Fate of Quinoxyfen Residues in Grapes, Wine, and Their Processing Products

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Quinoxyfen is a new fungicide that belongs to the family of the quinolines, recently introduced to control powdery mildew (*Uncinula necator*). In this paper the fate of quinoxyfen residues from vine to wine and in their processing products was studied. After the last of four applications at the recommended rate, 0.38 mg/kg of residue was found on the grapes, which is under the legal limit fixed in Italy (0.5 mg/kg). The degradation rate was according to a pseudo-first-order kinetics (r = 0.964) and the half-life was 7.24 days. Vinification was carried out with and without maceration. During the vinifications without maceration <50% of the residues passed from the grapes to the musts. Separation of the lees (8%) from the must by centrifugation caused no detectable residues in centrifuged must. At the end of fermentation with and without maceration no quinoxyfen residues were determinable in the wine. No effect on the alcoholic or malolactic fermentation was observed even in the presence of higher quinoxyfen concentrations than those found in the grapes at harvest time. During fermentation, the yeasts partially degraded the pesticides and completly adsorbed them. Bacteria, on the other hand, do not have any degradative effect on the pesticides. The raisins obtained by sun-drying did not contain any residues, whereas those obtained by oven-drying show the same amount of residues as in the fresh grapes. During the sun-drying process the fruit weight decreased by a factor of 4; the decrease in the oven-drying was equivalent. Samples of dregs and liquid lees, fortified with high levels of quinoxyfen. were double-distilled. The first dregs distillate, with an alcohol content of 32.1%, did not show any residues, whereas the first lees distillate, with an alcohol content of 34.5%, showed 7% of the initial residues. After the second lees distillation, the obtained product showed an alcoholic content of 81.2% and no residues of quinoxyfen (<0.01 mg/kg).

Keywords: *Residues; quinoxyfen; grapes; must; wine*

INTRODUCTION

Quinoxyfen is a new fungicide with specific activity against powdery mildew. This compound is highly active against strains resistant to the present pesticides and shows a low resistance-generating risk (Hollomon et al., 1996). Because its mechanism of action is different from that of other fungicides used to control powdery mildew (Longhurts et al., 1996), it could be used in new strategies in the event of resistances against traditional fungicides. It has recently been registered in Italy on vine, with a maximum residue level (MRL) of 0.5 mg/kg and a preharvest interval of 28 days. No paper has been found in the literature on the behavior of quinoxyfen residues on grapes in the field, during wine-making, during raisin production, or in the distillation products obtained from the vinification byproducts. Following our previous research with the same methodology on other pesticides used on vine (Cabras et al., 1995, 1997a−c, 1998a,b), we carried on a set of experiments to assess the behavior of quinoxyfen residues on grapes, raisins, wine, and its byproducts. Moreover, a simple rapid analytical procedure for the determination of the active compound in these matrices was also developed.

MATERIALS AND METHODS

Field Trials. The experiment was carried out on Chardonnay grapes in a vineyard at Ussana (Ca) with a 2.5 × 1.5 m plant density. Each block consisted of 60 plants, with four replications for each experiment. Quinoxyfen 250 SC at 25% active ingredient (ai) was the commercial formulation and was used at the doses recommended by the manufacturer (30 mL/hL of water with 7 hL/ha, corresponding to 52.5 g of ai/ha). The compound was sprayed with an AM 150 (Oleo-Mac, Reggio Emilia, Italy) manual sprayer at a 10 day interval. The fungicide treatments were carried out on June 18 and 28, 1999, and on July 9 and 19, 1999.

Two kilogram grape samples were collected before and after the last treatment and subsequently on the 3rd, 7th, and 14th days.

Meteorological data were collected by an agrometeorological station AD-2 (Silimet, Modena) located near the vineyard. During the experiments the maximum and minimum average temperatures were 32.2 and 16.7 °C, respectively. After the last treatment, it did not rain during all of the experiment time.

With regard to the raisins, the trial was conducted on a grape vineyard, cv. Sultanina, with the same commercial

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product and a single application at the same dose on September 7, 1999. The samples were collected on September 28 and dried.

Drying Procedure (Raisins). After damaged grapes had been removed, the samples were dried in the sun and in an oven according to the methods of Cabras et al. (1998a).

Vinification Process. The grape samples were processed with and without maceration according to the method of Cabras et al. (1995). The fermentation process was regular, and the obtained wines were analyzed after 15 days.

Distillation Process. The lees and the dregs from untreated grapes were fortified with different amounts of the pesticide and were distilled according to the method of Cabras et al. (1997c).

Distillation Apparatus. A Jaulmes (Lequeux, Parigi, France) apparatus was used to distill the lees, and a Camboni (Milano, Italy) apparatus was used to distill the dregs.

Reagents. Quinoxyfen was an analytical standard kindly provided by the manufacturer (Dow AgroSciences). Standard solutions (~500 ppm) were prepared in hexane. Working standard solutions were prepared by dilution; no matrix effect was observed in untreated samples. Hexane was the HPLC solvent (Carlo Erba, Milan, Italy).

Extraction Procedure Ten grams of the sample (grapes, must, wine, distillates, and raisins) was collected in a 40 mL screw-cap tube. Ten milliliters of hexane was added, and the mixture was agitated in a rotary stirrer (30 min). The grape mixture in particular should be stirred using a vortex to avoid partitioning of the grapes from the hexane. The phases should be left separate (centrifuged if necessary) and injected in a gas chromatograph for analysis. In the distillates the alcohol should be evaporated under nitrogen flow before extraction, and the above procedure should be followed.

Chromatographic Apparatus. An HRGC series Mega 2 (Carlo Erba), equipped with a split−splitless injector, an AS 800 autosampler, and an HP 3396 A integrator (Hewlett-Packard, Avondale, PA), was used. The column was a fused silica capillary CP Sil 8 CB (5% phenyl methyl silicone, 30 m × 0.25 mm, film = 0.10 μm, Chrompack, Middelburg, The Netherlands). The injector and the detector were at 200 and 280 °C, respectively. The sample (2 μL) was injected in the split mode (1:3). The oven was programmed as follows: 100 °C (1 min), raised to 280 °C (20 °C/min), held for 9 min. Helium was the carrier gas and nitrogen the makeup gas at 100 and 65 kPa, respectively. The detector was an ECD-80 at 300 °C, the current was 1.0 nA, and the pulse was 40 V.

Recovery Trials. Untreated samples of grapes, must, wine, raisins, and byproducts were fortified with 0.01, 0.1, and 1.0 mg/kg of pesticides and extracted as reported above.

Medium Culture. *(A) Yeast.* After the pesticide (0.45 g) had been dissolved in 5 mL of ethyl alcohol, it was added to 1 L of culture medium (180 g/L glucose and 7 g/L of yeast nitrogen base) at pH 3.6. The medium was sterilized using 0.22 μm PTFE membrane filters.

(B) Bacteria. After the pesticde (0.70 g) had been dissolved in 5 mL of ethyl alcohol, it was added to 1 L of Cagnulari wine of the following composition: 12% alcohol (v/v), volatile acidity = 0.45 g/L, total acidity = 6.2 g/L, malic acid = 5.0 g/L. The wine was sterilized using 0.22 μm Teflon membrane filters.

Inoculum and Growing Conditions. The trials were carried out on three different strains of *Saccharomyces cerevisiae*, 1043, 1090, and 1189, from the collection of winery yeasts of the Dipartimento di Scienze Ambientali Agrarie e Biotecnologie Agro-alimentari of Sassari University, and on the lactic bacterium *Lactobacillus plantarum*, kindly provided by Lallemand. The inoculation and fermentation procedures for the yeasts and bacteria were carried performed according to the methods of Cabras et al. (1999).

RESULTS AND DISCUSSION

Analytical Method. A review by Hänel et al. (1998) reported a scheme for the determination of quinoxyfen in grapes, must, and wine, based on unpublished data from the manufacturer. This method consists of an extraction with an 0.12 M acetone/HCl (8:2) solution, followed by an $NaHCO_3$ neutralization and a hexane partition. After SPE (aminopropyl) purification, the sample was injected in GC-MS or HPLC for analytical determination. Our method consists of an easy extraction of the active ingredient with hexane and a direct injection of the hexane extract in GC-ECD. The recoveries, obtained from four replicates, ranged between 88 and 102% with a maximum variation coefficient (CV) of 12.1%. The calibration curve was calculated between peak height and concentration using the external standard method. A good linearity was achieved in the range 0.01−1.00 mg/kg with a correlation coefficient of 0.9996. Cleanup was not necessary because there were no interference peaks (Figure 1). In Figure 1 only chromatograms of quinoxyfen in wine were reported because those of must and grapes were similar. The determination limit [according to Thier and Zeumer (1987)] was 0.01 mg/kg.

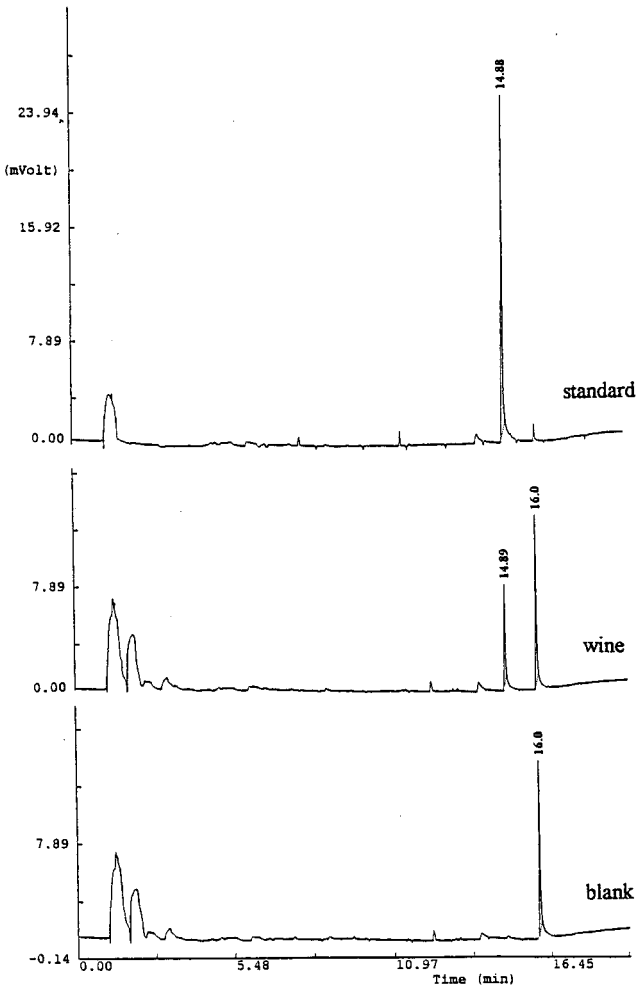

Figure 1. Chromatograms of quinoxyfen (t_R = 14.8) in wine under the operating conditions described under Chromatographic Apparatus: blank (untreated sample); wine (samples with 0.1 mg/kg of the fungicide); standard (solution in hexane at 0.5 mg/kg).

Grape Residues. Before the last treatments, the residues on the grapes were 0.09 mg/kg, suggesting a poor tendency to accumulate (Table 1). After the last of four applications at the recommended rate, the residues were 0.38 mg/kg and disappeared rapidly. The rate of disappearance of quinoxyfen in grapes follows a first-order kinetics (r = −0.964), with a half-life ($t_{1/2}$) of 7.2

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

days after last treatment	grapes	must	wine: centrifuged must	wine: vinification without skins	wine: vinification with skins
−0[a]	0.09 ± 0.02				
0	0.38 ± 0.02	0.18 ± 0.03	<0.01	<0.01	<0.01
3	0.20 ± 0.05	0.08 ± 0.02	<0.01	<0.01	<0.01
7	0.15 ± 0.02				
14	0.09 ± 0.03				

[a] Before treatment.

Table 2. Effect of Quinoxyfen on the Fermentative Action of Yeasts and Bacteria

	yeast control		pesticide control		
days after inoculation	cells/mL	alcohol %	cells/mL	alcohol %	pesticides, mg/L
0					0.47 ± 0.03
1					0.46 ± 0.03
4					0.47 ± 0.02
10					0.43 ± 0.04
	S. cerevisiae 1043				
0	5.0×10^6	0	5.0×10^6	0	0.45 ± 0.03
1	8.0×10^7	3.0	5.0×10^7	2.5	0.42 ± 0.03
4	8.7×10^7	7.8	3.1×10^7	7.5	0.26 ± 0.04
10	4.8×10^7	11.0	3.0×10^7	10.0	0.15 ± 0.04
	S. cerevisiae 1090				
0	5.0×10^6	0	5.0×10^6	0	0.43 ± 0.03
1	8.0×10^7	3.0	5.0×10^7	2.3	0.38 ± 0.03
4	10.0×10^7	7.8	7.5×10^7	7.3	0.25 ± 0.02
10	9.3×10^7	10.5	6.2×10^7	9.3	0.28 ± 0.04
	S. cerevisiae 1189				
0	5.0×10^6	0	5.0×10^6	0	0.40 ± 0.06
1	8.0×10^7	3.5	5.0×10^7	2.8	0.38 ± 0.03
4	8.1×10^7	9.0	5.6×10^7	7.8	0.27 ± 0.02
10	5.0×10^7	11.0	3.2×10^7	11.0	0.17 ± 0.04

	bacteria control		*L. plantarum*	
days after inoculation	malic acid, g/L	pesticides, mg/L	malic acid, g/L	pesticides, mg/L
0	5.00 ± 0.04	0.70 ± 0.03	5.00 ± 0.04	0.71 ± 0.03
10	3.50 ± 0.06	0.66 ± 0.06	3.70 ± 0.05	0.68 ± 0.05
20	2.70 ± 0.04	0.53 ± 0.03	2.95 ± 0.02	0.55 ± 0.04
30	1.65 ± 0.02	0.43 ± 0.04	2.00 ± 0.03	0.41 ± 0.02

days. Residues at all sampling points were under the legal limit (in Italy, 0.5 mg/kg); therefore, the use of this fungicide should not create limit problems if used following good agriculture practices.

Effect on the Fermentative Action of Yeast and Lactic Bacteria. Table 2 shows that during alcoholic fermentation, the pesticide was stable in the control sample but degraded in the presence of three strains of *S. cerevisiae*. At the end of fermentation, the residues decreased by two-thirds, with strains 1043 and 1189, and by one-third with strain 1090. Separate analysis of the solid and liquid fractions of the fermentation medium showed that the residues were concentrated in the solid phase. This suggests that at the end of fermentation, quinoxyfen should not be present in the wine because it is partially degraded by the yeasts and significantly adsorbed to the yeasts deposited in the lees. The fermentation was regular for all strains. After fermentation, the alcoholic content in the samples obtained from strains 1043 and 1090 was lower than the controls by 1%. During the malolactic fermentation experiments, the concentration of quinoxyfen in the control sample was constant for 10 days and later gradually decreased by ∼40% in 1 month. The same behavior was observed in the presence of *L. plantarum*. This suggests that, unlike yeasts, bacteria do not degrade this pesticide. Moreover, the regular disappearance of the malic acid showed that the pesticide did not affect fermentation.

Wine Residues. During vinification with maceration, grapes are fermented in the presence of skins. After fermentation, no residues were found in the wine (Table 1).

In vinification without maceration, the must is separated from the skins by pressing. The must with or without lees is then analyzed. After pressing, grape residues tend to distribute between the liquid phase (must) and the solid phase (skins) (Cabras and Angioni, 2000). In the case of quinoxyfen, in both experiments, 45% of the residue passes from the grapes to the must (Table 1), but if the lees is separated by centrifugation (8% of lees), the amount of residues in the must is no longer determinable. These data showed that, during wine-making, quinoxyfen tends to distribute completely in the solid fraction (skins and lees). At the end of fermentation, no determinable residues were found in the wine, irrespective of the amount of initial residue in the grapes.

Raisins. At harvest time the residues on grapes were 0.021 ± 0.010 mg/kg, and the average weight of a berry was 2.26 g. The grapes dried under the sun and in the oven decreased in weight by a factor of 4. Theoretically, residues in raisins should be higher by the same factor, but no residues were found in the sun-dried sample (<0.01 mg/kg). In the oven-dried sample, the amount of residues (0.018 ± 0.009 mg/kg) was comparable with that found in the fresh grapes. The lower degradation in the oven-dried sample could be explained by the absence of the degradation effect due to solar radiation.

Distillates. In the wine-making industry to produce brandy, cognac, and alcohol, wine and its byproducts (dregs and lees) are usually submitted to a first distillation in a stripping column, which produces a spirit at ∼35% alcohol, followed by a second distillation in a rectifying column to give a spirit at ∼80% alcohol. Samples of fermented dregs and liquid lees were analyzed to assess the absence of pesticide residues and interfering peaks. The samples were fortified with high levels of quinoxyfen, 4 and 10 mg/kg, respectively, for dregs and lees. A double distillation was carried out on these samples. The first dregs distillate, with an alcohol content of 32.1%, did not show any residues, whereas the first lees distillate, with an alcohol content of 34.5%, showed a residue of 0.68 ± 0.15 mg/kg (7% of the initial residues). After the second lees distillation, the obtained product showed an alcoholic content of 81.2% and no residues of quinoxyfen (<0.01 mg/kg). No experiments were carried out on the wines because no quinoxyfen residues had been found after wine-making.

Conclusions. After treatment, the residue levels of quinoxyfen on the grapes were already under the legal

limit. No effect on the alcoholic or malolactic fermentation was observed, even in the presence of higher concentrations than those found in the grapes at harvest time. During fermentation, the yeasts partially degraded quinoxyfen and adsorbed them totally. Bacteria, on the other hand, do not have any degradative effect on quinoxyfen. During wine-making, all residues pass in the lees, and after fermentation, no residues were found in the wine. These data showed that this compound could be used to produce wine free of detectable amount of residues. Distillates obtained from vinification byproducts (dregs and lees) do not show any determinable residues, even when fortified with high amounts of quinoxyfen. The raisins obtained by sun-drying did not present any residues, whereas those obtained by oven-drying showed the same amount of residues as in the fresh grapes despite a drying concentration factor of 4.

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