Fate of Some New Fungicides (Cyprodinil, Fludioxonil, Pyrimethanil, and Tebuconazole) from Vine to Wine

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The fate of four new fungicides (cyprodinil, fludioxonil, pyrimethanil, and tebuconazole) from the treatment on vine to the production of wine was studied. The influence of clarifying agents (bentonite, charcoal, potassium caseinate, gelatin, and polyvinylpolypyrrolidone) on residue concentrations in wine was also studied. The fungicide residues on grapes showed different decay rates after treatment, with first-order kinetics and half-lives ranging from 8 to 57 days. Grape processing into wine caused considerable residue reduction with cyprodinil (*ca*. 80%), fludioxonil (*ca*. 70%), and tebuconazole (*ca*. 50%) and no reduction with pyrimethanil. The two wine-making techniques employed (with and without maceration) had the same influence on the residue concentrations in wine, except for fludioxonil which showed maximum residue reduction with vinification with maceration. Among the clarifying agents tested, only charcoal showed effective action on the elimination of residue content in wine, proving complete elimination, or almost, of fungicide residues.

Keywords: *Fungicides; residues; wine-making*

INTRODUCTION

The active ingredients (AI) customarily used to control grey mold (*Botrytis cinerea*) on vine belong to different chemical classes (phthalimides, benzimidazoles, and dicarboximides). The prolonged, frequent use of these AI has contributed to reduce their effectiveness thanks to the development of phenomena of resistance. Recently new fungicides (Cyprodinil, Fludioxonil, pyrimethanil, and tebuconazole) of other chemical families are commercially available. These compounds show a high level of activity against *B. cinerea* strains, resistant to commonly used fungicides. Cyprodinil and pyrimethanil are anilinopyrimidines whose mechanism of action consists of the inhibition of methionine biosynthesis (Mastner et al., 1994). Fludioxonil, a phenylpyrrole, is a nonsystemic fungicide, which affects the transportation processes in the plasmatic membrane (Jespers et al., 1993). Tebuconazole is a systemic fungicide belonging to the triazole class which inhibits the biosynthesis of ergosterol (FAO, 1995).

Many researchers have studied residues of fungicides belonging to the phthalimides, benzimidazoles, and dicarboximides and their fate from vine to wine. The results of these studies are reported in some reviews (Cabras et al., 1988; Farris et al., 1992). Further studies have been carried out on the persistence in grapes and the fate from vine to wine of some insecticides (Cabras et al., 1995) and folpet (Cabras et al., 1997a). In this paper, we studied the fate of four new fungicides (cyprodinil, fludioxonil, pyrimethanil, and tebuconazole) from vine to wine.

EXPERIMENTAL PROCEDURES

Field Trials. The trial was carried out in a grape vineyard (cv. Vermentino), located at Ussana, near Cagliari, Italy. A random-block scheme was used, with four replications for each test, and each block contained 100 plants. Treatment was carried out on September 3, 1996. Folicur (25% tebuconazole), Scala (37.4% pyrimethanil) and Switch (37.5% cyprodinyl and 25% fludioxonil) were the commercial formulations applied (6 hL/ha) at the dose recommended by the manufacturer (375, 748, 360, and 240 g(AI)/hL, respectively) with an F-320 portable motor sprayer (Fox Motori, Reggio Emilia, Italy). Samplings (on dry plants) started about 1 h after 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, total rainfall was 14.8 mm in 11 rainy days, and the maximum and minimum average temperatures were respectively 27.0 and 15.4 °C.

Wine Making. Wine-making scheme described in a previous paper (Cabras et al., 1995) was used. Briefly, all four grape samples (ca. 20 kg) were pressed and stemmed together; 200 mg of sodium metabisulfite and 200 mg of dry yeast/kg of grapes was added, and the mixed sample was divided into 2 equal parts. One part was allowed to ferment with the skins (vinification with maceration); the other was dripped, and the resulting must was let to ferment (vinification without maceration). A 100 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. 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 assessed wine after addition of 0.25 mg/L of the studied fungicides. The clarifying agents and the doses were those usually applied in oenological practice. 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). 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. Tebuconazole was obtained from Dr. Ehren-

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Table 1. Residues (Milligrams per Kilogram ± Standard Deviation) of Fungicides in Grapes, Must, and Wine

fungicide	grapes: days after treatment	grapes: (mg/kg ± SD)	must	centrifuged must	wine: without maceration	wine: with maceration
cyprodinil	0	5.54 ± 0.28	4.01	0.18	0.70	0.74
	7	2.27 ± 0.48	1.67	<0.02	0.33	0.35
	14	1.69 ± 0.73	1.07	<0.02	0.21	0.39
	21	1.08 ± 0.24	0.47	<0.02	0.20	0.33
	28	1.03 ± 0.24	0.36	<0.02	0.18	0.21
fludioxonil	0	1.86 ± 0.09	1.79	1.20	0.71	0.50
	7	1.59 ± 0.26	1.25	0.24	0.44	0.11
	14	1.46 ± 0.19	0.60	<0.05	0.30	<0.05
	21	1.20 ± 0.22	0.54	<0.05	0.26	<0.05
	28	0.78 ± 0.13	0.39	<0.05	0.23	<0.05
pyrimethanil	0	1.62 ± 0.40	1.66	1.29	1.04	1.56
	7	1.31 ± 0.30	1.39	1.09	1.03	1.21
	14	1.24 ± 0.11	1.20	1.16	1.01	1.04
	21	1.19 ± 0.10	0.99	0.98	1.01	1.05
	28	1.11 ± 0.18	1.03	0.94	1.02	1.01
tebuconazole	0	4.84 ± 0.49	4.10	1.57	1.51	1.57
	7	3.16 ± 0.39	3.13	1.35	0.96	0.98
	14	2.69 ± 0.57	1.33	0.51	0.36	0.53
	21	0.68 ± 0.25	0.40	0.06	0.19	0.25
	28	0.42 ± 0.06	0.20	<0.05	0.16	0.22

storfer (Augsburg, Germany), cyprodinil, fludioxonil from Ciba-Geigy, and pyrimethanil from Hoechst Schering AgrEvo Italia. Acetone and hexane were of HPLC grade, and petroleum ether was of pesticide grade (Carlo Erba, Milan, Italy). Sodium chloride and anhydrous sodium sulfate were reagents for analysis (Carlo Erba, Milan, Italy). Triphenylphosphate (99%) was used as the internal standard (i.s.) and was of analytical grade (Janssen, Geel, Belgium). Standard stock solutions (ca. 200 mg/L) were prepared in methanol. Working standard solutions, containing the i.s. at 0.3 mg/kg, were obtained by dilution with an acetone/hexane mixture (1:1 v/v).

Pesticide Analysis. For the determination of cyprodinil, fludioxonil, pyrimethanil, and tebuconazole in grapes, must, and wine, a method previously described by Cabras et al. (1997b) was used.

Apparatus and Chromatography. A gas chromatograph HRGC Mega 5160 (Carlo Erba, Milano, Italy) equipped with nitrogen-phosphorus detector (NPD-40), an AS 550 autosampler (Carlo Erba), and a split-splitless injector, connected to an HP 3396-A reporting integrator (Hewlett-Packard, Avondale, CA) were used. The capillary column was a Durabond fused silica column (30 m × 0.25 mm i.d.) (J&W Scientific, Folsom, CA) DB 17 liquid phase (film thickness = 0.25 μm). The injector and detector were operated at 250 and 280 °C, respectively. The sample (2 μL) was injected in the splitless mode (60 s) and the oven temperature programmed as follows: 110 °C for 1 min, raised to 280 °C (20 °C/min) and held for 10 min. Helium was the carrier and make-up gas at 120 and 130 kPa, respectively. Calibration graphs for the active ingredients were constructed with the i.s. method by measuring peak heights *vs* concentrations. Good linearities were achieved in the 0.25−15 mg/kg range, with correlation coefficients between 0.9987 and 0.9995.

Sample Preparation. Grape samples were chopped and homogenized using a blender.

Extraction Procedure. A 5 g aliquot of sample was weighed in a 30 mL screw-capped tube; 2 g of NaCl and 10 mL of acetone/petroleum ether (50/50, v/v) were added, and the tube was agitated for 20 min in a rotatory shaker. The phases were allowed to separate, and the organic layer was poured into another tube containing 1 g of anhydrous sodium sulfate. An aliquot of 3 mL (1 mL for the highest concentrations) of dried extract was evaporated to dryness under nitrogen stream, the residue was dissolved in 0.3 mL (0.15 mL for the lowest concentrations) of internal standard solution and then injected for GC analysis.

Table 2. Residue Reduction (Percent)[a] of Fungicides in Wine Samples after Treatment with Different Clarifying Agents

fungicide	bentonite	charcoal	k caseinate	gelatin	PVPP
cyprodinil	42	100	0	0	0
fludioxonil	0	100	18	0	0
pyrimethanil	0	92	0	0	11
tebuconazole	0	100	0	0	0

[a] Mean data with CVs between 3 and 7.

RESULTS AND DISCUSSION

The data relating to the residues from the experiments carried out on grapes, must, and wine are reported in Table 1, while Table 2 shows the results obtained using different clarifying substances.

Cyprodinil. Among the fungicides studied, this AI showed the highest initial residue on grapes (5.54 mg/kg) which decreased to 1.03 mg/kg at harvest. The decay rate was quick, showing a half-life ($t_{1/2}$) of 12 days calculated by a pseudo 1st order kinetics ($r = 0.95$). The residue in the must was considerably lower than in the grapes. During wine making, the residues transferred from the grapes to the liquid phase (must) decreased on decreasing the residues present on the grapes. In fact, the residue in the must at first sampling was about 72% of that found in the grapes; during further samplings the residue decreased progressively, and at harvest it was 34%.

After the separation of the lees (*ca*. 6%) from must by centrifugation, a further reduction of residue occurred providing the complete elimination of cyprodinil.

The wines obtained with the two vinification techniques (with and without maceration) showed the same residue. The wines obtained at harvest showed residue of 0.2 mg/kg, which was *ca*. 20% of that found in the grapes.

No residue was detectable in wine when charcoal was used for clarification, while the other clarifying agents showed moderate or no effectiveness. Bentonite caused a residue reduction of *ca*. 42%.

Fludioxonil. This AI showed a lower decay rate in grapes than cyprodinil. In fact, $t_{1/2}$ calculated as a pseudo-first-order kinetics ($r = 0.95$) was 24 days. Also this fungicide showed a lower residue in the must on decreasing the residue on the grapes. After first sampling, the amount of fludioxonil in the must was 96% of that in the grapes and, at harvest, 50%. The elimination of the lees by centrifugation determined an analogous behavior to that observed for cyprodinil, providing a complete elimination of residue at concentrations below 0.60 mg/kg.

At the end of fermentation, the residue in the wine was lower than those found in the grapes and in the must. The wine obtained at harvest without maceration showed 29% of residue in the grapes and 59% in the must. The wines obtained by vinification with maceration showed lower fludioxonil residues than those without maceration.

The behavior of this AI during clarification with charcoal was analogous to that of cyprodinil. The other clarifying agents tested had no action on residue reduction, except potassium caseinate which showed scarce effectiveness (18%).

Pyrimethanil. Despite the similar structure, this AI showed a completely different behavior from cyprodinil both in the degradation in grapes and in the vinification process. Among the fungicides studied, this AI showed the lowest decay rate in grapes, with a pseudo-first-order kinetics ($r = 0.94$) and a $t_{1/2}$ of 57 days.

During wine making, this fungicide did not show great affinity for suspended matter. Residues in the must, in the centrifuged must, and in the wine obtained with the two different vinification techniques were analogous to those in the grapes. In fact, no remarkable residue reduction was observed on passing from grapes to wine.

During clarification tests, charcoal led to almost complete elimination the initial AI concentration (*ca*. 92%) while scarce effectiveness was only shown by PVPP (*ca*. 11%).

Tebuconazole. Among the fungicides studied, this AI showed the fastest decay rate in grapes, with a $t_{1/2}$ of 8 days calculated by a pseudo-first-order kinetics ($r = 0.96$). Despite the high initial residue (4.84 mg/kg), after 4 weeks the residue was only 0.42 mg/kg. Analogous results were obtained from supervised trials performed in France, Germany, and Portugal (FAO, 1995).

During wine making, this fungicide showed great affinity for the suspended matter. In fact, at harvest on passing from grapes to must, the initial residue (0.42 mg/kg) decreased ca. 50% and the entire residue of must was eliminated by centrifugation. The two different vinification techniques showed an analogous influence on residue reduction. At harvest, the residue in the wine was *ca*. 50% that found in the grapes. Among the clarifying agents tested, only charcoal showed great efficacy, with complete absorption of the residue.

CONCLUSIONS

All the fungicides studied showed very different decay rates in grapes, with pseudo-first-order kinetics and $t_{1/2}$ ranging from 8 to 57 days. Although cyprodinil and pyrimethanil have a similar structure, they showed distinct decay rates with $t_{1/2}$ of 12 and 57 days, respectively.

At harvest, the transformation of grapes into wine caused a noteworthy residue reduction with cyprodinil (*ca*. 80%), fludioxonil (*ca*. 70%), and tebuconazole (*ca*. 50%) and no reduction with pyrimethanil.

The two wine-making techniques employed (with and without maceration) had the same influence on residue concentrations, except for fludioxonil which showed maximum residue reduction with vinification with maceration. Among the clarifying agents used, bentonite, gelatine, polyvinylpolypyrrolidone, and potassium caseinate showed scarce or no action on residue contents in wine, while charcoal proved very effective and led to the complete elimination, or almost, of fungicide residues.

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LITERATURE CITED

Cabras, P.; Meloni, M.; Pirisi, F. M. Pesticide fate from vine to wine. *Rev. Environ. Contam. Toxicol.* **1987**, *99*, 83−117.

Cabras, P.; Garau, V. L.; Pirisi, F. M.; Cubeddu, M.; Cabitza, F.; Spanedda, L. Fate of some insecticides from vine to wine. *J. Agric. Food Chem*. **1995**, *43*, 2613−2615.

Cabras, P.; Angioni, A.; Garau V. L.; Melis, M.; Pirisi, F. M.; Farris, G. A.; Sotgiu, C.; Minelli, E. V. Persistence and metabolism of folpet in grapes and wine. *J. Agric. Food Chem*. **1997a**, *45*, 476−479.

Cabras, P.; Angioni, A.; Garau, V. L.; Minelli, E. V. Gas chromatographic determination of cyprodinil, fludioxonil, pyrimethanil and tebuconazole in grapes, must and wine. *J. AOAC Int.* **1997b**, in press.

FAO/WHO. *Pesticide residues in food*−*1994 Evaluations*; paper 131/2, FAO: Rome, 1995, pp 1055−1198.

Farris, G. A.; Cabras, P.; Spanedda, L. Pesticide residues in food processing. *Ital. J. Food Sci.* **1992**, *3*, 149−169.

Jespers, A. B. K.; Davidse, L. C.; De Waard, M. A. Biochemicals effects of the phenylpyrrole fungicide fenpiclonil in *Fusarium sulphreum* (Sclecht). *Pestic. Biochem. Physiol.* **1993**, *45*, 116−129.

Masner, P.; Muster, P.; Schmid, J. Possible methionine biosynthesis inhibition by pyrimidinamine fungicides. *Pestic. Sci*. **1994**, *42*, 163−166.

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