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Soil and highbush blueberry responses to fertilization with urea phosphate

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ABSTRACT

The low availability of soils with an appropriate pH value (4.2-5.2) is a factor limiting an increase in highbush blueberry acreage. The experiments examined the influence of a physiologically acidic urea phosphate fertilizer on the pH change of the soil and the mineral content in the soil, leaves, and fruit as well as polyphenols in the fruit of two highbush blueberry cultivars – 'Sunrise' and 'Brigitta Blue'. The fertilizer, at doses of 30 and 60 kg of nitrogen per hectare, was used in the experiments in each of the three years of the study.

After using 60 kg N ha^{-1}, urea phosphate caused a reduction in soil pH. It also significantly influenced the total soil nitrogen content – the average for the cultivars was 7.40 mg in 2015, while in the control plots – 1.85 mg 100 g^{-1}. These quantities are above the optimum recommended for highbush blueberry. At the same time, low amounts of this ingredient were found in the leaves ('Sunrise' – 1.83 mg; 'Brigitta Blue' – 1.77 mg 100 g^{-1}).

Even after the application of 30 kg of fertilizer in the second year, the phosphorus content in the soil was at a high level (> 4 mg 100 g^{-1}). The amount of phosphorus also increased in the leaves and fruit. Despite considerable quantities of available magnesium in the soil after urea phosphate application, a significant reduction of this component was observed in the leaves and fruit compared to the unfertilized control bushes. The applied fertilizer reduced the amount of polyphenols in the fruit of the tested cultivars.

Key words: macroelements, phenolics, soil pH, *Vaccinium corymbosum*

INTRODUCTION

The shortage of soils that are suitable for highbush blueberry cultivation is a factor limiting an increase in the number of bushes planted and the production of highbush blueberry fruits, both in Poland and in Europe (Chmielewski and Chmielewski, 2010; Chambers et al., 2013). The highbush blueberry plant forms a shallow root system with almost all roots distributed less than 0.4 m deep (Bryla and Strik, 2007). The role of exchanging nutrients and water between the plant and soil is fulfilled by fine roots, on the surface of which mycorrhizal fungi develop (Schilder et al., 2002). The bush requires specific

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soils with a low pH value (Jiang et al., 2017). The pH of soils determined in H_2O should fall within the range of 4.2-5.2, but the optimal value ranges from 4.5 to 4.8 (Williamson et al., 2006). Peat soils are the most appropriate for this species, especially highmoor peat or podsolic soils with a stabilized level of groundwater (Starast et al., 2002). Peat bogs are on the decrease all over Europe nowadays (Chmielewski and Chmielewski, 2010; Chambers et al., 2013). This is caused by the draining of areas intended for meadows and croplands, landscape modernization and also mineral mining for heating purposes. Also, other kinds of less suitable soils can be adapted to cultivating highbush blueberry. Usually, their pH must be changed by using soil sulphurization (Komosa, 2007) or by enriching the soil with acidic peat or sawdust from coniferous trees (Ochmian et al., 2009).

Highbush blueberry cultivars are derived from species of the genus *Vaccinium* with bushes growing naturally on soils with low nutrient levels. As a result, highbush blueberry fertilization requirements, as compared to other fruit plants, are relatively low (Pormale et al., 2009). Current N recommendations for blueberry range from 20 to 140 kg N per ha per season, varying with the age of the planting and bush vigour, location, soil type, organic mulching, and inherent soil fertility (Hanson, 2006). Kozinski (2006) obtained higher yields with up to 60 kg N ha^{-1}. On poorer soils the dose can be increased, but it should not exceed 120 kg. Increasing doses of fertilizers cause intense vegetative growth, reducing the yield (Koszański et al., 2008), most likely as a result of ammonium toxicity or salt stress (Banados et al., 2012). Fertilization of highbush blueberry with such high doses of nitrogen is not justified, especially that the assimilation of N-NO_3 ions is quite difficult in an acidic soil (Moro et al., 2017). Also, due to the fast rate of nitrogen transformation to N-NO_3, the fertilizer should be divided into several doses (Krzebietke and Benedycka, 2006).

Plants quickly respond to the lack of available minerals. This has a negative impact on plant growth and development (Ruan et al., 2010). The content of mineral ingredients in the leaves indicates the nutritional condition of the plant. However, it is difficult to define the optimal concentration of mineral ingredients in the soil or leaves. The recommended optimal macronutrient content for highbush blueberry is different depending on the authors (Eck, 1988; Sadowski et al., 1990; Pliszka et al., 1993; Glonek and Komosa, 2006). Pliszka et al. (1993) regarded the nitrogen content in the leaves at 1.3-1.4% as optimal, while Hanson and Hancock (1996) considered the content of < 1.7% to be insufficient. Excess of nutrients can also be disadvantageous. Nitrogen can extend the growing season of plants, but in the ammonium form in large amounts it is toxic (Hachiya et al., 2012).

Firmness is one of the most important features of highbush blueberry fruit. It determines the suitability of the fruit for transport and storage, and is mostly determined by the calcium content (Ochmian, 2012; Ochmian et al., 2015b). An increased calcium content in fruits increases firmness, while nitrogen-containing fertilizers reduce it (Ochmian and Kozos, 2015). The use of urea phosphate, especially at a dose of 60 kg N per hectare (1 UP), also causes a reduction in fruit firmness (Kozos and Ochmian, 2016).

The aim of the study was to determine the influence of the application rate of a nitrogen-phosphate fertilizer on the pH value of the soil and on the mineral content in the leaves and fruits of two highbush blueberry cultivars. In addition, its effect on the concentration of polyphenols in the fruit was also measured.

MATERIAL AND METHODS

Experimental conditions

The three-year (2013-2015) study was conducted at the Department of Horticulture of the West Pomeranian University of Technology in Szczecin. The research station is located in subzone 7A (Heinze and Schreiber, 1984) in the North-Western part of Poland in the Szczecin Lowland at a distance of approx. 65 km from the Baltic Sea (53°40' N, 14°88' E). The research was conducted on a production plantation specializing in the cultivation of highbush blueberry, located in the Szczecin Lowland. In this area, there are numerous hills of 40-60 m a.s.l., remnants of the frontal moraine. This affects the distribution of rainfall, the number of sunlight hours, temperature, and wind speed (Tab. 1). The climate of this area is also significantly affected by the presence of large water basins (the Szczecin Lagoon, Lake Dąbie, the Odra River), which provide additional moisture in the period of plant vegetation. The average temperature during the growing season (April-October) between 1951 and 2012 was 13.7°C, and rainfall 391 mm (Mijowska et al., 2017). Generally, in May and June of 2015, the temperatures recorded were lower, while in August of 2015 significantly higher, compared

Table 1. Weather conditions during the vegetative season (April-October) in the years 2013-2015 in relation to the average growing season during the multi-year period (1951-2012)*

Year	Month							
	IV	V	VI	VII	VIII	IX	X	
	Average temperature (°C)							Mean
2015	8.7	12.5	15.6	18.6	21.1	14.1	13.7	14.9
2014	10.8	13.4	16.3	21.3	17.5	15.4	11.8	15.2
2013	8.4	14.4	16.9	19.3	18.7	13.0	10.9	14.5
1951-2012	8.0	13.0	16.4	18.2	17.6	13.8	9.2	13.7
	Rainfall (mm)							Total
2015	29.0	48.0	32.8	62.0	14.7	34.4	22.1	242
2014	47.5	85.3	26.5	70.8	104.6	80.9	32.8	448
2013	20.8	88.1	112.5	50.4	35.9	43.9	45.8	397
1951-2012	39.7	62.9	48.2	69.6	74.2	58.7	37.3	391

*Source: Own work (data from a meteorological station in Ostoja operation within the measurement network of the West Pomeranian University of Technology in Szczecin)

with the years 2013 and 2014 and the mean value for the multi-year period. Additionally, the rainfall during the 2015 growing period was significantly lower in relation to the other experimental years and the mean for the years 1951-2012. The basic weather conditions, such as average temperature and rainfall, during the vegetative season (April-October) in the years 2013-2015 in relation to the multi-year period (1951-2012) had been thoroughly described previously (Mijowska et al., 2016 and 2017).

The plantation in the immediate vicinity of a forest was established on sandy soils – sandy silts, where a natural process of acidification had occurred because of long-term fallow. The organic matter content was 4.1-4.2% (determined by the weight method of Nelson and Sommers, 1982).

Plants were planted at a spacing of 2.3 × 1.2 m on raised beds covered with strips of nonwoven fabric for nurseries, which protected them from weeds. The bushes were watered using a drip-line and the intensity of watering was determined using soil tensiometers – two in one row. Soil moisture was maintained within the optimal range of 10-30 kPa (IRROMETER Company, Inc)

The fertilization treatments were arranged in a randomized block design with three replicates per treatment. Each treatment plot consisted of one row of 25 plants. The full dose of fertilization was 60 kg N ha^{-1} – 94 g of urea phosphate per plant (1 UP). Also, the full nitrogen dose reduced by half was applied, i.e. 30 kg N ha^{-1} – 47 g of urea phosphate per plant (½ UP). Plants that grew in the control fields were only irrigated (0 UP – control, without fertilizing – 0 kg N).

The fertilizer was applied in four equal doses at the beginning of vegetation (bud break – first leaves), at the end of blossoming, during the first ten days of June, and also at the end of June. The fertilizer consisted of 17.7% N and 44.6% P_2O_5 [$CO(NH_2)·H_3PO_4$]. The pH value of a 10% solution was 1.2, with an EC of 34 μS cm^{-1}. The fertilizer has a very good solubility in water. The electrical conductivity of the water used for watering was 0.36 μS cm^{-1}, and that of the solution used to fertilize the bushes – 1.4 μS cm^{-1}.

Tests were carried out on the cultivars 'Sunrise' and 'Brigitta Blue'. They are very productive cultivars, with large fruits attractive to the consumer. Bushes grow very well and yield annually in the climatic conditions of north-western Poland.

Soil and plant analysis

Plant and soil material was collected in mid-July. Bulk soil samples were prepared, which were collected from the humus layer (0-30 cm) using an Egner's sampling stick. The leaves were collected from the central part of one-year-old shoots. Then, they were dried at a temperature of 105°C. From each harvest (from mid-July to the beginning of August – 'Sunrise'; in August – 'Brigitta Blue'), fruits were taken and stored at a temperature of -29°C. For research purposes, the required amount of berries was defrosted.

The pH of the soil was measured potentiometrically with a CX 742 multifunction meter (ELMETRON, Poland). The estimation of the total mineral content in dry weight was carried out in accordance with the Polish Standard (IUNG, 1972) using certified reagents. After mineralization in

H_2SO_4 (96%) and $HClO_4$ (70%), the amounts of N, P, K, and Mg were estimated in berries and leaves. Soil K and P were determined by extracting in $C_6H_{10}CaO_6$, and the available Ca and also Mg by extracting in $C_2H_3O_2NH_4$. Total N was estimated after mineralization in H_2SO_4 (96%) and $HClO_4$ (70%). Nitrogen concentration was determined by the Kjeldahl distillation method using a Gerhardt 30 apparatus. The concentrations of potassium and calcium were measured by atomic emission spectrometry, whereas magnesium content with flame atomic absorption spectroscopy using an iCE 3000 Series spectrometer. Phosphorus content was determined with the Egner-Riehm method at a wavelength of 660 nm, employing a Marcel s 330 PRO spectrophotometer. Tests were performed each year in three replications. Measurements of phenolic compounds in blueberry fruits were performed by the UPLC-PDA–MS method (Mijowska et al., 2016).

Statistical analysis

A two-factor analysis of variance was performed separately for each cultivar, followed by an assessment of the significance of differences using Tukey's test. The statistical analyses were performed using the Statistica 12.0 software (StatSoft, Polska).

RESULTS AND DISCUSSION

Nitrogen is the basis for blueberry fertilization (Smolarz, 2009). Ammonia sulfate, urea and highly acidic urea phosphate are the most often recommended fertilizers due to their acidic pH value and influence on soil acidity (Vargas and Bryla, 2015; Kozos and Ochmian, 2016). Depending on the form of nitrogen ($N\text{-}NO_3$, $N\text{-}NH_4$, $N\text{-}NH_2$), fertilizers are subject to various processes of transformation and have a varying influence on the pH value of the soil (Diatta and Grzebisz, 2006).

The fertilizer used in the experiment reduced the pH value of the soil in which the highbush blueberry bushes grew (Tab. 2). The greatest dynamic of changes was observed after the application of 60 kg N ha^{-1} (1 UP). In this case, after three years, the pH value of the soil was reduced from the initial value of 5.35 (measured in April 2013) to 4.49 ('Sunrise') and 4.68 ('Brigitta Blue'). This is within the range of the optimal soil pH for highbush blueberry bushes (Williamson et al., 2006; Jiang et al., 2017).

Krzebietke and Benedycka (2006), too, observed a pH reduction after using urea phosphate. Changes in soil pH are associated with an increase in the nitrogen content (Ahmed et al., 2006; Butterly et al., 2011). The removal of two H+ ions causes a pH increase in the rhizosphere during $N\text{-}NO_3$ uptake (von Wiren et al., 2001). Because of the presence of H_3PO_4 in its composition, UP causes acidification of the soil environment (Cao et al., 2009).

During 2013-2015, no significant changes were observed in the pH value in the unfertilized control (0 UP). The experiment showed a significant

Table 2. Changes in the pH value of soil after fertilization with three levels of Urea-Phosphate (UP) applied over three years to two cultivars. Two methods for determining pH were used – in H_2O and KCl

		Cultivar							
		Sunrise				Brigitta Blue			
		Fertilizer							
		0 UP*	½ UP	1 UP	Mean	0 UP	½ UP	1 UP	Mean
Year		pH in H_2O							
August	2015	5.49 f**	4.87 bc	4.49 a	4.95 A	5.37 efg	4.97 bc	4.68 a	5.01 A
August	2014	5.41 f	5.06 cd	4.64 ab	5.04 A	5.58 g	5.13 cd	4.83 ab	5.18 AB
August	2013	5.33 ef	5.32 ef	5.13 de	5.26 B	5.55 fg	5.26 de	5.04 bc	5.28 BC
April	2013	5.29 def	5.24 def	5.35 ef	5.29 B	5.41 efg	5.44 efg	5.35 ef	5.40 C
Mean		5.38 C	5.12 B	4.90 A		5.48 C	5.20 B	4.98 A	
		pH in KCl							
August	2015	5.05 g	4.29 bc	3.86 a	4.40 A	4.89 def	4.37 b	3.98 a	4.41 A
August	2014	4.87 fg	4.40 cd	4.08 b	4.45 AB	5.02 f	4.62 c	4.40 b	4.68 B
August	2013	4.74 ef	4.65 ef	4.57 de	4.65 BC	4.94 ef	4.68 cd	4.64 c	4.75 B
April	2013	4.71 ef	4.72 ef	4.69 ef	4.71 C	4.81 c-f	4.77 cde	4.75 cde	4.78 B
Mean		4.84 C	4.51 B	4.30 A		4.91 B	4.61 A	4.44 A	

*0 UP = unfertilized, ½ UP = 30 kg N ha^{-1}, and 1 UP = 60 kg N ha^{-1}

**Means followed by the same letter do not differ significantly at $p < 0.05$ according to Tukey's multiple range test; lower-case letters indicate interaction and capital letters the main factors

Table 3. Nitrogen and potassium content in the soil, leaves and fruits after fertilization with three levels of Urea-Phosphate (UP) applied over three years to two blueberry cultivars

	Cultivar							
	Sunrise				Brigitta Blue			
	Fertilizer							
	0 UP*	½ UP	1 UP	Mean	0 UP	½ UP	1 UP	Mean
Year	nitrogen in soil – mg 100 g^{-1} (optimum 2.5-5.0[a]**)							
2015	1.97 a	4.43 cd	7.35 f	4.58 A	1.74 a	4.40 d	7.46 g	4.53 B
2014	2.31 a	4.07 bcd	6.16 ef	4.18 A	1.98 ab	3.91 d	5.77 f	3.89 AB
2013	2.64 ab	3.56 abc	5.61 de	3.94 A	2.37 b	3.02 c	5.11 e	3.50 A
Mean	2.31 A	4.02 B	6.37 C		2.03 A	3.78 B	6.11 C	
	nitrogen in leaves – g 100 g^{-1} (optimum 1.8-2.1[c], 2.25-2.75[d])							
2015	1.19 ab	1.64 de	1.83 e	1.55 B	1.17 ab	1.48 cd	1.77 d	1.47 A
2014	1.33 bc	1.58 cde	1.77 de	1.56 B	1.12 ab	1.34 bc	1.59 d	1.35 A
2013	0.96 a	1.35 bc	1.52 cd	1.28 A	1.01 a	1.26 abc	1.54 cd	1.27 A
Mean	1.16 A	1.52 B	1.71 C		1.10 A	1.36 B	1.63 C	
	nitrogen in fruits – g 100 g^{-1}							
2015	0.43 bc	0.52 de	0.59 e	0.40 A	0.30 ab	0.44 cde	0.51 e	0.42 B
2014	0.38 ab	0.46 cd	0.55 e	0.46 B	0.32 ab	0.39 bcd	0.45 de	0.39 AB
2013	0.33 a	0.37 ab	0.51 cde	0.51 B	0.26 a	0.35 abc	0.44 cde	0.35 A
Mean	0.38 A	0.45 A	0.55 B		0.29 A	0.39 B	0.47 C	
	phosphorus in soil – mg 100 g^{-1} (optimum 2.0-4.0[a], 3.0-6.0[b])							
2015	2.51 ab	4.78 c	8.11 e	5.13 B	2.40 a	5.62 e	8.93 g	5.65 C
2014	2.38 ab	4.39 c	6.36 d	4.38 B	2.57 ab	4.63 d	6.94 e	4.71 B
2013	2.20 a	3.12 b	4.35 c	3.22 A	2.36 a	3.40 bc	4.16 cd	3.31 A
Mean	2.36 A	4.10 B	6.27 C		2.44 A	4.55 B	6.68 C	
	phosphorus in leaves – g 100 g^{-1} (optimum 0.12-0.40[c], 0.20-0.30[d])							
2015	0.08 a	0.16 bc	0.23 cd	0.16 AB	0.07 a	0.15 bc	0.25 d	0.16 B
2014	0.12 ab	0.19 bcd	0.26 d	0.19 B	0.10 ab	0.16 c	0.22 d	0.16 B
2013	0.13 ab	0.15 b	0.15 b	0.14 A	0.07 a	0.12 abc	0.11 abc	0.10 A
Mean	0.11 A	0.17 B	0.21 C		0.08 A	0.14 A	0.19 A	
	phosphorus in fruits – g 100 g^{-1}							
2015	0.065 a	0.084 bc	0.109 d	0.086 A	0.051 a	0.094 cd	0.113 ef	0.086 A
2014	0.073 ab	0.087 bc	0.098 cd	0.086 A	0.054 ab	0.099 cde	0.121 f	0.091 A
2013	0.081 abc	0.095 cd	0.112 d	0.096 A	0.067 b	0.088 c	0.106 de	0.087 A
Mean	0.073 A	0.089 B	0.106 C		0.057 A	0.094 B	0.113 C	

*Explanations: see Table 2
**Optimal mineral content of the soil by [a]Sadowski et al. (1990) and [b]Komosa (2007), and in the leaves by [c]Eck (1988) and [d]Bal (1997)

influence of the fertilizer used on the changes in the concentration of the selected minerals in the soil and plants. The soil mineral nitrogen content in the control area (0 UP) was at a low level (Tab. 3). The application of fertilizer caused a significant increase in the nitrogen content in the soil. Fertilization with urea phosphate at a dose of 30 kg N ha^{-1} caused an increase in the nitrogen content up to the upper limit of the optimal content (2.5-5.0 mg 100 g^{-1}). A twofold increase in the application of the fertilizer (60 kg ha^{-1}) substantially increased the soil N content up to a mean value of 6.24 mg 100 g^{-1} ('Sunrise' 6.37, 'Brigitta Blue' 6.11 mg 100 g^{-1}). The soil N content changed each year. Despite the high soil N content, the N content in the leaves did not reach optimal values – which, according to the cited authors, should be from 1.80 to 2.75 mg 100 g^{-1} (Eck 1988; Bal 1997). In Poland, it has been found that the vast majority of highbush blueberry plantations have a low average concentration of available soil nutrients (Komosa et al., 2017). This indicates that errors in fertilization or the standards to which we refer need to be corrected or reprocessed. The application of 60 kg N ha^{-1} (1 UP) increased the leaf N content in the 'Sunrise' cultivar, but only to the lower limit of the optimal values recommended for highbush blueberry grown in the climatic conditions of the US and Canada (Eck, 1988). The 'Brigitta Blue' cultivar showed a lower leaf N content, which was below the optimal content. According to Bal's recommendations (1997), the optimal N content in the leaves should be higher than 2.25%. In other studies, the nitrogen content in the leaves of several highbush blueberry cultivars (Ochmian et al., 2009; Ochmian, 2012; Leitzke et al., 2015), and also of blueberry bushes from an organic plantation in optimal habitat conditions (Strik and Vance, 2015), was also below the optimal level. In the research conducted in Canada by Burkhard et al. (2009), the nitrogen content in the fruit of the cultivar 'Duke' was variable over the years and depended on the cultivation method (mulch application), but it did not exceed 1.52%. The experimental plants did not show any external symptoms of nitrogen

Table 4. Potassium and magnesium content in the soil, leaves and fruits after fertilization with three levels of Urea-Phosphate (UP) applied over three years to two blueberry cultivars

	Cultivar							
	Sunrise				Brigitta Blue			
	Fertilizer							
	0 UP*	½ UP	1 UP	Mean	0 UP	½ UP	1 UP	Mean
Year	potassium in soil – mg 100 g^{-1} (optimum 5-8^a, 6-8^b)							
2015	4.67 a	5.49 bcd	5.81 de	5.32 A	5.23 a	6.07 bc	6.35 cd	5.88 B
2014	5.37 bc	6.12 ef	6.78 f	6.09 B	5.96 b	6.63 d	6.94 e	6.51 C
2013	5.30 b	5.68 cd	5.47 bcd	5.48 A	5.36 a	5.46 a	5.16 a	5.33 A
Mean	5.11 A	5.76 B	6.02 B		5.52 A	6.05 B	6.15 B	
	potassium in leaves – g 100 g^{-1} (optimum 0.35-0.65^c, 0.45-0.75^d)							
2015	0.28 a	0.40 bc	0.34 ab	0.34 A	0.29 a	0.37 abc	0.44 c	0.37 A
2014	0.32 ab	0.38 abc	0.45 c	0.38 A	0.36 abc	0.41 bc	0.42 bc	0.40 A
2013	0.37 abc	0.35 abc	0.42 bc	0.38 A	0.33 ab	0.30 a	0.39 bc	0.34 A
Mean	0.32 A	0.38 AB	0.40 B		0.33 A	0.36 A	0.42 B	
	potassium in fruits – g 100 g^{-1}							
2015	0.28 ab	0.25 a	0.30 ab	0.28 A	0.41 ab	0.45 ab	0.44 ab	0.43 A
2014	0.42 c	0.38 bc	0.45 c	0.42 B	0.45 ab	0.43 ab	0.48 b	0.45 A
2013	0.44 c	0.43 c	0.38 bc	0.42 B	0.48 b	0.51 b	0.32 a	0.44 A
Mean	0.38 A	0.35 A	0.38 A		0.45 A	0.46 A	0.41 A	
	magnesium in soil – mg 100 g^{-1} (optimum 2.5-4^a, 3-6^b)							
2015	3.12 cd	2.61 ab	2.38 a	2.70 A	3.26 c	2.72 b	2.18 a	2.72 A
2014	3.64 e	3.40 de	2.94 bc	3.33 B	3.74 c	3.35 c	3.29 c	3.46 B
2013	3.57 e	3.16 cd	3.23 cde	3.32 B	3.63 c	3.50 c	3.36 c	3.50 B
Mean	3.44 C	3.06 B	2.85 A		3.54 B	3.19 AB	2.94 A	
	magnesium in leaves – g 100 g^{-1} (optimum 0.12-0.25^c, 0.15-0.25^d)							
2015	0.14 bc	0.12 abc	0.10 ab	0.12 A	0.14 bc	0.08 a	0.07 a	0.10 A
2014	0.17 c	0.13 abc	0.10 ab	0.13 A	0.15 c	0.09 ab	0.06 a	0.10 A
2013	0.10 ab	0.08 a	0.08 a	0.09 A	0.11 abc	0.09 ab	0.07 a	0.09 A
Mean	0.14 B	0.11 AB	0.09 A		0.13 B	0.09 A	0.07 A	
	magnesium in fruits – g 100 g^{-1}							
2015	0.025 ab	0.023 ab	0.020 a	0.023 A	0.019 ab	0.019 ab	0.017 a	0.018 A
2014	0.028 b	0.026 ab	0.019 a	0.024 A	0.022 ab	0.024 b	0.018 ab	0.021 A
2013	0.023 ab	0.022 ab	0.025 ab	0.023 A	0.019 ab	0.017 a	0.022 ab	0.019 A
Mean	0.025 B	0.024 AB	0.021 A		0.020 A	0.020 A	0.019 A	

*,**Explanations: see Table 2 and 3

deficiency, although the nitrogen level in the leaves was low. This confirms the opinion about the low demand for nitrogen by the highbush blueberry plant (Pormale et al., 2009). However, in order to increase productivity, higher doses of fertilizer should be used, e.g. 60 kg N ha^{-1} (Kozos and Ochmian, 2016). Increased fertilization also had a significant influence on an increase in the nitrogen content in the fruit. Higher nitrogen content was found in the fruit of the cultivar 'Sunrise'. In other studies, the fruit of this cultivar also had a higher nitrogen content compared to 'Brigitta Blue', regardless of the cultivation site. The nitrogen content in the fruit collected from bushes fertilized with 1 UP was similar to that in the unfertilized fruit collected from bushes cultivated under optimal habitat and soil conditions (Strik and Vance, 2015). A significant increase in the nitrogen content in the fruit in other studies was found after the application of nitrogen-containing foliar fertilizers (Ochmian and Kozos, 2015).

The soil phosphorus content increased after the application of 60 and 30 kg N ha^{-1} of urea phosphate. The phosphorus content in the leaves and fruit of those bushes also increased. Despite the low pH of the soil, the phosphorus content increased significantly. With such a low soil pH, it was accumulated in limited quantities. However, this form of fertilizer could have an impact on the accumulation of this nutrient by plants, despite the very low soil pH. The interpretation of the results and determination of soil fertility and leaf nutrition level are a problem. The amount of phosphorus in the soil in the control plots was optimal according to Sadowski et al. (1990); however, it was low according to the guidelines provided by Komosa (2007). As a result of full fertilization, the phosphorus content in the soil increased and exceeded the optimal range (3.0-6.0 mg 100 g^{-1}) in 2014 and 2015 according to Komosa (2007), but its content in the leaves was optimal according to Eck (1988) and too low according to Bal (1997).

The use of ½ UP increased the phosphorus content in the soil to the optimal level; however, its content in the leaves was insufficient according to Bal (1997). The phosphorus content in the leaves was at a level similar to that in the research conducted by Bryla et al. (2012) and Ochmian et al. (2009). According to Eck's recommendation (1988), it is not necessary to use phosphorus fertilization for the cultivars 'Sunrise' and 'Brigitta Blue'. In another experiment, applications of phosphorus-potassium fertilizers over many years had increased the phosphorus content up to 10.6 mg 100 g^{-1}, which is much higher than the recommended value (Smolarz, 2009). This also considerably reduced the soil pH. During the present experiment, the average value of potassium content in both the control and the fertilized soil fell within the optimal range (Tab. 4). During the first year of the experiment, the magnesium content exceeded the optimal values. However, high concentration of these elements in the soil did not influence their content in the fruits and leaves. Application of the fertilizer significantly reduced Mg content in both leaves and fruits. The Mg content was reduced below the optimal level specified by both Eck (1988) and Bal (1997). Research by Fageria (2001) showed that NH_4^+ ions inhibit Mg intake by plants. The Mg content in the leaves of the control plants was lower than that shown in the research by Bryla et al. (2012) and Ochmian et al. (2010).

Thirty-three phenolic compounds were determined in the analyzed samples. Application of the fertilizer, regardless of the 30 or 60 kg N ha^{-1} dose, contributed to the reduction in the concentration of polyphenols in the fruit of both cultivars (Tab. 5). It was found that there were significantly smaller amounts of anthocyanins and flavonols, particularly after the application of 60 kg N ha^{-1} (1 UP). The use of the fertilizer lowered the soil pH to the optimum for highbush blueberry. This could have led to the lower polyphenol content, as was most evidently shown in the last year of the study. Other studies have shown that the fruit harvested from highbush blueberry plants grown under optimal soil conditions contains more polyphenolic compounds (Ochmian et al., 2015a).

The use of nitrogen fertilization causes different responses of blueberry bushes in terms of the concentration of polyphenolic compounds in the fruit (Leitzke et al., 2015). Application of a foliar fertilizer also reduces the amounts of polyphenolic compounds in the fruit (Ochmian and Kozos, 2015). The concentration of polyphenols, especially anthocyanins, is also affected by the size of the fruit. Smaller fruit contains more of these compounds (Ochmian and Kozos, 2014).They are accumulated mainly in the fruit peel and directly underneath it (Fernandez-Pachon et al., 2004). For the same mass (e. g 100 g), the surface area of small-fruit peel is much larger than in large fruit. The applied urea phosphate fertilizer increased the yield of the bushes, and the harvested fruits were significantly larger (Kozos and Ochmian, 2016). The concentration of polyphenols varied over the three years of the study.

Table 5. Concentration of polyphenolic compounds in fruit after fertilization with three levels of Urea-Phosphate (UP) applied over three years to two blueberry cultivars

	Cultivar							
	Sunrise				Brigitta Blue			
	Fertilizer							
	0 UP*	½ UP	1 UP	Mean	0 UP	½ UP	1 UP	Mean
Year	anthocyanins – mg 100 g^{-1}							
2015	291 c	257 ab	224 a	241 A	388 bc	362 ab	335 a	362 A
2014	334 d	305 cd	276 bc	291 B	559 e	447 e	398 c	468 C
2013	252 ab	270 bc	244 ab	257 A	432 de	406 cd	449 e	429 B
Mean	292 B	277 B	248 A		460 B	405 A	394 A	
Year	phenolic acid – mg 100 g^{-1}							
2015	211 bc	203 bc	194 abc	199 B	173 cd	161 b-d	180 d	171 B
2014	183 ab	170 a	162 a	166 A	142 ab	156 bc	128 a	142 A
2013	204 bc	182 ab	221 c	202 B	165 cd	178 d	157 bc	167 B
Mean	199 A	185 A	192 A		160 A	165CA	155 A	
Year	flavonols – mg 100 g^{-1}							
2015	38.9 cd	32.5 ab	28.3 a	33.2 A	28.7 bc	26.8 ab	24.5 a	26.7 A
2014	42.2 cde	37.8 bc	36.6 bc	38.9 B	35.6 e	31.5 cd	28.4 abc	31.8 B
2013	42.5 cde	45.1 e	43.6 de	43.7 B	37.8 ef	39.7 f	34.4 de	37.3 C
Mean	41.2 B	38.5 AB	36.2 A		34.0 B	32.7 B	29.1 A	
Year	flavan-3-ols – mg 100 g^{-1}							
2015	40.3 c	37.0 c	31.5 ab	36.3 B	53.4 d	45.1 b	42.9 a	47.1 A
2014	48.6 de	45.4 d	51.3 e	48.4 B	61.2 e	50.3 cd	52.4 d	54.6 B
2013	32.7 ab	29.8 a	33.6 b	32.0 A	44.5 b	48.9 c	47.4 bc	46.9 A
Mean	40.5 B	37.4 A	38.8 AB		53.0 B	48.1 A	47.6 A	
Year	total – mg 100 g^{-1}							
2015	581 c	530 b	478 a	530 A	651 b	600 a	592 a	614 A
2014	608 c	558 b	526 b	564 B	790 c	680 b	597 a	689 B
2013	531 b	527 b	542 b	533 A	679 b	673 b	688 b	680 B
Mean	573 B	538 A	515 A		707 B	651 A	626 A	

*,**Explanations: see Table 2 and 3

Their highest level was found in the fruit in 2014, when the weather was extremely hot and rainy in July, followed by a cool and rainy period in August (Tab. 1). The effects of weather on the amounts of polyphenolic compounds have also been reported by other authors (Wawro et al., 2013). Plants which are grown at a low temperature generally contain smaller amounts of phenolic acid, flavonols, and anthocyanins (Wang and Zheng, 2001).

CONCLUSIONS

1. Regardless of the fertilizer dose, urea phosphate resulted in a significant reduction in soil pH.
2. The twofold increase in fertilization rate (60 kg N per hectare) significantly increased the soil N content to a level higher than the optimum level recommended for blueberry production. However, the leaf N remained low. The soil P content increased after the application of the fertilizer. The P content in the leaves and fruit also increased. Despite the considerable soil Mg content, the leaf and fruit Mg concentrations were substantially reduced by the application of urea phosphate fertilization.
3. Reducing the soil pH to the optimum for the cultivation of highbush blueberry decreased the fruit polyphenol content.

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AUTHOR CONTRIBUTIONS

I.O., M.S. and B.J. – contributed to the entire experimental process, chemical and data analyses, interpretation, literature search and writing; J.O. – was involved in the determination of polyphenolic compounds.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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