EFFECT OF NITROGEN FERTILIZATION ON THE CONTENT OF TRACE ELEMENTS IN CV. BIANCA GRAPEVINE (VITIS SP.)*

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Abstract

The knowledge of interactions between nitrogen and other nutrients and trace elements is the key to improving the uptake of nutrients. A study on a grapevine cultivar called Bianca was carried out in the Garlicki Lamus vineyard located in Garlica Murowana (near Krakow, Poland) in 2010-2011. The plants were treated with three nitrogen doses (0,50 and 100 kg N ha⁻¹) supplied as ammonium nitrate in a single application at three weeks pre-flowering. Samples of leaf petioles and blades, as well as grapes were taken. After wet microwave digestion in HNO3, some nutrient elements: B, Cu, Fe, Zn, Mn, Mo and Na, as well as trace elements: Al, Ba, Cd, Cr, Li, Ni, Sr, Ti and V were measured using the ICP-OES technique. Concentrations of microelements in the grapevine tissues were in the optimum (B, Cu, Fe, Zn and Mo) or high (Mn) range of content reported for 'full bloom' plants. N fertilizers enhanced leaf accumulation of trace elements such as Ti and V or depressed the uptake of some elements like B, Mn, Ba, Cd and Sr. Analyzed leaf blades contained higher amounts of Fe, Mn, Al, Ni, Pb, Ti and V than petioles. In contrast, petioles had more B, Zn, Mo, Cd, Ba, Li and Sr. Increased N fertilization diminished Cd and Ti (only at 50 kg N ha-1) in grape must; the reverse was true for Ba and Sr. The vintage strongly influenced grape mineral content. During warmer and wet year 2010, higher amounts Al, Cu, Fe and Ti were measured in fruits. The dry season in 2011 increased the content of Mn, B, Cd, Cr and Ni in fruits.

Key words: leaf analysis, nutrient status, microelements, environmental factors.

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WPŁYW NAWOŻENIA AZOTEM NA ZAWARTOŚĆ PIERWIASTKÓW ŚLADOWYCH W WINOROŚLI ODMIANY BIANCA

Abstrakt

Doświadczenie prowadzono w latach 2010-11 w winnicy Garlicki Lamus położonej w Garlicy Murowanej k. Krakowa. Krzewy winorośli nawożono trzema dawkami azotu (0, 50 i 100 kg N ha⁻¹) w postaci saletry amonowej. Nawóz podawano jednorazowo na 3 tygodnie przed kwitnieniem roślin. Do oznaczeń pobierano próbki liści (osobno blaszki i ogonki) oraz owoce. Po mineralizacji mikrofalowej w HNO_3 oznaczono, z wykorzystaniem spektrometrii ICP-OES, następujące mikroelementy: B, Cu, Fe, Zn, Mn, Mo i Na oraz pierwiastki śladowe: Al, Ba, Cd, Cr, Li, Ni, Sr, Ti i V. Zawartość mikroelementów w liściach była optymalna (B, Cu, Fe, Zn i Mo) lub wysoka (Mn) w porównaniu z liczbami granicznymi dla owocujących winnic. Nawożenie azotem wpłynęło na zwiększenie akumulacji Ti i V w liściach lub ograniczenie zawartości niektórych pierwiastków: B, Mn, Ba, Cd i Sr. Analizowane blaszki liściowe zawierały więcej Fe, Mn, Al, Ni, Pb, Ti i V niż ogonki liściowe. Natomiast ogonki akumulowały większe ilości B, Zn, Mo, Cd, Ba, Li i Sr. Poziom nawożenia azotowego wpłynąl na zmniejszenie zawartości Ba i Sr w moszczu winogron. Warunki podczas wegetacji (rocznik) miały istotny wpływ na skład mineralny zarówno liści, jak i owoców. Podczas cieplejszego, ale równocześnie mokrego sezonu 2010 stwierdzono wyższą zawartość Al, Cu, Fe i Ti w owocach. Suchy rok 2011 wpłynął na zwiększenie poziomu Mn, Ba, Cd, Cr i Ni w gronach.

Słowa kluczowe: analiza części wskaźnikowych, status odżywienia, mikroelementy, czynniki środowiskowe.

INTRODUCTION

Nutrition is an important aspect of vineyard management. Nitrogen fertilization increases vegetative growth and crop yield (Bell, Robinson 1999, Spayd et al. 2000, Ekbic et al. 2010). It also influences various parameters in vine production, such as fruit set, fruit quality and the quality of vine (Treeby et al. 2000, Rodrigue-Lovelle, Gaudillere 2002, Amiri, Fallahi 2007). Excess nitrogen leads to high vigour, increased fruit yield and modified juice composition (i.e. pH and concentrations of organic acids and esters), but may also create favorable conditions for diseases such as bunch stem necrosis and *Botrytis cinerea* bunch rot (Keller et al. 2004).

The fundamentals of nitrogen nutrition in grapevine are well known (Wade et al 2001). Nitrogen is a critical nutrient during grapevine rapid shoot growth in the spring, through bloom and early development of berries. Schaller (2000) indicates that N use efficiency in vineyard is low. The researcher demonstrated that the maximum N demand is reached after bloom, mainly at the end of June/beginning of July. Shifting of the fertilization date from early spring to the phenological stage "3–5 leaves unfolded" resulted in good synchronization between N applied and demanded by the plant. Grapevine has alow nitrogen demand compared with other fruit crops (Van Leeuwena, Seguin 2006, Schreiner et al. 2006). Spayd et al. (2000) showed

that application of 112 kg N ha⁻¹ was sufficient to obtain juice N concentrations suitable for healthy yeast fermentation. However, this level of fertilization resulted in excessive vine growth, fruit shading and delayed fruit ripening. Thus, application of no more than 50 kg N ha⁻¹ is recommended for most vines varieties (Robinson 1999). However, amounts of N fertilizer differ in respect of the variety, yield, soil type, crop residues and irrigation efficiency. For white wine production, nitrogen supply should be at least moderate to obtain high aroma potential in grapes (Peyrot des Gachons et al. 2005).

A change in one of the soil nutrient levels can interfere with the plant availability and uptake of other nutrients. Therefore, the effects of one nutrient on uptake or use of another, as well as their interactions should be considered in a complete nutrient management program (Fageria, Baligar 2005, Fageria 2009). Nitrogen application in a vineyard can modify absorption of other nutrients by grapevine. High N level can reduce the availability of B, K and Cu. On the other hand, increased N levels create a demand for more magnesium (Bell, Robinson 1999). Application of N-fertilizers to soils can also affect bioavailability of trace elements in soil (Basta et al. 2005). Nitrogen fertilizers induce some direct and/or indirect changes, which affect the dynamics of availability of metals in soils. Mineral N fertilizers containing ammonium can acidify the soil solution and depress the pH of the rhizosphere, thus enhancing the availability of metals (Diatta, Grzebisz 2006).

Plant nutrient analysis is an important tool used to determine the status of plant nutriention and fertilizer requirements. Fallahi et al. (2005) demonstrated that the French Diagnostic Foliar Laboratory at Montpellier relied on the status of leaf tissue status (blade and petiole) sampled twice: at bloom and at the end of veraison. In California, petiole tissues alone sampled at bloom time are often used for assessment of the plant nutritional status. Robinson (2000) reported that petiole nitrate-N determination is recommended in the Australian grape industry. However, the author concluded that petiole analysis does not give a good picture of nitrogen status except in very high yielding vineyards. In Poland, petiole or blade sampling are currently used and standards for these methods are adapted from other countries. These analytical techniques and standards may not be appropriate under Polish conditions with a relatively severe climate, considered marginal or unsuitable for growing grapes of European origin for wine production. There is a regional effect on quantities of nutrients grapevine needs (Mackenzie, Christy 2005, Greenough et al. 2005, Pacheco et al. 2010). The uptake of nutrients by plants is strongly influenced by environmental factors (rainfall, temperature and soil condition) and controlled by the plant's metabolic demand (MARCHNER 1995). Nevertheless, little is known about the uptake of trace elements by grapevine under different environmental condition and management practice. Microelements and other trace elements, for

example copper, chromium, molybdenium, cadmium or mercury, at high concentrations may be toxic to humans (McLaughlin et al. 1999). We lack information on the relationship between leaf and fruit mineral status. Such knowledge could help to choose appropriate sites for vineyards and to understand better the soil and plant interactions. These interactions as well as the specific nutrient needs of particular cultivars would enable us to establish sitespecific fertilization plans to promote vineyard sustainability.

As grapevine cultivation becomes increasingly popular in colder regions of Europe, we have decided to assess the influence of different nitrogen fertilization levels on content of micro- and trace elements in leaves and fruits of grapevine in southern Poland.

MATERIAL AND METHODS

Site characteristic

The study was carried out at the Garlicki Lamus vineyard located in Garlica Murowana (near Krakow, Poland, coordinates: 19°56'E oraz 50°08'N) in 2010-2011. The site of the experimental vineyard had long-term average annual precipitation of 576 mm with an average minimum temperature of -2.9°C (in January) and the maximum temperature of 17.8°C (in July). Onfarm rooted grapevine (Vitis sp. L.) shoots of cv Bianca planted in 2007 were used for this investigation. The cultivar is a hybrid of Seyve Villard × Bouvier. This Hungarian variety is moderately vigourous and highly productive. It bears white grapes, with high sugar content and good acidity. As the grapevine is resistant to fungal diseases, the cultivar is recommended for establishing organic vineyards. Grapevines were planted along the northsouth orientation, with 3.5 m inter-row and 0.9 in-row spacing, respectively. They were trained as Casenave's horizontal cordon with one arm. Plants were cane-pruned to 10 nodes per 1 meter of canopy, with vertically positioned shoots. The average yield ranged from 2.5 to 4.0 t ha⁻¹ in 2010 and 2012, respectively.

The experiment was arranged in a randomized complete-block design with four replications of five vines per block. The plants were treated with three nitrogen doses (0, 50 and 100 kg N ha⁻¹) as ammonium nitrate in a single application at three weeks pre-flowering. Fertilizers were applied within a radius area around each vine. No other macro- and micronutrients were added to the vines in this experiment.

The vineyard soil was characterized as silty clay loam (18% of sand, 43% of silt, 39% of clay) with the pH of about 5.6 and total organic matter content equal 1.68%. The soil available content of phosphorus and magnesium (measured according to the universal method) was in the medium to optimum range (20.8-45.7 mg P dm⁻³, 56.4-79.0 mg Mg dm⁻³). The soil avail-

able potassium (128.4-163.7 mg K dm $^{-3}$) and calcium content (463.7-630.4 mg Ca dm $^{-3}$) was below the optimum ranges (200-250 mg K dm $^{-3}$ and 1000-2000 mg Ca dm $^{-3}$ of soil, respectively). Average amounts of available soil microelements (extracted with 1 M HCl) were within the optimum range for manganese, copper and zinc. However, soil samples contained less available boron than considered as optimal (0.49 to 0.59 mg B kg compared to recommended 1.3-4.3 mg B kg $^{-1}$ soil). The content of other available soil elements varied from: 837 to 1003 mg Al kg $^{-1}$, 33.5-35.9 mg Ba kg $^{-1}$, 0.47-0.57 mg Cd kg $^{-1}$, 0.79-0.92 mg Cr kg $^{-1}$ and 13.9-15.1 mg Pb kg $^{-1}$.

Weeds between grapevine plants were controlled by application of glyphosat (Roundup, Monsanto) in mid-June every year. The plant protection was carried out according to the recommendations for commercial vineyards. In the wet year 2010, an incident of *Botrytis cinerea* infection was observed, but it was not severe enough to affect the grapevines.

Tissue analysis

During the subsequent seasons, samples of leaf petioles, leaf blades as well as grapes were taken. Ten leaves per plant were sampled from a cordon on both sides of a vine plant, from the inner and outer canopy layers. Collected samples were taken from recently matured, full-size leaves at the full bloom period in each year, around 15 June in 2010 and 13 June in 2011. Leaves of five vine plants in each block were combined to make a composite sample of 50 leaves. Petioles were separated from blades and their dry matter content was measured. Plant samples were washed in distilled water before forced-air oven drying at 60°C. Samples were comminuted in a grinder. After wet microwave digestion in HNO $_3$ (CEM 5-Express microwave), the samples were analysed for the following nutrients: B, Cu, Fe, Zn, Mn, Mo, and trace elements: Al, Ba, Cd, Cr, Li, Ni, Sr, Ti and V, using the ICP-OES technique (Prodigy Teledyne, Leeman Labs. spectrometer).

Grapes were harvested on 15/20 October in 2010 and 2011, respectively. Berries showing average growth and maturation were sampled for each treatment. The grapes were washed in distilled water and stalks were removed after drying at room temperature. Must was made in a laboratory press. The same procedure (wet digestion) was employed for fruit and tissues analyses.

Soil analysis

For determination of soil nutrients, soil samples were collected at 0-20 cm depth. Samples were dried at 60°C for 48 hours and passed through a 1 mm mesh sieve. The grain-size distribution was analyzed by Casagrande's aerometric method modified by Prószyński (Ostrowska et al. 1991). This procedure is regulated by the PN-R-04032 standard (Polish norm) applied mainly for agricultural soil analysis in Poland. Total organic carbon (TOC) with

wet oxidation followed by titration with ferrous ammonium sulfate was measured by Tiurin's method (Ostrowska et al. 1991). Soil pH was determined by adding deionized water at the soil to water ratio equal 1:2. The available microelements (Cu, Fe, Zn, Mn, B) and trace elements (Al, Ba, Cd, Cr, Li, Ni, Sr, Ti and V) were measured in 1 M HCl extractant (Ostrowska et al. 1991) using the ICP-OES technique. This soil extractant and procedure are currently used to estimate availability and critical levels for micronutrient cations in Poland.

Climatic condition

In 2010, air temperatures were near the average for Kraków area from April to September (Figs 1a and 1b). Rainfall during the growing season in 2010 was high, especially in May and September. This vintage was warmer and much wetter than the following year. The vintage of 2011 grew in a dry, especially in May, and colder season. In 2011, the average temperature from April to September was 12.9°C against 14.7°C in 2010.

Statistical analysis

We tested the data and found some significant interactions between years and N doses and leaf samples. Data were analyzed using Statistica 9.0 software (Statsoft Inc.). All results were subjected to 3-way analysis of variance (MANOVA). The least significant difference (LSD) multiple range test was used to compare means for significant main effects.

RESULTS AND DISCUSSION

Tissue analysis in a vineyard is a very effective tool for monitoring mineral nutrition. In the present work, concentrations of all microelements in the grapevine tissues were in the optimum (B, Cu, Fe, Zn, Mo) or high range (Mn) as compared with the content reported for 'full bloom' plants (Table 1). High Mn levels, especially in leaf blades, indicate low pH values and increased Mn availability in acid soil.

Strong impact of the climatic conditions on mineral acquisition, transport, and finally plant accumulation was observed. Extremely wet but warmer year 2010 favoured higher accumulation of B, Al, Cd, Li, Sr, Ti and V as measured in blades and petioles (Tables 1 and 2). On the contrary, colder but dryer year 2011 stimulated higher content of Fe, Mn, Zn, Mo, Ba, Cr and Ni. The process of absorption and root metabolism is undoubtedly conditioned by ambient temperature. High temperature activates chemical and biological processes in soil and increases solubility of trace elements. According to Greenough et al. (2005), concentrations of trace elements are correlated strongly with Degree Days, indicating that more heat results in increased evaporation, water uptake and higher elemental concentration in

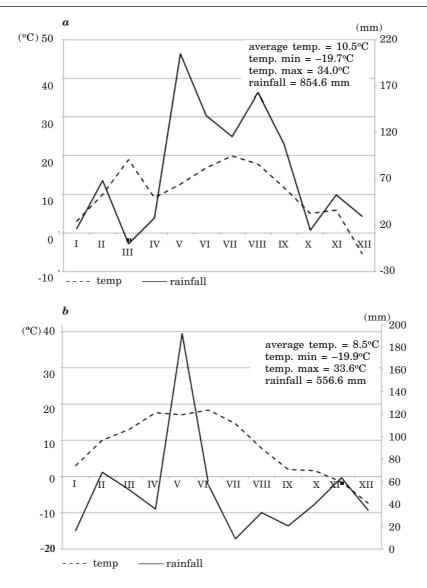


Fig. 1. Climate diagrams for 2010 (a) and 2011 (b), according to Walther and Lieth (1970)

grapevine. Similarly to temperature, rainfall has an effect on the composition of grapevine. Low soil moisture diminishes ions diffusion. On the other hand, the influence of soil moisture on the plant's elemental uptake is dependent on soil nutrient availability. Generally, plants take up trace elements dissolved in the soil solution (Kabata-Pendias 2011). In the study of Porro et al. (2010), the Ca, Mg, S, Mn and B content in leaves and in berries was significantly changed due to water stress. Low levels of these

 $\label{thm:continuous} Table~1$ Leaf blade and petiole micronutrient content (mg kg $^{-1}$ d.m.) in grapevine grown under different N fertilization in 2010-2011

Year	N dose (kg ha ⁻¹)	Leaf sample	В	Cu	Fe	Mn	Zn	Mo
	0		42.3	12.4	100.0	156.3	20.7	0.000
2010	50	blades	31.2	10.3	97.3	111.0	17.3	0.006
	100		30.3	10.5	101.3	144.5	20.7	0.043
2010	0		36.0	11.5	27.5	68.1	37.1	0.129
	50	petioles	32.4	12.1	30.6	63.6	39.1	0.098
	100		32.7	12.2	27.0	87.6	32.5	0.133
	0		29.2	12.0	117.3	396.7	48.7	0.233
2011	50	blades	29.8	10.6	121.2	289.6	51.9	0.198
	100		29.9	11.4	109.5	370.0	50.9	0.120
	0		33.2	12.1	30.6	135.7	62.2	0.316
	50	petioles	32.9	11.8	30.6	63.1	62.9	0.243
	100		33.0	12.0	30.4	99.0	63.3	0.10
	******	2010	34.1	11.5	63.9	105.2	27.9	0.068
	year	2011	31.4	11.6	73.3	225.7	56.7	0.237
Means		0N	35.2	12.0	68.8	189.2	42.2	0.170
	N dose	50N	31.6	11.2	69.9	131.8	42.8	0.136
		100N	31.5	11.5	67.1	175.3	41.9	0.151
	loof comple	blades	32.1	11.2	107.8	244.7	35.0	0.100
	leaf sample petioles		33.4	12.0	29.4	86.2	49.5	0.205
	year (A)		2.16	ns	4.01	24.21	3.00	0.027
	N doses (B)		2.65	ns	ns	29.65	ns	ns
	leaf sam	ple (C)	ns	0.67	4.01	24.21	3.00	0.027
LSD $p=0.05$	AxI	3	3.75	ns	ns	ns	ns	ns
μ=0.00	Ax(C	ns	ns	5.67	34.23	ns	ns
	BxC	C	ns	ns	ns	ns	ns	ns
	AxBx	кC	ns	ns	ns	ns	ns	0.066

elements were found both in leaves and in berries of water-stressed plants. Fallahi et al. (2005) found differences in the Fe blade and petiole concentration between years. The authors explained this phenomenon as an effect of

Leaf blade and netiole trace element content (mg kg⁻¹ d.m.) in granevine grown under different N fertilization in 2010-2011

Table 2

$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	Vecau	Note and periode trace element content ing ag u.m.) in grape the grown under under the trace and the content in 2010-2011	I and the second	۷۱	D. 0	Ì	arriada a	1 :	N.	10	2	Ë	17
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	<u>;</u>	N dose (kg na -)	real samble	TE	Da	Ca	Ċ	ij	INI	ΡD	Or	11	>
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		0		33.1	13.7	0.157	0.16	0.067	2.32	1.18	48.6	2.42	0.148
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		50	blades	29.9	10.8	0.134	0.11	090.0	2.17	1.38	43.0	2.30	0.125
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	9	100		31.3	15.7	0.139	0.17	0.065	3.15	1.33	52.4	2.59	0.131
50 petioles 7.93 18.0 0.285 0.20 0.099 1.26 0.61 59.4 0.36 100 9.31 24.5 0.347 0.05 0.126 1.45 0.75 666 0.55 0 100 18.5 0.044 0.83 0.040 3.78 1.75 46.9 0.75 100 24.0 18.5 0.044 0.83 0.040 3.78 1.75 46.9 0.73 100 21.9 14.4 0.068 0.72 0.052 2.69 1.73 34.6 1.34 100 9.21 10.44 0.094 0.74 0.029 2.69 1.76 0.78 100 4.60 30.8 0.221 1.04 0.048 1.73 1.46 0.74 0.099 2.89 1.46 0.72 0.059 2.69 0.49 0.73 0.099 1.48 0.71 0.049 0.74 0.029 1.48 0.72 0.89 0.49	01	0		7.73	23.2	0.417	0.07	0.097	0.97	0.67	59.3	0.51	0.082
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		50	petioles	7.93	18.0	0.285	0.20	0.099	1.26	0.61	59.4	0.36	0.080
0 0 44.0 18.5 0.094 0.83 0.040 3.78 1.75 46.9 0.73 50 blades 27.7 12.6 0.068 0.72 0.052 1.97 1.50 38.7 1.34 100 21.9 14.4 0.094 0.74 0.029 2.69 1.73 34.6 1.44 0 4.60 30.8 0.251 1.04 0.096 2.26 0.49 5.89 1.74 1.44 1.04 0.050 2.26 0.49 5.89 1.74 1.44 0.094 0.74 0.099 2.26 0.49 5.89 1.74 1.44 0.094 0.74 0.099 2.26 0.49 0.75 1.46 0.27 0.89 1.49 0.28 0.89 0.8		100		9.31	24.5	0.347	0.05	0.126	1.45	0.75	9.99	0.55	0.104
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		0		24.0	18.5	0.094	0.83	0.040	3.78	1.75	46.9	0.73	0.013
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		50	blades	27.7	12.6	0.068	0.72	0.052	1.97	1.50	38.7	1.34	0.025
$\begin{tabular}{ l l l l l l l l l l l l l l l l l l l$	111	100		21.9	14.4	0.094	0.74	0.029	2.69	1.73	34.6	1.46	0.091
$\begin{tabular}{ l l l l l l l l l l l l l l l l l l l$	11,	0		4.60	30.8	0.251	1.04	960.0	2.26	0.49	56.8	0.32	0.049
$\begin{tabular}{l l l l l l l l l l l l l l l l l l l $		50	petioles	4.29	21.2	0.121	0.87	0.033	1.46	0.27	48.3	0.28	0.033
$\begin{tabular}{ l l l l l l l l l l l l l l l l l l l$		100		3.89	26.4	0.182	1.40	0.048	2.19	0.26	47.0	0.14	0.027
$\begin{tabular}{ l l l l l l l l l l l l l l l l l l l$		\$ 000	2010	19.9	17.7	0.246	0.12	0.086	1.89	66.0	54.9	1.45	0.111
N dose N dose		year	2011	14.4	20.7	0.135	0.93	0.050	2.39	1.00	45.4	0.71	0.040
$N \ dose \ \ N \ \ \ \ N \ \ \ \ \ \ \ \ \ \ \ $			N0	17.4	21.5	0.230	0.52	0.075	2.33	1.02	52.9	1.00	0.073
	ans	N dose	50N	17.5	15.6	0.152	0.47	0.061	1.71	0.94	47.3	1.07	990.0
			100N	16.6	20.3	0.190	0.59	0.067	2.37	1.02	50.2	1.19	0.088
		Josephan Joseph	blades	28.0	14.3	0.114	0.45	0.052	2.68	1.48	44.0	1.81	0.089
year (A) 1.66 2.67 0.0493 0.182 0.0199 0.307 ns 3.51 0.120 N doses (B) ns 3.26 0.0604 ns ns 0.376 ns 4.30 0.147 Park (S) 1.66 2.67 0.0493 ns ns 0.307 0.131 3.51 0.120 AxB ns ns ns ns 0.698 0.257 ns ns 0.185 ns 0.170 AxBxC ns ns ns ns ns ns ns ns 0.208		ıeai sampie	petioles	6.29	24.0	0.267	09.0	0.083	1.60	0.51	56.2	0.36	0.062
N doses (B) ns 3.26 0.0604 ns ns 0.376 ns 4.30 0.147 leaf sample (C) 1.66 2.67 0.0493 ns 0.0199 0.307 0.131 3.51 0.120 AxB ns ns ns ns ns 0.631 ns 6.08 0.208 BxC ns ns ns ns ns ns ns 0.170 AxBxC ns ns ns ns ns ns ns ns ns 0.294		year (4	(A)	1.66	2.67	0.0493	0.182	0.0199	0.307	su	3.51	0.120	0.0139
leaf sample (C) 1.66 2.67 0.0493 ns 0.0199 0.307 0.131 3.51 0.120 AxB ns ns ns ns ns ns 6.08 0.208 ns ns ns ns ns ns ns ns ns AxBxC ns ns ns ns ns ns ns ns ns		N doses	(B)	su	3.26	0.0604	su	su	0.376	su	4.30	0.147	0.0170
AxB ns ns ns ns ns 6.631 ns 6.08 0.208 AxC ns ns 0.0698 0.257 ns ns 0.185 ns 0.170 AxBxC ns ns ns ns ns ns ns 0.208	Ę	leaf sampl	le (C)	1.66	2.67	0.0493	su	0.0199	0.307	0.131	3.51	0.120	0.0139
AxC ns ns 0.0698 0.257 ns ns 0.185 ns 0.170 BxC ns ns ns ns ns ns ns 0.208 AxBxC ns ns ns ns ns ns ns ns 0.294	ر ا ا	AxB		su	ns	ns	ns	ns	0.531	ns	80.9	0.208	ns
ns ns ns ns ns ns 0.208 ns ns ns ns ns 0.294	2	AxC		su	su	0.0698	0.257	su	su	0.185	su	0.170	0.0197
ns ns ns ns ns ns ns ns ns 0.294		BxC		su	su	su	su	su	su	su	su	0.208	su
		AxBx(C	ns	su	su	ns	su	su	su	su	0.294	0.0341

efficient Fe uptake by a more extensive root system created in a year with favourable growth conditions.

In this study, differences in the grape must from the two seasons were found for boron (4.72 and 3.88 mg B kg⁻¹ f.w.), copper (0.63 and 0.41 mg Cu kg⁻¹ f.w.), iron (2.14 and 1.54), manganese (1.28 and 1.82), aluminium (1.10 and 0.44 mg Al kg⁻¹ f.w.), chromium (2.41 and 27.7 µg Cr kg⁻¹ f.w.), nickel (58.3 and 56.3) and titanium (36.4 and 28.3 µg Ti kg⁻¹ f.w. in the wet 2010 versus the cold but drier 2011, respectively. The content of lead and vanadium in grape juice was under the limit of detection with an ICP spectrometer (Pb <1 ppb, and V <0.07 ppb).

Interactions between nutrients in crop plants are probably among the most important factors affecting yields of plant crops (MARCHNER 1995). The nitrogen fertilization applied in our experiment significantly influenced the content of microelements and trace elements in leaf blades and petioles. No N-treatments (control with 0 N) resulted in the highest boron and manganese content. In addition, a tendency towards increased copper and molybdenum levels in blades and petioles in control plants was noted. Surprisingly, also the highest cadmium amount was measured for the control in both years and for both petioles and blades. Similar observations albeit on Brassica plants were reported by Domagała-Świątkiewicz et al. (2009). N fertilizer containing ammonium significantly decreased the B and Mo content, although the environmental factors considerably modified this tendency. Wolf et al. (1983) showed that increasing N concentration caused increased iron levels in grape tissues. Fallahi et al. (2005) found positive correlation between nitrate-N and Cu blade content. Amiri, Fallahi (2007) showed that nitrogen application did not affect B in grapevine petioles, despite increasing the Mn content. Generally, the lowest content of the trace elements (except Al) was proven for a fertilization treatment with 50 kg N ha⁻¹, which could be attributed to the worst yielding in this treatment and consequently the strongest vegetative growth of grapevine, which in turn might have affected the other patterns of elemental accumulation.

In the present study, significant interaction was determined between climatic conditions during the years of the experiment and N fertilization versus the nickel, strontium and titanium concentrations in tissue samples. In the wet year 2010, an N-dose of 100 kg ha⁻¹ significantly increased these elements in blades and petioles in relation to the other treatments. The same trend was observed in 2010 for Mo and Ba in both blades and petioles, and for Mn, Al, Li, Pb and V in grapevine petioles. In the dry 2011, two elements only, that is Cr in petioles and V in blades, increased under the effect of 100 kg N ha⁻¹ fertilization (Tables 1 and 2). Rodriguez-Otriz et al. (2006) reported that N fertilizers which contain ammonium can strongly affect accumulation of heavy metals in yield. The acidification of the rhizosphere induced by N supply and by plants (enhanced net excretion of protons or of organic acid) are of particular importance in the acquisition of Fe,

Zn, Mn and Ni in soil (Kabata-Pendias 2011). The nickel concentration in leaves of plants grown on uncontaminated soil ranges from 0.05 to 5 mg Ni kg⁻¹ d.m. and is the lowest of any element. In our study, the Ni measured content ranged from 0.97 to 3.78 mg kg⁻¹ d.m. (Table 2). The content of strontium in edible plants is highly variable and seems to be the highest in vegetables leaves (45-74 mg kg⁻¹ f.w.). In cv. Bianca grapevine, the Sr concentration in leaf tissues varied from 34.6 to 66.6 mg kg⁻¹ d.m. Strontium occurs in soils as a bivalent cation and is easily taken up by plants (Kabata-Pendias, Pendias 1999). The solubility of Ti in soils is very limited, and the phytoability of this element is low. The titanium content in food plants ranges from 0.13 to 6.7 mg kg⁻¹ d.m. The lowest values are in prepared cereals and fruits (Kabata-Pendias 2011). In our study, the Ti concentration in grapevine leaves ranged between 0.14 to 2.59 mg kg⁻¹ d.m.

The analysed leaf blades contained higher amounts of iron (107.8 mg), manganese (244.7 mg), nickel (2.68 mg), lead (1.48 mg), titanium (1.81 mg) and vanadium (0.09 mg V kg⁻¹ d.w.) as compared to petioles (29.4 mg, 86.2 mg, 1.60 mg, 0.51 mg, 0.36 mg and 0.06 mg V kg⁻¹ d.w., respectively) – Tables 1 and 2. In contrast, petioles had more copper (12.0 mg vs. 11.2 mg), zinc (49.5 mg vs. 35.0 mg), molybdenum (0.205 mg vs. 0.100 mg), Cd (0.267 mg vs. 0.114 mg), lithium (0.084 mg vs. 0.052 mg) and strontium (56.2 mg vs. 44.0 mg kg⁻¹ d.w. for petioles and blades respectively). A similar effect was obtained Romero et al. (2010), who found higher concentration of micronutrients in grapevine leaf blades, except Zn. Also Fallahi et al. (2005) demonstrated that concentrations of blade Fe and Mn were higher, while blade Zn was lower than in petioles in all of the six examined cultivars. These researchers showed positive correlations between micronutrient concentrations in leaf blades and concentrations of the same elements in petiole tissues.

The chemical composition of grapes depends of many factors, including the cultivar, climatic condition (rainfall, temperature), soil parameters, and vineyard management (Van Leeuwena, Seguin 2006). Environmental factors such temperature and available water have a significant effect on the nutrient concentration in grapes (Cozzolino et al. 2010). Trace elements in grapevine fruits are very important, especially for the quality of wine and wine authenticity determination (Baxter et al. 1997, Taylor et al. 2002, Greenough et al. 2005). Generally, the concentration of these elements in fruits and wines is a result of their uptake by plants from soil (Galganoa et al. 2008). However, several factors besides climatic conditions, such as viticultural techniques and vine production process can modify the chemical composition of wine and alter the relationship between wine and soil composition. In this context, nitrogen nutrition of grapevine is of great importance (Bell, Henschke 2005).

In the present study, the N fertilization influenced composition of elements in grape juice, although the results were inconsistent. The differen-

 $\label{eq:Table 3} \mbox{Mean microelement concentration (mg kg$^{-1}$ d.m.) in the must from grapevine grown under different N fertilization in 2010-2011}$

Year	N dose (kg ha ⁻¹)	В	Cu	Fe	Mn	Zn	Мо
	0	4.50	0.648	2.46	1.40	0.783	0.008
2010	50	4.70	0.584	1.98	1.14	0.701	0.008
	100	4.98	0.653	1.98	1.31	0.689	0.010
	0	3.36	0.415	1.55	1.75	0.888	0.003
2011	50	4.08	0.325	1.55	1.94	0.898	0.014
	100	4.22	0.477	1.53	1.76	0.877	0.027
Means	2010	4.72	0.628	2.14	1.28	0.724	0.008
year	2011	3.88	0.406	1.54	1.82	0.888	0.015
Means	0	3.92	0.531	2.00	1.57	0.836	0.005
N-dose	50	4.38	0.455	1.77	1.54	0.852	0.011
	100	4.60	0.565	1.76	1.54	0.782	0.018
	year (A)	0.80	0.061	0.429	0.342	ns	ns
LSD p =0.05	N-dose (B)	ns	0.074	ns	ns	ns	ns
	AxB	ns	ns	ns	ns	ns	ns

ces should not be only attributed to environmental factors, but also to the vigour and yielding of grapevine in response to different nitrogen fertilization regimes. While the Cd accumulation in fruits was enhanced by an increased N rate, a reverse tendency was proven for Ba and Sr. Also, the Cu and Ti level in must was differentiated. For plots fertilized with 50 kg N ha⁻¹, the highest Ti and the lowest Cu amounts in grape must were measured (Tables 3 and 4). However, for the other investigated elements, no influence of a nitrogen rate was recorded. The vintage had great impact on the mineral content of grapes. The year 2010 favoured fruit accumulation of Cu, Fe, Al and Ti, whereas the climatic conditions in 2011 increased the content of Mn, Ba and the investigated heavy metals (Cd, Cr and Ni; Table 4). Mac-KENZIE, CHRISTY (2005) found that grape juice properties including Baumé level and titratable acidity are clearly correlated with several plant available trace elements in soil. Most notable of these were Ca, Sr, Ba, Pb and Si. These authors concluded that soil chemistry had some influence on wine grape composition and such knowledge could be taken advantage of for better vineyard management.

 $\label{eq:Table 4} \begin{tabular}{ll} Table 4 \\ Mean trace elements concentration (µg kg$^{-1} d.m., except for Al) in grapevine juices under different N fertilization in 2010-2011 \\ \end{tabular}$

Year	N dose (kg ha ⁻¹)	Al	Ba	Cd	Cr	Ni	Sr	Ti
	0	1.12	95.3	6.42	4.16	65.5	225.8	42.6
2010	50	1.18	63.3	5.16	1.00	47.9	150.5	32.9
	100	1.01	58.3	6.15	2.08	61.8	151.9	33.8
	0	0.52	127.1	5.72	28.93	52.4	263.9	11.9
2011	50	0.40	86.9	7.62	31.19	114.7	188.6	56.3
	100	0.39	76.6	12.83	22.85	121.8	139.2	16.8
Means	2010	1.10	72.3	5.91	2.41	58.3	176.1	36.4
year	2011	0.44	96.9	8.72	27.66	96.3	197.2	28.3
Means	0	0.82	111.2	6.07	16.54	59.0	244.9	27.3
N-dose	50	0.79	75.1	6.39	16.10	81.3	169.5	44.6
	100	0.70	67.5	9.50	12.46	91.8	145.6	25.3
	year (A)	0.283	26.6	1.52	0.9	32.6	ns	6.53
$\mathrm{LSD}p{=}0.05$	N-dose (B)	ns	32.6	1.96	ns	ns	30.5	8.28
	AxB	ns	ns	2.77	ns	ns	43.6	11.55

CONCLUSIONS

Balanced nutrient supply is one of the most important factors in increasing crop yields and their quality. Plant productivity is directly linked with nutrient availability and uptake. The knowledge of interactions of nutrients and trace elements can help to improve efficiency of nutrient uptake. The present study focused on the effect of nitrogen fertilization on microelements and trace elements in grape plants (leaves and berries). The Cu, Zn, Fe, B and Mo concentrations in the leaves (blades and petioles) of cv. Bianca grapevine were within an optimal range (Mn - high) of content reported for Vitis vinifera plants. This experiment showed that on slightly acid soils mineral N fertilizers containing ammonium can enhance the uptake of trace elements such as Ti and V. Nitrogen fertilization depressed the uptake of some elements like B, Mn, Ba, Cd and Sr. The analysed leaf blades contained higher amounts of Fe, Mn, Ni, Pb, Ti and V than petioles. In contrast, petioles had more Zn, Mo, Cd, Li and Sr. Increased N fertilization diminished Ba and Sr must accumulation. Moderate fertilization enhanced Ti and decreased Cu fruit content. The vintage strongly influenced

leaf as well grape mineral content. During the warmer and wetter year 2010, higher amounts of B, Cd, Sr, Ti and V in leaves and Al, Cu, Fe and Ti in grape must were determined. The dry season in 2011 increased the leaf Fe, Mn, Zn, Mo, Ba, Cr and Ni content as well as the amounts of Mn, Ba and heavy metals (Cd, Cr, Ni) in fruits.

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