

LONG-TERM EFFECTS OF ORGANIC FERTILIZERS ON MACROELEMENTS STATUS IN GRAPEVINE LEAF ON CALCAREOUS SOIL

Tomislav KARAŽIJA¹, Mihaela ŠATVAR¹, Tihana KEŠER², Sanja SLUNJSKI¹,
Boris LAZAREVIĆ¹, Marko PETEK¹

¹University of Zagreb, Faculty of Agriculture, Department of Plant Nutrition, Svetošimunska cesta 25, Zagreb, Croatia

²University of Zagreb, Faculty of Agriculture, student, Svetošimunska cesta 25, Zagreb, Croatia

Corresponding author email: Tomislav KARAŽIJA, tkarazija@agr.hr

Abstract

Growing grapevines on calcareous soils often leads to plant nutrition disorders. Vineyard fertilization practices on such soils differ significantly from the fertilization on acid soils because of influence of free calcium and high pH on the availability of nutrients. Three year trial was set up according to randomized complete block design with 6 treatments (unfertilized, farmyard manure 20 t ha⁻¹ and 40 t ha⁻¹, peat 20 000 L ha⁻¹ and 40 000 L ha⁻¹, NPK (5-20-30) 500 kg ha⁻¹+200 kg UREA kg ha⁻¹) in 4 repetitions. Samples of grapevine leaves were taken three times during the growing period: at the flowering, two weeks after flowering and at the veraison. Total nitrogen was determined by the modified Kjeldahl method, phosphorus was determined spectrophotometrically, potassium flamephotometrically and calcium and magnesium atomic absorption spectrometrically. Significant effect of fertilization was determined in the first year of the study on the amount of calcium (two weeks after flowering) and in the third year on the amount of potassium (flowering). Fertilization did not significantly affect the amount of nitrogen, phosphorus and magnesium in grapevine leaves.

Key words: grapevine, nitrogen, phosphorus, potassium, organic fertilizers.

INTRODUCTION

Soils are conditionally renewable natural resources and their sustainable use is possible only with appropriate agrotechnics (cultivation and fertilization), regulation of the air regime, decomposition of plant residues, and prevention of degradation and pollution (Varallay, 2007). In the last few decades intensive agriculture showed negative effects on soil and environment (loss of organic matter, soil erosion, pollution of groundwater, etc. (Zhao et al., 2009). Calcareous soils are characterized by a high proportion of carbonates with high pH values and thus high concentrations of HCO₃⁻ ions in soil solution (Mengel & Kirkby, 2001). Soil carbonates are derived from calcium and magnesium minerals (calcite CaCO₃ and dolomite CaCO₃ x MgCO₃). Their decomposition releases calcium and magnesium, which are important plant nutrients. In contrast, calcareous soils are associated with a soil reaction higher than 7.0 pH (pH_{KCl}), which leads to disruption in the

uptake of some plant nutrients (Čoga et al., 2011).

Soil organic matter is a basic indicator of soil quality (Bouajila and Sanaa, 2011) and has two basic roles: nutritional, which arises from the mineralization of organic nitrogen, sulfur and phosphorus (Kaur et al., 2005) and physical, because it improves soil properties such as water capacity, drainage, structure. In sandy soils, it is the only stable source of most available plant nutrients (Gladstones, 1994).

Use of modern agricultural techniques, with the use of grapevine cultivars with high-yielding, potential causes numerous problems in grapevine nutrition. So, fertilization of vineyards is an agro-technical practice that has a great impact on the yield and quality of must and wine. Fertilizer application increases yields, however excessive or unbalanced fertilization can have a negative impact on quality (Delgado, 2004). On the other hand, the use of organic fertilizers is a traditional agricultural practice (Tagliavini, 2001), and in the last few decades their influence on the

physical, chemical and biological properties of soil has been updated. This is the result of increased production and the desire to safely dispose of organic waste (manure from fattening yards and farms, composted municipal waste and sludge), at higher doses, which far exceed the application rates in traditional agronomic practice (Haynes i Naidu, 1998). According to Herencia et al. (2008), application of organic materials affects the distribution of biogenic elements and has positive effect on availability of microelements to plant. Furthermore, the use of organic fertilizers is associated with desirable soil characteristics, including higher water capacity, cation exchange complex, and lower soil bulk density. It also promotes the growth and development of beneficial microorganisms (Bulluck et al., 2002). The beneficial effect of organic fertilizers on the amount of nitrogen, phosphorus and potassium in grapevine leaves is probably result of an increase in soil organic matter (El-Wahab, 2011). Plant nutrition based on organic fertilizers as a source of plant nutrients has a different dynamics of nutrient availability compared to plant nutrition involving the use of mineral fertilizers, because the biomass of microorganisms in organic matter is relatively small, but very important in the circulation of nutrients in soil (Ladd et al., 2004; Kaur et al., 2005). According to El-Wahab (2011) fertilization of vineyards with organic fertilizers is a good alternative for supplying macro and microelements. However, only a small fraction of mineralized nitrogen, phosphorus and sulfur from organic matter is absorbed from depth more than 50 cm. However, some authors consider that distinctive character of wines obtained from older vineyards is the result of absorption of calcium, magnesium, iron and manganese from greater depths (layers) of the soil (White, 2009). On the other hand, the positive effects of the combination of organic and mineral fertilizers on increasing the yield, mass and length of the cluster are probably the result of meeting needs of the vines on nutrients from different sources over a longer period, as well as their impact on better availability of soil nutrients, resulting in better nutrient status in the vine (El-Wahab, 2011). Therefore, according to some authors (Efthimiado, 2010;

Islam et al., 2011) integrated plant nutrition represents the best approach for improving or maintaining soil fertility and productivity on a sustainable basis.

Soils of Plešivica wine region have heavy mechanical composition, low air capacity, and a high content of activated lime (CaO), which favors the formation of HCO_3^- ions, which can decrease or inhibit absorption of plant nutrients. Under such conditions, various chlorosis often occurs (Petek et al., 2008). Methods of fertilization of vineyards on these soils vary considerably from fertilization on acid soils due to the significant impact of free calcium and high pH values on the availability of nutrients, and chemical reactions that encourage permanent loss or fixation of plant nutrients (Ksouri et al., 2005).

Nitrogen is one of the main plant nutrients, as part of proteins, enzymes, amino acids, polypeptides and many biochemical compounds in the plant system. It is essential for cell division and development of meristem tissue (Mengel & Kirkby, 1987; El-Wahab, 2011). Nitrogen leaf concentration is often not correlated with increased growth and yield. Lack of other nutrients with water or temperature stress can cause an increase in total nitrogen in the tissue with a simultaneous decline in yields (Barker & Pilbeam, 2007). Decreasing of nitrogen leaf concentration begins at the beginning of ripening (veraison), when starts decomposition of proteins into amino acids and their transfer into the berries of clusters (Wermelinger, 1991). Excessive nitrogen supply causes increased vegetative growth, which competes with sugar translocation and pigment accumulation in grapes. It also interferes with the metabolic pathways of synthesis of compounds responsible for aroma (Bravdo & Hepner, 1987; Delgado, 2004). According to (El-Wahab, 2011), organic fertilizers are considered as more desirable source of nitrogen due to mineralization that allows slower release of nitrogen over a longer period. Moreover, the use of organic fertilizers (slow available nitrogen) has the effect on reducing the accumulation of nitrates in the plant compared to mineral fertilizers (fast available nitrogen) (EL-Sisy, 2000).

Phosphorus, along with nitrogen and potassium, is the most important plant nutrient. For high yields it is often lacking in the soil, so it is added by fertilizers. Phosphorus plays an important role in the process of plant growth. It is a constituent of phosphatides, nucleotides, nucleic acids, enzymes, and as a reserve in the plant, phosphorus is most commonly bound in phytic acid (Čoga et al., 2011). According to Čoga et al. (2008), the basic factor that determines the solubility and availability of phosphorus to plant is the soil pH or saturation of the adsorption complex by the bases. Under neutral and alkaline pH conditions, various forms of Ca-phosphates are dominated, which are more readily soluble than aluminum and ferric phosphates in acid soils. According to (Eghball et al., 2002), a large proportion of phosphorus (> 75%) in organic fertilizers is in inorganic form, which indicating high utilization after application.

Potassium is the only monovalent cation essential for plant growth. It is dominant inorganic cation in the plant cell, and makes 0.5 to 6% weight of plant dry matter (Pavalek-Kozlina, 2003). Unlike nitrogen and phosphorus, potassium does not a constituent of organic matter. Its importance arises from its activity in ionic form and high mobility in the plant (Bergman, 1992). Grapevine is a large consumer of potassium, and it is most widely uptake from the beginning of the vegetation to flowering, 5/8, and the rest of from veraison to harvest (Mirošević and Karlogan Kontić, 2008). According to Brancadoro et al. (1994) the highest amount of potassium in leaves and musts was determined on rootstocks 44-53 M and SO4, and the lowest amount of potassium on rootstocks 140 Ru, 420 A and 1202 C. This effect is especially pronounced in the dry years. The effect of potassium fertilization on the status of potassium in the grapevinevine depends on the type of soil, as well. In soils with a heavy mechanical composition, its fixation is greater and the risk of leaching is reduced, while the water retention capacity is much higher (Kuchenbuch, 1986). According to Čoga et al. (2011), the highest concentrations of K in grapevine leaves were determined on strongly acidic soil at flowering stage, while the lowest concentration was determined on calcareous soil at harvest.

Magnesium is required for a large number of processes for organic matter synthesis. It has a positive effect on the metabolism of carbohydrates, proteins and fats, Together with other cations, influences protoplasmic colloids and activates a large number of enzymes (Vukadinović & Vukadinović, 2011). Although in calcareous soils the amounts of total magnesium are sufficient to supply the plants, due to the high amount of calcium in the soil, an imbalance of the Ca-Mg cation is often obtained. Furthermore, in calcareous soils more than 80% of exchangeable cations is calcium, while the amount of exchangeable magnesium is less than 4%, which often leads to magnesium deficiency in the plant (Hugin & Tucker, 1982). Calcium is a component of a small number of organic molecules, but it participates in the structure of the cell membrane, reducing the hydration of the protoplasm and increasing its viscosity, and in cell division. In the central lamella, calcium is bound to the pectate, which is essential for cell wall strength (Bergman, 1992).

MATERIALS AND METHODS

Three years fertilization trial was set up on Plešivica wine-growing region, Borička location (northwestern Croatia), in a 10-year old vineyard, cv. Sauvignon White grafted on Kobber 5BB rootstock, planted on soil with quite high pH for grapevine growing (pH_{H2O} 8.02), containing 2 mg P₂O₅ 100 g⁻¹ soil, 14 mg K₂O 100 g⁻¹ soil and 13.5% CaO. The trial was set up according to randomize complete block design with 6 treatments: unfertilized (C), farmyard manure 20 t ha⁻¹ (FM 1) and 40 t ha⁻¹ (FM 2), peat 20 000 L ha⁻¹(P 1) and 40 000 L ha⁻¹ (P 2), NPK (5-20-30 500 kg ha⁻¹ + 2 x 100 kg UREA kg ha⁻¹) in 4 repetitions. Samples of grapevine leaves were taken three times during the growing period: at the flowering, two weeks after flowering and at the veraison. Average leaf samples were formed from 80 healthy, fully developed and undamaged leaves, taken opposite to clusters from 40 vinestocks (4 replicates x 10 vinestocks). Dried (105°C) homogenized grapevine leaf samples were analyzed in triplicate and the results are presented as mean values.

Total nitrogen was determined by the Kjeldahl method. After digestion with concentrated HNO₃

phosphorus was determined spectrophotometrically, potassium flamephotometrically and calcium and magnesium atomic absorption spectrometrically (AOAC, 1995). Statistical data analyses were performed using the SAS 8.2 System (2002-2003).

RESULTS AND DISCUSSIONS

Nitrogen leaf content (Table 1) is affected by a large number of factors, however, fertilization with organic fertilizers had no a significant effect in any year of research by individual sampling (flowering, two weeks after flowering, veraison).

Table 1. Amount of nitrogen (% N in DW) in grapevine leaves under different fertilization treatments in three year experiment

% N DW				
2008				
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	2.47	1.61	1.46	1.85
FM 1	2.52	1.58	1.31	1.80
FM 2	2.53	1.63	1.56	1.90
P 1	2.42	1.50	1.29	1.74
P 2	2.49	1.58	1.45	1.84
NPK	2.35	1.55	1.45	1.78
Average	2.46 a	1.57 b	1.42 b	
2009				
C	3.63	2.36	1.90	2.63 b
FM 1	3.70	2.46	1.93	2.69 b
FM 2	3.93	2.71	2.11	2.91 a
P 1	3.65	2.46	1.98	2.70 b
P 2	3.76	2.45	1.91	2.70 b
NPK	3.72	2.51	2.03	2.75 ab
Average	3.73 a	2.49 b	1.97 c	
2010				
C	3.51	2.32	2.26	2.70 ab
FM 1	3.39	2.28	2.18	2.61 b
FM 2	3.47	2.61	2.33	2.80 ab
P 1	3.59	2.43	2.31	2.77 ab
P 2	3.74	2.47	2.26	2.82 ab
NPK	3.73	2.59	2.50	2.94 a
Average	3.57 a	2.45 b	2.30 b	

Factor level means accompanied by different letters are significantly different, with error $p \leq 0.05$ according to Tukey's HSD test. Means without any letter indicate no significant differences.

In the first year of the study, nitrogen concentrations (% N) in leaf dry matter ranged from 2.35 to 2.53% N, in 2008 from 3.63 to 3.93% N, and in 2009 from 3.39 to 3.74% N. The average values of nitrogen in the veraison stage ranged from 1.29 to 1.56% N in 2008, from 1.90 to 2.11% in 2009, and from 2.18 to 2.50% in 2010. In the first year of the study, the average values in the veraison stage were below the optimal range, because according to

Fregoni (1998), the optimal concentrations of nitrogen in the veraison is between 1.60 and 2.65% N. This is probably due to the early ripening of the grapes (remobilization of nitrogen from the leaves into the berries) as explained by Wermelinger (1991). However, according to the average annual values of the nitrogen leaf content of individual treatments, in 2009 and 2010 statistically significant differences were determined. The highest annual average values in 2009 were determined on treatment with 40 t ha⁻¹ farmyard manure (2.91% N) and in 2010 on treatment with NPK fertilization (2.94% N), although according to Barker and Pilbeam (2007), nitrogen concentration in leaves is often not correlated with the increase in growth and yield, and lack of other nutrients with water or temperature stress can cause an increase in total tissue nitrogen. In all three years (Figure 1), the average concentration values decreased from the first to the third sampling, as confirmed by some authors (Löhnertz et al., 1988; Wermelinger, 1991).

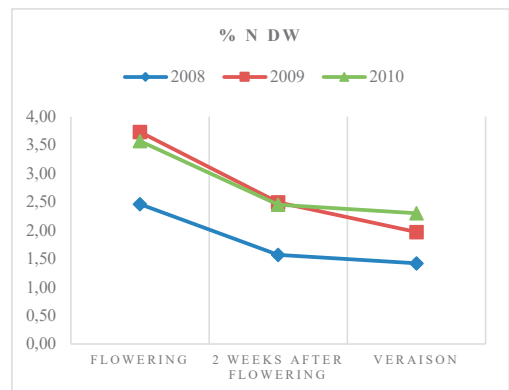


Figure 1. Average nitrogen content (% N in DW) in grapevine leaves by sampling

Grapevine is a crop that requires large quantities in the annual production cycle potassium and nitrogen, and significantly less phosphorus (Villa, 2005). Dynamics of phosphorus in grapevine leaves during the growing season (Figure 2) is similar to the dynamics of nitrogen, i.e. the amount in leaves falls towards the end of the growing season (Petek et al., 2008), while Skinner and William (1988) were found the highest

amounts of phosphorus at the harvest stage and the lowest at the flowering stage.

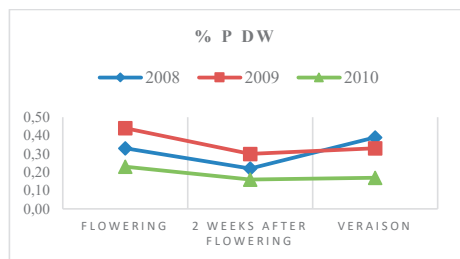


Figure 2. Average phosphorus content (% P in DW) in grapevine leaves by sampling

The results in this study varies (Table 2), in the second and third year of research, the highest average amount of phosphorus in the grapevine vine leaves according to the sampling was determined at flowering stage (0.44 and 0.23% P), while in the first year, the highest amount was recorded in the veraison stage (0.33% P).

Table 2. Amount of phosphorus (% P in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

% P DW				
2008				
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	0.31	0.22	0.39	0.31
FM 1	0.33	0.22	0.39	0.31
FM 2	0.32	0.22	0.42	0.32
P 1	0.33	0.22	0.40	0.32
P 2	0.34	0.22	0.38	0.31
NPK	0.32	0.21	0.37	0.30
Average	0.33 b	0.22 c	0.39 a	
2009				
C	0.45	0.31	0.33	0.36 ab
FM 1	0.42	0.26	0.27	0.31 b
FM 2	0.48	0.33	0.38	0.40 a
P 1	0.42	0.28	0.32	0.34 ab
P 2	0.46	0.32	0.37	0.39 a
NPK	0.42	0.30	0.32	0.34 ab
Average	0.44 a	0.30 b	0.33 b	
2010				
C	0.24	0.16	0.16	0.19
FM 1	0.23	0.15	0.18	0.19
FM 2	0.24	0.16	0.16	0.18
P 1	0.23	0.16	0.16	0.18
P 2	0.22	0.17	0.19	0.19
NPK	0.22	0.16	0.16	0.18
Average	0.23 a	0.16 b	0.17 b	

Factor level means accompanied by different letters are significantly different, with error $p \leq 0.05$ according to Tukey's HSD test. Means without any letter indicate no significant differences.

According to Fregoni (1998), the optimum phosphorus content in leaves is 0.15 to 0.38% P at flowering stage, while (Bergmann, 1992) states a general range of 0.25-0.45% P/DW.

The percentage of phosphorus in the leaf at flowering stage ranged from 0.31 to 0.34% in 2008, then from 0.42 to 0.48% in 2009, and from 0.22 to 0.24 in 2010. So, average values in the first and third years are in the range of optimal, while in 2010 they were slightly higher. In the second year of research in all sampling, the relatively highest amount of phosphorus in leaves was determined on treatment with 40 t ha⁻¹ farmyard manure (0.48, 0.33, and 0.38% P), which is similar to Masoud (2012), who found the highest average percentage of phosphorus in grapevine leaves on treatment with compost.

In the this research (Table 3), fertilization with organic fertilizers had significantly influenced the amount of potassium in the third year at flowering stage, when the highest amount of potassium was determined with fertilization of 40 t ha⁻¹ of farmyard manure (1.22% K), while the lowest amount was recorded on control treatment (0.79% K).

Table 3. Amount of potassium (% K in DW) in grapevinevine leaves under different fertilization treatments in three year experiment

% K DW				
2008				
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	0.74	0.75	0.79	0.76
FM 1	0.71	0.65	0.76	0.71
FM 2	0.72	0.69	0.68	0.70
P 1	0.72	0.65	0.73	0.70
P 2	0.72	0.71	0.73	0.72
NPK	0.82	0.91	0.77	0.83
Average	0.74	0.73	0.75	
2009				
C	0.79	0.76	0.63	0.72 b
FM 1	0.85	0.74	0.64	0.74 b
FM 2	0.82	0.87	0.74	0.81 ab
P 1	0.85	0.77	0.69	0.77 ab
P 2	0.80	0.81	0.69	0.77 ab
NPK	0.89	0.94	0.84	0.89 a
Average	0.83 a	0.81 a	0.73 b	
2010				
C	0.79 b	0.87	0.78	0.81 b
FM 1	0.85 b	0.80	0.72	0.79 b
FM 2	1.22 a	1.20	0.95	1.12 a
P 1	0.97 ab	1.04	0.79	0.93 ab
P 2	1.00 ab	0.96	0.78	0.92 ab
NPK	0.87 ab	1.10	0.82	0.93 ab
Average	0.95 a	0.99 a	0.80 b	

Factor level means accompanied by different letters are significantly different, with error $p \leq 0.05$ according to Tukey's HSD test. Means without any letter indicate no significant differences.

The same trend was observed in the second and third sampling, although no statistically significant differences were found. Therefore, the

average annual value was statistically significantly highest for fertilization with 40 t ha⁻¹ of manure (1.12% K).

According to Fregoni (1998), the optimum amount of potassium in grapevine leaves during development of berries ranges between 0.65 and 1.70% K and during the veraison from 0.50 to 1.60% K, while Vercesi et al. (1993) state that potassium deficiency occurs when the concentration in leaves is lower than 0.57% K, while a toxic effect can be expected when concentration is higher than 1.46% K.

Determined potassium values for treatment with 40 t ha⁻¹ of farmyard manure in Sauvignon Blanc grafted on SO4 were in accordance Herak Custić et al. (2008), for calcareous soils. Results are also in line with studies (Morlat & Saymoneaux, 2008), which state that the highest amount of potassium in a multi-year fertilization experiment is regularly determined on the treatment with the highest amount of organic fertilizer (20 t ha⁻¹ of manure).

According to the average values of each sampling in 2009 and 2010 (Figure 3), statistically significant highest values of potassium were determined at flowering stage (0.83 and 0.82% K), respectively, and two weeks after flowering (0.81 and 0.99% K). The lowest values were determined at veraison stage (0.70 and 0.80% K). This is in accordance with Čoga et al. (2011) who found the highest concentrations of K at flowering stage on very acidic soil, while the lowest concentration on calcareous soil was determined at harvest.



Figure 3. Average potassium content (% K in DW) in grapevine leaves by sampling

On the other hand, the results are in contrary to Schreiner and Scagel (2006) because potassium

concentrations in leaves of Pinot noir cultivar increased from the beginning of the vegetation up to the harvest during the two-year trial.

Results show that fertilization with organic fertilizers did not significantly affect the change in the percentage of calcium in leaves (Table 4).

According to Fregoni (1998), the optimal percentage of calcium in grapevine leaves is 1.70% of 3.80% Ca in dry matter, and a deficit occurs when it falls below 1.41% Ca, as reported by Vercesi et al. (1993) who state that calcium deficiency occurs when the concentration in leaves is less than 1.41% Ca.

Table 4. Amount of calcium (% Ca in DW) in grapevine leaves under different fertilization treatments in three year experiment

% Ca DW				
2008				
Phenophase	Flowering	After flow.	Veraison	Average
Treatments				
C	1.88	1.78 ab	2.25	1.97
FM 1	1.97	1.84 a	2.91	2.24
FM 2	1.88	1.73 ab	2.80	2.13
P 1	1.96	1.80 ab	2.69	2.15
P 2	1.81	1.51 ab	3.09	2.13
NPK	1.92	1.44 b	2.78	2.05
Average	1.90 b	1.68 c	2.75 a	
2009				
C	2.98	3.73	6.04	4.30
FM 1	2.82	4.04	5.51	4.10
FM 2	2.99	3.84	6.10	4.30
P 1	2.77	3.97	5.50	4.01
P 2	2.88	4.02	6.06	4.30
NPK	3.06	4.17	5.90	4.40
Average	2.92 c	3.96 b	5.85 a	
2010				
C	1.56	1.67	2.89	2.04
FM 1	1.55	1.73	2.86	2.05
FM 2	1.61	1.74	2.74	2.03
P 1	1.55	1.73	2.87	2.05
P 2	1.58	1.55	2.81	1.98
NPK	1.79	1.66	2.87	2.11
Average	1.60 b	1.68 b	2.84 a	

Factor level means accompanied by different letters are significantly different, with error $p \leq 0.05$ according to Tukey's HSD test. Means without any letter indicate no significant differences.

In these study, at the flowering stage the percentage of calcium in leaves in 2008 ranged from 1.81 to 1.96% Ca, in 2009 from 2.77 to 3.06% Ca, and in 2010 from 1.55 to 1.79% Ca, which means that in the first and second years the values were in the range of optimal values.

According to average values of individual sampling (Figure 4), in all three years of research statistically significant lowest amounts were determined at flowering stage (1.90, 2.92 and 1.60% Ca), while statistically significant highest values were determined at veraison

stage (2.75, 5.85, 2.84% Ca), which is in accordance to Gluhic et al. (2009), who state that amount of calcium in grapevine leaves depends of amount of active lime in the soil and sampling time, so that the amount in leaves increases through the vegetation and reaches very high values at the end of the vegetation (first sampling 1.66% Ca - fourth sampling 4.85% Ca).

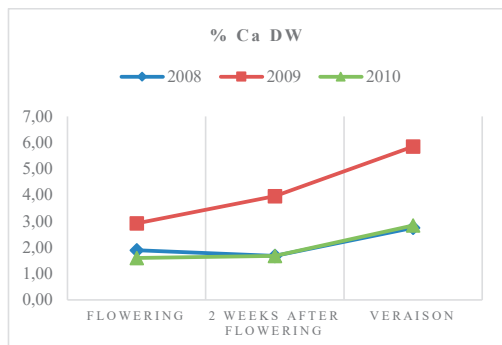


Figure 4. Average calcium content (% Ca in DW) in grapevine leaves by sampling

Although fertilization did not significantly affect the magnesium content in grapevine leaves, according to annual average values, in all three years of research, the relatively highest magnesium content (annual average of treatment) was determined on treatment with 40 t ha⁻¹ of farmyard manure (0.36; 0.47 and 0.41% Mg).

In this study (Table 5), at the flowering stage, the percentage of magnesium in leaves in 2008 ranged from 0.25 to 0.29% Mg, in 2009 from 0.21 to 0.31% Mg, and in 2010 from 0.28 to 0.34% Mg, which is in line with Fregoni (1998), who states that for optimal plant growth magnesium requirements range from 0.15 to 0.35% Mg in the dry matter of vegetative part of the plant, and for grapevine at flowering stage 0.15 to 0.45% Mg and 0.17 to 0.60% Mg at veraison stage.

Furthermore, according to average sampling values, in the second and third year statistically significant the lowest values were determined at flowering stage (0.25 and 0.30% Mg), and the highest values at veraison stage (0.60 and 0.55%).

Average values have increased from the beginning of the vegetation to ripening (Figure 5), which is in accordance with Conradie

(1981), who concludes that magnesium uptake increases around flowering, when is translocated to growth sites, and continues until ripening when increase magnesium reserves in roots, shoots and leaves.

Table 5. Amount of magnesium (% Mg in DW) in grapevine leaves under different fertilization treatments in three year experiment

% Mg DW				
Phenophase	2008			
	Flowering	After flow.	Veraison	Average
Treatments				
C	0.25	0.26	0.26	0.26
FM 1	0.29	0.31	0.34	0.31
FM 2	0.29	0.32	0.48	0.36
P 1	0.29	0.37	0.35	0.33
P 2	0.29	0.31	0.32	0.30
NPK	0.27	0.36	0.37	0.33
Average	0.28	0.32	0.35	
	2009			
C	0.21	0.39	0.59	0.40
FM 1	0.23	0.45	0.56	0.41
FM 2	0.31	0.45	0.66	0.47
P 1	0.25	0.44	0.60	0.43
P 2	0.26	0.47	0.62	0.43
NPK	0.26	0.44	0.56	0.42
Average	0.25 c	0.44 b	0.60 a	
	2010			
C	0.30	0.34	0.54	0.39
FM 1	0.28	0.34	0.52	0.38
FM 2	0.31	0.31	0.60	0.41
P 1	0.29	0.33	0.52	0.38
P 2	0.28	0.31	0.56	0.38
NPK	0.34	0.31	0.55	0.40
Average	0.30 b	0.32 b	0.55 a	

Factor level means accompanied by different letters are significantly different, with error $p \leq 0.05$ according to Tukey's HSD test. Means without any letter indicate no significant differences.

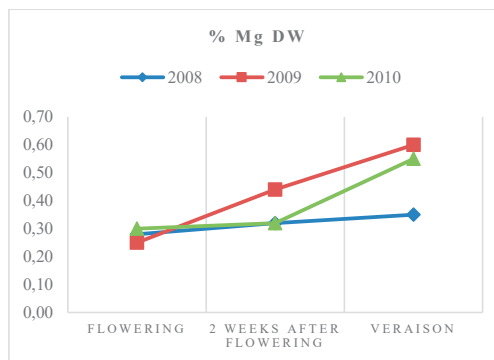


Figure 5. Average magnesium content (% Mg in DW) in grapevine leaves by sampling

Results are also in accordance with Gluhic et al. (2009) who states that magnesium concentration in grapevine leaves ranges between 0.25% at the beginning of vegetation and 0.64% Mg at the end of vegetation. However, the results obtained are in collision to

Schreiner and Scigel (2006), who states that magnesium concentration in leaves of Pinot noir cultivars decreases during vegetation.

CONCLUSIONS

Significant effect of fertilization was determined in the first year of the study on the amount of calcium (two weeks after flowering) and in the third year on the amount of potassium (flowering). Fertilization did not significantly affect the amount of nitrogen, phosphorus and magnesium in grapevine leaves.

REFERENCES

- Abd El-Wahab, M.A. (2011). Reducing the Amount of Mineral Nitrogen Fertilizers for Red Globe Grapevines by Using Different Sources of Organic Fertilizers. *Journal of American Science*, 2011;7(8):810-819.
- AOAC (1995). Official method of analysis of AOAC International, 16th Edition, Vol. I, Arlington, USA.
- Barker A.V., Pilbeam David J. (2007). *Handbook of plant nutrition*. Taylor & Francis Group, LLC, USA.
- Bouajila K., Sanaa M. (2011). Effects of organic amendments on soil physico-chemical and biological properties. *J. Mater. Environ. Sci.* 2 (S1):485-490.
- Brancadoro L., Valenti L., Reina A., Scienza A. (1994). Potassium Content of Grapevine during the Vegetative Period: The Role of the Rootstock. *Journal of Plant Nutrition*, 17(12), pp. 2165-2175.
- Bravdo B., & Hepner Y. (1987). Irrigation management and fertigation to optimise grape composition and vine performance (Review). *Acta Hort.*, 206: 49-67.
- Bulluck L.R., Brosius M., Evanylo G.K., Ristaino J.B. (2002). Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. *Applied Soil Ecology*, 19:147-160.
- Conradie W.J. (1981). Seasonal Uptake of Nutrients by Chenin blanc in Sand Culture II. Phosphorous, Potassium, Calcium and Magnesium. *South African Journal of Enology and Viticulture*, 2(1), pp. 7-13.
- Čoga, L.; Slunjski, S.; Herak Čustić, M.; Gunjača, J.; Cosić, T. (2008). Phosphorus dynamics in grapevine on acid and calcareous soils. *Cereal research communications* (0133-3720) 36, S5, Part 1; 119-122.
- Čoga, L.; Slunjski, S.; Pavlović, I.; Jurkić, V.; Benčić, Đ. (2011). Leaf Zn concentrations of grapevine (*Vitis vinifera* L.) on acid and calcareous soils. *Proceedings of the 11th International Conference on the Biogeochemistry of Trace Elements*, Firenze, Italija.
- Delgado R., Martín P., Álamo M., González M.-R. (2004). Changes in the phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilisation rates. *Journal of the Science of Food and Agriculture*. 84: 623-630.
- Efthimiadou A., Bilalis D., Karkanis A., Froud-Williams B. (2010). Combined organic/inorganic fertilization enhance soil quality and increased yield, photosynthesis and sustainability of sweet maize crop. *AJCS*. 4(9):722-729.
- Eghball B., Wienhold, B. J., Gilley J.E., Eigenberg R. A. (2002). Mineralization of manure nutrients. *Journal of soil and water conservation*. 57:469-473.
- EL-Sisy, L.M.H. (2000). Assessing the pollution caused by excessive nitrogen fertilization. *J. Agric. Sci., Mansoura Univ.*, 25(11): 7297-7313.
- Fregoni M. (1998). *Viticultura di quqlita*. In: *La nutrizione minerale della vite*. 493-579.
- Gladstones J. (1994). *Viticulture and Environment*, Paris, 33-45.
- Gluhic D., Herak Čustić M., Petek M., Čoga L., Slunjski S., Sinčić M. (2009). The Content of Mg, K and Ca Ions in Vine Leaf under Foliar Application of Magnesium on Calcareous Soils. *Agriculturae Conspectus Scientificus* (1331-7768) 74, 2; 81-84.
- Haynes R.J. i Naidu R. (1998). Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. *Nutrient Cycling in Agroecosystems*, 51:123-137.
- Herak Čustić, M.; Gluhic, D.; Čoga, L.; Petek, M.; Gošćak, I. (2008). Vine plant chlorosis on unstructured calcareous soils and leaf Ca, Mg and K content. *Cereal research communications* (0133-3720) 36, Part 1. Suppl.; 439-442.
- Herencia J. F., Ruiz J. C., Morillo E., Melero S., Villaverde J., Maqueda C. (2008). The effect of organic and mineral fertilization on micronutrient availability in soil. *Soil Science*, 69-80.
- Hugin J., Tucker B. (1992). *Fertilization of drayland and irrigated soils*, Srpinger Verlag, New York, USA
- Islam M.M., Karim A.J.M.S., Jahiruddin M., Majid N.M., Miah M.G., Mustaque Ahmed M., Hakim M.A. (2011). Effects of organic manure and chemical fertilizers on crops in the radish-stem amaranth-Indian spinach cropping pattern in homestead area. *AJCS*. 5(11):1370-1378.
- Kaur K., Kapoor K. K., Gupta A. P. (2005). Impact of organic manures with and without mineral fertilizers on soil chemical and biological properties under tropical conditions. *J. Plant Nutr. Soil Sci.*, 168:117-122.
- Ksouri R., Gharsalli M., Lachaal M. (2005). Physiological responses of Tunisian grapevine varieties to bicarbonate-induced iron deficiency. *Journal of Plant Physiology*. 162:335-341.
- Kuchenbuch R., Claasen N. , Jungk. A. (1986). Potassium availability in relation to soil moisture. *Plant Soil* 95:221-231.
- Ladd J. N., Amato M., Veen H.A. (2004). Soil microbial biomass: its assay and role in turnover of organic matter C and N. *Soil Biol. Biochem.* 36, 1369-1372.
- Löhnertz O. (1988.) *Untersuchungen zum zeitlichen Verlauf der Nährstoffaufnahme bei Vitis vinifera*. 228 pp. Thesis, University of Giessen. Geisenheimer Berichte.

- Masoud A. A. B. (2012). Effect Of Organic And Bio Nitrogen Fertilization On Growth, Nutrient Status And Fruiting Of Flame Seedless And Ruby Seedless Grapevines. *Research Journal of Agriculture and Biological Sciences*, 8(2): 83-91.
- Mengel K., Kirkby E. A. (2001). *Principles of Plant Nutrition*. Kluwer Academic Publishers. London.
- Mengel K., Kirkby E. A. (1987). *Principles of Plant Nutrition*. International Potash Institute, Bern.
- Mirošević N., Karoglan Kontić J. (2008). *Vinogradarstvo*, Zagreb, Hrvatska.
- Morlat R., Symoneaux R. (2008). Long-Term Additions of Organic Amendments in a Loire Valley Vineyard on a Calcareous Sandy Soil.III. Effects on Fruit Composition and Chemical and Sensory Characteristics of Cabernet franc Wine.*Am. J. Enol. Vitic.* 59:4.
- Pavalek-Kozlina B. (2003). *Fiziologija bilja*, Zagreb.
- Petek, M.; Gluhić, D.; Herak Čustić, M.; Čoga, L.; Čosić, T.; Slunjski, S. (2008). Leaf Content of Macro and Microelements in *Vitis vinifera* cv. Sauvignon Blanc. Book of Abstract. VI International ISHS Symposium on Mineral Nutrition on Fruit Crops, Faro, Portugal.
- SAS Inc. Copyright © 2002-2003 by SAS Institute Inc., Cary, NC, USA.
- Schreiner R.P., Scagel C.F. (2006). Nutrient Uptake and Distribution in a Mature 'Pinot noir' Vineyard. *HortScience*. 41(2):336-345.
- Skinner-William P. (1988). Phosphorus nutrition of grapevines (*Vitis vinifera* L.). University of California, Davis, 1988, 72 pages; AAT 8821725.
- Tagliavini M., Rombola A. D. (2001). Iron deficiency and chlorosis in orchard and vineyard ecosystems. 15: 71-92.
- Varallay Gy. (2007). Soil resilience (Is soil a renewable resource?). *Cereal Research Communications*, 35.2. 1277-1280.
- Vercesi, A., Bozzala, L., Fregoni, M. (1993). Diagnostica fogliare in viticoltura. 20 anni di esperienze, *Vignevini* 3:30-36.
- Villa, P. (2005). *Coltivare la Vite*, De Vecchi Editore. Milano, Italia.
- Vukadinović V., Vukadinović V. (2011). *Ishrana bilja*, Poljoprivredni fakultet Osijek.
- W., Bergmann. (1992). *Nutritional disorders of plants*. Gustav Fischer Verlag Jena, Stuttgart, New York.
- Wermelinger B. (1991). Nitrogen dynamics in grapevine: physiology and modeling. *Proc. Int. Symp. Nitrogen in grapes and Wine*, Seattle, USA (Ed. J.M. Rantz). 23-31.
- White R. E. (2009). *Understanding Vineyard Soils*. Oxford University Press, New York.
- Zhao Y., Wang P., Li J., Chen Y., Ying X., Liu S. (2009). The effect of two organic manures on soil properties and crop yields on a temperate calcareous soil under a wheat-maize cropping system. *Eur. J. Agron.* 31: 36-42.

