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# Fate of three Insect Growth Regulators (IGR) Insecticides (Flufenoxuron, Lufenuron and Tebufenozide) in grapes following field application and through the wine making process

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**Fate of three Insect Growth Regulators (IGR) Insecticides (Flufenoxuron, Lufenuron and Tebufenozide) in grapes following field application and through the wine making process**

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4 **Fate of three insect growth regulators (IGR) insecticides**  
5 **(flufenoxuron, lufenuron and tebufenozide) in grapes following field**  
6 **application and through the wine making process**  
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**Abstract**

The dissipation of three insecticide flufenoxuron, lufenuron and tebufenozide residues in grapes after field treatments and during the wine making process was assessed. Residues were determined in grape, must, centrifuged must and wine samples by HPLC-UV after cyclohexane extraction and clean-up on silica phase cartridges. Vines in vineyards with white and red grapes located in Central Greece were sprayed once with commercial formulations of each insecticide at the recommended doses in experiments carried out in 2004 and repeated in 2006. The insecticide residues in grapes showed slow reduction for a period of 42 days after application following first-order kinetics with dissipation rates ranged from 0.011 to 0.018 mg kg<sup>-1</sup> d<sup>-1</sup>. However, at the recommended pre-harvest interval (PHI) residues did not exceed 0.27±0.03 mg kg<sup>-1</sup> for flufenoxuron and lufenuron and 0.68±0.07 mg kg<sup>-1</sup> for tebufenozide and they were clearly lower than the maximum residue limits (MRLs) set by EU for grape (2 mg kg<sup>-1</sup> for flufenoxuron, 1 mg kg<sup>-1</sup> for lufenuron and 3 mg kg<sup>-1</sup> for tebufenozide). Grape processing into wine caused an almost complete reduction for flufenoxuron and lufenuron as their residues in wine were below the method LOQs (<0.01 mg L<sup>-1</sup>), but only a moderate reduction for tebufenozide with concentrations from 0.13 to 0.26 mg L<sup>-1</sup> measured in the produced wines. Mean transfer factors for tebufenozide of 0.45 for white 'Roditis' and 0.34 for red 'Cabernet Sauvignon' were found from grapes into wine for the wines processed without maceration. The wine-making technique (with or without maceration) had the same influence on tebufenozide residues in wine. Of the various clarifying agents studied, charcoal was found to be the only one to be effective in removing tebufenozide residues from wine.

**Keywords:** IGR insecticides, pesticides, residues, grapes, wine, vinification.

## Introduction

The use of insecticides in viticulture is essential for grape protection against pest diseases, and their use is therefore important on grape productivity and on wine quality.

The grape moth (*Lobesia botrana*) is a wide-spread and harmful pest of grapes. During one season in Greece, this insect has three or four generations, if environmental conditions are favourable. This fact leads to the need for pesticide treatments as near as possible to harvest. The use of these products, particularly when the dose and /or the established pre-harvest time is not observed, can lead to hazardous residues (Fernandez et al. 2005a). It is thus of particular interest to assess the presence of residues of these compounds in grapes and their processed products, such as wine. Among the different chemical classes of new insecticides which have been available the last years to control insects on vine, the benzoylureas (flufenoxuron, lufenuron and other active substances) and the diacylhydrazine tebufenozide are the most promising (Tomlin 2000). These insecticides belong to the family of growth control, so they have the lowest risks for the non-target organisms and their use helps in respecting the environmental balances. Put on the market in the 1990's to replace the toxic insecticides, they were included in plans for integrated protection.

Since the amount of residues is greatly affected by the pre-harvest interval, high residues could be present in grapes at harvest time, especially when the active substance applied shows high persistence. Consequently, pesticides residues could also be present in wine if the effect of the wine-making technique on residue reduction is poor (Cus et al. 2010, Cabras and Conte 2001). Although data for fungicide residues on grapes and their fate during wine-making are widely available (Garcia-Cazorla and Xirau-Vayreda 1994, Cabras et al. 1997, Cabras et al. 1998, Garcia et al. 1999, Cabras and Conte 2001, Cabras et al. 2001, Fernandez et al. 2005a, Mirlean et al. 2005, Vaquero-Fernandez et

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2  
3 al. 2009, Garau et al. 2009), data for insecticide residues are quite limited (Cabras et al.  
4  
5 1995, Sala et al. 1996, Goodwin and Ahmad 1998, Navaro et al. 1999 and 2001) and  
6  
7 even less as far as benzourea and other insect growth regulator insecticides are  
8  
9 concerned (Tsiropoulos et al. 1999). However, the type of wine-making process and the  
10  
11 different oenological steps (with or without maceration, clarification, filtration) carried  
12  
13 out can influence the reduction of pesticide residues (Fernandez et al. 2005a and b,  
14  
15 Oliva et al. 2007, Cabras and Angioni 2000, Ruediger et al. 2004).

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20 This paper aims to contribute to the knowledge of the fate of three common  
21  
22 insecticides (flufenoxuron, lufenuron and tebufenozide) residue levels in grapes of two  
23  
24 vine cultivars (Roditis and Cabernet Sauvignon) after field applications and during the  
25  
26 winemaking process, for which there are very few data concerning the persistence of  
27  
28 these active ingredients in grapes and their behaviour during vinification. Furthermore,  
29  
30 the influence of different clarifying substances on the removal of residues from wine  
31  
32 was studied.  
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## 41 **Materials and methods**

### 42 43 *Chemicals and standards solutions*

44  
45  
46 Analytical standards of flufenoxuron (purity 99.3%) and lufenuron (99.7%) were  
47  
48 obtained from Cyanamide (Princeton NJ, USA) and Novartis (Basel, Switzerland),  
49  
50 respectively, while tebufenozide (99.9%) was purchased from Dr Ehrenstorfer GmbH.  
51  
52 Individual analytical standard stock solutions 1000 mg L<sup>-1</sup> for all insecticides were  
53  
54 prepared in acetone and stored at -18 °C in glass vials. An intermediate mixture  
55  
56 standard solution containing all compounds, at 100 mg L<sup>-1</sup> each, was prepared in  
57  
58 acetone from the individual stock solutions and stored at -18 °C. Spiking solutions were  
59  
60

1  
2  
3 prepared by dilution from the intermediate solution and stored at 4°C. Calibration  
4  
5 standard solutions at concentrations 0.05 to 10 mg L<sup>-1</sup> (9 solutions) in acetonitrile-water  
6  
7 (1/1, v/v) were prepared from the intermediate solution.  
8  
9

10 Cyclohexane and dichloromethane were pesticide residue grade, water and  
11  
12 tetrahydrofurane were HPLC-grade and acetonitrile was HPLC-far UV grade. All  
13  
14 solvents were purchased from Labscan (Dublin, Ireland). Silica SPE cartridges (500  
15  
16 mg/3 mL) used for the extracts clean-up were purchased from Isolute (IST Ltd.  
17  
18 International Sorbent Technology, Mid Glamorgan, UK). Bentonite, charcoal,  
19  
20 polyvinylpyrrolidone (PVPP), cellulose, potassium caseinate, gelatine and colloidal  
21  
22 silicon dioxide were commercial grade products.  
23  
24  
25

26  
27 Insecticides formulations Cascade 10 DC (10% flufenoxuron w/v), Match 5 EC (5%  
28  
29 lufenuron w/v) and Mimik 24 SC (24% tebufenozide w/v) were purchased from  
30  
31 commercial market.  
32  
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### 34 35 36 *Field experiment* 37

38  
39 The experimental trials were carried out in two vineyards, located at Nea Aghialos,  
40  
41 Magnesia, central Greece. The first vineyard was planted with the white grape cv.  
42  
43 Roditis and the other with the red grape cv. Cabernet Sauvignon. The vines in both  
44  
45 vineyards were spaced 1.20 m on the row and 2.70 m between the rows, and, during the  
46  
47 experimental periods, they received routine horticultural practices.  
48  
49

50  
51 There were 3 trials in each vineyard for studying the insecticides' dissipation on  
52  
53 grapes. In each trial an insecticide formulation was sprayed on 27 August 2004 at the  
54  
55 recommended by the manufacturers rates (of 66 mL hL<sup>-1</sup> water for Cascade 10 DC  
56  
57 corresponding to 0.066 kg ai ha<sup>-1</sup> of flufenoxuron, 100 mL hL<sup>-1</sup> water for Match 5 EC  
58  
59 corresponding to 0.05 kg ai ha<sup>-1</sup> of lufenuron and 60 mL hL<sup>-1</sup> water for Mimik 24 SC  
60

1  
2  
3 corresponding to 0.144 kg ai ha<sup>-1</sup> of tebufenozide), using an automated high pressure  
4 mechanically driven applicator (Euro spray Ecology 2000 type 1100 L). Each trial was  
5 divided into 4 randomized plots of 15 vines each. Three of them, used as replicates,  
6 were treated with the insecticide and the fourth was left untreated to be used as control.  
7  
8 Meteorological data were collected by an agrometeorological station, located near the  
9 vineyard. During the experimental period (42 days) the average daily air temperature  
10 was 20.6 °C, average relative humidity was 64.2% and total rainfall was 18.8 mm.  
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20 Field dissipation study of insecticides in grapes was performed during the 2004  
21 period. Grape samples were collected at 0 (3h after application, when the spraying  
22 mixture had dried), 2, 4, 7, 14, 21, 28, 35 and 42 days after application (DAA). The  
23 samples consisted of randomly collected parts of at least 12 bunches of separate vines  
24 and the overall sample weight was 1,5-2 kg with 1 kg the minimum weight  
25 recommended in the FAO/WHO guidelines (FAO/WHO, 1986). Grape samples for  
26 residues determination were forwarded to the laboratory, blended after removal of the  
27 stems, subdivided into 50 g aliquots as analytical replicates and stored in individual  
28 bags at -18 °C until extraction.  
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41 Application of the insecticides' formulations in the above vineyard was repeated on  
42 2006 with the same spraying conditions and doses.  
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#### 50 *Vinification Process.*

51 Determination of insecticides was performed on grapes before vinification, in crude  
52 must, clear must, racked and filtered wine and clarified wine.  
53  
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55 For studying the fate of insecticides residues from vine to wine, samples of grape  
56 were processed to produce must and wine. Wine was also produced from grapes  
57 collected from the control plot to be used as control sample. Vinification experiments  
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3 were performed with grapes collected for each cultivar and each experimental plot at 21  
4 and 30 DAA from the field trials of 2004 and at 21 and 40 DAA from those of 2006  
5  
6 and the vinification was performed at laboratory scale. The collected grape samples  
7  
8 (~50 kg) were divided into two equal parts, pressed and stemmed. The one part was  
9  
10 allowed to ferment with the skins (vinification with maceration) while the other was  
11  
12 separated from the skin and the resulting must was allowed to ferment (vinification  
13  
14 without maceration). One hundred mg of sodium metabisulfite and 200 mg of dry yeast  
15  
16 were added per L of must in each part. After the completion of alcoholic fermentation  
17  
18 (20-25 days) at room temperature, the obtained wine was racked and filtered. A 2x50 g  
19  
20 aliquot of the above cloudy must was taken and centrifuged at 4000 rpm for 5 min in  
21  
22 order to quantify residues in the clear must.  
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29 Clarification tests were carried out on 500 mL of the filtered wine samples. The  
30  
31 clarifying agents and the doses employed (usually applied in oenological practice) in  
32  
33 separate triplicate samples were as follows: bentonite at 0.4 g L<sup>-1</sup>, charcoal at 0.2 g L<sup>-1</sup>,  
34  
35 polyvinylpolypyrrolidone (PVPP) at 0.4 g L<sup>-1</sup>, colloidal silicon dioxide 0.5 g L<sup>-1</sup> plus  
36  
37 gelatine at 0.025/0.05 g L<sup>-1</sup> or potassium caseinate at 0.4 g L<sup>-1</sup>. After clarification, clear  
38  
39 wine and unclarified wine samples were analyzed for insecticide residues.  
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43  
44 The samples of grapes before vinification, prepared as described for grape samples  
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46 in the precedent section, must and clear must, unclarified and clarified wine of sprayed  
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48 grapes, as well as control wines were conserved at -18 °C until analysis.  
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#### 51 52 *Sample extraction and clean-up procedure*

53  
54 The investigated insecticides were extracted from the matrices (grapes, must and  
55  
56 wine) by a simple and one step extraction using a mixture of cyclohexane-  
57  
58 dichloromethane (9/1, v/v) as the extraction solvent described by Likas and Tsiropoulos  
59  
60 (2009) and presented briefly below. An aliquot (10 g of previously homogenized grape

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3 sample or 5 mL of wine) was mixed in a centrifuge tube with the extraction solvent  
4  
5 mixture (10 mL for grapes or 5 mL for wine). The tubes were well agitated for 30 min  
6  
7 in orbital shaker and centrifuged. After centrifugation, an appropriate volume of  
8  
9 organic layer (5 mL and 3 mL for grape and wine samples, respectively) was  
10  
11 transferred to a pear shape flask, carefully evaporated to dryness with a rotary  
12  
13 evaporator at 40°C and the residue was quantitatively transferred with about 1 mL  
14  
15 cyclohexane to preconditioned with 10 mL cyclohexane Silica SPE cartridges. After  
16  
17 loading of the sample extract, the cartridge was rinsed with 10 mL cyclohexane  
18  
19 followed by 3 mL of a cyclohexane-tetrahydrofuran (90/10, v/v) solution. The  
20  
21 pesticides were eluted with 2 mL tetrahydrofuran and the eluent was dried under a  
22  
23 gentle stream of nitrogen. Residues were re-dissolved in acetonitrile-water (50/50, v/v)  
24  
25 solution (in 1 mL for grape and in 0.5 mL for wine) and the resulting solutions were  
26  
27 filtered prior to injection into the HPLC system. Unprocessed must and centrifugal  
28  
29 must samples were extracted as grape or wine samples, respectively.  
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### 36 37 *Instrumentation and Chromatographic Conditions*

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39 Chromatographic analysis for the determination of flufenoxuron, lufenuron and  
40  
41 tebufenozide was performed with an HP 1100 liquid chromatograph (Hewlett-Packard  
42  
43 GmbH, Waldbronn, Germany) equipped with a ternary-delivery system, a variable-  
44  
45 wavelength UV detector and an HP ChemStation LC 3D chromatography manager data  
46  
47 acquisition and processing system with the ability to obtain UV spectra at selected  
48  
49 retention time of chromatograms. The analytical column was a Thermo Hypersil HS  
50  
51 C<sub>18</sub> column (250 x 2.1 mm I.D with 5 µm particle size) with a guard column and was  
52  
53 maintained at 30 °C. The mobile phase was acetonitrile-water delivered at a flow of  
54  
55 0.26 mL min<sup>-1</sup> with a gradient composition; from acetonitrile-water (55/45, v/v), held  
56  
57 for 5 min, to acetonitrile-water (80/20, v/v) in 10 min, held for 10 min, and finally a  
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3 decrease at acetonitrile-water (55/45, v/v) over 10 min to stabilize the HPLC system  
4 before starting the next run, giving 35 min as total run time. The injection volume was  
5  
6 20  $\mu\text{L}$ . Before injection, samples were filtered through Titan 2 HPLC nylon membrane  
7  
8 filters (17 mm, 0.2  $\mu\text{m}$  pore size). The optimum detection was obtained at 210 nm.  
9  
10 Under these chromatographic conditions tebufenozide, flufenoxuron and lufenuron  
11  
12 were well separated and their concentrations were determined by the external standard  
13  
14 technique by comparing the peak heights in the samples with those found in the  
15  
16 calibration solutions.  
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## 23 **Results and discussion**

### 24 **Analysis**

25  
26 The screening method used for pesticides residues determination is simple and  
27  
28 suitable for routine analysis. The cyclohexane-dichloromethane solution at 9/1 ratio  
29  
30 effectively extracted lufenuron as well as flufenoxuron and tebufenozide. Accuracy  
31  
32 data were provided for flufenoxuron, lufenuron and tebufenozide by recovery  
33  
34 experiments (n=5) with grape and wine samples at three different levels (0.05-1.00 mg  
35  
36  $\text{Kg}^{-1}$  and 0.02-0.25 mg  $\text{L}^{-1}$ ). The mean recovery percentages for grapes analysis ranged  
37  
38 from 88 to 103% for flufenoxuron, 85 to 104% for tebufenozide and 92 to 107% for  
39  
40 lufenuron with relative standard deviations (RSDs) <10%. The mean recovery values  
41  
42 for wine analysis ranged from 91 to 105% for flufenoxuron, 87 to 99% for tebufenozide  
43  
44 and 85 to 106% for lufenuron with relative standard deviations (RSDs) <12%. The  
45  
46 limits of quantitation of the method (LOQs), calculated as a signal to noise from  
47  
48 untreated grape and wine samples equal to 10. The LOQs for grape analysis was 0.02  
49  
50 mg  $\text{Kg}^{-1}$  for flufenoxuron and tebufenozide and 0.05 for lufenuron and for wine  
51  
52 analysis 0.005 mg  $\text{L}^{-1}$  for flufenoxuron and 0.01 mg  $\text{L}^{-1}$  for lufenuron and tebufenozide,  
53  
54 depending on the final concentration factor for each extract and the sensitivity of each  
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1  
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3 compound. Figure 1 shows chromatograms of a mixture standard solution as well as of  
4  
5 extracts of grape sample and of the obtained wine sample from the field experiments.  
6  
7  
8  
9

### 10 **Field sample analysis and wine-making**

11  
12 Dissipation of flufenoxuron, lufenuron and tebufenozide residues in “Roditis” and  
13  
14 “Cabernet Sauvignon” grape samples from the field experiments is presented in  
15  
16 Figures 2, 3 and 4. It should be noticed that the insecticides were applied when grapes  
17  
18 had attained their final size and any diluting effect was negligible thereafter. In  
19  
20 addition, no residues of the studied insecticides were detected in any of the analysed  
21  
22 control grape or wine samples.  
23  
24  
25

26  
27 The data relating to the residues in grapes, must and wine for the vinification  
28  
29 experiments carried out are reported in Tables 1, 2, 3 and 4.  
30  
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33

34 **Flufenoxuron.** After treatment in 2004, flufenoxuron mean concentrations on grapes  
35  
36 ranged from  $0.33 \pm 0.04$  to  $0.39 \pm 0.06$  mg kg<sup>-1</sup> for “Cabernet” and “Roditis” grapes,  
37  
38 respectively. There after residues decreased very slowly with time (Figure 2) showing  
39  
40 pseudo-first-order kinetics ( $R^2 > 0.944$ ) with a dissipation rate of 0.011 d<sup>-1</sup>, and at 42  
41  
42 DAA ~60% of the initial deposited concentration remained in grapes. Similar behaviour  
43  
44 with very slow decay rate was also observed for teflubenzuron, an other benzoylurea  
45  
46 insecticide, on grapes of Roditis variety (Tsiropoulos et al. 1999). A different fate of  
47  
48 flufenoxuron residues was observed in dates where a reduction of 96% of initial  
49  
50 flufenoxuron residues was found after 60 days (Kamel et al. 2007)  
51  
52  
53

54  
55 At 35 DAA, which is the recommended by the manufacturer PHI, flufenoxuron  
56  
57 residues on grapes were  $0.24 \pm 0.03$  and  $0.26 \pm 0.03$  mg kg<sup>-1</sup> for “Cabernet” and  
58  
59 “Roditis”, respectively. For the 2006 experiment, grapes harvested 40 DAA contained  
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1  
2  
3 flufenoxuron at  $0.27\pm 0.03$  and  $0.26\pm 0.04$  mg kg<sup>-1</sup>, respectively. Concentration values  
4  
5 of flufenoxuron residues measured on all grape subsamples collected few hours after  
6  
7 treatment up to near the PHI are clearly below the recently established by EU (31  
8  
9 August 2008) MRL value (2.0 mg kg<sup>-1</sup>) for flufenoxuron on grapes. Considering our  
10  
11 results, we believe that, even after an additional flufenoxuron application 20-30 days  
12  
13 before our spraying (i.e. end of July or early August), residues in “Roditis” and  
14  
15 “Cabernet” grapes are unlikely to exceed the MRL value even the first day after the  
16  
17 second spraying.  
18  
19  
20  
21

22 Flufenoxuron residues’ partitioning during wine-making process was studied after  
23  
24 vinification of grapes collected at 21 and 30 DAA for 2004 (Tables 1 and 2) and at 21  
25  
26 and 40 DAA for 2006 (Tables 3 and 4). The concentration of residues in the must (0.11-  
27  
28 0.18 mg kg<sup>-1</sup>) were significantly lower than in grapes (0.23-0.34 mg kg<sup>-1</sup>), indicating  
29  
30 that flufenoxuron was distributed in the solid parts of grapes (skin) as well as in the  
31  
32 liquid part. The mean values of transfer factor of flufenoxuron from grapes to must  
33  
34 resulted from all vinification experiments were 0.48 and 0.52, for “Roditis” and  
35  
36 “Cabernet” grapes respectively. Centrifugation of the must resulted in major removal of  
37  
38 flufenoxuron residues as it was adsorbed by the suspended solids contained in the must  
39  
40 and residues in the clear must were 0.02-0.04 mg L<sup>-1</sup>. At the end of the wine-making  
41  
42 process, flufenoxuron residues were not determined in the produced wines (Table 4)  
43  
44 with both vinification techniques (with or without maceration) showing the same  
45  
46 influence of vinification technique on flufenoxuron residue reduction.  
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55 **Lufenuron.** Lufenuron showed analogous to flufenoxuron persistence on grapes during  
56  
57 the field experiments. During 2004, residues of lufenuron on grape samples at 0 DAA  
58  
59 ranged from  $0.39\pm 0.03$  to  $0.46\pm 0.09$  mg kg<sup>-1</sup> for “Cabernet” and “Roditis” grapes,  
60

1  
2  
3 respectively. Thereafter, lufenuron residues decreased very slowly with time to  
4  
5 0.38±0.03 and 0.39±0.04 mg kg<sup>-1</sup> at 7 DAA, and to 0.31±0.04 and 0.28±0.02 mg kg<sup>-1</sup> at  
6  
7 21 DAA for “Cabernet” and “Roditis” grapes, respectively. Thereafter, they decreased  
8  
9 further to 0.26±0.01 and 0.25±0.03 mg kg<sup>-1</sup> (representing 54-66% of the initial residues)  
10  
11 at 42 DAA, which is the recommended by the manufacturers PHI for lufenuron. For  
12  
13 2006, grapes harvested at 40 DAA contained lufenuron residues at 0.27±0.03 and  
14  
15 0.24±0.01 mg kg<sup>-1</sup> for “Roditis” and “Cabernet” cultivars, respectively. Residues of  
16  
17 lufenuron in all our grape subsamples both in 2004 and 2006 were clearly below the  
18  
19 recently established by EU (31/8/2008) MRL value (1.0 mg kg<sup>-1</sup>) for lufenuron on  
20  
21 grapes even few hours after the treatment (at 0 DAA).  
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26

27 Lufenuron dissipation on grapes (Figure 3) showed pseudo-first-order kinetics ( $R^2 >$   
28  
29 0.897) with low values of reduction rates ranging from 0.011 for “Cabernet” and 0.016  
30  
31 for “Roditis” grapes. Considering our data for flufenoxuron and lufenuron dissipation  
32  
33 and the published data for teflubenzuron dissipation in grapes, between the three  
34  
35 benzoylurea insecticides, teflubenzuron seems more persistent than the other two.  
36  
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39 The grape samples used to study lufenuron residues partitioning during vinification  
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41 process were harvested at 21 and 30 DAA for the wine-making process in 2004 and  
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43 from 21 and 40 DAA for that in 2006. During wine production without maceration and  
44  
45 before fermentation, a part of lufenuron residues was removed with the solid part of  
46  
47 grapes and the remaining residues in must ranged from 0.09 to 0.16 mg kg<sup>-1</sup> with mean  
48  
49 values of transfer factor from grapes to must at 0.45 and 0.43, for “Roditis” and  
50  
51 “Cabernet” grapes, respectively. After the must centrifugation, lufenuron residues in the  
52  
53 clear must were 0.01-0.03 mg L<sup>-1</sup> and the calculated transfer factor from must to wine  
54  
55 was <0.08, indicating that lufenuron was strongly adsorbed by the suspended solids in  
56  
57 the must. Wines obtained at the end of the wine-making process (with and without  
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3 maceration) were residue-free (below the method LOQ value of  $0.01 \text{ mg L}^{-1}$ ) and the  
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6 calculated transfer factor for lufenuron from grape to wine was  $<0.04$  for both  
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8 “Cabernet” and “Roditis” grapes.  
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12 **Tebufenozide.** After treatment, at 0 DAA, mean concentration of tebufenozide residues  
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14 on grapes were  $0.74 \pm 0.04$  to  $0.95 \pm 0.14 \text{ mg kg}^{-1}$ , for “Cabernet” and “Roditis” grapes,  
15  
16 respectively. Tebufenozide residues during the experimental trial dissipated slowly  
17  
18 according to pseudo-first-order kinetics ( $R^2 = 0.8809-0.8878$ ) with reduction rates  
19  
20 ranging from 0.011 for “Cabernet” to 0.018 for “Roditis” grapes (Figure 4). In  
21  
22 particular, tebufenozide residues decreased slowly to  $0.72 \pm 0.05$  and  $0.68 \pm 0.06 \text{ mg kg}^{-1}$   
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24 at 7 DAA, then to  $0.65 \pm 0.06$  and  $0.59 \pm 0.05 \text{ mg kg}^{-1}$  at 14 DAA reaching  $0.46 \pm 0.05$  and  
25  
26  $0.40 \pm 0.03 \text{ mg kg}^{-1}$  at 42 DAA (representing 62 and 42% of the initially deposited  
27  
28 amount on grapes) for “Cabernet” and “Roditis” grapes, respectively. Tebufenozide  
29  
30 residues determined in grapes 21 DAA, that is the recommended PHI, ranged from  
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32  $0.55 \pm 0.03$  and  $0.68 \pm 0.07 \text{ mg kg}^{-1}$ , values clearly lower than the proposed MRL value  
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34 for tebufenozide in grapes established by EU ( $3 \text{ mg kg}^{-1}$ ) and Codex Alimentarium ( $2$   
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36  $\text{mg kg}^{-1}$ ).  
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44 The grape samples used to study tebufenozide partitioning during vinification  
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46 process were harvested at 21 and 30 DAA for the wine making process in 2004 and at  
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48 21 and 40 DAA for that in 2006. Wine prepared with and without maceration from  
49  
50 treated grapes of both cultivars contained tebufenozide residues ranging from 0.13 to  
51  
52  $0.26 \text{ mg L}^{-1}$  for both vinification processes. The observed concentration of tebufenozide  
53  
54 residues in wines produced from grapes collected at or after the recommended PHI  
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56 exceed the tolerance value established for wine by Switzerland ( $0.1 \text{ mg kg}^{-1}$ ) but they  
57  
58 are in accordance with the MRL value for tebufenozide in wine ( $0.3 \text{ mg L}^{-1}$ ) proposed  
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3 by the International Organisation for Vine and Wine (OIV). The calculated transfer  
4 factors from grape to wine ranged from 0.45 for vinification of white 'Roditis' to 0.34  
5  
6 of red 'Cabernet' grapes for the wine-making process without maceration and from  
7  
8 0.31 to 0.32, respectively, for the wine-making process with maceration.  
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12 During wine production without maceration and before fermentation, residues were  
13 removed with the solid phase of grapes (Tables 1-4) and the remaining residues in must  
14 were 0.17 to 0.33 mg kg<sup>-1</sup> resulting in tebufenozide transfer factors from grape to must  
15  
16 0.48 for "Cabernet" and 0.52 for "Roditis", values almost similar to those observed for  
17  
18 the other two insecticides studied. After the must centrifugation, tebufenozide residues  
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20 in the clear must were 0.07-0.12 mg L<sup>-1</sup>, indicating that tebufenozide was only partially  
21  
22 adsorbed by the suspended solids of the must. The calculated mean values of transfer  
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24 factor for tebufenozide from must to wine were 0.73 for "Cabernet" and 0.86 for  
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26 "Roditis", values much higher than those calculated for flufenoxuron and lufenuron  
27  
28 (<0.08). The observed transfer factors of tebufenozide from grapes into must,  
29  
30 centrifuged must, and in wine in all cases indicated a similar pattern of tebufenozide  
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32 fate for both cultivars of white 'Roditis' and red 'Cabernet' grapes.  
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41 The clearly different fate of tebufenozide residues relatively to those of  
42 flufenoxuron and lufenuron during the wine-making process is due mainly to their  
43 different distribution capacity between the liquid and solid phase of the produced must  
44 and wine as found from the calculated high tebufenozide transfer factor values from  
45 must to centrifuged must and from must to wine.  
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52 Wine clarification experiments performed for wines containing tebufenozide  
53 residues (Figure 5) showed that among the tested clarifying agents, i.e. bentonite,  
54 potassium caseinate, gelatine-silicon dioxide and polyvinylpolypyrrolidone did not  
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56 presented significantly decrease the pesticide residue concentration compared with non-  
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3 clarified wine. On the other hand, clarification with charcoal significantly reduced (by  
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5 95%) tebufenozide residues in wine confirming that it is the most effective between  
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8 other clarifying agents for reducing various pesticides in wine (Cabras et al. 1995,  
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10 Tsiropoulos et al. 1999, Ruediger et al. 2004, Fernandez et al. 2005b, Oliva et al. 2007).

## 15 **Conclusions**

16  
17 All of the 'insect growth regulator insecticides' studied showed very slow  
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19 dissipation in grapes in the field described by pseudo-first-order kinetics and giving  
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21 dissipation rate values between 0.011 and 0.018 d<sup>-1</sup>. Their residues' concentrations at  
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23 the recommended PHI were clearly below the established MRLs in grape by EU (2 mg  
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25 kg<sup>-1</sup> for flufenoxuron, 1 mg kg<sup>-1</sup> for lufenuron and 3 mg kg<sup>-1</sup> for tebufenozide), while  
26  
27 the measured concentration were below the MRL value even just after application.  
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29 Therefore, the use of these insecticides should not create limit problems if used  
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31 following good agricultural practices.  
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36 The processing of treated grapes into wine almost eliminated residues for  
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38 flufenoxuron and lufenuron resulting to residues-free wine, whereas tebufenozide was  
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40 found in wine at concentrations up to near the 1/10 of the MRL value in grape. The two  
41  
42 wine-making techniques employed (with and without maceration) had the same  
43  
44 influence on tebufenozide residues in produced wine. Among the clarifying agents  
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46 used, bentonite, potassium caseinate, gelatine-silicon dioxide and  
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48 polyvinylpolypyrrolidone did not practically remove residues from wine, while  
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50 charcoal very effectively eliminated tebufenozide residues  
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### Figures captions

**Figure 1.** HPLC-UV chromatograms of tebufenozide (peak 1), lufenuron (peak 2) and flufenoxuron (peak 3) standard solution at 0.50 mg/L, of extracts from grape sample collected 30 days post application of tebufenozide, of extract of wine sample produced from the above grapes and of extracts from control grape and wine samples.

**Figure 2.** Field dissipation of flufenoxuron residues in 'Roditis' (● —) and 'Cabernet Sauvignon' (▲ ---) grapes for 2004 and decline curves as derived from the 1<sup>st</sup>-order kinetic model. The solid points show mean values of flufenoxuron residues and error bars represent 2xSD of three replicates.

**Figure 3.** Field dissipation of lufenuron residues in 'Roditis' (● —) and 'Cabernet Sauvignon' (▲ ---) grapes for 2004 and decline curves as derived from the 1<sup>st</sup>-order kinetic model. The solid points show mean values of lufenuron residues and error bars represent 2xSD of three replicates.

**Figure 4.** Field dissipation of tebufenozide residues in 'Roditis' (● —) and 'Cabernet Sauvignon' (▲ ---) grapes for 2004 and decline curves as derived from the 1<sup>st</sup>-order kinetic model. The solid points show mean values of tebufenozide residues and error bars represent 2xSD of three replicates.

**Figure 5.** Mean % overall reduction of tebufenozide residues in wines produced from 'Roditis' and 'Cabernet Sauvignon' grapes after clarification with different clarifying agents. Tables

**Table 1.** Flufenoxuron, lufenuron and tebufenozide residues from grapes (mg kg<sup>-1</sup>\*) to wine (mg L<sup>-1</sup>\*\*\*) for 'Roditis' grapes collected at different interval times (days after

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3 application, DAA) for 2004 experiments. Relative standard deviation of three  
4 replicates is shown in parenthesis  
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9 **Table 2.** Flufenoxuron, lufenuron and tebufenozide residues from grapes ( $\text{mg kg}^{-1*}$ ) to  
10 wine ( $\text{mg L}^{-1**}$ ) for 'Cabernet Sauvignon' grapes collected at different interval  
11 times (days after application, DAA) for 2004 experiments. Relative standard  
12 deviation of three replicates is shown in parenthesis  
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17 **Table 3.** Flufenoxuron, lufenuron and tebufenozide residues from grapes ( $\text{mg kg}^{-1*}$ ) to  
18 wine ( $\text{mg L}^{-1**}$ ) for 'Roditis' grapes collected at different interval times (days after  
19 application, DAA) for 2006 experiments. Relative standard deviation of three  
20 replicates is shown in parenthesis  
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26 **Table 4.** Flufenoxuron, lufenuron and tebufenozide residues from grapes ( $\text{mg kg}^{-1*}$ ) to  
27 wine ( $\text{mg L}^{-1**}$ ) for 'Cabernet Sauvignon' grapes collected at different interval  
28 times (days after application, DAA) for 2006 experiments. Relative standard  
29 deviation of three replicates is shown in parenthesis  
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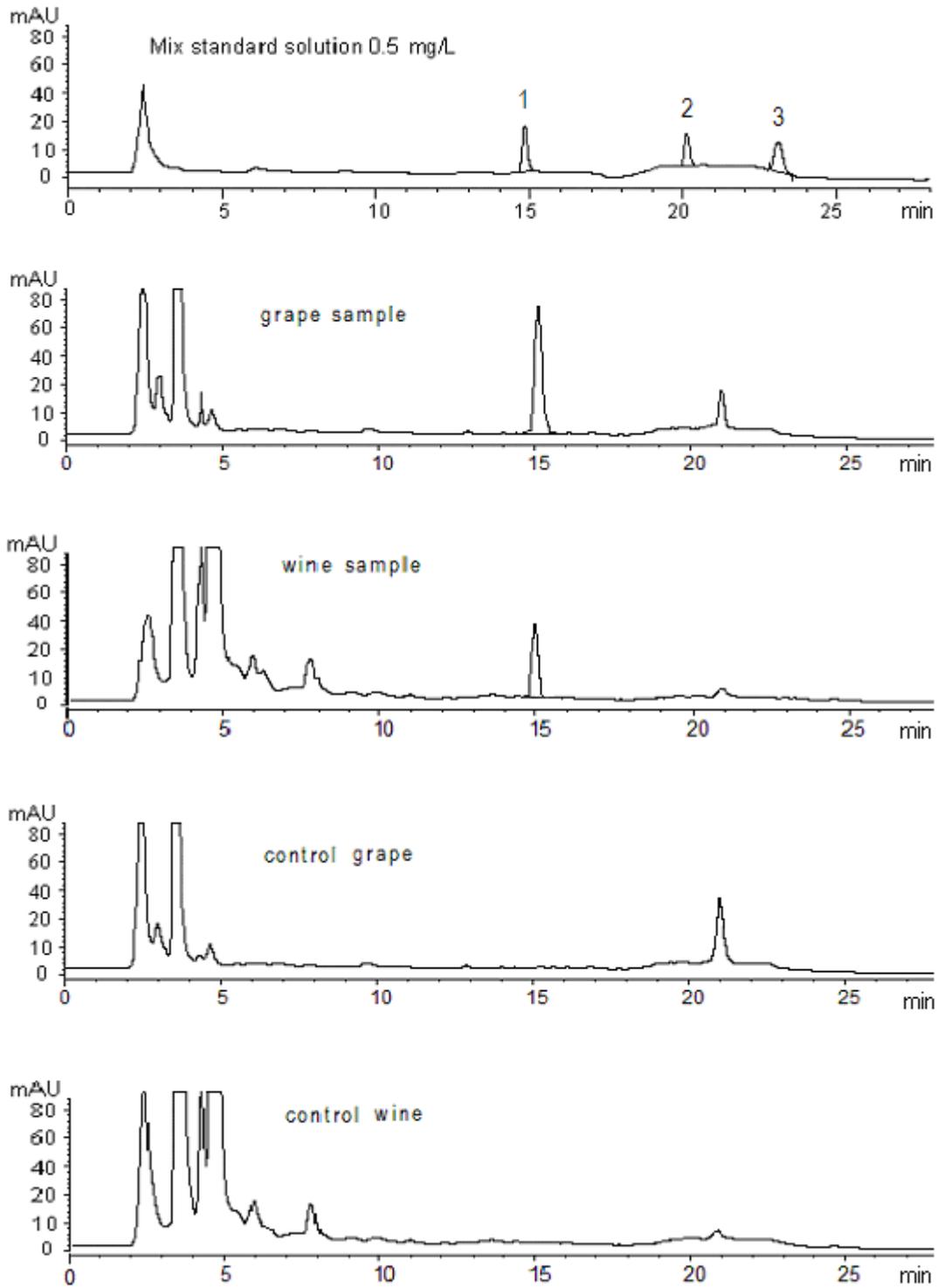


Figure 1

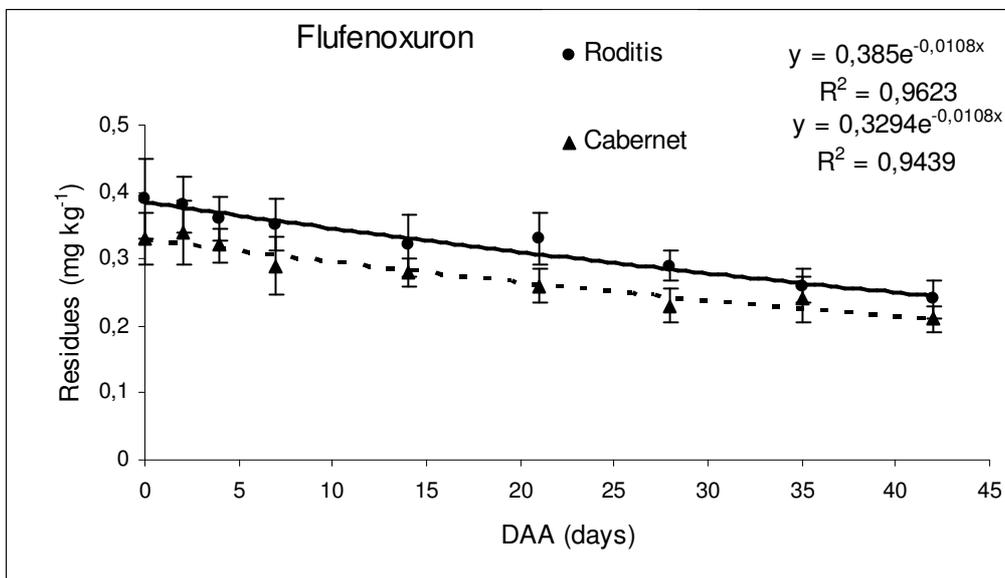


Figure 2

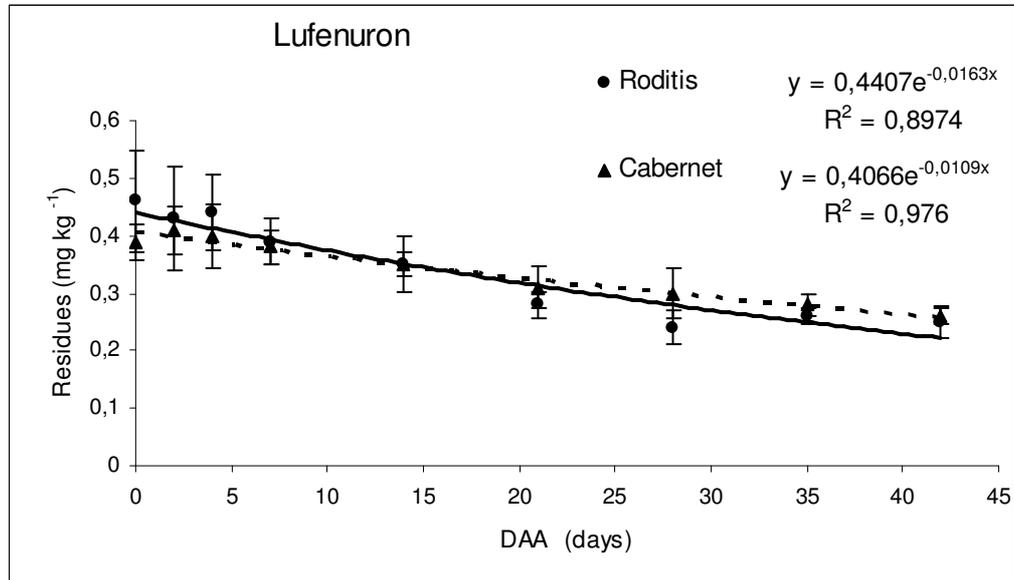


Figure 3

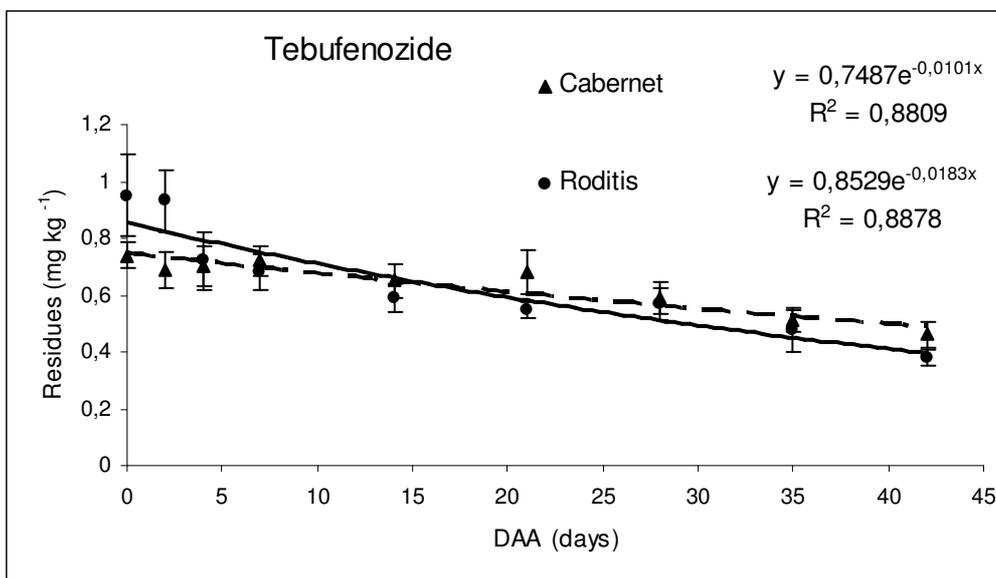


Figure 4

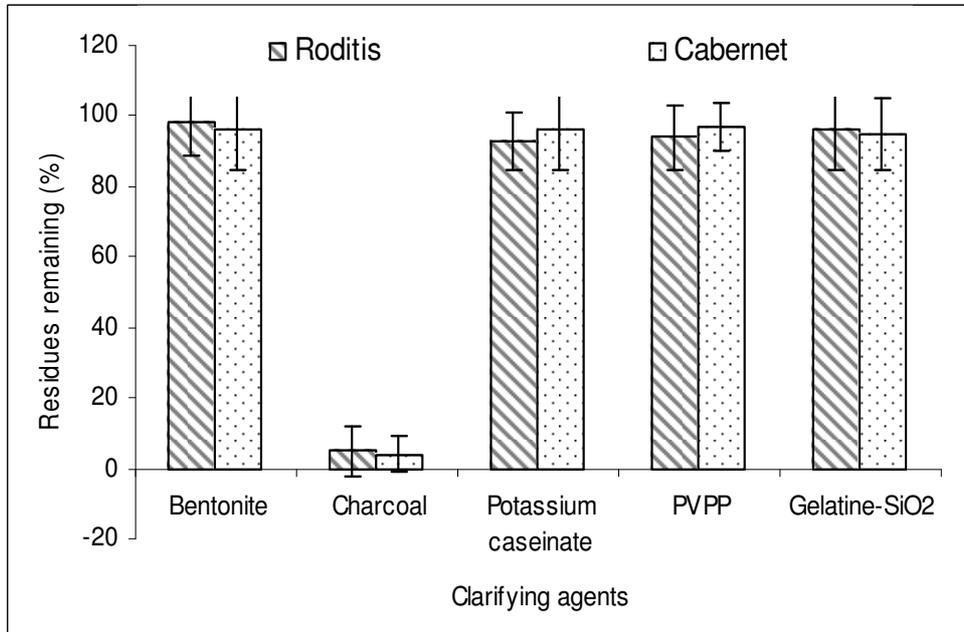


Figure 5

**Table 1.** Flufenoxuron, lufenuron and tebufenozide residues from grapes ( $\text{mg kg}^{-1}$ \*) to wine ( $\text{mg L}^{-1}$ \*\*\*) for 'Roditis' grapes collected at different interval times (days after application, DAA) for 2004 experiments. Relative standard deviation of three replicates is shown in parenthesis.

DAA	Flufenoxuron		Lufenuron		Tebufenozide	
	21	30	21	30	21	30
Grapes *	0.31 (14)	0.28 (11)	0.30 (10)	0.22 (12)	0.55 (11)	0.43 (15)
Must *	0.14 (10)	0.13 (9)	0.13 (12)	0.11 (16)	0.30 (14)	0.27 (17)
Centrifuged Must **	0.03 (12)	0.02 (13)	0.03 (11)	0.02 (15)	0.12 (12)	0.07 (14)
Wine with maceration **	<LOQ	<LOQ	<LOQ	<LOQ	0.14 (9)	0.13 (10)
Wine without maceration **	<LOQ	<LOQ	<LOQ	<LOQ	0.26 (12)	0.23 (12)

**Table 2.** Flufenoxuron, lufenuron and tebufenozide residues from grapes ( $\text{mg kg}^{-1}$ \*) to wine ( $\text{mg L}^{-1}$ \*\*\*) for 'Cabernet' grapes collected at different interval times (days after application, DAA) for 2004 experiments. Relative standard deviation of three replicates is shown in parenthesis.

DAA	Flufenoxuron		Lufenuron		Tebufenozide	
	21	30	21	30	21	30
Grapes *	0.24 (10)	0.23 (12)	0.31 (11)	0.27 (8)	0.68 (10)	0.56 (12)
Must *	0.13 (14)	0.11 (15)	0.16 (9)	0.12 (15)	0.27 (12)	0.33 (18)
Centrifuged Must **	0.03 (9)	0.02 (11)	0.01	0.02 (17)	0.08 (7)	0.11 (10)
Wine with maceration **	<LOQ	<LOQ	<LOQ	<LOQ	0.21 (12)	0.17 (9)
Wine without maceration **	<LOQ	<LOQ	<LOQ	<LOQ	0.23 (9)	0.19 (11)

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**Table 3.** Flufenoxuron, lufenuron and tebufenozide residues from grapes ( $\text{mg kg}^{-1*}$ ) to wine ( $\text{mg L}^{-1**}$ ) for 'Roditis' grapes collected at different interval times (days after application, DAA) for 2006 experiments. Relative standard deviation of three replicates is shown in parenthesis.

DAA	Flufenoxuron		Lufenuron		Tebufenozide	
	21	40	21	40	21	40
Grapes *	0.32 (9)	0.26 (14)	0.33 (8)	0.27 (10)	0.69 (12)	0.35 (9)
Must *	0.17 (11)	0.12 (10)	0.14 (11)	0.12 (5)	0.30 (9)	0.17 (10)
Centrifuged Must **	0.03 (8)	0.02 (7)	0.02 (12)	0.01	0.09 (12)	0.07 (7)
Wine with maceration **	<LOQ	<LOQ	<LOQ	<LOQ	0.19 (8)	0.14 (7)
Wine without maceration **	<LOQ	<LOQ	<LOQ	<LOQ	0.24 (10)	0.16 (11)

**Table 4.** Flufenoxuron, lufenuron and tebufenozide residues from grapes ( $\text{mg kg}^{-1}$ \*) to wine ( $\text{mg L}^{-1}$ \*\*\*) for 'Cabernet Sauvignon' grapes collected at different interval times (days after application, DAA) for 2006 experiments. Relative standard deviation of three replicates is shown in parenthesis.

DAA	Flufenoxuron		Lufenuron		Tebufenozide	
	21	40	21	40	40	
Grapes *	0.34 (9)	0.27 (11)	0.29 (8)	0.24 (6)	0.73 (12)	0.39 (9)
Must *	0.18 (8)	0.14 (7)	0.11 (10)	0.09 (9)	0.32 (11)	0.19 (7)
Centrifuged Must **	0.04 (12)	0.02 (9)	0.01	0.02 (10)	0.07 (5)	0.09 (10)
Wine with maceration **	<LOQ	<LOQ	<LOQ	<LOQ	0.25 (8)	0.13 (6)
Wine without maceration **	<LOQ	<LOQ	<LOQ	<LOQ	0.23 (5)	0.15 (9)