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Department of Horticulture, Faculty of Environmental Management and Agriculture,
West Pomeranian University of Technology Szczecin, Poland

Influence of rootstock on nutrients and heavy metals in leaves and berries of the vine cultivar 'Regent' grown in North-Western Poland

Kamila Pachnowska*, Ireneusz Ochmian

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Summary

The aim of this study was to evaluate nutrient contents and heavy metals of the leaves and berries of vine cultivar 'Regent' grafted on different rootstocks ('Couderc 161-49', 'Sori', 'Kober 125AA', 'Börner' and 'Kober 5BB') in comparison to control ('Regent' root; own-rooted vines). Leaf and berry samples were collected in three consecutive years (2013-2015) at the research station of West Pomeranian University of Technology Szczecin in Poland. In the experiment, Ca, Cu, Fe, K, Mg, Mn, N, P and Zn were analysed among mineral nutrients, whereas Cd and Pb were analysed among heavy metals. A substantial influence of tested rootstocks on analysed element contents in leaves and berries has been proven. All tested rootstocks enabled a higher concentration of Ca and Mg in leaves in comparison to 'Regent' root. The greatest concentrations of Ca, K and N were found in leaves and berries under 'Sori' rootstock. As the only rootstock, 'Couderc 161-49' was characterised by a higher level of iron in samples in relation to own-rooted vines. The 'Börner' rootstock was the most effective in inhibition of heavy metals uptake.

Introduction

Grafting is a widely used approach of plant propagation and growth control, which is of great importance for the adaptation of cultivars in specific areas (PINA and ERREA, 2005). Rootstock efficiency highly depends on its interactions with scion and environmental influence; therefore, these factors should be taken into consideration for rootstock selection. Possibly important rootstock genotype × environment interactions indicate that the results obtained with one specific rootstock-scion combination in one environment condition cannot be immediately extrapolated to other situations (KELLER et al., 2001).

Rootstocks may affect many aspects of vine development, such as plant growth and yield (IBACACHE et al., 2016; NELSON et al., 2016; SATISHA et al., 2010; SOMKUWAR et al., 2015), as well as fruit quality, e.g. total soluble solids (BASCUÑÁN-GODOY et al., 2017; PULKO et al., 2012), acids (GAO-TAKAI et al., 2017; JIN et al., 2016b) and phenols (CHENG et al., 2017; NEDELKOVSKI et al., 2017). So far, many papers have focused on the impact of rootstocks on mineral uptake and also its distribution in vines. The researchers observed that the differences occurring in tested mechanisms could be attributed to the rootstock genotype, which gives distinct absorption capability or tendency for some specific minerals. Moreover, translocation and distribution of nutrients may differ among rootstocks (for a more detailed discussion, please see RIZK-ALLA et al., 2011). In the preceding studies on the interactions between scions, rootstocks and mineral nutrient uptake, the authors reported that grafting shows various but significant impact on vine development (IBACACHE and SIERRA, 2009; SURIANO et al., 2016; WALKER et al., 2004; ZAMBONI et al., 2016).

As it has been mentioned, it is important to continue research on the influence of grafting on the grapevine growth and fruit quality under different climate conditions, with different rootstocks and scions. Current state of knowledge still lacks information about rootstock influence on mineral concentration in 'Regent' vine, which was the object of our study. Red grapevine 'Regent' is the most important new fungal-resistant cultivar. The interest in cultivation of the 'Regent' vine occurred recently in Denmark as well (SCHERNEWSKI, 2011).

As of today, there are some papers that involve vine cultivar 'Regent' as an object of study. In the papers of VRŠIČ et al. (2015) and VRŠIČ (2017), the authors conducted research on the 'Regent', which is in the process of introduction to the Slovenian grapevine list, by grafting vine on 'Börner', '5BB' and 'SO4' rootstocks. KONTIĆ et al. (2016) investigated grape polyphenolic composition of fungus-resistant varieties, including 'Regent' cultivated in the area of Croatia near Zagreb, which is characterised by a warm or hot summer and a cold or very cold winter. Moreover, in the cool climate of Denmark, LIU et al. (2017) studied the potential influence of sequential fermentations on flavour generation in cultivar 'Regent', which is valued due to its resistance to cold and fungi. However, none of the abovementioned research articles focused on the distribution of mineral elements in vine cultivar 'Regent' affected by different rootstocks, and there is still lack of detailed information on this matter.

The aim of this study was to evaluate the effect of five rootstocks ('Couderc 161-49', 'Sori', 'Kober 125AA', 'Börner' and 'Kober 5BB') on the mineral nutrition and heavy metals uptake of vine cultivar 'Regent' in comparison to control ('Regent' root; own-rooted vines). During the experiment, Ca, Cd, Cu, Fe, K, Mg, Mn, N, P, Pb and Zn were analysed in 'Regent' leaves and berries.

Materials and methods

Characteristics of the research area, plant material and soil

The study was carried out in the Department of Horticulture of the West Pomeranian University of Technology in Szczecin (Poland) in the years 2013-2015. The experiment involved grapevine cultivar 'Regent', which was planted in 2010 in the research station of the University. The research station is located in the North-Western part of Poland in the Szczecin Lowland at a distance of approximately 65 km from the Baltic Sea (53°40' N, 14°46' E). The soil in the orchard was an agricultural soil with a natural profile, that was characterised more in depth in our previous paper (MIJOWSKA et al., 2017). The vines were planted with a North-South row orientation at 2.3 × 1 m and grafted on five rootstocks, viz 'Couderc 161-49', 'Sori', 'Kober 125AA', 'Börner' and 'Kober 5BB', while own-rooted vines served as a control ('Regent' root). The plants were pruned with a Single Guyot training system and vertically positioned with eight shoots and two clusters on each. Each year in the spring at a stage of bud swelling (BBCH 01) was applied nitrogen fertilisation at 60 kg N and a magnesium sulfate in the dose of 80 kg Mg per hectare. The tillage was used as the soil management system in the rows. Other standard vineyard management practices, including pest treatment, were performed during all growing seasons.

The experimental treatments were arranged in a randomised com-

* Corresponding author

plete block design. Each experimental unit was comprised of six vines. Each year of the study, soil and leaf samples were taken in a first/second decade of July at the phonological stage of berry set. The soil samples were collected using an Egner's sampling stick from the humus level (0-50 cm). Leaves (blades and petioles) were collected from the central part of the one-year shoots, two-three leaves above second cluster. Grapes were collected on the first decade of October in successive seasons, with berry maturity ranging from an average of 19-20.5 °Brix.

Weather conditions

Basic weather conditions, such as average temperature and rainfall, during the growing season (April-October) in the years 2013-2015 with reference to the multi-year period (1951-2012) were previously thoroughly described (Mijowska et al., 2017). Generally, the growing period of 2015 was characterised by relevantly lower rainfall, while 2014 by higher rainfall in comparison to other years of study and mean of years 1951-2012. In addition, in May and June of 2015 the temperatures recorded were lower, while in August of 2015 significantly higher, comparing to the years of 2013 and 2014 and mean value of the multi-year period.

Elemental analysis

The estimation of the content of minerals in dry weight plant and soil was carried out in accordance with the Polish Standard (IUNG, 1972) using certified reagents, in three consecutive years of study (2013-2015). The averages of soil elemental composition and pH are presented in Tab. 1. K and P in soil were measured by extracting in $C_6H_{10}CaO_6$, Ca and Mg were determined by extracting in $C_2H_3O_2NH_4$. The contents of Cd, Cu, Fe, Mn, Pb and Zn were assessed after mineralisation in HNO_3 (65%) and $HClO_4$ (70%) in a ratio 1:1. The content of K was measured with the atomic emission spectrometry, whereas Ca, Cd, Cu, Fe, Mg, Mn, Pb and Zn contents with the flame atomic absorption spectroscopy using iCE 3000 Series (Thermo Fisher Scientific, UK). Phosphorus (P) was assessed by the colourimetric method on a Spekol 221 spectrophotometer (Carl Zeiss, Germany).

Leaves and berries were dried at a temperature of 65 °C and 105 ºC, respectively. Ca, K, Mg, N and P were measured after wet mineralisation in H_2SO_4 (96%) and $HClO_4$ (70%). The content of Cd, Cu, Fe, Mn, Pb and Zn was determined after mineralisation in HNO_3 (65%) and $HClO_4$ (70%) in a ratio 3:1. The total nitrogen concentration in plants was determined by the Kjeldahl distillation method using a Vapodest 30 (Gerhardt GmbH, Germany). The contents of Ca, Cd, Cu, Fe, K, Mg, Mn, P, Pb and Zn were measured using the same methods, as in the case of soil measurement. All tests were performed each year in three replications.

Statistical analysis

The obtained results were subjected to statistical analyses using Statistica 12.5 (Statsoft Polska, Cracow, Poland). The data were subjected to one-factor variance analysis (ANOVA), performed separately for each year. The average data of root influence on elements content in leaves and berries were analysed statistically by means of synthesis from years. Mean comparisons were performed using Tukey's least significant difference (LSD) test; significance was set at $p < 0.05$. The unprocessed data of plant and soil composition is available as comma separated value file (Pachnowska and Ochmian, 2018).

Results and discussion

General overview on the mineral content

The results of nutrients composition and heavy metals analyses in leaves and berries of cultivar 'Regent' are summarised in Tab. 2-3 and Fig. 1-2. All of the considered elements were significantly differentiated in the composition of leaves and berries influenced by various rootstocks. In this experiment, yearly weather conditions showed less relevant impact on mineral uptake than rootstocks. In case of grafting, each element was statistically differentiated between samples. However, considering years of study, only in a few cases of minerals, statistically significant changes occurred. Tab. 2 shows macronutrient composition (g/kg) of 'Regent' leaves and berries harvested from the own-rooted and grafted vines as the average of three years of study (2013-2015). Correspondingly, Tab. 3 presents micronutrient composition and heavy metals (mg/kg). Comparison of the consecutive experimental years shows, that in case of P and K in leaves and berries, and N in berries, the plant materials obtained in 2015 were the least abundant in macronutrients with statistical meaning. Comparing to 2013 and 2015, the Mg concentration in berries in 2014 was statistically higher. Among micronutrients and heavy metals, only Mn and Pb in leaves and berries, and Fe in berries were affected by weather conditions.

Macronutrients

Fig. 1 presents the average of macronutrient (Ca, K, Mg, N and P) content in leaves and berries of the 'Regent' cultivar from the own-rooted and grafted vines during three years of study (2013-2015). The most abundant element in leaves was nitrogen (N), followed by potassium (K), magnesium (Mg), phosphorus (P) and calcium (Ca). This sequence varied in berries as followed: K > N > P > Ca > Mg. It should be emphasised that the greatest differences in homogeneous groups under rootstocks occurred in the concentration of potassium in leaves, while the least differences occurred in phosphorus and magnesium in berries.

The results of our study did not indicate unambiguous influence of rootstock on the potassium amount in leaves and berries of cultivar 'Regent'. Only 'Sori' rootstock led to a statistically meaningful increase in K concentration in comparison to own-rooted vines, while 'Börner' and 'Kober 5BB' decreased ion level. In the outgoing reports are some papers about influence of plant grafting on potassium accumulation in grapes and wines. For example, Gong et al. (2010) reported that concentration of K increased in the pulp and berry skin as a result of vine grafting. Potassium in the grapes of 'Chardonnay' and 'Shiraz' was also affected by vineyard site, located at Merbein and Padthaway in Australia. In South Australia, Walker et al. (1998) also found grafting-enhanced K^+ concentration in grapes, with the exception of 'Chardonnay'. Similarly, a few years later Walker et al. (2004) found higher K^+ concentration in laminae and juice of cultivar 'Sultana' under grafting. In contradiction, other authors conducting research in Washington indicated that rootstock had a significant influence only on K^+ concentration in the skin of berries, these differences, however, were not enough to alter the significance of total K^+. Nevertheless, the wines from own-rooted vines tend to have slightly

Tab. 1: The average of elemental composition (mg/kg) and pH of the soil in vineyard during three years of study (2013-2015)

pH KCl	Ca	Cd	Cu	Fe	K	Mg	Mn	P	Pb	Zn
6.83	340.8	0.29	9.32	94.47	127.5	70.4	72.4	99.2	16.6	35.7

Tab. 2: The average of macronutrient composition (g/kg) in leaves and berries of 'Regent' during three years of study (2013-2015) from the own-rooted and grafted vines

Year	Ca	K	Mg	N	P
	Leaves				
2013	2.53±0.35 a	23.5±2.26 b	4.82±0.53 a	23.5±4.4 a	3.36±0.25 b
2014	2.62±0.32 a	25.5±2.54 b	4.83±0.49 a	25.5±4.5 a	3.31±0.33 b
2015	2.23±0.25 a	18.5±1.98 a	4.57±0.45 a	21.9±3.9 a	2.64±0.23 a
	Berries				
2013	0.76±0.11 a	11.06±1.19 b	0.54±0.06 a	5.88±0.74 b	2.23±0.19 b
2014	0.83±0.16 a	11.42±1.14 b	0.62±0.09 b	6.27±0.53 b	2.23±0.14 b
2015	0.79±0.14 a	7.99±0.46 a	0.56±0.08 a	4.98±0.35 a	1.28±0.12 a

±SD: standard deviation. Means having same letter were not significantly different by Tukey's comparison at $p < 0.05$ level.

Tab. 3: The average of micronutrient composition and heavy metals (mg/kg) in leaves and berries of 'Regent' during three years of study (2013-2015) from the own-rooted and grafted vines

Year	Micronutrients				Heavy metals	
	Cu	Fe	Mn	Zn	Cd	Pb
	Leaves					
2013	6.97±0.62 a	211±20.7 a	26.5±4.0 a	22.0±4.7 a	0.18±0.07 a	0.89±0.11 a
2014	6.76±0.80 a	210±21.3 a	40.9±4.2 c	22.2±5.1 a	0.17±0.05 a	0.97±0.04 ab
2015	6.90±0.68 a	199±17.9 a	33.7±5.3 b	22.4±4.2 a	0.14±0.03 a	1.05±0.07 b
	Berries					
2013	2.25±0.23 a	22.3±6.7 c	3.24±1.04 b	8.60±2.14 a	-	0.164±0.027 a
2014	2.09±0.19 a	18.5±5.3 b	5.73±0.95 c	8.75±1.57 a	-	0.167±0.016 ab
2015	2.22±0.24 a	13.4±2.2 a	2.11±0.46 a	8.46±0.76 a	-	0.195±0.03 b

"-" not detected. ±SD: standard deviation. Means having same letter were not significantly different by Tukey's comparison at $p < 0.05$ level.

higher potassium (Harbertson and Keller, 2012). In the study of Ibacache and Sierra (2009), potassium in petioles of three varieties showed a tendency to diminish under grafting. This was clearly shown in the case of ungrafted 'Superior Seedless' variety, which achieved the statistically-highest ion level.

The results of our experiment showed, that plant materials obtained from vines grafted on 'Sori' were characterised by the highest concentrations of N (28.8 and 6.32 g/kg), K (26.6 and 11.22 g/kg), and Ca (2.90 and 1.05 g/kg), for leaves and berries, respectively. In addition, berries harvested from vines grafted on 'Sori' were the most abundant in Mg (0.66 g/kg). Similar high amounts of nitrogen (leaves 29.3 g/kg; berries 6.31 g/kg) and magnesium (berries 0.64 g/kg) were found in samples of the 'Couderc 161-49' rootstock. However, this rootstock was characterised simultaneously by the lowest phosphorus uptake (leaves 2.56 g/kg; berries 1.74 g/kg), and, as well as own-rooted vines, by the lowest calcium level in berries (0.61 g/kg in both cases). In turn, Zamboni et al. (2016) reported that the best N nutrition in leaves of the '101-14' rootstock also corresponded to the lowest P and K accumulations. In addition, these results are not consistent with the observations of Ibacache and Sierra (2009) who found that, among four varieties, the 'Salt Creek' rootstock led to the statistically-highest accumulation of nitrogen and phosphorus with an average uptake of potassium.

'Börner' and 'Kober 5BB' rootstocks were characterised by similar or diminished uptake of N, K, and Mg in berries, compared to own-rooted vines. However, 'Börner' rootstock resulted in the highest levels of magnesium (5.15 g/kg), and, along with the 'Kober 125AA', phosphorus (3.46 g/kg) in leaves. In addition, our results of study on 'Regent' vine are consistent with Pulko et al.'s (2012) observation on grape juices of 'Sauvignon Blanc', that 'Börner' and 'Kober 5BB' rootstocks had statistically similar impact on the accumulations of potassium, magnesium and calcium. Furthermore, all the rootstocks tested in our experiment enabled a higher concentration of Ca and Mg in leaves in relation to the control. The results of Walker et al. (2004) research showed that rootstocks have a tendency to limit the concentration of Ca^{2+} in laminae in relation to own-root system; although in the case of grape juice, they tend to increase the amount of Mg^{2+}. Furthermore, the authors noted that grafting has an ambiguous impact on calcium in juice and on magnesium in laminae.

Referring to the Miele et al. (2009) we may compare the influence of 'Couderc 161-49' and 'Kober 5BB' rootstocks on N, P, K, Ca and Mg nutrients. Resulting from the authors' observation, these rootstocks showed mainly a statistically similar impact on the accumulations of minerals in leaves and grapes. In contradiction, our result showed statistically higher concentration of nitrogen and potassium in general, and magnesium in berries under 'Couderc 161-49' rootstock. Moreover, 'Kober 5BB' led to a higher amount of phosphorus and magnesium in leaves, and calcium in berries.

Micronutrients

Fig. 2 shows the average data obtained during three years of study (2013-2015) from the analyses of micronutrient (Cu, Fe, Mn and Zn) and heavy metals (Cd, Pb) contents in leaves and berries of the 'Re-

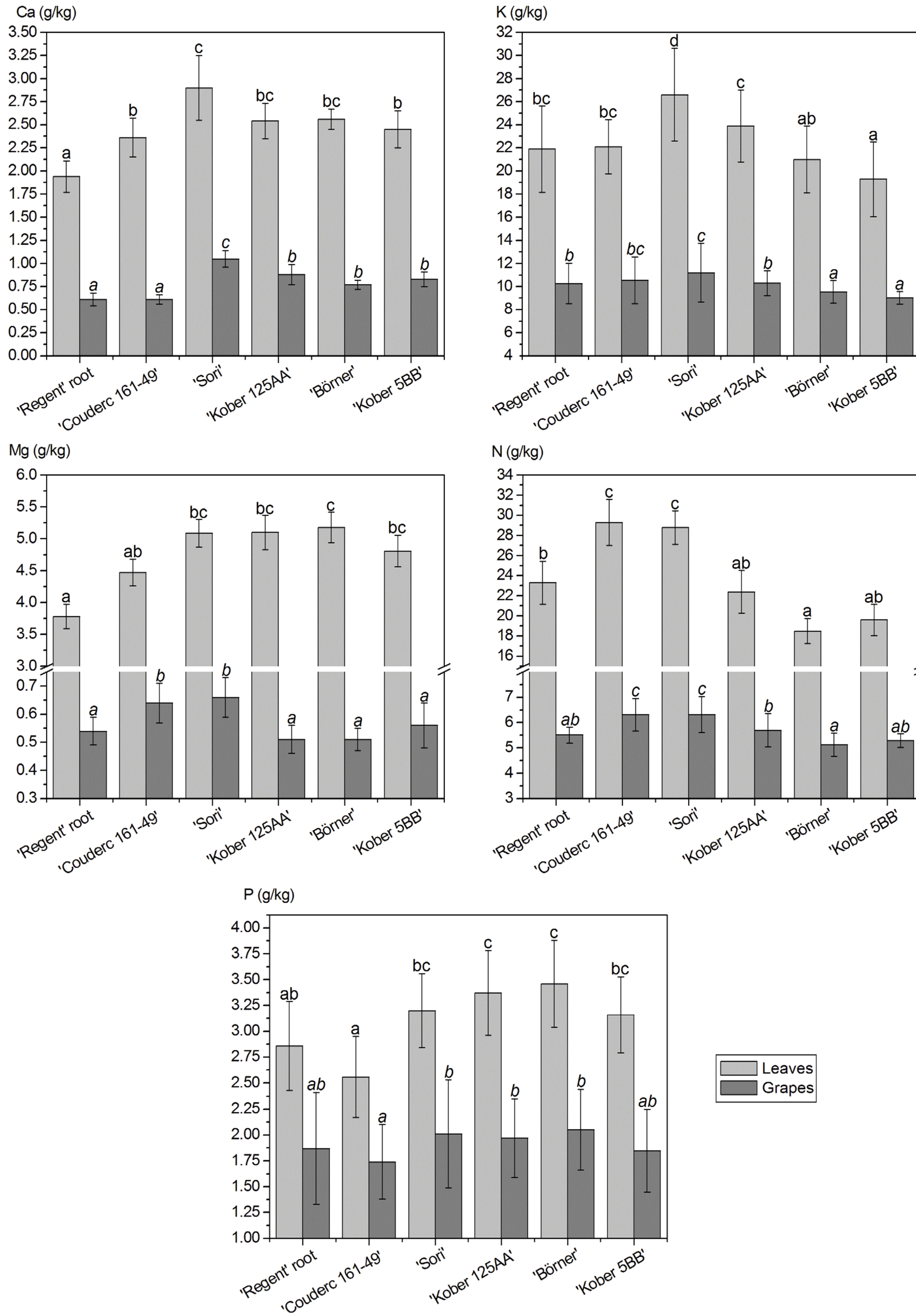

Fig. 1: The average of macronutrient content (g/kg) in leaves and berries of the 'Regent' cultivar from the own-rooted and grafted vines during three years of study (2013-2015). Means having same letter were not significantly different by Tukey's comparison at $p < 0.05$ level. Lowercase letters (a) indicate the means of leaves, and italic letters (*a*) of berries.

gent' cultivar, harvested from the own-rooted and grafted vines. The sequence of elements in leaves occurred in the following order: Fe > Mn > Zn > Cu > Pb > Cd. In case of berries, the only difference between element concentrations was between zinc and manganese (Zn > Mn). Cadmium was not detected in berries of grafted vines due to its level being below device sensitivity of <0.01 mg/kg. In addition, in berries from the own-rooted vines, Cd was measured at the concentration of 0.018 mg/kg.

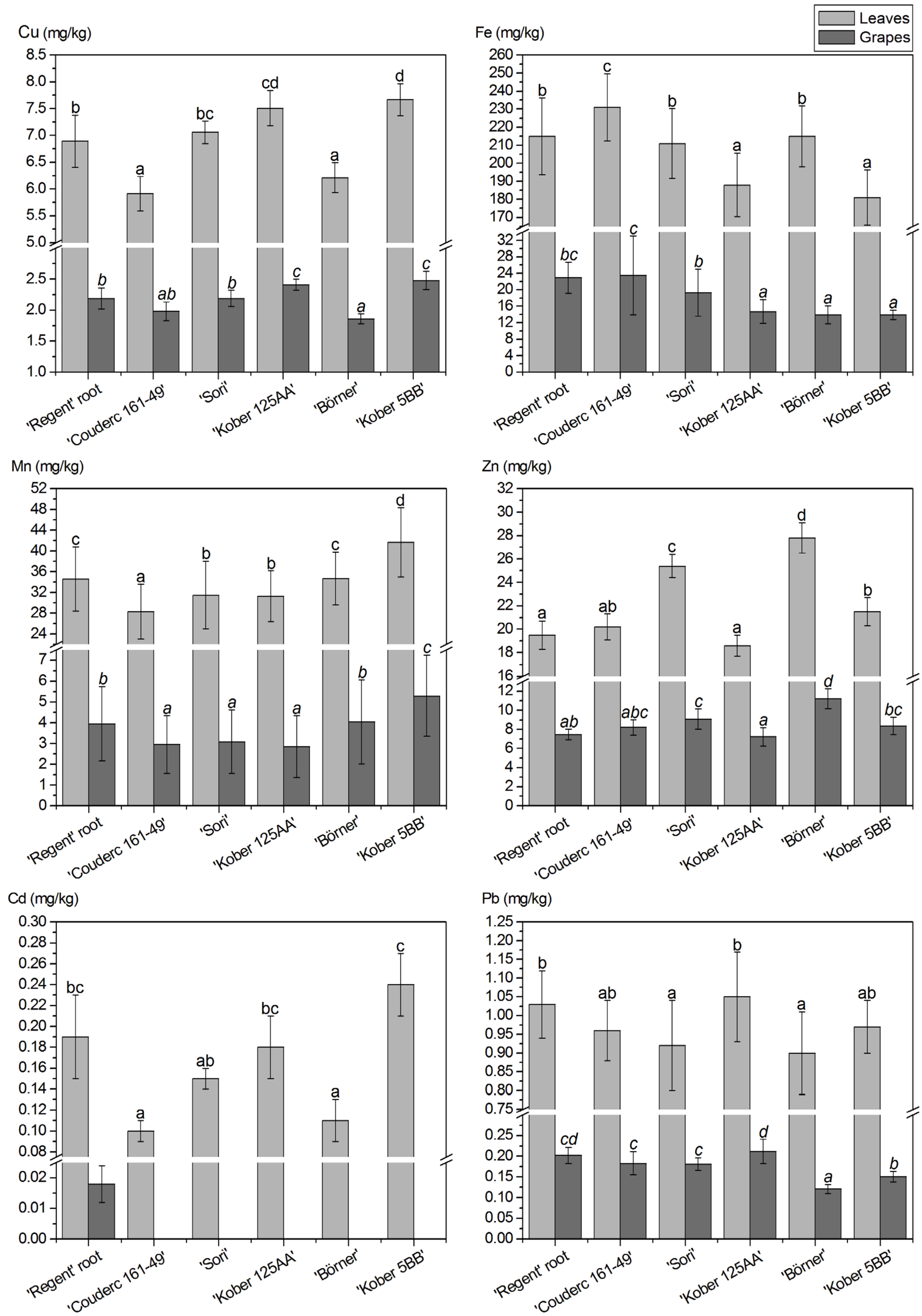

Fig. 2: The average of micronutrient content and heavy metals (mg/kg) in leaves and berries of the 'Regent' cultivar from the own-rooted and grafted vines during three years of study (2013-2015). Means having same letter were not significantly different by Tukey's comparison at $p < 0.05$ level. Lowercase letters (a) indicate the means of leaves, and italic letters (*a*) of berries.

Plant materials obtained from own-rooted vines and 'Kober 125AA' were characterised by statistically similar uptake of Zn and Pb, with the lowest concentration of zinc and the highest of lead among tested samples. In contradiction, the highest level of Zn was found in samples of 'Börner' (leaves 27.8 mg/kg, berries 11.24 mg/kg), which was also marked by one of the lowest accumulation of Cu and Pb in all plant samples, Fe in berries, and Cd in leaves. In case of Fe and Mn, three of the studied rootstocks diminished nutrients accumulation in berries, in comparison to 'Regent' root. Fe amount in leaves and berries was higher only under 'Couderc 161-49' rootstock. In turn, statistically higher level of Mn occurred only under 'Kober 5BB' rootstock, which also led to the highest uptake of Cd in leaves and Cu in all plant samples. According to the findings of Köse et al. (2016), 'Kober 5BB' was characterised by an average Cu, Fe, Mn and Zn nutrient contents in *Vitis labrusca* L. leaf blades at harvest, in comparison with two other rootstocks.

In the experiment of Jin et al. (2016a), berries from the 'Gold Finger' vine grafted on 'Kober 5BB' rootstock were charecterised by a statistically similar Fe concentration as berries from own-rooted plants. In addition, the authors reported that this rootstock led to the lowest Zn level among all tested samples. In contradiction to the abovementioned research, our result showed a diminished iron and enhanced zinc accumulations in leaves and berries under 'Kober 5BB' rootstock, in comparison to the control. In other experiment of Jin et al. (2016b), conducted on the 'Summer Black' cultivar, berries from vines grafted on 'Kober 5BB' achieved approximately 1.53 times higher Zn accumulation than samples from own-rooted plants.

Conclusions

In this study, five tested rootstocks ('Couderc 161-49', 'Sori', 'Kober 125AA', 'Börner' and 'Kober 5BB') showed a great influence on the nutrients and heavy metals in leaves and berries of cultivar 'Regent', comparing to samples obtained from an ungrafted vines. All tested rootstocks enabled a higher levels of Ca and Mg in leaves. The 'Sori' rootstock led to the statistically greatest concentrations of Ca, K and N in general and Mg in berries. A similarly high nitrogen and magnesium amounts was obtained under 'Couderc 161-49', which, as the only rootstock, led to a higher concentration of Fe in leaves and berries in comparison with 'Regent' root. However, this rootstock also led to the lowest P uptake, and, next to own-rooted vines, to the lowest Ca level in berries. The 'Börner' rootstock resulted in the highest accumulation of phosphorus (along with 'Kober 125AA') and magnesium in leaves, and zinc in general. In addition, this rootstock showed the greatest inhibition of Cu, Cd and Pb uptake. In turn, only the 'Kober 5BB' rootstock enabled statistically-higher level of Mn compared to own-rooted vines, and the highest concentration of Cu in leaves and berries.

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Conflict of interest

The authors declare that they have no competing interests.

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Address of the authors:

Kamila Pachnowska, Ireneusz Ochmian: Department of Horticulture, Faculty of Environmental Management and Agriculture, West Pomeranian University of Technology Szczecin, Słowackiego 17, 71-434 Szczecin, Poland

E-mail corresponding author: kamila.mijowska@zut.edu.pl