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Pot and Ridge Production of Three Highbush Blueberry (*Vaccinium corymbosum* L.) Cultivars under High Tunnels

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Abstract: In recent years, new approaches to intensive blueberry (*Vaccinium corymbosum* L.) production have become necessary, in terms of protected environments and planting systems. These are designed to avoid numerous production difficulties, such as market saturation, damage from hailstorms, bird attacks, and spring frosts, and specific soil property requirements. Use of high tunnels and planting in a custom substrate (e.g., pots, along ridges) have gained interest among growers in recent years. As in our previous study, we determined the performance of three blueberry cultivars, 'Duke', 'Aurora', and 'Brigitta', when planted in pots and along a ridge under a high tunnel. Substrate water content was maintained at the same level for the pots and the ridge, although the substrate temperature fluctuations were greater for pots. Plant growth in pots was significantly lower for 'Duke' and 'Aurora' compared to the ridge. Additionally, for 'Aurora', the fruit yield was significantly lower for pots (103.4 g/plant), compared to the ridge (315.2 g/plant), although the opposite was seen for 'Brigitta' (122.4 vs. 93.5 g/plant, respectively). Individual sugar and organic acid contents mostly coincided with total contents, with lower total sugars for 'Duke' and higher total organic acids for 'Aurora' and 'Brigitta' for pots. For 'Duke' and 'Brigitta' fruit, the contents of some individual phenolics showed significant differences between treatments for phenolic acids and flavonols. These data show that growth in pots can be a useful planting method for the blueberry cultivars 'Duke' and 'Brigitta', and high yields and good fruit quality can be attained by following correct technological measures.

Keywords: blueberry; substrate water content; substrate temperature; plant volume; yield; fruit quality

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1. Introduction

Blueberries can be found on the market all year round due to importation from other countries. However, the harvesting periods of individual areas and countries often overlap, which can lead to saturation of the market and consequently a lower price for the fruit [1]. Earlier fruit ripening and availability on the market can instead provide higher marketable prices, which can be achieved by production under protected environments, such as high tunnels [2]. The increased air temperature maintained under high tunnels provides earlier appearance of the individual phenophases, and thus ripening. High tunnels also modify the relative humidity and light quantity and quality [2].

High tunnel introduction into intensive blueberry production can extend the growing season of the plants, provide higher yields, and offer protection against birds, rain, hail, and frost damage, while also allowing production to expand towards cooler zones of the world [3]. Despite the advantages, high tunnel production has certain flaws, particularly as the maximum air temperature in the summer can rise above 40 °C, causing heat stress for the plants. This can lead to the inhibition of photosynthesis [4], damage to cell membranes, and cell senescence and death [5]. At the same time, additional irrigation is required as natural precipitation cannot reach the plants.

To achieve optimal growth and yield, blueberry plants have specific requirements for soil properties. An acidic substrate with pH 4.5 to 5.5 must be achieved for successful blueberry production. Too high a pH can lead to limited plant growth and yield, and limited intake of individual nutrients, such as iron, manganese, and copper [1,6]. At the same time, organic matter of approximately 7–10% and adequate drainage properties must be provided [7]. All these requirements can be difficult to maintain for growers, due to the great diversity in soil properties. Therefore, increased interest in alternative planting methods in custom substrates has been seen in recent years. Planting along a ridge is one alternative, as is planting in pots. Planting in pots represents an alternative to soil production, where the substrate properties can be sustained through selected substrate composition and controlled fertilization and irrigation [8]. The plants can also be moved around easily, allowing for adjustments to planting density based on plant volume [7]. Pots are also suitable for areas with inappropriate soil properties, such as high salinity, the presence of toxic compounds, and soil pests [9,10]. The great disadvantage of the pot production method is the limited growth of roots, and consequently the lower absorption area for water and nutrients, due to limited substrate capacity. Questions about long-term production and the possibly shorter life for plants in pots also arises [7]. Pots also represent an additional cost [11].

Nowadays, growers in intensive blueberry production face many limitations and difficulties, such as market saturation, low fruit prices, damage from hailstorms and bird attacks, and maintenance of substrate properties at optimal levels. Therefore, interest in alternative planting and production methods has emerged. The aim of this study was to define which planting method (i.e., pots vs. ridge) is best suited for three different blueberry cultivars regarding ripening time, i.e., 'Duke', 'Aurora', and 'Brigitta', under high tunnels.

2. Materials and Methods

2.1. Experimental Setup and Plant Material

The experiment took place in 2020 in a laboratory field of the Biotechnical Faculty of the University of Ljubljana (Slovenia; latitude, 46°05′ N; longitude, 14°47′ E; altitude, 295 m a.s.l.). Uniform, one-year old blueberry plants of 'Duke', 'Aurora', and 'Brigitta' cultivars were selected and planted in 60-L black pots along a ridge, with both under a high tunnel, in May 2018 (two treatments per cultivar). 'Duke' is an early-ripening cultivar (beginning of June), with moderate vigor. 'Aurora' is a late-ripening cultivar (August) with a vigorous bush. 'Brigitta' is a late-mid-season ripening cultivar (July), with an upright bush. The planting distance was 0.6 m along each row and 2 m between each row. The substrate was a mixture of soil, peat, and pine sawdust (one-third each, $v/v/v$). White polypropylene foil was placed over the ground to prevent soil overheating, weed growth, and increased light reflection from the ground. A drip irrigation system was installed, with four drippers for each plant and a flow of 900 mL h^{-1}. The plants were fertigated with mineral fertilizers through the irrigation system nine times through the growing season (end of March to mid-June). The high tunnel (Schwarzmann, Polhov Gradec, Slovenia) was 6 m wide and 24 m long, covered with white polypropylene foil, positioned with a south-to-north orientation, naturally ventilated, and equipped with air temperature sensors.

2.2. Monitoring of Substrate Properties

Substrate water content and temperature were monitored with continuous measurements, using soil moisture sensors (EC5; Decagon Devices, Pullman, WA, USA) and soil temperature sensors (DS18b20; Dallas Semiconductor, Dallas, TX, USA). The data were automatically sent to the Internet of Things cloud, thus allowing for constant access to and control of substrate water content and temperature measurements and conditions.

2.3. Plant Growth and Berry Harvest

Plant growth was calculated as the difference in plant size measurements between spring (16 March 2020) and autumn (9 October 2020). Plant heights and two diameters (row

direction, perpendicular to row) were determined with a measuring tape. Plant volumes were calculated according to Equation (1):

$$V\ (dm^3) = (\text{height (cm)} \times \frac{1}{2}\ \text{width 1 (cm)} \times \frac{1}{2}\ \text{width 2 (cm)} \times \pi)/1000. \quad (1)$$

Fruits were harvested five to eight times for each cultivar and planting method, starting on 1 June 2020. At each harvest, the number of fruits per plant was counted and the total yield per plant was weighed in the laboratory using a precision balance. The mean single fruit weight was calculated by dividing the total plant yield by the number of fruits per plant. At the same time, fruit color and firmness were measured. The fruits were then frozen in liquid nitrogen and stored in a freezer at −20 °C.

2.4. Fruit Color and Firmness Measurements

For fruit color and firmness, 15 randomly chosen berries from the harvest with the highest yield under each treatment were selected for the measurements. Fruit color was measured once for each (portable colorimeter, CR-10 Chroma; Konica Minolta, Tokyo, Japan). The parameters used to describe color were L* (lightness), C (chroma), and h° (hue angel) (CIELAB; Commission Internationale d'Eclairageusing, Vienna, Austria). L* is measured from 0 to 100, where 0 represents black and 100 represents white. For C*, higher values represent more intense colors. The h° characterizes the color, expressed in degrees, from 0° to 360° (0°, red; 90°, yellow; 180°, green; 270°, blue). Fruit firmness was measured once on each (digital penetrometer, TR; Turin, Italy) with a 1-mm-diameter tip.

2.5. Primary Metabolite Extraction and Identification

For sugar and organic acid extraction, the method previously described by Mikulic-Petkovsek et al. [12] was used, with some modifications. Extractions for each cultivar and planting method were carried out as five replicates. The thawed berries were finely chopped with a knife, to form a puree. Then, 1 g was added to 4 mL bi-distilled water, mixed by vortexing, and left at room temperature for 30 min with constant stirring (Unimax 1010 shaker; Heidolph, Schwabach, Germany) for extraction of the primary metabolites. After the extraction, the samples were centrifuged at 9000× *g* for 10 min at 4 °C (5810 R; Eppendorf, Hamburg, Germany), then filtered (0.2-µm cellulose filters; Chromafil A-20/25; Macherey-Nagel, Düren, Germany) into vials and stored at −20 °C until analysis.

Individual sugars were analyzed by HPLC (Vanquish; Thermo Fisher Scientific, Waltham, MA, USA) connected to a refractive index plus detector (ReafractoMax520; Thermo Fisher Scientific) [13]. Their separation was achieved using a Rezex column (RCM-monosaccaride Ca+ 2%; 300 mm × 7.8 mm; Phenomenex, Torans, CA, USA), at 65 °C. The mobile phase was bi-distilled water, with a flow rate of 0.6 mL min^{-1}. Each injected sample (20 µL) was analyzed over 30 min. Individual sugars were determined by comparing retention times with external standards for fructose, glucose, and sucrose (Fluka Chemie GmBH, Buch, Switzerland). They were expressed as mg g^{-1} fresh weight (FW).

Organic acids separation was performed by HPLC (Vanquish; Thermo Fisher Scientific) on a Rezex column (ROA-Organic acid H+ 8%; 150 mm × 7.8 mm; Phenomenex), at 65 °C, with detection using a UV detector, at 210 nm. Each injected sample (20 µL) was analyzed over 15 min. The mobile phase used was 4 mM sulfuric acid in bi-distilled water, with a flow rate of 0.6 mL min^{-1}. The organic acids were identified using external standards for citric, tartaric, malic, and shikimic acids, and by comparisons of retention times, and are expressed as mg g^{-1} FW.

2.6. Individual Phenolics Extraction and Identification

The individual phenolics were extracted from the same samples as the primary metabolites, as five replicates, according to the method of Mikulic-Petkovsek et al. [14]. Here 2 g of the finely chopped fruit was added to 4 mL extraction solution, as 70% methanol, 3% formic acid in bi-distilled water. The samples were mixed by vortexing, left in a cooled ultrasonic

bath (0 °C) for 1 h, and then centrifuged at 9000× *g* for 10 min at 4 °C (5810 R; Eppendorf, Hamburg, Germany). The supernatants were filtered (0.2 µm; Chromafil AO-20/25 polyamide filters; Macherey-Nagel, Düren, Germany) into vials and stored at −20 °C.

The phenolics were analyzed by HPLC (Dionex UltiMate 3000; Thermo Fisher Scientific), with detection by absorbance at 280 nm, 350 nm, and 530 nm. The phenolics were separated on the column (Gemini C18; 150 mm × 7.8 mm, 3 µm; Phenomenex), at 25 °C. Each sample was injected (20 µL) under a flow rate of 0.6 mL min^{-1}, and the autosampler temperature was held at 10 °C. Two mobile phases were used: solvent A, 3% acetonitrile, 0.1% formic acid in bi-distilled water (*v*/*v*/*v*); solvent B, 3% bi-distilled water, 0.1% formic acid in acetonitrile (*v*/*v*/*v*). The discontinuous mobile phase gradient used was: 0–15 min, 5% B; 15–20 min, 5–20% B; 20–30 min, 20–30% B; 30–35 min, 30–90% B; 35–45 min, 90–100% B; 45–50 min, 100–5% B.

Identification of the phenolics was performed by a comparison of retention times with external standards and using linear ion trap mass spectrometry (LTQ XL; Thermo Fisher Scientific) with electrospray ionization, based on mass fragmentation patterns. The mass spectrometry operated in negative or positive ion mode, depending on the compound of interest. The analysis conditions were: flow rate, 0.6 mL min^{-1}; injected sample volume, 10 µL; capillary temperature, 250 °C; sheath gas, 20 units; auxiliary gas, 8 units; source voltage, 4 kV; and scanning from *m*/*z* 115 to 1600. The phenolic contents were calculated from standard curves from corresponding external standards or similar compounds, and are expressed as mg (100 g)$^{-1}$ FW.

2.7. Statistics

Statistical analysis of the data was performed using R commander i386 3.5.2. Significant differences in the data were estimated using *t*-tests and one-way analysis of variance (ANOVA), with least significant difference (LSD) tests, and with significance set to $p < 0.05$.

3. Results

3.1. Substrate Properties

The substrate water contents are shown in Figure 1. In general, similar water distributions were seen throughout the growing season for pots and the ridge, with minor increases at the beginning of May 2020, by approximately 30 mV. From then on, only minor fluctuations in water contents were noted for both treatments.

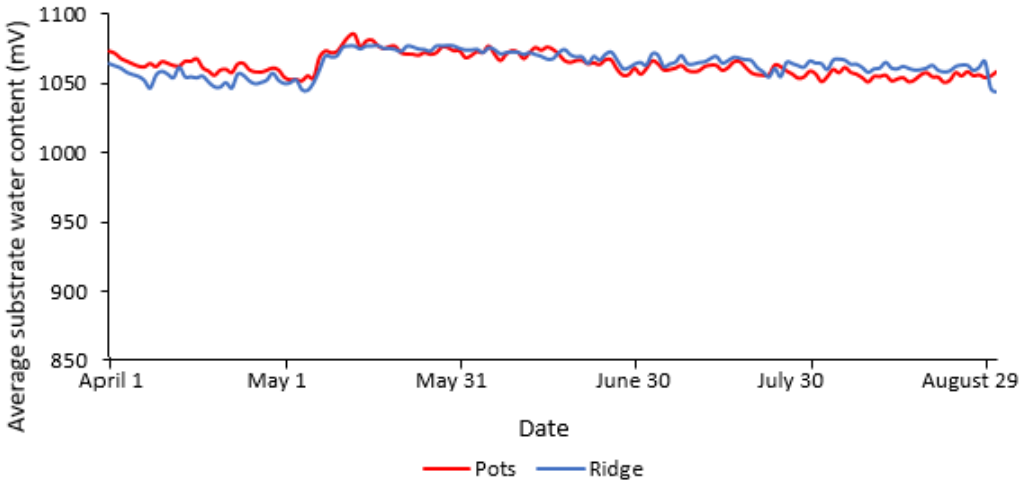

Figure 1. Substrate water content (mV) for the pots and ridge from 1 April 2020 to 31 August 2020.

Figure 2 shows the maximum air and substrate temperatures from the beginning of April 2020 to the end of August 2020. Differences were seen between the pot and ridge treatments, with consistently higher values for pots. The highest substrate temperature for pots was 36 °C and that for the ridge was 28 °C, while the highest air temperature was

46 °C. At the same time, higher temperature fluctuations were noted in the substrate for pots compared to that for the ridge.

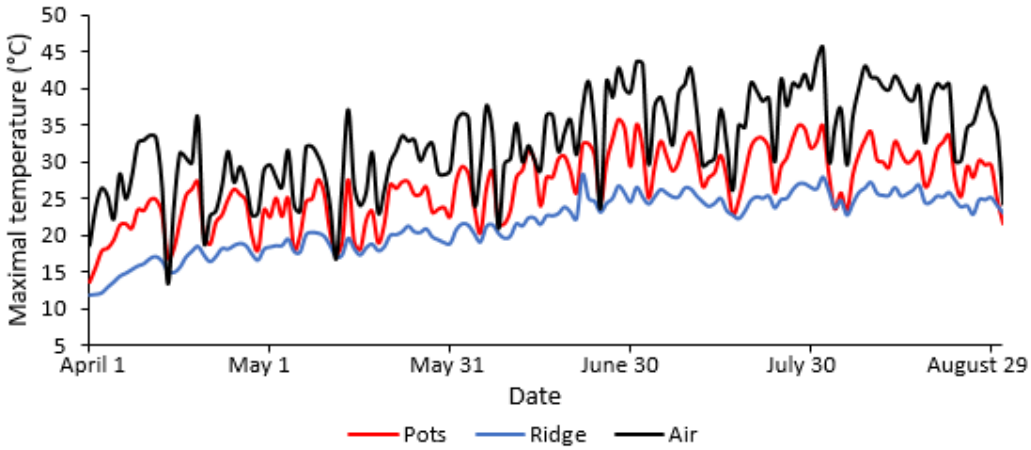

Figure 2. Maximum air temperatures and substrate temperatures for the pots and ridge (as indicated) from 1 April 2020 to 31 August 2020.

3.2. Plant Growth and Yield

'Duke' and 'Aurora' plants grown in pots showed significantly lower growth than those grown along the ridge (Figure 3). For 'Brigitta', no significant differences were seen. 'Duke' showed the highest growth for both pots and the ridge, with 'Aurora' and 'Brigitta' was similarly low for pots, and 'Brigitta' the lowest for the ridge.

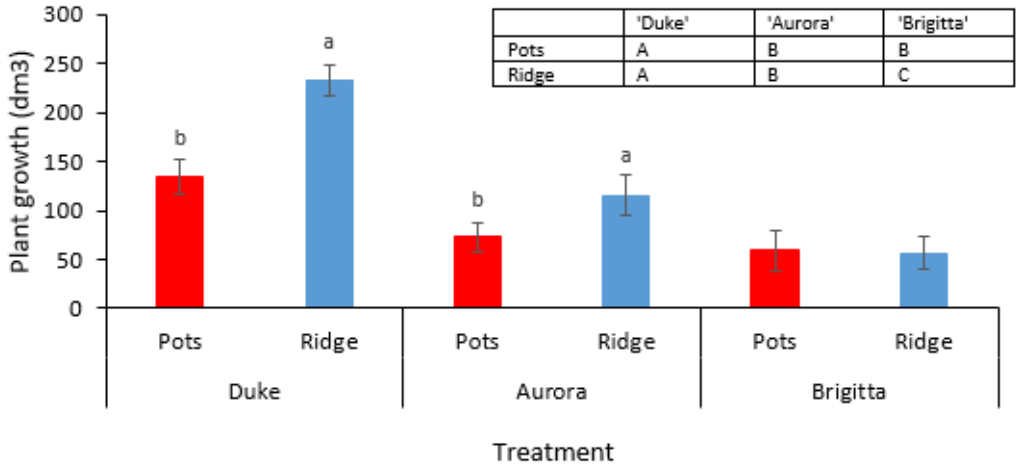

Figure 3. Plant growth for the 'Duke', 'Aurora', and 'Brigitta' blueberry cultivars grown in pots and along the ridge. Different lowercase letters indicate statistically significant differences between planting methods within cultivars (*t*-tests, $\alpha < 0.05$), and different uppercase letters (inset) between cultivars within planting methods (LSD tests, $\alpha < 0.05$).

Different responses to the changed growth conditions were noted for the yields between the cultivars (Figure 4). 'Duke' showed no significant differences between pots and the ridge, while 'Aurora' and 'Brigitta' had significantly lower yields for pots and the ridge, respectively. Taking pots and the ridge separately, a similar trend was seen for yield to that for plant growth, with 'Duke' showing the highest yield across the cultivars for both pots and the ridge, with the lowest yield for pots with 'Aurora' and for the ridge with 'Brigitta'.

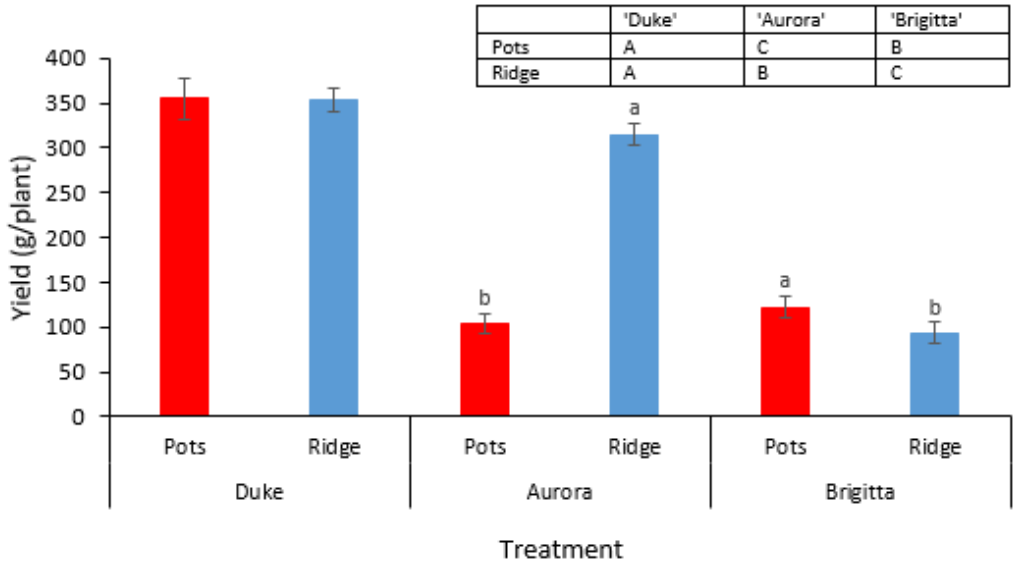

Figure 4. Fruit yield for the 'Duke', 'Aurora', and 'Brigitta' blueberry cultivars grown in pots and along the ridge. Different lowercase letters indicate statistically significant differences between planting methods within cultivars (*t*-tests, $\alpha < 0.05$), and different uppercase letters (inset) between cultivars within planting methods (LSD tests, $\alpha < 0.05$).

3.3. Blueberry Skin Color and Fruit Firmness

Across all three of these blueberry cultivars, there were no significant differences for skin color and fruit firmness (Table 1). Significant differences were only seen for h° between cultivars along the ridge, and for fruit firmness between cultivars in pots.

Table 1. Blueberry skin color and fruit firmness for blueberry 'Duke', 'Aurora', and 'Brigitta' fruit harvested from plants grown in pots and along the ridge.

Cultivar	Planting	Skin Color			Firmness
	Method	L*	C*	h°	(N)
'Duke'	Pots	31.37 ± 1.97	3.33 ± 0.73	253.00 ± 1.99	0.21 ± 0.05 B
	Ridge	32.25 ± 2.49	3.50 ± 0.70	252.20 ± 1.08 B	0.21 ± 0.03
	Significance	NS	NS	NS	NS
'Aurora'	Pots	32.20 ± 2.76	2.76 ± 1.31	259.60 ± 12.02	0.22 ± 0.03 B
	Ridge	30.70 ± 2.02	2.61 ± 1.01	256.60 ± 4.49 B	0.20 ± 0.05
	Significance	NS	NS	NS	NS
'Brigitta'	Pots	31.02 ± 1.97	3.03 ± 0.62	259.80 ± 6.05	0.16 ± 0.06 A
	Ridge	29.95 ± 2.38	3.08 ± 0.97	265.00 ± 10.35 A	0.20 ± 0.07
	Significance	NS	NS	NS	NS
Significance	Pots	NS	NS	NS	*
Significance	Ridge	NS	NS	***	NS

Data are the mean ± standard error of 15 replicates. Different uppercase letters indicate statistically significant differences between cultivars within planting method (LSD tests, $\alpha < 0.05$). * $p < 0.05$; *** $p < 0.001$; NS, not significant.

3.4. Total and Individual Sugar Contents

The total sugar contents in the blueberries are shown in Table 2. The 'Duke' and 'Aurora' fruit had significantly higher total sugar contents when harvested from plants along the ridge, while for 'Brigitta', this was significantly higher when harvested from the pots. For pots in particular, the highest total sugar contents were seen for the fruit of 'Brigitta', and for the ridge, for the fruit of 'Aurora'.

Table 2. Total sugar and organic acid contents and sugar/organic acid ratios for 'Duke', 'Aurora', and 'Brigitta' blueberry fruit harvested from plants grown in pots and along the ridge.

Cultivar	Planting Method	Total Sugar Content (mg g^{-1} FW)	Total Organic Acid Content (mg g^{-1} FW)	Sugar/Organic Acid Ratio
'Duke'	Pots	52.07 ± 2.00 C	8.74 ± 0.91 C	6.00 ± 0.62 A
	Ridge	57.25 ± 1.51 C	8.48 ± 0.54 B	6.78 ± 0.62 B
	Significance	**	NS	NS
'Aurora'	Pots	61.05 ± 1.79 B	16.87 ± 0.57 A	3.62 ± 0.09 B
	Ridge	67.20 ± 1.90 A	15.75 ± 0.79 A	4.27 ± 0.09 C
	Significance	***	*	***
'Brigitta'	Pots	73.52 ± 1.77 A	11.46 ± 0.53 B	6.42 ± 0.34 A
	Ridge	63.98 ± 1.19 B	8.62 ± 0.53 B	7.45 ± 0.49 A
	Significance	***	***	**
Significance	Pots	***	***	***
Significance	Ridge	***	***	***

Data are the mean ± standard error of five replicates. Different uppercase letters indicate statistically significant differences between cultivars within planting method (LSD tests, $\alpha < 0.05$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, not significant. FW, fresh weight.

Figure 5 shows the individual sugar contents in the blueberry fruit according to the cultivars and planting methods. For 'Duke' and 'Aurora', the sucrose contents did not differ between pots and the ridge, while significantly higher contents of glucose and fructose were seen for the fruits from the ridge. 'Brigitta' berries harvested from plants in pots contained the highest contents of all three sugars. For pot production, no particular trends were seen for the individual sugar contents across the cultivars. The highest sucrose content was seen for the 'Aurora' fruit, while the glucose and fructose contents were highest for the 'Brigitta' fruit. These were similar for the pot and ridge production technologies.

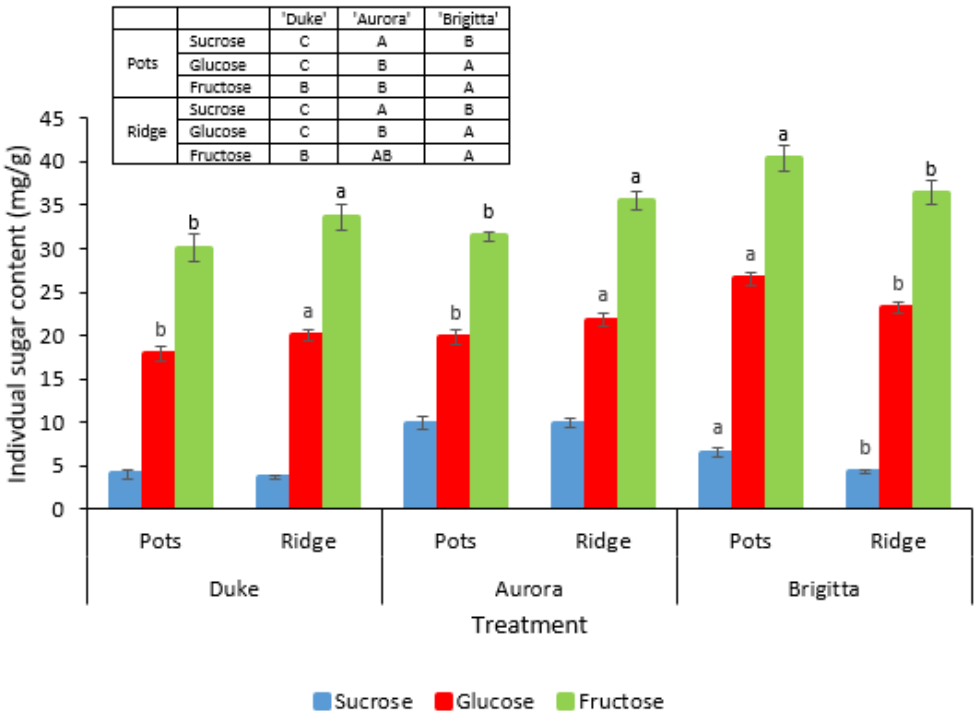

Figure 5. Sucrose, glucose, and fructose contents for the 'Duke', 'Aurora', and 'Brigitta' blueberries harvested from plants grown in pots and along the ridge. Different lowercase letters indicate statistically significant differences for each sugar between planting methods within cultivars (*t*-tests, $\alpha < 0.05$), and different uppercase letters (inset) for each sugar between cultivars within planting methods (LSD tests, $\alpha < 0.05$).

3.5. Total and Individual Organic Acid Contents

The total organic acid content in the 'Duke' fruit was not affected by the different growth conditions (Table 2). On the contrary, growth in pots significantly increased the organic acid contents in the fruit of the other two cultivars. When comparing cultivars within individual planting methods, 'Aurora' showed the highest total organic acid contents for pots and the ridge. The lowest total organic acid content for pots was seen for 'Duke', while for the ridge, this was seen for 'Duke' and 'Brigitta'.

For the individual organic acids, citric acid predominated, while the others represented only minor components of the total organic acids (Figure 6). The fruit of 'Duke' did not differ between the treatments for the citric and shikimic acids, while for tartaric and malic acids, minor, although significant, differences were seen in favor of the ridge and pots, respectively. The same was seen for the 'Aurora' fruit, with the exception of tartaric acid, which was higher in the fruit from pots. The opposite was seen for the 'Brigitta' fruit, where significant differences between treatments were detected for all four of these organic acids, with the highest contents in the fruit from pots, with the exception of shikimic acid. Of the cultivars in pots, 'Aurora' had the highest contents of citric, tartaric, and malic acids. For the ridge production, citric and malic acids were the highest in 'Aurora' again, with the lowest in 'Duke' and 'Brigitta', respectively. Across all of the cultivars and production conditions, the 'Duke' fruit contained the most tartaric and shikimic acids.

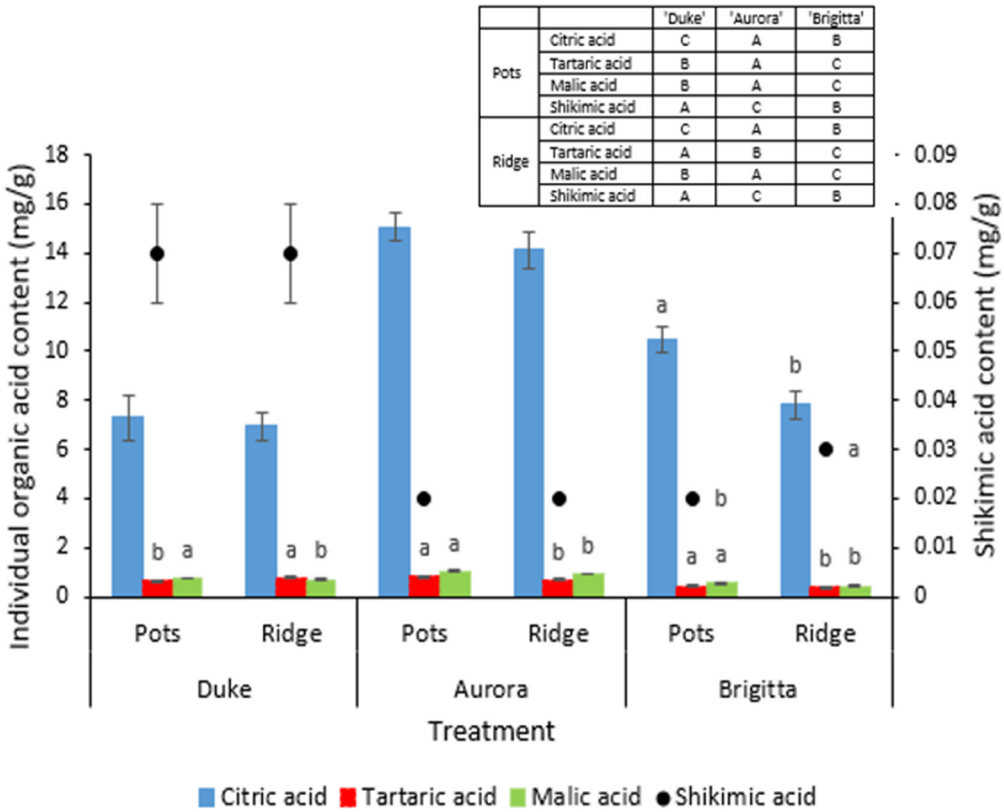

Figure 6. Citric, tartaric, malic, and shikimic acids contents for the blueberry 'Duke', 'Aurora', and 'Brigitta' fruit harvested from plants grown in pots and along the ridge. Different lowercase letters indicate statistically significant differences for each organic acid between planting methods within cultivars (*t*-tests, $\alpha < 0.05$), and different uppercase letters (inset) for each organic acid between cultivars within planting method (LSD tests, $\alpha < 0.05$).

3.6. Sugar/Organic Acid Ratios

The total sugar/organic acid ratios are given in Table 3. For the 'Duke' fruit, no significant differences were seen between pots and the ridge. On the contrary, 'Aurora' and 'Brigitta' fruit harvested from pots showed significantly lower sugar/organic acid

ratios compared to the ridge. For growth in pots, the 'Duke' and 'Brigitta' fruit showed the highest sugar/organic acid ratios, while for the ridge, the highest sugar/organic ratio was seen for the 'Brigitta' fruit. For both pots and the ridge, the lowest ratios were seen for the 'Aurora' fruit.

3.7. Phenolic Contents

The contents of the individual phenolics identified in these blueberry fruit cultivars are given in Table 2. For 'Duke', the total phenolics content did not differ between pots and the ridge. Significant differences between the planting methods were seen for the phenolic acids and flavonol groups, with higher values in the fruit from the ridge. For the phenolic acids, most of the compounds contributed to this result, and although 3-caffeoylquinic acid and the ellagic acid derivative had the highest contents among the phenolic acids, no significant differences were seen between the treatments. For the flavonol group, most of the compounds showed significant differences in favor of the ridge; however, the isorhamnetin-3-*O*-galactoside content, which represented almost half of the flavonols content, did not significantly differ between the treatments. Despite showing significant differences, the procyanidin B2 and epicatechin contents (flavan-3-ols) did not affect the total flavan-3-ols content; no significant differences were seen between the treatments. Most of the anthocyanin contents did not differ between the planting methods, with the exception of delphinidin-3-*O*-galactoside, delphinidin-3-*O*-glucoside, and acetylated forms of malvidin (malvidin-3-[6″-acetyl] galactoside, malvidin-3-[6″-acetyl] glucoside).

For the different groups of phenolics, the 'Aurora' fruit did not significantly differ between pots and the ridge, as for the total phenolics content. Some exceptions can be noted, however, such as quercetin-3-*O*-arabinofuranoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, and syringetin-3-*O*-glucoside in the flavonols group, with significantly higher contents in the fruit from pots, and procyanidin B2 in the flavan-3-ols, with significantly higher contents in the fruit from the ridge.

For the 'Brigitta' fruit, similar total phenolic contents were seen for the fruit from both of the planting methods. Significant differences were seen only for the phenolic acids and flavonols, with the highest values for the fruit from pots. For the phenolic acids, significant differences were also seen for 5-caffeoylquinic acid contents and among the flavonols quercetin-3-*O*-rutinoside and quercetin-3-*O*-galactoside. These were the compounds with the highest contents in their group, and therefore contributed the most to the total contents. Individual, and consequently total, flavan-3-ols did not differ between the treatments. For some of the individual anthocyanins, significantly different contents were measured between pots and the ridge; however, those values did not affect the total anthocyanin contents.

For the pot production, the highest contents of all of the groups of phenolics (with the exception of the flavonols) were seen for 'Duke'. The lowest contents of phenolic acids and flavonols were seen for 'Brigitta', while the lowest flavan-3-ols, anthocyanins, and total phenolic contents were detected for the 'Aurora' and 'Brigitta' fruit. Similarly for the ridge (except for the flavonols), the highest contents were seen for the 'Duke' and 'Aurora' fruit.

Table 3. Individual phenolics identified and quantified in the 'Duke', 'Aurora', and 'Brigitta' blueberries harvested from the plants grown in pots and along the ridge.

Phenolic Compound	'Duke'			'Aurora'			'Brigitta'			*p*-Value	
	Pots (mg [100 g]$^{-1}$ FW)	Ridge (mg [100 g]$^{-1}$ FW)	*p*-Value	Pots (mg [100 g]$^{-1}$ FW)	Ridge (mg [100 g]$^{-1}$ FW)	*p*-Value	Pots (mg [100 g]$^{-1}$ FW)	Ridge (mg [100 g]$^{-1}$ FW)	*p*-Value	Pots	Ridge
Ellagic acid derivative	72.83 ± 3.47	78.81 ± 5.60	NS	50.13 ± 4.17	50.19 ± 2.95	NS	42.72 ± 2.29	40.71 ± 3.45	NS		
Caffeic acid derivative 1	0.55 ± 0.04 b	0.63 ± 0.05 a	**	0.52 ± 0.03	0.54 ± 0.04	NS	0.25 ± 0.02	0.24 ± 0.02	NS		
Caffeic acid derivative 2	2.23 ± 0.18 b	2.76 ± 0.30 a	**	8.49 ± 0.32	8.47 ± 0.25	NS	5.39 ± 0.22 b	5.95 ± 0.22 a	***		
Caffeic acid derivative 3	0	0		0	0		0.33 ± 0.03 b	0.39 ± 0.02 a	***		
3-caffeoylquinic acid	100.1 ± 0.01	100.1 ± 0.01	NS	0	0		0	0			
4-caffeoylquinic acid	3.29 ± 0.36 b	3.91 ± 0.19 a	**	3.84 ± 0.23	3.69 ± 0.35	NS	2.48 ± 0.21	2.55 ± 0.30	NS		
5-caffeoylquinic acid	52.16 ± 1.60 b	59.95 ± 1.94 a	***	123.1 ± 3.39	118.4 ± 4.26	NS	80.90 ± 3.60 a	68.89 ± 2.44 b	***		
Caffeoylquinic acid dimer	0.79 ± 0.04 b	0.86 ± 0.03 a	*	1.17 ± 0.07	1.16 ± 0.08	NS	1.37 ± 0.07	1.43 ± 0.07	NS		
di-Caffeoylquinic acid	1.01 ± 0.07	1.09 ± 0.05	NS	0	0		0	0			
5-feruloylquinic acid	0.18 ± 0.04 b	0.24 ± 0.01 a	**	0.46 ± 0.04	0.43 ± 0.03	NS	0.10 ± 0.02	0.11 ± 0.01	NS		
Ferulic acid derivative	5.93 ± 0.41	6.27 ± 0.32	NS	3.29 ± 0.42	3.43 ± 0.37	NS	2.82 ± 0.30 b	3.21 ± 0.19 a	*		
Feruloyl-glucoside	0.20 ± 0.03	0.17 ± 0.05	NS	0	0		0.11 ± 0.01	0.10 ± 0.01	NS		
Total phenolic acids	**239.3 ± 6.25 A**	**254.8 ± 8.55 A**	***	**191.0 ± 8.67 B**	**186.3 ± 8.33 B**	**NS**	**136.5 ± 6.77 C**	**123.6 ± 6.73 C**	**	***	***
Procyanidin B1	58.31 ± 7.83	60.86 ± 3.25	NS	32.75 ± 1.90	30.83 ± 1.79	NS	39.87 ± 2.08	38.35 ± 0.31	NS		
Procyanidin B2	30.85 ± 2.41 a	26.82 ± 0.78 b	**	15.05 ± 0.67 b	16.78 ± 0.82 a	**	12.04 ± 1.87	13.37 ± 0.44	NS		
Catechin	42.92 ± 2.35 b	47.67 ± 2.10 a	**	20.98 ± 1.42	22.84 ± 1.93	NS	20.78 ± 1.37	23.12 ± 1.93	NS		
Epicatechin	2.91 ± 0.36	2.83 ± 0.39	NS	2.45 ± 0.36	2.62 ± 0.51	NS	1.53 ± 0.39	1.76 ± 0.38	NS		
Total flavan-3-ols	**135.0 ± 12.95 A**	**138.2 ± 6.52 A**	**NS**	**71.23 ± 4.35 B**	**73.07 ± 5.05 B**	**NS**	**74.22 ± 5.71 B**	**76.60 ± 3.06 B**	**NS**	***	***
Myricetin-3-*O*-pentoside	0.22 ± 0.02 b	0.29 ± 0.02 a	***	0.54 ± 0.02	0.53 ± 0.03	NS	0.49 ± 0.01 a	0.38 ± 0.02 b	***		
Myricetin-3-*O*-hexoside	1.88 ± 0.13 b	2.54 ± 0.31 a	***	0.98 ± 0.08	1.05 ± 0.09	NS	1.14 ± 0.08	1.20 ± 0.11	NS		
Myricetin-rhamno-hexoside	0	0		3.10 ± 0.25	2.97 ± 0.67	NS	0.21 ± 0.01	0.23 ± 0.03	NS		
Laricitrin-3-*O*-glucoside	2.40 ± 0.25 b	3.04 ± 0.36 a	**	0.76 ± 0.05	0.78 ± 0.08	NS	0.37 ± 0.03	0.33 ± 0.04	NS		
Quercetin-3-*O*-rutinoside	0.08 ± 0.01	0.09 ± 0.01	NS	2.33 ± 0.14	2.25 ± 0.24	NS	1.95 ± 0.12 a	1.66 ± 0.20 b	*		
Quercetin-3-*O*-galactoside	2.24 ± 0.18 b	2.85 ± 0.24 a	**	1.83 ± 0.18	1.98 ± 0.21	NS	2.26 ± 0.18 a	1.77 ± 0.15 b	**		
Quercetin-3-*O*-glucoside	0	0		1.62 ± 0.10	1.63 ± 0.56	NS	1.18 ± 0.19	1.10 ± 0.14	NS		
Quercetin-3-*O*-glucuronide	0.07 ± 0.01	0.08 ± 0.01	NS	2.84 ± 0.17	2.78 ± 0.23	NS	0.15 ± 0.01	0.13 ± 0.02	0.0662		
Quercetin-3-*O*-arabinopyranoside	0.34 ± 0.04 b	0.41 ± 0.02 a	**	0.50 ± 0.03	0.48 ± 0.03	NS	0.33 ± 0.02 a	0.25 ± 0.02 b	0.0006		
Quercetin-3-*O*-arabinofuranoside	0	0		0.53 ± 0.04 a	0.46 ± 0.04 b	*	0	0			
Quercetin-3-*O*-rhamnoside	0.25 ± 0.01 b	0.28 ± 0.02 a	**	0	0		0.76 ± 0.04 a	0.66 ± 0.01	0.0005		
Kaempferol-3-*O*-rutinoside	0	0		0.54 ± 0.04 a	0.43 ± 0.02 b	***	0.46 ± 0.02 a	0.37 ± 0.03 b	0.0005		
Isorhamnetin-3-*O*-galactoside	5.38 ± 0.41	6.15 ± 0.92	NS	1.64 ± 0.11	1.68 ± 0.12	NS	0.98 ± 0.07	0.95 ± 0.17	0.6650		
Isorhamnetin-3-*O*-rutinoside	0.12 ± 0.01	0.13 ± 0.03	NS	1.26 ± 0.15 a	1.03 ± 0.08 b	*	0.19 ± 0.02	0.17 ± 0.04	0.4440		
Syringetin-3-*O*-glucoside	0.61 ± 0.04 b	0.70 ± 0.06 a	*	0.24 ± 0.02 a	0.21 ± 0.02 b	*	0.63 ± 0.03	0.64 ± 0.04	0.5490		
Total flavonols	**13.59 ± 1.11 B**	**16.58 ± 2.00 A**	***	**18.71 ± 1.38 A**	**18.27 ± 2.42 A**	**NS**	**11.08 ± 0.83 C**	**9.83 ± 1.02 B**	**	***	***
Delphinidin-3-*O*-galactoside	2.88 ± 0.28 b	3.95 ± 0.26 a	***	1.37 ± 0.21	1.39 ± 0.17	NS	4.15 ± 0.19 a	3.18 ± 0.35 b	0.0006		
Delphinidin-3-*O*-glucoside	143.7 ± 7.62 b	156.4 ± 7.93 a	*	103.8 ± 7.00	104.0 ± 7.95	NS	100.9 ± 3.40	94.15 ± 6.78	0.0816		
Delphinidin-3-*O*-arabinoside	67.08 ± 2.43	70.64 ± 2.90	NS	31.10 ± 2.00	28.87 ± 2.39	NS	35.67 ± 2.62	34.45 ± 2.13	0.4410		
Cyanidin-3-*O*-galactoside	28.91 ± 1.29	30.45 ± 1.73	NS	21.22 ± 1.65	20.77 ± 1.74	NS	19.42 ± 1.43	18.75 ± 1.37	0.4720		
Cyanidin-3-*O*-arabinoside	3.34 ± 0.29	3.29 ± 0.44	NS	1.43 ± 0.17	1.35 ± 0.10	NS	1.47 ± 0.17	1.31 ± 0.16	0.1600		
Petunidin-3-*O*-galactoside	393.2 ± 14.13	404.6 ± 18.22	NS	260.1 ± 16.75	259.7 ± 24.64	NS	255.6 ± 3.57	243.9 ± 7.59	0.1840		
Petunidin-3-*O*-arabinoside	92.13 ± 5.64	96.12 ± 9.13	NS	40.84 ± 1.17	39.71 ± 2.04	NS	43.06 ± 1.89 b	48.61 ± 1.45 a	0.0008		
Peonidin-3-*O*-galactoside	8.69 ± 0.66	9.17 ± 0.54	NS	2.95 ± 0.17	2.87 ± 0.27	NS	3.31 ± 0.31 b	3.72 ± 0.21 a	0.0397		
Peonidin-pentose	9.51 ± 0.82	9.63 ± 0.65	NS	8.16 ± 0.36	7.97 ± 0.43	NS	6.41 ± 0.44	6.21 ± 0.21	0.3820		
Malvidin-3-*O*-hexoside	127.1 ± 3.88	123.3 ± 11.39	NS	77.55 ± 3.30	80.37 ± 4.86	NS	64.36 ± 3.55	69.13 ± 3.31	0.0589		
Malvidin-3-*O*-arabinoside	13.29 ± 0.87	13.75 ± 1.13	NS	6.74 ± 0.46	7.07 ± 0.38	NS	5.78 ± 0.46	5.95 ± 0.37	0.5350		
Malvidin-3-*O*-xyloside	50.85 ± 2.41	52.61 ± 2.99	NS	33.13 ± 2.00	32.51 ± 2.25	NS	27.11 ± 1.65	29.00 ± 1.19	0.0700		
Malvidin-3-(6″-acetyl) galactoside	3.47 ± 0.29 b	4.68 ± 0.39 a	***	0	0		0	0			
Malvidin-3-(6″-acetyl) glucoside	3.44 ± 0.14 b	4.70 ± 0.55 a	**	0	0		0	0			
Total anthocyanins	**947.6 ± 40.75, A**	**983.2 ± 58.25 A**	**NS**	**588.4 ± 35.24 B**	**586.6 ± 47.22 B**	**NS**	**567.2 ± 19.68 B**	**558.4 ± 25.12 B**	**NS**	***	***
Total phenolic contents	**1335 ± 61.06 A**	**1393 ± 75.32 A**	**NS**	**869.4 ± 49.64 B**	**864.3 ± 63.02 B**	**NS**	**789.0 ± 32.99 C**	**768.4 ± 35.93 C**	**NS**	***	***

Data are the mean ± standard error of five replicates. Different lowercase letters indicate statistically significant differences between planting methods within cultivar and uppercase letters between cultivars within planting method (LSD tests, $\alpha < 0.05$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS, not significant. FW, fresh weight.

4. Discussion

Production of blueberries in pots has been studied in recent years to determine the optimal substrate composition [8,15] and pot properties (e.g., volume, color) [7,16,17]. Irrigation regimes and fertilization have also been studied [8,15]. In the present study, differences between substrate conditions in pots and a ridge were evaluated, together with the blueberry plant responses to growth in a custom substrate, i.e., in pots and along a ridge. The similar distributions of the substrate water contents for pots and the ridge suggest the efficiency of the irrigation system here, where equal amounts of water were added to all of the plants. As the substrate in smaller pots dries out more quickly [7,18], 60-L pots were used to satisfied the water requirements of the plants. These results are in agreement with our previous report [19], where similar water contents during the growing season were measured for pots and a ridge located under a hail net. However, in this previous study, the values were approximately 20 mV higher, probably due to natural precipitation and lower air temperatures, which dried out the substrate to a lesser extent.

According to the temperature measurements, the substrate temperature variations coincided with the maximum air temperature, which was rising towards the summer. However, there were higher substrate temperatures in pots, and a wider range between the minimum and maximum temperatures, compared to the ridge, which indicated higher temperature fluctuations on a daily level [19]. According to Markham et al. [17], the cooler substrate in white pots, or in the present study, with white foil on the ridge, was most likely caused by the greater albedo. At the same time, pots have a higher surface area exposed to the sun in relation to the total substrate volume, compared to the ridge covered with white foil [16].

For 'Duke' and 'Aurora', more intense growth was seen for the plants along the ridge, due to the sensitivity of the individual cultivars to high substrate temperatures [20]. Nevertheless, the total yield per plant was not affected by the planting method for 'Duke', while for 'Aurora', the yield followed the plant growth measurements. Spiers [20] reported that substrate temperatures above 27 °C can significantly reduce shoot growth in some southern highbush and rabbit-eye blueberry clones. In the present study, the substrate in pots reached over 30 °C during the warmer months, which will have negatively affected plant growth, as individual cultivars have multiple growth flushes throughout the growing season [1]. Additionally, Markham et al. [17] stated that warmer substrates reduce the aboveground biomass for red maple, while Keever et al. [21] reported that the greater temperature variability of the substrate explained their reduced plant growth. On the other hand, 'Brigitta' plants did not show differences in growth; however, minor, but still significant, differences were noted between pots and the ridge. This suggests lower susceptibility of 'Brigitta' to changes in substrate properties, and especially to elevated temperatures [20]. In addition, substrate volume in pots was probably equivalent with that along the ridge, especially as the plants were only three years old. According to Cantliffe [18], plant growth increases with increased pot volume. These results contradict our previous findings [19], which might have resulted from the higher air temperatures, and consequently substrate temperatures, under the high tunnel. The similar growth rates between cultivars within the individual planting methods suggest that growth is primarily a cultivar, and secondarily an environmental, trait. 'Duke' originates from Maryland, USA, 'Aurora' from Michigan, USA, and 'Brigitta' from Victoria, Australia [22,23].

Blueberry skin color and fruit firmness primarily depend on the ripening stage [24,25]. Consequently, similar values for these two measured parameters indicate that all of the fruits were harvested at full maturity. Although 'Brigitta' showed a significantly higher h° value and lower fruit firmness, these differences were negligible and could not be detected by the naked eye. These results are also in agreement with our previous study [23].

Sugars provide fruit with sweetness, and organic acids with acidity. Their contents depend first on the genetics and the ripening stage, and then on the environmental conditions [26]. For 'Duke' and 'Aurora', the fruit sugar contents corresponded to the plant

growth, where the values were in favor of the ridge. Higher growth rates provided the fruit with sufficient sucrose contents, which is transported from the leaves as a product of photosynthesis and later transformed into other forms of soluble sugars [26]. The sucrose contents did not differ between the treatments for the 'Duke' and 'Aurora' cultivars; however, due to its minor content in blueberries, sucrose contributed the least to the total sugar content. Glucose and fructose are the most represented sugars in most fruit, including blueberries [26]. Therefore, their contents coincided with the total sugar contents, where the values were significantly higher for the fruit from the ridge. For the organic acids, 'Duke' did not differ between the treatments, while the 'Aurora' and 'Brigitta' fruit contained significantly higher values when grown in pots. This is partly in agreement with our previous study [19]. For the 'Aurora' fruit, inverse values were noted between sugars and organic acids within the same treatment; higher organic acids and lower sugars were seen for the fruit from pots, while the fruit from the ridge contained higher sugars and lower organic acid contents, which is common for ripe fruit [26]. The 'Brigitta' fruit harvested from pots had the highest contents of all three of the individual and total sugars, which might have resulted from the higher yields. Contradictory data were obtained in our similar study with blueberry plants under a hail net, with no significant differences in the total sugar contents between pots and a ridge for the 'Brigitta' fruit [19].

The sugar/organic acid ratio determines the fruit taste and hence whether it is liked by consumers. Usually a high sugar/organic acid ratio is desirable, which can result from high sugar or low organic acid contents. This is clear in the present study, where the higher sugar/organic acid ratio in the fruit from the ridge resulted from the higher sugar content for 'Aurora' and from the lower organic acid content for 'Brigitta'. The 'Aurora' berries are known for their acidic taste, which is due to the organic acid content, which remains high throughout the ripening period. Consequently, it is necessary to harvest 'Aurora' fruit at their full ripeness, so after 5–7 days of completely blue coloration [27].

Stress conditions accelerate secondary metabolite synthesis in plants, such as phenolics, which serves as a defense mechanism [1]. Except for the higher temperatures and temperature fluctuations seen for the substrate in pots, there were no other differences between pots and the ridge for environmental conditions that could cause stress conditions for these plants. Therefore, similar total phenolic contents would be expected between the planting methods for all of the cultivars. Anthocyanins are most represented among phenolics in blueberry fruit, and these determine the fruit color [19]. According to the present study, there were no differences for anthocyanin contents between the treatments, so we can conclude that the berries were harvested at their full ripeness, as those ready for harvest are visually determined according to their color. Across both treatments, the highest phenolic content was seen for 'Duke', and the lowest for 'Brigitta', which indicates the strong influence of genetics on the total phenolic content [24].

5. Conclusions

This study provides the first evaluation of blueberry plant and fruit responses to the pot and ridge planting methods under a high tunnel. Planting blueberry plants in pots might represent a suitable production method for 'Brigitta' in terms of plant growth, and for 'Duke' and 'Brigitta' in terms of fruit yield. Higher substrate temperatures and greater temperature fluctuations in pots were probably the main reasons for the lower growth and yield for 'Aurora'. At the same time, lower sugar/organic acid ratios were detected for the 'Aurora' and 'Brigitta' fruit harvested from pots. The phenolic contents were not affected by the planting methods.

From the present study, we can conclude that the blueberry 'Duke' and 'Brigitta' cultivars are suitable for pot production, while 'Aurora' is not recommended for this planting method. Despite promising data, further studies are needed to determine the responses of these three cultivars to these production methods in following years, for older plants. Additionally, it would be useful to explore the responses of other blueberry cultivars.

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