

Microelements content in leaves of raspberry cv. Willamette as affected by foliar nutrition and substrates

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Abstract

KARAKLAJIĆ-STAJIĆ Ž., GLIŠIĆ I.S., RUŽIĆ DJ., VUJOVIĆ T., PEŠAKOVIĆ M., 2012. **Microelements content in leaves of raspberry cv. Willamette as affected by foliar nutrition and substrates.** Hort. Sci. (Prague), 39: 67–73.

Raspberry (*Rubus idaeus* L.) cultivar Willamette has long been the most commonly grown raspberry cultivar in Serbia, which is owing to high adaptability of the cultivar to respective agro-environmental conditions. Massive dieback of full bearing plantings is a major problem in raspberry growing hence quality planting material is a must when establishing new raspberry plantings. The study was conducted under protected conditions (in greenhouse) on plants obtained by micropropagation *in vitro*. In order to achieve optimal vegetative potential, plants were grown for two consecutive years (2004–2005) on two substrates (Steckmedium and Seedling) using three foliar fertilizers (Wuxal, Murtonik and Ferticare). The study revealed optimal vegetative growth in plants studied, excess manganese (150.60–214.52 mg/g), optimum iron content (94.00–123.50 mg/g), and zinc (28.60–31.00 mg/g) and copper (3.10–4.00 mg/g) deficiencies, based on the referent values of microelements content. The assessment of nutritional status of plants by the DOP index suggested significant differences in microelements imbalance when different foliar fertilizers and substrates are applied.

Keywords: *Rubus idaeus* L.; foliar fertilizer; growing medium; nutrients; DOP index

From the economic aspect, red raspberry (*Rubus idaeus* L.) is a major soft fruit in Serbia (Central Serbia), the total production of fresh fruits amounting to 58,000 tons (FAOSTAT 2011). According to NIKOLIĆ et al. (2008), cultivar Willamette predominates in the Serbian raspberry growing, accounting for 95% of the total area.

In recent years, a dieback of raspberry plants was observed in raspberry plantings of Serbia, and species of the genus *Phytophthora* spp. are believed to be the primary causal agent of the phenomenon (KOPRIVICA et al. 2002). However, the fact that raspberry plantings were established with shoots originating from commercial plantings has considerably contributed to the dieback.

In order to ensure steady and quality yields and extend exploitation period in raspberry plantings, virus-free, true-to-type planting material propagated in mother plantings is a must for planting establishment. This type of planting material is obtained by micropropagation *in vitro* (RUŽIĆ, LAZIĆ 2004), and optimal and balanced plant nutrition ensures high plant vegetative potential.

Well-balanced nutrients ratio and their favorable content in soil (the uptake of which can be influenced by adverse conditions) ensure optimal minerals supply (LANAUSKAS et al. 2006). Therefore, foliar nutrition plays an important role in increasing mineral content in the aboveground plant parts (SWIETLIK, FAUST 1984). Plants have very low mi-

croelements requirements, contrary to their need for macroelements, which does not diminish their importance for plant growth and reproduction (KIRKBY, ROEMHELD 2004).

Generally, comparatively little is known about essential processes in the plant, such as nutrient uptake in leaves, nutrients processing in the plant and their effect on different physiological processes in leaves and fruits (WEINBAUM 1988; SCHLEGEL, SCHÖNHER 2002). Similarly, little is known about the effects of foliarly applied macro- and microelements in red raspberry (REICKENBERG, PRITTS 1996).

The objective of this investigation was to study indirect impact of foliar nutrition and different substrates on microelements content in raspberry leaves which meet the criteria in terms of health, true-to-typeness and quality of plants.

MATERIAL AND METHODS

Plant material and experimental design

Plants of raspberry cv. Willamette obtained by micropropagation *in vitro* were used for planting establishment. The research was conducted in screenhouse (30 × 6 m) at a site of the Fruit Research Institute, Čačak (43°53'N latitude, 20°20'E longitude, 225 m altitude) over 2004–2005. The screenhouse is protected by anti-insect net that provides full plant isolation and protection. The trial was done using a randomized block design and it included four replications of each foliar fertilizer and substrate. Some 640 plants were potted on 12th May 2004 in plastic 0.5 dm³ vessels and transferred into 2 dm³ plastic pots in 2005.

Treatments

The study involved three foliar fertilizers [Wuxal 0.3%, Murtonik 0.2%, Ferticare (Kemira 0.5%), and no treatment in the control variant] and two plant growth substrates (Steckmedium, Seedling). Given the three foliar fertilizers and the control, the trial involved four variants of treatment (80 plants) in four replications with 20 plants. Wuxal is a foliar fertilizer that comprises microelements in the form of chelate complexes, which ensures high utilization and good mobility of adopted elements. Wuxal includes: N 8%, P₂O₅ 8%, K₂O 6%, B 0.01%, Mn 0.013%, Cu 0.007%, Fe 0.0015%, Mo 0.001% and Zn 0.005%.

Murtonik contains N 19%, P₂O₅ 9%, K₂O 27% and microelements (Fe, Zn, B, Mo, Cu and Mg). Ferticare (Kemira) has high nitrogen content, and its microelements are also in the form of chelate complexes. This fertilizer contains: N 24%, P₂O₅ 8%, K₂O 16%, S 5.0%, Mg 3.7%, Fe 0.1%, Mn 0.05%, B 0.03%, Cu 0.01%, Zn 0.025% and Mo 0.004%.

Foliar fertilizers above were applied periodically, every 15 days during the period of intensive growth (June, July and August). First treatment in 2004 was done on the 1st June (two weeks after planting), and the last on the 15th July. In 2005, the first treatment was done on the 10th June, and the last on the 25th July.

In addition, the research involved two Klasmann substrates (Steckmedium, Seedling), with total of 320 plants studied per substrate. Steckmedium (0.7 g/l nutrient content) is a mixture of fine white sphagnum peat (0–10 mm) and perlite (25%), whereas Seedling (0.5 g/l nutrient content) is a mixture of double-sifted white peat, frozen black sphagnum peat and lime. Both substrates are slightly acid (pH = 6), enriched with water-soluble nutrients, microelements and wetting agents.

Nutrient measurements

Raspberry leaf samples used to determine microelements content were collected from all the studied treatments (late July, 2005). Leaves were taken proportionally from all plant parts and adequately prepared for chemical analysis, which involved rinsing in a solution of nitric acid, air drying, and drying at 65°C (in a drier). Contents of Cu, Fe, Zn and Mn were checked by atomic absorption spectrophotometer (Pye Unicam SP 191, London, UK) and expressed in µg/g leaf dry matter.

Deviation from optimal content

The deviation from optimum percentage (DOP index) was estimated for the diagnosis of mineral content status in leaves (MONTAÑÉS et al. 1991). The DOP index of leaf microelements was calculated for each substrate and foliar fertilizer by:

$$\text{DOP} = \frac{C \times 100}{C_{\text{ref}}} - 100$$

where:

C – stands for nutrient content in sample studied

C_{ref} – nutrient content considered as optimum

both values being given on a dry matter basis. The C_{ref} was taken from microelements optimum values, as proposed by KESSEL (2003). The ΣDOP is obtained by adding the DOP index values irrespective of sign. The larger the ΣDOP , the greater the nutrients imbalance (ZARROUK et al. 2005).

Analysis of morphological traits of plants

Growth intensity of the aboveground plant parts was examined during intensive growth period by checking height of the aboveground part and plant thickness at the plant base. The former was determined by a ruler, and the latter by the 'Inox' vernier scale (± 0.005 mm accuracy).

Data analysis

Experimental data were subjected to analysis of variance (ANOVA) using MSTAT-C statistical

computer package (Michigan State University, East Lansing, USA). The Dunett's test (d') at $d' \leq 0.05$ and $d' \leq 0.01$ was used for mean separation of experimental data referring to the application of foliar fertilizers. The Duncan's multiple range test was used for mean separation of data concerning application of substrates as well as for analysis of interaction among substrates and foliar fertilizers. Testing of ΣDOP mean values was based on the LSD test at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS

Micronutrients content

Application of different substrates and foliar fertilizers revealed significant differences in Cu and Fe content, while the Mn content varied markedly among different substrates (Table 1). Analysis of variance suggests a major influence of substrate/foliar fertilizer interaction on Cu content.

Table 1. Microelements content in leaves of raspberry cv. Willamette in the second year after planting (2005)

Treatment		Microelements content ($\mu\text{g/g}$)				
		Cu	Fe	Zn	Mn	
A	Seedling	3.25 ± 0.12^b	112.75 ± 0.12^a	30.30 ± 0.47^a	150.60 ± 2.60^b	
	Steckmedium	3.55 ± 0.13^a	94.00 ± 0.13^b	29.55 ± 0.58^a	214.52 ± 2.99^a	
B	Wuxal	$3.10 \pm 0.08^{**}$	95.00 ± 0.08^{ns}	29.90 ± 0.64^{ns}	178.00 ± 11.15^{ns}	
	Murtonik	$4.00 \pm 0.08^{**}$	$123.50 \pm 0.08^{**}$	30.20 ± 0.63^{ns}	181.00 ± 12.96^{ns}	
	Ferticare	$3.00 \pm 0.07^{**}$	97.00 ± 0.07^{ns}	31.00 ± 0.87^{ns}	188.50 ± 11.42^{ns}	
	control ¹	3.50 ± 0.20	98.00 ± 0.20	28.60 ± 0.73	182.75 ± 14.84	
A \times B	Seedling	Wuxal	3.00 ± 0.11^b	104.00 ± 0.10^a	30.20 ± 1.07^a	150.00 ± 5.49^a
		Murtonik	4.00 ± 0.12^a	131.00 ± 0.12^a	30.40 ± 0.81^a	148.00 ± 4.19^a
		Ferticare	3.00 ± 0.11^b	104.00 ± 0.11^a	31.00 ± 1.22^a	160.00 ± 5.21^a
		control ¹	3.00 ± 0.11^b	112.00 ± 0.11^a	29.60 ± 0.96^a	144.40 ± 4.04^a
	Steckmedium	Wuxal	3.20 ± 0.12^b	86.00 ± 0.12^a	29.60 ± 0.83^a	206.00 ± 5.49^a
		Murtonik	4.00 ± 0.12^a	116.00 ± 0.12^a	30.00 ± 1.08^a	214.00 ± 4.19^a
		Ferticare	3.00 ± 0.12^b	90.00 ± 0.12^a	31.00 ± 1.41^a	217.00 ± 5.21^a
		control ¹	4.00 ± 0.12^a	84.00 ± 0.12^a	27.60 ± 0.93^a	221.10 ± 4.04^a
ANOVA	A	**	**	ns	**	
	B	**	**	ns	ns	
	A \times B	**	ns	ns	ns	

¹the control is a plant not treated with a foliar fertilizer; A – substrate, B – foliar fertilizer; * and ** indicate significant difference between means of the control and other foliar fertilizers at $d' \leq 0.05$ and $d' \leq 0.01$ by the Dunett's test, respectively; values within each column followed by the same small letter are not significantly different at the ($P \leq 0.05$) by the Duncan's multiple range test; ns – non-significant differences

Table 2. Microelements content in cv. Willamette raspberry plants in the second year after planting (2005) based on DOP index

Treatment		DOP index				ΣDOP
		Cu	Fe	Zn	Mn	
Substrate	Seedling	-74.00	+0.22	-47.30	+36.91	158.43 ^b
	Steckmedium	-71.60	-16.44	-48.51	+95.02	231.57 ^a
Foliar fertilizer	Wuxal	-75.20	-15.55	-48.00	+68.82	207.57 ^c
	Murtonik	-68.00	+9.78	-47.48	+64.55	189.81 ^a
	Ferticare	-76.00	-13.77	-46.09	+71.36	207.22 ^c
	control ¹	-72.00	-12.88	-50.26	+66.14	201.28 ^b

¹the control is a plant not treated with a foliar fertilizer; (-) indicate lower microelements supply as compared to the optimal content; (+) indicate higher microelements supply as compared to the optimal content; values within each column followed by the same small letter are not significantly different at $P \leq 0.05$ and $P \leq 0.01$ by LSD test

Cu content in raspberry leaves ranged from 3.00 ± 0.07 to 4.00 ± 0.08 µg/g, and was significantly higher in leaves of plants grown on Steckmedium.

Application of different foliar fertilizers revealed the highest Cu content in leaves of plants treated with Murtonik (4.00 ± 0.08 µg/g), while it was the lowest in treatments with Ferticare (3.00 ± 0.07 µg/g). All variants of treatment with fertilizers above showed significant differences in Cu content compared to the control (Table 1).

Fe content ranged from 94.00 ± 0.13 to 123.50 ± 0.08 µg/g, while significantly high content of adopted Fe was evidenced in leaves of plants grown on the Seedling substrate. As for the application of different fertilizers, considerably higher Fe content was obtained using Murtonik compared to the control (123.50 ± 0.08 µg/g).

Zn content ranged from 28.60 ± 0.73 to 31.00 ± 0.87 µg/g, and no significant influence was observed on either substrate or foliar fertilizer applied (Table 1).

Mn content varied greatly among substrates, the highest Mn content being in leaves of plants grown on Steckmedium (214.52 ± 2.99 µg/g).

Deviation from the optimal content

Cu and Zn deficiency and Mn excess were evidenced on all substrates used, while Fe content varied among treatments (Table 2). Fe deficiency was found in plants grown on Steckmedium, whereas Fe content was optimal in those grown on the Seedling substrate. As for the application of different foliar fertilizers, plants treated with Wuxal and Ferticare, as well as those in the control vari-

ant, exhibited Fe deficiency, while treatments with Murtonik gave a slight Fe surplus.

Analysis of variance indicated a significant influence of applied substrates and foliar fertilizers on microelements imbalance (Table 2). Maximum deviation from optimal microelements balance was observed in plants grown on Steckmedium ($\Sigma\text{DOP} = 231.57$), whereas it was lowest in those grown on Seedling ($\Sigma\text{DOP} = 158.43$).

Morphological traits of plants

Applied foliar fertilizers affected differently plant thickness at the plant base. Plants were the thickest at the basal section (compared to the control) when treated with Murtonik (2.69 ± 0.04 mm), while Ferticare did not have a similar effect, thickness values being 2.51 ± 0.03 mm (Table 3).

As for plant thickness, no significant differences were identified among years and substrates used. In addition, ANOVA showed a significant effect of substrate/foliar fertilizer interaction and substrate/year interaction on plant thickness.

The data shown in Table 3 suggest a significant effect of the substrate, foliar fertilizer and year on height of the studied plants. Similarly, substrate/foliar fertilizer interaction on the one hand, and substrate/fertilizer/year interaction on the other play a significant role on plant height at $P \leq 0.05$. Plants grown on the Seedling substrate were significantly higher than those grown on Steckmedium. Untreated plants were the shortest (20.66 ± 1.67 cm) and were significantly lower compared to those treated with Ferticare at $d' \leq 0.05$, and Murtonik and Wuxal at $d' \leq 0.01$. In the first year of study plants were considerably higher.

Table 3. Morphometric characteristics of cv. Willamette raspberry plants over 2004–2005

Treatment		Plant thick- ness (mm)	Plant height (cm)		
A	Seedling	2.58 ± 0.04 ^a	23.79 ± 1.18 ^a		
	Steckmedium	2.55 ± 0.03 ^a	20.25 ± 1.20 ^b		
B	Wuxal	2.57 ± 0.06 ^{ns}	23.03 ± 1.82 ^{**}		
	Murtonik	2.69 ± 0.04 ^{**}	22.73 ± 1.81 ^{**}		
	Ferticare	2.51 ± 0.03 ^{ns}	21.67 ± 1.73 [*]		
	control ¹	2.51 ± 0.03	20.66 ± 1.67		
C	2004	2.54 ± 0.04 ^a	28.47 ± 0.45 ^a		
	2005	2.59 ± 0.03 ^a	15.57 ± 0.41 ^b		
A × B	Seedling	Wuxal	2.70 ± 0.06 ^a	25.11 ± 2.26 ^a	
		Murtonik	2.67 ± 0.09 ^{ab}	24.94 ± 2.70 ^a	
		Ferticare	2.49 ± 0.05 ^c	22.90 ± 2.00 ^b	
		control ¹	2.46 ± 0.05 ^c	22.21 ± 2.75 ^b	
	Steckmedium	Wuxal	2.43 ± 0.07 ^c	20.95 ± 2.80 ^c	
		Murtonik	2.70 ± 0.02 ^a	20.52 ± 2.33 ^c	
		Ferticare	2.53 ± 0.04 ^{bc}	20.44 ± 2.90 ^c	
		control ¹	2.56 ± 0.04 ^{abc}	19.10 ± 1.93 ^d	
A × C	Seedling	2004	2.52 ± 0.06 ^b	30.14 ± 0.48 ^a	
		2005	2.65 ± 0.03 ^a	17.45 ± 0.45 ^a	
	Steckmedium	2004	2.57 ± 0.04 ^{ab}	26.81 ± 0.48 ^a	
		2005	2.54 ± 0.04 ^b	13.70 ± 0.20 ^a	
	B × C	Wuxal	2004	2.58 ± 0.08 ^a	29.66 ± 0.65 ^a
			2005	2.55 ± 0.09 ^a	16.40 ± 1.10 ^a
Murtonik		2004	2.71 ± 0.08 ^a	29.32 ± 1.10 ^a	
		2005	2.67 ± 0.03 ^a	16.14 ± 0.69 ^a	
Ferticare	2004	2.47 ± 0.05 ^a	28.11 ± 0.28 ^a		
	2005	2.54 ± 0.03 ^a	15.24 ± 0.98 ^a		
control ¹	2004	2.41 ± 0.03 ^a	26.79 ± 1.07 ^a		
	2005	2.61 ± 0.03 ^a	14.52 ± 0.29 ^a		
ANOVA	A	ns	**		
	B	**	**		
	C	ns	**		
	A × B	**	*		
	A × C	*	ns		
	B × C	ns	ns		
	A × B × C	ns	**		

¹the control is a plant not treated with a foliar fertilizer; A – substrate, B – foliar fertilizer, C – year; * and ** indicate significant difference between means of the control and other foliar fertilizers at $d' \leq 0.05$ and $d' \leq 0.01$ by the Dunnett's test, respectively; values within each column followed by the same small letter are not significantly different at $P \leq 0.05$ by Dunnett's multiple range test; ns – a non-significant difference

DISCUSSION

Evaluation of microelements content

KIRKBY and ROEMHELD (2004) found that low contents of microelements are crucial for plant growth since they not only participate in building cell walls (B), membranes (B, Zn) and enzymes (Fe, Mn, Cu, Ni) but also affect enzymes activity (Mn, Zn) and photosynthesis (Fe, Cu, Mn, Cl). During growth and development phase, raspberry and other fruits have specific microelements requirements although, according to KOWALENKO (2004), little is known about the accumulation and distribution of microelements in raspberry.

Our results showed that Cu and Fe contents in raspberry leaves varied greatly among plants treated with different substrates and foliar fertilizers. Mn content varied only among different substrates, while Zn content was not significantly affected by varying treatments.

Plant growth substrate used for plants transferred from tissue culture laboratory to greenhouse or in open field for plant acclimatization usually needs to be composed of sterile earth, sterilized sand, or should include their mixtures and vermiculite (MIŠIĆ 1998). HEIBERG and LUNDE (2006) reported that plants of high-bush blueberries are very sensitive to chemical composition of substrate. The results obtained in our study also indicate a significant effect of substrate composition on Cu, Fe and Mn content in raspberry leaves. Studying mineral composition of vegetative organs and fruits of two raspberry cultivars, Haida and Willamette, KOWALENKO (2006) reports on more intense accumulation of microelements as growing period progresses. In addition, microelements content of raspberry in different parts of plant varied considerably through months and years (KOWALENKO 2004).

Foliar nutrition includes mineral fertilizers and special products manufactured for this purpose (EL-FOULY 2002). Studying the effect of foliar fertilizers, SWIETLIK and FAUST (1984) discovered their compensating or alleviating effect on nutrient deficiencies in plants.

Given the low mobility of Ca, S and Fe (WEINBAUM et al. 2002), our data infer that Fe uptake is promoted by chelate complexes contained in the applied foliar fertilizers, which corresponds to reports of TAKKAR and WALKER (1993). On the other hand, BUKOVAC and WITTEWER (1957) report on

moderate mobility of Cu, Zn, Mn and Mo when foliarly applied. This was also confirmed by our study in which both the treatments applied had a considerable effect on the increase of Cu content and its enhanced mobility accordingly.

Evaluation of deviation from optimum percentage (DOP)

Results of the study on microelements content in raspberry plants reveal Cu and Zn deficiency, Mn surplus and optimal Fe content. Similar results in terms of Mn content were obtained by GLIŠIĆ (2004) who studied the influence of Agrozol on mineral composition of blackberry leaf. The accumulation of Mn is due to the higher adoption of the microelement in plants grown on soils with anaerobic prevalence and higher acidity. Also, interrelations of certain nutrients may affect unfavorably mineral nutrients balance (LUČIĆ et al. 1996), as confirmed by the results of PERKINS-VEAZIE (2004) that reveal that excess P content can lead to a deficiency in some microelements, Zn in particular. Optimal Fe content in raspberry leaves resulted from the presence of chelate complexes (UBAVIĆ et al. 2001), while weak remobilization (mobility in the phloem) of Cu, according to KASTORI (1986), led to the deficiency of this microelement.

DOP index values and Σ DOP provide similar information to the Diagnosis and Recommendation Integrated System (DRIS) (DAVEE et al. 1986; SANZ 1999; JIMÉNEZ et al. 2007). The presented microelements imbalance, i.e. the deviation from optimal balance induced by application of different substrates and foliar fertilizers can be accounted for by a hindered establishment of a balanced nutrition in fruit trees compared to other agricultural crops (VELIČKOVIĆ 2006).

Evaluation of morphological traits of plants

According to MILOŠEVIĆ (1997), raspberry develops underground (geophilic) and aboveground (photophilic) shoots. During the early stages of growth (which usually takes a year), under favorable conditions, vegetatively propagated raspberry plants (by shoots, root cuttings, rooted shoot tips) develop from 2 to 4 shoots (MIŠIĆ, NIKOLIĆ 2003).

Results of our study, in terms of vegetative potential of raspberry plants, are in agreement with reports on

rapid adoption of ions in leaves of plants with intense developmental processes, because younger leaves have a thinner cuticle, which allows faster transmission of ions into the leaf (UBAVIĆ et al. 2001).

CONCLUSION

The analysis of mineral composition of raspberry cv. Willamette leaves points to the excess Mn, optimal Fe content and Zn and Cu deficiencies.

The highest deviation from optimal microelements balance was observed in plants grown on Steckmedium, while it was lowest in those grown on Seedling.

Treating raspberry plants with foliar nutrients and growing them on suitable substrates contributes to more intense vegetative growth, which further ensures meeting criteria in terms of quality of raspberry planting material.

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Received for publication April 27, 2011

Accepted after corrections October 12, 2011

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