ACTA Acta Sci. Pol., Hortorum Cultus 12(2) 2013, 87-96

GROWTH, YIELD AND FRUIT QUALITY TWO CULTIVARS LOWBUSCH BLUEBERRY

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Abstract. The hybridisation of *Vaccinium angustifolium* with *Vaccinium corymbosum* has led to several cultivars which are also known as Lowbush or Half-highbush blueberry. The appearance, taste and chemical composition of their fruits are reminiscent of those from wild varieties of *Vaccinium angustifolium*. The research was conductedat the Research Station of the Laboratory of Orchard at the West Pomeranian University of Technology in Szczecin. The plant height, size, yield, quality and the chemical composition of fruits of Lowbush blueberry bushes ('Emil' and 'Putte') were determined. Lowbush blueberry bushes were planted at distances of 1.0 × 2.5 m in acid muck soil (peat) at the Research Station of the Fruit Farming Department in 2005. Six-year-old bushes were characterized by a relatively low height, 70 cm on average; however, 'Putte' cultivar plants were slightly bigger. They also rendered a higher yield, totalling to 1401 g over the 4 years of research. On average, 100 g of fruits were collected from one bush in the first year and 600–700 g in the last year. Moreover, fruits from these bushes were characterized by a higher content of extract, organic acids and polyphenolic compounds, among which anthocyanins constituted the largest group. 'Emil' cultivar fruits were bigger and firmer than fruits of 'Putte' cultivar.

Key words: *V. angustifolium* × *V. corymbossum,* chemical composition, firmness, phenolics

INTRODUCTION

Vaccinium angustifolium, known as the Lowbush blueberry or Canadian blueberry is native to the north-eastern regions of North America. In Europe, this name is assigned to plants from the species *Vaccinium myrtillus* L., which commonly occur in the wild in Eastern European forests [Konovalchuk and Konovalchuk 2009]. Several cultivars, including Swedish 'Putte' and 'Emil', have been created by crossing the Lowbush blueberry (*Vaccinium angustifolium*) with the Highbush blueberry (*Vaccinium corymbosum*). The

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plants created during this process are sometimes also referred to as Lowbush or Half-highbush blueberries, and the appearance, taste and chemical composition of their fruits remind of wild varieties of *Vaccinium angustifolium.* This plant is relatively new to farming and is of interest in northern European countries [Hjalmarsson 2006] due to its low climatic requirements. Indeed, research conducted to date shows that it can be cultivated in cooler regions due to its high frost resistance [Karp et al. 2000]. Its fruits are characterised by a high nutritional and health value [Martineau et al. 2006], being a rich source of antioxidants, flavonoids [Hakkinen and Torronen 2000].

Lowbush blueberry is a plant with similar requirements to Highbush blueberry [Vander Kloet 1988], although it is more resistant to changes in the reaction and moisture. Furthermore it needs light soils, with low pH and a high humus content [Starast et al. 2002]. The bushes, which are quite small (up to 100 cm tall) and therefore recommended for amateur growers, bear black, aromatic and sweet fruits with a slightly blue waxy coating. They mature in late July/early August and tend to be larger than for wild blueberry varieties but smaller than for Highbush blueberry varieties. In full fructification, over one kilogram of fruit can be collected from a single bush [Krzewińska et al. 2007].

The experiment described herein was aimed at comparing the growth, yield, firmness and chemical composition of fruits from two Lowbush blueberry cultivars cultivated in the West Pomerania region.

MATERIALS AND METHODS

Materials. This experiment was conducted in the period 2007–10 at the Research Station of the Laboratory of Pomology at the Department of Horticulture West Pomeranian University of Technology in Szczecin (53°26'17" N 14°32'32" E). Lowbush blueberry bushes of the 'Emil' and 'Putte' cultivars were planted following a random sub-block scheme. The bushes were planted in the trenches, 35 cm deep and 80 cm wide, which were then filled up to the ground level with the acid muck soil (peat). The bushes were spaced 1.5 m apart in the row and 2.5 m apart between the centres of the beds and watered by drip irrigation using water acidified with sulfuric acid to a pH of 3.5 (EC 2.0–2.3) to maintain the optimal reaction of the substrate. Ammonium nitrate was applied at a dose of 30 kg every year.

Methods. The vegetative growth, the size of annual increments and the height and width of bushes were determined. Measurement of leaf surface was performed by means of a DIAS scanner connected to a computer. Physical features of fruits and soluble solids, titratable acidity, vitamin C, nitrate content were measured on fresh berries immediately after the harvest. The **fruit weight** was measured with Radwag WPX 4500 electronic scales (0.01 g accuracy). **The fruit diameter, firmness and puncture the skin** was measured by means of non-destructive device FirmTech2 combined with a computer (BioWorks, USA). The firmness of 100 randomly selected berries from every replicate was expressed as a Gram-force causing fruit surface to bend 1 mm. Soluble solids, L-ascorbic acid and nitrates were measured in the juice. For juice extraction efficiency fruit were homogenized with a blender and heated up to 50°C. Then, after cooling, 1.5 ml of pectinase per 500 g of pulp were added. The pulp was left to stand in a room temperature for 1 hour. Afterward, the pulp was filtered using a vacuum pumpthe pulp. **Soluble solids** content was determined with a digital refractometer PAL-1 (Atago, Japan). **L-ascorbic acid** and **nitrates** content was measured with a RQflex 10 reflectometer (Merck). **Titratable acidity** was determined by titration of a water extract of Lowbush blueberry homogenate with 0.1 N NaOH to an end point of pH 8.1 (measured with an multimeter Elmetron CX-732) according to PN-90/A-75101/04 p.2. **Total polyphenol** content was estimated spectrophotometrically (at 760 nm) in 70% methanol extracts of fruit with the Folin-Ciocalteau reagent [Singleton and Rossi 1965]. The data is expressed as gallic acid equivalents (GAE) g·100 g^{-1}. **Phenolics composition** of berries was determined in fruit samples that were kept frozen (-35°C) in polyethylene bags (250–300 g) until analyzed. The 2 g aliquots of fruit (after thawing) were extracted three times with ~8 mL of 80% MeOH acidified with a glacial acetic acid (1 ml of 100% acetic acid per 1 l 80% MeOH) in an ultrasonic bath for 15 min. The samples were filtered and transferred to the flasks and made up to the final volume 25 ml. Further, the extracts were centrifuged twice at 12 000 g and 20 μl of supernatants were injected into the HPLC system. The HPLC apparatus consisted of a Merck-Hitachi L-7455 diode array detector (DAD) and quaternary pump L-7100 equipped with D-7000 HSM Multisolvent Delivery System (Merck-Hitachi, Tokyo, Japan). The separation was performed on a Cadenza CD C18 (75 × 4.6 mm, 5 mm) column (Imtakt, Japan). Column oven temperature was set at 30°C. The mobile phase was composed of solvent A (4.5% formic acid, pH 2.2) and solvent B (acetonitrile). The program began with a linear gradient from 0% B to 21% B (0–30 min), followed by washing and reconditioning the column. The flow rate was 1 ml min^{-1} and the runs were monitored at the following wavelengths: phenolic acids at 320 nm, flavonol glycosides at 360 nm, and anthocyanin glycosides at 520 nm. The Photo Diode Array spectra were measured over the wavelength range 200–600 nm in steps of 2 nm. Retention times and spectra were compared to those of pure standards within 200–600 nm. Standards of anthocyanidin glycosides were obtained from Polyphenols Laboratories (Norway), flavonols, and phenolic acids from Extrasynthese (France). **Statistical analysis** was done by using Statistica software package version 10 (Statsoft, Poland). The data were subjected to one-way analysis of variance. Values of $P < 0.05$ according to Tuckey test for physical features estimation were considered significant.

RESULTS AND DISCUSSION

The results obtained show that several-year-old bushes of the cultivars under study are quite small (fig. 1). Thus, although the bushes grew by over 10 centimetres per year, in terms of both length and width, in the first three years, from 2009 onwards growth of the bushes was reduced. They reached a height of 65 cm ('Emil') and 71 cm ('Putte') in the fifth year after planting. The height-to-width ratio in a previous study by Starast et al. [2009] was close to 1, whereas in our experiment it never reached such a value as the plants were more slender.

On the older plants was observed the length of one-year shoots which average length amounted to 4.6 and 6.9 cm, (adequately 'Putte' and 'Emil') depending on the cultivar, and up to 13.5 cm on young plants ('Emil'). The average shoot length for bushes cultivated under different conditions was 17 cm, although it should be noted that shoots shorter than 5 cm were not included in the calculations [Krzewińska et al. 2009]. A slight reduction in the number of leaves was observed on older plants (tab. 1), although this is most likely due to the growing yield and the fact that the bushes were not trimmed, which results in an increase in the yield and the weight of fruits [Smolarz and Chlebowska 2002].

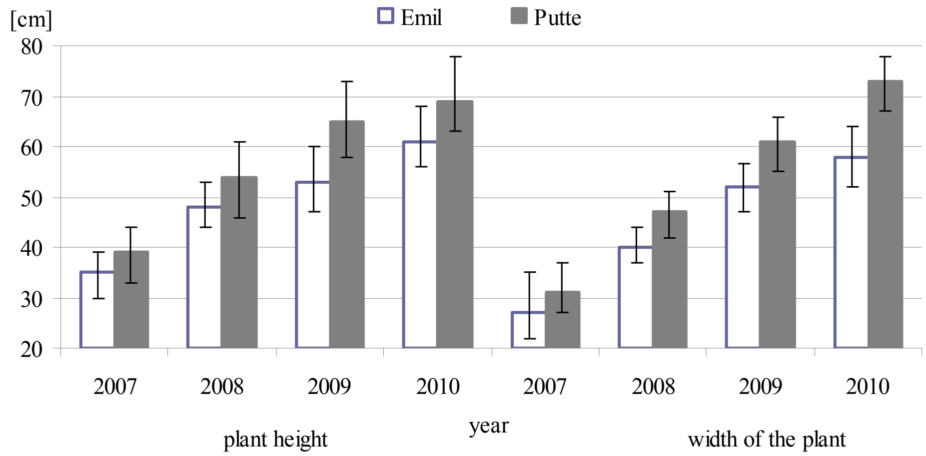

Fig. 1. Width and height of Lowbush blueberry 'Emil' and 'Putte' in particular years of research

The first fruits were collected as early as 2005, although the yield was low and it proved impossible to fully evaluate the quality of the fruits. The plants bore fruit every year thereafter and the yield increased systematically. No damage to the plants was observed in winter, and no damage to the flowers was observed after spring frosts, thereby confirming the high resistance of these cultivars to low temperatures [Hjalmarsson 2006]. Consumption of the maturing fruits by birds proved to be a problem, therefore the plants were protected by nets. In 2007, less than 100 g of fruits were collected from the bushes, and the 'Putte' cultivar already gave a yield of 728 g and 'Emil' a yield of 575 g (fig. 2). Generally speaking, more fruits were collected from the 'Putte' cultivar (average: 1401 g) than the 'Emil' cultivar (average: 1055 g) during the experimental period (tab. 1). However, this increased yield resulted in a decrease in the weight of single fruits. The largest fruits were collected from the youngest bushes (in 2007) for both cultivars, with an average weight of 100 fruits from the 'Emil' cultivar of 92 g and 84 g for the 'Putte' cultivar. In previous studies, Krzewińska et al. [2009] obtained fruits with an average weight of 105 g for this latter cultivar, with Starast et al. [2009] reporting an average weight as high as 130 g. In 2010, the fruits of the 'Emil' cultivar were 22% lighter, and those of the 'Putte' cultivar 25% lighter, with respect to those obtained in 2007. A similar tendency was observed by Krzewińska et al. [2009], although the unit weight of the fruits from young bushes in that study was some 50% higher. The cultivars under analysis were characterized by small fruits, which were relatively firm, especially along the height axis, with this value being around twice as high as for the diameter axis. The fruits from the 'Emil' cultivar were firmer (364 G·mm^{-1}) but more susceptible to skin damage than 'Putte' fruits (331 G·mm^{-1}; Table 1). These values are similar to those obtained in other studies, where the firmness, which ranged from 321 to 396 G·mm^{-1}, was found to depend on the cultivation method [Ochmian and Grajkowski 2010, Ochmian at al. 2009 a].

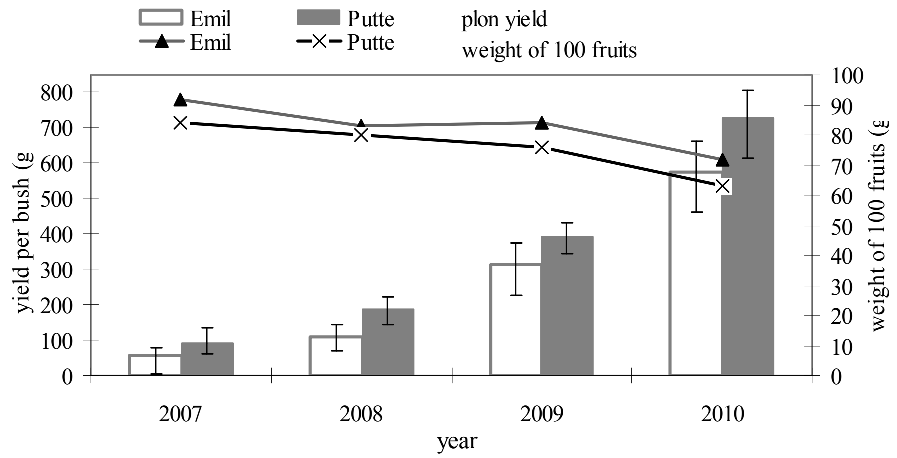

Fig. 2. Yield and fruit weight in particular years of research

The extract and acidity, and their relationship, determine the flavour properties of fruits, in other words the sweet and sour taste desired by the consumer. The extract is positively correlated with the sugar content in fruits, thus meaning that those with a higher extract have a less perceivable sour taste. Testers rate the Lowbush blueberry fruits collected in our study did not have a sour taste. Indeed, according to the persons who ate them, they were bland, indistinctive and too sweet, although they were well liked by children. The extract content obtained was 12.4% ('Emil') and 13.8% ('Putte'), although values as high as 15.5% have been reported previously [Krzewińska et al. 2009]. The acid level was less than 1 g per 100 g of fruit (tab. 1). Fruits from wild Low-bush blueberries contain even less acid (only 0.48 g per 100 g) [Jackson et al. 1999], with an acid-sugar ratio of 28.7. The sugar to acid ratio in the 'Putte' fruits grown in our study was 18.6, whereas in fruits from the Highbush blueberry used during the cultiva-tion process to obtain Lowbush blueberries, the acid to sugar ratio was approx. 14, simi-lar to that for Emil cultivar fruits [Ochmian et al. 2009a, Skupień 2006]. Noormets et al. [2006] estimated the range 6.2–14.3 mg·100 g^{-1} for vitamin C in *Vaccinium myrtillus*, *Vaccinium corymbosum* × *Vaccinium angustifolium* and *Vaccinium angustifolium*. The vitamin C content for the 'Putte' fruits studied herein (33.5 mg·100 g^{-1}; tab. 1) was higher than that reported previously (25 mg·100 g^{-1}) by Starast et al. [2007].

Table 1. Growth, yield and some characteristics Lowbush blueberry 'Emil' and 'Putte' fruits – mean of 2007–2010

Characteristics		'Emil'		'Putte'	
		mean of the years			
		2007–2008	2009–2010	2007–2008	2009–2010
Mean length of one-year shoots (cm)		13.5 b	6.9 a	11.2 b	4,6 a
Leaf area (cm^2)		5.27 a	4.32 a	4.48 a	4.11 a
Total yield per bush z lat 2007–2010 (g)		1055 a		1401 b	
Weight of 100 fruits (g)		82.8 a		75.8 a	
Fruit size (mm)	height	9.6 b		8,7 a	
	diameter	10.8 a		11.1 a	
Firmness fruits ($G\cdot mm^{-1}$)	at fruit height	364 b		331 a	
	at fruit diameter	152 a		168 a	
Puncture (cracking) the skin at fruit diameter		246 a		302 b	
Soluble solids (%)		12.4 a		13.8 b	
Titratable acidity (g citric acid in 100 g		0.92 b		0.74 a	
Vitamin C ($mg\cdot 100\ g^{-1}$)		25.0 a		33.5 b	
Nitrate ($mg\cdot kg^{-1}$)		24 a		47 b	
Polyphenols ($mg\ GAE\cdot 100\ g^{-1}$)		212 a		271 b	

Explanation: the means in rows signed the same letter not differ significantly at the 5% level of significance

Lowbush blueberry fruits are known to be a rich source of polyphenolic compounds (tab. 1 and 2). It was observed that the antioxidant capacity in *Vaccinium* species and diverse small fruits is more highly correlated to total phenolics than to anthocyanins [Moyer et al. 2002], with Lowbush blueberries having higher antioxidant capacity than Highbush blueberries [Kalt et al. 2001]. The polyphenol content determined herein was found to be higher for 'Putte' fruits (271 mg $GAE\cdot 100\ g^{-1}$) than for 'Emil' fruits (212 mg $GAE\cdot 100\ g^{-1}$). In a previous study, Taruscio et al. [2004] found polyphenol contents of between 1.43 and 1.74 $mg\cdot 1\ g^{-1}$ in lowbush blueberry fruits, depending on the cultivar. Anthocyanins were found to be the largest group of compounds responsible for giving the fruits their blue and red colour, accounting for 57.6% of all polyphenolic compounds determined in 'Emil' cultivar fruits and 62.6% in 'Putte' fruits. A value of up to 73% has been reported previously by Taruscio et al. [2004]. Delphinidin 3-galactoside was found to account for up to 30% of the anthocyanins contained in 'Putte' cultivar fruits and 18.7% of the polyphenolic compounds. The Delphinidin 3-galactoside content was lower for 'Emil' cultivar fruits (13.43%). Delphinidins are the most common anthocyanins in blueberry fruits, accounting for 50% in the cultivars studied herein and 39% in the study reported by Taruscio et al. [2004]. In the Northcountry cultivar fruits, the anthocyanin content in the fruits was over 200 mg·100 g^{-1} [Starast et al. 2007]. Flavonols, especially Quercetin 3-galactoside, were another group of compounds. Quercetin 3-galactoside was at a similar level as Delphinidin 3-galactoside in ‘Emil’ cultivar fruits.

Table 2. The content of phenolic compounds in fruits

Phenolic compounds		‘Emil’	‘Putte’
		mean for 2007–2010 (%)	
Delphinidin 3-arabinoside		6.77	7.18
Delphinidin 3-galactoside		13.43	18.74
Delphinidin 3-glucoside		9.25	9.45
Cyanidin 3-arabinoside		3.22	3.67
Cyanidin 3-galactoside		3.71	6.09
Cyanidin 3-glucoside		3.96	1.51
Petunidin 3-arabinoside		4.89	5.31
Petunidin 3-galactoside		1.94	3.26
Petunidin 3-glucoside		3.44	1.26
Peonidin 3-arabinosiade		0.32	0.24
Peonidin 3-galactoside		3.89	3.66
Peonidin 3-glucoside		1.75	1.29
Malvidin 3-arabinoside		0.36	0.34
Malvidin 3-galactoside		0.39	0.37
Malvidin 3-glucoside		0.30	0.21
Anthocyanins		**57.62**	**62.58**
Quercetin 3-galactoside		14.21	9.42
Quercetin 3-glucoside		2.28	2.09
Quercetin 3-ramnoside		1.96	0.85
Kaempferol 3-rutinoside		0.67	0.75
Flavonols		**19.12**	**13.11**
Chlorogenic acid		**23.26**	**24.30**
Polyphenols (mg·100 g^{-1})	year 2007	174	237
	year 2008	159	225
	year 2009	196	258
	year 2010	211	249
	mean	**185 a**	**242 b**

Lowbush blueberry fruits are a particularly rich source of chlorogenic acid, a strong, natural antioxidant. Its content was the highest in ‘Putte’ cultivar fruits – 65.8, which constitutes as much as 24.3% of all polyphenolic compounds. In black blueberries, its content can reach up to 200 mg in 100 g [Budryn and Nebesny 2006].

CONCLUSIONS

1. Lowbush blueberry plants are characterised by a small size; the height of 5-year-old bushes does not exceed 70 cm. Bushes from the 'Putte' cultivar are taller and broader than 'Emil' cultivar bushes.

2. In older bushes, one-year-old shoots are twice as short as shoots in young plants. As the bushes grow older, the size of leaves and fruits decreases slightly.

3. The 'Putte' cultivar yields more fruit than the 'Emil' cultivar; on average, 700 g of fruits is collected from a five-year-old bush of the 'Putte' cultivar, while approx. 600 g of fruit is collected from the 'Emil' cultivar.

4. The fruits of the 'Emil' cultivar are larger and firmer, but more susceptible to skin damage. 'Putte' cultivar fruits are characterised by a higher content of the extract, vitamin C and polyphenols; however, they also contain more harmful nitrates. The fruits of the cultivars under analysis are characterised by a very low content of organic acids, below 1 g in 100 g of fruits.

5. Lowbush blueberry fruits are a rich source of polyphenolic compounds, among which anthocyanins constitute the largest group. They make up as much as 62.6% of all determined polyphenols (with delphinidin-3-galactoside occurring in the largest amount) in the 'Putte' cultivar and 57.6% in the 'Emil' cultivar. Lowbush blueberry fruits are also a rich source of chlorogenic acid, which constitutes approx. 24% of all polyphenolic compounds.

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WZROST, PLON ORAZ JAKOŚĆ OWOCÓW DWÓCH ODMIAN BORÓWKI NISKIEJ

Streszczenie. Krzyżówka *V. angustifolium* z *V. corymbosum* dała początek odmianom, które nazywamy borówką niską lub półwysoką. Krzewy są niewielkie, a owoce smakiem i wyglądem przypominają dzikie formy *V. angustifolium.* W Sadowniczej Stacji Badawczej Katedry Sadownictwa Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie przeprowadzono badania, w których porównano wzrost, plonowanie oraz skład chemiczny owoców dwóch odmian borówki niskiej ('Emil' i 'Putte'). W 2005 r. posadzono krzewy w glebie murszowej (torf) o odczynie kwaśnym w rozstawie 1,0 × 2,5 m. Sześcioletnie krzewy charakteryzowały się niewielkim wzrostem (wysokość krzewów wynosiła średnio 60 cm, rośliny odmiany 'Putte' były nieznacznie wyższe). Uzyskano z nich większy plon, którego suma z 4 lat badań wyniosła 800 g. W pierwszym roku badań średnio z jednego krzewu zebrano 100 g owoców, w ostatnim 400 g. Ponadto owoce z tych krzewów charakteryzowały się większą zawartością ekstraktu, kwasów organicznych oraz związków fenolowych, wśród których największą grupę stanowiły antocjany. Owoce odmiany 'Emil' były większe i jędrniejsze od owoców odmiany 'Putte'.

Słowa kluczowe: *V. angustifolium* × *V. corymbossum,* skład chemiczny, jędrność, związki fenolowe

ACKNOWLEDGEMENTS

This work was supported by the Polish Ministry of Science and Higher Education under grant No. N N310 205337.

Accepted for print: 2.08.2012