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Soil and foliar fertilization affects mineral contents in *Vitis vinifera* L. cv. 'rebula' leaves

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Abstract

Grapevine nutrient oversupply as well as shortage can both result in unbalanced vine growth and poor grape production. Mineral fertilization is a powerful tool also in viticulture in order to increase yields and improve grape quality. The trial carried out in Slovenian winegrowing region investigated the effects of basic fertilization coupled with soil or foliar applications of fertilizers containing Mg and Fe on the concentration of K, Mg, Fe and Zn in the grapevines leaves at berry set and veraison. The results obtained in a 2-years study (2008-09) with seven different fertilization treatments (along with control) showed that fertilization with K decreased Mg uptake almost on a half comparing to untreated vines, resulting in basal leaves chlorosis. High K/Mg ratio, determining low Mg uptake, was not lowered in our trial neither by foliar spraying nor by fertirigation with $MgSO_4$ solution. On the other hand, Mg application in addition to Fe supply had same effect on soil Zn uptake and its accumulation in the leaf petioles. Moreover, foliar Fe fertilization enhanced Fe levels in blades at berry set and to a lesser extent also at veraison.

Keywords: Fertilization, iron, magnesium, potassium, *Vitis vinifera* L., zinc

1. Introduction

All plants need an adequate supply of macro- and micro-elements in order to match their normal physiological and biochemical function. Besides basic mineral nutrients (nitrogen, phosphorus and potassium), some other elements (magnesium, iron, zinc, boron, etc.) are considered to be essential for plant metabolic processes because they are cofactors and/or activators of many metabolic enzymes (Bergmann, 1992; Marschner, 1995). The nutrients mentioned above are required for vine life cycle too from budburst to leaf senescence, and generally they limit grape production in world viticulture (Mullins *et al*., 1992).

Both nutrient deficiencies and excessive supply can both lead to physiological disorders. Nutrient deficiencies occur when plants cannot reach sufficient availability of nutrients for their basic metabolism in the surrounding environment, while in case of abundance of minerals, especially trace metals (e.g. zinc, copper, manganese), sometimes toxicity phenomena can occur (He *et al*., 2005). Nutrient deficiency affects the physiology of different plant tissues, but normally leaf chlorosis is the most common symptom that can be observed (Bergmann, 1992).

Less mobile nutrients (e.g. Fe and Zn) are less available in new leaf tissues and shoot tips causing deficiency symptoms visible in the terminal younger part of the canopy. As opposite, mobile nutrients (e.g. Mg and K) can rapidly reach new tissues being less available for older leaves that normally highlight chlorosis in case of deficiency (Bergmann, 1992; Marschner, 1995).

The application of nutrients contributes to manipulate environmental variables when properly integrated in a soil management program. It can be used as supplement to compensate for shortcomings of soils: that is to provide adequate nutrients when nature does not supply them during the critical stages of the seasonal growth cycle (Keller, 2005). However, despite the obvious importance of soil fertilization in plant growth and production, the knowledge and understanding about nutrient availability, the actual uptake from different fertilizers and how they are affecting grapevine physiology and productivity is surprisingly poor.

Generally nutrients can be applied directly into the soil (in the solid form or dissolved in water) or sprayed on the leaves. Soil feeding is the most ancient normal fertilization practice, however it depends on many factors from soil type to plant characteristics and its physiological state and therefore cannot be generalized. For example the availability of nutrients, especially micronutrients, is deeply limited in alkaline soils with high carbonate content (Fregoni, 1997). On the other hand, foliar fertilization, which has been developed in the last 60 years, does not totally replace soil fertilization on crops with large leaf area, but may improve the uptake and the efficiency of the nutrients applied to the soil (Kannan, 2010; Tejada and Gonzales, 2004). Foliar fertilization is increasingly adopted in order to alleviate micro- and macro-nutrient deficiency, but the resulting changes in the distribution of other nutrients may have significant adverse effects on plant growth and yield. However, there are not many data available in literature as regard the effects of applied elements on the concentration of other micro- and macro-elements within the plant (Kaya and Higgs, 2002).

Thus, the aim of this experiment was to study the effects of basic N, P and K fertilization coupled with soil or foliar applications of fertilizers containing Mg and Fe on the concentration of macroelements K and Mg and microelements Fe and Zn in the blades and petioles of grapevines variety 'Rebula' (*Vitis vinifera* L.), planted on alkaline and high carbonate soil from Goriška Brda region (Slovenia) and to examine the interactions among them at two sampling times - berry set and veraison.

2. Materials and Methods

2.1. Location and experimental design

A pot trial was carried out during two consecutive growing seasons (2008 and 2009) in Goriška brda (Western Slovenian winegrowing district), area characterized with a typical sub-Mediterranean climate with frequent dry periods in summer and with an average annual rainfall of 1200 mm (Rusjan *et al.,* 2006).

Meteorological data (Table 1) were recorded at a weather station located in Capriva del Friuli (ARPA-OSMER FVG, Italy), close to the experimental station. During the experiment, the annual rainfall was 1856 and 1437 mm in 2008 and 2009, respectively. In addition, no great differences in temperatures between these two years were recorded. During the growing cycle, the temperature range was more or less the same in the summer months, with lower temperatures in April, in May and in September 2008 as compared with the following year. One-year old grapevines variety 'Rebula' (*Vitis vinifera* L.), grafted on SO_4, were planted in 21-L-plastic pots in April 2007.

Table 1. Distribution of monthly rainfall and average temperatures ($T_{average}$) in 2008 and 2009 (Weather Station of Capriva del Friuli, ARPA-OSMER FVG, Italy).

	Year 2008		**Year 2009**	
Month	**Rainfall (mm)**	**$T_{average}$ (°C)**	**Rainfall (mm)**	**$T_{average}$ (°C)**
January	145	5.86	96	4.42
February	53	5.43	112	4.63
March	110	8.43	252	8.44
April	143	12.3	93	15.0
May	161	18.1	24	19.5
June	146	21.5	128	20.4
July	243	23.2	71	23.5
August	111	23.9	62	25.4
September	65	18.0	68	21.2
October	90	14.8	156	14.0
November	248	9.59	128	9.84
December	342	5.63	247	5.37

After planting, vines were sprayed periodically in order to avoid diseases, following a fungicide strategy plan based on integrated pest management rules. Pots were put outdoor and the plants were covered with net to protect them from hail. A drip irrigation system was also set up and water was applied to avoid drought conditions in the summer time.

1.5 cm^2-net sieved soil taken from the surface layer (ca. 0-10 cm) of typical vineyard of Goriška brda was used to fill the pots where the grapevines were planted. The physical and chemical parameters of the soil are presented in (Table 2) (analysis performed by YARA Italia S.p.a., Italy).

In the winters 2007-08 and 2008-09, the grapevines were pruned retaining a shoot with 4 buds (where possible) that was trained vertically. In 2008 and 2009 eight treatments were imposed with 3 replicates of 4 plants each (Table 3). Seven fertilization treatments were applied in comparison with the untreated control (without fertilization). The amounts of mineral fertilizers additions (50 N, 90 P_2O_5 and 140 K_2O in kg/ha) was concluded from to soil nutrient analysis before planting (20 mg kg^{-1} of ammonlactate (AL) soluble-P_2O_5 and 100 mg kg^{-1} of AL-K_2O, results from Agricultural and Veterinary Institute Nova Gorica, Slovenia) and recommendations for yearly side-dressing (Vršič and Lešnik, 2005). Nitrogen as ammonium sulphate (20.6% N; Italy) was applied in both years in the spring, while phosphorus as phosphate (26% P_2O_5; Austria) and potassium as potassium sulphate (50% K_2O; Italy) were applied in 2008 only.

Table 2. Average physical and chemical characteristics of the soil (YARA Italia S.p.a., Italy).

Parameter	Mean value
pH (in water 1:1.25)	8.0
EC (dS m^{-1})	0.80
OM (%)	1.9
CEC (cmolc kg^{-1})	24.9
$CaCO_3$ total (%)	33.0
Active lime (%)	9.0
N total (mg kg^{-1})	1039
Available P (mg kg^{-1}) [a]	8
Available K (mg kg^{-1}) [b]	174
Available S (mg kg^{-1}) [c]	7
Available Ca (mg kg^{-1}) [b]	4793
Available Mg (mg kg^{-1}) [b]	43
Available B (mg kg^{-1}) [d]	1.34
Available Cu (mg kg^{-1}) [e]	17.5
Available Fe (mg kg^{-1}) [e]	143
Available Mn (mg kg^{-1}) [f]	104
Available Mo (mg kg^{-1}) [f]	0.05
Available Zn (mg kg^{-1}) [e]	3.9
Clay (%)	32.7
Silt (%)	47.2
Sand (%)	20.1
Texture	Clay loam

Methods/extractants used for determination of plant available fraction: Olsen procedure (a), 1 M ammonium nitrate (b), calcium tetrahydrogen diorthophosphate (c), hot water (d), 0.05 M EDTA (e), and ammonium acetate (f).

In addition, fertilizers containing Mg - "Bittersalz" ($MgSO_4$ x 7 H_2O, 16% MgO; Germany), Fe - Foliacon Fe (Fe complexed to amino acids, 5% (w/w) Fe; Italy) have been added before bloom (in May), individually or combined. Mg and Fe applications were calculated according to producer recommendations. Since the amounts of nutrient are usually given per hectare of vineyard, the rate of each element was calculated per pot, considering a field with 5000 vines/ha. In order to understand the effectiveness of Mg and Fe application to soil by fertirigation vs. leaf fertilization, a comparison between foliar spraying (L; leaves) and fertirigation (S; soil) was applied to the experimental vines. A completely randomized experimental design was set up with three replicates of four plants.

2.2. Mineral leaf analysis and measurements

Leaf analysis was made up selecting eight-to-twelve leaves per replicate (2-3 leaves x vine for each plant), sampled at berry set (end of June; leaf opposite to the cluster) and at veraison (beginning of August; mid-shoot leaves).

After sampling, the blades were separated from the petioles, washed up with tap water first and deionised water thereafter, in order to remove dust and other residues on the leaf surface; then, leaves/petioles were oven-dried at 105 °C for three days. After homogenisation and grinding (28 Hz s^{-1}, 2.30 min; Mixer mill MM 400, Retsch, Germany) blades (0.5 g) and petioles (0.3 g) were digested with HNO_3 (left overnight covered with lid) and H_2O_2 (65% and 30%, respectively; Suprapure, Merck, Germany) in the PTFE beakers on a sand-bath (Gestigkeit, Germany) according to Hoenig *et al.* (1998) without HF step. The dry residues were re-dissolved in 0.5 ml of HNO_3 with 1-2 min heating and filled to a final volume of 25 mL with double-deionised water in PP centrifuge tubes. Mineral concentrations were determined by atomic absorption spectrophotometry (SpectrAA-55, Varian, Australia), in an air-acetylene flame at the following wavelengths (nm): 766.5 (K), 285.2 (Mg), 248.3 (Fe), and 213.9 (Zn). Appropriate quality controls (standard reference materials (SRMs) from National Institute of Standards & Technology - NIST (SRMs 1572 -citrus, 1547 - peach, 1573 and 1573a - tomato leaves) were performed for each set of measurements.

Table 3. Description of treatments applied in the pot experiment with 'Rebula' grapevines.

Ex.	Treatment	Concentration/quantity of fertilizer (typology of application)
1	Untreated	No added nutrients
2	NPK	10 g N, 18 g P_2O_5, and 28 g K_2O per pot (into the soil)
3	NPK + Mg L	3% (w/v) "Bittersalz" (foliar spraying)
4	NPK + Mg S	3% (w/v) "Bittersalz" (fertirigation)
5	NPK + Fe L	0.15% (w/v) "Foliacon Fe" (foliar spraying)
6	NPK + Fe S	0.15% (w/v) "Foliacon Fe" (fertirigation)
7	NPK + Mg + Fe L	3% (w/v) "Bittersalz" + 0.15% (w/v) "Foliacon Fe" (foliar spraying)
8	NPK + Mg + Fe S	3% (w/v) "Bittersalz" + 0.15% (w/v) "Foliacon Fe" (fertirigation)

N, P, K were added in pots 2-8 (a).

2.3. Statistical analysis

All experimental data were analysed throughout ANOVA using a STATGRAPHICS Plus (Version 4.0) statistical package. When differences at ANOVA were significant (P < 0.05), means were separated using Duncan's multiple range test.

3. Results

3.1. K and Mg concentration in grapevine leaves

At berry set, K concentration in untreated vines was 1.0 ± 0.1 vs. 1.2 ± 0.3% in blades and 2.6 ± 0.3 vs. 2.7 ± 0.5% in petioles in 2008 and 2009, respectively (Figure 1).

The measured K concentration was quite similar at verasion, being 1.0 ± 0.04 vs. 1.3 ± 0.2% in blades and 2.4 ± 0.3 vs. 2.9 ± 0.5% in petioles, in the same years respectively. Treatments (#2-8) with added K showed significantly higher concentrations of K in comparison with untreated vines at both sampling times and in both years. On the overall average, the concentration of K was enhanced by 1.6-fold in all treated samples in comparison with untreated ones.

In the contrast, the concentration of Mg was much higher (Figure 2) in the leaf samples of untreated vines in both years and at both sampling times (except for blades at berry set). At berry set, the measured Mg concentration was 0.08 ± 0.02 vs. 0.10 ± 0.01% in blades and 0.19 ± 0.02 vs. 0.14 ± 0.01% in petioles in 2008 and 2009, respectively. Moreover at veraison, the Mg content in both leaf samples of untreated vines was on the average almost two-time (1.9-fold) higher than in K-added treatments (with mean values 0.078-0.101 vs. 0.063-0.073% in blades and 0.11-0.15 % in petioles, in 2008 and 2009 respectively). Moreover at veraison, the Mg content in both leaf samples of untreated vines was on the average almost two-time

(1.9-fold) higher than in K-added treatments (with mean values 0.078-0.101 vs. 0.063-0.073% in blades and 0.11-0.15 % in petioles, in 2008 and 2009 respectively). In the control vines, the concentration of Mg increased by 1.6-fold (averaging both years) from berry set to veraison in both blades (0.14 ± 0.01 vs. 0.14 ± 0.02%, in 2008 and 2009 respectively) and petioles (0.26 ± 0.02 vs. 0.27 ± 0.02%, respectively). A comparable enhancement was seen also in the petioles treated with Mg and Fe fertilizers (#4, #6-8) in 2009. In other cases, some changing in Mg contents in blades and petioles were observed during the growing seasons.

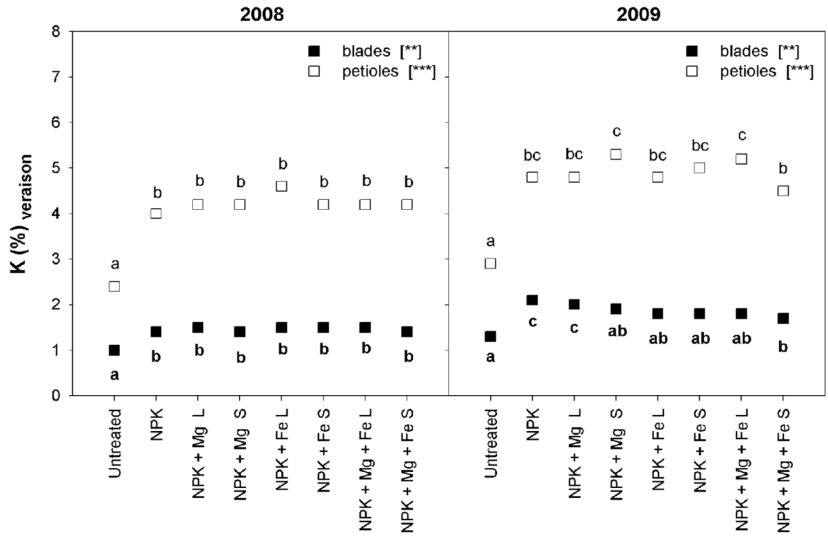

Figure 1. Measured K concentrations in blades and petioles of differently treated grapevines at veraison 2008, 2009 (on a dry matter basis). Within each graph, means (n = 3) followed by a different letter are significantly different at $p < 0.05$ using Duncan`s MR test. Asterisks indicate significance of ANOVA test (* $p< 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant).

3.2. Effects of different fertilization treatments on Fe concentration in grapevine leaves

At berry set 2008, the amounts of Fe were found to be much higher than at veraison of the same year and at both sampling times in the year after (with mean values ranging between 201 and 264 mg kg^{-1}; in the treatments #5 and #7 even > 300 mg kg^{-1} in blades; and 41-63 mg kg^{-1} in petioles; Figure 3). Moreover, comparing values from berry set and veraison 2008 samples, Fe concentrations were reduced by 60% in both blades and petioles (76-105 and 18-22 mg kg^{-1}, respectively).

In 2009, the concentration of Fe in blades was significantly enhanced by all fertilization treatments (with mean values 76-96 mg kg^{-1}) as compared with control (56 ± 8 mg kg^{-1}). At berry set, a significant higher amount of Fe was observed in blades and petioles when the same element was added by foliar spraying both alone alone (#5; 120 ± 7 and 24 ± 2 mg kg^{-1}, respectively) or in combination with Mg (#7; 106 ± 6 and 23 ± 2 mg kg^{-1}, respectively). The same enhancement was observed at berry set in 2008 but the differences were not significant because of the high standard deviations among the three parallels of the same treatment (#5: 323 ± 69 mg kg^{-1} and #7: 306 ± 42 mg kg^{-1}).Moving on veraison, Fe concentrations measured in the petioles were very low (closed to the Fe quantification limit for FAAS; measured means were 17-22 mg kg^{-1}) and statistically identical in all treatments also in control.

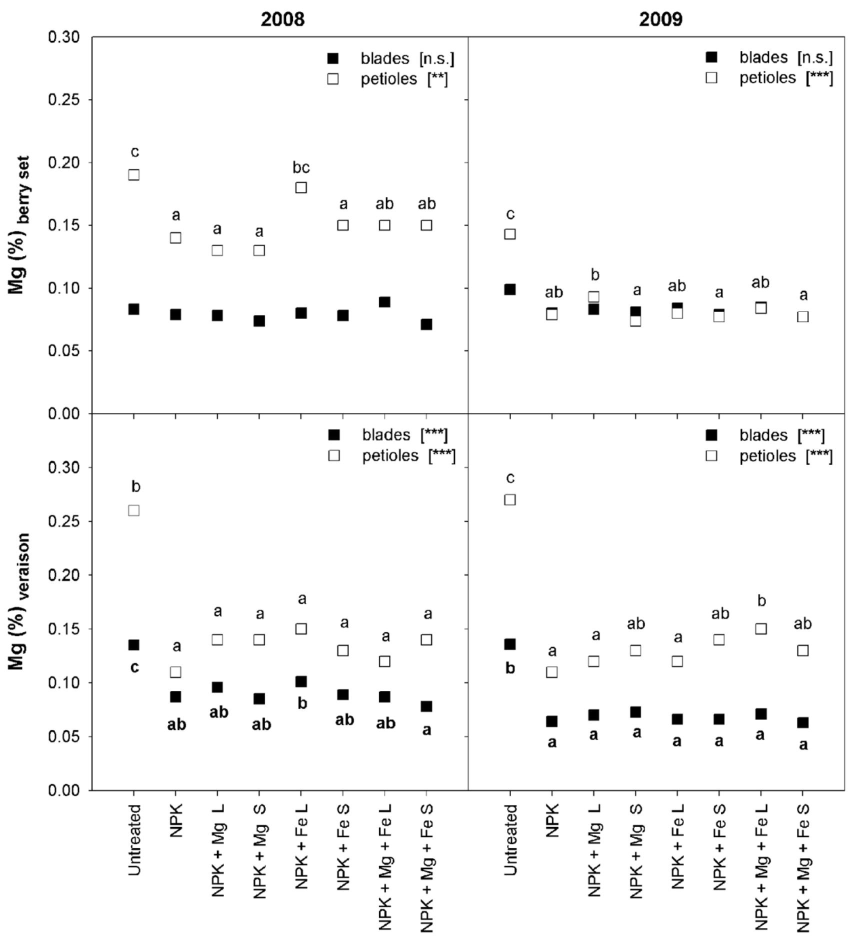

Figure 2. Measured Mg concentrations in blades and petioles of differently treated grapevines at berry set and veraison 2008, 2009 (on a dry matter basis). Within each graph, means (n = 3) followed by a different letter are significantly different at $p < 0.05$ using Duncan`s MR test. Asterisks indicate significance of ANOVA test (* $p < 0.05$; ** $P < 0.01$; *** $p < 0.001$; ns: not significant).

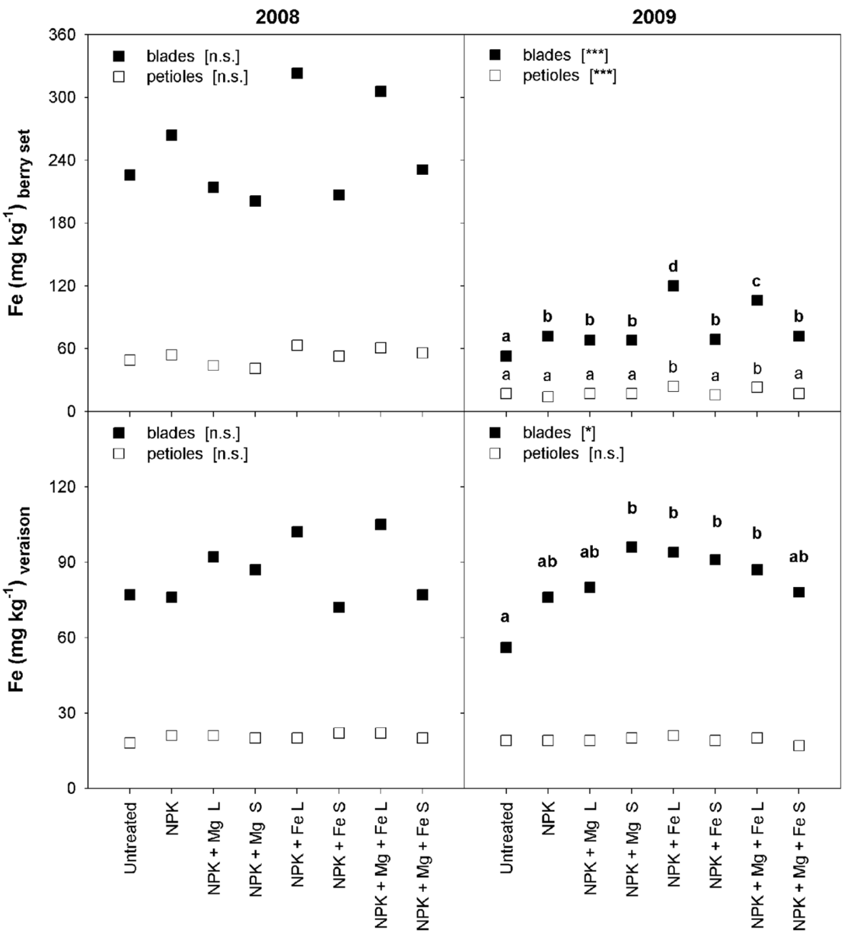

Figure 3. Measured Fe concentrations in blades and petioles of differently treated grapevines at berry set and veraison 2008, 2009 (on a dry matter basis). Within each graph, means (n = 3) followed by a different letter are significantly different at $p < 0.05$ using Duncan`s MR test. Asterisks indicate significance of ANOVA test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant).

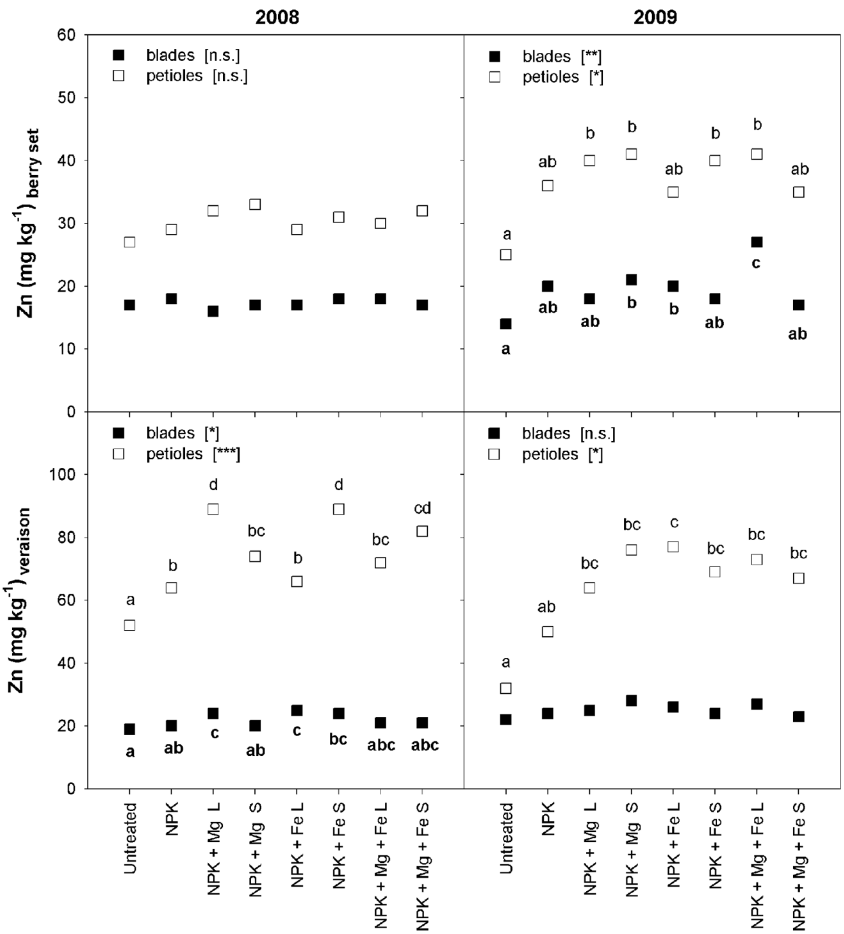

Figure 4. Zn concentrations in blades and petioles of differently treated grapevines at berry set and veraison 2008, 2009 (on a dry matter basis). Within each graph, means (n = 3) followed by a different letter are significantly different at $p < 0.05$ using Duncan`s MR test. Asterisks indicate significance of ANOVA test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant).

3.3. Effects of Mg and Fe fertilizers on Zn contents in grapevine leaves

In both seasons, Zn concentration was in almost all cases the lowest in blades (17 ± 1 vs. 14 ± 1 mg kg^{-1} at berry set; and 19 ± 2 vs. 22 ± 4 mg kg-1 at veraison in 2008 and 2009, respectively) and petioles (27 ± 3 vs. 25 ± 4 mg kg^{-1} at berry set; and 52 ± 2 vs. 32 ± 5 mg kg^{-1} at veraison, respectively) of untreated vines and the differences were also statistically confirmed (Figure 4).

The Zn concentration was increased in the time from berry set to veraison in the petioles of all sampled grapevine leaves. Moreover, Zn contents were enhanced in the petioles of vines treated either with Mg or Fe alone or in combination (from 2.2 to 2.9-fold vs. 1.6-2.2–fold in 2008 and 2009, respectively) in comparison with control vines (1.9-fold vs. 1.3-fold in 2008 and 2009, respectively) or with vines in which only N, P and K have been added (2.2-fold vs. 1.4-fold in 2008 and 2009, respectively). At veraison, the petiole Zn concentrations were statistically higher in the treatments #3-8 (66-89 vs. 64-77 mg kg^{-1} in 2008 and 2009, respectively) in comparison to untreated vines and vines of the treatment #2.

3. Discussion

Leaf analysis (blades or petioles) is widely recognized as the most reliable laboratory method to determine the nutritional status in grapevines (Christensen, 1984), and during the growing season the best periods for leaf sampling are berry set and veraison since several reference range in literature are focusing these stages (Capps and Wolf, 2000; Christensen, 1984; Fregoni, 1998; White, 2009).

At berry set, K concentration (Figure 1) in untreated vines was nearly matching the values found in the literature (Fregoni, 1998; White, 2009), low to marginal in blades (1.0 vs. 1.2% in 2008 and 2009, respectively) and in the optimal range in petioles (2.6 vs. 2.7%, respectively). The situation was similar at veraison, being K concentration slightly below the minimal threshold in 2008 (1.0 vs. 2.4% in blades and petioles, respectively) while in the reference range in 2009 (1.3 vs. 2.9% in blades and petioles, respectively). In contrast, values of K contents in the treatments with added K fall into high-to-excessive range (> 1.4 vs. > 3.5% in blades and petioles, respectively; Fregoni, 1998) at both sampling times and in both years.

As opposite to K, measured Mg concentrations (Figure 2) were much lower than optimal (Bigot *et al*., 2009; Fregoni, 1998; White, 2009) in both blades and petioles in all studied plants. As reported by Bergmann (1992) and Romic *et al.* (2012) increased K contents in grapevines my decrease the uptake of Mg. Another aspect to take into account is related to the slow mobility of Mg in soil (Marschner, 1995) and its root absorption, since Stefanini *et al*. (1994) already reported that SO_4 rootstock poorly uptakes Mg from the soil.

The addition of K to the soil resulted in higher soil K availability and final K concentration in blades, as reported by Poni *et al*. (2003). Throughout K fertilization (side-dressing) which is regular annually viticulture practise in many countries, the Mg uptake decreases almost on an half with a consequent lack of Mg, which was also seen in both years with basal leaves chlorosis. In the trial neither foliar spraying nor fertirrigation with 3% $MgSO_4$ solution could overcome this problem. According with Capps and Wolf (2000), Haefs *et al.* (2002) and Stefanini *et al*. (1994), foliar or soil application of $MgSO_4$ could be very effective in enhancing Mg levels in grapevine blades and petioles or on the other hand, no measurable effects could be obtained depending on experimental conditions (e.g. application rate and distribution timing and frequency). In the present study, some differences in Mg contents between treatments were showed (#2-8; Figure 2), that were slightly modified between blades and petioles and much affected by the season. Thus, the effects of Mg fertilization cannot be surely described.

Relatively strong correlation between K and Mg ($r > -0.5$; $p = 0.05$) confirmed the already known antagonistic relationship of these two elements (Bergmann, 1992; Fregoni, 1998). The K/Mg ratio in petioles, which is very important factor in determining Mg uptake, was very high in all treatments (on average 30-40) except in untreated vines (9-10) where it was slightly below the minimal threshold comparing to Fregoni (1998).

If fertilization treatments resulted in correlations between K and Mg, not so clear and reliable relationships were found as regard other two elements, in our study Fe and Zn. The amounts of Fe at berry set 2008 were found to be adequate or even a bit in excess both in blades and petioles. At veraison 2008 and in both sampling times in 2009, Fe concentrations were comparable and in agreement with Fregoni (1998) and with Bigot et al. (2009), low to marginal (< 100 vs. < 25 mg kg^{-1} in blades and petioles, respectively; Figure 3). The same results were described by Díaz *et al.* (2010) with very high Fe contents in petioles of one-year-old grapevines and much lower Fe concentrations in the following years. According to Tagliavini and Rombolà (2001), SO_4 rootstock is moderately tolerant to iron chlorosis and despite low to marginal Fe concentration, young vines does not show chlorotic symptoms as reported also by Bergmann (1992).

Moving on veraison 2009, the differences between soil and foliar applications of Fe disappeared in blades, thus suggesting that plants need long time in order to acquire Fe from the soil and really short to get it from foliar fertilisers. The results obtained at berry set by foliar spraying are suggesting that Fe-ions in the commercial "Foliacon Fe" solution could penetrate through the leaves significantly enhancing foliar Fe concentration. Despite to not significant differences, at veraison 2008 the differences among treatments were reduced as compared with berry set, but some more content of Fe was still observed when the same element was added throughout foliar fertilization. Fe and Zn enhancement could be partly due to nitrogen, because it is well known that N-ions may increase the concentration of other elements such as P, Mn, Cu and thereby Fe and Zn (Assimakopoulou, 2006; Clark *et al*., 2003).

In many experiments, the effectiveness of Fe application was widely variable because of several plant-related, environmental and physico-chemical factors that in different ways may affect plant physiology and growth (Fernández et al., 2006; 2008; 2009; Fernández and Ebert, 2005; Abadía et al., 2011). Problems of reproducibility and interpretation of results from foliar Fe-application studies have been described elsewhere (Fernández and Ebert, 2005). Fernández et al. (2008) showed that foliar application of different Fe-containing compounds [Fe(III)-citrate, many Fe(III)-chelates] markedly increased Fe concentration in peach leaves. On the opposite, Díaz et al. (2010) working with one to three-years-old potted grapevines, reported that Fe contents in petioles of vines sprayed with Fe chelate (FeEDDHA) and synthetic vivianite were not significantly enhanced as compared with untreated plants that even showed chlorosis symptoms.

When analysing blades at berry set and petioles at veraison, more than nitrogen and sulphur, Bigot et al. (2009) did not provide significant correlations between nutrients in different growing periods and comparing years, thus approving that micro-nutrients are deeply affected by many factors not easily to standardise.

The effects of various treatments on Zn concentration have also been evaluated, and many contrasting results within years and among treatments, not easy to explain, were highlighted. In the petioles of untreated vines, the measured Zn content was low-to-marginal at berry set and within an optimal range at veraison of both years (30-60 mg kg^{-1}; Bigot *et al.*, 2009; Figure 4). On the opposite, in all treated vines the measured Zn content falls into the optimal range at first sampling, but at veraison, Zn concentrations exceed the maximal threshold for this nutrient in the treatments #3-8. The measured concentrations of Zn in the grapevine leaves during two-year experiment revealed that application of Mg and/or Fe resulted in a comparable effect on soil

Zn uptake and its accumulation in the leaves, mainly in petioles.

Contrary to our findings, Díaz *et al.* (2010) found that the application of Fe-chelate fertilizers and synthetic vivianite in one to three-years-old potted grapevines had no effect on Zn concentration or even a reduction was shown in the first year of application. Similar experiments in other plants revealed that application of Fe either as a foliar spray or used in irrigation system decreased Zn concentration in the tomato and spinach leaves, respectively (Assimakopoulou, 2006; Kaya *et al.*, 1999). Additionally, antagonistic relationship was reported by Kaya *et al.* (2002) also by foliar application of Zn which decreased Fe levels in tomato leaves.

As far as we know, the increased concentration of Zn in plant tissues (e.g. in the grapevine petioles) due to the effect of Mg and Fe fertilizers applications was not seen before. Since our conclusions are based on the two years study, further research in this way has to be carried out to verify these statements.

4. Conclusions

The results of the trial carried out with potted 'Rebula' grapevines showed that K fertilization is a critical factor decreasing Mg concentration in grapevine leaves causing Mg chlorosis also for 'Rebula' grapevines. Due to very high ratio between K and Mg and its well known antagonistic relationship, neither foliar spraying nor fertirigation with 3% $MgSO_4$ solution could overcome this problem. Looking at the main differences, under the experimental conditions studied, the treatments with addition of Mg and Fe fertilizers ("Bittersalz" and "Folicacon Fe") resulted in increased concentration of Zn in the petioles and Fe in blades in larger extend than in vines exposed to N fertilization alone. Since we cannot give the clear explanation of the phenomena we can speculate that the application of Fe and Mg stimulates the absorption Thus the relationships among Mg, Fe and Zn should be studied more in detail in order to ascertain the relative importance of both foliar and soil fertilization.

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