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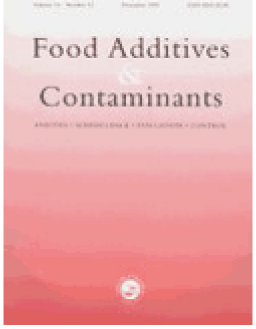

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COPPER CONTENT OF GRAPE AND WINE FROM ITALIAN FARMS

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Abstract

The copper content of grape and wine from 16 wine-farms in Italy was studied during the harvest of 2003. The influence of the number of copper applications, the time period between the last application and harvest, and the total amount of copper applied was examined. Of the total number of samples analysed, 13% of grape samples and 18% of wine samples exceeded the maximum residue level (MRL). The total amount of copper applied and the number of days between the last application and harvest explained 44% of the concentration of copper in grape. This low correlation may be due to other influencing factors, such as meteorology and application method. In 2003, conditions were unusually dry and the recommended safety interval for copper application (20 days) was not sufficient to guarantee a residue level in grape below the MRL (20 mg/kg). In order to reduce the probability of copper residues being close to the MRL, a time period of 40 – 50 days between the last application and harvest is suggested. Furthermore, the copper content of grape and wine was not dependent on the pest management strategy of the farm (conventional, integrated or organic). A more important factor influencing copper residue levels may be that copper applications are made in response to the prediction of a disease outbreak rather than the being dependent on the pest management strategy in place. No difference in copper content was observed between red and white grape or wine.

Keywords: Copper residue, wine, grape, pest management strategy

Introduction

Fungal diseases represent a large threat to wine growers, causing significant damage and severely reducing product quality. In European viticulture, downy mildew (*Plasmopara viticola*) is one of the most dangerous diseases affecting grapes (Pearson and Goheen 1988). Other fungal diseases include oidium (*Uncinula necator*), botritis (*Botrytis cinerea*) and black-rot (Boubals 1984, Martelli 1984). Besides organic pesticides (for example, Mancozeb, Zineb, Dimetomorf, Metalaxyl), copper either alone, or formulated with other agrochemicals, is an important measure in the prevention of the outbreak of fungal diseases. However, cupric products are readily removed from treated crops by rainfall and only protect organs sprayed with the product. Therefore, in the case of high disease pressure, fast growing vines and/or rains, it may become necessary to spray more frequently. Copper has traditionally been used to protect vines against fungal disease since the end of the eighteenth century (Lafforgue 1928). Above all, European organic viticulture is predominantly based on copper and sulphur treatments for the control of downy mildew, in line with European Regulation (EC) nº 2092/91 amended by the regulation (EC) nº 473/2002. The aforementioned regulation limits the use of copper to 8 kg/ha/year until 31 December 2005, and up to 6 kg/ha/year from 1 January 2006.

The intensive and long-term use of copper has raised concerns regarding negative effects on the environment, through toxicity to aquatic and soil organisms (Beltrami and Capri 1999, Capri et al. 1999), and impacts on human health (Araya et al. 2003; Turnlund et al. 2004). High levels of copper can also damage vine plants, through phytotoxicity (Fleming and Trevors 1989), and ruin the quality and processing of wine (Gennaro et al. 1986). Concentrations above 1 mg/l in white wine may cause copper casse, resulting in a turbid wine (Ribereau-Gayon 2000). For these and other reasons the European Regulation (EC) nº 2092/91 has fixed a maximum permitted field dose of copper at 8 kg/ha/year, and a MRL in grape and wine of 20 mg/kg and 1mg/L respectively(reference?).

Copper applied in the field in the form of the bivalent ion (Cu ++) does not dissipate. Following application to the vine plants, the sole route for the decrease of concentrations on the grapes is by wash-off. By this means, copper enters the soil where it is accumulated as a result of strong binding to soil particles (Flores-Vélez et al. 1996; Müller 2000). As transport of copper from the roots appears to be of minor importance in determining concentrations in the aerial parts of the plant (Brun et al. 2001), the variables that determine the concentration of copper in grape at the time of harvest are presumably the total amount of copper applied, the number of applications made and the amount precipitation in the period between the last application and harvest.

The concentration of copper in wine may be decreased compared to that in the grape due to the elimination of copper during fermentation. Copper is reduced, forming insoluble sulphides, which are removed through sedimentation together with yeasts and lees (Tromp and Klerk 1988, Ribéreau-Gayon et al. 2000). Furthermore, copper content may differ between red and white wine, as a result of differences in the winemaking process. In a study performed by Amati (1984) the concentration of copper was higher in red must than in white must. This was explained by the fact that, during the initial winemaking process for red must, the skin of the grapes is not removed. An understanding of the overall fate of copper in the field is necessary in order to assess the key factors influencing the concentration of copper on grape for human risk assessment, and to enable the proposal of measures to minimize residue levels in grape and wine. In this study we have considered the influence of *(1)* the number of copper applications, *(2)* the period of time between the last application and harvest and *(3)* the total mass of copper applied, on copper residue levels in grape and wine.

Materials and methods

Data collection

A total of 38 grape and 34 wine samples were collected from sixteen farms dedicated to grape cultivation for winemaking, during the harvest period of 2003. All samples were obtained by random sampling techniques in the cultivated crop area. Different pest management strategies were used on the farms (conventional (n=5), integrated (n=8) and organic (n=3)). The farms sampled were located in Lombardia (LO), Trentino-Alto Adige (TN), Emilia-Romagna (ER), Friuli-Venezia-Giulia (FV) and Lazio Regions (LZ) in Italy.

The use of pesticides differs according to the viticulture production system. In the conventional pest management strategy, the application of active ingredients does not follow rules established by the regional authority. In the integrated pest strategy, the use of pesticides is restricted and their application is allowed only when pests and diseases exceed defined limits. In organic viticulture, diseases can be managed using a combination of cultural strategies and acceptable, mineral-based fungicides, such as sulphur and copper.

Usually, in conventional and integrated pest management strategies, copper is formulated with organic pesticides to improve the efficacy of cupric products. Copper is included in fungicidal formulations as oxychloride, sulphate or hydroxide. The advantage of these formulated products is their adhesiveness to guarantee an adequate deposit on the treated vegetable. In our study, the different formulations used by farmers (excluding organic farms) included copper with Dimetomorf, Fosetyl-Al, Iprovalicarb, Cimoxanil and Metalaxyl. These favour the penetration of the active ingredient into the plant, and this may increase the time for which the compound remains in the vineyard.

It is important to know the level of disease and damage in order to use the most suitable active ingredients. Disease pressure may be especially high during wet years, and under such conditions grapes could receive increased fungicide applications. In the year of our study (2003), climatic conditions were unusual (dry and slightly rainy). In 2003, rainfall ranged between 700 and 890 mm in Trentino-Alto Adige (TN) and Friuli-Venezia-Giulia (FV) regions. In Lombardia (LO), Emilia-Romagna (ER), and Lazio Regions (LZ) rainfall ranged between 250 and 594 mm.

In the present study, the collaboration of farmers was fundamental. Besides the samples of grape and wine, they provided important information regarding the pest management strategy in place, the number of copper applications made during the vegetative period, the time elapsed between the last application (DLA) of copper and harvest, and the dose of copper (kg/ha) used in each application.

One of the most important factors in the study of pesticide residues, is the number of applications during the vegetative period. In our study, this factor ranged from one to eleven, and the number of applications made did not follow established rules for the different pest management strategies in place. The statutory period of time between the last application (DLA) of copper and harvest is 21 days. All of the selected farms respected this legal condition, and the DLA ranged from 21 to 112 days. The quantity of copper used in vine crops is limited to 8 kg/ha/year until December 2005 (EC 2092/91). The total mass of copper applied for all farms considered in this study was between 0.525 kg/ha and 7.8 kg/ha. The highest application rates of copper correspond to farms located in the north of Italy (TN and FV) where problems with downy mildew are greater.

Table 1 summarises the farms and their location, the number of samples of grape and wine collected from each farm, the pest management strategy, the number of applications, the time between last application and sampling, the total mass of copper (kg/ha) applied and rainfall (mm/year) during 2003. Total copper is calculated by multiplying its fraction in the formulation by the applied dose.

[insert table 1]

Extraction method and apparatus

Each unwashed grape sample (sin raspon) consisted of 1 kg fruit, which was minced to a homogenous paste in a stomacher (Stomacher® 400, Seward Ltd). Approximately 10 g of the paste was dried in an oven for about 10 hours at 105ºC, after which 1 g was weighed into a glass test tube. The wine samples were collected from 1 l bottles. A volume equivalent to 1 g was transferred to a test tube. 5 ml of HNO_3 and 2ml of $HClO_4$ were added to the tubes to facilitate the mineralization of the samples, which was performed using a digestion apparatus (Model: Büchi 425). The digestion tubes are heated consistently from all sides. This guarantees optimum boiling characteristics without boiling delay. The temperature is easily adjustable up to approx. 250°C. Due to the temperature controls, samples tending to foam can be digested without any problems. The digestion unit is suitable for four samples with a tight suction module and with a continuously adjustable IR-heating. The metal components of the equipment are made of acid-resistant steel. The digestion times are typically 20 to 60 minutes depending on quantity and type of sample.

Following mineralization, samples were washed with 10-15 ml of Mili-Q water and were filtered through 150 Ø mm filters (Schleicher & Schuell). Blank samples were prepared as controls for the procedure. All reagents and chemicals were of analytical grade. High-purity water from a Mili-Q apparatus was used to prepare standard solutions. A THGA graphite furnace mounted on a Perkin-Elmer (mod. AA600) atomic absorption spectrophotometer (AAS) was used with an auto-sampler for the determination of copper concentration at 324.8 nm. Argon was the inert gas used, and a copper cathode lamp was used for the measurements of copper. For calibration purposes, standard solutions were prepared by diluting reference standard solutions of copper for the atomic absorption spectrophotometer

(AAS), certified by the manufacturers (Aldrich Chemical Company, Icn.). An average of three readings of absorbance were taken for all samples(including controls). The calibration curve was based on 4 concentrations, giving a correlation coefficient of 0.996 and a percentage residue standard deviation of 10.19 % from the analytical means of different samples. The average value for blank samples was 0.011 ± 0.007 mg/l .

Statistical analysis

Analysis of variance (ANOVA) and non parametric Kruskal-Wallis (with SPSS version 11.5 for PC) were carried out to asses differences in copper content for the number of applications, the total dose applied and the interval between the last application and harvest. Multiple regression analysis was also performed for these determining factors and levels of copper residue, in order to assess the contribution of each factor to the regression. The effect of pest management strategies on copper levels and differences between white and red grape and wine were also tested with ANOVA. To fulfil the assumptions of the ANOVA, the data was log-transformed.

Results and discussion

The analysis found that the average copper concentration for red grape (n= 22) and white grape (n=16) was 11.32 ± 8.61 mg/kg and 7.54 ± 7.50 mg/kg respectively. These concentration are lower than those obtained by Fregoni and Corallo (2001) in which the total copper residue concentration in grape from several italian regions ranged from 10 to 56 ppm. Out of the 38 grape samples analysed, 13 % exceeded the established MRL (20 mg/kg). No significant difference in copper content (p>0.05) was found between red and white grape.

The mean copper content found in red and white wine (n=20; n= 14) was 0.71 ± 0.52 mg/L and 1.01 ± 0.95 mg/L respectively. Of the total number of wine samples analysed, 18% exceeded the MRL (1 mg/L). No significant difference (p>0.05) was found between red and white wine. The results found here were similar those obtained by Marengo and Aceto (2003) who analysed 68 red wine samples from the Piedmont region and found copper concentrations that ranged from 0.001 mg/l to 1.34 mg/l. Our results also agree with another study where the copper content of white wines ranged from 0 to 1.8 mg/l (Sauvage et al. 2002). The values in the present work show that there may be a risk of turbidity and quality reduction in white wine as a result of copper casse due to copper concentrations in excess of 1 mg/L in almost one of every five bottles.

The number of grape and wine samples exceeding the relevant MRL are relatively high considering the small number of samples analysed. The recommended limit for total daily intake of copper for adults is 10-12 mg/day (WHO 1996). Considering that the dietary intake of copper can be increased from the regular consumption of certain foods, such as shellfish, organ meats (e.g., liver and kidney), legumes, nuts and drinking water, the total daily intake of copper could represent a health risk. The number of copper applications made at the 16 farms studied ranged from 1 to 11 events. Most commonly, between 2 and 6 applications were made. The correlation between the number of applications and copper residues in grape is very weak (r^2= 0.16). Upon grouping the data by number of applications (1-2 (n=6), 3 (n=14), 4 (n=8) and 5-11 (n=10) applications) a statistical difference was found (Kruskal-Wallis; p= 0.006) between the group in which 4 applications were made and those groups with lower and higher numbers of applications, further confirming the weak correlation. Of the five data-points that exceeded the MRL, two were samples from farms where 4 applications were made, and three from farms where 5-11 applications were made (figure 1 a & b). Perhaps, rather than the number of applications, the total dose applied and the time at which the applications are made may better describe copper residue levels on grape. If, for example, five applications with a high total dose are made at the beginning of the growth period, the likelihood of the copper being washed off in the period between application and harvest is greater than for three applications at a lower dose made at the end of the growing season, close to harvest. Copper residue level in wine did not appear to be affected by the number of applications. In a similar study, no positive correlation was found between copper residues in wine and the number of applications made (Taccheo Barbina 1992).

[insert Figure 1]

A further factor that can affect copper content in grape is the total dose applied during the vegetative period. For our data set, correlating total applied dose and copper residue level in grape gave a correlation coefficient, r^2, of 0.26. Upon grouping the same data into six groups based on application rate (0-2000 g/ha (n=6), 2000-3000 g/ha (n=9), 3000-4000 g/ha (n=8), 4000-5000 g/ha (n=9), 5000-8000 g/ha (n=5)) a significant difference was observed between the 3000-4000 g/ha and 4000-5000 g/ha groups (Kruskal-Wallis; p= 0.000). One of the data-points exceeding the MRL was from the 2000-3000g/ha group, while two were in the 4000-

5000g/ha group and two in the 5000-8000 g/ha group (figure 2 a & b). For wine samples, no relationship was observed. The average concentration of copper in wine samples does not depend on the quantity applied in the vineyard. During the wine making process, copper concentrations decrease by between 84% and 98% due to yeast consumption (Tromp and Klerk 1988) or precipitation as copper-sulphides (Amati 1984). Similar results were obtained by Rodríguez et al. (1999). The authors show a clear influence of the fermentation processes on metallic cation content. The copper concentration fell by 96% during the winemaking process.

[insert Figure 2]

The importance of time in reducing levels of plant protection products on crops is firmly established. To reflect this, safety times after which application is not permitted are defined for each plant protection product, and are often based on the degradability of the compound. This limit is intended to guarantee a safe level of plant protection products in crops. The safety time for copper and copper-products is 20 days. Since copper does not degrade like organic plant protection compounds, this safety time is considered sufficient for copper concentrations to decrease to an acceptable level by wash-off. Correlation between the time interval between the last application and harvest and the copper residue level in grape gave a r^2 value of 0.27. Most samples had a safety time limit of 20-60 days, and a small group of samples had a 112 day safety limit (n = 8). The data were grouped into four categories, based on the safety time limit (20-30 days (n = 8), 31-40 days (n = 9), 41-60 days (n = 13) and 112 days (n = 8)). A significant difference was observed between the 112 day and the 20-30 and 31-40 day safety time limit groups (Kruskal-Wallis; p = 0,000). No difference was observed between the wine samples. Of the three grape samples exceeding the MRL, one each was from the 20-30 day, 31-40 day and 41-60 day safety time limit groups (figure 3 a & b). The average values (average ± stdev) for the concentrations of the groups are, 18.6 ± 10.7; 10.5 ± 6.3; 7.8 ± 5.6; 3.2 ± 1.4 mg/kg for the 20-30 day, 31-40 day, 41-60 day and 112 day groups respectively. The average residue concentration for the 20-30 day safety limit group is close to the MRL of 20 mg/kg, with an upper 95% confidence limit as high as 29.3 mg/kg. By contrast, the 31-40 day group has a lower residue level with an upper 95% confidence limit of 16.8 mg/kg (i.e., below the MRL). This dataset indicated that a period of 20 days may not be a sufficient safety time for copper. Rather, the data suggest that a safety time of at least double the current recommendation is needed, recalling that only residues in the 112 day group were statistically lower than those in the 20-30 day and 31-40 day groups. However, it is acknowledged that the grape samples in this study were collected in 2003 which was a particularly dry year. Precipitation was lower than the annual average, especially in the northern regions. Had the study had been performed in a more normal year with regards to precipitation, then the copper residue levels may have been lower, and may have been below the MRL in the 20 day group. In light of the unusual climatic conditions in the study year, the findings of this study may be considered a worst case scenario. Thus, a safety limit of 40 or 50 days could be adequate, even for very dry years.

[insert Figure 3]

Multiple regression analysis of the three factors considered revealed that the combination of time between last application and harvest, and total dose, best explain the residual level of copper in grape with a correlation coefficient (r^2) of 0.44. Including the number of applications in the regression does not increase correlation coefficient. The low correlation illustrates that predicting copper residue levels on grape is complicated. Many additional factors not considered here may be influential, for example, meteorological conditions and application practices.

In the study presented here, we have observed that the number of applications, the total dose and time between last treatment and harvest to some extent influence the final copper concentration in grape. These aspects all form part of the management regime implemented by the farmer, and may differ depending upon the pest management strategy used. In Italy, integrated, conventional and organic farms differ from each other with regards to regulations and guidelines which, among other things, govern the use of pesticides. Organic farms only use sulphur and copper, while integrated farms follow a list of recommended pesticides proposed by the authorities. Conventional farms can follow the minimal requirements posed by law. One could perhaps hypothesise that in organic farms larger quantities of copper are used as copper is the only permitted control for fungal disease, and this may result in higher residual levels of copper. The mean copper residue values and standard deviation in grape and wine for conventional farms were 10.62 ± 12.44 mg/kg (n=7) and 1.17 ± 1.34 mg/L (n=7). The values for integrated farms were 11.84 ± 7.36 mg/kg (n=21) for grape and 0.76 ± 0.39 mg/L (n = 19) for wine. Grape and wine from organic farms had copper residue levels of 4.70 ± 4.34 mg/kg (n= 10) and 0.70 ± 0.55 mg/L (n= 8) respectively. No significant difference in copper levels for grape and wine were observed between the different pest management strategies (figure 4). It is important to note that in our study there exists an imbalance in the number of samples analysed for each pest management type and the number of farms that utilise each pest management strategy. For this reason, a larger study focusing on this aspect could better clarify the impact of pest management strategies on copper residue levels on grape. However, considering that the limit for copper application (8 kg/ha/year) is general, regardless of the pest management strategy, and considering that the total copper applied varied widely between strategies (table 1), it is not unreasonable to expect that the residual levels would not differ between farm management types.

[insert Figure 4]

Conclusion

In the work presented here, the influence of the number of applications, total applied dose and time between the last application and harvest on copper residue levels in grape and wine were studied. The two most influential factors were the total amount of copper applied and the time between the last application and harvest which together explain 44% of the regression. This is a weak correlation, and additional factors not investigated here, such as meteorology and application methods, may also influence residue levels. In order to decrease the probability of copper residue levels being close to the MRL under meteorological conditions similar to those prevalent during the experiment, (i.e., dry conditions representing a worst case scenario), the recommended time between the last application and harvest is 40-50 days. Furthermore, the copper content in grape and wine did not appear to be dependent on the pest management strategy of the farm. Standard deviations have high values suggesting heterogeneity among the practices considered.. It is therefore important to investigate alternative methods to decrease the application of cupric products. Perhaps the most important factor influencing the amount of copper used and, ultimately, the level of residue is, that applications are made depending upon the prediction of a disease outbreak rather than the pest management strategy in place. With regards to the risk to human health from exposure to copper, 13% of the grape samples and 18% (i.e., one in five) of the wine bottles sampled exceeded the MRL. It is therefore important to regularly monitor copper residue levels in grape for wine-making as they may also be consumed as grape juice or unprocessed grape.

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Table 1.

Farms and location	Pest Strategy	Grape samples	Wine samples	No. Applications	DLA	Copper applied (kg/Ha)	Rainfall (mm/year)
C1 (TN)	Conventional	1	1	4	21	7.8	800
C2 (ER)	Conventional	2	2	2	45	1.6	350
C3 (LO)	Conventional	2	2	4	52	2.8	500
C4 (LO)	Conventional	1	1	1	36	0.5	540
C5 (FV)	Conventional	1	1	6	55	7.4	730
I1 (TN)	Integrated	1	1	7	41	5.5	780
I2 (ER)	Integrated	4	4	6	23	4.9	520
I3 (ER)	Integrated	3	3	3	22	4.2	500
I4 (ER)	Integrated	2	2	5	41	5.5	520
I5 (LO)	Integrated	2	2	4	37	4.7	580
I6 (LO)	Integrated	3	3	3	41	2.7	590
I7 (LO)	Integrated	3	3	2	36	1.1	590
I8 (LO)	Integrated	3	1	4	39	2.7	590
O1 (ER)	Organic	1	1	5	47	2.8	290
O2 (TN)	Organic	1	1	11	41	7.7	870
O3 (LZ)	Organic	8	6	3	112	3.0	250

Locations: TN(Trentino-Alto Adige Region); ER (Emilia-Romagna Region); LO (Lombardia Region); FV (Friuli-Venezia-Giulia Region); LZ (Lazio Region)

DLA: Days elapsed from the last application to the harvest

Figure 1

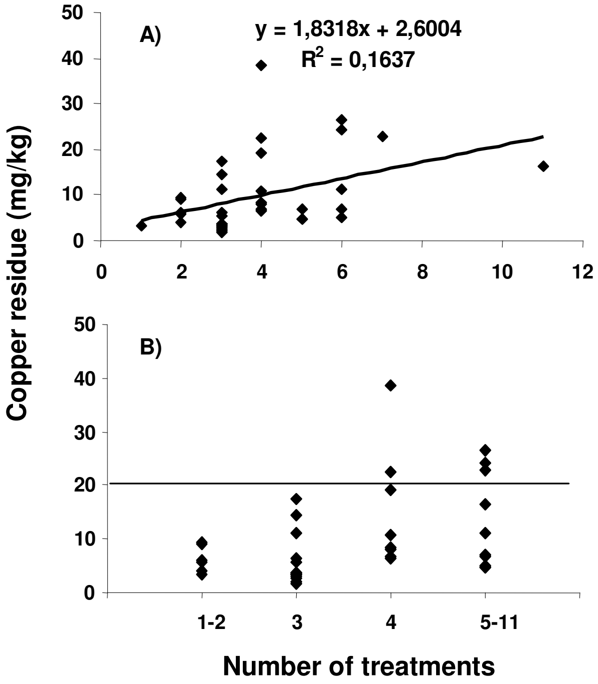
A)
y = 1,8318x + 2,6004
R^2 = 0,1637
B)
Copper residue (mg/kg)
Number of treatments
0
10
20
30
40
50
0
2
4
6
8
10
12
1-2
3
4
5-11

Figure 2

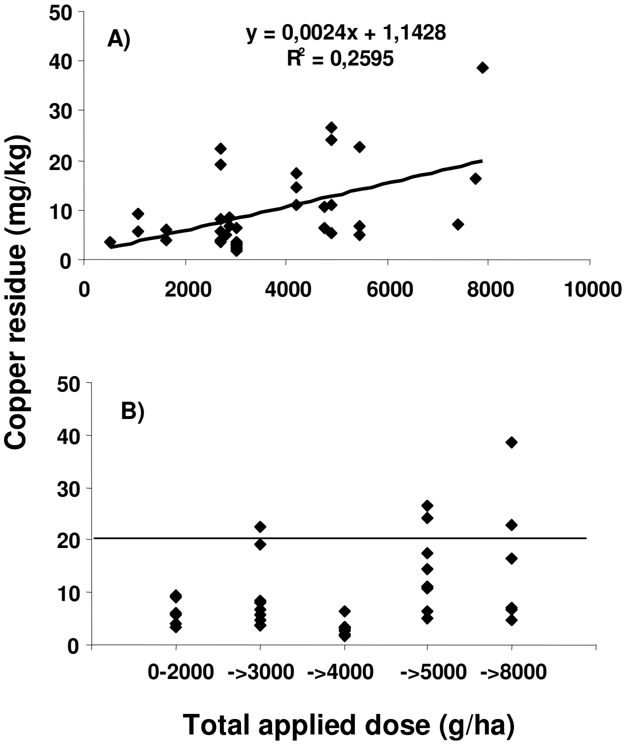
A)
y = 0,0024x + 1,1428
R² = 0,2595
50
40
30
20
10
0
0
2000
4000
6000
8000
10000
Copper residue (mg/kg)
B)
50
40
30
20
10
0
0-2000
->3000
->4000
->5000
->8000
Total applied dose (g/ha)

Figure 3

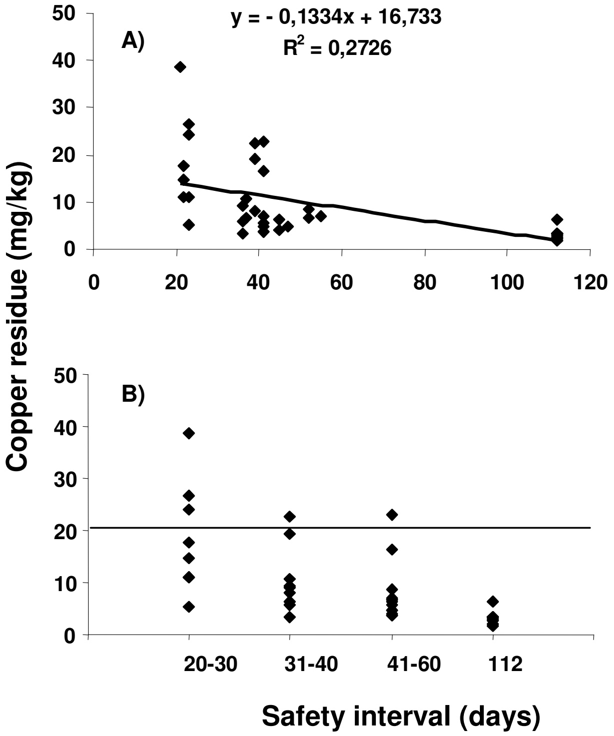
A)
y = - 0,1334x + 16,733
R² = 0,2726
50
40
30
20
10
0
0
20
40
60
80
100
120
B)
50
40
30
20
10
0
20-30
31-40
41-60
112
Copper residue (mg/kg)
Safety interval (days)

Figure 4

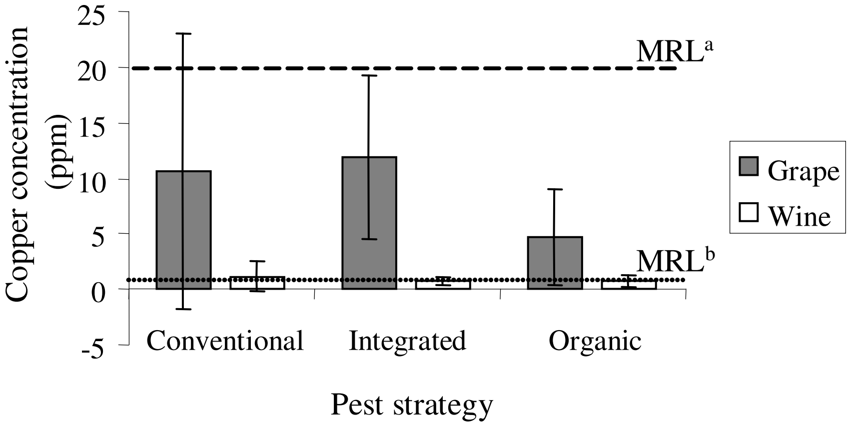
25
20
15
10
5
0
-5
Copper concentration (ppm)
MRL^a
MRL^b
Grape
Wine
Conventional
Integrated
Organic
Pest strategy

Table 1

Main characteristics of the studied data-set.

Figure 1

Copper residue level in grape (mg/kg) for different number of treatments, A) presented with the linear regression equation and correlation coefficient (R^2) and B) in groups. The line at 20 mg/kg indicates the MRL.

Figure 2

Copper residue level in grape (mg/kg) for different total applied doses, A) presented with the linear regression equation and correlation coefficient (R^2) and B) in groups. The line at 20 mg/kg indicates the MRL.

Figure 3

Copper residue level in grape (mg/kg) for different safety intervals between last application and harvest, A) presented with the linear regression equation and correlation coefficient (R^2) and B) in groups. The line at 20 mg/kg indicates the MRL.

Figure 4

Average copper concentration in grape (mg/kg) and wine (mg/l) for different pest strategies. Error bars signify Standard Deviation. Maximum Residual Level, MRL[a] (limit by European Community) and MRL[b] (used by Italian authorities), for grape and wine respectively.