

Fate of Some Insecticides from Vine to Wine

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The fate of residues of five insecticides (chlorpyrifos methyl, fenthion, methidathion, parathion methyl, and quinalphos) from the treatment on vine to the production of wine was studied. The influence of clarifying agents (bentonite, charcoal, potassium caseinate, gelatin, polyvinylpyrrolidone, and colloidal silicon dioxide) on residue concentrations in wine was also studied. The insecticide residues on grapes showed high decay rates after treatment, with first-order kinetics and half-lives ranging from 0.97 to 3.27 days. Grape processing into wine caused considerable residue reduction (>80%) for chlorpyrifos methyl, parathion methyl, and quinalphos, moderate reduction (ca. 50%) for methidathion, and almost no reduction for fenthion. The wine-making technique (with or without maceration) had the same influence on the residue concentrations in wine. The clarifying agents tested showed no or moderate influence on the residue contents in wine with the exception of charcoal, which allowed complete or almost complete elimination of insecticide residues.

Keywords: *Insecticides; residues; wine-making*

The grape moth (*Lobesia botrana*) is the most widespread and harmful parasite of grapes, after cryptogams such as downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*), and gray mold (*Botrytis cynerea*). Usually, during one life cycle this insect can reproduce itself three times, but when environmental conditions are favorable (which is quite frequent in the south of Italy), a fourth generation can be reached. This fact leads to the need for pesticide treatments as near as possible to harvest. Since the amount of residues is greatly affected by the preharvest interval, high residues could be present on grapes at harvest time, especially when the active ingredient (ai) applied shows high stability. Consequently, high residues could also be present in wine if the effect of the wine-making technique on residue reduction is poor.

Fungicide residues on grapes and their fate during wine-making have been widely investigated. In the current literature there are numerous papers that give a sufficiently clear picture of the residues of the most extensively used fungicides in grapes and wine. Moreover, the results of studies carried out in this field are reported in some recent reviews (Cabras *et al.*, 1987; Flori and Cabras, 1990; Zironi *et al.*, 1991; Farris *et al.*, 1992). On the other hand, since data on insecticide residues in grapes and wine are quite limited, further studies are needed. This paper aims to contribute to the knowledge of the fate of five insecticides (chlorpyrifos methyl, fenthion, methidathion, parathion methyl, and quinalphos) from vine to wine. The persistence of these active ingredients in grapes, their behavior during wine-making (with the simplest technique, Figure 1), and adsorption by the most widely used clarifying substances were studied.

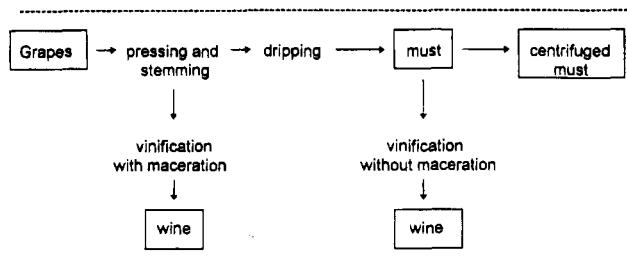


Figure 1. Wine-making scheme.

EXPERIMENTAL PROCEDURES

Materials and Methods. The trial was carried out in a red grape vineyard (cv. Cannonau), located at Ussana, near Cagliari, Italy. A random-block scheme was used, with four replications for each test, and each block contained 100 plants. Treatments were carried out on September 1, 1994; the insecticides were applied (6 hL/ha) with an F-320 portable motor sprayer (Fox Motori, Reggio Emilia, Italy). The following commercial formulations were used at the doses recommended by the manufacturers: Tumar (containing 22.1% chlorpyrifos methyl), 150 g/hL; Lebaycid (24.4% fenthion), 250 g/hL; Supracaffaro (19% methidathion), 200 g/hL; Polisar (16% parathion methyl), 200 g/hL; Ekalux (25% quinalphos), 150 g/hL. Samplings started about 1 h after treatment (on dry plants): random 5-kg samples of grapes were collected from each plot and analyzed immediately for insecticide residues. The samplings and analyses were repeated 1, 5, and 7 days after treatment. The environmental conditions were continuously recorded with an AD-2 automatic weather station (Silimet, Modena, Italy). During the experiments there was no rainfall and maximum and minimum average temperatures were, respectively, 28.5 and 15.8 °C.

Wine-Making. After residue analysis, all four grape samples per ai (ca. 20 kg) were pressed and stemmed together (Figure 1); 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). A 100-g aliquot of cloudy must was taken and centrifuged at 4000 rpm

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Table 1. Residues (Parts per Million \pm SD) of Insecticides in Grapes, Must, and Wine

insecticide	grapes		must	centrifuged must	wine	
	days after treatment				without maceration	with maceration
chlorpyrifos methyl	0	0.16 \pm 0.07	0.06	0.02	0.03	0.03
	1	0.06 \pm 0.02	<0.01	<0.01	<0.01	<0.01
	5	0.01 \pm 0.00	<0.01			
fenthion	0	0.28 \pm 0.07	0.22	0.20	0.21	0.24
	1	0.18 \pm 0.07	0.11	0.11	0.11	0.13
	7	0.06 \pm 0.02	0.04	0.04	0.03	0.04
methidathion	0	0.56 \pm 0.13	0.26	0.25	0.21	0.21
	1	0.25 \pm 0.07	0.16	0.12	0.12	0.11
	5	0.04 \pm 0.01	<0.01			
	7	0.02 \pm 0.01	<0.01			
parathion methyl	0	0.37 \pm 0.04	0.13	0.05	0.05	0.05
	1	0.07 \pm 0.03	0.03	<0.01	0.01	0.01
	5	0.01 \pm 0.00	<0.01			
quinalphos	0	0.39 \pm 0.04	0.16	0.04	0.07	0.07
	1	0.18 \pm 0.02	0.06	0.02	<0.01	<0.01
	5	0.05 \pm 0.02	<0.01			

Table 2. Residues (Parts per Million \pm SD) of Insecticides in Wine Samples after Treatment with Different Clarifying Agents

insecticide	control	B ^a	C ^a	K ^a	S ^a	G ^a	PVPP ^a
chlorpyrifos methyl	0.11	0.09 \pm 0.01	<0.01	0.07 \pm 0.00	0.08 \pm 0.01	0.08 \pm 0.01	0.09 \pm 0.01
fenthion	0.24	0.20 \pm 0.00	<0.01	0.20 \pm 0.00	0.19 \pm 0.03	0.17 \pm 0.03	0.19 \pm 0.01
methidathion	0.12	0.10 \pm 0.00	0.03 \pm 0.00	0.12 \pm 0.01	0.10 \pm 0.02	0.10 \pm 0.01	0.11 \pm 0.00
parathion methyl	0.10	0.10 \pm 0.00	<0.01	0.10 \pm 0.00	0.11 \pm 0.01	0.09 \pm 0.01	0.10 \pm 0.00
quinalphos	0.05	0.04 \pm 0.00	<0.01	0.04 \pm 0.00	0.04 \pm 0.00	0.04 \pm 0.01	0.04 \pm 0.00

^a B, bentonite; C, charcoal; K, potassium caseinate; S, colloidal silicon dioxide + gelatin; G, gelatin; PVPP, polyvinylpyrrolidone.

for 5 min 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 insecticide residues.

Wine Clarification. Clarification tests were carried out on 1-L samples of residue-free assessed wine. The clarifying agents and the doses employed (usually applied in oenological practice) were as follows: bentonite, 100 g/hL; charcoal, 20 g/hL; potassium caseinate, 100 g/hL; gelatin, 20 g/hL; polyvinylpyrrolidone, 80 g/hL; and colloidal silicon dioxide plus gelatin, 50 and 5 g/hL, respectively. Two days after the clarifying treatment, clear wine and control samples (without clarification) were analyzed for insecticide residues. Each clarification test was performed in quadruplicate.

Extraction Procedure. A 25-g aliquot of grapes was weighed in a 100-mL screw-capped flask; 25 mL of benzene containing triphenyl phosphate as the internal standard (i.s.) was added and the flask agitated in a flask shaker (Stuart Scientific) for 15 min. The phases were allowed to separate, and the organic layer was poured into another flask containing 5 g of anhydrous sodium sulfate and injected for gas chromatographic (GC) analysis. Extraction from must and wine (5-mL samples) was carried out in 20-mL screw-capped test tubes, with benzene containing the i.s.. After the test tubes were shaken in a rotary stirrer, the organic layer was separated, dehydrated, and injected for GC analysis.

Recovery Assays. Untreated grapes, must, and wine samples were fortified with the insecticides tested and processed according to the above-described procedure. Recovery assays, carried out at 0.01, 0.50, and 2.00 ppm, showed values obtained from four replicates ranging between 93 and 101%, with a maximum coefficient of variation (CV) of 11.

Apparatus and Chromatography. A gas chromatograph HRGC Mega 5160 (Carlo Erba, Milano, Italy) was employed, fitted with an N-40 nitrogen-phosphorus detector, an AS 550 autosampler (Carlo Erba), and a split-splitless injector, connected to an HP 3396-II reporting integrator (Hewlett-Packard, Avondale, PA). A Durabond fused silica column (30 m \times 0.32 mm i.d.) (J&W Scientific, Folsom, CA) was employed, with DB-1701 liquid phase (film thickness 0.25 μ m). The injector and detector were operated at 180 and 295 $^{\circ}$ C, respectively. The sample (2 μ L) was injected in the splitless

mode (60 s), and the oven temperature was programmed as follows: 90 $^{\circ}$ C for 1 min, raised to 190 $^{\circ}$ C (30 $^{\circ}$ C/min), then to 270 $^{\circ}$ C (5 $^{\circ}$ C/min) and held for 5 min, and finally raised to 290 $^{\circ}$ C (20 $^{\circ}$ C/min) and held for 5 min. Helium was the carrier and makeup gas at 50 and 130 kPa, respectively. Calibration graphs for the five insecticides were constructed with the i.s. method by measuring peak heights vs concentrations. Good linearities were achieved in the range 0–2.5 ppm, with correlation coefficients between 0.9992 and 0.9995.

Chemicals. Chlorpyrifos methyl, fenthion, methidathion, parathion methyl, quinalphos, and triphenyl phosphate were analytical standards purchased from Ehrenstorfer (Augsburg, Germany); benzene was an HPLC grade solvent (Carlo Erba); anhydrous sodium metabisulfite and sodium sulfate were of analytical grade (Carlo Erba). Stock standard solutions of the insecticides (ca. 100 ppm each) were prepared in benzene. Working standard solutions were obtained by dilution with benzene containing the i.s. at 0.3 ppm. Bentonite (Dal Cin, Milano, Italy), charcoal (AEB, Brescia, Italy), gelatin (AEB), polyvinylpyrrolidone (Fluka, Milano), potassium caseinate (Marescalchi, Alessandria, Italy), and colloidal silicon dioxide (Bayer AG, Leverkusen, Germany) were commercial grade products.

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. Though wine-making was performed on the laboratory scale, the resulting data were analogous to those from experiments carried out on the semi-industrial scale (Cabras *et al.*, 1991).

Chlorpyrifos Methyl. Among the insecticides studied, this ai showed the lowest initial deposit on grapes (0.16 ppm). The decay rate was very quick, showing a pseudo-first-order kinetics ($R^2 = 0.97$) with a half-life ($t_{1/2}$) of 1.28 days. These data agree with those from supervised trials performed in France and Italy (Dagna

et al., 1992; FAO/WHO, 1991). The residue in the must was considerably lower than in the grapes, indicating a noteworthy affinity of chlorpyrifos methyl for the suspended matter. This was confirmed by the further reduction that occurred after the lees (*ca.* 8%) were separated from must by centrifugation. The wines obtained with the two vinification techniques (with and without maceration) showed the same residue (0.03 ppm), which was less than 20% that found in the grapes. No residue was detectable in wine when charcoal was used for clarification, while moderate or scarce effectiveness was shown by the other clarifying agents; among these, potassium caseinate caused the highest residue reduction (*ca.* 40%).

Fenthion. This insecticide also showed a high decay rate, though it was less than that of the other active ingredients studied; in fact, $t_{1/2}$ calculated as a pseudo-first-order kinetics ($R^2 = 0.98$) was 3.27 days. Seven days after treatment the residue was very low (0.06 ppm). These data are in agreement with those published by FAO/WHO (1973). During wine-making the residue almost completely passed from the grapes to the liquid phase. The scarce affinity of fenthion for the suspended matter was confirmed by the fact that the elimination of lees (*ca.* 9%) by centrifugation did not cause a decrease in residue. At the end of fermentation the residue in the wine was analogous to that in the grapes. The behavior of this ai during clarification tests was analogous to that of chlorpyrifos methyl.

Methidathion. This ai showed modest persistence since its residue was initially 0.56 ppm and was 0.02 ppm after 7 days. Also in this case the decay rate showed a pseudo-first-order kinetics ($R^2 = 0.99$), with a $t_{1/2}$ of 1.46 days. The data from evaluations of methidathion residues in grapes carried out in numerous countries (FAO/WHO, 1992a) are not always in accordance with those of our research. In detail, results obtained in Germany, Switzerland, and South Africa show decay rates lower than determined by us, while data from Australia and Israel are analogous to ours. This could probably be ascribed to different environmental conditions. During wine-making almost half of the initial residue was transferred to the liquid phase (must) and did not undergo further reduction during fermentation. Analogous results were obtained by Kawar *et al.* (1979) with must samples fortified with large amounts of methidathion (26 ppm). They also verified that this insecticide tends to degrade with the aging of wine. In FAO/WHO 1992 evaluations (FAO/WHO, 1992a) much lower residues in wine are reported. Since information about the age of the wines at the time of analytical determination of methidathion is lacking, it could be supposed that the samples had been analyzed several months after wine-making. After clarification treatment, no significant residue reduction was observed except when using charcoal, which reduced the initial ai concentration by *ca.* 75%.

Parathion Methyl. Among the insecticides studied, this ai showed the highest decay rate in grapes, with a pseudo-first-order kinetics ($R^2 = 0.97$) and a $t_{1/2}$ of 0.97 days. Analogous behavior was observed in Spain by Cascolla *et al.* (1988), while, as for methidathion, experiments carried out in Germany (FAO/WHO, 1992b) showed lower decay rates. Also in this case the difference could be ascribed to the different environmental conditions. During wine-making this insecticide showed great affinity for the suspended matter. In fact, on passing from grapes to wine the residue concentration was reduced by *ca.* 65%; in the centrifuged must it further decreased by *ca.* 62%. The two different vini-

fication techniques showed an analogous influence on residue reduction, which was *ca.* 86% in both cases. Among the clarifying agents tested, only charcoal showed great efficacy, with complete absorption of the residue.

Quinalphos. This insecticide also showed a noteworthy decay rate, with a pseudo-first-order kinetics ($R^2 = 0.96$) and a $t_{1/2}$ of 1.73 days. To our knowledge, no data about quinalphos residues in grapes are available in the current literature. The behavior of this ai during wine-making and clarification tests was analogous to that of parathion methyl.

Conclusions. All of the insecticides studied showed very quick decay rates in grapes, with pseudo-first-order kinetics and $t_{1/2}$ ranging from 0.97 and 3.27 days. The transformation of grapes into wine caused a noteworthy residue reduction (*ca.* 80%) for chlorpyrifos methyl, parathion methyl, and quinalphos; a moderate reduction (*ca.* 50%) for methidathion, and almost no reduction for fenthion. The two wine-making techniques employed (with and without maceration) had the same influence on residue concentrations. Among the clarifying agents used, bentonite, gelatin, polyvinylpyrrolidone, potassium caseinate, and colloidal silicon dioxide showed scarce or no action on residue contents in wine, while charcoal proved to be very efficacious and led to the complete or almost complete elimination of insecticide residues.

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Received for review December 23, 1994. Revised manuscript received April 11, 1995. Accepted July 11, 1995.*

JF940724S

* Abstract published in *Advance ACS Abstracts*, September 1, 1995.