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RESEARCH ARTICLE

Influence of overliming vineyard acid soils on the macro-nutritional status of grapevines

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Abstract

Aim of study: The main aim of this study was to evaluate the effect of overliming with dolomitic lime on the topsoil and grapevine macro-nutritional levels (both petiole and grape tissues), as well as on berry weight and must quality properties in grapevines growing on an acid soil.

Area of study: The study was carried out in the viticultural region of El Bierzo (Spain), one of the main wine protected designation of origin in the northwest of Spain.

Material and methods: The effects of overliming were studied in soil parameters, petiole and grape tissues, as well as in must quality during three years (2014-2016). Data analysis was performed using factorial ANOVA (both parametric and non-parametric tests have been used).

Main results: The results found on the soil levels of magnesium and phosphorus were mirrored by those shown in petiole and grape tissues. Data suggest that insufficient Mg supply in vineyard acid soils could lead to a lower P vascular movement in vines. Additionally, our findings suggest that a great decrease of K levels in vine tissues as a consequence of overliming, could lead to changes in harvest quality.

Research highlights: Overliming with dolomitic limestone in large quantities decreased soil exchangeable K, as well as improved supply of exchangeable Mg and available P. Additionally Mg and P levels in both petiole and grape tissues were significantly affected by overliming.

Additional key words: nutrient interaction; phosphorus; potassium; grape seeds; grape skins

Abbreviations used: AAS (atomic absorption spectrometry); AlCEC (Al saturation of the cation exchange capacity); C (control); Ca-CEC (Ca saturation of the cation exchange capacity); Ca_P (Ca content in petioles); Ca_S (Ca content in seeds); Ca_{Sk} (Ca content in skins); CCE (calcium carbonate equivalent); CEC (cation exchange capacity); d (effect size); D (liming dose); EC (soil electrical conductivity); ICP-AES (coupled plasma atomic emission spectroscopy); K_P (K content in petioles); K_S (K content in seeds); K_{Sk} (K content in skins); KCEC (K saturation of the cation exchange capacity); MA (malic acid); Mg_P (Mg content in petioles); Mg_S (Mg content in seeds); Mg_{Sk} (Mg content in skins); MgCEC (Mg saturation of the cation exchange capacity); OD3 (overliming three-fold dose); OD9 (overliming nine-fold dose); P_P (P content in petioles); P_S (P content in seeds); P_{Sk} (P content in skins); pH (real acidity); pHs (pH in soils); SOM (soil organic matter); T (treatment); TA (total acidity); TcA (tartaric acid); TSS (total soluble solids); UV-V (ultraviolet-visible spectroscopy); W (berry weight); Y (sampling year).

Authors' contributions: Conceived, designed and performed the experiments: MAO, MJQ and EG. Analyzed the data: MAO and PAO. Wrote the paper: MAO, MJQ and MC.

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Introduction

Soil acidification is caused by a number of factors. Among the main ones on agricultural land are the application of ammonium-based fertilizers and urea, elemental sulfur fertilizer and the growth of legumes, cause the loss of base cations, an increase in aluminium (Al) saturation and a decline in crop yields (Goulding, 2016). Thus, this degradation process can be alleviated, or largely avoided by effective crop production practices. Liming is one of the main methods used by farmers to enhance the fertility of acid soils because it decreases the contents of exchangeable Al^{3+} by replacement with calcium (Ca^{2+}) and magnesium (Mg^{2+}), and also the contents of soluble Al^{3+}

by precipitation with the hydroxyl anions generated by carbonate hydrolysis in the soil solution. The application of adequate quantities of liming materials to acid soils encourages various beneficial physical, chemical and biological changes in the soil. In this sense, liming improves the structural conditions (aeration) and increases the bioavailability of phosphorus (P), Ca, Mg, and molybdenum (Mo) nutrients (Olego & Garzón, 2014).

Among the base cation lost as a result of soil acidification is the potassium (K), which is essential for vine growth and yield. Potassium, the major cation in grape juice (in such a way that high juice K decreases free acids and increases overall wine pH), has significant physiological-biochemical roles in vines like osmotic potential regulation, and although other cations may replace K in some of its functions, it plays a major role because plant membranes are highly permeable to K and it is the most abundant cation in plant tissues (Mpelasoka *et al.*, 2003).

On the other hand, although most soils contain abundant phosphorus (P), it is considered as a major limiting factor of crop production (naturally, including the production of grapes), playing an important role in photosynthesis, respiration, and the regulation of many enzymes and signal receptors. Insufficient P supply appears to restrict Mg transport in the xylem (Skinner & Matthews, 1990), a nutrient which, on the other hand, is already limiting in many acid soils. Thus, in vineyard acid soils ($pH<5.5$), P is one of the major limiting nutrients (Kochian *et al.*, 2004), which gives another good reason to ameliorate these soils through liming.

Despite the beneficial effects of liming over soil acidity (eliminating toxicity to Al^{3+}, Mn^{2+}, Fe^{3+} or hydronium ions (H_3O^+) toxicity, improving soil physical fertility or enhancing the availability of Ca, Mg, P and Mo), inadequate liming rates, *i.e.* overliming, could create deficiencies of micronutrients (Fageria & Baligar, 2008). Additionally, the consequences of such overliming could be yield reduction and decreased availability of P (Sanchez, 2019). This P deficiency could be induced through the formation of insoluble Ca phosphates. On the other hand, overliming with dolomitic limestone, which comprises mainly the mineral dolomite, which is made of a Ca and Mg double carbonate ($CaMg(CO_3)_2$), could result in higher Mg^{2+}/K^+ rates and so poor K^+ availability (Goulding, 2016).

Although extensive research has been carried out on the effects of liming on the properties of acid soils, overliming and its effects on soil fertility and plant nutrition have been poorly reported. Specifically, the impact of overliming on the nutritional status of vines cultivated under acidic soils, as well as its nutrient content of grape tissues, crop yield and must quality, have not been investigated until today. Since limitations in a particular nutrient often also impacts the uptake and transport of other nutrients, overliming and its effects on both vine nutritional status and harvest quality should be an important concept in the management of the vineyard under soil acidic conditions. We believe this is the first study to address this knowledge gap. The aim of this work was to investigate the different effects caused by overliming, with dolomitic limestone in a vineyard acid soil with a very low Mg content, on K and P nutrients at three levels: (i) soil bioavailability, (ii) petiole and (iii) grape berry tissues (seeds and skins). Finally, the impact of overliming on both berry weight and harvest quality was evaluated.

Material and methods

Study site

A commercial vineyard located around 550 meters above sea level in the municipality of Villafranca del Bierzo (León; Spain) with geographic coordinates of 42°37'N latitude and 6°45'W longitude, was selected as the study site (Fig. 1). From a climatic point of view, the grape growing region would be classified as Region I ($\leq$ 1,390 Celsius degree-days) based on the system devised by Amerine & Winkler (Jackson, 2020). The mean reference evapotranspiration (FAO Penman-Monteith) and rainfall were, respectively, 907.7 and 648.3 mm yr^{-1} during 2014-2016 (SIAR, 2020). The parent material of the viticultural soil under study (order Inceptisol; USDA, 2017) and its common clay minerals, as well as the bioclimatic characteristics where is located, have been previously described in Olego *et al.* (2016).

The research was conducted on the *Vitis vinífera* L. subsp. Mencía variety>50-year-old grafted on a Rupestris du Lot rootstock, which has been classified as highly sensitive to soil acidity (Fráguas, 1999). Planting lines displayed an east-west orientation; vines were spaced 0.5 m

Figure 1. Map of the location of the research area (North orientation is shown in the lower right corner). The thick black line in the upper left corner reflects the province of León, whereas the grey area in the lower left corner reflects the municipality of Villafranca del Bierzo (Spain).

between them, whereas the width of the rows was 0.6 m. Plants were head trained with 3–4 arms, with 6–8 nodes retained per plant at winter pruning. Winter pruning left a thumb-sized arm with two buds. The vineyard had no irrigation system support and a no-tillage system was applied during the research period. Finally, no fertilizers or extra amendments other than those used in this research were applied to the investigated vineyard.

Characterisation of the liming materials and liming doses

The composition of the liming material used in this study had the following composition: 31.1% CaO and 18.4% MgO (information suministrated by the company manufacturer (Calfensa®)), with a Ca carbonate equivalent (CCE) of 101.2%. A hypothetical liming rate was calculated with the aim of decreasing the Al saturation of the cation exchange capacity (CEC) down to 20%. In this regard, 20% exchangeable Al can be considered to represent a critical value to ensure an adequate degree of base saturation, *i.e.* 80%, required by most crop plant species (Fageria & Baligar, 2008). Specifically, the lime requirement was calculated using the known Cochrane's formula (Cochrane *et al.*, 1980), and resulted to be around 4120 kg CCE ha^{-1}, which would correspond to 4000 kg of dolomitic lime ha^{-1} according to its CCE. Taking into account the lime requirement calculated, and given that a single lime applications above 3500 kg CCE ha^{-1} is not very advisable for an approximate soil depth of 30 cm (Porta *et al.*, 2019), as well as the previous noted lack of studies regarding the effects of overliming, it was decided to apply three and nine-fold overliming doses compared with that calculated to achieve an Al saturation of the effective CEC of 20% (thus, 12000 and 36000 kg of dolomitic lime ha^{-1}). Effective CEC, which was determined with a solution buffered using ammonium as the exchanger cation (MAPA, 1993), was obtained as the arithmetic sum of the concentrations of the exchangeable Ca, Mg, K and Al (sodium (Na) concentrations were negligible). The dolomitic limestone was in a powdery state, and was uniformly spread onto the entire surface of the subplots, and were incorporated with one-pass tillage on January 2014.

Experimental design

The overliming factor was applied in four levels or treatments: control untreated with lime (C), liming with adequate dose (D), and overliming with three (OD3) and nine-fold dose (OD9), with three replications per treatment. The study plot was split into twelve subplots with six vines in each one (with two buffer vines and one buffer row between subplots). Because of the homogeneity of the soil area under study, the treatment replications were distributed among the twelve subplots in a completely random design with three treatments per row.

Soil sampling and analyses

Before the amendments were added, the following soil properties: texture, soil organic matter (SOM), soil pH in 0.01 M $CaCl_2$ (pHs), soil electrical conductivity (EC), Ca, Mg, K and Al content, as well as P content, were evaluated at 0-30 cm soil depth using the methods indicated below. The choice of this soil study depth was based on the fact that a large proportion of the vine rhizosphere was found to be developing at this depth.

After the amendments were added in January 2014, the effects of liming on the following soil properties in each subplot were monitored for three years (2014, 2015 and 2016): pHs, exchangeable CaCEC, MgCEC, KCEC and AlCEC (by dividing the sum of Ca, Mg, K and Al by the effective CEC), as well as P contents. This monitoring was conducted by sampling the soil at 0-30 cm depth at the senescence phenological stage (end of leaf fall).

The soil samples (before and after liming) were collected using an auger. Then they were sealed in plastic bags, transported to the laboratory and air-dried at room temperature. Next, they were disaggregated to pass a 2-mm mesh sieve, and analysed. Textural classes according to USDA were determined by the Bouyoucos (1962) hydrometer method. Next, the following methods of analysis were used for the determination of (i) SOM by wet oxidation followed by titration with ferrous ammonium sulfate (MAPA, 1993), (ii) pH determination in 0.01 M $CaCl_2$ (pHs) (Benton, 2001), (iii) soil EC at 25°C in a soil:water (1:2.5) suspension (MAPA, 1993), (iv) the content of exchangeable cations (Ca, Mg and K) by extraction with successive aliquots of 1 M ammonium acetate ($NH_4C_2H_3O_2$) (MAPA, 1993), and subsequent analysis of the displaced cations by atomic absorption spectrometry (AAS) (v) the exchangeable Al determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) using 1 M KCl as the extraction solution (Little, 1964), and (vi) the P levels were determined by ultraviolet-visible spectroscopy (UV-V) after successive extraction with sodium bicarbonate 0.5 M at pH 8.5 (Benton, 2001).

Leaf sampling and analyses

The Ca, Mg, K and P content in petioles (Ca_P, Mg_P, K_P and P_P respectively), were annually monitored at the veraison phenological stage (berries begin to colour and enlarge). Specifically, around 20 basal petioles opposite bunches were randomly collected per subplot each year. They were sealed in paper bags and transported to the laboratory. The leaves were carefully rinsed with abundant deionized water, and then dried for three days at 70ºC (Bavaresco *et al.*, 2010). Next, they were wet digested with an acid mixture of perchloric, sulphuric and nitric acid at 420 ºC during 20 minutes (Calleja, 1978), and the nutrient contents in the extracts determined by ICP-AES.

Grape sampling and analyses

The grapes were sampled at harvest every year (at the second half of September). From each subplot, one hundred grape berries were randomly chosen to determine the average berry weight (W) as well as the must quality parameters. The grape must of each subplot was obtained manually from the one hundred berries by gently pressing the grapes, using rubber gloves to avoid sample contamination. In the must thus obtained, the following harvest quality properties were determined (i) real acidity (pH), (ii) total soluble solids (TSS), that were measured using a refractometer (iii) total acidity (TA), that was determined by titration of the grape must with sodium hydroxide (0.1 M) to an endpoint of pH 7, and expressed as the equivalent content of tartaric acid in g/L, and finally, malic acid (MA) and tartaric acid (TcA) that were determined by enzymatic methods (340 and 492 nm respectively) (OIV, 2018). The seeds and skins from one hundred grapes were manually separated from flesh and immediately dried at 60 ºC to constant weight. Ca, Mg, P, and K content in both dried seeds (Ca_S, Mg_S, KS and P_S respectively), and skins (Ca_{Sk}, Mg_{Sk}, K_{Sk} and P_{Sk} respectively), were determined by ICP-AES after wet digestion with an acid mixture of perchloric, sulphuric and nitric acid at 420 ºC during 20 minutes (Calleja, 1978).

Comparisons between treatments

Statistical analyses were performed using R software (R Core Team, 2019). Several ANOVAs were carried out to study the effect of liming and overliming, with four levels or groups, namely: C, D, OD3 and OD9, i) on the soil chemical properties, ii) on the petiole nutrient contents, iii) on the berry weight and must quality properties and finally, v) on the grape tissue nutrient contents.

In all the ANOVAs the year of sampling with three levels (2014, 2015 and 2016) was also included as a block factor. Therefore, a two-way ANOVA (treatment (T) and sampling year (Y)) was used for the soil chemical properties, nutrient levels in petioles, berry weight, harvest quality properties, and grape nutrient contents. If the interaction between factors presented a significant effect, we did not interpret any main effects, because the higher-order interaction supersedes it. In that case, the effect of treatment was split independently for each year of the research and studied using post hoc contrasts to determine which groups differed significantly. On the other hand, if the interaction between factors did not present a significant effect, the main effect of treatment dose was interpreted independently of the factor year, whereas the main effect of the factor year was ignored.

To carry out an ANOVA the hypotheses of univariate normal distribution and homocedasticity of the data have to be tested in advance. However, before that, an outlier analysis of the data was developed. The univariate normality hypothesis for every variable, as a function of T and Y, was tested using the Shapiro-Wilk test. Although the analysis of variance (ANOVA) is fairly robust in terms of the error rate associated to violations of the assumption of homogeneity of variance (homocedasticity) when sample sizes are equal (Field *et al.*, 2012) as occurs in the present study, the null hypothesis that the variances of the groups are not different was tested using Levene's tests (again as a function of T and Y). When normality and homoscedasticity (equal variances) were violated, robust statistical methods were used, because violating these two assumptions is a serious practical concern (Mair & Wilcox, 2020). When the F statistic of ANOVAs was large enough to be statistically significant, post hoc contrasts (Bonferroni as parametric contrasts or trimmed means (Mair & Wilcox, 2020) as non-parametric ones) were carried out to find out which groups significantly differed (*significant at the $p<0.05$ level; **significant at the $p<0.01$ level; ***significant at the $p<0.001$ level). Throughout the research, despite the significant effect of liming treatment showed by ANOVA on some of the study parameters, contrasts did not reveal significant differences. A possible explanation for the above is the control of the family-wise error rate (Type I error rate) of the post-hoc procedures. Finally, to assess the importance of the potential significant effect in ANOVAs for the differences between pairs of groups, effect sizes (d) were calculated. Rather than rely on significance, we also quantified the effect of both liming and overliming in a standard way as an effect size which can be helpful in gauging the importance of that effect (Field *et al.*, 2012).

Soil initial characterization before liming and overliming

In Table 1 the baseline characteristics of the acid soil under study (at 0-30 cm depth) before liming are shown. The Al saturation of the CEC was 58% in the 0-30 cm layer (this exchangeable Al content was clearly above the 20% threshold established). Very low exchangeable Ca and Mg contents were also found. Assuming that the soil contains 26.2% clay and 1% organic matter, with a pHs=4.12, it is reasonable to estimate that the clays in this soil consist mainly on iron (Fe) and Al oxides and kaoilinite, which

Table 1. Average characteristics before liming and overliming in the 0-30 cm soil layer (Number of samples: 3).

Soil parameter	Value
Sand (%)	32.1
Silt (%)	41.6
Clay (%)	26.2
Textural class (USDA)	Loam
pHs	4.12
EC (dS/m)	0.04
SOM (%)	1.00
Ca (cmol(+)/kg)	0.85
Mg (cmol(+)/kg)	0.06
K (cmol(+)/kg)	0.25
Al (cmol(+)/kg)	1.60
P (mg/kg)	10.0

have a great affinity for phosphate ions. However, P levels did not show extreme low values; perhaps previous P management strategies are the reason for that.

Results

Soil properties

In Fig. 2 the time evolution of means and standard errors (SE) for pHs, CaCEC, MgCEC, P, KCEC and AlCEC for the treatment and control subplots, throughout the three years of monitoring, are showed. There are obvious differences in performance efficiency between liming doses, and specifically OM9 stands out for its ability to drop drastically KCEC and AlCEC levels, as well as to enhance MgCEC and P levels for the first two years. Similarly, it is necessary to highlight the effect of this overliming treatment in the soil pH levels.

ANOVAs were used to determine if the differences between liming treatments (T) were statistically significant, and furthermore, if they depended on the year of sampling (Y), and the interactions between both (Table 2). Because violations of parametric assumptions, robust ANOVA methods were used in pHs, CaCEC and P levels. According to the ANOVAs there was a significant effect of the liming treatment on all soil properties (pHs, CaCEC, MgCEC, P, KCEC and AlCEC). Besides, the effect of year of sampling was non-significant on every of the soil properties, whereas the effect of liming treatments significantly change with the year of sampling, which is revealed by the significant interaction between both factors (T × Y), for the CaCEC and MgCEC levels. To know where significant differences between liming doses lie out, *i.e.*, which means are significantly different, we must compare all pairs through post hoc methods (Bonferroni's or robust pairwise post hoc contrasts (trimmed means); Table 3). Besides there was a significant effect of the liming treatment on CaCEC, post hoc contrasts did not reveal significant differences. A possible explanation for the above is the control of the Type I error rate of the post-hoc procedures in the robust method.

Petiole nutrient contents

The evolution of Ca_P, Mg_P, K_P and P_P levels in petioles for the liming and overliming treatments, and control subplots throughout the research was evaluated. Fig. 3 shows the time evolution of means and SE for petiole nutrients for the treatment and control subplots, throughout the three years of monitoring. Leaving aside the differences between treatments, it is remarkable the low levels showed by CaP (optimal range: 1.86-2.09 %), and the appropriate levels showed by Mg_P (optimal range: 0.78-0.95 %) in all those subplots that were limed or overlimed, as well the above optimal ranges showed by K_P (optimal range: 1.14-1.68 %) in control subplots.

As in the case of soil parameters, ANOVA was used to investigate if the differences between treatments were statistically significant, and furthermore, if they depended on the year of sampling, and the interactions between these two factors (Table 4). Because violations of parametric assumptions, robust ANOVA method was used in P_P levels. According to the ANOVAs there was a significant effect of the liming treatment on Mg_P, K_P and P_P. Additionally, year of sampling was significant on K_P and P_P, whereas the effect of liming treatments significantly did not change in any of the petiole parameters, whereas the effect of liming treatments did not significantly change with the year of sampling (non significant interaction between both factors T × Y). Bonferroni's or robust pairwise post hoc contrasts (trimmed means), revealed significant differences (Table 5). Specifically P_P contrasts did not reveal significant differences. Again, a possible explanation for the above is the control of the Type I error rate of the post-hoc procedures in the robust method.

Berry weight, must quality and grape nutrient levels

The evolution of the berry weight (W), harvest quality parameters (pH, TSS, TA, MA and TcA) and grape nutrient levels (in seeds and skins), in the treatment and control subplots, were evaluated throughout the three years of monitoring. Figs. 4, 5 and 6 show the time evolution of means and SE for harvest data as well as nutrient content in grape skins and seeds respectively.

Again, an ANOVA was carried out to investigate if the differences in harvest parameters, as well as in the grape tissue nutrient contents, between liming treatments were

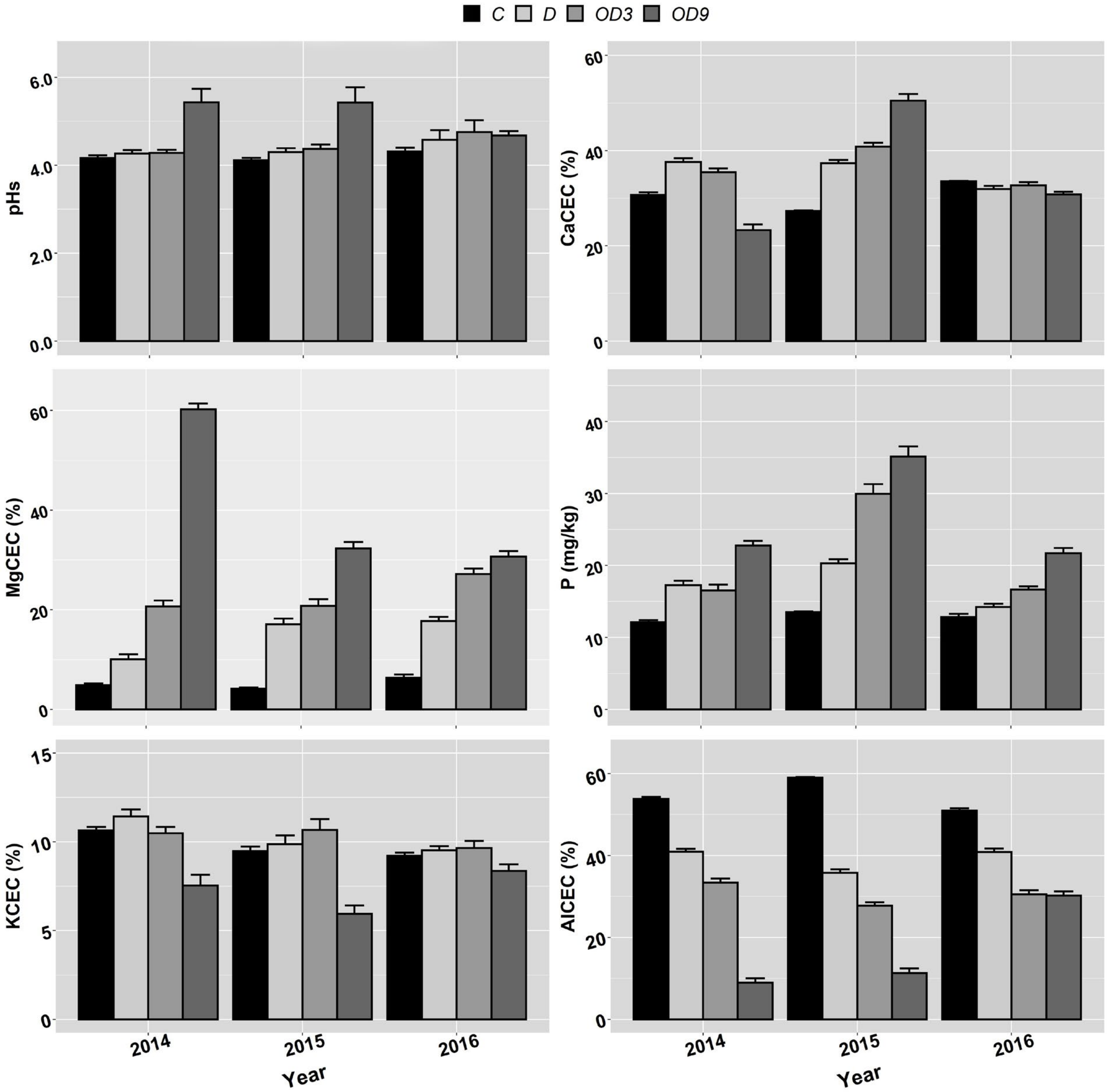

Figure 2. Mean values of the soil parameters pHs, CaCEC, MgCEC, P, KCEC and AlCEC for each treatment (2014-2016). Treatments: control (C), liming dose (D) and overliming with three (OD3) and nine-fold dose (OD9). Means values are showed above error bars. Error bars reflect the SE of the mean (+ 1 SE mean).

Table 2. Factorial analysis of variance performed on soil parameters (pHs, CaCEC, MgCEC, P, KCEC and AlCEC) at leaf fall stage.

Soil parameter	F-value (T)	F-value (Y)	F-value (T×Y)
pHs	22.4 ($2.20 \cdot 10^{-2}$*)	0.07 (0.97)	12.0 (0.32)
CaCEC	19.1 ($1.20 \cdot 10^{-2}$*)	5.64 (0.15)	31.0 ($2.50 \cdot 10^{-2}$*)
MgCEC	18.9 ($1.68 \cdot 10^{-6}$***)	0.81 (0.46)	2.53 (0.04*)
P	38.6 ($2.00 \cdot 10^{-2}$**)	5.63 (0.13)	9.03 (0.45)
KCEC	5.77 ($4.07 \cdot 10^{-2}$**)	1.15 (0.34)	0.69 (0.66)
AlCEC	36.0 ($4.84 \cdot 10^{-9}$***)	1.19 (0.32)	2.31 (0.07)

p values in parentheses, *i.e.* *, ** and *** denote a significant difference between treatments at $p<0.05$, $p<0.01$ and $p<0.001$, respectively. The variability in the soil parameters pHs, CaCEC and P were evaluated through robust ANOVA (F-distributed Welch-type test statistic), whereas MgCEC, KCEC and AlCEC were evaluated through parametric ANOVA (F-test). T: treatment. Y: year.

Table 3. Post hoc methods performed on soil parameters (pHs, CaCEC, MgCEC, P, KCEC and AlCEC) at leaf fall stage.

Soil parameter	C	D	OD3	OD9
pHs	4.20±0.03 a	4.38±0.09 a	4.47±0.14 a	5.18±0.26 a
CaCEC (%) (2014)	30.7±1.65 a	37.6±3.12 a	35.5±3.04 a	23.3±7.13 a
CaCEC (%) (2015)	27.3±0.08 a	37.3±2.35 a	40.8±3.32 a	50.5±10.2 a
CaCEC (%) (2016)	33.5±0.04 a	31.9±2.55 a	32.7±2.32 a	30.8±1.74 a
MgCEC (%) (2014)	4.84±0.72 a	10.1±5.00 a	20.7±7.28 a	60.2±7.27 b
MgCEC (%) (2015)	4.18±0.27 a	17.1±7.11 a	20.8±8.86 a	32.3±9.00 a
MgCEC (%) (2016)	6.35±2.10 a	17.7±3.52 ab	27.2±6.41 ab	30.7±6.53 b
P (mg/kg)	12.8±0.38 a	17.3±1.19 a	21.0±3.65 ab	26.5±3.74 b
KCEC (%)	9.78±0.25 a	10.3±0.53 a	10.3±0.67 a	7.28±0.75 b
AlCEC (%)	54.6±1.39 a	39.2±1.97 b	30.5±2.71 b	16.8±4.68 c

Means ± SE followed by a different letter indicate significant differences between treatments at least $p<0.05$. When effect of both treatment and interaction between year and treatment was significant (*i.e.*, $p<0.05$), post hoc contrasts were performed separately for each year (in parentheses). Treatments: lime (C), liming with adequate dose (D), and overliming with three (OD3) and nine-fold dose (OD9). Effect sizes (d) in absolute value of significant comparisons: MgCEC (2014; C<OD9 (d=6.18), D<OD9 (d=4.63), OD3<OD9 (d=5.52)); MgCEC (2016; C<OD9 (d=2.90)); P (C<OD9 (d=1.72); D<OD9 (d=1.11)); KCEC (C>OD9 (d=1.50), D>OD9 (d=1.55)), OD3>OD9 (d=1.42)); AlCEC (C>D (d=0.47), C>OD3 (d=3.74), C>OD9 (d=3.64), D>OD9 (d=2.07), OD3>OD9 (d=1.19)).

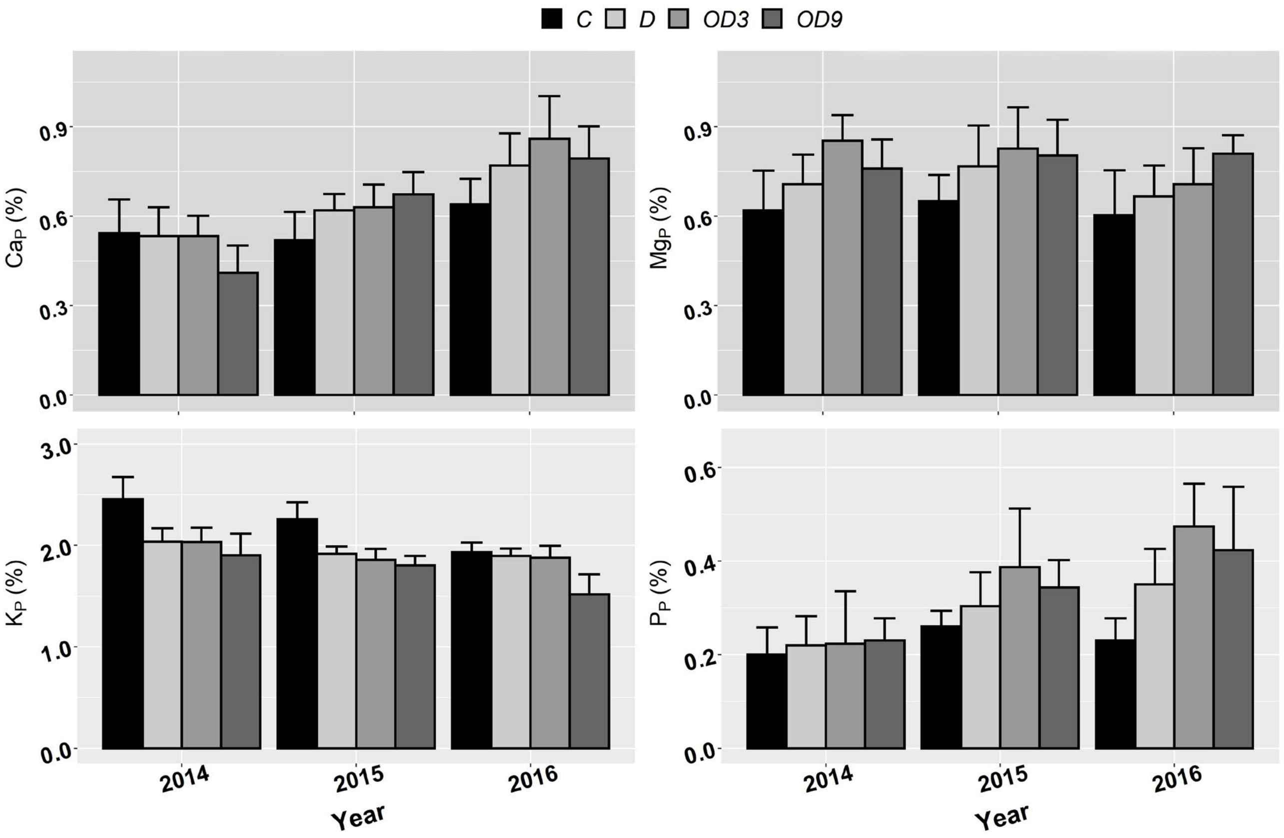

Figure 3. Mean values of the petiole nutrients (Ca_P, Mg_P, K_P and P_P) for each treatment at veraison time (2014-2016). Treatments: control (C), liming dose (D) and overliming with three (OD3) and nine-fold dose (OD9). Means values are showed above error bars. Error bars reflect the SE of the mean (+ 1 SE mean).

Table 4. Factorial analysis of variance performed on petiole nutrients (Ca_P, Mg_P, K_P and P_P) at veraison stage.

Petiole nutrient	F-value (T)	F-value (Y)	F-value (T×Y)
Ca_P	2.15 (0.12)	24.8 (1.43·10-6***)	1.94 (0.12)
Mg_P	3.56 (0.03*)	0.78 (0.47)	0.35 (0.91)
K_P	6.50 ($2.25 \cdot 10^{-3}$**)	5.15 ($1.38 \cdot 10^{-2}$*)	0.64 (0.70)
P_P	30.2 ($3.00 \cdot 10^{-3}$**)	26.6 (3.00·10-3**)	15.4 (0.17)

p values in parentheses, *i.e.* *, ** and *** denote a significant difference between treatments at $p<0.05$, $p<0.01$ and $p<0.001$, respectively. The variability in the petiole parameter P_P was evaluated through robust ANOVA (F-distributed Welch-type test statistic), whereas CaP, MgP and K_P were evaluated through parametric ANOVA (F-test). T: treatment. Y: year.

Table 5. Post hoc methods performed on petiole nutrients (Ca_P, Mg_P, K_P and P_P) at veraison stage.

Petiole nutrient	C	D	OD3	OD9
Ca_P (%)	0.57±0.03 a	0.64±0.04 a	0.67±0.06 a	0.63±0.06 a
Mg_P (%)	0.62±0.05 a	0.71±0.04 ab	0.80±0.04 b	0.79±0.03 b
K_P (%)	2.21±0.11 a	1.95±0.04 ab	1.92±0.05 ab	1.74±0.11 b
P_P (%)	0.23±0.01 a	0.29±0.02 a	0.36±0.05 a	0.33±0.04 a

Means ± SE followed by a different letter indicate significant differences between treatments at least $p<0.05$. Treatments: lime (C), liming with adequate dose (D), and overliming with three (OD3) and nine-fold dose (OD9). Effect sizes (d) in absolute value of significant comparisons: Mg_P (C<OD3 (d=1.33), C<OD9 (d=1.58)); K_P (C>OD9 (d=1.42)).

statistically significant, and furthermore, if they depended or interact with the year of sampling. For berry weight and must quality parameters, only TcA required robust ANOVA because of violation of parametric assumptions, while in the case of the berry nutrient levels, only differences between means of Ca_S were assessed using robust ANOVA methods. According to the ANOVA none of the treatments had any significant effect on berry weight and must quality parameters, but, as might be expected, there was a significant effect of year of sampling (a vintage effect) on all harvest parameters (Tables S1 and S2 [suppl.]).

According to the ANOVAs in grape tissue nutrient contents (Table 6), there was a significant effect of the liming treatment on nutrient content in seeds (Mg_S and K_S) and skins (Mg_{Sk} and P_{Sk}). Additionally, year of sampling was significant on Ca_S, P_S, K_S and K_{Sk}, whereas the effect of liming treatments did not significantly change with the year of sampling. Bonferroni's or robust pairwise post hoc contrasts (trimmed means) revealed where significant differences lied out in seeds and skins (Table 7). As in the case of the soil and plant data, overliming seems to have a remarkable effect on Mg, K and P content in grape tissues.

Correlations between nutrient levels

With regard to P and K nutrients, relationships between soil and vine tissues levels were investigated to evaluate potential links. Additionally, relationships between K/Mg levels in both soil and vine tissue levels have also been evaluated (Fig. 7). There were no strong overall relationships (Pearson correlation ≥ ±0.50), between KCEC and K content in vine tissues, while two of them (KCEC - K_P (*) and K_P - K_S (*)) were moderate (Pearson correlation ± 0.30 - ±0.49). Regarding the P nutrient, one strong relationship between petioles and grape skins was observed (P_P - P_{Sk} (***)), whereas another moderate one between grape seeds and skins (P_S - P_{Sk} (*)) also was observed.

Stronger relationships were observed when K/Mg levels were evaluated. This way, the following strong relationships were observed: KCEC/MgCEC - K_P/Mg_P (***), K_P/Mg_P - K_S/Mg_S (***) and K_P/Mg_P - K_{Sk}/Mg_{Sk} (***). Additionally, moderate relationships between KCEC/MgCEC - K_S/Mg_S (**), KCEC/MgCEC - K_{Sk}/Mg_{Sk} (*) and K_S/Mg_S - K_{Sk}/Mg_{Sk} (*) were also observed.

Discussion

As expected, overliming was more effective than liming in increasing soil CaCEC and MgCEC levels as well as decreasing soil AlCEC levels. However, only optimal petiole nutritional levels (García-Escudero *et al.*, 2013) for the nutrient magnesium (Mg_P) were achieved in limed or overlimed subplots. At first, there is no doubt that the application of dolomite has promoted both an

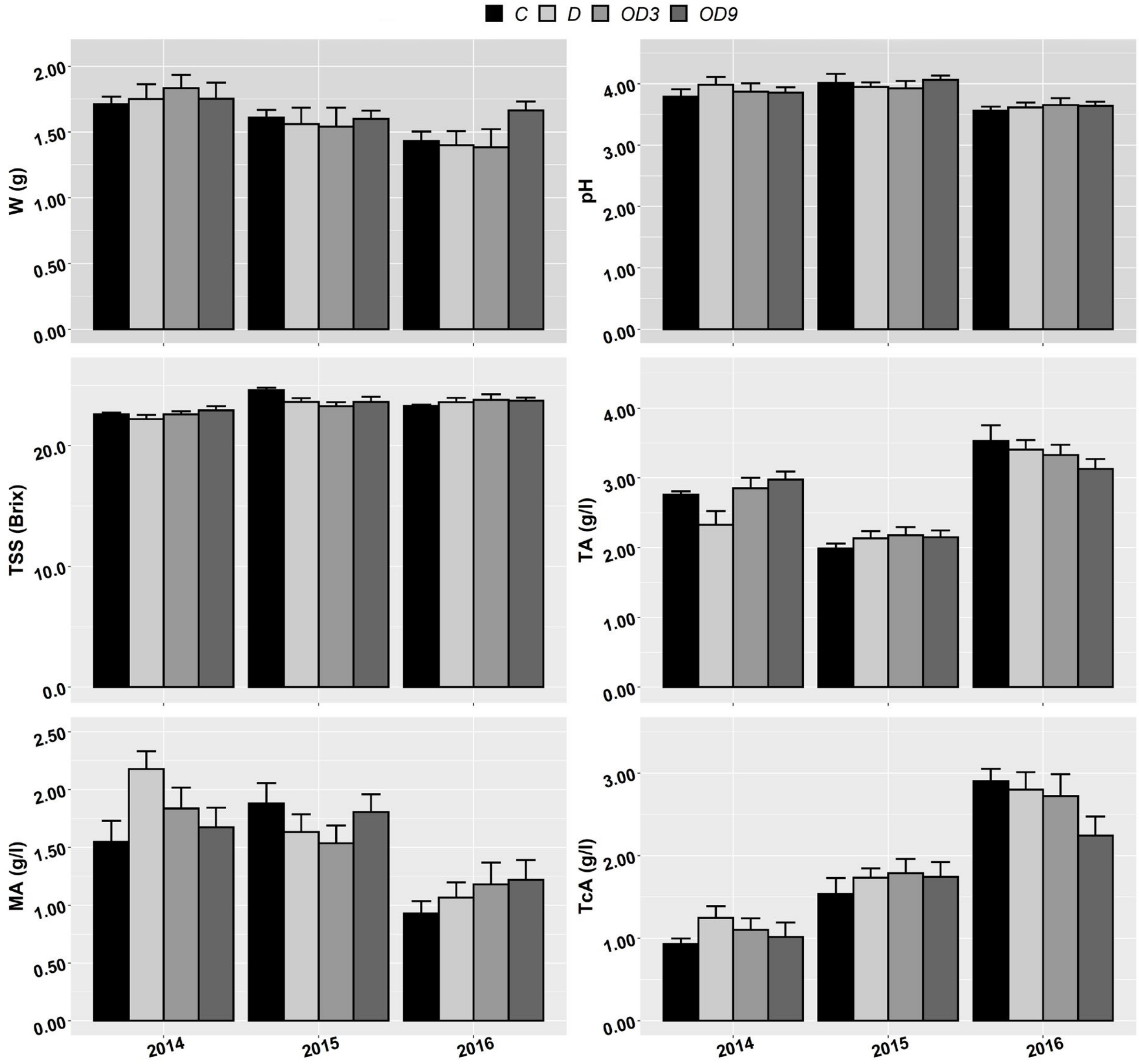

Figure 4. Mean values of the harvest parameters (W, pH, TSS, TA, MA and TcA) for each treatment (2014-2016). Treatments: control (C), liming dose (D) and overliming with three (OD3) and nine-fold dose (OD9). Berry weight (W) is expressed per berry basis whereas total acidity (TA) is expressed as g tartaric acid per l. Means values are showed above error bars. Error bars reflect the SE of the mean (+ 1 SE mean).

increase on P availability as the dose of dolomite increased and a decrease on KCEC. In our research, overliming did not induce soil P deficiency, which suggests that the vineyard soil under study does not have high P-fixation capacity, as well as that the overliming doses in this research have not promoted such a great solubilization of Ca and Mg that due to its affinity to those basic cations (Ca^{2+} and Mg^{2+}), the formation of insoluble complexes were induced. Specifically, a high concentration of Ca relative to that of phosphate outside the roots might counteract a temporary uptake of phosphate (Jakobsen, 1979). The lowered in KCEC could be explained by an opening up of K selective exchange sites that were blocked by Al at low pH or as lowered percent K saturation caused by the increased CEC (Magdoff & Bartlett, 1980).

Although Busenberg & Plummer (1989) suggested that the $CaCO_3$ component of dolomite dissolves faster than the $MgCO_3$ component, the findings on MgCEC of the current study do not support this previous research. Apparently, CaCEC observed in this investigation only shows a marked increase in its levels for liming and overliming in the second year of research. While the increase in Ca_P levels in liming and overliming with respect to control subplots observed in both 2015 and 2016, suggests that this inconsistency in our findings may be due to the soil sampling procedure, it should not be surprising that even if there are high CaCEC levels in soils, this is not the case for vine tissues, because Ca could be sequestered in vacuoles present in the root system (Storey *et al.*, 2003).

With regard to Mg as a competing ion in cation exchange reactions, and taking into account the well documented

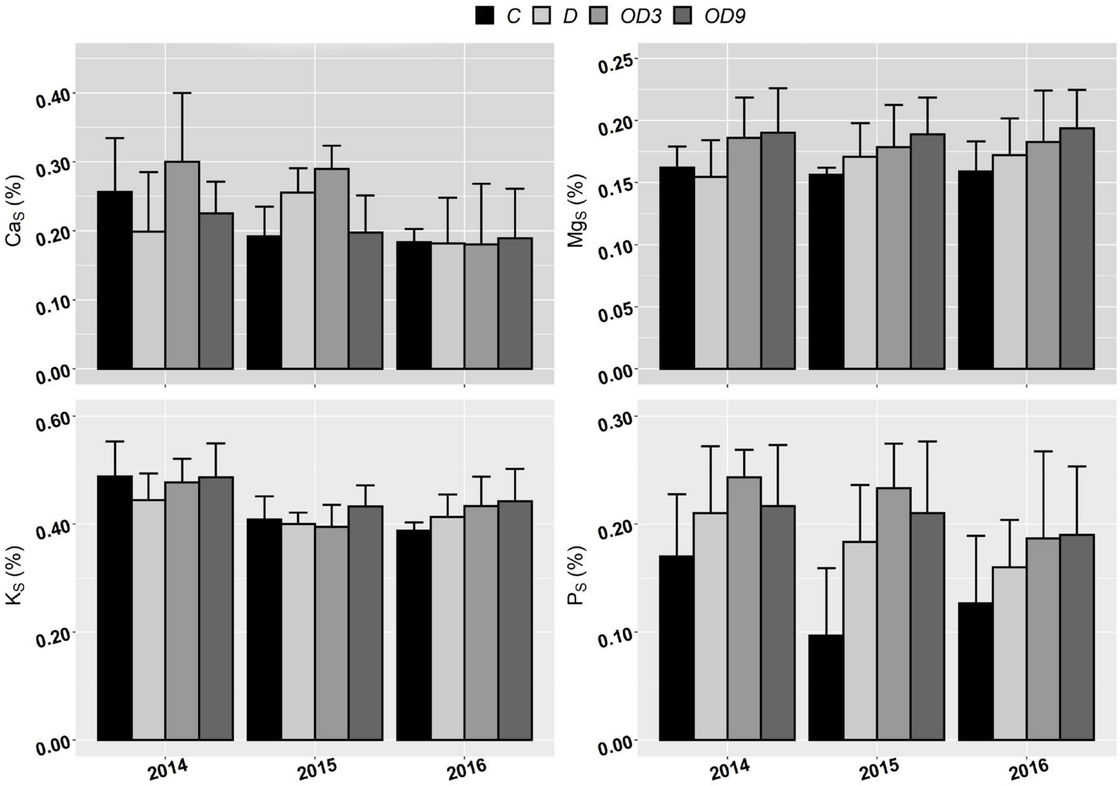

Figure 5. Mean values of the nutrient levels in seeds (CaS, MgS, KS and PS) for each treatment at harvest time (2014-2016). Treatments: control (C), liming dose (D) and overliming with three (OD3) and nine-fold dose (OD9). Means values are showed above error bars. Error bars reflect the SE of the mean (+ 1 SE mean).

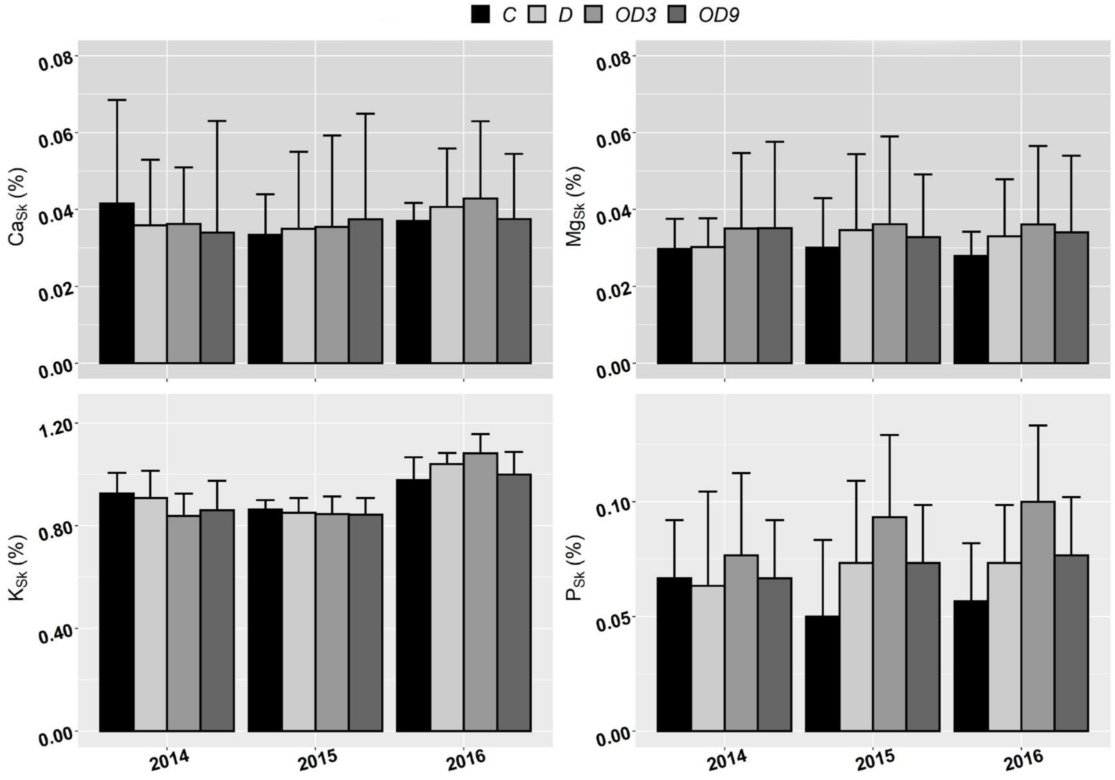

Figure 6. Mean values of the nutrient levels in skins (Ca$_{Sk}$, Mg$_{Sk}$, K$_{Sk}$ and P$_{Sk}$) for each treatment at harvest time (2014-2016). Treatments: control (C), liming dose (D) and overliming with three (OD3) and nine-fold dose (OD9). Means values are showed above error bars. Error bars reflect the SE of the mean (+ 1 SE mean).

Table 6. Factorial analysis of variance performed on nutrient level in grape tissues (seeds and skins) at harvest stage.

Grape nutrient	Grape tissue	F-value (T)	F-value (Y)	F-value (T×Y)
Ca	Seeds	4.72 (0.32)	13.2 (0.02*)	16.1 (0.18)
	Skins	0.29 (0.83)	2.91 (0.07)	1.57 (0.20)
Mg	Seeds	25.8 ($1.11\cdot10^{-7}$***)	0.62 (0.55)	1.45 (0.24)
	Skins	7.79 ($8.42\cdot10^{-4}$***)	0.30 (0.74)	0.81 (0.57)
K	Seeds	3.80 (0.02*)	28.5 ($4.59\cdot10^{-7}$***)	1.84 (0.13)
	Skins	0.39 (0.76)	25.4 ($1.19\cdot10^{-6}$***)	1.23 (0.32)
P	Seeds	12.9 ($3.23\cdot10^{-5}$***)	5.62 ($9.98\cdot10^{-3}$**)	1.00 (0.45)
	Skins	18.4 ($2.01\cdot10^{-6}$***)	2.42 (0.11)	2.38 (0.06)

p values in parentheses, *i.e.* *, ** and *** denote a significant difference between treatments at $p<0.05$, $p<0.01$ and $p<0.001$, respectively. The variability of the Ca levels in seeds, was evaluated through robust ANOVA (F-distributed Welch-type test statistic), while the variability in the seed and skin levels of the other nutrients were evaluated through parametric ANOVA (F-test). T: treatment. Y: year.

Table 7. Post hoc methods performed on seed (Ca_P, Mg_P, K_P and P_P) and skin nutrients (Ca_{Sk}, Mg_{Sk}, K_{Sk} and P_{Sk}) at harvest stage.

Berry nutrient	C	D	OD3	OD9
Ca_S (%)	0.05±0.02 a	0.05±0.02 a	0.08±0.03 a	0.03±0.01 a
Mg_S (%)	3.91·10-3±$1.30\cdot10^{-3}$ a	$0.01\pm3.56\cdot10^{-3}$ a	$0.01\pm3.63\cdot10^{-3}$ b	$0.01\pm2.82\cdot10^{-3}$ b
K_S (%)	0.43±0.02 a	0.42±0.01 a	0.44±0.01 a	0.45±0.01 a
P_S (%)	0.13±0.01 a	0.18±0.01 b	0.22±0.01 b	0.21±0.01 b
Ca_{Sk} (%)	$0.04\pm1.62\cdot10^{-3}$ a	$0.04\pm1.20\cdot10^{-3}$ a	$0.04\pm1.66\cdot10^{-3}$ a	$0.04\pm1.84\cdot10^{-3}$ a
Mg_{Sk} (%)	$0.03\pm4.32\cdot10^{-3}$ a	$0.03\pm9.37\cdot10^{-3}$ ab	$0.04\pm1.17\cdot10^{-3}$ b	$0.03\pm1.09\cdot10^{-3}$ b
K_{Sk} (%)	0.92±0.02 a	0.93±0.03 a	0.92±0.04 a	0.90±0.03 a
P_{Sk} (%)	$0.06\pm3.23\cdot10^{-3}$ a	$0.07\pm3.73\cdot10^{-3}$ ab	$0.09\pm4.71\cdot10^{-3}$ c	$0.07\pm2.22\cdot10^{-3}$ b

Means ± SE followed by a different letter indicate significant differences between treatments at least $p<0.05$. Treatments: lime (C), liming with adequate dose (D), and overliming with three (OD3) and nine-fold dose (OD9). Effect sizes (d) in absolute value of significant comparisons: Mg_S (C<OD3 (d=2.63), C<OD9 (d=4.74), D<OD3 (d=1.50), D<OD9 (d=2.76)); P_S (C<D (d=1.41); C<OD3 (d=2.25); C<OD9 (d=2.26)); Mg_{Sk}: C<OD3 (d=2.33), C<OD9 (d=2.24); P_{Sk} (C<OD3 (d=2.49), C<OD9 (d=1.42), D<OD3 (d=1.59), OD3>OD9 (d=1.63)).

effect of K/Mg interaction, since in this research we have overlimed with dolomitic limestone, one of the main risks of vineyard soil Mg in excess may be interfere with K uptake. In this sense, since interactions between nutrients depending on the nutrient supply, the potential antagonisms between nutrients resulting from soil overliming could modify vine growth and yield. To the above, it should be added that Ca should not be disregarded as another source of antagonistic interaction with K. On the other hand, it is possible to hypothesise that both liming and overliming have improved soil structure, which in turn had improved root penetration and internal drainage (van Leeuwen *et al.*, 2018), and consequently, better nutritional conditions for the vines (because soil physical properties affect nutrient movement and uptake in grapevines; Cass, 2005).

Although in the third year of the investigation, both liming and overliming showed a decrease in the intensity of their effect on soil properties the residual effect of dolomite on MgCEC is still very remarkable. If we take into account that the length of the residual effect of liming can be estimated by decreases in aluminum saturation (which will determine when additional lime should be applied), data seem to show that four (or perhaps five) years after the initiation of the research it would be necessary to liming again. In any case, three years after the start of the investigation, the residual effect of overliming (OD3 and OD9) on soil MgCEC, P and AlCEC levels was clearly higher compared to liming dose (D).

Results of relationships between K levels in soil and vine tissues indicate the complexity in regulating K uptake by the roots, and to partition K between the vegetative and reproductive tissues (Xiao *et al.*, 2020). Since our vineyard had no irrigation system support, it could conceivably be hypothesised that because an increase in

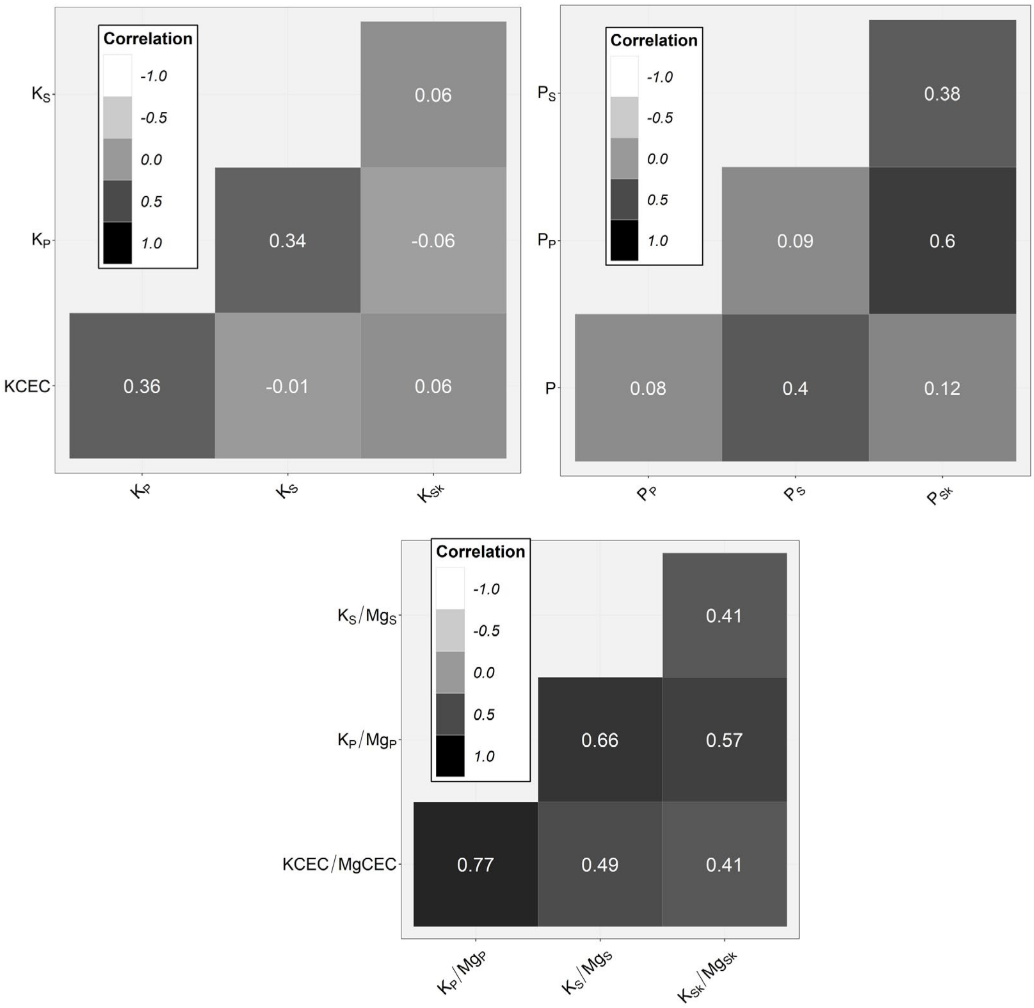

Figure 7. Pearson correlations between contents in soil, petiole and grape tissues (seeds and skins) for both K and P nutrients, as well as for K/Mg ratio.

the root uptake of K under irrigated conditions account for elevated grapevine and berry K accumulation (Mpelasoka *et al.*, 2003), stronger relationships between K pools could have showed if overliming had been develoved under irrigation system. P and K relationships seem to confirm the idea that a model in which nutritional composition of compartments connected in series are related to and influenced by the next compartment (soil - root - xylem - leaves - fruits) does not work directly proportional, and other environmental conditions may play a more important or confounding role (Peuke, 2009). Further studies, which take these variables into account, will need to be undertaken to provide more evidence on nutrient transport and distribution in the grapevine.

The positive correlations found between K/Mg rates seem to indicate that whereas the rate KCEC/MgCEC does not reach extreme levels in either direction, K and Mg accumulated in the petiole and grape tissues at similar rates. In this sense, overliming doses used in this research don't seem to have been high enough, at least under the growing conditions of this study, to cause this effect. The above, is consistent with that of Xiao (2019), who in its research about concentrations of soil, petiole and grape K, Ca and Mg cations of an established rootstock trial, found positive correlations between whole berry Mg and K content. Even more, since K uptake by the plant is affected by the antagonistic effect of Mg (Zlámalová *et al.*, 2015), it can be therefore assumed that as overliming dose increases, a greater decrease on KCEC could be showed and, finally, a lower levels in vine and grape tissues. An implication of the above is the possibility that overliming would have negative effects on key roles of K in vine (photosynthesis and stomatal control, biotic and abiotic stress resistance or pollen tube growth) and grape maturation (cell expansion and growth, phloem unloading, seed reserve storage, stomatal control or regulation of berry turgor) (Rogiers *et al.*, 2017).

Since the increase of Mg availability has increased P content in petiole and grape tissues, and because insufficient P supply appears to restrict Mg transport in the xylem, leading to a Mg deficiency (Skinner & Matthews, 1990), it could conceivably be hypothesised that insufficient Mg supply in vineyard acid soils could restrict in vines P vascular movement. On the other hand, the effect of overliming on K concentration in petiole and grape tissues is less evident. While in the petioles overliming seems to cause a decrease in K levels, in grape tissues this antagonistic effect doesn't seem so obvious. This could be related to the heterogeneous nature of leaves and its nutritional demands and, consequently, regulation of ionic uptake which are remarkably different in various cells, even of one leaf (Shabala, 2003), as well as with the differential mobility of this nutrient in the phloem and xylem, although the phloem is the predominant route for K entry into the berry after veraison (Rogiers *et al.*, 2006). As almost all K found in plant tissues is taken up by roots, it is not surprising that K transport systems in roots are the most studied, although the majority of K in vines is found in the stems, leaves (Shabala, 2003), being the most abundant cation in the grape berry (Rogiers *et al.*, 2017). Thus, several questions remain unanswered at present with regard to long distance transport of K in vines. In any case, since not all nutrient deficiencies are caused by insufficient nutrient availability, our findings suggest that overliming could be a way to decrease the amount of exchangeable K in vineyard acid soils, and therefore, a source of nutritional imbalance in the vine. The above is, if possible, even more important taking into account the importance of mineral nutrition and nutrient balance on grapevine, and within a climate change context, it is of crucial importance to know how changes in environmental factors can affect plant nutritional status and the K concentration of must, which is central to wine quality (Leibar *et al.*, 2017).

Both liming and overliming did not significantly affect berry weight and must quality. Our results could suggest that the Al saturation (AlCEC, *i.e.* exchange acidity) reached with both liming and overliming is still a little high and so even after the treatments, the vines are still somewhat constrained by the acidic growing conditions. In other words, even after overliming some Al dissolution can occur. Additionally, due to the influence of several other biophysical factors (such as soil type and climate), liming can lead to the absence of significant differences in yield quality (Holland *et al.*, 2018). Because of the significant seasonal effect on the yield-pH relationship showed by Holland *et al.* (2019), in their long-term liming experiment dedicated to several crop yields response to soil acidity, it would be appropriate a greater number of years of data to understand both lime and overlime crop response better (in terms of both yield and quality). Despite the fact that previous studies showed that liming did not cause significant differences in grape harvest quality properties (Quiroga *et al.*, 2017), our results also suggest that although the practice of liming (and by extension overliming) may affect them, the potential for extensive storage and mobilization of nutrients within the woody parts of the vine may explain the delay in vine harvest response even in the case of overliming.

The anthocyanin levels in berries, which have not studied in this research, are protected, apparently, from degradation in cell vacuoles by high amounts of Mg (Sinilal *et al.*, 2011). It can therefore be assumed that overliming with a liming material without $MgCO_3$ in its composition could materialize detrimental effects in the development of the color of the grape. Even more so, previous studies evaluating P forms in leaves and their relationships with must composition and yield in grapevine, have showed a positive correlation between P content in leaves and total anthocyanin content in berries (Piccin *et al.*, 2017). Therefore, overliming and its links with Mg and P availability could be a major factor, if not the only one, in the color quality of harvest under soil acidity conditions. This is an important issue for future research.

Perhaps, detrimental effects in both yield and/or harvest quality as a result of overliming can be seen most commonly noted in wine-growing variable-charge soils when overliming increases pH values greater than those showed in this research. With regard to this subject, it is possible to hypothesise that more clear trends would appear on yield and harvest quality, as well as on soil availability levels and its content in vine tissues, developing the research with younger vines and/or using higher overliming doses. Further work is required to establish the overliming doses that could induce substantially changes in both quantitative and qualitative harvest parameters. The generalisability of our results is subject to certain limitations, like the overliming dose as well as the nature of soil acidity. Additionally, understanding of nutrient partitioning in vines and the effects of overliming on it, may be further elucidated by research into the relationship between soil and vine nutrient status as well as nutrient-nutrient interactions.

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References

Bavaresco L, Gatti M, Fregoni M, 2010. Nutritional deficiencies. In: Methodologies and results in grapevine research; Delrot S, Medrano H, Or E, Bavaresco L and Grando S (eds.). pp: 165-191. Springer: Dordrecht, The Netherlands. https://doi.org/10.1007/978-90-481-9283-0_12

Benton J (ed), 2001. Laboratory guide for conducting soil tests and plant analysis. CRC Press, Boca Raton, FL, USA. 384 pp.

Bouyoucos G, 1962. Hydrometer method improved for making particle size analyses of soils. Agron J 544:

464-465. https://doi.org/10.2134/agronj1962.00021962005400050028x

Busenberg E, Plummer LN, 1989. Thermodynamics of magnesian calcite solid-solutions at 25ºC and 1 atm total pressure. Geochim Cosmochim Acta 53: 1189-1208. https://doi.org/10.1016/0016-7037(89)90056-2

Calleja A, 1978. La mineralización de muestras vegetales para el análisis de minerales por espectrofotometría y colorimetría. An Fac Vet León 24: 175-177.

Cass A, 2005. Effects of soil physical characteristics on mineral nutrient availability, movement, and uptake; Christensen P & Smart DR (eds.). Proc Soil Environment and Vine Mineral Nutrition Symp 2004. ASEV 2005, pp: 3-11.

Cochrane TT, Salinas JG, Sánchez PA, 1980. An equation for liming acid mineral soils to compensate crop aluminium tolerance. Trop Agr 57: 133-140.

Fageria NK, Baligar VC, 2008. Ameliorating soil acidity of tropical oxisols by liming for sustainable crop production. Adv Agron 99: 345-399. https://doi.org/10.1016/S0065-2113(08)00407-0

Field A, Miles J, Field Z, 2012. Discovering statistics using R, 1st ed. SAGE Publ Ltd, NY, USA. 957 pp.

Fráguas JC, 1999. Tolerância de porta-enxertos de videira ao aluminio do solo. Pesqu Agropec Bras 34: 1193-1200. https://doi.org/10.1590/S0100-204X1999000700011

García-Escudero E, Romero I, Benito A, Domínguez N, Martín I, 2013. Reference levels for leaf nutrient diagnosis of cv. Tempranillo grapevine in the Rioja appellation. Commun Soil Sci Plant Anal 44 (1-4): 645-654. https://doi.org/10.1080/00103624.2013.745385

Goulding KWT, 2016. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. Soil Use Manage 32 (3): 390-399. https://doi.org/10.1111/sum.12270

Holland JE, Bennett AE, Newton AC, White PJ, McKenzie BM, George TS, *et al.*, 2018. Liming impacts on soils, crops and biodiversity in the UK: A review. Sci Total Environ 610-611: 316-332. https://doi.org/10.1016/j.scitotenv.2017.08.020

Holland JE, White PJ, Glendining MJ, Goulding KWT, McGrath SP, 2019. Yield responses of arable crops to liming-An evaluation of relationships between yields and soil pH from a long-term liming experiment. Eur J Agron 105: 176-188. https://doi.org/10.1016/j.eja.2019.02.016

Jackson R, 2020. Wine science. Principles and applications, 5th ed. Acad Press, London. 1030 pp.

Jakobsen ST, 1979. Interaction between phosphate and calcium in nutrient uptake by plant roots. Commun Soil Sci Plant Anal 10: 141-152. https://doi.org/10.1080/00103627909366884

Kochian LV, Hoekenga OA, Piñeros MA, 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annu Rev Plant Biol 55: 459-493. https://doi.org/10.1146/annurev.arplant.55.031903.141655

Leibar U, Pascual I, Aizpurua A, Morales F, Unamunzaga O, 2017. Grapevine nutritional status and K concentration of must under future expected climatic conditions texturally different soils. J Soil Sci Plant Nutr 17 (2): 385-397. https://doi.org/10.4067/S0718-95162017005000028

Little I, 1964. The determination of exchangeable aluminium in soils. Aust J Soil Res 2: 76-82. https://doi.org/10.1071/SR9640076

Magdoff FR, Bartlett RJ, 1980. Effect of liming acid soils on potassium availability. Soil Sci 129 (1): 12-14. https://doi.org/10.1097/00010694-198001000-00003

Mair P, Wilcox R, 2020. Robust statistical methods in R using the WRS2 package. Behav Res Meth 52: 464-488. https://doi.org/10.3758/s13428-019-01246-w

MAPA, 1993. Métodos oficiales de análisis. Tomo III. Secretaría General Técnica, Ministerio de Agricultura, Pesca y Alimentación, Madrid. 662 pp.

Mpelasoka BS, Schachtman DP, Treeby MT, Thomas MR, 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust J Grape Wine Res 9: 154-168. https://doi.org/10.1111/j.1755-0238.2003.tb00265.x

OIV, 2018. Compendium of international methods of wine and must analysis, Vol 1. Int Org of Wine and Vine, Paris. 770 pp.

Olego MA, Garzón JE, 2014. Predictive modelling of magnesium concentration in grapevine petioles as a basis for liming recommendations in vineyard acid soils. Vitis 53 (1): 29-32.

Olego MA, Visconti F, Quiroga MJ, de Paz JM, Garzón-Jimeno E, 2016. Assessing the effects of soil liming with dolomitic limestone and sugar foam on soil acidity, leaf nutrient contents, grape yield and must quality in a Mediterranean vineyard. Span J Agric Res 14 (2): e1102. https://doi.org/10.5424/sjar/2016142-8406

Peuke AD, 2009. Nutrient composition of leaves and fruit juice of grapevine as affected by soil and nitrogen fertilization. J Plant Nutr Soil Sci 172: 557-564. https://doi.org/10.1002/jpln.200625205

Piccin R, Couto RDR, Sartori RJS, Gatiboni LC, Conti LD, Rodrigues LAT, *et al.*, 2017. Phosphorus forms in leaves and their relationships with must composition and yield in grapevines. Pesqu Agrop Bras 52 (5): 319-327. https://doi.org/10.1590/s0100-204x2017000500005

Porta J, López-Acevedo M, Poch RM, 2019. Edafología: uso y protección de suelos, 4th ed. Ed Mundi-Prensa, Madrid. 624 pp.

Quiroga MJ, Olego MA, Sánchez-García M, Medina JE, Visconti F, Coque JJR, Garzón Jimeno JE, 2017. Effects of liming on soil properties, leaf tissue cation composition and grape yield in a moderately acid vineyard soil. Influence on must and wine quality. OENO One 51 (4): 342-362. https://doi.org/10.20870/oeno-one.2017.51.4.2039

R Core Team, 2019. R: A language and environment for statistical computing. R Found for Statist Comput. http://www.R-project.org/ [accessed 06/2020].

Rogiers SY, Greer DH, Hatfield JM, Orchard BA, Keller M, 2006. Solute transport into Shiraz berries during development and late-ripening shrinkage. Am J Enol Viticult 57 (1): 73-80.

Rogiers SY, Coetzee ZA, Walker RR, Deloire A, Tyerman SD, 2017. Potassium in the grape (*Vitis vinifera* L.) berry: transport and function. Front Plant Sci 8: 1629. https://doi.org/10.3389/fpls.2017.01629

Sanchez PA, 2019. Properties and management of soils in the tropics. Cambridge Univ Press, Cambridge, UK. 666 pp.

Shabala S, 2003. Regulation of potassium transport in leaves: from molecular to tissue level. Ann Bot 92: 627-634. https://doi.org/10.1093/aob/mcg191

SIAR, 2020. Sistema de información agroclimática para el regadío. Ministerio de Agricultura, Pesca y Alimentación: Madrid. http://eportal.mapa.gob.es/ /websiar/SeleccionParametrosMap.aspx?dst=1 [accessed 06/ 2020].

Sinilal B, Ovadia R, Nissim-Levi A, Perl A, Carmeli-Weissberg M, Oren-Shamir M, 2011. Increased accumulation and decreased catabolism of anthocyanins in red grape cell suspension culture following magnesium treatment. Planta 234 (1): 61-71. https://doi.org/10.1007/s00425-011-1377-0

Skinner PW, Matthews, MA, 1990. A novel interaction of magnesium translocation with the supply of phosphorus to roots of grapevine (*Vitis vinifera* L.). Plant Cell Environ 13 (8): 821-826. https://doi.org/10.1111/j.1365-3040.1990.tb01098.x

Storey R, Jones G, Schachtman P, Treeby M, 2003. Calcium-accumulating cells in the meristematic region of grapevine root apices. Funct Plant Biol 30: 719-727. https://doi.org/10.1071/FP02212

USDA, 2017. Soil survey manual. Agriculture Handbook No. 18. United States Dept. of Agriculture, Washington, DC. 603 pp.

van Leeuwen C, Roby JP, de Rességuier L, 2018. Soil-related terroir factors: a review. OENO One 52 (2): 173-188. https://doi.org/10.20870/oeno-one.2018.52.2.2208

Xiao Z, 2019. How can we influence potassium (K) levels in the vineyard? Nat Wine and Grape Indust Centr, New South Wales, Australia. 32 pp.

Xiao Z, DeGaris KA, Baby T, McLoughlin SJ, Holzapfel BP, Walker RR, Schmidtke LM, Rogiers SY, 2020. Using rootstocks to lower berry potassium concentrations in 'Cabernet Sauvignon' grapevines. Vitis 59 (3): 117-126.

Zlámalová T, Elbl J, Baroň M, Bělíková H, Lampíř L, Hlušek J, Lošák T, 2015. Using foliar applications of magnesium and potassium to improve yields and some qualitative parameters of vine grapes (*Vitis vinifera* L.). Plant Soil Environ 65 (10): 451-457. https://doi.org/10.17221/437/2015-PSE