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EFFECT OF SUBSTRATES ON GROWTH, YIELD, AND PHENOL CONTENT IN LOWBUSH BLUEBERRY (*Vaccinium angustifolium* AIT.) FRUIT 'EMIL'

WPŁYW PODŁOŻY NA WZROST, PLONOWANIE ORAZ ZAWARTOŚĆ POLIFENOLI W OWOCACH BORÓWKI NISKIEJ (*Vaccinium angustifolium* AIT.) ODMIANY 'EMIL'

Abstract: The experiment was carried out in the years 2007–2008 in the Experimental Pomological Station at Rajkowo near Szczecin. In 2005 the plants of lowbush blueberry, 'Emil' cv. were planted in peat (acid muck soil), sawdust (previously composted), and cocoa husk substrate (a by-product from chocolate confectionary plant) at spacing 1.0 × 2.5 m. Plant growth, quantity, quality, and chemical composition of yield were assessed. No effect of substrate was observed regarding plant height tested in the substrates however, the bushes planted in peat and cocoa husk had bigger leaves and one-year shoots. Further, the bushes grown in these substrates yielded best and their fruits were largest, showed highest firmness as well as highest content of soluble solids, organic acids, and phenol compounds.

Keywords: *Vaccinium angustifolium* AIT., substrates, yield, fruit quality, phenols

Vaccinium angustifolium AIT., dubbed lowbush blueberry, Canadian blueberry occurs in the wild in north-east regions of North America [1]. The crossings of *Vac. angustifolium* with *Vac. corymbosum* yielded a few cultivars also named lowbush blueberry or half-highbush blueberry. Regarding the taste, appearance and chemical composition of hybrid berries they resemble wild berries of *Vac. angustifolium*. The half-highbush blueberry is relatively new orchard species but due to its low climatic requirements gets a growing interest in the countries of northern Europe [2]. The berries have high nutritional value [3] and both berries and other parts of plant exhibit health promoting properties [4]. Their high antioxidant activity [5] results from high concentration of flavonoids [6], and especially anthocyanins [7]. The lowbush blueberry has specific inhabitat requirements. Similarly to highbush blueberry [1], lowbush blueberry thrives and yields on light soils with optimum soil humidity, low pH, and

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high humus content [8]. The lack of suitable soils enforces usage of substrates matching the needs of the species.

The aim of the experiment was to test usefulness of selected organic substrates bedded in the alkaline reaction orchard soil for lowbush blueberry cultivation and estimation of effect of the substrates on the quality of the berries.

Material and methods

The experiment was carried out in 2007–2008 in the Pomological Experimental Station at Rajkowo, near Szczecin. The purpose of field trial was to evaluate suitability of the peat, a composted conifer sawdust (obtained from a local sawmill), and the cocoa husk (a by-product obtained from Chocolate Confectionary Plant 'Gryf' in Szczecin) (Table 1) for growing of lowbush blueberry, 'Emil' cv. In spring of 2005 the bushes were planted in the trenches 35 cm deep and 100 cm wide, and filled with the medium at spacing 1.0 m (in the row) × 2.5 m (between the rows).

The fertilization was limited to nitrogen supply only, because chemical analyses both of the soil and the substrates showed high and/or middle content of other nutrients. Each type of media was fertilized with the ammonium nitrate on three occasions at a total dose of 30 kg · N ha^{-1}. The fertilizer was spread evenly on the bed tops at the width of 1 m.

Table 1

Mineral composition, pH, and water capacity of the soil and substrates used in the experiment (mean of 2007–2008)

Bedding	pH	Field water capacity	Full water capacity	N	P	K	Ca	Mg
		[% v · v^{-1}]		[mg · 100 g^{-1}]				
Orchard soil	6.9	—	—	12	12	36	71	6
Peat	3.8	44.8	80.6	11	11	38	157	36
Cocoa husk	5.7	36.9	85.3	24	16	33	162	34
Sawdust	4.9	31.3	82.6	6	9	73	94	25

The supplemental irrigation was applied through the drip line type T-Tape with using water acidified with H_2SO_4 up to pH = 3.5 (as measured in H_2O). The intensity of water supply was adjusted to the substrate moisture by means of the tensiometer monitoring twice a week, according to pF units (the pF, soil suction being the common logarithm of water height in centimeters). Measuring tubes (30 cm) were installed 15 cm below the soil surface and pF 2.2 was used as a threshold value for irrigation. Having reached the threshold, the soil was irrigated to approximately pF 1.0.

Each year total yield, fruit size, fruit mass, and firmness was measured. The mass and firmness of berries was done with a FirmTech 2 apparatus (BioWorks, USA). The firmness of 50 randomly selected berries from each harvest was expressed as a gram-force causing fruit surface to bend 1 mm. Moreover, in fresh fruit soon after the harvest titratable acidity, soluble solids, and L-ascorbic acid content was measured. The titratable acidity was determined by titration of water extract of blueberry homogenate with 0.1 mol · dm^{-3} NaOH to the end point of pH = 8.1, according to PN-90/A-75101/04.

The soluble solids content was determined in the berry juice by means of the digital refractometer Atago (Japan). The L-ascorbic acid content was determined with the iodimetric method. The fruits meant for phenol analysis were kept each harvest. The HPLC analyses of polyphenols were carried out on combined samples of berries kept frozen after each harvest (–36 ^{o}C) prior to thawing. The HPLC apparatus consisting 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 Synergi Fusion RP-80A 150 × 4.6 mm (5 mm) Phenomenex (Torrance, CA USA) column. Column oven temperature was set at 30 ^{o}C. Aliquots of 1 g fruit tissue were extracted with methanol acidified with 0.1 % HCl. The extraction was performed in an ultrasonic bath for 20 min. Next, the slurry was centrifuged at 19000 × g for 10 min and the supernatant was used for HPLC analysis. The supernatant was recovered and filtered through a 0.45 μm cellulose syringe filter before analysis. The mobile phase was composed of solvent A (2.5 % acetic acid, pH = 2.9) and solvent B (acetonitrile). The program began with a linear gradient from 0 % B to 25 % B (0–36 min), followed by washing and reconditioning the column. The flow rate was 1 $cm^3 \cdot min^{-1}$ and the runs were monitored at the following wavelengths: chlorogenic acid at λ = 320 nm, flavonol glycosides at λ = 360 nm, and anthocyanin glycosides at λ = 520 nm. The Photo Diode Array spectra were measured over the wavelength range λ = 200–600 nm in steps of 2 nm. Retention times and spectra were compared with those of pure standards within 200–600 nm.

The values were evaluated by the Duncan test and for phenolics by the t-Student test. The differences between the means at $p < 0.05$ were considered significant.

Results and discussion

Data referring to lowbush bluberry growth, yield and chemical composition of berries are presented in Table 2.

Table 2

The growth of bushes and fruit quality of lowbush blueberry 'Emil' in dependence on the substrates (mean of 2007–2008)

Parameter		Peat	Cocoa husk	Sawdust
Plant height [cm]		53.5 a	46.0 a	49.5 a
Mean length of one-year shoots [cm]		22.5 b	20.7 ab	18.5 a
Leaf area [cm^2]		5.4 b	5.6 b	4.8 a
Total yield per bush [g]		189 b	175 b	124 a
Mean mass of 100 fruits [g]		86.8 b	85.3 b	71.5 a
Fruit firmness [$G \cdot mm^{-1}$]	at fruit height	375 b	396 b	321 a
	at fruit diameter	147 ab	169 b	139 a
Soluble solids [%]		12.9 b	12.6 b	11.8 a
Titratable acidity [g citric acid · 100 g^{-1}]		0.98 b	0.94 b	0.85 a
Vitamin C [$mg \cdot 100\ g^{-1}$]		23.5 a	25.0 ab	26.5 b

Explanation: The means signed with the same letter do not differ significantly at the 5 % level of significance, according to Duncan t-test.

The substrates showed no significant effect on height of plants, however longer one-year shoots produced bushes grown in peat compared with that of sawdust. Three-year old bushes tested in the experiment of [9] were lower. The berries obtained from plants grown in peat and cocoa husk had larger leaf-area, yield per bush, mean weight of 100 fruits, and fruit firmness measured at fruit height compared with the berries originating from sawdust substrate. At full fruiting stage it is possible to obtain one kg of fruits per bush [2]. The highest soluble solids and titratable acidity was found in berries grown in peat and cocoa husk, whereas berries originating from peat showed higher vitamin C content than that of sawdust medium. In the finding of Starast et al [10] other cultivars showed similar amount of vitamin C, whereas acidity ranged from 0.2 to 2.3 g citric acid per 100 g of fruit and soluble solids content 11.5–14.9 %.

The data on phenolics composition of lowbush blueberry 'Emil' are presented in Table 3.

Table 3

Phenols pattern for lowbush blueberries, 'Emil' cv. [mg · 100 g^{-1}] – mean for 2007–2008

Phenols	Peat	Cocoa husk	Sawdust
Delphinidin 3-galactoside	30.29	36.50	18.71
Delphinidin 3-glucoside	4.92	29.17	24.84
Delphinidin 3-arabinoside	15.17	18.44	9.46
Cyanidin 3-arabinoside	8.43	7.11	4.98
Cyanidin 3-galactoside	8.41	10.34	4.90
Cyanidin 3-glucoside	10.76	8.00	6.44
Petunidin 3-galactoside	3.59	6.19	2.56
Petunidin 3-arabinoside	12.61	9.37	9.17
Petunidin 3-glucoside	9.30	7.17	5.47
Peonidin 3-galactoside	8.97	9.53	6.27
Peonidin 3-glucoside	4.01	4.06	3.05
Peonidin 3-arabinoside	0.30	0.94	0.79
Malvidin 3-galactoside	0.94	1.07	0.46
Malvidin 3-glucoside	1.25	0.40	0.26
Malvidin 3-arabinoside	0.90	0.66	0.73
Anthocyanins	**119.85 a**	**148.95 b**	**98.09 a**
Quercetin 3-galactoside	34.46	29.81	26.21
Quercetin 3-glucoside	5.28	5.16	4.04
Quercetin 3-ramnoside	3.92	3.70	4.90
Kaempferol 3-rutinoside	0.64	2.09	1.53
Flavonols	**44.30 a**	**40.76 a**	**36.68 a**
Chlorogenic acid	**79.03 c**	**40.86 b**	**28.21 a**
Total	**243.14 b**	**230.56 b**	**162.96 a**

The predominant anthocyanin identified in berries was delphinidin-3-galactoside followed by delphinidin-3-glucoside (for berries grown in cocoa husk and sawdust) and delphinidin-3-arabinoside. Further, the decreasing anthocyanin order was: cyanidin

glycosides > petunidin glycosides > peonidin glycosides > malvidin glycosides. Berries originating from plants grown in cocoa husk showed the highest content of total anthocyanin. Similar values were noted by Starast et al [10] for *Vac. angustifolium* and the hybrids of *Vac. corymbosum* × *Vac. angustifolium*. Quercetin-3-galactoside was predominat among identified flavonols and kaempferol-3-rutinoside content was lowest. However, no influence of substrate was observed regarding total flavonols content. Riihinen et al [11] determined 531 $\mu g \cdot g^{-1}$ quercetin for 'Northblue' (*Vac. corymbosum* × *Vac. angustifolium*) in berry peels, whereas no flavonols were detected in berry pulps. The substrates tested in this finding significantly affected chlorogenic acid content in 'Emil' berries (from ~28 mg · 100 g^{-1} for berries of bushes grown in sawdust to ~79 mg · 100 g^{-1} for peat-originating berries). Regarding total phenol, blueberries obtained from plants cultivated in peat and cocoa husk showed significantly higher amounts (>240 and 230 mg · 100 g^{-1}, respectively) compared with berries originating from sawdust bedding (>162 mg · 100 g^{-1}).

Conclusions

1. The plants of lowbush blueberry, 'Emil' cv., growning in peat and cocoa husk substrate had larger leaves and longer one-year shoots.
2. The lowbush blueberry, 'Emil' cv., cultivated in three organic substrates (peat, cocoa husk and sawdust) started to yield in the second year after planting. The best crop and the biggest berries were obtained from plants grown in peat and cocoa husk.
3. The bushes planted in peat and cocoa husk produced fruits of highest content of soluble solids, acidity, and phenol compounds.

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Abstrakt: Doświadczenie przeprowadzono w latach 2007–2008 w Sadowniczej Stacji Badawczej Katedry Sadownictwa, gdzie w 2005 roku posadzono krzewy borówki niskiej odmiany 'Emil' w rozstawie 1,0 × 2,5 m w glebie murszowej (torf) o odczynie kwaśnym, przekompostowanych trocinach z drzew iglastych oraz w łusce z ziarna kakaowego, która jest odpadem przy produkcji czekolady. Określano wzrost roślin oraz ilość, jakość oraz skład chemiczny plonu.

Nie stwierdzono wpływu zastosowanych podłoży na wysokość roślin, jednak krzewy posadzone w torfie oraz łusce kakaowej miały większe liście oraz dłuższe pędy jednoroczne. Krzewy, które rosły w tych podłożach, również plonowały najlepiej a owoce z nich uzyskane były największe i najbardziej jędrne. Ponadto owoce z tych krzewów charakteryzowały się największą zawartością ekstraktu, kwasów organicznych oraz związków polifenolowych.

Słowa kluczowe: *Vaccinium angustifolium*, plon, jakość owoców, polifenole, podłoża