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