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Foliar Application of Some Macronutrients and Micronutrients Improves Yield and Fruit Quality of Highbush Blueberry (*Vaccinium corymbosum* L.)

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Abstract: Foliar fertilization makes it possible to quickly provide plants with essential nutrients, mainly micronutrients, which can significantly improve the quality of yields. The aim of this study was to evaluate the effect of foliar fertilization with fertilizers containing calcium and microelements on yielding and fruit quality of highbush blueberry (*Vaccinium corymbosum* L.). A two-year study was carried out in western Poland in an experimental highbush blueberry production plantation. During the growing season the bushes were treated several times with the following foliar fertilizers: Armurox, BioCal, and Stymjod. The experiment assessed bush growth vigor, yield, fruit quality characteristics, sugar, organic acid, and health-promoting substance content. It was found that as a result of fertilizing highbush blueberry bushes with foliar fertilizers, the leaf blade area and plant yield increased significantly. The fruits collected from those bushes were characterized by a higher mass, firmness, and TSS content. This also applies to blueberry fruit after storage. Foliar fertilization had no significant effect on the content of chlorophyll a and b in the leaves of northern highbush blueberry, on fruit coloration, the content of sugars, ascorbic and citric acids, and the phenolic compounds in them.

Keywords: highbush blueberry; foliar fertilization; yield; fruit firmness; extract content (TSS); organic acids; sugars; phenolic compounds

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1. Introduction

The main blueberry species grown on a mass scale is highbush blueberry (*Vaccinium corymbosum* L.), in Poland it also known as "American blueberry". According to statistics from the Food and Agriculture Organization of the United Nations (FAOSTAT), in 2020, its largest producers worldwide were the United States and Canada (approximately 35% and 17% of world production, respectively). There is a growing worldwide interest in the cultivation of northern highbush blueberry. While in the mid-1960s the global production of this fruit was approximately 33 thousand tons, in 2020 it was already approximately 850 thousand tons. In addition, in Polish fruit farming over the last few decades, highbush blueberry has been one of the fastest growing products [1]. According to FAOSTAT, in 2020, domestic production of this fruit was around 55 thousand tons (6.5% of global production), which places Poland 6th in the world.

The high nutritional and health-promoting properties of highbush blueberry are one of the reasons for its popularity. Its fruits contain vitamins A, B1, B2, and B3 as well as phosphorus, calcium, sodium, folic acid, and phytoestrogens. Blueberries are an excellent source

of health-promoting compounds, mainly polyphenols [2–4]. One serving of blueberries, similar to a serving of cranberries or grapes, provides the body with 200–400 mg of polyphenols [5], which may be crucial in reducing the risk of developing type 2 diabetes [6,7] or other chronic diseases [8].

Shrubs of the Vaccinium genus, from which highbush blueberry originates, grow wild in soils with low nutrient content. This is why the fertilization requirements of highbush blueberry are relatively low in comparison to other fruit farming species [9]. However, high yields depend on the use of mineral fertilization to maintain the sufficiently high microelement and macroelement contents of the soil. Soil fertilization is common and effective for nutrients required in large quantities. However, doing so not only increases the number of nutrients in the soil but also changes the soil structure, enzymatic activity, and diversity of soil microorganisms [10]. Nitrogen fertilizers, in particular, can have a limiting effect on soil fungal communities by lowering soil pH. High fertilizer doses accelerate the vegetative growth of blueberry, thus reducing their yield [11].

In addition to standard soil fertilization, especially under intensive cultivation conditions, plants sometimes require additional nutrient-supplementing treatments during the growing season, i.e., foliar fertilization. It is particularly recommended when macroelements—and especially microelements—must be provided to plants. Micronutrients are part of most enzymes regulating biochemical and physiological processes in plants, and their deficiency may lead to disorders of these processes [12]. In blueberry cultivation, foliar calcium fertilization is particularly recommended [13]. This is because the blueberry is a specific species that requires a low pH soil [14], the optimum value of which should be between 4.5 and 4.8 (pH H_2O). In such a substrate, high concentrations of Al^{3+}, Fe^{2+}, and Mn^{2+} inhibit calcium ion absorption. Apart from the standard macronutrients (the aforementioned calcium), foliar fertilizers can contain ingredients that are difficult to extract from soil (e.g., silicon) or that are present in trace amounts (e.g., iodine). Both iodine and silicon activate plants' natural defense mechanisms, allowing them to mitigate the effects of stress [15]. An advantage of foliar fertilization is the rapid utilization by plants of nutrients supplied in this way, which has a positive effect on yield and fruit quality. Maintaining high quality after harvest and extending the storage life of the fruit is particularly important in the case of highbush blueberry, mainly due to the ongoing global fruit overproduction of this species. Overproduction is facilitated by, among other things, high purchase prices combined with production automation and mechanization [16]. It is assumed that properly conducted foliar fertilization can improve blueberry fruit quality both after harvest and after storage.

The aim of the study was to assess the effect of foliar fertilization with preparations containing macronutrients and micronutrients on the amount and quality of yield of northern highbush blueberry (*Vaccinium corymbosum* L.).

2. Materials and Methods

2.1. Study Sites and Experimental Design

The study was conducted between 2020 and 2021 at the experimental station of the Poznań University of Life Sciences in western Poland (52°31′ N; 16°38′ E). The research object covered highbush blueberry bushes of the Bluecrop cultivar growing on an experimental production plantation at a distance of 2.5 × 1.5 m (2667 pcs. per ha). The bushes grew in podzolic soil, formed from loamy sands with a slightly acid reaction (pH 5.6), humus content of 4.78%, and salinity of 0.59 g NaCl dm^3. The content of macroelements in the soil was (in mg dm^3): N-NO_3—62; P—61; K—194; Ca—952; Mg—230; Cl—41. The soil nitrogen and phosphorus contents were optimal, the potassium and calcium contents were very high, and the magnesium contents were low.

The experiment consisted of four treatments: (1) foliar spraying with water (control combination); (2) foliar spraying with Stymjod; (3) foliar spraying with BioCal; (4) foliar spraying with Armurox. They were established in a randomized block design in four plots, where one plot consisted of 6 bushes. The total number of plants used in the experiment was 96.

In each year of the experiment, the highbush blueberry bushes were fertilized with foliar fertilizers three times (i.e., during flowering, fruit setting, and ripening) in a total dose of 4 L ha for Stymjod and Armurox and 1.5 L ha for BioCal. The BioCal preparation used in the experiment was a liquid foliar fertilizer containing calcium in the form of water-soluble calcium oxide at 8% *w*/*w* and water-soluble zinc at 3% *w*/*w*. Another fertilizer, Armurox, contained silicon oxide (8%), free amino acids (3%), and total nitrogen (1%). Armurox forms a physical barrier under the plant cuticle and activates plants' endogenous defense mechanisms. Stymjod is a liquid fertilizer in the form of a concentrate, produced in nanotechnology using the cold plasma effect. It contains the optimum macronutrient composition for plants (i.e., N—6.3%; P—4.58%; K—6.42%; Mg—1.69%; S—1.6%), micronutrients (i.e., B—0.46%; Cu—0.17%; Fe—0.14%; Mn—0.16%; Mo—0.028%; Zn—0.42%) as well as humic and amino acids, increasing the resistance of plants to unfavorable environmental conditions. Information regarding the composition of the preparations was obtained from their manufacturer. During the vegetation period, standard agrotechnical practices recommended for highbush blueberry cultivation were applied. In the spring period (March–April) of each year of the study and for the highbush blueberry bushes in all treatments, the soil was fertilized with nitrogen at 50 kg ha with the addition of ammonium sulphate ($(NH_4)_2SO_4$) at 20% N and 24% S.

Climatic conditions (mean monthly and mean annual air temperature and precipitation) were evaluated on the basis of measurements taken at the meteorological station located at the place of the experiment. Spring 2020 was relatively cool (especially May) with numerous frosts (down to −8 °C). Summer saw frequent prolonged water shortages (Table 1). Compared to the multiyear mean measurements, the total precipitation during the growing season in 2020 was approximately 70 mm lower. The spring of 2021 was also relatively cold. During the summer months, there was high intensity precipitation. Over the entire growing season of that year, the amount of precipitation was approximately 25 mm higher compared to the multiyear average (Table 1).

Table 1. The course of climatic conditions in the years 2020 and 2021.

Years	Months							Sum
	IV	V	VI	VII	VIII	IX	X	
Precipitation (mm)								
2020	42.6	49.4	48.6	78.2	57.4	41.6	53.2	279.8
2021	29.2	36.8	78.6	96.7	43.8	60.7	32.4	378.2
1982–2012 [1]	28.0	48.0	63.5	78.8	61.9	41.0	32.0	353.2
Air temperature (°C)								
2020	8.9	11.6	18.2	18.5	20.3	15.1	10.5	103.1
2021	6.2	12.3	20.1	19.0	17.5	16.4	9.1	100.6
1982–2012 [1]	9.3	14.6	17.2	19.5	18.9	14.1	9.0	102.6

[1]—average.

2.2. Measurements and Observations

In the experiment, the following measurements and analyses were performed: blueberry leaves (i.e., leaf blade area and chloroplast pigment content), content of macroelements (i.e., N-NO_3; P_2O_5; K_2O; CaO; MgO), and microelements (i.e., Zn; Cu; Mn; B) of leaves and fruit; yield level (kg per bush); fruit weight as well as their height and width; fruit coloration; their quality parameters (i.e., firmness, extract, organic acid, and sugar contents); content of phenolic compounds and anthocyanins in fruit.

2.2.1. Measurements and Analyses of Blueberry Leaves

In the experiment, the highbush blueberry leaf area was measured. For this purpose, in both years of the study, 50 leaves were randomly collected from each treatment. They

were collected from the central part of shoots in the second half of July, at the time recommended for assessing the nutritional status of blueberries [17]. The collected leaves were scanned, and then the area of their blades was calculated using DigiShape 1.9 software (ver.1.9.19, Cortex Nowa, Bydgoszcz, Poland).

The content of chloroplast pigments, chlorophyll a and b, and carotenoids (in mg kgf.m.) was determined in 2020 in 25 sample leaves from the treatment. They were collected in the summer, after the last fruit harvest. Determinations of chloroplast pigments were performed using the extraction method according to Hiscox and Israel [18]. Leaf blade fragments weighing 0.5 g were cut with a cork borer. The prepared material was covered with 5 mL of dimethyl sulfoxide and placed in a water bath at 65 °C. After 20 min, using a spectrophotometer, absorbance was measured at 470 nm for β-carotene, 645 nm for chlorophyll b, and 663 nm for chlorophyll a. The calculations were based on the equation:

- Chlorophyll a = $(12.7 \times A663 - 2.7 \times A645) \times V \times (1000 \times W)^{-1}$;
- Chlorophyll b = $(22.9 \times A645 - 4.7 \times A663) \times V \times (1000 \times W)^{-1}$;
- Sum of chlorophyll a + b = $(20.2 \times A645 + 8.02 \times A663) \times V \times (10{,}000 \times W)^{-1}$.

where V—total extract volume cm^3; W—sample weight in g.
Carotenoid contents in fruits (in mg kg f.m.) were calculated based on the equation:

- Carotenoids = $(1000 \times A470 - 1.9$ chlorophyll a $- 63.14$ chlorophyll b$) \times 214^{-1}$.

2.2.2. Macro- and Microelement Contents in Leaves and Fruits

Analyses of the macroelement and microelement contents in the leaves and fruits of highbush blueberry were performed in 2021. For this purpose, 200 leaves and 100 fruits were collected from each combination, which were then dried in the dryer (Binder, Tuttlingen, Germany) (at 45–50 °C), ground, and mineralized in the presence of sulphuric acid in a mineralizer. The content of N in plant material was determined by the distillation method according to Kjeldahl; P by the vanadomolybdenum method; K, Ca, and Mg by the atomic absorption method on Zeiss Jena AAS-5 apparatus (Oberkochen, Germany). All determinations were made in the laboratory of the Chemical and Agricultural Station, in accordance with the Polish Standard, using certified reagents.

2.2.3. Yielding and Quality of Fruits

In 2020 and 2021, fruit for analysis was harvested three times: at the end of July (first date), in early August (second date), and in the second half of August (third date). The yield was determined on the basis of the weight of ripe fruit collected from a bush (kg per bush). To assess average fruit weight, 100 fruits were randomly collected from each treatment after each harvest and weighed with an accuracy to 0.01 g. Fruit height and width (mm) were measured for a sample of 100 fruits from each treatment using an electric caliber. The extract content (TSS—total soluble solids) was measured using the PR-101a refractometer (Atago Co., Ltd., Fukaya-shi, Japan) for a sample of 100 fruits from each treatment. The values are expressed in %. Fruit firmness was assessed for the same sample (g mm^{-1}). Measurements were taken using a firmness tester "Fruit Pressure Tester mod. 327" by Facchini, Alfonsine, Italy), mounted on a stand. This test is known as the Magness–Taylor test and consists of piercing the fruit flesh with a 1.5 mm pin. In both years of the study, the post-harvest stability of the fruits was also evaluated by analyzing their firmness and extract after 6 days of storage at 4–6 °C.

For the assessment of fruit coloration, a Minolta colorimeter was used in the Lab color space, based on the so-called trichromatic theory of color vision. Coloration was expressed in the color space of L * a * b *, where L * denotes the brightness from black (0) to white (100), a * the color from green (−60) to red (60), and b * the color from blue (−60) to yellow (60). Measurements were made on 50 fruits from one treatment. Four measurements were made on one fruit and then the obtained results were averaged.

The content of organic acids (i.e., ascorbic, citric, and malic acids), sugars (i.e., total, fructose, glucose, and sucrose), phenolic compounds, and anthocyanins in the blueberry

fruit was tested in 2020 on a sample of 100 fruits randomly harvested from each treatment and each of the three harvest dates.

The HPLC method developed by Bozan et al. [19] was used to determine the organic acid content. For organic acids extraction, 0.25 g of the sample and 4 mL of 3% metaphosphoric acid was mixed. The mixture was placed in the ultrasonic water bath at 80 °C for 15 min, and it was sonicated and centrifuged at 5500 rpm for 15 min. Afterward, the mixture was filtered. The extract of organic acids was analyzed using a high-performance liquid chromatographic apparatus HPLC (Shimadzu LC 20A VP, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD 20A VP), and we used an 87 H column (5 µm, 300 × 7.8 mm). The identified acids were evaluated according to the relevant standard calibration curves.

Changes in glucose, fructose, sucrose, and total sugar content in homogenized blueberry samples were determined using the HPLC technique according to the method developed by Crisosto [20]. One milligram of blueberry fruit powder was added to 4 mL of ultrapure water (Millipore Corp., Bedford, MA, USA). The reaction mixture was placed in an ultrasonic bath and sonicated at 80 °C for 15 min and then centrifuged at 5500 rpm for 15 min, and it was filtered before HPLC analysis. Sugar contents were determined using HPLC (Shımadzu, Prominence LC-20A) RID (Refractive Index) detector and Coregel-87C (7.8 × 300 mm). Separations were performed at 70 °C at a flow rate of 0.6 mL min^{-1}. Elution was isocratic ultrapure water. The individual sugars were calculated according to their standards. Calibration curves of the references used were created and content was determined according to these external standard calibration curves.

The total phenolic content was evaluated using Folin–Ciocalteu reagent in the modified method of Spanos and Wrolstad [21]. Briefly, methanol extract was added on 1 g of samples. Water, Folin–Ciocalteu, and 20% sodium carbonate were added to the samples taken from the supernatant of this extract and then kept in the dark for 2 h. The absorbance of all samples were measured at 760 nm with the use of a Thermo Scientific Multiskan GO microplate spectrophotometer. Quantifications were calculated through a calibration curve daily prepared with known concentrations of gallic acid (GA) standards, and the results are expressed as milligrams of GA equivalents per 100 g of dry weight (DW) of fruits.

The pH-differential absorbance method of Wrolstad [22] was employed to quantify monomeric anthocyanin pigment content of the methanol-blueberry fruit powder extract within buffers at pH 1.0 (hydrochloric acid–potassium chloride, 0.2 mol) and 4.5 (acetate acid–sodium acetate, 1 M). A UV-spectrophotometer and 1 cm disposable cell were utilized for spectral measurements at 510 and 700 nm. Anthocyanin content was calculated as mg (cyanidin-3-glucoside).

2.3. Statistical Analysis

All results obtained were subjected to one-way classification using the STATISTICA 7 program (StatSoft, Inc., Tulsa, OK, USA). The significance of the differences between the means for individual treatments was assessed using Duncan's test for the significance level of $\alpha = 0.05$. The chemical analyses were performed in four repetitions.

3. Results and Discussion

3.1. Parameters of Highbush Blueberry Leaves

Leaf shape and size are important factors affecting plant yield. To absorb enough light energy, leaves must be as wide as possible. The blueberry leaf area in the experiment, depending on the year and treatment, varied from 7.69 cm^2 in the control treatment to 13.12 cm^2 in the treatment where Stymjod was applied (Figure 1). The leaf blade area of highbush blueberry increased significantly after foliar fertilization. In the first year of the study, BioCal and Stymjod fertilizers were the most effective in this respect, with leaf area increasing by 60% and 64%, respectively, compared to the control treatment. In 2021, the increase in the leaf blade area in the treatments with foliar fertilization compared to the control treatment was smaller, on the order of a few percentage units.

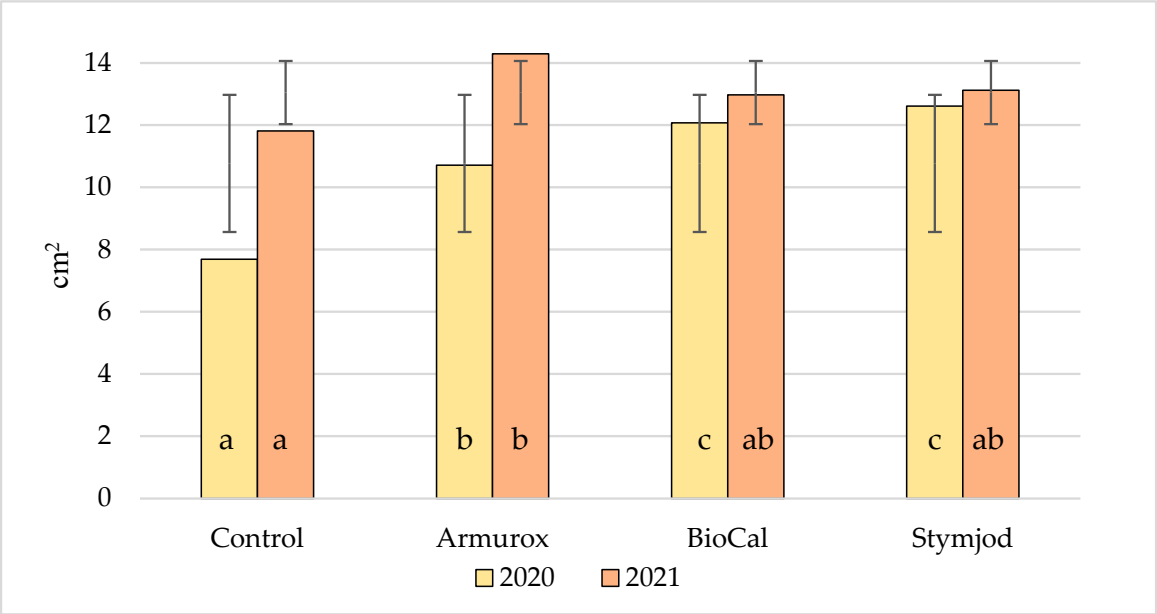


Figure 1. Effect of foliar fertilization on leaf blade area (cm^2) of highbush blueberry in 2020 and 2021. The mean values marked with the same letters do not differ significantly at a = 0.05.

Photosynthesis is the main factor responsible for the process of biomass accumulation by plants. In the experiment, there was no significant variation in the chlorophyll content of highbush blueberry leaves. Two of the three tested foliar fertilizers did not affect its content either. Only in the treatment with the bushes sprayed with the Stymjod fertilizer did the total content of chlorophyll a and b in the leaves increase significantly (Table 2). The authors also did not obtain confirmation of previous results by other researchers [23,24] showing an increase in chlorophyll content in plants growing under stress condition under the influence of silicon. In the highbush blueberry leaves sprayed with Armurox (8% Si), the chlorophyll content did not differ significantly from that in leaves of bushes from the other treatments. Carotenoids perform a protective function for chlorophyll. In the experiment, the differences in their content in the leaves of highbush blueberry foliar fertilized were recorded. The content of carotenoids in leaves from bushes treated with BioCal and Stymjod fertilizers was higher than the control.

Table 2. Content of chloroplast pigments in highbush blueberry leaves (mg kg^{-1} f.m.) in 2020.

Treatments	Chlorophyll a	Chlorophyll b	Chlorophyll a + b	Carotenoids
Control	54.56 ± 0.10a [1]	101.46 ± 0.54a	156.13 ± 0.62ab	750.30 ± 3.16a
Armurox	54.60 ± 0.08a	100.50 ± 1.03a	155.20 ± 1.09a	757.43 ± 6.61ab
BioCal	54.85 ± 0.32ab	101.65 ± 0.76ab	156.61 ± 1.08bc	790.34 ± 4.70b
Stymjod	54.95 ± 0.12b	102.76 ± 0.51b	157.82 ± 0.14c	997.42 ± 8.97c

[1] The mean values marked with the same letters did not differ significantly at a = 0.05.

3.2. Macroelements and Microelements Content of Leaves and Fruit

The mineral content of leaves is an indicator of the nutritional status of the plant. In the experiment, in the leaves of highbush blueberry, depending on the treatment, the contents of macroelements were (in % d.m.) N_{tot}—from 1.53 to 1.70; P_2O_5—0.26; K_2O—0.53; CaO—from 0.92 to 1.12; MgO—from 0.41 to 0.49 (Table 3).

Table 3. The content of macro- and microelements of highbush blueberry leaves in 2021.

Treatments	Macroelements (% d.m.)					Microelements (mg kg^{-1} d.m.)			
	Ntot	P_2O_5	K_2O	CaO	MgO	Zn	Cu	Mn	B
Control	1.56 ± 0.011b	0.26 ± 0.010a	0.53 ± 0.012a	1.04 ± 0.07c	0.44 ± 0.017a	7.75 ± 0.10a	2.96 ± 0.36a	213 ± 12.4d	33.60 ± 2.6c
Armurox	1.53 ± 0.019a	0.26 ± 0.017a	0.53 ± 0.009a	0.92 ± 0.04a	0.41 ± 0.021a	8.86 ± 0.35a	3.68 ± 0.54c	173 ± 18.7a	31.05 ± 1.9b
BioCal	1.67 ± 0.013c	0.25 ± 0.021a	0.52 ± 0.017a	1.12 ± 0.11d	0.46 ± 0.011a	32.0 ± 0.98c	3.24 ± 0.31b	204 ± 25.4c	30.05 ± 3.8a
Stymjod	1.70 ± 0.021d	0.26 ± 0.019a	0.53 ± 0.011a	0.96 ± 0.09b	0.49 ± 0.018a	17.0 ± 0.67b	7.67 ± 0.72d	186 ± 17.6b	49.30 ± 2.9d

The mean values marked with the same letters did not differ significantly at a = 0.05.

The literature contains numerous and often varied results of macronutrient content in highbush blueberry leaves. The contents obtained in this experiment were higher than in earlier studies by the authors of the article, where the macronutrient contents in blueberry leaves under various conditions, including replant disease [25], were analyzed, or similar to the results obtained in experiments by other researchers. These contents were (in % d.m.) N—from 1.80 to a maximum of 2.29 [17,26–28]; P—from 0.49 [17] to 0.90 [28]; K—minimum of 0.13; Mg—from 0.17 to 0.19 [17]. In the experiment, the content of N-NO_3 and Mg (BioCal and Stymjod fertilizers) significantly increased in the leaves of highbush blueberry after foliar fertilization. In treatment with BioCal (8% Ca), an especially high content of calcium was also found in blueberry leaves compared to the control treatment.

The contents of microelements in the leaves of highbush blueberry were (in mg kg^{-1} d.m.) Zn—from 7.75 to 32.0; Cu—0.29; K—0.53; Ca—from 0.92 to 1.12; Mg—from 0.41 to 0.49 (Table 3). In blueberry leaves from foliar-fertilized bushes, the content of Zn and Cu was significantly higher and Mn was lower than in the control treatment. The greatest differences were demonstrated for BioCal and Stymjod. It should be added that the composition of the latter one, besides amino acids, included a number of microelements. Considerable differences in Zn content were also found. The content of this element in leaves in the treatments with bushes sprayed with BioCal and Stymjod, as compared to the control, was over four times higher (7.75 and 32.0 mg kg^{-1} d.m., respectively) (Table 3). As Raj and Raj [12] pointed out, Zn is essential for plant nutrition. It participates in all major plant functions, increases nitrogen uptake and, at later stages, activates CO_2 fixation.

In the fruits of highbush blueberry, depending on the treatment, the contents of macroelements (in % d.m.) were N—from 0.60 to 0.76; P—0.26; K—from 0.69 to 0.80; Ca—0.15; Mg—0.08 (Table 4). The contents of macroelements in the blueberry fruit was less varied than in the leaves. The amount of Ca and Mg in fruits was identical, regardless of the treatments. In the blueberry fruit from the bushes sprayed with Armurox and Stymjod, the contents of N, P, and K were lower than in the fruit from the bushes that were not foliar fertilized (Table 4). The position of Ochmian et al. [29,30] regarding an increase in Ca and P contents in blueberry fruit as a result of spraying bushes with Ca- and P-containing fertilizers was not confirmed.

Table 4. The content of macro- and microelements of highbush blueberry fruits in 2021.

Treatments	Macroelements (% d.m.)					Microelements (mg kg^{-1} d.m.)			
	N-NO_3	P_2O_5	K_2O	CaO	MgO	Zn	Cu	Mn	B
Control	0.74 ± 0.020c	0.26 ± 0.030b	0.80 ± 0.12c	0.15 ± 0.07a	0.08 ± 0.021a	6.13 ± 0.45b	2.94 ± 0.57b	30.4 ± 2.45d	5.28 ± 0.74c
Armurox	0.64 ± 0.017b	0.23 ± 0.027a	0.69 ± 0.09a	0.15 ± 0.03a	0.08 ± 0.016a	5.40 ± 0.26a	2.48 ± 0.23a	26.8 ± 1.98b	4.18 ± 0.95a
BioCal	0.76 ± 0.061d	0.26 ± 0.019b	0.74 ± 0.15b	0.15 ± 0.02a	0.08 ± 0.009a	24.7 ± 1.34d	2.97 ± 0.19c	21.3 ± 0.86a	4.43 ± 0.56b
Stymjod	0.60 ± 0.019a	0.26 ± 0.036b	0.74 ± 0.17b	0.15 ± 0.03a	0.08 ± 0.010a	6.52 ± 0.29c	4.04 ± 0.36d	28.9 ± 1.73c	8.80 ± 1.03d

The mean values marked with the same letters did not differ significantly at a = 0.05.

In the fruits of highbush blueberry, depending on the treatment, the contents of microelements were (in mg kg^{-1} d.m.) Zn—from 5.40 to 24.7; Cu—from 2.48 to 4.04; Mn—from 21.3 to 30.4; B—from 4.18 to 8.80 (Table 4). Significant differences in micronutrient contents occurred in the treatment with Stymjod. In fruits from the bushes sprayed with this preparation, significantly higher contents of Zn, Cu, Fe, and B were found in

comparison to the control. It should be added that the composition of this fertilizer included a whole range of microelements. The content of Zn in blueberry fruit increased evenly several times. This was true for the treatment with the foliar application of BioCal.

3.3. Yielding of Blueberry Bushes

A high yield is the determining factor for profitable production. In the first year of the experiment, spraying highbush blueberry bushes with each foliar fertilizer resulted in a significant increase in their yield. The difference compared to the control treatment ranged from approximately 30% (Stymjod) to over 40% (Armurox) (Figure 2). In the second year of the experiment, the yielding of blueberry bushes in each treatment was higher than in the previous year. However, in that period, no significant effect of foliar fertilization on the yielding of highbush blueberry bushes was found.

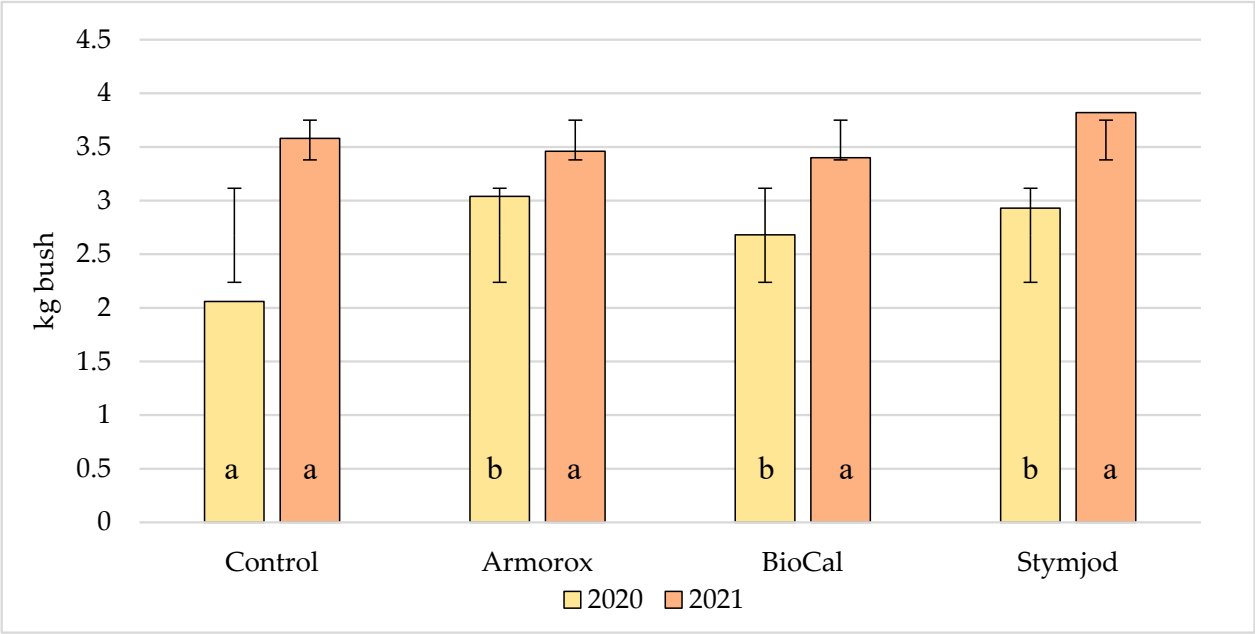


Figure 2. Yielding of blueberry bushes (kg bush) in 2020 and 2021. The mean values marked with the same letters did not differ significantly at a = 0.05.

The high effectiveness of foliar fertilization in increasing the yield of highbush blueberry bushes only in 2020 may be due to the fact that in that period there were hardly favorable weather conditions for cultivation. For example, the amount of precipitation was low (Table 1), which resulted in long periods of drought. Under such conditions, the effectiveness of foliar fertilization may have increased. The characteristics of the foliar fertilizers used in the experiment show that one of their important properties is the mitigation of stress conditions.

3.4. Biometric and Quality Parameters of Highbush Blueberry Fruit

Biometric parameters (i.e., height and width) and fruit weight are important physical indicators affecting fruit attractiveness. Fruit size is vital for economic reasons. Large fruit is much easier to sell than smaller fruit. In the experiment, the smallest highbush blueberry fruit was collected from the bushes that were not foliar fertilized (control). In the experiment, the range of fruit weight measurements was quite considerable, from 1.39 g in the control treatment to 2.19 g in the treatment with BioCal fertilizer application in 2021 (Table 5). The available literature shows that such differences in highbush blueberry fruit weight were demonstrated in the works of many authors. Fluctuations may range from 1.12 to 2.11 g according to Aliman et al. [31]; from 1.4 to 2.4 g according to Correia et al. [32];

from 1.76 to 1.94 g according to Zorenc et al. [33]; from 1.29 to 1.80 g. These should be explained, among other things, by varietal differences or weather conditions.

Table 5. Effect of foliar fertilization on highbush blueberry fruit weight and biometric parameters in 2020 and 2021.

Treatments	Weight (g)		Height (mm)		Width (mm)	
	2020	2021	2020	2021	2020	2021
Control	1.39 ± 0.46a [1]	1.63 ± 0.43a	9.91 ± 1.12a	9.98 ± 0.97a	13.30 ± 1.44a	14.29 ± 1.34a
Armurox	1.92 ± 0.35b	2.14 ± 0.37b	10.85 ± 1.13c	10.81 ± 0.80b	15.46 ± 0.97d	15.73 ± 1.09b
BioCal	1.89 ± 0.45b	2.19 ± 0.43b	10.80 ± 0.85c	11.17 ± 0.75c	15.19 ± 1.38c	15.92 ± 1.28b
Stymjod	1.91 ± 0.39b	2.14 ± 0.29b	10.58 ± 0.86b	11.32 ± 0.62c	14.94 ± 1.23b	15.75 ± 0.94b

[1] The mean values marked with the same letters did not differ significantly at a = 0.05.

In both years of the study, the bushes sprayed with foliar fertilizers produced fruit with significantly higher weight and biometric parameters (i.e., height and width) compared to the bushes without foliar fertilization. All three foliar fertilizers were equally effective in this respect. The effectiveness of foliar fertilization with calcium-containing fertilizers in increasing highbush blueberry fruit weight was also reported by Ochmian et al. [29].

With the growing health consciousness of the public, consumer attention is increasingly focused on the nutritional quality of foodstuffs. Fruit firmness is considered one of the most important quality characteristics, determining, among other things, its commercial value. The resistance of fruit to mechanical damage largely depends on the condition of cell membranes. Silicon and calcium play a leading role in their stabilization. The latter determines the suitability of fruit for transport and storage by stabilizing pectins that form the central lamina of the cell walls. As Ochmian and Kozos [29] pointed out, an increase in the calcium content of fruit increases its firmness. The blueberry fruit firmness shown in the experiment ranged from 176.0 g mm^{-1} in 2020 to 293.55 g mm^{-1} a year later (Table 6). In both years of the study, fruits with the lowest firmness were harvested from bushes in the control treatment, i.e., without foliar fertilization. As a result of foliar spraying with each of the three fertilizers, the firmness of blueberry fruit increased significantly. Compared to the control treatment, the difference ranged from approximately 32% in 2020 (Armurox) to almost 50% in 2021 (Stymjod) (Table 6). The effectiveness of BioCal in this respect, despite its significant calcium content, was not the highest. This may have been due to the difficult transport of this element into the cell during foliar spraying [34].

Table 6. Effect of foliar fertilization on blueberry fruit firmness (g mm^{-1}) and TSS content (%) in 2020 and 2021 (TSS—total soluble solids).

Treatments	2020		2021			
	Firmness	TSS	Firmness		TSS	
			H [1]	S [2]	H [1]	S [2]
Control	176.00 ± 30.59a [3]	14.29 ± 2.75a	196.95 ± 21.29a	176.6 ± 25.85a	11.85 ± 1.51a	12.75 ± 1.29a
Armurox	232.53 ± 41.62b	13.20 ± 1.62a	264.20 ± 34.81b	242.3 ± 26.35b	14.43 ± 1.44c	14.46 ± 1.43b
BioCal	242.40 ± 34.66c	13.88 ± 1.35a	285.20 ± 41.92c	241.4 ± 20.13b	13.96 ± 1.73c	13.90 ± 1.47a
Stymjod	245.07 ± 36.42c	14.19 ± 1.71a	293.55 ± 38.02d	249.10 ± 22.21c	14.54 ± 1.94c	16.46 ± 1.54b

[1] H—fruit after harvest. [2] S—fruit after storage. [3] The mean values marked with the same letters did not differ significantly at a = 0.05.

The dessert value of the fruit is determined by its high extract content (TSS) [35]. Similar to firmness, the TSS value in highbush blueberry fruit was the lowest in the control treatment (11.85%) and the highest (14.54%) in the treatment where Stymjod was applied.

This TSS content in fruit was higher than in the experiment of Shevchuk et al. [36] and similar to the results obtained by Zenkov and Pinchykov [37], from 13 to 15.3%, or by Celik et al. [38]—approximately 13.3%. In 2020, there was no variation in extract content in blueberry fruit. The causal factor in this case could have been weather conditions. As Ersoy et al. [39] pointed out, the physicochemical properties of fruit can be affected by factors such as variety, cultivation method, and weather conditions. The TSS content in blueberry fruit varied in 2021. The bushes fertilized with the foliar fertilizer had a higher extract content than the bushes without foliar fertilization. Differences ranged from a few percent (BioCal) to over 20% (Stymjod and Armurox) (Table 6).

Producers not only want to maintain the high quality of their fruit after harvest, but also after storage. The experiment showed that regardless of the treatment, the firmness of highbush blueberry fruit after six days of storage was lower than after harvest. The difference ranged from a few percent for the treatment with the application of Armurox to 17–18% for treatments with BioCal and Stymjod (Table 6). The firmness of stored blueberry fruit from the foliar-fertilized bushes was significantly higher than that of fruit from the bushes to which foliar fertilizers were not applied. The Stymjod fertilizer was the most effective in this respect. The firmness of stored blueberry fruit from this treatment (249.10 g mm^{-1}) was over 40% higher than that of the control (176.6 g mm^{-1}). Differences in the firmness of stored blueberry fruit from bushes to which Armurox and BioCal were applied were approximately 37% (Table 6).

The extract content of highbush blueberry fruit after storage, regardless of the treatment, was higher than after harvest. The effect of foliar fertilizers on the extract content of blueberry fruit after storage was less than on their firmness. The differences ranged from less than one percent in the treatment with Armurox to 13% in the treatment with Stymjod. After application of the latter one, the TSS content in stored highbush blueberry fruit (16.46%) was about 30% higher than in the control treatment (12.75%) (Table 6).

The color of the fruit is an important indicator of its maturity and harvest date [40]. The foliar fertilizers used in the experiment modified blueberry fruit coloration to a small extent. L and b values in post-harvest fruit were not significantly different (Table 7). Blueberry fruits sprayed with Stymjod were the brightest (L = 26.65), while those from the treatment without foliar fertilization were the darkest (L = 28.08). However, statistical analysis did not show the significance of differences between the measurements. Greater changes in fruit coloration occurred after storage. The fruits with the darkest color were those of the control treatment (L 27.76), and those with the lightest color were those of the control treatment (L value 26.38) (Table 7).

Table 7. Highbush blueberry fruit coloration in 2021.

Treatments	After Harvest			After Storage		
	L	a	b	L	a	b
Control	28.08 ± 3.13a [1]	0.86 ± 1.58b	5.15 ± 0.44a	27.76 ± 3.27c	1.29 ± 0.25b	4.79 ± 0.60a
Armurox	27.38 ± 2.73a	0.27 ± 1.36a	4.93 ± 0.61a	27.43 ± 3.56bc	0.87 ± 0.17a	4.16 ± 0.21a
BioCal	28.08 ± 3.22a	1.03 ± 1.66b	5.38 ± 0.53a	26.38 ± 3.11a	1.14 ± 0.13ab	4.68 ± 0.25a
Stymjod	26.65 ± 2.69a	0.12 ± 1.42a	5.42 ± 0.61a	26.65 ± 3.15ab	0.99 ± 0.12ab	4.54 ± 0.34a

[1] The mean values marked with the same letters did not differ significantly at a = 0.05.

3.5. Sugar Content and Acidity of Highbush Blueberry Fruits

Consumers initially make their choice of fruit based on its appearance and are then guided by other sensory factors—taste and sugar content [41]. It is the amount and ratio of soluble sugars and organic acids in the fruit that determines its flavor [42]. Sugars are the basic products of photosynthesis, providing energy for all biochemical processes in the cells [43]. In highbush blueberry fruits, it is the sugars that determine their organoleptic quality [44], in particular the taste [45]. The total sugar content of fruit can depend on

a number of factors: plant species and cultivar, age of the plant, soil properties, climatic conditions, agrotechnical practices, and occurrence of biotic and abiotic stress [46,47]. In the experiment, the total sugar content varied from 12.96 to 15.22 mg 100 g^{-1} depending on the treatments (Table 8). This was more than in the experiment of Aliman et al. [31], from 9.73 to 9.94 mg 100 g^{-1}, and comparable to the results obtained by Kirin et al. [48], from 10.15 to 14.8 mg 100 g^{-1}.

Table 8. Effect of foliar fertilization on the sugar content of highbush blueberry fruits in 2020.

Treatments	Fructose	Glucose	Sucrose	Total Sugar
Control	6.03 ± 1.21b [1]	9.48 ± 1.17b	0.19 ± 0.02a	15.22 ± 1.38b
Armurox	4.89 ± 0.11a	7.92 ± 0.70a	0.18 ± 0.02a	12.96 ± 1.03a
BioCal	5.50 ± 0.61ab	8.83 ± 0.13ab	0.18 ± 0.07a	14.51 ± 0.76b
Stymjod	5.39 ± 0.79ab	8.95 ± 0.86ab	0.19 ± 0.42a	14.53 ± 0.98b

[1] The mean values marked with the same letters did not differ significantly at a = 0.05.

The experiment did not show any significant differentiation in total sugar content in highbush blueberry fruits harvested from bushes fertilized with and without foliar fertilizers. Blueberry bushes sprayed with BioCal and Stymjod yielded fruit with a sugar content comparable to that of the control treatment. Regardless of the treatment, glucose was the most abundant sugar and sucrose the least (Table 8). Glucose is considered the most important sugar found in blueberry fruits [49]. The content of fructose and glucose in fruit harvested from bushes sprayed with these fertilizers was also not significantly different from their content in the control treatment.

In the experiment, the authors detected little variation in the organic acid content of highbush blueberry fruit in different treatments. Foliar fertilization had very little effect on this parameter. The content of ascorbic and citric acids in all treatments was not significantly different. The greatest differences were in the content of malic acid. In the treatment with the foliar application of Stymjod, its amount (1.00 mg 100 g^{-1}) was approximately 30% lower than in the treatment without foliar application (1.42 mg 100 g^{-1}) (Table 9). The differences in malic acid content in fruit from bushes treated with BioCal and Armurox were not significant.

Table 9. Effect of foliar fertilization on organic acids content (mg 100 g^{-1}) of highbush blueberry fruits in 2020.

Treatments	Ascorbic Acid	Citric Acid	Malic Acid
Control	44.32 ± 6.16ab [1]	9.12 ± 1.73a	1.42 ± 0.39b
Armurox	60.12 ± 3.36b	8.75 ± 2.42a	1.08 ± 0.16ab
BioCal	36.58 ± 2.99a	8.38 ± 2.46a	1.06 ± 0.31ab
Stymjod	51.99 ± 3.34b	9.65 ± 2.37a	1.00 ± 0.52a

[1] The mean values marked with the same letters did not differ significantly at a = 0.05.

Ascorbic acid refers to bioactive compounds that act as antioxidants in the human body [38]. Its varying content in blueberry fruit is a varietal characteristic [50]. Fluctuations in ascorbic acid content depending on habitat conditions can range from 6 to 162 mg 100 g^{-1} [32]. The content of ascorbic acid in blueberry fruits in the experiment varied from 35.58 mg 100 g^{-1} (treatment with BioCal fertilizer) to 60.12 mg 100 g^{-1} (treatment with Armurox fertilizer) (Table 9). This was more than in the experiment by Rupasova et al. [51] at 11.8 mg 100 g^{-1} and comparable to the results of Zenkova and Pinchykova's [37] experiment at 60.5–72.2 mg 100 g^{-1}. The content of the other two acids—citric and ascorbic acids—in highbush blueberry fruit did not differ significantly regardless of the treatment (Table 9).

3.6. The Content of Health-Promoting Compounds in Highbush Blueberry Fruit

Phenolic compounds constitute the largest group of antioxidants that inhibit the oxidation reaction and reduce the amount of so-called oxygen free radicals, which reduces the risk of, for example, coronary heart disease [52]. They can be found in numerous plants. Their content in the fruit depends, among other things, on the variety, coloration and degree of ripeness. In the experiment, the total content of phenolic compounds ranged from 1606.18 mg GA 100 g^{-1} d.m. (BioCal treatment) to 1779.63 mg GA 100 g^{-1} d.m. (Armurox treatment) (Table 10). These values are similar to the results obtained by Sadowska et al. [53] at 1768.63 mg GA 100 g^{-1} d.m phenolic compounds in blueberry fruit and 1776.18 mg GA 100 g^{-1} d.m. in chokeberry fruit [54].

Table 10. Effect of foliar fertilization on the content of health-promoting compounds in blueberry fruit in 2020.

Treatments	Total Phenolic Compounds (mg GA 100 g^{-1} d.m.)	Anthocyanins (mg 100 g^{-1} d.m.)
Control	1716.25 ± 103.69a [1]	38.76 ± 3.94b
Armurox	1779.63 ± 139.09a	32.46 ± 1.67a
BioCal	1606.18 ± 220.86a	32.32 ± 1.45a
Stymjod	1631.84 ± 190.88a	32.93 ± 1.94a

[1] The mean values marked with the same letters did not differ significantly at a = 0.05.

The authors of the experiment did not detect any significant differentiation in the total content of phenolic compounds in the fruit of highbush blueberry from bushes fertilized with and without foliar nutrition. According to Ochmian et al. [30], increasing the doses of foliar fertilizers can even lead to a reduction in their content in blueberry fruit. The anthocyanin content of blueberry fruit depends on variety, size of the fruit, degree of ripeness, and climatic conditions and can vary from 25 to 490 mg 100 g^{-1} d.m. [55]. In the experiment, the anthocyanin content of highbush blueberry fruit ranged from 32.32 to 38.76 mg 100^{-1} d.m. (Table 10). These results were higher than in Shevchuck et al.'s [36] experiment, which were from 35 to 68 mg 100^{-1} d.m. in blueberry fruit, and much lower than in cranberry fruit at 370 mg 100^{-1} d.m. [52]. In the experiment, the authors did not show any significant effect of foliar fertilization on anthocyanin content in highbush blueberry fruit. In fruit from bushes in treatments with such fertilization, the anthocyanin content was lower than from bushes in the control (Table 10).

3.7. Impact of Harvesting Dates on the Tested Parameters of Highbush Blueberry

Fruit harvest dates, season, temperature differences, and amount of precipitation have an impact on quality features of blueberry fruit, including its firmness, weight, sugar and acid content [56,57]. The experiment showed such significant effect of fruit harvesting dates on a number of the tested parameters. This concerns, among other things, the yield of highbush blueberries. The highest yield, both in the first and second years of the experiment, was harvested on the second date, in early August (1.08 and 1.67 kg per bush, respectively), and the lowest on the third date, in mid-August (0.45 and 1.02 kg per bush) (Figure 3).

The biometric parameters of highbush blueberry fruit also differed significantly depending on the harvest date. Regardless of the study year, fruits with significantly higher size parameters were collected from blueberry bushes at the beginning of the harvest than at the other dates. This applied to the average weight as well as the height and width of the fruit (Table 11). In the last third of the harvesting season, especially in 2020, the fruit with the lowest weight and the smallest height and width were collected from the blueberry bushes. In 2020, the amount of precipitation was much lower than a year later, and as Zorenc et al. [33] showed, blueberry fruit weight increases with an increase in rainfall.

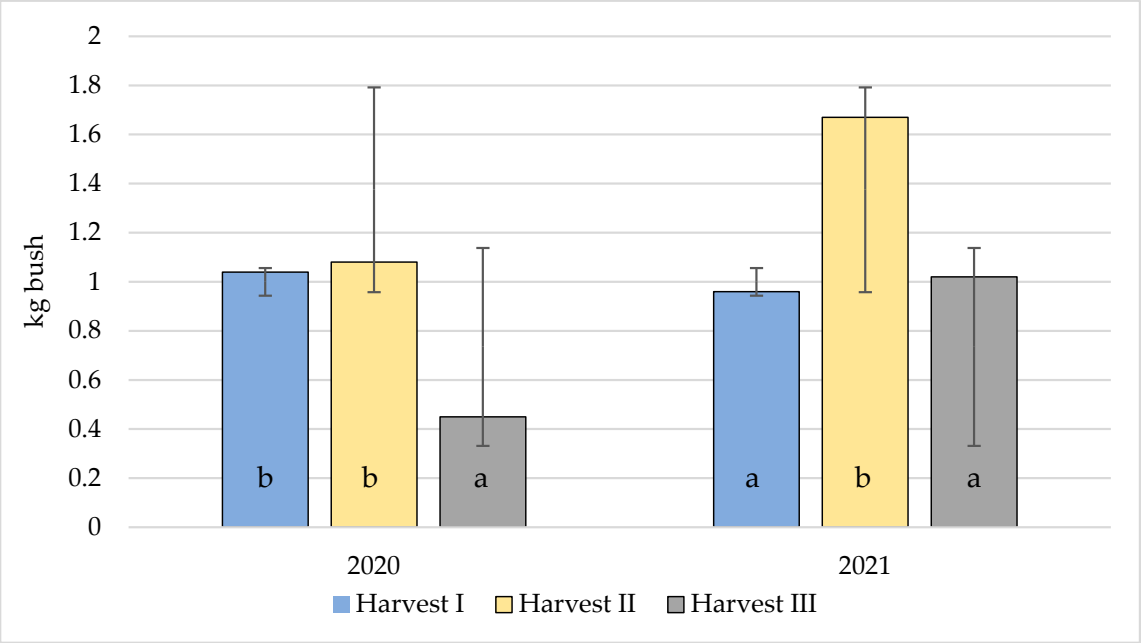


Figure 3. Impact of harvest time on the yielding of highbush blueberry bushes (kg bush) in 2020 and 2021 (harvest I—end of July; harvest II—beginning of August; harvest III—mid-August). The mean values marked with the same letters did not differ significantly at a = 0.05.

Table 11. Impact of harvest time on highbush blueberry fruit weight and their biometric parameters in 2020 and 2021.

Harvest Dates	Weight (g)		Height (mm)		Width (mm)	
	2020	2021	2020	2021	2020	2021
End of July (I)	2.11 ± 0.37c [1]	2.09 ± 0.31c	11.27 ± 0.62b	11.49 ± 0.65c	15.57 ± 1.03c	15.73 ± 1.05c
Beginning of August (II)	1.69 ± 0.30b	1.74 ± 0.29a	10.20 ± 0.47a	10.47 ± 0.67b	14.50 ± 1.02b	16.29 ± 0.98a
Mid-August (III)	1,54 ± 0.29a	1.88 ± 0.30b	1013 ± 0.82a	10.09 ± 0.68a	14.16 ± 1.08a	15.51 ± 15.51a

[1] The mean values marked with the same letters did not differ significantly at a = 0.05.

Another characteristic showing visible variation depending on the harvest dates was the quality of the fruit. In this case, there was not as much repeatability as in the analysis of yielding and fruit size. This may have been due to the mentioned variation in weather conditions occurring in the two years of the experiment (Table 1). In 2020, at the beginning of August (second harvest date), blueberry fruits were the least firm (211.8 g mm^{-1}) (Table 12). In the following research year, the firmness of fruit harvested at this date was highest (281.45 g mm^{-1}) and lowest at the end of the harvest. Greater reproducibility in both years of the study was shown with TSS content analysis in highbush blueberry fruit. In both years of the study, the TSS value was the highest (14.73 and 13.77%) in blueberry fruits harvested in early August (second term).

Table 12. Impact of harvest time on several quality parameters of highbush blueberry fruit in 2020 and 2021.

Harvest Dates	Firmness (g mm^{-1})		TSS (%)	
	2020	2021	2020	2021
End of July (I)	223.26 ± 37.67b [1]	286.30 ± 25.50b	12.81 ± 1.07a	12.65 ± 0.96a
Beginning of August (II)	211.80 ± 31.51a	281.45 ± 40.61c	14.73 ± 1.44b	13.77 ± 1.36c
Mid-August (III)	227.55 ± 25.02b	225.50 ± 27.05a	14.15 ± 1.37ab	13.17 ± 1.44b

[1] The mean values marked with the same letters did not differ significantly at a = 0.05.

In fruits harvested on the first date (end of July), the total sugar content (15.08 mg 100^{-1}) was the highest, and on the last date, mid-August, it was the lowest (13.45 mg 100^{-1}) (Table 13). The same can be said for the fructose and glucose content of fruit. The highest content of these sugars was found in fruit harvested on the first date (glucose) or the first and second dates (fructose). The content of organic acids in highbush blueberry fruit varied considerably depending on the harvest date. The contents of ascorbic (68.12 mg 100 g^{-1}), citric (12.47 mg 100 g^{-1}), and malic (1.47 mg 100 g^{-1}) acids in fruits from the third harvest were significantly higher than those from the first harvest (Table 13). These results are different from those obtained by Zorenc et al. [33]. In their experiment, at the end of the vegetation period the sugar content in highbush blueberry fruits was the highest, and the acid content-the lowest. The highest total content of phenolic compounds and anthocyanins (1762.71 mg GA 100 g^{-1} and 37.32 mg 100 g^{-1}, respectively), was detected in fruits harvested on the last date–mid-August, and the lowest (1535.89 mg GA 100 g^{-1} and 30.98 mg 100 g^{-1}) on the second date (Table 13).

Table 13. Impact of harvest time on the content of sugar, organic acids, and health-promoting compounds in blueberry fruit (harvest I—end of July; harvest II—beginning of August; harvest III—mid-August).

Studied Characteristic	Harvest I	Harvest II	Harvest III
Sugar content (mg 100 g^{-1})			
Fructose	5.97 ± 1.17b [1]	5.64 ± 0.32b	4.74 ± 0.58a
Glucose	9.29 ± 1.16b	8.57 ± 0.33a	8.53 ± 0.74a
Sucrose	0.19 ± 0.03ab	0.17 ± 0.03a	0.20 ± 0.06b
Total sugar	15.08 ± 1.41c	14.38 ± 1.01b	13.45 ± 1.33a
Organic acids (mg 100 g^{-1})			
Ascorbic acid	28.86 ± 7.51a	40.29 ± 26.92b	68.12 ± 27.57c
Citric acid	8.26 ± 1.33b	6.12 ± 1.69a	12.47 ± 2.06c
Malic acid	1.01 ± 0.42a	0.94 ± 0.29a	1.47 ± 0.29b
Health-promoting compounds			
Phenolic compounds	1751.81 ± 92.77b	1535.89 ± 199.21a	1762.71 ± 134.34c
Anthocyanins	34.06 ± 1.28b	30.98 ± 3.99a	37.32 ± 3.22c

[1] The mean values marked with the same letters did not differ significantly at a = 0.05.

4. Conclusions

In the experiment, the authors obtained confirmation of the previous assumption of a positive effect of foliar fertilization of highbush blueberry bushes on its vegetative growth, yield, and fruit quality. In treatments with the application of foliar fertilizers, the leaf area of blueberries increased significantly compared to unfertilized bushes. Fruit yields also increased by several tens of percent. The effect of foliar fertilizers on yielding, especially BioCal and Armurox, was most evident in the first year of their application, under unfavorable climatic conditions for blueberry cultivation (water deficiencies). The blueberry bushes that were foliar fertilized yielded fruit with better quality parameters than those not fertilized. This applies to average fruit weight, firmness and extract content (TSS). Foliar fertilizers also improved blueberry fruit quality after storage. Stored fruit from bushes treated with foliar fertilizers had a higher extract content and kept their firmness longer, compared to bushes without foliar fertilization.

The authors showed no significant effect of foliar fertilization on the contents of chlorophyll a and b in the leaves of highbush blueberry or on its fruit coloration, total sugar content, and ascorbic and citric acids. There was also no significant effect of the tested foliar fertilizers on the content of health-promoting compounds in highbush blueberry fruit. Yielding of highbush blueberry bushes, biometric parameters of fruit, their quality

characteristics, and content of health-promoting substances significantly differed depending on the harvest date. The highest yields of blueberry fruit were harvested in early August (second term). The fruits with the highest weight and biometric parameters (i.e., height and width) were harvested at the beginning of the harvest, at the end of July. Fruits harvested on this date also had the highest total sugar content. On the other hand, in blueberry fruits from the last harvest date (mid-August), the content of all tested organic acids was the highest. The obtained results of research about foliar fertilization of blueberries can be used to increase its yield and improve fruit quality also after their storage.

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