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Effects of soil characteristics and leaf thinning on micronutrient uptake and redistribution in 'Cabernet Sauvignon'

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Summary

This study investigated the uptake and distribution in grape tissues of the micronutrients copper (Cu), zinc (Zn) and manganese (Mn), in a 16 year-old 'Cabernet Sauvignon' vineyard and their relationship with soil characteristics and management. The analysis was carried out in two plots with differences in vigour, grown in a calcareous soil. Two different management treatments (with and without leaf thinning after bloom) were applied in each plot. Partitioning and distribution of micronutrients (Cu, Zn and Mn) in petiole, seeds skin and flesh were evaluated from veraison to harvest. The relationship between micronutrients and grape parameters such as pH, total acidity and berry weight were also evaluated. Differences in nutrient concentration were found between the areas with differences in vigour, but concentration in petiole did not present good correlation with the soil fraction extracted with $CaCl_2$+DTPA+Trietanolamine. The results showed that micronutrient concentrations varied in a different way between organs during ripening. Cu and Zn in petioles had higher concentrations at veraison than at harvest, while for Mn the concentrations were higher at the end of the cycle. Zn and Mn were within the acceptable levels, while Cu levels were above them. Mn and Zn were mainly concentrated in seeds and skins. Cu and Mn concentrations in petiole, skins and seeds were higher in the leaf tinning treatment, but the results were opposite for Zn. Zn and Cu in flesh increased with berry weight while Mn decreased. Acidity and pH affected Zn in skins and flesh and Mn in seeds.

K e y w o r d s : grape quality parameters; grape tissues; nutrients (Cu, Zn, Mn); plant vigour; soil metal extractable fraction.

Introduction

Soil characteristics, such as pH, redox potential, texture and organic matter, condition the partition and availability and uptake of minerals from the soil by the grapevine (Pradubsuk and Davenport 2011). The form in which trace elements can be associated with various soil components (organic matter, clays, Fe and Mn oxides and carbonate or silicate minerals) differs among metals and it conditions their release or fixation to soil (Tessier *et al.* 1979). Cu is mainly associated with the organic matter while Zn and Mn may be found mainly bound to oxides (Ramos 2006, Chopin *et al.* 2008). The availability of micronutrients in calcareous soils, in most cases with high pH, is usually limited (Peralta-Videa *et al*. 2002).

Some of these elements are in soils as a result of pesticides and fertilizers' application or other additional practice during the growing season and they may increase the amounts of these metals in wines (Mirlean *et al.* 2007, Ibañez *et al.* 2008, Ruyters *et al.* 2013). These nutrients are mainly taken up by grapevines from the soil and absorbed during bloom and veraison, and redistributed and stored within the plant (Pradubsuk *and* Davenport 2011). However, rootstocks, variety and management also affect metal content in the plant (Wooldridge *et al.* 2010, Likar *et al.* 2015). Several studies carried out in different cultivars reported that the uptake of micronutrients such as Fe, Mn, B, Zn, and Cu presents high variability from vine to vine (Schreiner *et al.* 2006) and that they have different mobility depending on each specific metal and on plant nutritional status and environmental conditions (Marschner 2002, Vystavna *et al.* 2015). In addition, the uptake of soil metals may be also dependent on rootstock and variety (Cugnetto *et al.* 2014).

Each micronutrient has its function within the plant and in many cases deficiencies are the problem more than an excess, although high concentrations could also produce toxicity in some cases. Cu, Mn and Zn are considered essential to plants and play important roles in regulating plant growth and development. They are involved in many metabolic and cellular functions (Hänsch and Mendel 2009) and they influence fruit yield and fruit quality, as they may determine factors such as Baumé/Brix, pH, total acidity and phenolic content as well as the distinctive but difficult-to-quantify flavors of the wine (Mackenzie and Christy 2005). Cu is used in several enzymes and proteins involved in oxidation and reduction (Salisbury and Ross 1992) and it is also involved in the lignification or hardening of canes and shoots. Plants need very small amount of Cu and generally Cu levels in vineyards are usually high due to its use as fungicide. Deficiency symptoms in vineyards occur as dark green and twisted leaves. Zn is involved in the production and functioning of many enzymes as well as many growth

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hormones (Salisbury and Ross 1992). It is important for cell division, especially at fruit set and improves the retention of bunches onto the branches although available Zn levels over 15 mg kg^{-1} turn out to be phytotoxic. On the other hand, deficiencies in Zn levels (< 0.8 mg·kg^{-1}) also affect growth. They produce irregular growth of young leaves and can cause poor fruit set, although the effect varies widely on grape varieties and rootstocks. Deficiency symptoms can appear in sandy soils and in high pH soils. Mn is important in the photosynthetic split of water and also as an activator of many enzymes (Salisbury and Ross 1992). It is readily taken and transported from the roots to the shoots. This is probably the main reason why Mn is classified as less toxic to roots compared to other metals, and why the toxicity symptoms first occur on shoots. Under excessively high Mn concentration alterations in shoots could appear (Graham *et al.* 1988). On the other hand, deficiencies in Mn (available Mn levels below 1 mg·kg^{-1}, Marcet *et al.* 2003) are seen as interveinal chlorosis of younger and older vines. The deficiency symptoms are more likely to occur on alkaline, sandy soils high in organic matter or on limey soils that are deficient in Mn (Pearson and Goheen 1988).

The objective of this research was to investigate the influence of soil characteristics on the uptake of Cu, Zn and Mn and their distribution in grape tissues as well as the effect of leaf thinning (LT) on final metal concentrations. The relationship between micronutrient concentrations and grape quality parameters such as pH, total acidity, total soluble solids and berry weight were also evaluated. The research was carried out in the variety 'Cabernet Sauvignon' cultivated in calcareous soils in a continental Mediterranean climate.

Material and Methods

Study area: The study was conducted in two vineyard fields (P1 and P2) located in Raimat (Costers del Segre Designation of Origin), Lleida, NE Spain (290735.572 E, 4619350.362 N, 320 m, UTM 31 T). This is a semi-arid area with continental Mediterranean climate. Mean annual maximum and minimum temperatures are 20.8 ± 0.9 and 8.8 ± 0.6 °C, respectively. Mean annual precipitation is 362 ± 95 mm, with about 65 % recorded during the growing season. Soils in these fields are classified as *Calcic Haploxerepts* and *Typic Haploxerepts*. Both vineyards are on gentle slopes, ranging between 10 and 15 %. The vineyard plots, which were planted in 1997, consisted of trained vines with a space of 2 m between plants and 3 m between rows. Vine rows were orientated north-northwest/east-southeast in both plots in slopes that ranged between 3 and 5.5 %. Vines followed a unilateral single cordon with vertical shoot positioning and with three vertical catch wires. In both fields, the rootstock was SO4. Herbaceous ground cover between the rows was maintained during the growing cycle. Both plots had sprinkle irrigation and similar fertilization and irrigation doses. In each plot, LT was carried out in alternated rows 20 d after fruit set. Approximately half of the basal leafs were removed on the northeastern side. The differences in vigour between both plots (P1 and P2) were confirmed with the NDVI map corresponded to the analysed year obtained from ICC (Institut Cartografic de Catalonia). In P1, the NDVI ranged between 0.4 and 0.6 whereas in P2, NDVI values ranged between 0.2 and 0.4.

Soil sampling and analysis: Soil samples were taken in each plot at different points from 0-20 cm, 20-40 cm, 40-60 cm and 60-80 cm, depths which corresponded to boundaries of soil horizons (Ap (0-20 cm), Bw (20-61cm), Bw1 (61-80cm)). Three composed samples were obtained in each plot. Each sample was homogenized, air dried and sieved through a 2 mm mesh. Soil properties such as pH, organic matter content, electrical conductivity, carbonate content and soil particle distribution (van Reeuwijk 2002) were analysed. In addition, some micronutrients (Cu, Zn and Mn) were analysed in the soil. The available fraction, extracted with $CaCl_2$ 0.01M+ DTPA 0.1M + Trietanolamine 0.1M at pH = 7.3 (DTPAE content), as well as the aqua regia extractable content (ARE content) (extracted with HNO_3 65 % Merck + 37 % HCl) were analysed by Atomic Absorption Spectroscopy.

Plant sampling and analysis: Petioles were sampled from early veraison to harvest (from July, 30th to October 10th) in both plots every 15 d. In each plot, samples were taken in 50 vines within 6 rows, including rows with and without LT. Between 25-50 petioles were collected at each sampling from leaves opposite to basal cluster in the first sampling time and then from the most recently developed mature leaf, following the recommendation of OIV(1996). Additionally, berries were sampled at the same dates, from the central and lower part of the clusters, according to the criteria proposed by Jordan and Crosser (1983). In order to have a good representativity, about 600 berries were sampled at each date from each treatment.

Leaves were cleaned with distilled water and dried at 70 °C for 48 h and stored in plastic containers until analysis. From berries, grape skins and seeds were separated and dried (70 °C, 48 h). All tissues were finely ground and digested with nitric acid and hydrogen peroxide in a closed vessel microwave digestion system (Touch control equipment, Rotor: SK-12T), with temperature control. For each analysis, 0.5 g of dry mass was digested with 8 mL HNO_3 + 1 mL H_2O_2. One blank was prepared with the same conditions. The extracts were diluted to 25 mL and used for the nutrient analysis using Absorption Atomic Spectrometer.

In addition, berry weight, total acidity (titration with NaOH, 0.1N with phenolftalein as indicator), sugar concentration (°Brix- measured by refractometry) and pH were analysed in the sampling day following the methods proposed by the OIV (OIV 2012).

Data analysis: Micronutrient concentrations were individually determined for each vineyard, tissue, and sampling time. Differences between micronutrient in soil and in different grape tissues, between plots and treatments, were analysed for the whole pooled data using Fisher's LSD test. In addition the relationship between each micronutrient concentration in different tissues with other grape parameters analysed at ripening were analysed using Principal component analysis (PCA). All statistical analyses were carried out using Statgraphics.

Results

The mean maximum and minimum temperature during the growing cycle were 26.2 and 15 °C, respectively, with 50 d in which maximum temperatures > 30 °C were recorded. Most of them took place in veraison or at the beginning of the ripening period. Precipitation during the growing period was 183.2 mm, most of them recorded before veraison. Due to these climatic conditions ripening was very irregular and harvest was extended more than usual in the area.

Soil characteristics: The main difference among both plots was referred to texture and organic matter content. Regarding soil texture, the soils were silty-loam and loam, respectively in P1 and P2, with higher clay and sand contents in P2 than in P1, and high silt content in P1. The organic matter content ranged between 0.9 and 1.8 % in P1 and between 1.1 and 2.8 % in P2. The pH values ranged between 8.4 and 8.65, without differences between both plots. The electrical conductivity ranged between 0.24 and 0.26 dS·m^{-1} in P1 and between 0.23 and 0.33 dS·m^{-1} in P2. The measured EC values in the extract 1:5 imply slightly saline soils, which may affect own-rooted vines. The values of calcium carbonate equivalent (CCeq) averaged at 35 ± 0.77 % in P1 and 45 in P2, and it was slightly greater in the top layer of the soil than in subsoil in P1 but opposite in P2. Mean soil characteristics of the analysed soils are shown in Tab. 1.

Soil micronutrient content extracted with aqua regia and available fraction extracted with $CaCl_2$+DTPA+trietanolamine: The ARE and the DTPAE contents of the analysed nutrients are shown in Tab. 2. The Cu ARE content was slightly greater in the whole profile in P2 than in P1. For Zn and Mn, the ARE contents were slightly greater in P1 than in P2. For the three elements, greater levels were observed on the surface and they decreased progressively with depth.

The DTPAE content also varied with depth and represented different fractions from the total content for the three micronutrients. For Cu, the DTPAE content was higher in the upper 40 cm (up to 9.7 mg·kg^{-1}) than in deeper layers (up to 1.1 mg·kg^{-1}). These concentrations represented between 25.1 and 18.5 % of total Cu in the soil surface and between 4.1 and 5.7 % in deeper layers, respectively in both plots. For Zn, the DTPAE content was slightly higher in P1 than in P2 on the surface, although the levels were similar in both plots in deeper layers. These fractions represented low percentages of the Zn ARE content, ranging between 6.3 and 4.4 % for the soil surface and less than 3 % for deeper layers. Similarly, the DTPAE contents of Mn were higher on the surface (up to 19.2 mg·kg^{-1}) than in deeper layers (up to 6.2 mg·kg^{-1}), which represented very low percentage of the ARE contents (between 4.3 % and 5.8 % on average in the profile respectively in P1 and P2).

Micronutrient concentration in petiole: Fig. 1 shows the evolution of Cu, Zn and Mn concentration in petiole for both plots and for both treatments (with and without LT). Petiole Cu ranged between about 20 and 65 mg·kg^{-1} (dry matter) in P1 and between 15 and

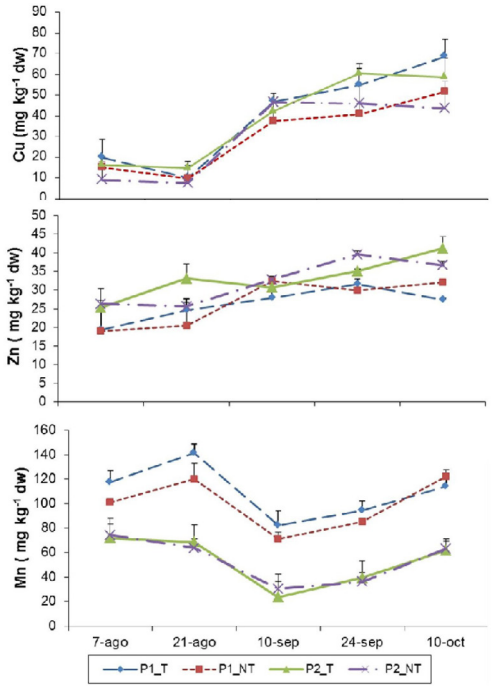

Fig. 1: Cu, Zn and Mn concentrations in petioles during ripening in both plots (P1 and P2) and treatments (T: with leaf thinning; NT; without leaf thinning).

Table 1

Mean soil characteristics of the study plots

Variable	Plot	Soil depth			
		0-20 cm	20-40 cm	40-60 cm	60-80 cm
pH	P1	8.46±0.09	8.39±0.22	8.65±0.09	8.56±0.09
	P2	8.41±0.09	8.42±0.06	8.40±0.06	8.41±0.05
CE (d·m^{-1})	P1	0.24±0.05	0.26 ±0.04	0.25±0.02	0.26±0.03
	P2	0.23±0.04	0.28±0.04	0.33 ±0.09	0.29 ±0.08
OM (%)	P1	1.8±0.2	1.2±0.15	0.9±0.1	0.8±0.3
	P2	2.8±0.5	2.1±0.7	1.2±0.4	1.1±0.2
$CaCO_3$ (g·100 g^{-1})	P1	35.0±2.5	36.0±1.5	33.0±2.5	33.4±2.5
	P2	37.0±2.5	41±1.5	58.0±2.5	35.4±2.5
		Texture (%)			
P1	Clay	17.0±1.0	11.0±0.8	13.8±1.5	12±3
	silt	56.4±2.0	61.6±1.9	53.5±2.0	55.0±3.0
	sand	26.5±2.0	27.3±2.3	32.6±2.5	30.0±2.1
P2	clay	25.8±1.3	25.9±0.8	22.3±1.5	21.2±3.0
	silt	32.3±3.0	32.5±1.9	34.4±2.0	33.2±2.2
	sand	41.8±2.1	41.7±1.9	43.3±1.8	40.2±2.4

Table 2

Mean aqua regia acid extractable and DTPAE metal content in the analysed soils at different soil depths in each plot

Nutrient	plot	Soil depth 0-20 cm	Soil depth 20-40 cm	Soil depth 40-60 cm	Soil depth 60-80 cm
Aqua regia acid extractable soil metal content					
Cu (mg·kg^{-1})	P1	37.6±0.4	27.6±0.9	22.1±1.5	20.0±1.9
	P2	52.7±1.2	41.0±0.5	36.1±0.6	29.0±0.5
Zn (mg·kg^{-1})	P1	60.6±0.8	56.2±1.5	46.2±2.3	23.6±1.3
	P2	58.2±1.2	39.3±0.4	26.2±0.9	23.6±1.9
Mn (mg·kg^{-1})	P1	346±20	320±23	250±21	222±25
	P2	280±15	274±7.6	255±15	112±20
CaCl2+DTPA+TE extractable soil metal content					
Cu (mg·kg^{-1})	P1	9.2±0.19	5.0±0.9	1.7±0.09	1.1±0.09
	P2	9.7±0.15	6.2±0.5	4.8±0.06	2.1±0.05
Zn (mg·kg^{-1})	P1	3.8±0.8	1.3±0.5	0.8±0.2	0.4±0.2
	P2	2.6±1.2	1.2±0.4	0.6±0.09	0.4±0.1
Mn (mg·kg^{-1})	P1	16.1±2	13.7±2.3	12.5±0.1	7.4±0.3
	P2	19.2±2.1	14.4±3.0	13.7±1.5	6.2±0.2

55 mg·kg^{-1} in P2. Significantly higher values were found in the plot under the LT treatment for 4 of the 5 samplings. Cu concentration was smaller at the beginning of veraison than at harvesting.

Mn concentrations in petiole were also greater in P1 than in P2, with values that ranged between 71 and 103 mg·kg^{-1}, and between 23.5 and 74.2 mg·kg^{-1}, respectively. Despite the differences in levels, concentrations were lower in the middle of the analysed period (from early veraison to harvest), with similar pattern in both plots. In P1, Mn levels in petiole were greater in the LT treatment in four of the five samplings. However, there was not significant effect of LT in P2.

Zn concentrations in petiole, however, were greater in P2 than in P1, with values that ranged between 19 and 32 mg·kg^{-1}, and between 25 and 41 mg·kg^{-1}, respectively in P1 and P2. In both plots, Zn levels were increasing from early veraison during ripening, but Zn levels in the last sampling decreased in both plots. No clear effect of LT was observed in any plot.

Cu and Mn concentrations in petiole correlated with total soil content, but poor correlations were found with the extractable fraction. However, Zn concentration was correlated with the extractable fraction but poorly correlated with the total soil content. The Pearson correlation coefficients between metal in petiole and total soil metal content were $R^2 = 0.67$ and 0.30, respectively for Mn and Cu; and 0.64 for Zn between petiole concentration and the soil extractable fraction.

Redistribution of metals within the berry: seed, skin and flesh micronutrient levels: Micronutrients in grapes were mainly concentrated in skins and seeds. The analysis of each micronutrient in these two tissues along the ripening period confirmed some differences between plots and treatments, which were not uniform for the three elements. Micronutrient concentration in flesh decreased during ripening, being Zn the micronutrient that recorded the lowest concentrations. The average values for each micronutrient are shown in Tab. 3.

Cu levels in seeds and skins increased during ripening and were greater in the most vigourous vines (P1) and without significant differences between treatments. At ripening, the average values were 11.9 *vs*. 9.8 mg·kg^{-1} in P1 and 6.6 *vs.*7.4 mg·kg^{-1} in P2. Cu concentrations in skins also increased during ripening, reaching greater values in P2 than in P1. Cu levels in skins at the end of ripening were higher in the non LT treatment in both plots (17.9 *vs.* 14.6 mg·kg^{-1} in P1 and 23.5 *vs.* 22.3 mg·kg^{-1} in P2). Cu concentrations in flesh decreased during ripening without significant differences between plots. Zn concentrations in seeds increased during ripening. There were no differences between plots but slightly greater values were observed in the LT treatment (12.8 *vs.*10.8 mg·kg^{-1} in P1 and 12.0 *vs*. 10.07 mg·kg^{-1} in P2). Similarly, Zn concentrations in skins increased during ripening reaching greater values in P2 than in P1 at the end of ripening. Zn concentrations in skins were higher in the LT treatment in both plots (19.55 *vs.* 17.62 mg·kg^{-1} in P1 and 21.35 *vs.* 19.75 mg·kg^{-1} in P2). In flesh, Zn concentrations were slightly lower in P1 than in P2 and higher in the LT treatment in both plots (3.13 *vs* 2.35 mg·kg^{-1}in P1 and 3.98 *vs* 2.73 mg·kg^{-1} in P2).

For Mn, both the concentration in skins and seeds increased during ripening but the greatest increase was observed in seeds. Mn concentrations in seeds were higher in P1 than in P2 and also higher in the LT treatment in both plots (36.85 *vs* 31.07 mg·kg^{-1} in P1 and 27.99 *vs.* 25.59 mg·kg^{-1}

Table 3

Mean values of Cu, Zn and Mn in seed and skins at veraison and mid and end ripening in each plot (P1 and P2) and treatment (T: with leaf thinning; NT; without leaf thinning)

Micronutrient	Plot	Seed			Skin			Flesh		
		Aug-7th	Sep-10th	Oct-10th	Aug-7th	Sep-10th	Oct-10th	Aug-7th	Sep-10th	Oct-10th
Cu	P1T	6.18	12.62	11.94	6.18	13.76	14.58	14.08	8.50	6.86
(mg·kg^{-1})	P1NT	3.60	9.97	9.84	3.60	14.27	17.95	15.36	6.20	12.13
	P2T	13.11	16.25	6.59	13.11	21.99	22.37	8.19	13.39	6.31
	P2NT	9.69	11.15	7.45	9.69	22.56	23.52	9.73	6.92	4.48
Zn	P1T	7.13	10.65	12.85	11.45	12.67	19.55	10.59	6.6	3.13
(mg·kg^{-1})	P1NT	6.39	11.97	10.84	10.21	16.18	17.62	8.20	5.6	2.35
	P2T	7.04	10.56	11.05	12.03	15.11	21.35	11.90	8.5	3.98
	P2NT	7.99	10.90	10.20	10.07	15.35	19.75	10.46	7.8	3.73
Mn	P1T	12.65	22.0	36.85	11.45	13.34	13.60	5.98	4.5	1.39
(mg·kg^{-1})	P1NT	17.51	14.31	31.07	10.21	11.01	10.97	5.07	3.9	5.95
	P2T	17.66	25.81	27.99	12.03	13.87	16.59	11.05	6.5	3.72
	P2NT	16.70	23.02	25.59	10.07	10.33	16.52	5.09	4.6	3.63

in P2). Mn concentrations in skins, at the end of ripening, were greater in P2 than in P1. Despite this fact, differences between treatments were only found in the most vigourous vines, in which lower concentration were recorded (final values of 13.6 *vs.* 10.9 mg·kg^{-1} in P1 and 16.5 mg·kg^{-1} in P2). Mn concentrations in flesh showed clear trends in three of the four plots, and values were lower than those in seeds and skins.

Grape quality parameters: The size of the berries increased from about 0.7 g at early veraison to about 1.3 g at ripening, with the highest increase after veraison. LT had a positive effect on the increase of berry weight, which was more evident at the end of ripening in the less vigourous vines (higher in P2 than in P1). However, due to the large variability among samples, the differences were not significant. This fact could be related to the increased nutrient input to the berry. Berry weight is an important parameter in defining the quality of future musts, because the high concentration of grape colour depends on the maturation of the variety and the size of the berries.

The weight of skins by berry increased from about 0.025 g to values greater than 0.09 g in the most vigourous vines (P1) and up to 0.055 g in P2. There were some differences between tretaments in P1, with greater values in the LT treatment, while no differences between treatments were in P2. Skin weights represented between 2.8 and 6.9 % of berry weight at ripenning. The pH values reached at the end of ripening ranged between 3.18 and 3.23, with the lowest values in P1 without thinning, and without differences between both treatments in P2. Total acidity, however, did not show differences between plots and treatments. Total acidity at the end of ripening was 9 g·L^{-1}. The pH values achieved are considered very low in comparison with the optimal values, that range between 3.6 and 3.8. The total soluble solids content at early veraison expressed in °Brix was about 5 °Brix, increasing fast during the following 20 d. After that time period, 18-20 °Brix were reached, which are equivalent to 11 probable alcoholic degrees. It indicated the migration of sugar to the berries. After that moment, the increase was smaller, even lower than the average which produced a delay in the harvest dates. There were differences between plots, with smaller values in P2 than in P1 (Fig. 1) and with slightly higher values in the areas with LT.

Relationship between grape quality parameters and micronutrients: The results of the PCA based on the grape parameters analysed (acidity and pH, °Brix) and micronutrients in seeds, skins and flesh are shown in Fig. 2. The analysis was done for each tissue (skin, seed and flesh). Two components were retained in each case, which represented 60.67 and 22.54 % (for skin), respectively; 68.03 and 21.11 % (for seeds) and 76.1 and 15.12 % (for flesh). The results showed that Mn in seeds and Zn in flesh were positively correlated with berry weight, °Brix and pH and negatively correlated with AcT, while the correlations of Zn in skins and Cu in flesh with the same parameters were opposite. The rest of parameters (Mn levels in flesh and skins, Cu in skins and seeds and Zn in seeds) were not correlated with the grape quality parameters included in the analysis.

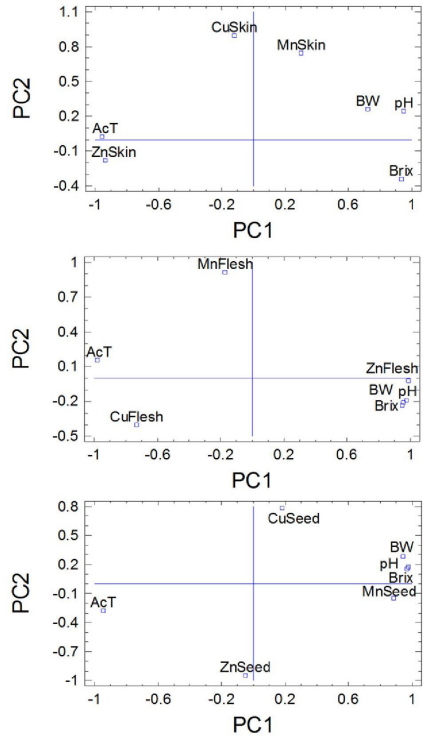

Fig. 2: Results of the Principal component analysis for skin, seed and flesh based on micronutrient concentration in the tissue and grape quality parameters (Brix: sugar concentration expressed in °Brix; BW: berry weight; AcT: tartaric acidity).

Discussion

The main difference among both plots was referred to the organic matter content and the texture, which condition to vigour response of the plants. The organic matter contents in P1 were below the desirable ranges for grapes, established in about 2 % (Gómez-Miguel 2011). The less vigourous vines were cultivated in soils with higher clay and organic matter content, which are two soil characteristics that affect metal fixation to soil. However, in the most vigourous vines, the organic matter contents were below 2 %, indicated by Gómez-Miguel (2011) as the desirable value for grape growing. The high soil pH levels found in the plots of the study could also favour adsorption processes and could justify the low available fraction of the micronutrients analysed. Metal adsorption may occur into positions from which they are not readily displaceable (Barber 1995) and reaction becomes irreversible (Yaron *et al.* 1996). In addition, the moderately high values of calcium carbonate equivalent could also contribute to fix part of the nutrients analysed. Vines are moderately sensible to electrical conductivity and these observed values may cause yield reductions between 15-25 % (Cass *et al.* 1995).

Cu concentration on the soil surface was relatively high in comparison with the levels observed in deeper layers. This may be due to vine treatments carried out for years, which increase soil copper concentration (Toselli *et al.* 2009, Wightwick *et al.* 2010). A great range of Cu values can be found in vineyard soils depending on soil characteristics and management (Angelova *et al.* 1999, Mirlean *et al.* 2007,

Chopin *et al.* 2008, Duplay *et al.* 2014, Likar *et al.* 2015, among others). The available fraction was higher than the value considered as high values (0.2 ppm; Rosen 2008) in all layers but particularly on the surface. It was about 10 times higher in the 20-40 cm layer and more than 25 times in the 0-20 cm layer. The results agree with those found in other vineyards soils (Mirlean *et al.* 2007, Dos Santos *et al.* 2013). Cu is mainly associated with the organic matter. In this respect, the results showed that the ARE fraction of Cu was highly correlated with the organic matter and clay content. The ratio between the DTPA and the ARE metal content in the surface layers (0-40 cm) was lower in the plot with higher organic matter and higher clay content.

Soil Zn and Mn levels were of the same order of magnitude that those found in other viticultural areas (Ramos 2006, Amorós *et al.* 2013, Dos Santos *et al.* 2013), particularly in the upper layers. Nevertheless, these Mn levels are relatively low compared with the average values found in other soils (ranging between 200 and 3,000 $mg \cdot kg^{-1}$, Kalanquin *et al.* 2013).The available fractions represented low percentage of the metal content that may be extracted with aqua regia. However, they can be considered as high levels for vine cultivation (established in 1 ppm for Zn and 6 ppm for Mn; Rosen 2008). The high soil pH may be the main reason of this behaviour. High soil pH decreases the exchangeable fractions and increased the more tightly bound fractions of Cu and Zn, but particularly of Mn (Longbottom 2009). The available fraction for Zn was slightly higher in most layers in the soil with higher clay organic matter content. However, for Mn, this behaviour was only observed in the surface layer (0-20 cm).

Although soil test levels of micronutrients (Mn, Fe, B, Zn, Cu) have not been correlated to grapevine vigour and yield in other studies (Schloemann and Allen 2013), the results observed in these plots showed that Cu and Zn levels in P1, where higher vigour was found, were greater than in P2. However, no significant differences were found for Mn.

Cu levels in petiole at early veraison were of the same order of magnitude than those proposed as suitable values for a correct crop development (optimal ranges proposed by some authors: between 5 and 15 $mg \cdot kg^{-1}$ (Rosen 2008); and between 6 and 11 $mg \cdot kg^{-1}$ considered optimum value in California and Australia (Gómez-Miguel and Sotés 2014)). However, at the end the ripening period, the observed values were much higher than the optimum, being higher in the most vigourous vines. Nevertheless, the values were similar to that observed in other studies (Benito *et al.* 2013*)*. LT did not affect in similar way to both plots. While the highest Cu levels in the most vigourous vines were found in the LT treatment, in the plot with less vigour the results were opposite at the end of ripening. The ratio between Cu concentration in petiole and in the soil was greater than 1 in both plots, but it was higher in P1 than in P2. Higher ratios have been observed in other viticultural areas (Huzum *et al.* 2013, Amorós *et al.* 2013).

For Zn, the levels reached in petiole were within the acceptable interval (30-50 $mg \cdot kg^{-1}$ proposed by Rosen (2008) and 26 $mg \cdot kg^{-1}$ adopted as suitable values in California and Australia). Similar values were found in other studies (Chopin *et al.* 2008, Duplay *et al.* 2014, Benito *et al.* 2013). The greater levels in petiole in P2 (less vigourous vines) than in P1 were in agreement with the greater proportion of Zn extracted from the soil profile at depths in which maximum root development exist. Greater Zn availability for depths > 20 cm was observed, although the greatest levels were on top of the surface. No clear effect of LT was observed in any plot. The ratio between Zn concentration in petiole and in the soil ranged between 0.5 and 0.7, which agree with the ratios found by other authors (Amorós *et al.* 2013).

Mn levels in petiole reached values slightly higher than the proposed as optimum for California and Australia (Gómez-Miguel and Sotés 2014) but within the acceptable interval (30-150 $mg \cdot kg^{-1}$) proposed by Rosen (2008). The highest values were found in the most vigourous vines. The levels reached were smaller than the ones found by Benito *et al.* (2013). Mn content evolution during the study period differed from the expected trend indicated by Pradubsuk and Davenport (2011) and Colugnati *et al.* (1995). These authors indicated that Mn content increased during the vegetative cycle, then remained steady before veraison, and then progressively increased from veraison to fruit maturity. However, Benito *et al.* (2013) showed that Mn in petiole increased during the first stages, but during ripening presented oscillations around the steady value reached in the previous stages or even decreased, while Mn in leaves increased. In the case of study, the climatic conditions experienced during the analysed cycle could have contributed to the stop or decrease of the uptake at mid ripening, due to the dry and hot recorded conditions. The ratio between Mn concentration in petiole and in soil ranged between 0.26 and 0.29. This low value is similar to that indicated by Amorós *et al.* (2013), and in agreement with the low availability on Mn in those soils due to the high pH.

The micronutrient levels observed in petiole, which in most cases were above the recommended levels, indicated that these metals were extracted by the plant in a significant way. However, in that conditions the extractable fraction obtained using DPTA+$CaCl_2$+TEA did not correlate with that measured in petiole for all these micronutrients.

Regarding the micronutrient concentrations in grape tissues, Cu, Zn and Mn were mainly accumulated in seeds and skins and the lowest metal content was established in the berry flesh. These results agree with that found by Angelova *et al.* (1999). However, the levels recorded in the case of study in all plots were higher than those found for Cu and Zn in that study (4 and 4.8 $mg \cdot kg^{-1}$ in skins; 11.3 and 16.6 $mg \cdot kg^{-1}$ in seeds, and 0.9 and 1.01 $mg \cdot kg^{-1}$ in pulp, respectively for Cu and Zn). Yang *et al.* (2010) in a study with 'Cabernet Sauvignon', however, found higher concentrations in seeds than in skins and flesh for the three elements, although Cu concentrations in flesh was greater than Zn and Mn. Metal concentrations in seeds and skins increased during ripening while concentration in flesh decreased. The increase was more pronounced for Cu and Zn in skins and for Mn in seeds. Mn accumulation in seeds and Zn accumulation in skins agree with the results found by Rogiers *et al.* (2006) in 'Syrah'. The same authors also indicated that Cu accumulated throughout berry growth and ripening and that it was mainly accumulated in flesh. In the case of study, despite Cu was the micronutrient that showed

greater concentration in flesh, the trend during ripening was to decrease. Regarding Mn and Zn distribution in grapes, Bertoldi *et al*. (2011), in a study carried out in 'Chardonnay', indicated that Zn and Mn were mainly accumulated prior to veraison, and Hradzina *et al.* (1984) also confirmed that Mn only increased during growth. They linked this result to the fact that the xylem contribution to berry growth diminished after veraison. The accumulation of Zn in skins and of Mn in seeds during the whole ripening period was affected by LT. However, for Cu and Mn in skins there were only differences during the first stages after verasion, but the effect disappeared at the end of ripening. The ratios between metal concentration in the tissue and in soil differed between metals and tissues. At ripening, the ratio for Cu ranged between 0.18 and 0.45 for seeds; between 0.55 and 0.67 for skins and between 0.12 and 0.45 for flesh, with higher values in P1 than in P2 for seeds and flesh. For Zn, the ratios varied between 0.07 and 0.3 for seeds; between 0.12 and 0.58 for skins and between 0.02 and 0.11 in flesh, with higher values in P2, but without differences between treatments. For Mn, the ratios were similar in both plots: about 0.12 for seeds, 0.05 for skins and 0.02 for flesh, without differences between treatments. The average translocation ratios for each element were of the same order of magnitude as the ratio grape/soil indicated by Vystavna *et al.* (2015) in other varieties for Mn and Cu but much lower for Zn.

The effect of leaf thinning on skin weight was in agreement with the expected results. Leaf removal favoured the increase of total soluble solids in agreement with the practice of the shading of clusters used to reduce the graduation in power and maintain a high pH in grape must and wine. Nevertheless, it is difficult to separate the study of the effects of exposure to light and temperature. For example, Di Profio *et al.* (2011) detected in a study of handling multi-farming techniques a significant increase in the concentration of sugars by reducing the basal leaves in 'Cabernet Sauvignon' in only one of three campaigns studied. LT allows more air flow and favour skin thickening. Regarding acidity and soluble solids, LT gave rise to lower acidity during ripening but the differences disappeared at maturity. Titratable acidity generally declined as sunlight exposure of the canopy increased (Bergqvist *et al.* 2001). Nevertheless, higher temperature may also occur as a result of increased sunlight exposure, and its effects on fruit composition are dependent upon the increase in berry temperature. The results in the case of study partially agree with those of King *et al*. (2012) in a study carried out in 'Merlot'. They indicated that leaf removal had no effect on ripening (unchanged Brix, TA, or pH). Similarly, Risco *et al.* (2014) in a study for 'Tempranillo' found that defoliation increased berry total soluble solids. However, Beslic *et al.* (2013) indicated that defoliation treatments increased the content of total phenols in comparison with control, while the content of total anthocyanins was not significantly changed in 'Cabernet Sauvignon'. Several authors confirmed that the effect depended on leaf removal timing. In this respect, Risco *et al.* (2014) concluded that defoliation at fruit set was the most effective treatment for increasing TSS while maintaining must acidity. The results obtained in the PCA analysis confirmed the negative relationship between Mn and Zn and Cu in some grape tissues with tartatic acid as well as the positive relationship with total soluble solids and berry weight. Although very few information exist related to the relationships between metals and grape quality, El-Razek *et al*. (2015) found that treatments, which include Zn and Mn enhanced the accumulation of total soluble solids and yield, but decreased the titratable acidity.

Conclusions

The analysed micronutrients in grape tissues were mainly driven by soil properties such as texture and organic matter content, which affect vine vigour. However, the micronutrient levels found in the tissues were not well correlated with the soil available fraction in a similar manner for the three elements, and the response was not directly related to vine vigour. While Cu and Mn levels increased during ripening and were greater in petiole in the most vigourous vines, Mn levels in petiole did change significantly and were greater in the less vigourous vine. These elements increased during ripening in skins and seeds and decreased in flesh. The effect of LT affects the uptake and redistribution of Cu and Mn, being greater in the leaf tinning management while no clear effect of LT was observed for Zn.

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