

Yield and Mineral Content of Stinging Nettle as Affected by Nitrogen Fertilization

S. Radman^{1*}, I. Zutic¹, L. Coga², S. Fabek¹, B. Benko¹, and N. Toth¹

ABSTRACT

Stinging nettle (*Urtica dioica* L.) is a valuable multifunctional plant species, mainly collected from natural habitats, but, quality of such plant material is rather variable. Cultivation of the plant allows for controlling some environmental factors and enhances the quality of the product. The goal of this research was to determine the influence of different doses of nitrogen fertilization (0, 100, and 200 kg N ha⁻¹) on yield, dry matter content, crude proteins and mineral content in stinging nettle herbage collected at flowering time. Results of the study showed that nitrogen fertilization had a negative effect on the amount of dry matter, content of phosphorus, potassium, and trace elements. Crude proteins significantly increased with use of larger amounts of nitrogen fertilizer and the highest value was recorded in the last harvest at 200 kg N ha⁻¹ (180.0 g kg⁻¹). The amount of iron measured in this research was very variable (0.62-2.96 g kg⁻¹) and much higher compared to the other studies on stinging nettle and similar leafy vegetables rich in iron. The highest total yield of fresh stinging nettle herbage was achieved at 200 kg N ha⁻¹ (15.18 t ha⁻¹), however, in the absence of nitrogen fertilization, the highest values of mineral composition and dry matter content were recorded.

Keywords: Crude proteins, Dry matter, Multiple harvests, *Urtica dioica*.

INTRODUCTION

Stinging nettle (*Urtica dioica* L.) is a medicinal plant which has also widespread use in cosmetic, textile, and food industry. It is an ample source of bioactive compounds such as amino acids, vitamins, and minerals (Rutto *et al.*, 2013), chlorophylls (Hojnik *et al.*, 2007) and carotenoids (Guil-Guerrero *et al.*, 2003). The pharmacological effects observed for the aerial parts of *U. dioica* mainly come from flavonol glycosides and phenolic acids (Grevsen *et al.*, 2008; Otles and Yalcin, 2012). In recent times, number of medical and pharmacologic researches on nettle has increased (Otles and Yalcin, 2012). The aerial parts of the plants are used as a blood purifier and diuretic, and infusion

is used for the treatment of diabetes, rheumatism, eczema and against diarrhea as well as in the treatment of arterial hypertension (Grevsen *et al.*, 2008). Extract of stinging nettle herb has hypotensive, antiallergic, hemostatic, antimicrobial, immunotropic, antitumor, hepatoprotective, antidiabetic (Otles and Yalcin, 2012; Yıldız *et al.*, 2008; Kopyt'ko *et al.*, 2012), anti-inflammatory, local analgesic, diuretic (Upton, 2013), antioxidant, antimicrobial, antiulcer and analgesic activities (Gülçin *et al.*, 2004; Bisht *et al.*, 2012). Similar to spinach in flavor, tasty and rich in vital microelements, nettle leaves are commonly used in human nutrition (Nica *et al.*, 2012).

This perennial herbaceous plant species from *Urticaceae* family is naturally found in

¹ Department of Vegetable Crops, Faculty of Agriculture, University of Zagreb, Svetošimunska Cesta 25, 10000 Zagreb, Croatia.

² Department of Plant Nutrition, Faculty of Agriculture, University of Zagreb, Svetošimunska Cesta 25, 10000 Zagreb, Croatia.

*Corresponding author; email: sradman@agr.hr



pathway, fields, and wildwood (Otles and Yalcin, 2012). Although stinging nettle is considered as a weed, there are many reasons to cultivate this valuable plant. According to Weiss (1993), if stinging nettle is gathered from natural habitats, the control of quality standards is difficult and expensive. As a result of degradation and destruction of its natural habitats due to over growth of invasive plants, this important plant needs conservation through plantation (Bisht *et al.*, 2012). However, the largest percentage of stinging nettle herb is still wild-harvested (Upton, 2013). So far, several studies related to its introduction in agricultural production have been conducted (Weiss, 1993; Biesiada and Wołoszczak, 2007; Grevsen *et al.*, 2008; Biesiada *et al.*, 2009; Biesiada *et al.*, 2010; Çalışkan and Ayan, 2011; Rutto *et al.*, 2012).

Stinging nettle production has low input requirements, could promote the biodiversity of local flora and fauna, and can be a new high-quality agricultural raw material for dyeing, textile, and energy sectors (Di Virgilio *et al.*, 2014). It can be grown as a medicinal herb or vegetable with agronomic focus on leaf/shoot yield (Rutto *et al.*, 2012). Harvest time depends on the final product (Rutto *et al.*, 2012; Di Virgilio *et al.*, 2014). If the products are fresh leaves, harvest is carried out before flowering, in the phase of intensive growth. For the pharmaceutical industry, harvest should be done in the flowering stage, and if the objectives of production are fibers, stinging nettle is harvested at the stage of mature seeds.

Current studies on stinging nettle mineral nutrition are part of efforts to establish agronomic guidelines for cultivation management (Rutto *et al.*, 2012). In the literature, different rates of nitrogen for stinging nettle cultivation are reported: 160 to 300 kg N ha⁻¹ (Vogl and Hartl, 2003), 300 to 400 kg N ha⁻¹ (Grevsen *et al.*, 2008) and 440 kg N ha⁻¹ (Weiss, 1993). Although application of nitrogen improves plant growth and yield, high concentrations could contaminate environment and cause NO₃⁻ accumulation in the leaves (Martínez-

Ballesta *et al.*, 2010). Nitrate content in vegetables depends on different factors such as biological properties of plant culture, plant maturity, ecological factors (light conditions, type of soil, temperature, humidity), planting time, plant density, fertilization and harvesting time (Kmecl and Žnidarčič, 2015). Nitrogen fertilizer increases water use efficiency, the herbage yield, and crude protein content in some forage crops (Mohsenabadi *et al.*, 2008). In order to achieve higher yield, producers often apply large amounts of nitrogen fertilizer which can result in accumulation of nitrate, reducing the quality of plant material (Fabek *et al.*, 2012). Conventional production requires procedures that pose a risk to the environment and human health (Oplanić *et al.*, 2009). However, the effect of nitrogen fertilizers on the mineral content of plants is variable depending on the doses applied, the nutrient analyzed, the studied species, and the plant part to be consumed (Martínez-Ballesta *et al.*, 2010). Rutto *et al.* (2012) state that through judicious supply of fertilizer, planting time, and selective harvesting of different plant parts, growers could manage stinging nettle yield and quality.

The aim of this research was to determine the effect of nitrogen fertilization (0, 100, 200 kg N ha⁻¹) on the content of crude proteins, some minerals (phosphorus, potassium, calcium, magnesium and iron) and fresh herbage yield during multiple harvests.

MATERIALS AND METHODS

Field Experiment and Plant Material

The field experiment was conducted in Zagreb, at Maksimir (45°49' N, 16°02' E) during 2013. The seed of *U. dioica* population (B and T World Trade, France; mass of 1000 seeds 0.11 g) was used for seedlings production in polystyrene trays with 209 cells in unheated greenhouse, and sowing was on March, 4. Seedlings were

planted in the field on April 15, 2013, at 0.5 m×0.3 m spacing (6.67 plants m⁻²). Monofactorial field experiment was set up according to the randomized block design with four replications; Experimental treatments were 3 levels of nitrogen application (0, 100, and 200 kg ha⁻¹). The area of the main plot was 8.75 m² and included 48 plants (four rows, each with 12 plants). Nitrogen was applied in the form of calcium ammonium nitrate (27% N; Petrokemija, Croatia) given as a split application: 50% of the planned dose of fertilizer was added at planting and the other half 60 days later. The crop was not irrigated, simulating the field production in humid and semi-humid climate of southwestern (SW) continental part of Croatia. Harvest time was defined by the final product (herbage for pharmaceutical industry). Three harvests were conducted: June 26, August 7, and October 10 (72, 113 and 177 days after planting). Plant cuttings were done during the flowering stage, and plants were cut at least above the two lower nodes to enable the regeneration. In the

representative samples (20 plants from each plot) the concentration of dry matter, crude proteins, and content of essential macro and micro elements (P, K, Ca, Mg, Fe, Zn, Mn, Cu) were determined.

Meteorological Conditions

Abiotic stresses (low water availability and extreme temperatures) can severely modify the mineral and nutritive quality of vegetables for human consumption (Martínez-Ballesta *et al.*, 2010). Mean, minimum, and maximum air temperatures and precipitation sum per decade during stinging nettle growing period in 2013 are shown in Table 1.

Harvests were conducted on June 26, August 7, and October 10, which means that Production Cycles (PC) between harvests lasted 72, 42 and 64 days. Literature says that optimal temperatures for nettle growth are between 15 and 25°C, while temperatures higher than 30°C may cause early flowering and lower yield (Radman *et al.*, 2014). The lowest values of minimal,

Table 1. Average temperatures and precipitation during stinging nettle vegetation period.

Month	Decade	Temperature			Precipitation
		Minimum	Mean	Maximum	
				(°C)	
April	Second	7.8	14.9	20.9	7.1
	Third	10.3	17.7	24.5	6.7
	First	13.6	19.0	24.0	29.6
May	Second	10.1	16.2	21.3	24.3
	Third	9.7	14.1	18.8	40.1
	First	11.9	17.0	21.9	18.8
June	Second	16.3	23.3	29.0	7.4
	Third	14.3	19.9	25.0	22.5
	First	16.4	22.5	28.3	28.9
July	Second	15.0	21.7	27.6	1.2
	Third	17.6	25.3	32.1	3.1
	First	19.1	26.9	34.0	2.2
August	Second	15.7	21.9	28.4	19.8
	Third	15.3	19.2	24.2	123.2
	First	13.2	18.4	24.5	19.5
September	Second	10.4	15.0	20.4	53.1
	Third	10.3	14.4	19.6	39.3
October	First	7.0	11.0	15.1	0.8



mean, and maximal temperatures during stinging nettle vegetation period (7.0, 11.0 and 15.1°C, respectively) were measured in the last decade of the third PC. The highest values of the observed temperatures (19.1, 26.9 and 34.0) along with lack of precipitation were recorded in the shortest cycle i.e. second PC.

During stinging nettle vegetation period, the precipitation was more affluent in the third PC (255.7 mm), than in the first PC (156.5 mm). In the second PC, there was a lack of precipitation, especially in the two last decades of July (1.2 and 3.1 mm). The distribution of rainfall was unfavorable during vegetation, particularly in the third PC (0.8-123.2 mm per decade).

Determination of Soil Quality

The soil of the experimental field was classified as hortisol originating from coluvium, dominated with fractions of sand and silt (70%), and with 18% of clay, having considerable portion of micro-pores whereupon it is capable to retain water. Water-air regime in the humid part of the year is influenced by underground water of a nearby brook.

Chemical properties of the soil were analyzed in the Analytical Laboratory of the Department of Plant Nutrition (Faculty of Agriculture University of Zagreb, Croatia), and are shown in Table 2. The following methods were used: Gravimetric method for dry matter (HRN ISO 11465, 2004), dry combustion method for total nitrogen (HRN ISO 13878, 2004), oxidation method with chromium sulfuric acid for organic matter (HRN ISO 14235, 2004) and AL method (extraction with Ammonium Lactate acetic acid) for available phosphorus and potassium (Egener et al., 1960). Soil pH was measured in 1M KCl (HRN ISO 10390,

2004).

Determination of Crude Proteins and Minerals in Nettle Herb

Mineral content of stinging nettle after each harvest was determined in the Analytical Laboratory of the Department of Plant Nutrition at Faculty of Agriculture in Zagreb. Samples were prepared and homogenized by the following methods: digestion with concentrated HNO₃ and flame photometer for K, digestion with concentrated HNO₃ and spectrophotometry for P, digestion with concentrated HNO₃ and atomic absorption spectrophotometer for Ca, Mg, Zn, Mn, Cu, and Fe (AOAC, 1995). Nitrogen was determined by dry combustion method (HRN ISO 13878, 2004), and crude proteins were calculated according to the formula: %N×6.25 (Vajić, 1964). Gravimetric method (HRN ISO 11465, 2004) was used to determinate dry matter content in plants.

Statistical Analysis

For each harvest, data were analyzed separately. The effect of nitrogen fertilization was determined using the analysis of variance and average values were tested by the Least Significant Difference (LSD). For statistical analysis of the data, statistical program SAS® Software ver. 9.3 (2010) was used.

RESULTS AND DISCUSSION

Yield of Fresh Herbage

Stinging nettle is a perennial crop with satisfactory yields of fresh herbage for 10–

Table 2. Chemical properties of the soil in the study site in Zagreb–Maksimir.

pH		g kg ⁻¹				
H ₂ O	1M KCl	Organic matter	N	P ₂ O ₅	K ₂ O	CaCO ₃
8.13	7.10	17.2	0.9	0.42	0.19	25.0

15 years of production (Di Virgilio *et al.*, 2014), but according to Weiss (1993) and Kleitz *et al.* (2008), in the first year of cultivation lower yields can be expected, with significant improvements in the second and third year. In this research, after three harvests, a satisfactory total yield of fresh herbage was achieved (7.6, 8.7 and 15.18 t ha⁻¹ at 0, 100, and 200 kg N ha⁻¹) already in the first year of cultivation (Table 3). The highest rate of nitrogen fertilization (200 kg N ha⁻¹) had significant influence on stinging nettle fresh yield in all harvests (4.07, 4.06, and 7.05 t ha⁻¹), compared to other treatments. This is in accordance with Grevsen *et al.* (2008), who claim that *Urtica dioica* is Nitrophilic plant and higher amount of nitrogen will therefore increase the yield of aerial part substantially.

Regardless the fertilization, the highest total yield of fresh herbage was achieved at the third harvest, which amounted to 48% of total yield. The lowest yield was recorded at the second harvest (23% of total yield), when uptake of nitrogen was difficult due to lack of precipitation.

Dry Matter Content

Yield and plant quality are affected by soil composition, fertilization, and agronomic conditions (Martínez-Ballesta *et al.*, 2008). Dry matter allocation is affected by mineral nutrition and could be manipulated by adjusting the quantity and

timing of nutrient supply (Rutto *et al.*, 2012). During stinging nettle cultivation, dry matter content was in the range of 197.4 to 263.2 g kg⁻¹ (Table 4).

In each harvest, the highest dry matter values were measured when nitrogen was not applied (263.2, 252.6 and 226.1 g kg⁻¹, respectively), *i.e.* nitrogen fertilization had negative influence on dry matter content. Santamaria *et al.* (1999) explain that this could be correlated with the replacement of nitrate with organic acids and sugars. However, in the first harvest there was no significant difference between control plants (263.2 g kg⁻¹) and those fertilized with 200 kg N ha⁻¹ (261.9 g kg⁻¹). In the same harvest, significantly less dry matter was measured in plants fertilized with 100 kg N ha⁻¹ (247.4 g kg⁻¹). According to Grevsen *et al.* (2008), the dry matter content of stinging nettle is significantly negatively correlated to nitrogen application and decreases from an average of 235.0 g kg⁻¹ at 0 kg N ha⁻¹ to 210.0 g kg⁻¹ at 400 kg N ha⁻¹. ČustiĆ *et al.* (2002) claim that application of nitrogen fertilizer in head chicory growing leads to depression in dry matter content and proteins. According to Ugrinović (1998), dry matter of red beet decreases with nitrogen fertilization from 119.0 g kg⁻¹ (0 kg N ha⁻¹) to 107.0 g kg⁻¹ (225 kg N ha⁻¹). Regardless of the nitrogen fertilization, dry matter content of stinging nettle herb in this research decreased with number of harvests, meaning that it depends on harvest time, which also has been confirmed by Biesiada

Table 3. The yield of fresh stinging nettle herbage (t ha⁻¹) depending on the dose of nitrogen fertilization.^a

Fertilization (kg N ha ⁻¹)	Harvest			Total yield
	First	Second	Third	
0	2.38 ^b	1.56 ^b	3.66 ^b	7.60
100	2.60 ^b	1.71 ^b	4.39 ^b	8.70
200	4.07 ^a	4.06 ^a	7.05 ^a	15.18
□ □	3.02	2.44	5.03	
LSD	0.96	1.75	1.82	

^a Mean values followed by the same letter within each column do not differ significantly at $P \leq 0.05$ (a) according to the LSD test



Table 4. Effect of nitrogen fertilization on dry matter, crude proteins and content of macroelements in singing nettle herb, g kg⁻¹ dry matter.^a

Fertilization (kg N ha ⁻¹)	Dry matter	Crude proteins	Phosphorus	Potassium	Calcium	Magnesium
First harvest						
0	263.2 ^A	96.9 ^C	3.7	19.1 ^a	56.1	3.2 ^a
100	247.4 ^B	128.1 ^B	3.6	17.9 ^{ab}	57.7	2.5 ^b
200	261.9 ^A	160.0 ^A	3.4	17.4 ^b	56.0	2.6 ^b
□□	257.5	128.3	3.6	18.1	56.6	2.6
LSD	7.7	1.4	NS	1.2	NS	0.5
Second harvest						
0	252.6 ^A	137.5 ^C	2.5 ^a	19.1 ^a	69.8 ^B	4.4
100	242.2 ^B	146.3 ^B	2.4 ^a	16.6 ^b	78.9 ^A	4.0
200	204.6 ^C	165.0 ^A	1.9 ^b	16.1 ^b	70.3 ^B	4.2
□□	233.1	149.6	2.3	17.3	73.0	4.2
LSD	5.4	1.1	0.4	1.5	3.8	NS
Third harvest						
0	226.1 ^A	135.6 ^C	6.5 ^A	20.2 ^A	76.6 ^A	3.9
100	204.6 ^B	154.4 ^B	5.6 ^B	17.9 ^B	58.0 ^B	3.7
200	197.4 ^C	180.0 ^A	4.9 ^C	17.3 ^B	50.8 ^C	4.1
□□	209.4	156.7	5.7	18.5	61.8	3.9
LSD	6.9	1.4	0.6	2.1	3.5	NS

^a Mean values followed by the same letter within each column do not differ significantly at $P \leq 0.05$ (a) and $P \leq 0.01$ (A) according to the *LSD* test.

et al (2010).

Crude Proteins

Nitrogen is an essential element for growth and development and plays a significant role in the nutrition of plants (Maryam et al., 2007; Petek et al., 2012). Higher rates of nitrogen fertilization in plants implicate higher crude proteins (Ćustić et al., 2002; Herak Ćustić et al., 2007), which are important for the nutritive quality of vegetables. Therefore, lack of nitrogen always slows down the synthesis of proteins and, thus, generally inhibits plant growth (Bergmann, 1992).

Contrary to the dry matter, the content of crude proteins increased with applied fertilization in every harvest (Table 4). Congruently, the lowest values of crude proteins in Dry Matter (DM) were recorded in the first harvest of control plants (96.9 g kg⁻¹ DM at 0 kg N ha⁻¹), and the highest

content was in the last harvest at fertilization rate of 200 kg N ha⁻¹ (180.0 g kg⁻¹ DM). According to Petek et al. (2012) the highest content of crude proteins in fresh weight in beetroot in a two-year research was obtained in fertilization treatment with 1000 kg ha⁻¹ NPK 5-20-30.

The content of crude proteins gradually increased with the subsequent cuts, as was confirmed according to Biesiada et al. (2010). In this experiment, the highest values were recorded in the last harvest (135.6, 154.4 and 180.0 g kg⁻¹ DM at 0, 100 and 200 kg N ha⁻¹), compared to previous two harvests. This could be correlated with a better exploitation of nitrogen, due to a favorable amount of precipitations. The lowest values in the first harvest (96.9, 128.1 and 160.0 g kg⁻¹ DM at 0, 100 and 200 kg N ha⁻¹) could be explained by early stage of plant growth, i.e. less developed root system, which lowered the uptake of nitrogen.

Mineral Content

Nutritional value of plants is reflected not only in their energy content but also in the presence of proteins and minerals (Petek *et al.*, 2012). Martinez-Ballesta *et al.* (2010) stated that humans need more than 22 mineral elements for maintenance of health and proper organ function. Nitrogen, P and K are the most important biogenic macroelements, because they enter into the composition of phosphatides, nucleotides, nucleic acids and enzymes (Petek *et al.*, 2008). These elements are required in large amounts, and others such as Fe, Zn, and Cu are required in trace amounts. Stinging nettle leaves are rich in Ca, K, P, Mg and Fe (Guil-Guerrero *et al.*, 2003; Nica *et al.*, 2012; Upton, 2013), and contain 34.8 g Ca. kg⁻¹, 17.7 g K. kg⁻¹, 4.0 g P. kg⁻¹, and 3.4 g Mg. kg⁻¹ (Biesiada *et al.*, 2010) in DM.

High amount of N, P, and Fe concentrations in plant material could be explained by the influence of agricultural inputs (fertilizers) on the level of N, P and Fe in the soil (Koniecznyński and Wesołowski, 2007). According to the soil analysis (Table 2), it was poorly supplied with total nitrogen (0.9 g kg⁻¹), which resulted in significant influence of fertilization on most measured parameters.

The content of macronutrients in stinging nettle herb is shown in Table 4. During the cultivation period, the content of phosphorus ranged from 1.9 to 6.5 g kg⁻¹ DM. It decreased with an application of nitrogen, with statistical significance in the second and third harvest (2.5, 2.4, 1.9 and 6.5, 5.6, 4.9 g kg⁻¹ DM at fertilization levels of 0, 100 and 200 kg N ha⁻¹, respectively).

Potassium content ranged from 16.1 to 20.2 g kg⁻¹ DM. The same as with P, N fertilization had adverse effect on K content. Regardless of the nitrogen fertilization, the highest values of P and K were measured in third harvest and the lowest in the second harvest. The ability to take up and transport the mineral nutrients differs in distinct crops and depends on the plant's tolerance to

drought, because lack of water can affect nutrient uptake and impair translocation of some nutrients (Martínez-Ballesta *et al.*, 2010). Since potassium uptake by plants depends largely on diffusion and mass flow, its uptake by plants is greatly inhibited by lack of precipitations (Bergmann, 1992). During stinging nettle's production cycles, distribution of precipitations was unequal (Table 1). Lack of precipitation in the second production cycle (the second and the third decade in July and the first decade in August) influenced the lower uptake of P and K. Negative impact of nitrogen fertilization on dry matter concentration and content of some minerals (P, K, and Ca) was also confirmed in other studies (Petek *et al.*, 2012; Sørensen, 1998; Ugrinović, 1998).

Content of Ca was in the range of 56.0 to 78.9 g kg⁻¹ DM, higher compared to Szewczuk and Mazur (2004) and Biesiada *et al.* (2010), who stated values of 4.3 to 5.1 and 31.1 to 39.0 g kg⁻¹ DM, respectively. Nitrogen fertilization had disparate effects on the calcium content in each harvest. The highest value of Ca was recorded in the second harvest of plants fertilized with 100 kg N ha⁻¹ (78.9 g kg⁻¹ DM). In the third harvest, negative influence of nitrogen fertilization on calcium content was recorded, whereas the highest amount was found in control plants (76.6 g kg⁻¹ DM). Regardless of the nitrogen fertilization, the highest values of calcium contents were recorded at second harvest (69.8, 78.9 and 70.3 g kg⁻¹ DM at 0, 100, and 200 kg N ha⁻¹). This could be caused by lack of precipitation, because transport of calcium is associated with the mass flow of water. A reduced water supply could lead to decreased contents of minerals, *i.e.* without sufficient water to cool the soil, plant roots may be subjected to heat stress, which affects the uptake of minerals (Fabek *et al.*, 2012). Also, higher amounts of calcium in this period could be the result of physiological aging of the plant, because water stress raises the level of abscisic acid synthesis, thus reducing the K and P concentration while increasing Ca and Fe concentration (Bergmann, 1992). According



to Biesiada *et al.* (2010) with increased number of harvests, the amount of calcium was higher, which was not confirmed in the present research.

Magnesium content varied from 2.5 to 4.4 g kg⁻¹ DM, wherein the lowest values were measured at the first harvest and the highest, the same as with calcium, at the second harvest. In the first and the second harvest, the highest contents of Mg were recorded for the control plants (3.2 and 4.4 g kg⁻¹ DM, respectively), with statistical significance only in the first harvest. However, difference in the amount of this nutrient depending upon the dose of nitrogen (100 or 200 kg N ha⁻¹) was not statistically justified.

Ability of stinging nettle to accumulate trace elements reflects its benefits to human nutrition and health as vector of essential trace elements (Nica *et al.*, 2012). According to Diaconu *et al.* (2012), essential heavy metals for humans are iron, zinc, manganese and copper. Increased nitrogen fertilization had negative impact on the amount of all observed microelements (Table 5).

The decrease in the content of macro- and microelements caused by increased nitrogen supply is explained by Sørensen (1998) with dilution effect, *i.e.* the uptake is relatively less than the growth rate. The lowest values of zinc, manganese and copper were recorded in the second harvest at fertilization rate of 200 kg N ha⁻¹ (14.50, 87.60 and 3.50 mg kg⁻¹ DM, respectively). In this period, the uptake of these minerals from the soil was lower due to lack of precipitation.

The content of Fe varied from 0.62 to 2.4 g kg⁻¹, what was much higher compared to stinging nettle collected from natural habitats, which contain only 0.23 g kg⁻¹ of iron, according to Koniecznyński and Wesółowski (2007). Nitrogen fertilization had negative influence on iron content, but these values were still much higher compared to the other studies. Therefore, stinging nettle, together with other leafy vegetables, is particularly rich in iron (spinach, lettuce, Chinese cabbage, parsley), classified as a ferrophilic plant (Bergmann,

Table 5. Effect of nitrogen fertilization on content of microelements in singing nettle herb (in dry matter).^a

Fertilization (kg N ha ⁻¹)	Iron (g kg ⁻¹)	Zink (mg kg ⁻¹)	Manganese (mg kg ⁻¹)	Copper (mg kg ⁻¹)
First harvest				
0	2.36 ^{A*}	25.40 ^A	173.90 ^A	6.50 ^A
100	2.06 ^B	22.30 ^B	112.10 ^B	4.20 ^C
200	1.71 ^C	20.30 ^C	108.90 ^C	5.60 ^B
□ □	2.04	22.67	131.63	5.43
LSD	0.03	0.55	1.61	0.53
Second harvest				
0	2.47 ^A	18.80 ^A	146.50 ^A	3.70 ^{ab}
100	2.17 ^B	16.40 ^B	96.40 ^B	4.10 ^a
200	0.98 ^C	14.50 ^C	87.60 ^C	3.50 ^b
□ □	1.87	16.57	110.17	3.77
LSD	0.02	0.06	1.39	0.56
Third harvest				
0	2.96 ^A	24.70 ^A	166.40 ^A	7.50 ^A
100	2.28 ^B	21.50 ^B	137.40 ^B	6.80 ^B
200	0.62 ^C	19.70 ^C	116.60 ^C	5.80 ^C
□ □	1.95	21.97	140.13	6.70
LSD	0.02	0.52	1.32	0.53

^a Mean values followed by the same letter within each column do not differ significantly at $P \leq 0.05$ (a) and $P \leq 0.01$ (A) according to the *LSD* test.

1992). With higher number of harvests, amount of Fe increased. An exception was fertilization level of 200 kg N ha⁻¹, where an increased number of harvests resulted in the reduction of iron in plant material (1.71, 0.98 and 0.62 g kg⁻¹ DM, respectively).

CONCLUSIONS

The results indicate significant influence of nitrogen fertilization on the yield of stinging nettle herb and chemical traits observed, depending on the time of harvest. Due to increased nitrogen supply, the fresh herbage yield and the content of crude proteins increased, while contents of dry matter, potassium, phosphorus and microelements considerably decreased. The fertilization with 100 kg N ha⁻¹ represents the compromise between quality and quantity of herbage yield.

REFERENCES

1. AOAC. 1995. Official Method 975.03: Metals in Plants and Pet Foods. Official Methods of Analysis of AOAC International. 16th ed. AOAC International, Washington DC, USA.
2. Bergmann, W. 1992. *Nutritional Disorders of Plants*. (Ed.): Fischer, G., Verlag Jena, Stuttgart, New York.
3. Biesiada, A., Kucharska, A. Z., Sokół-Łętowska, A. and Kuś, A. 2010. Effect of the Age of Plant, Plantation and Harvest Term on Chemical Composition and Antioxidant Activity of Stinging Nettle (*Urtica dioica* L.). *Ecol. Chem. Eng. S.*, **17(9)**: 1061-1067.
4. Biesiada, A. and Wołoszczak, E. 2007. The Effect of Method of Plantation Establishing on Yield and Chlorophyll Concentration of Stinging Nettle (*Urtica dioica* L.) in the First year of Cultivation. *Herba Pol.*, **53(3)**: 85-89.
5. Biesiada, A., Wołoszczak, E., Sokół-Łętowska, A., Kucharska, A. Z. and Nawirska-Olszańska, A. 2009. The Effect of Nitrogen Form and Dose on Yield, Chemical Composition and Antioxidant Activity of Stinging Nettle (*Urtica dioica* L.). *Herba Pol.*, **55(3)**: 84-93.
6. Bisht, S., Bhandari, S. and Bisht, N.S. 2012. *Urtica dioica* (L): An Undervalued, Economically Important Plant. *Agric. Sci. Res. J.*, **5**: 250-252.
7. Çalışkan, Ö. and Ayan, A. K. 2011. The Effects of Different Doses of Organic NPK Fertilizer on Yield and Yield Components in Stinging Nettle (*Urtica dioica* L.). *Anadolu J. Agr. Sci.*, **26(3)**: 217-220.
8. Ćustić, M., Horvatić, M. and Butorac, A. 2002. Effects of Nitrogen Fertilization upon the Content of Essential Amino Acids in Head Cichory (*Cichorium intybus* L. var. foliosum). *Sci. Hort.*, **92(3)**: 205-2015.
9. Diaconu, D., Diaconu, R. and Navrotescu, T. 2012. Estimation of Heavy Metals in Medicinal Plants and Their Infusions. *Ovidius Univ. Annal. Chem.*, **23 (1)**: 115-120.
10. Di Virgilio, N., Papazoglou, E. G., Jankauskiene, Z., Di Lonardo, S., Praczyk, M. and Wielgusz, K. 2014. The Potential of Stinging Nettle (*Urtica dioica* L.) as a Crop with Multiple Uses. *Ind. Crop. Prod.*, **68**: 42-49.
11. Egner, H., Riehm, H. and Domingo, W. R. 1960. Untersuchungen über die Chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Boden. II. Chemische Extraktions Methoden zur Phosphor- und Kaliumbestimmung. *Lantbrukshogskolan Annaler*, **25**: 199-215.
12. Fabek, S., Toth, N., Radojčić Redovniković, I., Herak Ćustić, M., Benko, B. and Žutić, I. 2012. The Effect of Nitrogen Fertilization on Nitrate Accumulation and the Content of Minerals and Glucosinolates in Broccoli Cultivars. *Food Technol. Biotech.*, **50(2)**: 183-191.
13. Grevsen, K., Frette, X. C. and Christensen L. P. 2008. Concentration and Composition of Flavonol Glycosides and Phenolic Acids in Aerial Parts of Stinging Nettle (*Urtica dioica* L.) Are Affected by Nitrogen Fertilization and by Harvest Time. *Europ. J. Hort. Sci.*, **73(1)**: 20-27.
14. Guil-Guerrero, J. L., Rebolloso-Fuentes, M. M. and Torija Isasa, M. E. 2003. Fatty Acids and Carotenoids from Stinging



- Nettle (*Urtica dioica* L.). *J. Food Comp. Anal.*, **16**(2): 111-119.
15. Gülçin, I., Küfrevioğlu, Ö. İ., Oktay, M. and Büyükokuroğlu, M. E. 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). *J. Ethnopharmacol.*, **90** (2): 205-215.
 16. Herak Ćustić, M., Petek, M., Toth, N., Poljak, M. and Ćosić, T. 2007. Effects of Organic and Mineral Fertilization on NPK Status in Soil and Plant, and Yield of Red Beet (*Beta vulgaris* var. *conditiva*). *Cereal Res. Commun.*, **35**(2): 449-452.
 17. Hojnik, M., Škerget, M. and Knez, Ž. 2007. Isolation of Chlorophylls from Stinging Nettle (*Urtica dioica* L.). *Sep. Purif. Technol.*, **57**(1): 37-46.
 18. HRN ISO 10390. 2004. Soil Quality: Determination of pH. Croatian Standard Institute, Zagreb.
 19. HRN ISO 11465. 2004. Gravimetric Method: Determination of Dry Matter and Water Content on a Mass Basis. Croatian Standard Institute, Zagreb.
 20. HRN ISO 13878. 2004. Elementary Analysis: Determination of Total Nitrogen Content with Dry Combustion. Croatian Standard Institute, Zagreb.
 21. HRN ISO 14235. 2004. Soil Quality: Determination of Organic Matter with Oxidation Method. Croatian Standard Institute, Zagreb.
 22. Kleitz, K. M., Wall, M. M., Falk, C. L., Martin, C. A., Remmenga, M. D. and Guldán, S. J. 2008. Stand Establishment and Yield Potential of Organically Grown Seeded and Transplanted Medicinal Herbs. *HortTechnol.*, **18**(1): 116-121.
 23. Kmecl, V. and Žnidarčič, D., 2015. Accreditation of the Analytical Method Used for Nitrate Determination in Vegetables. *Arch. Biol. Sci., Belgrade*, **67**(1): 295-302.
 24. Koniecznyński, P. and Wesołowski, M. 2007. Determination of Zinc, Iron, Nitrogen and Phosphorus in Several Botanical Species of Medicinal Plants. *Pol. J. Environ. Stud.*, **16**(5): 785-790.
 25. Kopyt'ko, Ya. F., Lapinskaya, E. S. and Sokol'skaya, T. A. 2012. Application, Chemical Composition, and Standardization of Nettle Raw Material and Related Drugs: Review. *Pharm. Chem. J.*, **45**(10): 622-63.
 26. Maryam, B., Ansari, N. A. and Dehcordie, F. S. 2007. Effect of Cultivars, Harvesting Time and Level of Nitrogen Fertilizer on Nitrate and Nitrite Content, Yield in Romaine Lettuce. *Asian J. Plant Sci.*, **6**: 550-553.
 27. Martínez-Ballesta, M. C., Domínguez-Perles, R., Moreno, D. A., Muries, B., Alcaraz-López, C., Bastías, E., García-Viguera, C. and Carvajal, M. 2010. Minerals in Plant Food: Effect of Agricultural Practices and Role in Human Health. *Agron. Sustain. Dev.*, **30**: 295-309.
 28. Martínez-Ballest, M. C., López-Pérez, L., López-Berenguer, M. H. C., Fernández-García, N. and Carvajal, M. 2008. Agricultural Practices for Enhanced Human Health. *Phytochem. Rev.*, **7**(2): 251-260.
 29. Mohsenabadi, Gh. R., Jahansooz, M. R., Chaichi, M. R., Rahimian Mashhadi, H., Liaghat, A. M. and Savaghebi, Gh. R. 2008. Evaluation of Barley-vetch Intercrop at Different Nitrogen Rates. *J. Agri. Sci. Tech.*, **10**: 23-31.
 30. Nica, D., Bordean, D. M., Gergen, I., Bura, M. and Vintila, T. 2012. Comparative Assessment of Trace Metal Accumulation in Celery and Nettle Leaves. *J. Hortic. Sci. Biotech.*, **16**(2): 217-220.
 31. Oplanić, M., Ban, D., Ilak-Peršurić, A., Žnidarčič, D., 2009. Profitability of Leek (*Allium porrum* L.) in Three Production Systems. *J. Food Agric. Environ.*, **7**(3/4): 376-381.
 32. Otles, S. and Yalcin, B. 2012. Phenolic Compounds Analysis of Root, Stalk and Leaves of Nettle. *ScientificWorld J.*, **2012**: 1-12.
 33. Petek, M., Herak Ćustić, M., Čoga, L. and Pecina, M. 2008. Phosphorus Content in Soil and in Fresh and Cooked Red Beet in Dependence on Different Fertilization. *Cereal Res. Commun.*, **36**: 435-438.
 34. Petek, M., Herak Ćustić, M., Toth, N., Slunjski, S., Čoga, L., Pavlović, I., Karažija, T., Lazarević, B. and Cvetković, S. 2012. Nitrogen and Crude Proteins in Beetroot (*Beta vulgaris* var. *conditiva*) under Different Fertilization Treatments. *Not. Bot. Horti. Agrobi.*, **40**(2): 215-219.
 35. Radman, S., Fabek, S., Žutić, I., Benko, B. and Toth, N. 2014. Stinging Nettle

- Cultivation in Floating Hydropon. *Contemp. Agric.*, **63** (3): 215-223.
36. Rutto, L. K., Ansari, M. S. and Brandt, M. 2012. Biomass Yield and Dry Matter Partitioning in Greenhouse-grown Stinging Nettle under Different Fertilization Regimes. *HortTechnol.*, **22** (6): 751-756.
 37. Rutto, L. K., Xu, Y., Ramirez, E. and Brandt, M. 2013. Mineral Properties and Dietary Value of Raw and Processed Stinging Nettle (*Urtica dioica* L.). *Int. J. Food Sci.*; **2013**. Article ID 857120, 9 PP; <http://dx.doi.org/10.1155/2013/857120>
 38. Santamaria, P., Elia, A., Serio, F. and Todaro, E. 1999. A Survey of Nitrate and Oxalate Content in Fresh Vegetables. *J. Sci. Food Agric.*, **79**(13): 1882-1888.
 39. SAS®/STAT 9.3. 2010. SAS Institute Inc., Cary, NC, USA.
 40. Sørensen, J. N. 1998. Nitrogen Effects on Vegetable Crop Production and Chemical Composition, *Acta Hortic.*, **506**: 41-50.
 41. Szewczuk, C. and Mazur, M. 2004. Effect of Different Rates of Nitrogen Fertilizers on Chemical Composition of Stinging Nettle (*Urtica dioica* L.) Plants Harvested at Three Development Stages. *Acta Sci. Pol., Agric.*, **3**(1): 239-248.
 42. Ugrinović, K. 1998. Effect of Nitrogen Fertilization on Quality and Yield of Red Beet (*Beta vulgaris* var. *conditiva* Alef.). *Acta Hortic.*, **506**: 99-104.
 43. Upton, R. 2013. Stinging Nettles Leaf (*Urtica dioica* L.): Extraordinary Vegetable Medicine. *J. Herb Med.*, **3**(1): 9-38.
 44. Vajić, B. 1964. *Food, Determination of the Main Ingredients*. University of Zagreb, Zagreb.
 45. Vogl, C. R. and Hartl, A. 2003. Production and Processing of Organically Grown Fiber Nettle (*Urtica Dioica* L.) and Its Potential Use in the Natural Textile Industry: A Review. *Am. J. Alternative Agric.*, **18**(03): 119-128.
 46. Weiss, F. 1993. Effects of Varied Nitrogen Fertilization and Cutting Treatments on the Development and Yield Components of Cultivated Stinging Nettles. *Acta Hortic.*, **331**: 137-144
 47. Yıldız, L., Başkan, K. S., Tütem, E. and Apak, R. 2008. Combined HPLC-CUPRAC (Cupric Ion Reducing Antioxidant Capacity) Assay of Parsley, Celery Leaves, and Nettle. *Talanta*, **77**(1): 304-313.

اثر کود نیتروژن بر عملکرد و کانی های موجود در گیاه گزنه

س. رادمن، ی. زوتیک، ل. کوگا، س. فابک، ب. بنکو، و ن. توس

چکیده

گیاه گزنه (*Urtica dioica* L.) گونه ای چند منظوره و با ارزش است که معمولاً از زیستگاه های طبیعی جمع آوری می شود، ولی، کیفیت این مواد گیاهی تا حدودی متغیر است. کاشتن این گیاه کنترل برخی عوامل محیطی را ممکن می سازد و موجب بهبود کیفیت محصول می شود. هدف این پژوهش تعیین اثر مقادیر مختلف کود نیتروژن (صفر، ۱۰۰ و ۲۰۰ کیلو گرم در هکتار) روی عملکرد، تولید ماده خشک، پروتئین خام، و کانی های موجود در توده علفی جمع آوری شده در مرحله گلدهی بود. نتایج پژوهش نشان داد که افزودن کود نیتروژن اثر منفی روی مقدار ماده خشک، و مقدار فسفر و پتاسم و عناصر کمیاب در گیاه داشت. از سوی دیگر، مقدار پروتئین خام با افزایش کود نیتروژن به طور



معنی داری زیاد شد و بیشترین مقدار آن در برداشت آخر در تیمار ۲۰۰ کیلو گرم نیتروژن در هکتار ثبت شد. مقادیر آهن اندازه گیری شده در این مطالعه بسیار متغیر بود (۲/۹۶-۰/۶۲ گرم در کیلو گرم) و بسیار بیشتر از مطالعات دیگر روی این گیاه و سبزیجات برگی مشابه که آهن فراوانی دارند بود. بالاترین عملکرد کل در تیمار ۲۰۰ کیلو گرم نیتروژن در هکتار (برابر ۱۵/۱۸ تن در هکتار) به دست آمد هر چند که ، بیشترین ماده خشک و بیشترین مقدار کانی ها در تیمار بدون مصرف کود نیتروژن ثبت شد.