

Trace Element Partitioning in ‘Sibera’ Grapevines as Affected by Nitrogen Fertilisation

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A study on grapevine cv. Sibera was carried out in a vineyard located near Kraków (Poland) in 2010 and 2011. The plants were treated with three nitrogen application rates (0, 50 and 100 kg N ha⁻¹), administered as ammonium nitrate in a single application three weeks before flowering. Samples of leaf petioles and blades, as well as grapes, were taken. After wet microwave digestion in HNO₃, the nutrient elements boron (B), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), molybdenum (Mo) and sodium (Na), and the trace elements aluminium (Al), barium (Ba), cadmium (Cd), chromium (Cr), lithium (Li), nickel (Ni), lead (Pb), strontium (Sr), titanium (Ti) and vanadium (V), were measured using the ICP-OES technique. Environmental factors such as temperature and available water had a significant effect on the nutrient concentration in the grapes. In the wet and warm 2010 vintage, higher amounts of B, Cu, Cd, Ti and V were measured in the leaves, and of Mn, Al, Ba and Ti in the grape juice. The dry season of 2011 increased the leaf Fe, Mn, Zn, Mo, Na, Ba, Cr, Li and Ni content, which was associated with a higher Zn, Mo, Na, Sr, Cd and Ni concentration in the grape must. The study showed that, in slightly acid soils, mineral N fertilisers containing ammonium can augment the uptake and accumulation of microelements such as Fe, Mn, Zn, Al and Ti by the grape must. In contrast, nitrogen fertilisation depressed the concentrations of some elements, such as B, Fe, Mn, Cd, Cr, Ni and Ti in the leaves. Correlations between the mineral content of the analysed plant tissues are also discussed.

INTRODUCTION

The transport of chemical elements from the soil to the food chain is a part of their natural cycling. Concentrations of trace elements in plants are often related to the content of these elements in the soil. Mackenzie and Christy (2005) showed that soil chemistry influences wine grape quality or composition. This vine-soil relationship is a key part of the concept of *terroir* (geochemical and other factors that may affect grapevines and the development of berries). Individual vineyard areas have unique trace element “signatures” that permit the identification of the provenance of a wine (Taylor *et al.*, 2002; Greenough *et al.*, 2005; Van Leeuwen & Seguin, 2006; Galgano *et al.*, 2008). This suggests that trace elements could play some role in determining the distinctiveness of wine that differentiates wines of one region from those of another.

Several anthropogenic factors, including agronomic ones, have changed the supply and availability of natural trace elements. Trace elements cycles are closely associated with major element cycles (Kabata-Pendias, 2011). Yield response is strongly altered by interactions between

nutrients, chemical elements and other growth factors. The application of fertilisers, lime and additional materials to soils can affect the bioavailability and redistribution of trace elements into different chemical pools or chemical “species” (Basta *et al.*, 2005). Nitrogen (N) fertilisers induce some direct and/or indirect changes that have an impact on the dynamic availability of metals in soils (Diatla & Grzebisz, 2006). The form of N supply has a strong but opposite impact on the uptake of other chemical elements, cellular pH regulation and the rhizosphere pH. N fertilisers containing ammonium can acidify the soil solution and decrease the pH of growing media (Marschner, 1995; Fageria & Baligar, 2005; Fageria, 2009). Rodriguez-Ortiz *et al.* (2006) reported that N fertilisers containing ammonium could have a strong effect on the accumulation of heavy metals in the yield. The rhizosphere acidification, which is affected by N supply as well as by the plant factors (enhanced net excretion of protons or of organic acid), is of particular importance for the acquisition of Fe, Zn, Mn and Ni by soils. On the other hand, growth enhancement due to nitrogen nutrition causes a ‘dilution’ of the mineral elements in plant tissues.

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Nitrogen nutrition is widely recognised as an efficient controlling factor in grapevine production. Among other nutrients, nitrogen is evidently the one that has the greatest impact on vine vigour, yield, berry ripening and composition. Many studies have demonstrated the effect of N nutrition on growth and yield parameters (Bell & Robinson, 1999; Spayd *et al.*, 2000; Treeby *et al.*, 2000; Rodriguez-Lovelle & Gaudillère, 2002; Ekbic *et al.*, 2010). Nitrogen rate and timing are important in grapevine management practices for improving N-use efficiency and crop yields (Schaller, 2000; Schreiner *et al.*, 2006). Crop response to applied N is an important criterion for evaluating N requirements for maximum economic yield and quality. Chloné *et al.* (2001) showed that limited N supply increases quality in red wine production due to reduced vine vigour, as well as increased berries and wine phenolic content. N-deficient grapevines will have a smaller crop, but the highest fruit maturity. A vine with a moderate N status will achieve optimum production and intermediate fruit maturation. Peyrot des Gachons *et al.* (2005) demonstrated that, for white wine production, nitrogen supply should be at least moderate to obtain high aroma potential in the grapes. The addition of N beyond the moderate rate will further delay fruit ripening and result in berries of a lower quality (Christensen & Peacock, 2000). Nitrogen is required for yeast growth and fermentation, and is one of the most important nutrients in must (Spayd *et al.*, 2000; Bell & Henschke, 2005).

The quantity of N removed from the vineyard ecosystem with the harvested fruits ranges from 8 to 30 kg/ha, depending on the crop load and variety (Schreiner *et al.*, 2006). The relatively wide variation in the rate of N use for grapevines, of between 30 and 80 kg N/ha, suggests that nutrient management recommendations are developed on a regional basis or on a single vineyard basis (Conradie, 2005). Rational fertiliser application requires information on the nutritional status of the plants. Leaves are usually used for plant analysis as a diagnostic tool. The nutrient content in dry matter may differ considerably between leaf blades and petioles (Christensen & Peacock, 2000; Fallahi *et al.*, 2005; Pacheco *et al.*, 2010; Romero *et al.*, 2010). Sometimes the petioles are thought to be a more suitable indicator of the nutritional status (Robinson, 2000). In Poland, petiole or blade sampling is used currently and standards for determining grapevine nutrient status by these methods are adapted from other countries. These analysis techniques and standards are probably not appropriate for Polish conditions, with a relatively severe climate.

At present, grapevine cultivation is becoming and more popular in the colder regions of Europe. Although these areas are subject to the risk of severe winter injury in some years, the grapes and wines obtained often are of outstanding quality. However, adaptability, plant mineral status and the fruit quality of wine grape cultivars in such regions should be studied before they are planted widely. The purpose of this study was to determine the interactive effects of rate of nitrogen x year on trace element content in *Vitis vinifera* L. cv. 'Sibera' tissues, and in the grape must. 'Sibera', with its high productivity, quality grapes and excellent winter hardiness (Lisek, 2010, 2012), is a very promising cultivar in cooler climatic conditions to produce wine of the Riesling type.

MATERIAL AND METHODS

Site characteristics

The study was carried out in the "Garlicki Lamus" vineyard located in Garlica Murowana (near Krakow, Poland, coordinates: 19°56'E, 50°08'N) in 2010 to 2011. Four-year-old grapevines cv. 'Sibera' (= Gm 6495-3) were used in this investigation. Grapevine rows were oriented north-south, with 3.5 m spacing between rows and 0.9 m in-row. The vines were trained on Casenave's horizontal unilateral cordons. Each plant was pruned to one nine-node cane. The vineyard soil was characterised as a silty clay loam (18% sand, 43% silt, 39% clay), with a pH of about 5.6 and total organic matter of 1.68%. Vineyard management was carried out according to the recommendations for commercial vineyards in Poland (Myśliwiec, 2006).

Tissues analysis

Leaf petioles, leaf blades and fruits were sampled for nutrient analyses. At full bloom (2010-06-15, 2011-06-13), ten mature, full-sized leaves per plant were sampled from each side of the cordon from both the inner and outer canopy layers. Petioles were separated from blades, and the dry matter of those tissues was measured. Plant samples were washed in distilled water, dried at 60°C in a forced-air oven and ground. After wet microwave digestion in HNO₃ (Pasałowski & Migaszkowski, 2006) the concentrations of B, Cu, Fe, Zn, Mn, Mo, Al, Ba, Cd, Cr, Li, Ni, Pb, Sr, Ti and V were determined using the ICP-OES technique (Ostrowska *et al.*, 1991). For elemental analyses, a Prodigy High Dispersion ICP-OES Spectrometer (Teledyne Leeman Labs, Hudson NH, USA) was used.

Grapes were harvested on 2010-10-10 and 2011-10-12, respectively. From each plot, 20 randomly chosen bunches were taken for analysis. The grapes were washed in distilled water and, after drying at room temperature, the pedicels were removed. Juices were obtained using a PH2 (Taco, Poland) laboratory vertical hydraulic press. Juice samples were wet-mineralised and the mineral analyses for leaf tissues were conducted.

Soil analysis

Soil samples were collected from a depth of 0 to 20 cm. Samples were dried at 60°C for 48 h and passed through a 1 mm sieve. The granulometric analysis was done according to the Casagrande aerometric method modified by Prószyński (Ostrowska *et al.*, 1991). This procedure is regulated by the Polish norm (PN-R-04032, 1998) – the standard most frequently used for agricultural soil analysis in Poland. Total organic carbon (TOC) with wet oxidation, followed by titration ferrous ammonium sulphate, was measured by the Tiurin method (Ostrowska *et al.*, 1991). Soil pH was determined by adding deionised water at a ratio of 1:2 (soil:water). Total soil nitrogen was detected by the Kjeldahl method (Ostrowska *et al.*, 1991). The available macroelements (P, K, Ca, Mg and S) were detected by the universal method with 0.03M CH₃COOH extractant (Nowosielski, 1974; Nowosielski *et al.*, 1984). The available microelements (Cu, Fe, Zn, Mn and 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.

Climatic conditions

The meteorological data are presented in climate diagrams (Figs 1a and 1b). The site of the experimental vineyard had a long-term average annual precipitation of 576 mm, with an average minimum temperature of -2.9°C (in January) and a maximum temperature of 17.8°C (in July). In 2010, the temperatures for the vegetation period were near the average, while the rainfall recorded for 2010 was heavy, especially in May and September. This vintage was warmer, with the average temperature recorded from April to September at 14.7°C against the 12.9°C recorded in 2011. Higher precipitation (757 mm and 346 mm for 2010 and 2011 respectively) was noted. The year 2011 was dry, except for May, and colder compared to multi-annual temperatures.

Experimental design

The study design was a randomised complete block with four replications of five vines per block. To separate plots, one buffer vine was planted between them. Vines were treated with one of three nitrogen application rates (0, 50 or 100 kg N per ha) as NH_4NO_3 in a single application at three weeks prior to flowering. The fertiliser was applied in a 90 cm radius around each vine. No other macro- and micronutrients were applied to the vines in this experiment.

Statistical analysis

The data were subjected to a three-way analysis of variance (MANOVA) using Statistica 9.0 (StatSoft Inc., Tulsa, USA). Orthogonal contrasts (Helmert mode for N rates) were

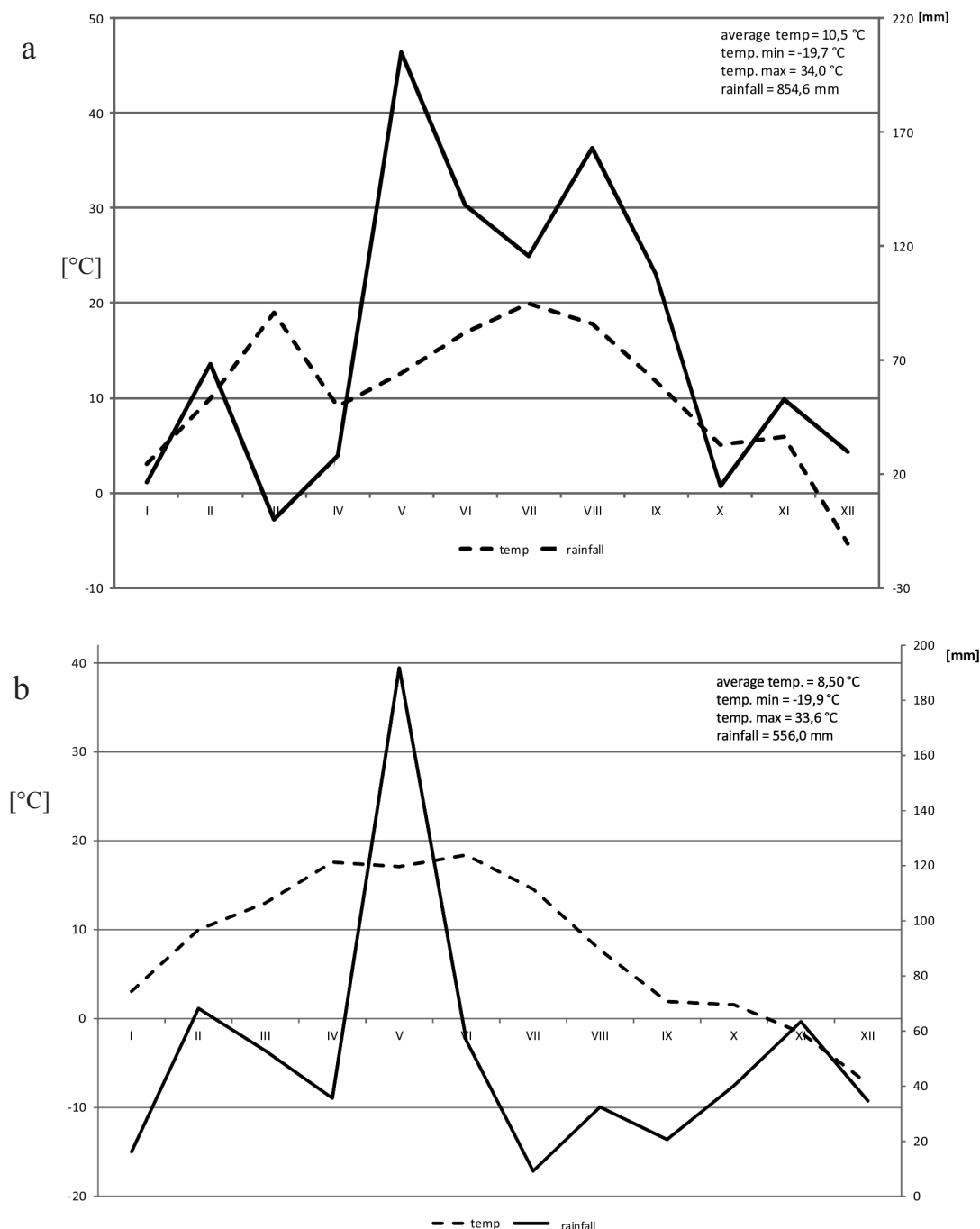


FIGURE 1

Climate diagrams for 2010 (a) and 2011 (b), according to Walter and Lieth (1960)

used for means separation. Moreover, the Pearson product-moment correlation coefficients between the content of the analysed elements were calculated. The correlations were ascertained both within the investigated tissues (leaf blades, petioles and grapes), as well as between them. The level of significance for the correlation coefficient was $p = 0.05$, and the number of pairs for the calculations was $N = 54$.

RESULTS AND DISCUSSION

Soil nutrient content as measured in the herbicide strips (0 to 20 cm depth) were in the medium to optimum level for phosphorus (20.8 to 45.7 mg P/dm³), as well as for magnesium (56.4 to 79.0 mg Mg/dm³) (Sadowski *et al.*, 1990). The measured available soil potassium (128.4 to 163.7 mg K/dm³) and calcium content (463.7 to 630.4 mg Ca/dm³) was below the optimum limits (200 to 250 mg K/dm³ and 1 000 to 2 000 mg Ca/dm³ of soil respectively). Average amounts of available soil microelements were in the optimum range for Mn, Cu and Zn as compared to the values presented by Fotyma and Mercik (1992). However, soil samples contained lower than the optimal content of available B (0.49 to 0.59 mg B/kg), as compared to the recommended 1.3 to 4.3 mg B/kg soil (Fotyma & Mercik, 1992). The content of the other available soil elements varied from 837 to 1003 mg Al/kg, 33.5 to 35.9 mg Ba/kg, 0.47 to 0.57 mg Cd/kg, 0.79 to 0.92 mg Cr/kg and 13.9 to 15.1 mg Pb/kg. The data on soil mineral content are presented in Table 1.

While the statistical analysis did not prove any effect of interactions between the investigated factors, the main effects were statistically significant. The vintage influenced the content of all the minerals analysed in the grapevine tissues (Tables 2 and 3). In 2010 (wetter and warmer season), a higher B, Cu, Cd and Ti content was observed in the leaves (both blades and petioles), while the Fe, Zn, Mo, Ba, Cr, Li and Ni content was lower than in 2011. In the case of the

grapes, the content of Mn, Al, Ba and Ti was higher, whereas the amounts of Zn, Mo, Na, Cd and Sr were lower in 2010 than in 2011. Environmental factors such as temperature (Greenough *et al.*, 2005) and soil moisture (Porro *et al.*, 2010) could affect soil nutrient availability and consequently the mineral nutrient status of the leaves or grapes (Cozzolino *et al.*, 2010). The negative effects of dry and cold weather on the dissolution of soil minerals, diffusion coefficient and plant nutrient uptake are well known (Marschner, 1995). Low soil water content also impairs root elongation, decreases root hair numbers, as well as mycorrhiza symbiosis development, which is crucial for efficient nutrient uptake (Wilson & Tommerup, 1992; Pregitzer & King, 2005). However, in dry conditions, root growth is usually much less depressed than shoot growth, leading to typical increases in the root-shoot dry weight ratio (Marschner, 1995). Therefore, vines can compensate to some extent for part of these negative effects, showing more efficient nutrient uptake than might be expected.

In the present study, nitrogen fertilisation influenced leaf boron content (Table 2). The trend of lower accumulation of B was proved for the rate of 100 kg N/ha. This tendency was true for both petioles and blades. Petiole B was not affected by nitrogen treatment in the study by Amiri and Fallahi (2007). We measured higher B concentration in petioles than in blades (26.9 and 23.9 mg B kg⁻¹ d.m. respectively). The 2010 season, with warmer weather and higher precipitation, favoured the higher accumulation of B in the leaves (Table 2). However, fruit B content was not affected by either N fertilisation or vintage. Tissues analysis is useful for determining both B deficiency and toxicity. The deficient vineyard will have levels of 25 ppm or less. Adequate B levels are above 30 ppm in grapevine petioles (Christensen & Peacock, 2000). In 2010, we measured B content in plant petioles from 27.4 to 29.7 ppm, and in 2011 from 23.7 to 26.4 ppm. Boron supply is known to be reduced by low soil

TABLE 1

Ranges of available macro-element, microelement and heavy metal concentrations in the experimental vineyard soil.

	Herbicide strip		Sward	
	0-20 cm	20-40 cm	0-20 cm	20-40 cm
P (mg/dm ³)	20.8-45.7*	5.30-6.30	21.7-22.3	5.40-6.20
K (mg/dm)	128-164	76-87	153-172	119-140
Ca (mg/dm)	464-630	419-480	558-625	489-552
Mg (mg/dm)	56.4-79.0	140-156	67.5-90.2	46.2-65.1
S (mg/dm)	5.80-6.20	3.50-3.70	6.20-6.50	3.80-4.10
B (mg/kg)	0.49-0.59	0.35-0.42	0.39-0.48	0.52-0.60
Cu (mg/kg)	4.94-5.35	4.39-4.90	6.32-6.40	2.87-3.20
Fe (mg/kg)	1 190-1 250	962-1 030	1 345-1 390	1 042-1 125
Mn (mg/kg)	148-161	142-150	165-178	114-120
Zn (mg/kg)	21.1-24.0	17.9-19.2	12.8-14.2	12.1-13.5
As (mg/kg)	1.96-2.01	2.09-2.15	2.34-2.45	1.89-1.94
Cd (mg/kg)	0.47-0.57	0.37-0.44	0.47-0.49	0.41-0.45
Cr (mg/kg)	0.79-0.92	0.59-0.67	0.81-0.95	0.69-0.71
Pb (mg/kg)	13.9-15.1	10.1-12.0	7.6-8.9	7.5-9.2

*soil samples were collected before establishing the experiment (April, 2010)

TABLE 2

Leaf blade, petiole and grape juice micronutrient content as influenced by different N application rates.

		B	Cu	Fe	Mn	Zn	Mo	Na
Leaf blade and petioles (mg/kg DM)								
N rate	0 N	25.6 ^b	7.6 ^{ns}	55.9 ^b	179.0 ^b	30.9 ^{ns}	0.090 ^{ns}	99.8 ^{ns}
	50 N	26.7 ^b	7.4	49.9 ^a	117.4 ^a	31.2	0.098	98.5
	100 N	23.9 ^a	7.7	50.5 ^a	152.9 ^b	31.6	0.101	89.0
Tissue type	Blades	23.9 ^a	9.3 ^b	86.9 ^b	220.4 ^b	31.6 ^{ns}	0.091 ^{ns}	15.1 ^a
	Petioles	26.9 ^b	5.9 ^a	17.2 ^a	79.2 ^a	30.8	0.101	176.5 ^b
Year	2010	26.6 ^b	8.3 ^b	45.0 ^a	90.5 ^a	20.3 ^a	0.044 ^a	77.3 ^a
	2011	24.2 ^a	6.8 ^a	59.2 ^b	209.1 ^b	42.2 ^b	0.148 ^b	114.3 ^b
Grape juice (mg/kg FM)								
N rate	0 N	2.38 ^{ns}	0.267 ^{ns}	0.66 ^a	1.87 ^b	3.84 ^a	0.033 ^{ns}	2.43 ^{ns}
	50 N	2.60	0.242	0.89 ^a	1.26 ^a	4.22 ^a	0.026	2.37
	100 N	2.51	0.249	1.51 ^b	2.15 ^b	7.47 ^b	0.025	2.15
Year	2010	2.66 ^{ns}	0.272 ^{ns}	1.03 ^{ns}	2.21 ^b	0.85 ^a	0.011 ^a	1.89 ^a
	2011	2.33	0.234	1.01	1.30 ^a	9.50 ^b	0.045 ^b	2.74 ^b

Means were pooled across replications and other variables

Means followed by the same letter do not differ significantly at $p = 0.05$

moisture. In the study of Porro *et al.* (2010), low levels of this element were found in both the leaves and berries of water-stressed plants. However, due to B mobility, it may be leached by heavy rainfall, what also may cause temporary B soil shortages.

The investigated N rates did not affect leaf and grape copper content (Table 2). Leaf blades contained more Cu compared to petioles (9.3 vs 5.9 mg Cu kg⁻¹ respectively). Similarly to boron, climatic conditions during 2010 enhanced Cu leaf accumulation (8.3 vs 6.8 mg Cu kg⁻¹). We found relatively low Cu concentrations in the grape juice, which is in accordance with USDA (2010) data and the results of Olalla *et al.* (2004). However, Cu leaf status suggests that the copper content in the petioles was optimal, with the exception of in the 2011 growing season, when we detected < 5 ppm of copper in the petioles. The optimal levels of Cu in the grapevine petioles at full bloom ranged from 5 ppm to 10 ppm, and from 5 ppm to 15 ppm in véraison (Christensen & Peacock, 2000).

Vines that did not receive N fertiliser accumulated more Fe in their leaves (Table 2). This is opposite to the findings of Wolf *et al.* (1983), who found that the graduated N concentration increased Fe levels in grape tissues. We noticed the reverse pattern during the grape analysis. Vines that did not receive N fertiliser had the lowest fruit Fe content. Leaf blades accumulated fourfold more Fe than petioles (86.9 vs 17.2 mg Fe/kg). The optimum range of the iron content in grapevine petioles ranges from 40 to 180 ppm at full bloom, and from 31 to 50 ppm for véraison (Christensen & Peacock, 2000).

The manganese leaf content was strongly linked with N application rates. The lowest Mn content (both for

leaves and grapes) was found for moderate N fertilisation (Table 2). Amiri and Fallahi (2007) found that petiole Mn was significantly higher in the N treatments (150 g per plant at bud break and 30 days after bloom). In the present study, blades contained much more Mn (220.4 mg Mn/kg) than petioles (79.2 mg Mn/kg) or grapes (1.75 mg Mn/kg). This confirms the results of Fallahi *et al.* (2005) and Romero *et al.* (2010). The Mn accumulation in leaves was higher in 2011, while the reverse was true for fruit. Generally, manganese shows the greatest variation in leaf tissues, because root uptake of this nutrient depends on the soil solution concentration of Mn²⁺ (Christensen & Peacock, 2000). Adequate Mn values for grape petioles are 18 to 100 ppm at full bloom and 31 to 150 ppm at véraison. In our experiment, high Mn levels, especially in the leaf blades, could be linked to an increased availability of Mn observed in acid soils.

No impact of N fertilisation was observed on Zn leaf content (Table 2). However, some differences were found in the case of fruit, although these were inconsistent from one season to another. Also, no significant differences were recorded between the analysed leaf tissues. Fallahi *et al.* (2005) demonstrated that the concentration of blade Fe and Mn was higher, while blade Zn was lower, than those of petioles in all of the six examined cultivars. Romero *et al.* (2010) found higher concentrations of micronutrients in grapevine leaf blades, except for Zn. The critical Zn levels established for petiole samples ranged from 20 to 100 ppm at full bloom and between 30 to 50 ppm from mid-July to mid-August (Christensen & Peacock, 2000). In our research, the Zn content in leaf blades varied from 18.3 to 48.0 ppm, while in petioles we detected from 19.7 to 43.1 ppm Zn (data not shown). Zinc deficiency can affect foliage as well as fruit.

TABLE 3

Leaf blade, petiole and grape juice trace element content as influenced by different N application rates.

		Al	Ba	Cd	Cr	Li	Ni	Pb	Sr	Ti	V
Leaf blade and petioles (mg/kg DM)											
N rate	0 N	16.6 ^{ns}	21.8 ^{ab}	0.287 ^b	0.394 ^b	0.577 ^c	2.34 ^b	1.02 ^{ns}	47.5 ^{ns}	1.22 ^b	0.074 ^{ns}
	50 N	14.2	19.7 ^a	0.219 ^a	0.254 ^a	0.318 ^a	1.56 ^a	0.83	45.1	0.94 ^a	0.056
	100 N	13.3	24.8 ^b	0.227 ^a	0.324 ^b	0.539 ^b	2.18 ^b	0.93	47.0	0.83 ^a	0.066
Tissue type	Blades	24.4 ^b	16.8 ^a	0.119 ^a	0.320 ^{ns}	0.286 ^a	2.68 ^b	1.32 ^b	40.8 ^a	1.60 ^b	0.084 ^b
	Petioles	5.08 ^a	27.4 ^b	0.370 ^b	0.328	0.669 ^b	1.37 ^a	0.53 ^a	52.3 ^b	0.39 ^a	0.046 ^a
Year	2010	14.4 ^{ns}	20.3 ^a	0.287 ^b	0.060 ^a	0.420 ^a	1.70 ^a	0.83 ^{ns}	47.1 ^{ns}	1.25 ^b	0.083 ^b
	2011	15.1	23.8 ^b	0.203 ^a	0.588 ^b	0.536 ^b	2.35 ^b	1.03	46.0	0.75 ^a	0.048 ^a
Grape juice (µg/kg FW)											
N rate	0 N	378.8 ^a	74.0 ^{ns}	9.5 ^{ns}	135.8 ^{ns}	< 1.0	356.3 ^{ns}	< 1.0	106.2 ^{ns}	378.8 ^a	< 1.0
	50 N	549.2 ^b	47.0	11.8	119.5	< 1.0	391.0	< 1.0	133.2	549.2 ^b	< 1.0
	100 N	606.6 ^b	76.2	14.7	133.4	< 1.0	454.4	< 1.0	251.6	606.6 ^b	< 1.0
Year	2010	683.0 ^b	96.2 ^b	8.3 ^a	124.2 ^{ns}	< 1.0	196.8 ^a	< 1.0	24.8 ^a	683.0 ^b	< 1.0
	2011	340.0 ^a	35.2 ^a	15.8 ^b	134.3	< 1.0	604.3 ^b	< 1.0	302.5 ^b	340.0 ^a	< 1.0

Means were pooled across replications and other variables

Means followed by the same letter do not differ significantly at $p = 0.05$

Mild deficiencies reduce fruit set and increase the presence of shot berries (small berries that always remain hard and green).

In the present experiment, fruit content of zinc was more variable than within leaves. The values noted for Zn content ranged from 0.66 to 14.16 mg Zn/kg. On the basis of the main food composition tables in general use, we found that the data proposed by the USDA (2010) give similar values to those we measured in 2010. However, the concentration of Zn found in must in 2010 was higher than that stated in the study of Olalla *et al.* (2004) (0.49 to 0.52 ppm for white and red grapes). The concentration of zinc in grape juice determined in 2011 was very high, reaching 9.50 ppm. In the case of Zn and Cu, pesticide usage may affect the mineral content of grapes and, consequently the wine micronutrient level (Orescanina *et al.*, 2003; Salvo *et al.*, 2003).

In the case of the Mo content of leaves and grapes, the only influence was observed when comparing the studied seasons. Higher values were noted in 2011 (Table 2). The optimal concentration of Mo in grape petioles ranges from 0.2 to 0.4 ppm at full bloom. Williams *et al.* (2004) observed that a level of Mo in the petioles at flowering of 0.05 to 0.09 ppm was associated with deficient vines. A deficiency of Mo can affect the occurrence of fruit set disorders, such as berry asynchrony (bunches with berries that have a great range of size and maturity at harvest) or millerandage (seedless, usually unripe berries at harvest). In the present research, low soil pH, along with less than optimum levels of available Mo, caused low Mo concentrations in the grapevine tissue, particularly in the wet 2010. It should be noted that, on acid soils (pH < 5.5) low in Mo, this element is hardly available for plants (Kabata-Pendias, 2011).

The sodium leaf and fruit content was not dependent on the N fertilisation rate. Big differences were found when comparing petioles (176.5 mg Na/kg) and blades (15.1 mg Na/kg) (Table 2). Moreover, the vintage strongly influenced the petiole and grape content of Na. As a beneficial element, sodium plays an important role in controlling turgor pressure and plant water-uptake capacity, and preventing water losses. Wang *et al.* (2008) and Sardans *et al.* (2008) suggest that the capacity to maintain Na in plant tissues under drought is a part of the water stress avoidance mechanism.

The barium level was not influenced by the N rate (Table 3). Petioles contained higher Ba concentration (27.4) than leaf blades (16.8 mg Ba/kg). The climatic conditions during 2011 favoured Ba leaf accumulation, and the reverse tendency was proved for fruits. Barium is not very mobile in soil because it is easily precipitated and strongly absorbed by clays. The Ba mean contents in most plants range from 2 to 13 mg/kg DW (Kabata-Pendias, 2011). In the Mackenzie and Christy (2005) study, Ba and Sr contents in the soil significantly decreased the Baumé level in berries. Moreover, they indicated a positive correlation between Ba and Sr and titratable acidity in grapes.

As far as Cd, Cr and Ni are concerned, the lowest leaf concentration for all of them was measured for the combination with 50N, whereas the highest was with 0N treatment (Table 3). These differences were probably linked to environmental factors, and also to the vigour and yield of vines with different nitrogen fertilisation regimes. In this study, we observed a significant effect of N rate on plant vigour measured as trunk cross-sectional area (TCSA). At the end of the study, TCSA was 4.04, 5.23 and 6.05 cm² for 0, 50 and 100 N respectively (data not presented). In all cases,

these effects could be explained by growth dilution, since nitrogen fertilisation is often accompanied by a dilution effect, i.e. a reduction in the percentage content of mineral nutrients in plants due to increased growth or yield (Gębski, 1998). The concentration and dilution effects of mineral nutrients in plants are common phenomena that must be considered carefully in the interpretation of nutrient contents (Jarrell & Beverly, 1981).

Petioles contained higher Cd concentration than blades, while the reverse was found for Ni (Table 3). In the fruits we observed a trend for Cd increasing along with the N application rate, but only in the 2011 growth season. The same relationship was noted for cv. Bianca (Domagała-Świątkiewicz & Gąstoł, 2012). No significant differences were found for nickel and chromium.

The only differences in the Pb content were ascertained by comparing blades (0.53) and petioles (1.32 mg Pb/kg). The amounts measured in grapes were under the detection limits (< 1 ppb Pb).

The increased N fertilisation lowered Ti leaf concentration, but increased fruit Ti level (Table 3). The higher contents were measured for blades (1.60), rather than petioles (0.39 mg Ti/kg). The 2011 vintage decreased Ti leaf and fruit content (Table 3). The solubility of Ti in soils is very limited, and the phyto-availability of this element is low. The titanium content in food plants ranged from 0.13 to 6.7 mg/kg/d.m. The lowest values are in prepared cereals and fruits (Kabata-Pendias, 2011).

Only a few relationships between leaf blade $\times$ fruits element content were statistically significant (coefficient significant at $p = 0.05$). These correlation coefficients were generally low (data not shown). The only relationship with higher correlation coefficient was found between leaf Zn $\times$ fruit Zn content ($r = 0.43$), as well as for leaf V $\times$ fruit V content ($r = -0.48$). The total N blade content was linked slightly to fruit amounts of B ($r = 0.45$), Mn ($r = 0.48$), Ba ($r = 0.49$) and Cr ($r = -0.40$). The obtained correlation coefficients indicate the stronger linkage between the petioles and the fruit mineral content. The total N petiole level was correlated with fruit B content ($r = 0.50$), Mn ($r = 0.59$) and Ba ($r = 0.61$). A significant correlation between petiole $\times$ fruit microelement content was proved for Al ($r = 0.32$), Cr ($r = 0.29$), Na ($r = 0.33$), Sr ($r = -0.38$), Ti ($r = -0.56$), V ($r = -0.34$), Cu ($r = 0.42$) and Zn ($r = 0.36$). This may imply that analysing petioles is a better predictor of the content of grape trace elements.

CONCLUSIONS

The present study focused on the effect of nitrogen fertilisation on the concentration of microelements and trace elements in grapevine tissues (leaves and berries). Environmental factors, such as temperature and available water, have a significant impact on nutrient concentration in grapes. In the wet and warm vintage of 2010, higher amounts of B, Cu, Cd, Ti and V were measured in the leaves, and higher amounts of Mn, Al, Ba and Ti in were measured in grape juices. The dry season of 2011 increased the Fe, Mn, Zn, Ba, Na, Mo, Cr, Li and Ni content in leaf blades, and was associated with a higher Zn, Mo, Na, Cd, Ni and Sr concentration in fruit must. The leaf tissues analysis provides a guideline for vineyard

nutrient management. As compared to other grapevine cultivars, cv. 'Sibera' revealed a high Mn leaf concentration (blades and petioles), while the concentration of Cu, Fe and Zn was optimal. The leaf B and Mo concentrations ranged from low to moderate. The study showed that, in slightly acidic soils, mineral N fertilisers can augment the uptake and accumulation of microelements such as Fe, Mn, Zn, Al and Ti in grapes. Nitrogen fertilisation depressed the uptake and concentration of some elements, such as B, Fe, Mn, Cd, Cr, Ni and V, by the leaves. The analysed leaf blades contained higher amounts of Cu, Fe, Mn, Al, Ni, Pb, Ti and V as compared to the petioles. In contrast, the petioles had more B, Na, Ba, Cd, Li and Sr. The obtained correlation coefficients indicate a stronger linkage between petioles and fruits than between leaf blades and fruit mineral content.

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