LATVIJAS LAUKSAIMNIECĪBAS UNIVERSITĀTE
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LATVIJAS LAUKSAIMNIECĪBAS UN MEŽA ZINĀTŅU AKADĒMIJA
LATVIAN ACADEMY OF AGRICULTURAL AND FORESTRY SCIENCES

AGRONOMIJAS VĒSTIS

LATVIAN JOURNAL OF AGRONOMY

Nr. 12/ No. 12

JELGAVA 2009

Agronomijas Vēstis. – Nr. 12. – Jelgava, LLU, 2009 – 148 lpp. ISSN 1691-3485
Latvian Journal of Agronomy.-No 12.-Jelgava, LLU, 2009 – 148 p., ISSN 1691-3485

VACCINIUM SPP. AND LESS KNOWN SMALL FRUIT: CHALLENGES AND RISKS

Issue of the International Scientific Conference and
NJF seminar N° 426
Jelgava, Latvia, October 6-9, 2009

Starptautiskas zinātniskas konferences izdevums un
NJF semināra N° 426

Izdevums publicēts ar Latvijas Republikas Zemkopības ministrijas finansiālo atbalstu.

Publication of the issue has been financed by the Latvia Ministry of Agriculture

ISSN 1691-3485

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Content/Saturs

CRANBERRY AND BLUEBERRY PRODUCTION IN LATVIA
DZĒRVEŅU UN KRŪMMELLEŅU AUDZĒŠANAS SITUĀCIJAS IZPĒTE LATVIJĀ

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Abstract
Large cranberries (*Vaccinium macrocarpon* Ait.) and highbush blueberries (*Vaccinium corymbosum* L.) are relatively new cultures in Latvia. The first commercial cranberry plantation was established in 1985. Nowadays, the area of cranberries is approximately 100 ha which makes it the third largest in the world. The blueberry plantations compared to other European states are not so large - 170 ha. The largest part of the area of high moss peat bogs was drained and is now being used as cranberry fields – a bogs area of approximately 10 000 ha. The examination of the situation of cranberry and blueberry production started in 2007/2008. Cranberry and blueberry plantations were surveyed in all four fruit-growing areas of Latvia. The following parameters were examined: varieties and plantation sizes, the physiological status of the plants of different varieties after overwintering, growing technologies - characterisation of the soil or substrate, plantation location, growing distances; yield and yield quality. The most popular varieties of cranberries are 'Stevens', 'Bergman', 'Ben Lear', etc. The most popular cultivars of blueberries are 'Bluecrop', 'Patriot' and 'Northland'. The newest cultivars in the Latvian plantations are 'Toro', 'Rubel', 'Blue Gold', 'Hanna', 'Klara', 'Drapers' and 'Bonus'. Planting technologies of highbush blueberries: 1) mineral soil (78 % of farmers – with specific preparation of the soil before planting; 2) peat – 22 % of farmers.

Kopsavilkums
Lielogu dzērvenes (*Vaccinium macrocarpon* Ait.) un krūmmellenes (*Vaccinium corymbosum* L.) ir salīdzinoši jauni kultūraugi Latvijā. Pirmie komerciālie dzērveņu stādījumi tika ierīkoti 1985. gadā. Šobrīd dzērveņu stādījumu platības sasniedz jau 100 ha, ieņemot trešo vietu pasaulē, bet krūmmelleņu stādījumu platības ir mazākas, salīdzinoši ar citām Eiropas valstīm – 170 hektāri. Lielākā daļa augstā sūnu kūdras purvi ir nosusināti un šobrīd tiek izmantoti kā dzērveņu lauki - purvu platību aptuveni 10 000 ha. Pētījumi par dzērveņu un krūmmelleņu audzēšanu Latvijā uzsākti 2007/2008 gados. Dzērveņu un krūmmelleņu stādījumi tika apsekoti visās četrās augļkopības zonās Latvijā. Tika vērtēts: audzētās šķirnes un to audzēšanas platības, dažādu šķirņu augu fizioloģiskais stāvoklis pēc ziemošanas, audzēšanas tehnoloģijas – raksturojot augsni vai substrātu, atrašanās vieta, audzēšanas attālumi, raža un ražas kvalitāte. Visvairāk audzētākās dzērveņu šķirnes 'Stevens', 'Bergman', 'Ben Lear', u.c. Visvairāk audzētākās krūmmelleņu šķirnes – 'Bluecrop', 'Patriot', 'Northland'. Jaunākās krūmmelleņu šķirnes Latvijā ir 'Toro', 'Rubel', 'Blue Gold', 'Hanna', 'Klara', 'Drapers' and 'Bonus'. Krūmmelleņu audzēšanas tehnoloģijas:
1) minerālaugsnē–78 % saimniecībās (pirms stādīšanas augsni speciāli sagatavojot); 2) kūdrā–22% saimniecībās.

Key words: *Vaccinium macrocarpon*, *Vaccinium corymbosum*, cultivars, area, technologies

Introduction
Cranberries, as it is with highbush blueberries, can play an important role in the economy of Latvia as these berries are sought in the world market for their medical and dietary properties and they are among the best paying berries.
The Latvia's climate and the vast marshland area is the main reason why from ancient times the European or the Latvian local (*Vaccinium oxycoccus* L.) cranberries are grown in the wild. Berry plantation is not mechanized due to their morphological properties. The yield was mostly used in the local market.
The American large cranberry (*Vaccinium macrocarpon* Ait.) and highbush blueberry (*Vaccinium corymbosum L.*.) are relatively new cultures in Latvia. The first commercial cranberry plantations

were established in 1985. Nowadays, the area of the large cranberries is approximately 100 ha and that is third place in the world. The blueberry plantations compared to other European countries are not so large- about 170 ha (after Latvian Fruitgrowers Association data).
The scientific research on the European or Latvian local cranberries was started in the 70ies of the last century by the Department of Horticulture under the Faculty of Agriculture of the Latvia University of Agriculture. It was found that the advantage of these cranberries lies in the growing season, it is shorter- the beginning of flowering is about two weeks earlier, it starts already in the middle of May. Besides, they require a lower sum of the effective temperatures than the large berry cranberries. The berries are better protected from autumn frosts, the berry texture is more gentle. For this species of cranberries it is not possible to use mechanization in harvesting (Abolins and Gurtaja, 2006). Producing shoots are upright, unable to detach the berries from the plant and the berries develope unequally.
Productive wild clones were found not only in the bogs of Latvia but also in Karelia (Russia) and Estonia using both physical and chemical mutagenesis. 172 cranberry genotypes were studied. Most of them -163 genotypes had been collected in Latvia, 7 - in Estonia, 1 - in Novosibirsk and 1 - in Petrozavodsk in Russia.
The research was carried out propagating woody and softwood cuttings, investigating planting density, substrates, morphological and biological characteristics of the plant and other issues (Gronskis and Liepniece, 2004.)
Improving less productive areas of cranberry bogs with high-value varieties as well as on recovering the cranberry degraded bog areas. It was decided to continue selection in cranberry clone test conditions in order to breed crops, to test their suitability in re-cultivated bogs. It was also decided to develop elaborated technologies for the propagation and cultivation of the specific genotype.
In the breeding work, the most valuable clones were chosen. Having analyzed the organic harvest of the best cranberry genotypes, it can be concluded that the greatest number of inflorescence - 800 m^{-2} , the largest flower number 1420 m^{-2} and berry mass are calibrated with the genotype V-63583 (Gronskis and Liepniece, 2004.)
According to the length of the vegetation period, the best cranberry genotypes are divided into: medium early B-83 and V-63383; medium late V-63583, but the late is -V-21682. The highest breeding ability for grass-like cuttings is noteworthy with the genotype V – 63583 and B – 83 (Abolins.and Gurtaja, 2006).
In the recultivated cranberry bogs areas - in total 40.8 ha of land, selected in different places of Latvia, the most valuable genotype seeds were sown. The seed sowing was done by plane. Studying the recultivated cranberry areas it was found that cranberry growing was very uneven, averaging from 10 to 30 %. In some places it was associated with deep groundwater levels, sulphur spring diffluence and open places in the array as well they were destroyed by frost.
However, in a more carefully prepared place - in the Experimental Plantation of Jelgava MRS (Jelgava Forestry), sowing the seeds in the area of 1 ha, the projective cover totaled to 100 % and planting seedlings in the growing area of 0.8 ha also totaled to 100 %. The yield reached 1.7 – 2.1 t ha^{-1} in the given stands.

Materials and Methods

Climate in Latvia. The northern part of Latvia is outside the fruit zone of the temperate climate. However, nearness to the ice-free Riga Gulf Stream avoids early autumn frosts, and during the winter becomes the reason for a relatively mild climate in the western part of Latvia. The average precipitation is 560 – 850 mm, the monthly average winter temperature (January) is -2.6 to – -6.6 ^{o}C and the summer temperature (July) is +16.8 to +17.6 ^{o}C with the day length 17 – 18 hours in June. In the growth season, temperature over +5 ^{0}C lasts 180 – 200 days. The sum of the active temperatures (> 10 ^{o}C) in the vegetative period is 1800 – 2100 ^{0}C. There are 120 – 140 active cyclones and 160 – 180 anticyclones per year.
The total area of peat bogs is 6401 km^2 or 9.9 % of all the land area of Latvia, which makes recourses of peat – 1.7 - milliard t. There are more than 5000 peat deposits: 7 exceeding an area of 5000 ha; 87 with the area of 1001- 5000 ha; 109 with the area of 501 – 1000 ha. Included are 49.3 % of low grass peat bogs; 41.7 % of high moss peat bogs, 9 % others. The average depth of

deposits is 2 – 5 m, but the maximum - up to 15 metres. The largest part of the area of high moss peat bogs is drained and used for establishing cranberry fields- could in the future be approximately 10 000 ha (Abolins and Gurtaja, 2006).
Despite the greatly varying climatic conditions, the winter of 2007/2008 was more favorable for the growth and development of large cranberries and highbush blueberries, rouhg the highbush plantings could suffer from spring frosts when temperatures fell below 0ºC. In such cases, the frost did not damage plantings where surface irrigation was available. The physiological observations carried out in 2008 showed that only single twigs of highbush blueberries were frost damaged. The after effect of the damage caused by the winter of 2006/2007 caused the delayed development of single twigs that later led to the twigs dying.
An evaluation of the situation of cranberry and blueberry production started in the season of 2007/2008 within the framework of Project No. 04.1 – 25/5. Cranberry and blueberry plantations were surveyed in all fruit-growing zones of Latvia – Central, Eastern, Southern and Western zones. The following parameters were examined: the used varieties and plantation sizes, growing technologies - characterisation of soil or substrate, plantation location, growing distances, the physiological status of plants of different varieties after overwintering, yield and yield quality.
In collaboration with the researchers of the Laboratory of Plant Mineral Feeding under the Institute of Biology of the Latvia University, in the farm „Strelnieki" (Riga District, in the mineral soil, the variety 'Patriot') and Lienama Ltd (Aluksne District, in the peat bog, the variety 'Northblue'). The testing of fertilizer systems: option 1 - the basic fertilizer NPK + Mg + S, a supplementary fertilizer N; Ca; S;option 2- leaf fertilizer Vito Silva; option 3- leaf fertilizer Vito Silva + B; Cu; Mo; option 4–leaf fertilizer Vito Silva + B; Cu; Mo + Caltrac.
The research on the development of plant phenology, the physiological status of plants, yield and its quality indicators is simultaneously carried out by the Training and Research Farm of the Institute of Agrobiotechnology under the Faculty of Agriculture of the Latvia University of Agriculture (www.llu.lv) in the City of Jelgava. On this base farm, the high bush blueberry and cranberry collection is structured. The high bush blueberries are planted in the mineral soil in peat cushions distributed in furrows. The plant age-7 years, biennial seedlings were planted. Cranberries are planted in peat providing the necessary growth conditions.

Results and Discussion
The **large cranberries** (*Vaccinium macrocarpon* Ait.)**.** The most popular varieties of cranberries are 'Stevens', 'Bergman', 'Ben Lear' and others, but less popular are 'Franklin', 'Pilgrim', 'Hoves' and 'Lemynion' (Figure 1).

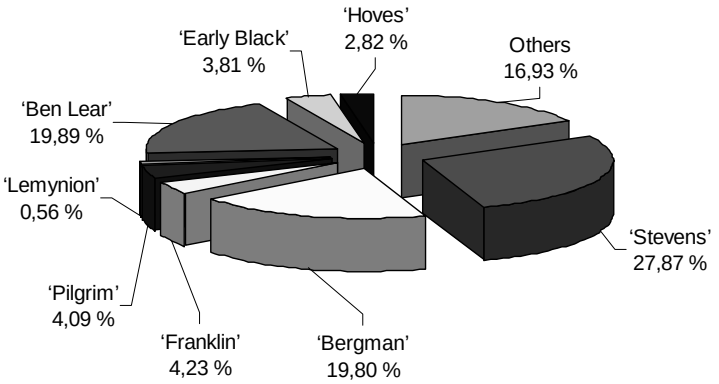

Figure 1. The division of cranberry varieties in farms, %

Due to the high costs involved to establish cranberry fields, most (65 %) of the farms are small - with 0.1 – 5.0 ha of land, but 21 % of farms – with 5.1 – 10.7 % with 10.1 – 15.0 and 7 % - with 15.1 – 20.0 ha of land (Figure 2).

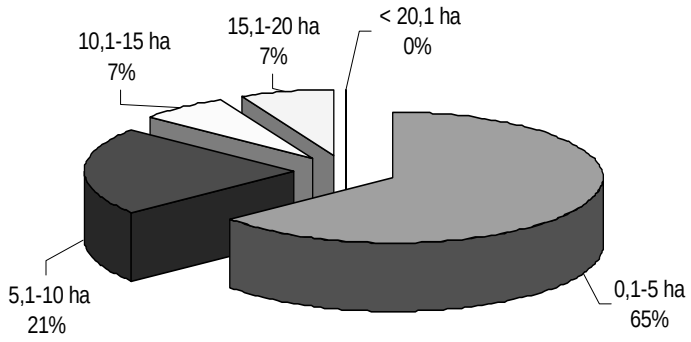

Figure 2. Farm division by cranberry plantation areas, %

There are three planting technologies for large cranberries used in Latvia.
First - in the prepared field, cranberry tendrils are evenly spread on the soil surface and imbedded in peat with a disc harrow. Cranberries are planted or seeded in straight lines. It is a widely used method with sand substrate abroad. The drawback of the method- all tendrils are not deeply the soil.
Second - imbedding tendrils by power harrow. In order not to damage the plants the speed of the power harrow should be reduced. One should not imbed them too deep or too shallow.
Third - planting by hand using a planting stick. The drawback - a laborious process, but it can be used for planting a small area.
The large cranberries unlike the local ones, start blooming later-in mid-June/early July (the local cranberries – in May/June). Thus, the harvest of the large cranberries is only affected by the late spring frosts (sprinkling should be provided). On the surveyed farms, in May maintenance work was started in the cranberry plantations-combing, tendril cutting, and in the end of June, cranberry plantations were at the flowering stage.
On the whole, in terms of weather conditions, in the second decade of September in Latvia it was cold but dry in 2008. The average air temperature of the decade was 3.7 degrees below the norm. The cold weather lasted the whole decade. During the coldest night of the decade (16 September), the first frosts were recorded -2 ºC. During the decade there was frost almost every night, during the coldest periods falling to -5 – -6 ºC.
The highest yield of 2007 was 4500 g m^{-2}. In 2008 there was a harvest of 3900 g m^{-2}. Also, 100-berry mass was higher in 2007 by an average of 180 g and in 2008. - 150 g. In the middle and eastern areas rainfall was lower.
Weed control as an essential part of cranberry management was done in two ways - by hand and by using glyphosate pesticides as a replant treatment by weed wipers on bicycle wheels.
Sand, used as a mulching material, was spread with a specially constructed spreader but there were problems with sand pH and weeds. Therefore, sanding is not popular. The farmers use high moss peat, but some -sawdust or sawdust mixed with peat especially those without a sprinkler irrigation system.
The highest cranberry harvest in the year 2007 was - 4500 g m^{-2}, but in the year 2008 – 3900 g m^{-2} (Figure 3). The large berries of cranberries (above 19 mm), were on average 1 – 2,5 %, but the main harvest was produced large berries (15-16 mm), 43 – 62 % in the year 2007 and 35 – 59 % in the year 2008, accordingly.

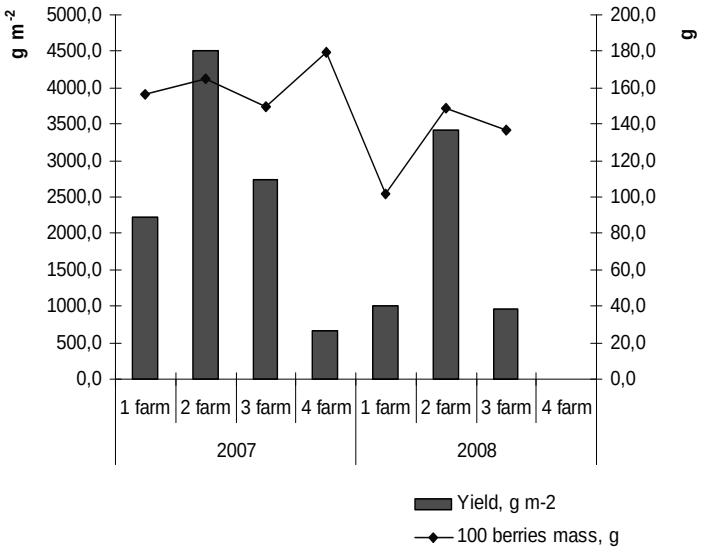

Figure 3. The average yield of the cultivar 'Stevens', g m^{-2}, and the mass of 100 berries, g (on three farms in the years 2007 and 2008).

The analysed late cranberries varieties in the autumn frost, up to 10 % of the harvest was lost in autumn frosts on the farms of the western area of Latvia.

Blueberries (*Vaccinium corymbosum* L.)**.** The most popular varieties of blueberries in Latvia are the northern highbush varieties adapted to quite cold mid-winter temperatures below -20 ºC 'Bluecrop', 'Patriot' and 'Northland' (Hancock, 2006). The newest varieties are 'Toro', 'Rubel', 'Blue Gold'.

In the collections of some farms, such varieties as 'Hanna', 'Klara', 'Drapers', 'Bonus' are starting to appear but are not popular in Latvia yet (Figure 4).

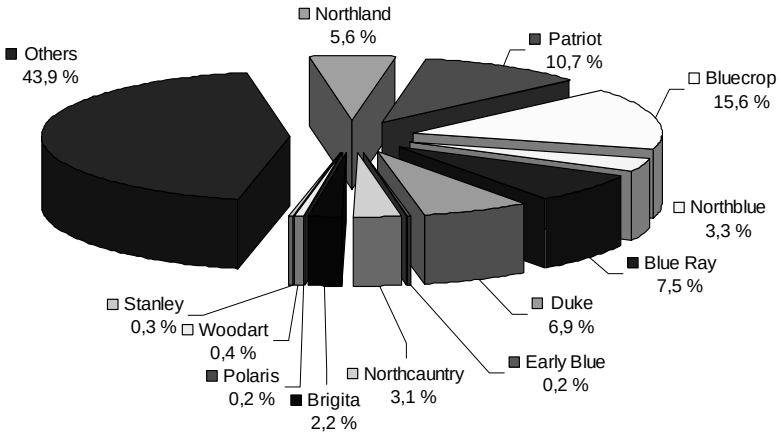

Figure 4. The division of highbush blueberry cultivars on farms, %

Like it was with cranberries, most blueberry farms - 80 % are small with 0.1 – 5.0 ha of land, but 11 % of the farms have 5.1 – 10 ha of land (Figure 5). The main reason why small farms are so many, is the high start up cost and lack of experience in highbush blueberries cultivation.

On the surveyed farms, the highbush blueberries are chosen for cultivation in one of the following ways: in mineral soil (78 % of the breeders) – the soil before planting is adequately prepared: the plants are planted in cushion peat pits (50 x 50 x 50 cm), which are filled with acidic high moss peat, some of the farmers fill the furrows with peat and chippings or sawdust (1:1).

The second option-in the peat bog (22 % of the farmers) they choose to breed highbush blueberries in worked out peat-moss bogs, planting plants on a level field or in the beds.

In less than half the surveyed farms, the highbush blueberry plantations were irrigated against frosts. Therefore, the spring frost damage was very slight. In determining soil acidity pH/KCl in all the surveyed farms it was within the norm from 4.3 to 5.15 (Nollendorfs, 2003).
Spring maintenance includes: fertilizing, the tree crown formation, excision of the dead twigs, bed maintenance (weeding, mulching) and irrigation. As a mulching material, moss bog peat is used as well as chipping mulch. The research carried out in the year 2007 shows that incompletely mulched plantations are destroyed by frosts.

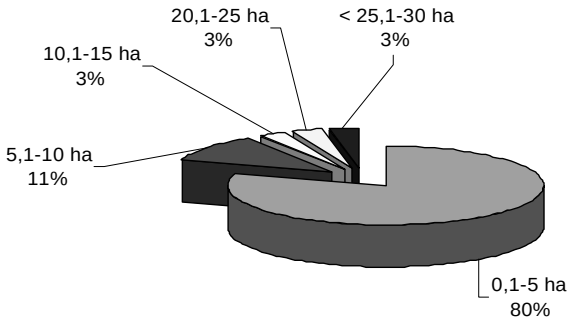

Figure 5. Farm division by the highbush blueberry plantation areas, %

Irrigation. The farms use both surface (sprinkling) and drip irrigation. Some farm irrigation is not yet available and this condition highly affects the further growth, development and production of highbush blueberries.
Fertilizers. Most farmers use soil analysis and adjust the required amount of fertilizers accordingly.
Yield, harvest and quality. The highbush blueberry varieties 'Northland' – 4.12 and 'Patriot' – 4.06 kg from a bush had the highest yields, the lowest the variety 'Chandlers' – 0.32 (Figure 6).

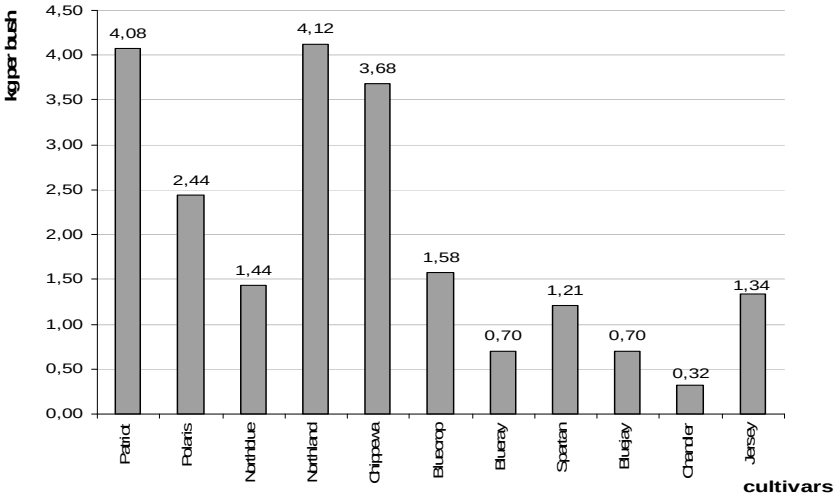

Figure 6. The average yield of highbush blueberry cultivars per bush, kg.
(Training and Research Farm of the Faculty of Agriculture under the LLU in Jelgava)

The largest 100 berry mass was achieved by the variety 'Northblue', using the supplementary fertilizer Vito Silva + B Cu Mo + Caltrac. For the variety 'Patriot', the effects of ferilizers are not essential. Depending on the fertilizer option, the variety 'Northblue' had the largest berries – the proportion of the very big berries was 2 – 7 % and of the big berries – 29 – 40 %. For the variety 'Patriot', proportion of the very big berries was on average - 5 %, but of the big berries on average – 50 % of the total number of berries. The fertilizers used did not essentially affect the berry size.

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SELECTION OF THE EUROPEAN CRANBERRY IN LITHUANIA
EIROPAS DZĒRVEŅU SELEKCIJA LIETUVĀ

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Abstract
A detailed evaluation of the morphological diversity and economical properties of the European cranberry was carried out in 2000 – 2008 in the Kaunas Botanical Garden of Vytautas Magnus University with the purpose of ascertaining the most valuable clones. The European cranberry clones were selected from the strictly protected areas of Čepkeliai, Žuvintas, and Kamanos in 1995 – 1999. These clones with distinctive morphological peculiarities were propagated and planted into acid peat (pH 3.5 – 4.0) in the field collection of the Kaunas Botanical Garden for further investigations. The results of the comprehensive evaluations of the phenotypic and genetic diversity of the European cranberry were crowned with the selection of the most valuable clones. These clones were compared with Estonian and Russian cultivars. Berry size, yield, and the amount of biologically active substances were evaluated. The selected clones are characteristic of exceptional biological and biochemical peculiarities. The five Lithuanian clones 'Amalva', 'Žuvinta', 'Vaiva', 'Vita, and 'Reda' were proposed to recieve the cultivar status.

Kopsavilkums
Novērojumi par Eiropas dzērveņu morfoloģisko daudzveidību un ekonomiskajām īpašībām tika veikti no 2000. – 2008. gadam Vitautas Magnus universitātes Kauņas botāniskajā dārzā ar mērķi noskaidrot vērtīgākos klonus. Eiropas dzērveņu kloni tika izraudzīti no īpaši aizsargājamām teritorijām Čapkeliai, Žuvintas un Kamanos, laikā no 1995. – 1999. gadam. Šie morfoloģiski atšķirīgie kloni tika pavairoti un iestādīti skābā kūdrā (pH 3.5 – 4.0) tālākai izpētei un novērojumiem kolekcijas stādījumos Kauņas botāniskajā dārzā. Pēc vispusīgiem pētījumiem par Eiropas dzērveņu fenotipisko un genotipisko daudzveidību rezultāti ir vainagojušies ar vērtīgāko klonu izlasi. Šie kloni tika salīdzināti ar Igaunijas un Krievijas dzērveņu šķirnēm. Tika vērtēts ogu lielums, raža un bioloģiski aktīvās vielas. Izvēlētajiem kloniem bija īpaši vērtīgas bioloģiskās un bioķīmiskās īpatnības. Pieci Lietuvas kloni 'Amalva', 'Žuvinta', 'Vaiva', 'Vita' and 'Reda' ir pieteiktas, lai saņemtu šķirnes statusu.

Key words: anthocyanins, cultivar, morphological diversity, phenolics, selection

Introduction
The European cranberry (*Vaccinium oxycoccos* L., syn. *Oxycoccus palustris* Pers.) belongs to the most valuable berry plants, whose natural habitats are found in moist boreal forests, ombrothrophic shagnum bogs and minerotrophic fens. This species has been intensively researched for its health properties in recent years. The cultivars of other species American cranberry (*Vaccinium macrocarpon* Aiton, syn. *Oxycoccus macrocarpus* (Aiton) Purs.) are known for their exceptional

economical and biochemical characteristics. This species has been fully introduced in Lithuania. The productive cultivars ‘Ben Lear‘, ‘Early Black, ‘Stevens, and ‘Pilgrim‘ were recommended for berry growers (Budriūnienė, 1998). The European cranberry is well adapted to Lithuanian climate conditions. The obviuos desirable traits of the European cranberry are productivity, fruit size and quality as well as disease resistance. The breeding of new cultivars set a task to enhance the levels of the before mentioned traits. As with most crops, productivity is a main factor for selection to culture.

At present the investigations on the introduction of the European cranberry are being carried out in Russia and other countries of Europe (Gorbunov, 1993).The selection of economically valuable clones of the European cranberry were carried out in the Kostroma Forest Research Station. The long-term research resulted in the selection of six *V. oxyccocos* clones. These clones were registered by the State Commission of the Russian Federation for Testing and Protection of Selection Achievements as the first Russian cultivars ‘Alaya Zapovednaya’, ‘Krasa Severa’, ‘Sazonovskaja’, ‘Severyanka’, ‘Sominskaja’, and ‘Khotavetskaya’ (Cherkasov *et al.*, 1998, Makeev *et al.*, 2000). Six cultivars suitable for garden cultivation have been selected from the wild clones in Estonia as well: ‘Kuresoo’, ’Nigula’, ‘Soontagana’, ‘Maima’, ‘Virussaare’, and ‘Tartu’. In distinguishing the different cultivars special characteristics including berry size and shape have been used (Ruus & Vilbaste, 1968).

The evalution of the European cranberry genetic resources started in Lithuania in 1995. About 120 different cranberry clones were selected from the strictly protected areas of Čepkeliai, Žuvintas, and Kamanos as well as in the raised bogs of the Ignalina, Jurbarkas and Švenčionys districts in 1995 – 1999. The clones of wild cranberry with different morphological properties of leaves, stems, and berries were described. The collected cranberry shoots were used for vegetative propagation. The collection of selected clones was planted in the Kaunas Botanical Garden of VMU. Investigations of seasonal development, morphological and genetic diversity as well as fruiting characteristics were ascertained (Daubaras *et al*., 2006; Areškevičiūtė *et al*., 2006). Five clones of the European cranberry were selected in consideration of productivity, berry size, disease resistance and were approved as suitable to cultivars.

Aim of this study was to evaluate the peculiarities of the five selected Lithuanian clones and compare them with the Russian and Estonian cultivars of the European cranberry.

Material and Methods

Five European cranberry clones as well as the Russian and Estonian cultivars were selected for evaluation. The clones 96-Ž-10, 96-Ž-11, and 99-Ž-10 were selected in the strictly protected reserve Žuvintas, whereas the clones 95-A-05 and 95-A-09 were collected in different mesotrophic bogs in the East Lithuania in 1995, where great morphological variation was noticed. These clones were named, respectively: ‘Vita‘, ‘Amalva‘, ‘Žuvinta‘, ‘Vaiva‘ and ‘Reda‘.The collected cuttings of these clones were planted into acid peat (pH 4.0 – 5.0) beds in the field collection of the Kaunas Botanical Garden of VMU.

A detailed evaluation of the morphological diversity of these clones was carried out in 2000 – 2008. For the morphological characterization berry properties per clone were used: berry size, shape, colour, and waxy layer intensity. The average weight of a berry was calculated by weighing 50 berries in three replications. A 1 – 5 point scale was used for berry weight estimation where 1 stands for very small (<0.3 g), 2 – small (0.3 – 0.5 g), 3 – medium (0.6 – 1.0 g), 4 – large (1.1 – 1.5 g), and 5 – very large berries (>1.5 g). The yield production of the clones was estimated. The distribution of fungal diseases were observed during the vegetation period.

Samples of berries for the estimation of total phenolics compounds and antocyanins amounts were gathered during the stage of berry mass ripening.

The amount of the total phenolics in the cranberry extracts was determined with the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard. The reagent was prepared by diluting a stock solution (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) with distilled water (1:10, v/v). Samples (1.0 ml, two replicates) were introduced into test cuvettes, and then 5.0 ml of Folin-Ciocalteu’s phenol reagent, and 4.0 ml of Na_2CO_3 (7.5 %) was added. The absorbance of all samples was measured at 765 nm using the Genesys10 UV/Vis spectrophotometer (Thermo Spectronic, Rochester, USA) after incubating at

20 °C for 1.0 h. The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 gram of fresh weight. The pigments were extracted from 5 g of frozen berries with acidified (0.1 N HCl, v/v) 95 % (v/v) food grade ethanol. The berries were ground with quartz sand and the extraction was continued with 20 ml portions of solvent until the sample became colourless. The extract was diluted with acidified ethanol. The absorption was measured on a spectrophotometer Genesys-5 (Thermo Spectronic, Rochester, USA) at 544 nm. The concentration of anthocyanins was determined from the calibration curve, which was constructed by measuring the absorption of cyanidin-3-rutinozide (MW 595.2, ε=28.800) reference solutions. The concentration of anthocyanins was calculated using the following formula and expressed in milligrams of cyanidin-3-rutinozide in 100 g of berries:

$$C = \frac{c \times V \times k}{m \times 10}$$

where C – the concentration of anthocyanins in mg/l obtained from the calibration curve;
V – the volume of the extract in ml;
k – the dilution factor;
m – the amount of berries used for the extraction in g.

Observations of seasonal development were made during the entire vegetation period. The collection of the European cranberry clones was observed and assessed twice a week on the same days of the week. The following main phases of seasonal development were recorded: the beginning of shoot growth, the beginning of budding, the beginning of flowering, the end of flowering, the beginning of ripening, the end of ripening, the end of vegetation.

Fort the mathematical - statistical assessment of the data program packet SELEKCIJA was used, which operates as applied to the basic program in the Excel packet. Specific differences were identified with LSD (Tarakanovas & Raudonius, 2003).

Results and Discussion

The selected five Lithuanian European cranberry clones were characteristic of significant phenological plasticity. In different years the beginning of the phenological phases possessed a wide amplitude (from 9 to 15 days) of variation. A comparison of the duration of the vegetation period in 1999 – 2008 revealed no reliable differences between the clones. It was estimated that differences between the clones in the phase of flower bud formation varied from 3 to 8 days and in the phase of the beginning of berry ripening – from 9 to 15 days. There is a risk of late frosts during the cranberry flowering period in late May through mid June, which could cause the loss of flowers and proportionately the berry yield. The flowering period persisted from 16 to 26 days. The berry ripening of the clone ‘Vita‘, ‘Amalva‘, and ‘Žuvinta continued from 32 to 35 days. The clones ‘Reda‘ were characteristic of the most concise ripening period, which continued 20 days, whereas the clone ‘Vaiva‘ was distinguish for long ripening period, i.e. 38 days. The ripening time of berries depended on prevailing meteorological conditions as well as on the clone peculiarities. The beginning of berry ripening was observed from mid to the end of August, whereas ripening ended mid September. The ripening of the Estonian and Russian cultivars were recorded from mid August to mid September. While assessing the adaptation possibilities of *V. oxycoccos* clones and cultivars the period of spring must be distinguished as being critical: late spring frost damage caused considerable decrease or the complete destruction of the berry yield of all clones in 2000 and 2004.

The determination of the berry shape of the European cranberry revealed high variability. The most common were clones and cultivars with oblate or oblong conical berries (Table 1). The average length and width of a berry varied from 0.89 to 1.35 cm and from 1.00 to 1.30 cm, respectively. The Lithuanian clone ‘Reda‘ and the Estonian cultivar ‘Soontagana‘ were conspicuous by their unique pyriform berries. The colour of berries was red or dark red at full maturation. Only the berries of the clone ‘Žuvinta‘ and the cultivar ‘Virussaare‘ were distinguish for the pink colour. The berries of the clone ‘Amalva‘ were covered with a waxy coat. The clone ‘Reda‘ was characteristic of large berries. The Russian cultivar ‘Dar Kostromy‘ ripened exceptionally large berries (1.84 g). Therefore, the European cranberry clones and cultivars with large or very large berries (4 – 5 points) could be compared to the large-fruited cranberry cultivars with medium berry size, such as ‘Searles’, ‘Crowley’, and ‘Franklin’ (Budriūnienė, 1998).

Table 1. Morphological characteristics of European cranberry cultivars and selected Lithuanian clones

Cultivar or clone	Yield, kg/m^2	Berry weight, g	Berry shape	Berry colour
'Vaiva'	2.33	0.81	oblate	red
'Reda'	1.34	1.13	pyriform	dark red
'Vita'	2.06	1.03	round	dark red
'Amalva'	1.80	1.01	oblate	dark red
'Žuvinta'	1.65	0.97	oblate	pink, unevenly coloured
'Nigula'	1.49	1.07	oblong-conical	red
'Kurresoo'	1.60	1.04	oblong-conical	red
'Soontagana'	1.23	1.07	pyriform	purple, strong wax coating
'Maima'	1.16	1.1	oblong-conical	light red
'Virussaare'	1.73	1.10	oblate	pinkish, unevenly coloured
'Krasa Severa'	0.86	1.30	egg-shaped	pink
'Severianka'	0.64	0.94	egg-shaped	dark red
'Dar Kostromy'	0.58	1.85	oblate	dark red
LSD_{01}	0.56	0.135		
S_x%	8.34	4.68		

LSD_{01}- the least significant difference, P<0.01
S_x%- the relative error of an average

The estimation of the berry yield *ex situ* indicated quite big differences among the Lithuanian clones. The most productive was the clone 'Vaiva' with an average yield 2.33 kg m^{-2}. The average yield of other clones varied from 1.34 kg m^{-2} (the clone 'Reda') to 2.06 kg m^{-2} (the clone 'Vita').
The largest damages to leaves, shoots and berries was caused by *Monilinia oxycocci* (Wor.) Honey., *Fusicoccum putrefaciens* Shear, *Botrytis cinerea* Pers., *and Phyllosticta elongata* Weid. The most intensive spread of fungal diseases was occured during the flowering and berry ripening phases. Berry rot diseases damaged about 5 – 15 % of the berry yield.
The differences in the total phenolics and anthocyanins amounts among the clones and cultivars of the European cranberry were ascertained (Table 2).

Table 2. Amounts of total phenolics and athocyanins in berries of the European cranberry.

Cultivar or clone	Total phenolics in berry extract, mg l^{-1}	Total phenolics, mg 100 g^{-1}	Anthocyanins in berry extract, mg l^{-1}	Anthocyanins, mg 100 g^{-1}
'Kuressoo'	401.34±8.65	521.74±10.12	99.58±0.84	129.45±1.11
'Soontagana'	275.84±6.23	349.40±7.56	72.89±0.96	92.34±1.23
'Virussaare'	343.06±7.36	445.98±8.15	64.70±0.53	84.12±0.79
'Nigula'	309.56±6.32	340.51±7.14	123.11±0.69	135.42±0.81
'Maima'	363.64±9.12	387.88±10.24	107.56±0.53	114.74±0.91
'Sazonovskaja'	255.53±8.14	459.95±9.25	135.92±0.35	244.66±0.42
'Dar Kostromy'	268.66±7.65	331.34±8.14	69.96±0.45	86.28±0.60
'Vaiva'	416.93±5.17	403.03±6.55	88.03±0.35	85.09±0.45
'Žuvinta'	297.47±8.52	386.71±11.07	51.26±0.44	66.64±0.57
'Amalva'	353.18±16.27	423.82±26.53	98.74±1.03	118.49±1.24
'Vita'	371.50±8.84	309.50±10.02	63.87±0.59	72.02±0.67
'Reda'	273.59±9.32	428.63±10.50	63.24±0.89	99.07±1.39

The largest amounts of total phenolics were found to be in berries of the Estonian cultivar 'Kuressoo' (521.74 mg 100 g^{-1}). The berries of the European cranberry accumulated from 66.64 to 244.66 mg 100 g^{-1} of anthocyanins. The cultivar 'Sazonovskaja' was characteristic of an exceptionally large amount of anthocyanins (244,66 mg 100 g^{-1}). Anthocyanins in berries of the

investigated European cranberry accessions comprised from 18.8 to 53.3 % of the total phenolic compounds.

As several authors have reported cranberries and these specific biochemical components are being associated with human health attributes, such as maintenance of urinary tract health and antioxidant status (Povilaitytė *et al*., 1998; Vorsa *et al*., 2002). The berries of European cranberry are one of the best sources of phenolic compounds as compared with the other berry plants, such as strawberry, black currant, raspberry etc. (Moyer *et al.*, 2002). The American cranberry has been increasingly researched for its health properties in recent years as well. Significant genetic variability was found for total phenolics, total anthocyanins, proanthocyanidins, soluble solids, quinic acid, citric acid etc. (Vorsa *et al*., 2002; Zeldin *et al*., 2007). At present large-fruited cranberries are successfully cultivated by amateur gardeners and even some commercial plantations are being established in Lithuania. Nevertheless, the wild cranberry is more suitable for cultivation on harvested peat bogs as well as for their renaturalisation.

Therefore, there is an evident need to conserve wild cranberries *ex situ* in order to complement their conservation *in situ* and facilitate the investigation and utilisation of this wild crop relative. The unique collection of genetic resources of the European cranberry was established at the Kaunas Botanical Garden of Vytautas Magnus University with the purpose to preserve the most valuable clones. In consequence of their comprehensive evaluations the selection of five prospective clones was achieved.

Conclusions

The estimation of the berry yield and berry size indicated statistically reliable differences among the selected Lithuanian clones. The clone 'Vaiva' was characteristic of the highest yield.

The largest damage was caused by the fungal dieseases *Monilinia oxycocci*, *Fusicoccum putrefaciens*, *Botrytis cinerea*, *and Phyllosticta elongata.* Berry rot diseases damaged about 5 – 15 % of the berry yield.

The berries of the European cranberry accumulated from 66.64 to 244.66 mg 100 g^{-1} of anthocyanins and from 309.50 to 521.74 mg 100 g^{-1} of the total phenolics.

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THE EVALUATION OF EUROPEAN CRANBERRYBUSH (*VIBURNUM OPULUS*) FOR BREEDING IN LITHUANIA
IRBENES (*VIBURNUM OPULUS*) PIEMĒROTĪBA AUDZĒŠANAI LIETUVĀ

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Abstract
The investigations were carried out at the European cranberrybush (*Viburnum opulus* L.) collection at the Kaunas Botanical Garden of Vytautas Magnus University in 1999 – 2005. The biological peculiarities of cranberrybush cultivars and clones were estimated according to the usual methods of the evaluation of horticulture plants. Reliable differences were determined between accessions with respect to productivity, the number of fruit per cluster and the mean weight of the fruit. The investigations of the biochemical composition of the fruit of different cultivars and clones revealed significant differences in the amounts of phenolic compounds (anthocyanins and flavonols), benzoic, and ascorbic acids. The accession of Lithuanian origin P1 was typical of the largest amount of ascorbic acid (46.1 mg 100 g^{-1}). The fruit of the cultivar 'Leningradskaja Otbornaja' accumulated the largest amounts of anthocyanins. The cultivars 'Krasnaja Grozd' as well as the clone P2 had the largest benzoic acid amounts. The value of the most productive European cranberrybush accessions were determined by the number of fruit in a cluster. The results of the biochemical investigations corroborated that the selection of European cranberrybush accessions in respect to benzoic acid, anthocyanins and ascorbic acid amounts could be advisable.

Kopsavilkums
Izmēģinājumi tika veikti irbeņu (*Viburnum opulus* L.) kolekcijas stādījumā Vitauta Magnus universitātes Kauņas botāniskajā dārzā no 1999. līdz 2005. gadam. Tika noteiktas dažādu irbeņu šķirņu un klonu bioloģiskās īpašības. Atrastas būtiskas atšķirības starp dažādu paraugu ražību, augļu skaitu ķekarā un augļa vidējo masu. Augļu bioķīmiskā sastāva pētījumi uzrādīja būtiskas atšķirības starp dažādu šķirņu un klonu fenolu (antocianīni un flavonoli), benzoskābes un askorbīnskābes saturu. Lietuvas izcelsmes paraugs P1 saturēja visvairāk askorbīnskābes – 46.1 mg $100g^{-1}$. Šķirnes 'Leningradskaja Otbornaja' augļi saturēja visvairāk antocianīnu, bet šķirne 'Krasnaja Grozd' un klons P2 – visvairāk benzoskābes. Produktīvāko irbenes vērtību noteica augļu skaits ķekarā. Bioķīmiskā sastāva pētījumu rezultāti rāda, ka būtu ieteicams veikt irbeņu selekciju attiecībā uz askorbīnskābes, benzoskābes un antocianīnu saturu.

Key words: evaluation, biochemical composition, cultivars, productivity, breeding.

Introduction
The species of genus *Viburnum* L. such as *Viburnum opulus* L. (European cranberrybush), *V. trilobum* Marsh. (American cranberrybush) as well as *V. sargentii* Koehne are widely used in traditional and folk medicine. The European cranberrybush (*Viburnum opulus* L.) is a native plant in Lithuania which is widely used in traditional and folk medicine. Its flowers, bark and leaves are an important medicinal raw because it possesses large amounts of the tanic substances, carotenoids, isovalerianic acid, saponines, and glycosides. The seeds contain up to 21 % of fatty oil (Bock *et al*., 1978). Fruit of this species accumulate significant amounts of biologically active substances. The

fruit of *V. opulus* has different amounts of ascorbic acid, pectin, carotenoids, flavonols, tocopherols, anthocyanins, different polysaccharides (Евтухова *et al*., 2000; Евтухова *et al*., 2002; Лобанова *et al*., 1999; Оводова *et al*., 2000; Velioglu *et al*., 2006; Jordheim *et al*., 2007).

The collection of *V. opulus* accessions at Kaunas Botanical Garden was started in 1998. The evaluation of collected samples was done during 1999 – 2008. The most comprehensive research work on *Viburnum sp*. was accomplished hitherto in Russia and Ukraine. Different cultivars were added to the Russian official register and recommended for growing (Куденков М. and Чурканенко Н., 1998). The Ukrainian selections have small bushes, dark red fruits with a weaker bitter quality and a yield of 8 – 9 t ha^{-1} (Panteev *et al*., 1995).

The investigation of productivity, fruit properties and biochemical components of *V. opulus* accessions and the selection of the most valuable clones and cultivars makes it possibility to use them for breeding.

The aim of present study was to investigate the variation of yield, fruit properties and biochemical components of *V. opulus* accessions and select the most valuable clones and cultivars for preservation and breeding in Lithuania.

Materials and methods

The European cranberrybush cultivars of Russian origin, the clones P1 (of Lithuanian origin) and P2 (of Ukrainian origin) were selected for evaluation. All accessions are proving easy to grow and exceptionally winter hardy. Fruit production began within two years after planting.

The yield and fruit properties (yield per bush, number of fruit in a cluster and mean weight of a fruit) were studied. The results were statistically analyzed and regression (R^2) and variation (CV) coefficients were calculated using STAT for Excel.

For the average fruit weight 100 fruits were collected and examined. 20 racemes were examined to evaluate fruit number per raceme and 10 bushes were examined to evaluate the yield per bush..

The investigations of the biochemical composition of the fruit were conducted in their mature stage. The amount of ascorbic acid was ascertained by the Tilmans reaction. It was titrated by a solution of 2.6-dichlorphenolindophenol sodium salt. The pigments were extracted with 95 % (v/v) grade ethanol acidified with 0.1 M HCl with the purpose to assay the total amount of anthocyanins. The total amount of anthocyanins was expressed by cyanidin 3-rutinoside. The amount of flavonols was expressed by rutin and determined spectrophotomerically at a wavelength of 440 nm. The method of benzoic acid determination was based on steam distillation. The spectrophotometric analysis was carried out by a reaction of benzoic acid with hydroxylamine-HCl and peroxide at a wavelength of 315 nm (Ермаков А. and Арасимович В., 1987; Helrich, 1990).

Results and Discussion

The investigation showed that accessions differed in their yield per bush, the number of fruit in a cluster and the mean weight of the fruit as well. Reliable differences between the cultivar ‘Leningradskaja Otbornaja’ and other accessions were determined. A constant large average yield was typical of the cultivars ‘Leningradskaja Otbornaja’, P3 and ‘Zarnica’ 8.5 – 6.2 kg per bush. The cultivars ‘Zarnica-2’, ‘Souzga’, and ‘Šukšinskaja’ had the lowest yields 2.6 – 3.2 kg per bush (Table 1).

The accessions of *V. opulus* were distinguished by significant differences in the size of a raceme. The number of fruit in a raceme varied from 28.1±5.83 (‘Upninkai’) to 51.0±6.1 (cv. ‘Krasnaja Grozd’).

The cultivars ‘Upninkai’ and ‘Leningradskaja Otbornaja’ produced the largest fruit (0.74±0.09 g and 0.66±0.05 g, respectively). The cultivar ‘Šukšinskaja’ and P3 were typical of the smallest fruit, 0.45±0.04 g and 0.47±0.04 g, respectively. Tge length of a cluster stalk varied from 2.9±0.10 (‘Leningradskaja Otbornaja’) to 4.6±0.21 cm (P3).

Statistically reliable differences were determined between the mean weight of a fruit. Estimates of variability indicated that the most stable trait was the mean weight of a fruit. The coefficient of variation did not exceed 20 %, except in the cv. ‘Krasnaja Grozd’ (22.7 %). The stability of fruit number in a cluster differed subject to the accession. The variation of fruit number in a cluster of accessions P1 and ‘Leningradskaja Otbornaja’ was low (CV < 10 %). The medium coefficient of variation (10 % < CV < 20 %) was determined for the clone P2 and the cultivars ‘Krasnaja Grozd’,

'Kijevskaja Sadovaja', and 'Zarnica'. The high variability of fruit number in a cluster was typical of the cultivars 'Souzga' and 'Šukšinskaja', respectively: 30.1 % and 25.4 %.
Regression analysis revealed that the response of yield per bush to the amount of fruit in a cluster could be best expressed by the exponential equation $y = 0.33685 \times e^{0.4969x}$ at the coefficient of determination $R^2 = 0.895$ (Fisher's criterion F=88.4**). The relationship between the yield and mean weight of a fruit was poor and not significant while the coefficient of regression $R^2 = 0.138$ (Fisher's criterion F=4.8*).
On the basis of the obtained results it is possible to state that the number of fruit in a cluster is one in the most important indexes for the selection of *V. opulus* accessions with predictable high yield.

Table 1**.** Comparison of *V. opulus* accessions fruiting characteristics, 2000 – 2007

Accession	Mean fruit weight, g	Fruit number per raceme	Stalk length of raceme, cm	Yield, kg/bush
'Krasnaja Grozd'	0.51 *abcd*	51.0 *e*	3.1 *ab*	5.8 *abc*
'Kijevskaja Sadovaja'	0.64 *efgh*	34.4 *abc*	3.8 *bcde*	4.1 *abc*
'Leningradskaja Otbornaja'	0.66 *fgh*	43.1 *bcde*	2.9 *a*	8.5 *c*
'Zarnica'	0.56 *cdef*	35.1 *abcde*	3.6 *abcd*	6.2 *abc*
'Souzga'	0.54 *bcde*	32.3 *ab*	3.9 *cde*	2.7 *a*
'Šukšinskaja'	0.45 *ab*	33.8 *abc*	3.6 *bcd*	3.0 *a*
'Zarnica-2'	0.51 *abcd*	33.8 *abc*	4.2 *de*	2.6 *a*
'Upninkai'	0.74 *h*	28.1 *a*	3.9 *cde*	4.3 *bc*
P1	0.58 *def*	37.8 *abcde*	4.2 *de*	3.2 *abc*
P2	0.56 *cdef*	35.0 *abcde*	3.2 *abc*	5.9 *abc*
P3	0.47 *abc*	42.5 *bcde*	4.6 *efg*	6.3 *abc*

Means followed by the same letter in columns are not significantly different at the P=0.05 (Duncan's multiple range test).

The evaluation of the fruits chemical composition showed that they accumulate quite large amounts of flavonols (Figure 1).
The largest amount of flavonols was found in the fruits of the cultivars 'Krasnaja Grozd' and P1 (on average about 18 mg 100 g^{-1}). The lowest content of flavonols was found in the fruits of the cultivar P2 – less than 14 mg 100 g^{-1}.

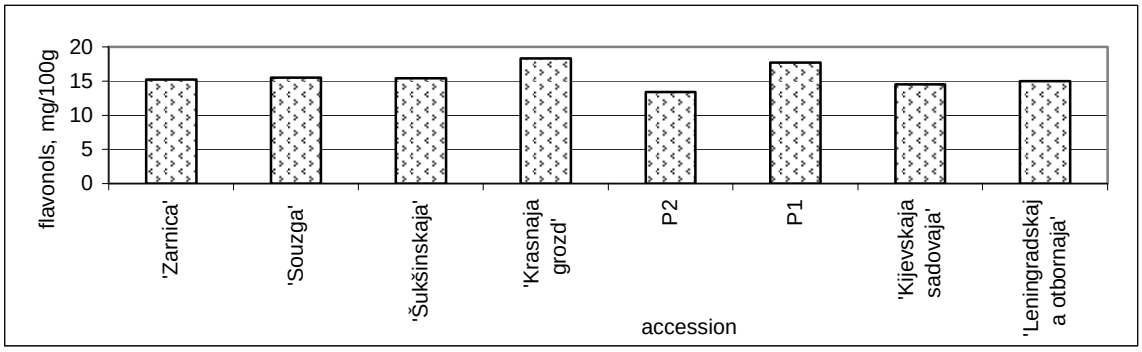

Figure 1. Amounts of flavonols in the fruits of *V. opulus,* accessions, 1999 – 2002

The accessions were compared according to the amounts of ascorbic acid, anthocyanins, polyphenolic compounds, and benzoic acid. Statistically reliable differences were ascertained (Table 2).
The fruit of different accessions accumulated from 22.9 mg 100 g^{-1} (P3) to 49.9 mg 100 g^{-1} ('Leningradskaja Otbornaja') of anthocyanins and from 732.5 mg 100 g^{-1} ('Souzga') to 1280.0 mg 100 g^{-1} (P3) of polyphenolic compounds. The smallest amount of anthocyanuins was noted in the fruits of the cultivar P3 (22.9 mg 100 g^{-1}) and the smallest amount of polyphenolic compounds in the fruits of the cultivar 'Souzga' (732.5 mg 100 g^{-1}). The amount of ascorbic acid content ranged from 13.0 mg 100 g^{-1} ('Souzga') to 44.7 mg 100 g^{-1} (P3).

As several authors have stated (Kozłowski *et al.*, 2006; Рупасова and Василевская, 1999), the berries of the *Ericaceae* family species accumulate benzoic acid on an average of 65 mg 100 g^{-1}. Benzoic acid has been regarded as the preserving agent responsible for the good preservative qualities of cranberry, cowberry and products manufactured from them (Shwartz and Medrek, 1968; Smolarz, 2003). The fruit of *V. opulus* accessions accumulated benzoic acid, with an average of 12.4±0.90 mg 100 g^{-1}. The amounts of benzoic acid in different V. opulus accessions are presented in the fig. 2. The cultivar P2 accumulated the highest amount of benzoic acid (16.3 mg 100 g^{-1}).

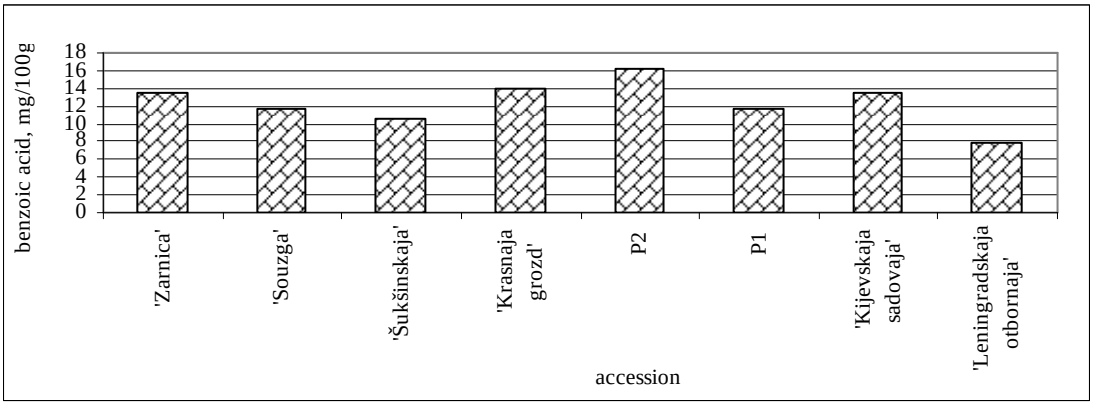

Figure 2. Amounts of benzoic acid in the fruits of *V. opulus,* accessions, 1999 – 2002

The statistical analysis of the biochemical components of the fruit revealed a high variability between the accessions investigated. The most stable biochemical characteristic was the amount of flavonols (CV=10.4 %). Very high variations in the amount of ascorbic acid, anthocyanins and benzoic acid amounts was ascertained (45.7, 24.1 and 20.4 %, respectively).

Table 2. Amount of biochemical compounds in the fruit of *V. opulus* accessions, 2007

Accession	Biochemical components, mg 100 g^{-1}					
	Polyphenolic compounds		Anthocyanins		Ascorbic acid	
'Krasnaja Grozd'	1136.0	*bc*	36.8	*defg*	20.9	*b*
'Kijevskaja Sadovaja'	1103.1	*bc*	35.7	*def*	26.6	*c*
'Leningradskaja Otbornaja'	962.0	*ab*	49.9	*i*	13.8	*a*
'Zarnica'	976.9	*ab*	39.0	*fg*	26.4	*c*
'Souzga'	732.5	*a*	34.0	*cd*	13.0	*a*
'Šukšinskaja'	1007.0	*bc*	31.0	*bc*	31.9	*d*
'Zarnica-2'	1002.5	*abc*	38.4	*def*	21.2	*b*
'Upninkai'	1065.6	*bc*	35.2	*de*	33.3	*de*
P1	1157.9	*bcd*	40.4	*f*	32.2	*g*
P2	1220.5	*bcdef*	37.9	*cdef*	35.3	*de*
P3	1280.0	*cdef*	22.9	*a*	44.7	*f*

Means followed by the same letter in columns are not significantly different at the P=0.01(Duncan's multiple range test).

As a result of the research the most valuable accessions ('Leningradskaja Otbornaja', P3, 'Zarnica' and 'Krasnaja Grozd') in regard to the largest productivity and significant amounts of biologically active substances could be selected for the breeding.

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PROPAGATION AND CULTIVATION OF VACCINIUM SPECIES AND LESS KNOWN SMALL FRUITS
VACCINIUM ĢINTS SUGU UN MAZĀK ZINĀMO AUGĻAUGU PAVAIROŠANA UN AUDZĒŠANA

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Abstract
The production of *Vaccinium* species crops has recently been the subject of much interest globally because of an improved understanding of the important role of dietary fruit in maintaining human health. Cloudberry (*Rubus chamaemorus* L., family *Rosaceae*), a less known small fruit of medicinal importance, and the Vaccinium species are genetically heterozygous and do not reproduce progeny from seed that are similar to the seed parent. Tremendous progress in plant tissue culture, resulting in great advances in micropropagation, has occurred in these crops. Of particular significance has been the evolution of the technology permitting multiplication of these plants through bioreactor micropropagation. The in vitro morphogenesis seems to be highly dependent on the plant growth regulators and media used for the culture, which is again genotype specific. Although the automation of micropropagation in bioreactors has been advanced as a possible way of reducing propagation cost, optimal plant production depends upon a better understanding of the physiological and biochemical responses of plants to the signals of the culture microenvironment and an optimization of specific physical and chemical culture conditions to control the morphogenesis of berry plants in liquid culture systems. Clonal fidelity can be a serious problem and molecular strategies have been developed to reduce the variation to manageable levels. The paper focuses on conventional and bioreactor systems used for the in vitro culture of the *Vaccinium* species and cloudberry, cultivation of micropropagules and the employment of molecular markers in micropropagated plants for the assessment of genetic fidelity, uniformity, stability and true-to-typeness among donor plants and tissue culture regenerants.

Kopsavilkums
Vaccinium ģints kultūraugu audzēšanai pēdējā laikā visā pasaulē pievērsta pastiprināta uzmanība, jo arvien labāk tiek izprasta šo augļu diētiskā nozīme cilvēka veselības saglabāšanai. Lācenes (*Rubus chamaemorus* L., *Rosaceae* dzimta) – mazāk zināmi augļi ar medicīnisku nozīmi un *Vaccinium* ģints sugas ir ģenētiski heterozigoti un, pavairojot ar sēklām, nedod vecākiem līdzīgus pēcnācējus. Milzīgais progress augu šūnu kultūru izpētē ir izraisījis arī lielus uzlabojumus šo kultūraugu mikropavairošanā. Īpaši nozīmīga ir bijusi tādas tehnoloģijas attīstība, kas ļauj šo augu mikropavairošanu veikt bioreaktorā. Morfoģenēze *in vitro* apstākļos ir ļoti atkarīga no kultivēšanā izmantotajiem augu augšanas regulatoriem un barotnes, kas ir atkarīga no genotipa. Lai gan mikropavairošanas automatizācija bioreaktoros ir attīstīta kā iespējamais pavairošanas izmaksu samazināšanas ceļš, optimāla stādu ražošana ir atkarīga no labākas izpratnes par augu bioķīmisko un fizioloģisko reakciju uz mikrovides signāliem un specifisku fizikālu un ķīmisku kultivēšanas apstākļu optimizācijas, lai kontrolētu ogaugu morfoģenēzi šķidrās kultivēšanas sistēmās. Klonālā mainība var būt nopietna problēma, tāpēc ir izveidotas molekulāras metodes, lai samazinātu šo mainību līdz iespējami zemākam līmenim. Šajā rakstā lielākā uzmanība pievērsta konvencionālajām un bioreaktora sistēmām *Vaccinium* ģints kultūraugu un lāceņu *in vitro* pavairošanai, mikropavairoto augu kultivēšanai un molekulāro marķieru izmantošanai ģenētiskās atbilstības, viendabīguma, stabilitātes un autentitātes novērtēšanai starp donora augiem un audu kultūrā iegūtajiem stādiem.

Key words: cloudberry, propagation, micropropagation, *in vitro*, genotype.

Introduction
The genus *Vaccinium* L. (family: *Ericaceae*) contains about 400 species, and one or more species are native to all continents except Antarctica and Australia (Vander Kloet, 1988; Ballington, 2001). It is typically characterized as having fleshy, more-or-less edible fruits with very high levels of vitamin C, cellulose, pectin and anthocyanins possessing antitumor, antiulcer, antioxidant and antiinflammatory activities (Wang *et al.*, 1999). The proanthocyanidins in cranberries have been shown to help prevent urinary tract infections through reduced adhesion of uropathogenic Escherichia coli (Howell *et al.*, 2005). Lingonberry fruits and leaves are used to lower cholesterol levels and treat stomach disorders, rheumatic diseases, and bladder and kidney infections (Novelli, 2003). Blueberry (*Vaccinium* spp.), cranberry (*V. macrocarpon* Ait.), and lingonberry (*V. vitis-idaea* L.) are three commercially cultivated *Vaccinium* fruit crops of economic importance. Although the majority of cultivated blueberry hectarage is in the United States and in Canada, they are also grown commercially in Europe, Asia, Africa, Australia, New Zealand and South America (Lehnert, 2008). While the leading countries in cranberry production are the United States, Canada, Latvia and Poland; its culture has also shown promise in Austria, Germany and Russia (http:////aesop.rutgers.edu/~bluecran/cranberrypage.htm).
Commercial lingonberry production primarily involves the harvesting of berries from wild populations in northern Europe, Asia and North America, with cultivated production still in its infancy compared with cranberries and blueberries (Ballington, 2001). The cloudberry (*Rubus chamaemorus* L., family *Rosaceae*), a less known small fruit crop, is a boreal circumpolar, rhizomatous dioecious perennial herb common to bogs. The berries and leaves of cloudberry are rich in vitamin C and tannins, and possess high ellagic acid content (Amakura *et al.*, 2000). Cloudberries are used medicinally to treat scurvey and diarrhea in traditional medicine (Thiem, 2003).
Although conventional vegetative propagation methods by cuttings or rhizome divisions are successful in these species, the micropropagation of selected germplasm can potentially multiply plants more rapidly than traditional propagation methods. Various culture conditions, basal media and growth regulators have been investigated for the micropropagation of these crops on semi-solid gelled media (for review, please see Debnath, 2003a, 2006a, 2007a).
However, these techniques are difficult to automate and the production cost is high. Automated bioreactors for large scale production of micropropagated plants are important for the micropropagation industry. Bioreactors are self-contained, sterile environments which capitalize on liquid nutrient or liquid/air inflow and outflow systems, designed for intensive culture and control

over microenvironmental conditions – aeration, agitation, dissolved oxygen, etc. (Paek *et al.*, 2005). This review provides an overview of in vitro culture and the production of micropropagated plants of blueberry, cranberry, lingonberry and cloudberry, and also highlights the research efforts of our programme at the Atlantic Cool Climate Crop Research Centre of Agriculture and Agri-Food Canada in St. John's, Newfoundland and Labrador.

Blueberry. There are five major groups of blueberry species which are commercially-grown: 1) lowbush (*V. angustifolium* Ait., *V. myrtilloides* Michx., V. boreale Hall and Aald.), 2) highbush (*V. corymbosum* L.), 3) half-high, which are hybrid or backcross derivatives of highbush-lowbush hybridizations; 4) southern highbush, which were developed from the hybridization of *V. corymbosum* with one or more species (mainly V. darrowi Camp and V. ashei Reade); and 5) rabbiteye (*V. ashei*). Micropropagation techniques using gelled media for axillary shoot production have been developed for lowbush (Debnath, 2004, 2007b), highbush (Gajdošová *et al.*, 2006; Litwińczuk and Wadas, 2008; Tetsumura *et al.*, 2008) and rabbiteye (Lyrene, 1980) blueberries.

Shoot cultures can be initiated from nodal segments or from shoot tips. Media with low ionic concentrations are suitable for *Vaccinium* culture (Debnath and McRae, 2001a). While the woody plant medium (WPM) (Lloyd and McCown, 1980) was the best for highbush blueberry micropropagation (Sedlak and Paprstein 2009), Debnath (2004, 2007b) established in vitro lowbush blueberry cultures on a modified cranberry tissue culture medium (BM-C) (Debnath and McRae, 2001a).

Tetsumura *et al.* (2008) observed a mixture of equal parts of Murashige and Skoog (1962) (MS) and WPM containing 20 µM zeatin was the best for in vitro shoot proliferation of highbush blueberry cultivars. Zeatin was effective for shoot initiation and proliferation of lowbush blueberries (Debnath, 2004) although Gonzalez *et al.* (2000) observed the best shoot multiplication of highbush blueberry with 25 µM N6-[2-isopentenyl] adenine (2iP) in the culture medium.

A low concentration of an auxin [5.7 µM 3-indolyl-acetic acid (IAA)] is beneficial when added to the induction medium (Morrison *et al.*, 2000). However, using low levels of zeatin (2-4 µM) and sucrose (20 g l^{-1}), Debnath (2004) reported an increased the in vitro-shoot multiplication rate of the lowbush blueberry by about 50 to 100-fold over a 12-week interval when shoots were exposed to lower irradiance (15 µmol m^{-2} s^{-1}). A major problem in blueberry micropropagation is the formation of unwanted callus at the base of the explants and the occurrence of spontaneous adventitious shoots (Litwińczuk and Wadas, 2008).

Cao *et al.* (2002) reported shoot regeneration in the highbush blueberry based on a two-step pre-treatment and regeneration on TDZ medium. Explants of 2-week-old shoot cultures were incubated the following regime: pretreatment medium # 1 containing 5 µM TDZ and 2.6 µM naphthalene acetic acid (NAA) for 4 days, pretreatment medium #2 containing 7 µM zeatin riboside and 2.6 µM NAA for 3 days, regeneration medium containing 1 µM TDZ for 6 weeks, and last on a medium without growth regulators for 10 days. Debnath (2009a) developed a two-step shoot regeneration protocol in lowbush blueberry where leaf cultures produced multiple buds and shoots on 2.3–4.5 µM TDZ within 6 wk of culture initiation. The greatest shoot regeneration came from young expanding basal leaf segments positioned with the adaxial side touching the culture medium and maintained for 2 weeks in darkness. TDZ-initiated cultures were transferred to a medium containing 2.3–4.6 µM zeatin and produced usable shoots after one additional subculture.

Application of bioreactor micropropagation in Vaccinium crops is still at the infancy stage. A protocol for Vaccinium micropropagation using a temporary immersion bioreactor (TIB) system in a liquid medium combined with a in vitro culture on a semi-solid gelled medium has been developed in the author's laboratory. Successful shoot regeneration and proliferation have been obtained in the lowbush blueberry (Figure 1.), cranberry and lingonberry (S. C. Debnath, unpublished).

In vitro-derived shoots are rooted either in vitro (Litwińczuk and Wadas, 2008; Tetsumura *et al.*, 2008) or, most frequently, in *ex vitro* conditions on an acidic substrate such as 1 peat : 1 perlite (v/v) (Gonzalez *et al.*, 2000) and 4 peat : 2 vermiculite : 1 perlite (v/v/v) (Morison *et al.*, 2000) without an auxin-pretreatment. An auxin-pretreatment was unnecessary for the *ex vitro* rooting of blueberries (Gonzalez *et al.*, 2000) although Debnath (2009a) found 80 % to 90 % rooting in lowbush blueberries when microshoots were dipped in 4.9 mM 3-indolebutyric acid (IBA) before planting in 3 peat : 2 perlite (v/v) medium. For *ex vitro* rooting, the microcuttings are generally

maintained in a mist chamber with very high relative humidity (95 %) and then transferred to a greenhouse (85 % relative humidity, RH) for acclimatization. In vitro rooting can be induced in the shoot proliferation medium containing 1-2 µM zeatin (S.C Debnath, personal communication) or without plant growth regulators (PGR) (Tetsumura *et al.*, 2008).

Figure 1. Shoot proliferation of wild lowbush blueberry 12 weeks after transfer to a bioreactor system containing liquid medium supplemented with 2 µM zeatin.

Cranberry. Marcotrigiano and McGlew (1991) and Smagula and Harker (1997) recommend a high 2iP concentration along with an auxin (IAA or IBA) in the culture media to increase cranberry shoot proliferation. Debnath and McRae (2001b) established *in vitro* cranberry cultures and maintained them in a medium containing low levels of cytokinin to avoid excessive callus formation at the base of explants and the formation of somaclonal variants.

Shoot organogenesis from cranberry explants has been reviewed by McCown and Zeldin (2005). A number of factors such as genotype, culture medium (including growth regulators and their combinations), the physical environment, the explant development stage, etc. can affect adventitious shoot regeneration. Qu *et al.* (2000) regenerated shoots from cranberry leaves by culturing on a basal medium supplemented with 10 µM TDZ + 5 µM 2iP. Elongation of adventitious shoots began 2 weeks after transfer to the basal medium without growth regulators.

Both *in vitro* and *ex vitro* methods have successfully been used to root and acclimatize micropropagated cranberry shoots (Qu *et al.*, 2000; Debnath and McRae, 2001b). For *in vitro* rooting, shoots are cut at the base and then placed onto an auxin-free medium (Qu *et al.*, 2000; Debnath and McRae, 2001b, 2005). *In vitro*-derived shoots (>1.5 cm long) can also be rooted *ex vitro* in shredded sphagnum moss in pots (Qu *et al.*, 2000).

Debnath and McRae (2005) developed a protocol that enables cranberry multiplication in one step, i.e. multiplying shoots and having them rooted in the same culture medium containing 2-4 µM zeatin. The main advantage of this protocol is that all the shoot tips of the *in vitro*-grown plantlets can be used for shoot proliferation and rooting, whereas basal rooted nodal segments can be transferred to the peat-perlite medium and acclimatized in the greenhouse (Debnath, 2008).

Lingonberry. Lingonberries grow wild in diverse habitats, ranging from lowland to upland and mountain areas, in largely acid soils to pure peat bogs (Gustavsson, 1997). Two subspecies of *V. vitis-idaea* have been recognized: the larger lowland race as *V. vitis-idaea* ssp. *vitis-idaea* (L.) Britton and the dwarf arctic-montane race as *V. vitis-idaea* ssp. minus (Lodd.) Hult. (Hulten, 1949).

Various culture conditions, basal media, and growth regulators have been investigated for axillary shoot proliferation of the lingonberry (Debnath and McRae, 2001a; Jaakola *et al.*, 2001; Debnath, 2005a, b). Debnath and McRae (2001a) compared four different media for the shoot proliferation of lingonberry cultivars: 'Regal', 'Splendor' and 'Erntedank', and found that a reasonable balance of shoot multiplication rate and desirable growth characteristics was attained in a new medium

(BM-C) formulated in the author's laboratory (Debnath and McRae, 2001a). Debnath (2005a) observed that TDZ supported shoot proliferation in lingonberries at low concentrations (0.1 to 1 µM) but inhibited shoot elongation. However, usable shoots were obtained within 4 weeks by transferring shoot clusters to the culture medium containing 1µM zeatin. In the lingonberry, shoot proliferation is greatly influenced by explant orientation, changing the orientation of explants from vertically upright to horizontal increases the axillary shoot number, but decreases shoot height and leaf number per shoot (Debnath, 2005a). Debnath (2005b) observed that the best response was afforded by sucrose at 20 g l^{-1} both in terms of explant response and shoot development potential, although glucose supported shoot growth equally well, and in a wild clone at 10 g l^{-1} it resulted in better in vitro growth than sucrose.

The first adventitious shoot regeneration from lingonberry leaves was described by Debnath and McRae (2002). Later, the regeneration efficiency has been much improved by Debnath (2005c) where leaf explants were cultured on the 1-5 µM TDZ-containing a nutrient medium for 8 weeks for bud and shoot regeneration followed by transferring on to the medium containing 1-2 µM zeatin for shoot elongation. Adventitious shoots have also been regenerated from hypocotyl segments of seedlings from open-pollinated seeds of lingonberry cultivars and a wild clone (Debnath, 2003b). Multiple bud and shoot regeneration can be obtained using apical segments of the hypocotyl from in vitro-grown lingonberry seedlings by incorporating 5-10 µM TDZ in the regeneration medium. Such TDZ-induced buds can be proliferated and elongated on a shoot proliferation medium containing 1-2 µM zeatin and 20 g l^{-1} sucrose. Callus, bud, and shoot regeneration frequency, callus growth, and the number of buds and shoots per regenerating explant depend not only on the specific segment of the hypocotyl, but also on the parental genotype (Debnath, 2003b).

For rooting, 3 to 4 cm long *in vitro*-derived shoots are excised just above the original explant, dipped in 39.4 mM IBA powder, planted in a 2 peat : 1 perlite (v/v) medium and maintained in a humidity chamber [(22 ∀ 2EC, 95 % RH, 16 h photoperiod, 55 µmol m^{-2} s^{-1} photosynthetic photon flux (PPF)]. *In vitro* proliferated shoots root easily within 4 weeks. Plantlets can be acclimatized by gradually lowering the humidity over 2 to 3 weeks and hardened-off plants can be maintained in the greenhouse at 20 ∀ 2EC, 85 % RH, and 16 h photoperiod at a maximum PPF of 90 µmol m^{-2} s^{-1} (Debnath and McRae, 2001a, 2002; Debnath, 2003a, b; 2005a, b, c).

Cloudberry. *In vitro* propagation of cloudberry has been reported in a gelled medium through axillary shoot initiation from seedling explants (Thiem, 2001) and through meristem cultures (Martinussen *et al.*, 2004). When meristem cultures were sub-cultured from clusters of 3 – 5 shoots, approximately 70 and 50 shoots were produced per cluster within 6 weeks at 8.9 µM BAP for the female cv. 'Fjellgull' and the male cv. 'Apollen', respectively. The addition of 5.5 µM gibberellic acid (GA3) reduced the number of shoots. Auxins (IBA, NAA) promoted root development *in vitro*, but inhibited the formation of new shoots (Martinussen *et al.*, 2004).

Debnath (2007c) established a protocol for the *in vitro* culture of wild cloudberry clones using a bioreactor system combined with a gelled medium. Cultures were established on a modified cranberry (*V. macrocarpon* Ait.) tissue culture medium containing 8.9 µM BAP. The addition of 5.8 µM GA3 in 8.9 µM BAP-contained medium improved shoot proliferation. TDZ supported rapid shoot proliferation at low concentration (1.1 µM) but induced a 20 to 30 % hyperhydricity in a plastic airlift bioreactor system containing a liquid medium. The bioreactor-multiplied hyperhydric shoots were transferred to a gelled medium containing 8.9 µM BAP and 5.8 µM GA3 and produced normal shoots within 4 weeks of culture. Proliferated shoots were rooted on a potting medium with a 65 % to 75 % survivability rate of rooted plants. Growth and morphology of micropropagated plants. Increased branching and vigorous vegetative growth are often noted in plants produced through *in vitro* culture. Morrison *et al.* (2000) observed that micropropagated lowbush blueberry plants from shoots that passed through several subcultures produced ten-fold more rhizomes than those of stem cuttings. The micropropagated lowbush blueberry plants produced longer and more stems with more leaves per stem than the conventional cuttings (Debnath, 2007b).

Softwood cutting-derived 'Herbert' highbush plants grew more slowly and produced less and shorter shoots than micropropagated ones, although the majority of cutting-propagated plants developed flowers earlier, flowered more abundantly and bore larger berries than those of tissue culture plants (Litwiczuk *et al.*, 2005). Micropropagated cranberry plants have an excellent juvenile

period and produce vigorous vegetative growth (mostly runners) during their first season but do not produce flowers until their third growing season (Serres and McCown, 1994). Micropropagated 'Bergman', 'Pilgrim' and 'Stevens' plants produced more runners and uprights with more leaves per upright than the conventional cuttings (Debnath, 2008).
Debnath (2005d, 2006b) observed that the *in vitro*-derived lingonberry plants produced more stems, leaves and rhizomes than the conventional cuttings. Under field condition, rhizome production and total plant weight were greater for tissue culture plants than for stem cuttings in the lingonberry cultivar, 'Sanna' (Gustavsson and Stanys, 2000). After 4 years of growth, the tissue culture plants of 'Splendor' and 'Erntedank' lingonberries produced berries with more antioxidant activity, although the berry diameter, number and yield per plant were higher in the stem cutting plants (Foley and Debnath, 2007).

Conclusions

The commercial propagation of the *Vaccinium* species and cloudberry by tissue culture is becoming increasingly common as it is a reliable and efficient method, especially for the rapid introduction of new cultivars. In breeding programs, the technique can provide advantages in: (i) the mass production of elite selections and for analysis in a replicated trial of new releases, (ii) germplasm conservation, (iii) accelerating the breeding process by in vitro selection.
Large-scale liquid cultures combined with automated bioreactors can eliminate most manual handling in micropropagation and decrease production costs significantly. Cultures in liquid medium are advantageous for several plant species but may limit the gas exchange of the plant materials and often cause asphyxia and hyperhydricity, resulting in malformed plants and loss of material.
True-to-type propagules and genetic stability are prerequisites for the application of micropropagation. Molecular markers are powerful tools in the genetic identification of clonal fidelity. Special classes of markers including restriction fragment length polymorphism (RFLP), random-amplified polymorphic DNA (RAPD), arbitrary primed PCR (AP-PCR), DNA amplified fingerprinting (DAF), simple (short) sequence repeat (SSR), short tandem repeat (STR), sequence characterized amplified region (SCAR), sequence-tagged sites (STSs), amplified fragment length polymorphism (AFLP) and inter simple sequence repeat (ISSR) are appropriate for genetic analysis of tissue culture-raised plants. RAPD and ISSR marker analyses have been developed in the author's laboratory to identify genetic diversity in the Vaccinium species (Debnath, 2007d, 2009b) and in cloudberry germplasm (Debnath, 2007e), and can be used to study the clonal fidelity of the micropropagated plants of these species.

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CLIMATICALLY DETERMINATE PROJECTIONS OF RESOURCES OF VACCINIUM SPECIES IN BELARUS TO 2050
KLIMATISKI NOTEIKTA PROGNOZE VACCINIUM ĢINTS SUGĀM BALTKRIEVIJĀ LĪDZ 2050. GADAM

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Abstract
Based on the predicted dynamics of the forest fund in terms of global climate change we have made a climatically determinate projection of the resources of major species of wild berry plants, namely, cowberry (*Vaccinium vitis-idaea* L.), bog blueberry (*Vaccinium uliginosum* L.), European cranberry (*Oxycoccus palustris* Pers.) and bilberry (*Vaccinium myrtillus* L.), in Belarus to the year 2050 at 5-year intervals. The amounts of European cranberries and bilberries may increase, while those of cowberries and bog blueberries may decrease towards the end of the forecast period. On the whole the amounts of all the principal wild berry plants may increase by 11 percent.

Kopsavilkums
Balsoties uz prognozēto meža fonda dinamiku sakarā ar globālajām klimata pārmaiņām, tika veikta klimatiski noteikta prognoze galveno savvaļas ogu sugu resursiem, tai skaitā brūklenēm (*Vaccinium vitis-idaea* L.), savvaļas zilenēm (*Vaccinium uliginosum* L.), Eiropas dzērvenēm (*Oxycoccus palustris* Pers.) un mellenēm (*Vaccinium myrtillus* L.) Baltkrievijā līdz 2050. gadam ar laika intervālu 5 gadi. Eiropas dzērveņu un melleņu ražas var palielināties, bet brūkleņu un savvaļas zileņu samazināties aprakstītā perioda gaitā. Kopumā apskatīto svarīgāko meža ogu ražas varētu palielināties par 11 procentiem.

Key words: *Vaccinium vitis-idaea* L., *Vaccinium uliginosum* L., *Vaccinium myrtillus* L., *Oxycoccus palustris* Pers., forecast.

Introduction

Nowadays forecasting is used on all levels of the national economy. Of prime importance is the environmental forecast. This is required to plan harmonious exploitation and protection of plant resources on the basis of scientific anticipation of environmental changes.

Recent trends in Belarus are towards reorientation of the environmental policy to suit requirements for steady development. In this regard, therefore, major problems are those associated with forecasts of environmental changes. The most intricate ecological forecasts are required to foresee changes in forest plant resources, including wild berry plant resources. In specific cases predicted environmental impact assessments may serve to devise scenarios of changes in plant resources in different regions of the country.

The last few years have witnessed a decline in the resource potential of wild berry plants due to the increasing scale of society's impact on the forest ecosystems. Cuttings, reforestation, forest fires are very detrimental to the berry fields. Also, this decline is associated with radioactive contamination, digression of forest phytocoenoses, drainage reclamation, violation of harvesting regulations and wanton consumption of berries. Management for timber production alone cannot promote reproduction of minor forest products (Grimashevich, 2002, 2005).

Forecasts for minor forest produce resources and their relationship with the dynamics of forest stands might ensure the rational use and protection of side products. A perfect forecast of the dynamics of forest stands, including berry fields, offers a means of carrying out silvicultural practices to prevent negative trends.

We recognise the following forecasts in relation to the forecast duration: seasonal (up to 1 year), short-range (1 to 5 years), extended-range (5 – 15 years), long-range (15 years to several decades) and very-long-range (several hundreds of years) (Loginov, 2004). Forty-two years being left to the year 2050, our investigation was based on the long-range forecast.

Materials and methods

Using data from forest surveys of all the stands of forest-forming species occurring in Belarus and each of the state production forestry associations (SPFA) and taking into consideration the geobotanical subdivision, we systematised the data obtained and analysed the condition and structures of the stands assayed. Principal parameter values and pre-existing dynamics of resources of the berry plants assayed were obtained on the basis of our earlier investigations and regularities revealed in the current research (Grimashevich, 2002, 2005).

Estimates of climate changes that may occur in Belarus over the period of the first fifty years of the 21st century were made on the basis of the results obtained through the use of the had CM2 atmospheric circulation model (Great Britain). The years 1960 – 1990 were taken as the base period. Climate changes, essential to forestry, according to this model, are a monthly rise in average temperature by 0.6 °C to 2.9 °C, a decrease in the transpiration rate and an insignificant increase of rainfall in winter months when its role as a source of moisture for the current-year vegetation period is slight (Loginov, 2004).

In forecasting changes in the resources of wild berry plants at 5-year intervals we used verified data obtained by us in the years 2003 – 2004; the data on variations in the typological structure of forest stands obtained by Lazareva (2007) in 2006 – 2007 being used as a basis for the data. Our forecast

of changes in the typological structure of forest stands is in close agreement with that made by the Russian investigator Minin (2000). Hence our forecast of changes in the resources of wild berry plants in Belarus correlates with the dynamics of areas of forest stands and swamps by forest type to the year 2050.

To develop the climatically determinate dynamics of pineries of different forest types Lazareva (2007) used two main principles of theoretical representation of typological relations, namely, the Sukachev net of edapho-phytocoenotic series of forest types and the Pogrebniak edaphic net of forest site types. The principle of the typological continuum of forest formation was used. The overlapping of some forest types points to the most commonly encountered edaphically conditioned associations of these types, which makes it possible to make a prediction about their dynamics in theory (Loginov, 2003; Alexandrovich, 2003). Each forest type is represented by a number of associations, of which the native one has the most significant place.

With the model of changes of climate parameter values for each of the geobotanical districts to the year 2050 and the relative values for 'shifts' in the air temperature change of 1 °C Lazareva (2007) succeeded in forecasting changes in the forest vegetation of the republic to the above mentioned year.

It is found that aridization may cause colonisation of mires by woody vegetation as a result of which over the forecast period sedge-grass downy birch forests (*P.-Betuleta caricosa*), sedge-grass-bog moss downy birch forests (*P.-Betuleta caricoso-sphagnosa*), black alder forests (*G.-Alneta*), pineries (*Pineta*) and willow woods (*Saliceta*) may cover 40,000 to 45,000 hectares. Young stands of small-leaved trees and bushes may cover 11,000 to 12,000 ha of idle hay fields and pastures by the year 2010 and 20,000 ha by 2020.

By the year 2010, about 30,000 ha of cutover peatlands and 1,300 to 1,400 ha of quarries will be turned over to the forest fund. Also, about 20,000 ha of used peatbogs and about 1,500 ha of quarries will be turned over to the forest fund in 2011-2020. It is expected that no less than 65 percent of cutover peatlands and no less than 80 percent of quarries will be afforested while the remainder will be transformed into wetlands (Loginov, 2004). Large unproductive agricultural lands have already been turned over to the forest fund. To date, most of these lands have been afforested.

As a consequence of the reforestation of unproductive forestry lands and the colonisation of forest-free areas by the year 2020 the forest cover percentages in Belarus will most likely be as high as 41 – 42 percent. In other words, the forest cover percentage may approach that recorded in Belarus in the latter half of the 19th century.

With respect to the stock and forest exploitation it is anticipated that future changes will be positive. By the year 2020, for instance, more than 1 million ha of forest stands will mature and the area of maturity stands will be almost doubled, which will account for 13.0 – 13.5 % of stocked forestlands. By 2015, young stands of the 1st age class will most probably cover 1.2 million ha, which will account for 15 % of stocked forestlands. It is anticipated that the years 2025-2030 will see the equalisation of the age structure (Loginov, 2004).

In all likelihood, the prescribed cut will increase by 70 – 75 % by the year 2020, principal felling volume will rise to 11.8 million m^3, felling volume of coniferous timber will increase by a factor of 2.2 and account for 6.2 million m^3 a year. By the year 2020 the percentage of environment-oriented partial clear cuttings will most likely run to 25 % of their total volume, which will amount to as much as 3.0 million m^3 a year (Loginov, 2004).

The above changes formed the basis of the climatically determinate projections of wild berry plant resources in Belarussian forests to the year 2050 (Grimashevich, 2001, 2002, 2005, 2008; Lazareva, 2007; National strategy of steady development of the Republic of Belarus, 1997; Strategic plan of development of the forestry in Belarus, 1997).

Results and Discussion

The most detailed forecast of environmental changes in Belarus for 2010 – 2020 was made in 2004 (Loginov, 2004). To forecast it was necessary to:

- develop appropriate procedures;
- reveal possible environmental changes;

- substantiate a complex of measures to prevent and minimise negative environmental changes.

We summed up and analysed the findings of researches on probable changes in plant resources to promote the development of a system of measures to be taken to prevent the decline in the resource potential of wild berry plants in Belarus.

It is speculated that the global threshold level of consumption of primary produce has already increased many-fold, which generated the biosphere depletion and global ecological crisis (Loginov, 2004). The data obtained are indicative of the distinct relationship between global changes in the biosphere and anthropogenous factors (Loginov, 2003; Alexandrovich, 2003).

About 11,500 plant species, including 2,100 higher plants and 9,000 to 9,400 lower plants, among which are algae, lichens and fungi, are found naturally in Belarus (Loginov, 2004). Forest vegetation covers more than 38 percent of the territory of the republic.

Belarus is located at the interface between two vegetation zones, namely, the boreal zone in which coniferous forests predominate and the nemoral one in which deciduous summer broadleaved forests predominate. Here, therefore, three geobotanical subzones are recognised, namely, the subzone of oak-dark coniferous southern taiga forests, the subzone of hornbeam-oak-dark coniferous sub-taiga forests and the subzone of mixed broadleaved-pine forests (Loginov, 2004).

The last few years have been characterised by a large-scale dieback of spruce, oak and ash stands brought on by droughts and heavy pest infestations. Forest fires, infectious tree diseases, windfalls, windbreaks, flooding and underflooding also cause grave damage to crops. Drainage reclamation that brought on aridity in Polesye was one of the main factors extremely detrimental to the ecosystems in Belarus. To date, the percentage of lichen, heath and bog moss forests has increased by 1.5 – 2.0 percent. And this process will build up in the future (Loginov, 2004).

In the context of global warming the monthly rise in temperature, the decrease in the depth of snow cover and freezing, and the increase in the probability of droughts are of paramount importance for forestry (Loginov, 2003; Alexandrovich, 2003). The increments therewith will most likely increase by 5 % by 2020 and 10 % by 2050, fruits and seeds of forest trees and wild berries will presumably ripen 7 to 10 days and over earlier, and the silvicultural season will apparently begin 10 to 15 days earlier (Loginov, 2004). Among the negative consequences of global warming are marked changes in the stand structure caused by the shift of the boundaries of the geographical ranges of spruce (*Picea*), hornbeam (*Carpinus*) and grey alder (*Alnus incana*), a longer fire danger period, favourable conditions for the reproduction of forest pests, the increase in probability of spring frosts, unfavourable growing conditions of stands owing to the decline of water table, higher rates of evaporation and transpiration and unfavourable wintering conditions for plants due to unstable snow cover. In Brest and Gomel Polesye the negative consequences will most likely be much more pronounced (Loginov, 2003; Loginov, 2004; Alexandrovich, 2003).

Within the past fifteen years the percentage of pine formations dominated by the *Vaccinium* species has decreased by 6.4 percent. According to the data of scientific projections, global warming may result in an expansion of the distribution of mixed and broadleaved forests and forest-steppe and a reduction in the area of coniferous stands.

Forecasts of changes in the composition, productivity and resource potential of vegetation cover are required to take timely measures to adapt forestry and related branches of the economy of Belarus to the projected climate changes.

The results of the 2006 – 2007 research done within the project on the climatically determinate forecasting of the typological structure of pineries suggest that by 2050 the transformation will very likely bring about a decline in productivity of about 800,000 ha of pine forests, mainly at the cost of upland pineries (Lazareva, 2007). More than 300,000 ha of mossy pine forests (*Pineta pleuroziosa*) will most likely grade into cowberry ones (*Pineta vacciniosa*); in this case the yield class may drop from 2 to 3. Around 700,000 ha of fresh mossy pine forests may grade into dry ones; in this case the yield class may drop from 1 to 2. Also, the research points towards a probable decline in productivity of around 40,000 hectares of bracken pineries (*Pineta pteridiosa*), particularly in the northern and central parts of the republic.

The yield class for about 15,000 ha of wood sorrel pine forests (*Pineta oxalidosa*) in the northern and central parts of the republic may drop moderately from 1a to 1; in this case increasing aridity is not a limiting factor for the growth of the pine stands. The yield class for cowberry pineries may

fall from 2 to 3 and that for heath pine forests (Pineta callunosa) may drop from 3 to 4 (Lazareva, 2007). The data of the forecast therefore indicate that productivity of around 200,000 ha of polytric (*Pineta polytrichosa*), bilberry (*Pineta myrtillosa*) and paludal pine forests, including sedge-grass (*Pineta caricosa*), sedge-grass-bog moss (*Pineta caricoso-sphagnosa*) and bog moss (*Pineta sphagnosa*) pineries will very likely increase with the increasing aridity (Lazareva, 2007).
The forecast suggests that warming may cause a shift of the southern limit of the spruce continuos distribution and replacement of the species by other pine forest-forming trees. In wood sorrel, bracken, bilberry and most mossy pineries spruce may be replaced by hornbeam and other types of the nemoral flora. Hence we predicted probable changes in the resources of wild berry plants from changes in the area of the pineries.
The data obtained indicate that by 2050 the amounts of cowberry may decrease by 3.3 % (from 2,528 to 2,444 tons). The decrease may be a maximum in the Brest (18 %), Gomel (15 %) and Mogilev (10 %) SPFAs. In the Grodno and Minsk SPFAs the amount of cowberry may decrease by 4 % and 2 %, respectively. The amounts in the Vitebsk SPFA may remain constant. Beginning in 2030, the cowberry amount may stabilise in all the SPFAs as the result of equalisation of the forest ecosystems.
By the year 2050 the amount of bog blueberry may decrease by 5.3 % (from 1,132 to 1,072 tons). In view of aridization in Polesye the decrease may be at a maximum in the Gomel (11 %), Mogilev (8 %), Grodno (7 %) and Brest (5 %) SPFAs. The amount of bog blueberry may decrease by 4 % in the Minsk SPFA and increase by 3 % in the Vitebsk SPFA. By the same reasoning as for cowberry, beginning in 2030 the bog blueberry amounts may stabilise in all the SPFAs.

Table 1. Forecast of climatically determinate dynamics of the berry resources of all the principal wild berry plants in Belarus

SPFA	Dynamics of wild berry resources by year in the form of tons : percent									
	2008	2010	2015	2020	2025	2030	2035	2040	2045	2050
Brest	5,816 100	5,810 100	5,739 99	5,672 98	5,599 96	5,533 95	5,533 95	5,533 95	5,533 95	5,533 95
Vitebsk	14,560 100	14,689 101	15,298 105	15,964 110	16,740 115	17,661 121	17,661 121	17,661 121	17,661 121	17,661 121
Gomel	8,062 100	8,045 100	7,987 99	7,819 97	7,700 96	7,763 95	7,763 95	7,763 95	7,763 95	7,763 95
Grodno	4,144 100	4,180 101	4,279 103	4,377 106	4,438 107	4,540 109	4,540 109	4,540 109	4,540 109	4,540 109
Minsk	13,602 100	13,718 101	14,172 104	14,848 109	15,407 113	15,968 117	15,968 117	15,968 117	15,968 117	15,968 117
Mogilev	2,806 100	2,805 100	2,850 102	2,870 102	2,946 105	3,022 107	3,022 107	3,022 107	3,022 107	3,022 107
TOTAL	48,990 100	49,247 101	50,325 103	51,550 105	52,830 108	54,387 111	54,387 111	54,387 111	54,387 111	54,387 111

By the year 2050 the amount of the European cranberry in the republic may increase by 0.4 % (from 7,552 to 7,583 tons). In view of aridization in Polesye, however, the decrease in the European cranberry amount may reach a maximum in the Gomel (12 %), Brest (11 %), Grodno (8 %), Mogilev (7 %) and Minsk (3 %) SPFAs. In the Vitebsk SPFA the European cranberry amounts may increase by 9 percent. Beginning in 2030, the cowberry amounts may stabilise in all the SPFAs.
By the year 2050 the resources of bilberry in the republic may increase by 15 % (from 37,778 to 43,288 tons). In view of aridization in Polesye, however, the bilberry resources may decrease by 4 % and 3 % in the Brest and Gomel SPFAs, respectively. The forecast suggests that the resources of

bilberry may increase by 30 % in the Vitebsk, 21 % in the Minsk, 15 % in the Mogilev and 12 % in the Grodno SPFAs. Beginning in 2030, the cowberry resources may stabilise in all the SPFAs.
According to the projections, by 2050 the amounts of all the principal wild berry plants in Belarus may increase by 11 % (from 48,990 to 54,387 tons; Table 1.). In view of aridization in Polesye, however, the decrease in the amounts may be a maximum in the Brest (5 %) and Gomel (5 %) SPFAs. In the Vitebsk, Minsk, Grodno and Mogilev SPFAs the berry amounts may increase by 21 percent, 17 %, 9 % and 7 %, respectively. Beginning in 2030, the amounts of the wild berry plants may stabilise in all the SPFAs.
The predicted stabilisation of berry resources by 2030 will be due not only to the levelling and improvement of the age and species structures of the forest fund but also to the measures taken to stabilise the general environmental situation in the republic. Among these are reswamping, repairs to drainage systems, the establishment of forest strips and the certification of harvesting berries.

Conclusions

Based on the principal parameter values for principal wild berry plant resources for 2008 and factors that govern their climatically determinate dynamics, we have forecasted the dynamics of the resources to the year 2050 at 5-year intervals.
The amounts of European cranberries and bilberries may increase, while those of cowberries and bog blueberries may decrease towards the end of the forecast period. On the whole the amounts of all the principal wild berry plants may increase by 11 percent.
A complex of silvicultural and other measures should be worked out to conserve and increase minor forest product resources. Silvicultural and special-purpose practices are being developed to overcome negative berry plant productivity trends.

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HIGHBUSH BLUEBERRY BREEDING
AUGSTKRŪMU MELLEŅU SELEKCIJA

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Abstract
Most of the blueberry cultivars now grown in the world came from the breeding programs of Frederick Coville (1908 – 1937), George Darrow (1938-1958) and Arlen Draper (1965 - present) of the United States Department of Agriculture, and Paul Lyrene (1978 - present) of the University of Florida. Until about 30 years ago, highbush blueberry cultivation was restricted to cold climates, as the original cultivated species, *Vaccinium corymbosum,* required about 800 – 1000 chilling hours below 7 ^{0}C for normal floral development. To expand the range of highbush blueberry cultivation into the southern USA, Ralph Sharp of the University of Florida and Darrow hybridized the original northern highbush types with native southern species. There are now three types of highbush blueberry varieties grown worldwide - Northern, Southern and Intermediate. These vary in the number of chilling hours they require for normal floral development and their level of tolerance to winter cold. The primary goals of today's southern and Intermediate highbush breeders are to obtain early ripening types with high plant vigor, disease resistance and a later bloom (particularly in Florida). Northern highbush breeders are concentrating on flavor, longer storing fruit, expanded harvest dates, disease and pest resistance and machine harvestability. Current trends in highbush blueberry breeding include speeding the cultivar release process, expanding the germplasm base of varieties and licensing.

Kopsavilkums
Lielākā daļa no krūmmelleņu šķirnēm, kas pašreiz tiek audzētas pasaulē ir izveidojuši Frederick Coville (1908 – 1937), George Darrow (1938 – 1958), Arlen Draper (1965 – pašlaik) un Paul Lyrene (1978 – pašlaik). Apmēram pirms 30 gadiem augstkrūmu melleņu audzēšanu ierobežoja aukstais klimats, jo kultivētām *Vaccinium corymbosum* sugām, lai ziedkopa normāli attīstītos, nepieciešamas 800 – 1000 aukstuma stundas zem + 7 ^{0}C. Lai paplašinātu diapazonu augstkrūmu melleņu audzēšanā ASV dienvidu daļā, R. Šarps no Floridas universitātes un G.Darovs krustoja vietējās ziemeļu augstkrūmu mellenes ar vietējām dienvidu sugām.
Šobrīd ir trīs veidu augstkrūmu melleņu šķirnes, ko audzē visā pasaulē – ziemeļu, dienvidu un starpformu. Tās atšķiras ar nepieciešamo aukstuma stundu skaitu, lai attīstītos normālas ziedkopas un krūmi būtu aukstumizturīgi. Galvenais selekcijas mērķis dienvidu un starpformu krūmmellenēm ir iegūt agrīnas, ar lielu augšanas spēku, pret slimībām izturīgas un vēlu ziedošas (īpaši Floridā) krūmmellenes. Ziemeļu augstkrūmu melleņu selekcionāri koncentrē uzmanību uz aromātisku ogu ieguvi, ilgāku augļu uzglabāšanos, ienākšanās laika pagarināšanu, slimību un kaitēkļu izturību un izturību pret mehanizētu vākšanu. Nozīmīgi augstkrūmu melleņu selekcijā ir virzība uz ātrāku šķirnes atzīšanas procesu, šķirņu gēnu banku paplašināšanu un licences izsniegšanu.

Key words: *Vaccinium corymbosum,* varieties, cultivars

Introduction
There are now three types of highbush blueberry varieties grown - Northern, Southern and Intermediate. These vary in the number of chilling hours they require for normal floral development and their level of tolerance to winter cold. Northern highbush varieties are adapted to quite cold mid-winter temperatures below -20 ^{0}C, but grow well anywhere there are 800 – 1000 hours of chilling. These are grown primarily in Australia, France, Germany, Michigan, New Jersey, New Zealand, the Pacific Northwest, Poland and Chile. Southern highbush varieties do not tolerate winter temperatures much below freezing and require chilling hours under about 350 hours. They are grown primarily in Australia, Argentina, California, Florida, Chile and southern Spain. Intermediate highbush varieties have a wide range in chilling requirements from 400 – 800 hours. They generally fail in the colder climates because they bloom too early and are too slow to harden

in the fall, resulting in freeze damage to the flower buds. The Intermediate highbush types are grown primarily in Arkansas, Chile, North Carolina and the Pacific Northwest.

History of blueberry breeding.

Blueberry breeding is a very recent development (Hancock, 2006a; Lyrene, 1998). Highbush breeding began in the early 1900s in New Jersey, with the first hybrid being released in 1908 by Frederick Coville of the United States Department of Agriculture (USDA). He conducted the fundamental life history studies of the blueberry that served as the basis of cultivation such as soil pH requirements, cold and day-length control of development, pruning strategies and modes of propagation. Working with Elizabeth White and others, he collected several outstanding wild clones of *V. corymbosum* and *V. angustifolium*, which he subsequently used in breeding improved types. Over 75 % of the current blueberry acreage is still composed of his hybrids, most notably 'Bluecrop', 'Jersey', 'Weymouth', 'Croatan', 'Blueray', 'Rubel' and 'Berkeley' (Mainland, 1998).

George Darrow took over the USDA program after Coville died in 1937 and made important contributions on the crossibility and phylogeny of the native *Vaccinium* species working with the taxonomist W. H. Camp (Hancock, 2006b). He formed a large collaborative testing network that encompassed both private growers and Agricultural Experiment Station Scientists (AES) scientists in Connecticut, Florida, Georgia, Maine, Massachusetts, Michigan, New Jersey and North Carolina. From 1945 to 1961, he sent out almost 200,000 hybrids to his cooperators for evaluation. Arlen Draper followed Darrow and focused on mixing the genes of most wild *Vaccinium* species into the cultivated highbush background. He maintained and strengthened Darrow's collaborative network and released a prodigious number of southern, intermediate and northern highbush cultivars, with improved fruit color and firmness, smaller pedicle scars and higher productivity (Hancock and Galletta, 1995). His Northern highbush 'Duke' and 'Elliott' have been major successes, along with his newer Intermediate release 'Legacy'. Mark Ehlenfedlt took over the USDA program in 1998.

Ralph Sharp began working in the 1950s in Florida on the development of Southern highbush types in collaboration with Darrow (Sharp and Darrow, 1959; Lyrene, 1998). To expand the range of highbush blueberry cultivation into the southern USA, they hybridized the original northern highbush types with native southern species. Sharp was the first collector of *V. darrowii* for breeding, and until very recently, all southern highbush cultivars contained genes from his wild clones. Sharp developed a number of successful cultivars, including 'Sharpblue', which was grown commercially until very recently. Paul Lyrene took over the breeding work in Florida in the late 1970s.

Stanley Johnson at Michigan State University spent a considerable amount of time in the 1950s and 1960s improving the cold tolerance of highbush by crossing it with *V. angustifolium*. Out of this work came the "half-high" cultivar Northland and the mostly pure Northern highbush type 'Bluejay', which was released by his successor Jim Moulton. The program was abandoned in 1978, but was renewed in 1990 by Jim Hancock.

Joseph Eberhart, in Washington released three Northern highbush cultivars, Pacific, Olympia, and Washington in the 1920s and 1930s. 'Olympia' is still grown today in the Pacific Northwest, but not planted.

Outside of the USA, blueberry breeding work was conducted in Australia, Germany and New Zealand. Johnston sent open pollinated seed to D. Jones and Ridley Bell in Australia in the 1960s that generated the important Northern highbush cultivar 'Brigitta Blue' along with several others. Narandra Patel at HortResearch in New Zealand released the Northern highbush cultivars Nui, Puru and Reka from breeding material initially provided by the University of Arkansas and the USDA at Beltsville in the 1960 and 1970s. Walter Heermann in Germany, working with seed provided by Frederick Coville, released several Northern highbush varieties in the 1940s and 1950s including 'Blauweiss-Goldtraube', 'Blauweiss-Zukertraube', 'Heerma', 'Rekord', 'Ama' and 'Gretha'.

Current Breeding Goals. The current goals of Southern and Intermediate highbush breeders are to obtain early ripening types with high plant vigor, disease resistance and a later bloom (particularly in Florida). Established breeding lines are being used for this purpose, along with hybrids derived from native *V. ashei*, *V. elliottii* and *V. darrowii*. There is also growing interest in developing very low chill, evergreen types that fruit in both the summer and fall (Lyrene, 2007; Darnell and Williamson, 1997).

Southern and Intermediate highbush cultivars are being developed at several locations, including Arkansas, Australia, California, Florida, Georgia, Mississippi, Chile and Spain. Paul Lyrene at the University of Florida has the most active program dealing with very low chill genotypes and has released many high impact cultivars including 'Emerald', 'Jewel', 'Misty' and 'Star'. Jim Ballington in North Carolina has the most significant program operating at the interface between Northern and Southern highbush types, and has generated a number of important cultivars including 'Lenore', 'New Hanover', 'O'Neal', 'Reveille' and 'Sampson'. O'Neal is a very low chill type, while the rest are intermediate. Jim Moore and now John Clark at the University of Arkansas have focused on mixing southern wild species with northern types and released 'Ozarkblue,' a late Intermediate type. Scott NeSmith at the University of Georgia has generated several new early Intermediate varieties including 'Rebel', 'Camelia' and 'Palmetto'. Steve Stringer, Arlen Draper and Jim Spears at the USDA in Mississippi have developed a number of Intermediate highbush types including 'Biloxi', 'Gupton' and 'Magnolia'. Several private breeding programs have also emerged that are developing Southern and Intermediate highbush types including Atlantic Blue in Spain, Berry Blue in Michigan and Chile, Driscoll Associates in California**,** Mountain Blue Orchard in Australia and Vital Berry in Chile.

Northern highbush breeders are concentrating on flavor, longer storing fruit, expanded harvest dates, disease and pest resistance and machine harvestability. Established breeding lines are being used in these efforts, along with complex hybrids made up of *V. darrowi*, *V. angustifolium*, *V. constablei* and most of the other wild species. Even though it has limited winter hardiness, *V. darrowii* has proven to be an interesting parent in colder climates, because it passes on a powderblue color, firmness, high flavor, heat tolerance and potential upland adaptations.

Northern highbush blueberries are currently being bred in New Jersey, Michigan, Oregon and Chile. Jim Hancock at Michigan State University is focusing on late maturing, long storing genotypes and has released three new Northern highbush cultivars that show high promise, 'Aurora', 'Draper' and 'Liberty'. Mark Ehlenfeldt of the USDA program in New Jersey is focusing on identifying genotypes with high disease resistance and tolerance to winter cold, and has released several cultivars including 'Chanticleer' and 'Hannah's Choice'. Nicholi Vorza at the Cranberry and Blueberry Research Station of Rutgers University has begun a program in New Jersey to develop locally adapted highbush cultivars with machine harvestability and high fruit quality. Chad Finn of the USDA in Oregon is active in identifying genotypes that are well suited to the Pacific Northwest. The HortResearch program has recently changed hands to Dave Brazelton and Fall Creek Nursery in Oregon. Other worldwide northern highbush breeding projects include 'Berry Blue' in Michigan and Chile, Driscoll Associates in California, the University of Talca and Vital Berry in Chile.

Recent trends in highbush breeding. There has been a recent trend to speed up the cultivar release process. While it used to take up to 25 years from the original cross to the farm, there is now a push to go from seed to release in 10 – 12 years, with 8 years being thought possible. In the early days, a plant was often evaluated for 6 – 12 years before selection and then the elites were evaluated for another 6 – 12 years in replicated trials before release. A good example is 'Elliott,' which was released 25 years after the cross. Today, the primary selection and replicated trial stages have each been reduced to 3 to 4 years in some programs. 'Draper' was released 12 years after the original cross, and 'Aurora' and 'Liberty' were evaluated only 10 years.

The rapid expansion of the industry and the need for new improved types has stimulated this acceleration, along with a need to keep evaluation costs low. The key to the success of this approach is to make sure that the replicated trials are conducted across a wide range of environments, so that the full potential of the selections is recognized as soon as possible. Still, today's growers must be more prepared to abandon a cultivar if it fails to live up to expectations or a better one appears.

Another trend which is to produce varieties with a blend of species genes. In the early days, virtually all the cultivars were pure derivatives of *V. corymbosum*, with at little bit of *V. angustifolium*. The southern breeders changed all of this when they used *V. darrowii* and to some extend *V. ashei* to reduce the chilling requirement of southern highbush. With increasing frequency, native species genes are finding their way into the blood of northern types. For example, Legacy is 25 % *V. darrowii* and 2 % *V. angustifolium*; 'Sierra' is 20 % *V. darrowii*, 15 %

V. ashei, 13 % *V. constablei* and 2 % *V. angustifolium*. Breeders are finding that the use of *V. darrowii* has dramatic impacts on fruit quality, and it only takes two or three generations to restore winter hardiness (Hancock et al, 1995). The complex genetic background of modern breeding populations also makes testing of superior genotypes across broad climatic zones imperative to finding their optimal adaptive zone, particularly for selections of southern and intermediate highbush families. For example, a few years ago we split our breeding families between Oregon and Michigan, and evaluated them independently at each location (Finn *et al.*, 2003). We used a diverse array of families with varying amounts of southern species blood in their heritage. The elite families that emerged in Michigan where also elite in Oregon, but there were a number of elite families in Oregon that proved poorly adapted to the heat and cold in Michigan. Had we relied on Michigan screens, we would have disregarded some important families.

One other important change in highbush breeding is the move towards patenting and licensing blueberry varieties. Today, only the USDA breeding program does not license their varieties, and they are headed in this direction. This move has come primarily as a means to support further breeding work, as State resources dwindle. Licensing may save some public programs from extinction due to diminishing state support, but it will also restrict the availability of new varieties.

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FRUIT DEVELOPMENT IN *VACCINIUM* SPECIES
VACCINIUM SUGU AUGĻU ATTĪSTĪBA

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Abstract

Fruit development and ripening represent one of the most complex developmental processes in plants. Functionally, the role of fruits is to cover the developing seeds and promote the dispersal of mature seeds through the production of attractive colour, flavour and aroma compounds. After fertilisation, the first phases of fruit development include the division and the expansion of the cells. The ripening phase is initiated after the completed seed maturation. Tissue softening and accumulation of flavour compounds, aromatic volatiles and pigments occurs during the ripening phase. The quality of fruits is determined by the different developmental steps via the signalling cascade that is responsible for the metabolic and structural changes during the ripening phenomenon. Genus *Vaccinium* is widespread over the world and it includes many economically important cultivated and wild berry species. Fruits of the *Vaccinium* species are non-climacteric

and anatomically they belong to false berries, many-seeded fleshy fruits in which the inferior ovary along with floral tube ripens into an edible pericarp. *Vaccinium* berries are especially rich with phenolic compounds that are known to possess antioxidative properties. This review focuses on characteristics of fruit development in both cultivated and the wild *Vaccinium* species.

Kopsavilkums
Augļa attīstība un nogatavošanās ir viens no sarežģītākajiem attīstības procesiem augos. Funkcionāli augļa uzdevums ir pasargāt jaunattīstītās sēklas un veicināt to nogatavošanos, reizē veicinot arī to iekrāsošanos, atbilstošai garšai un aromātam. Pēc apaugļošanās, pirmajās augļa attīstības fāzēs, tiek veicināta šūnu dalīšanās un augšana. Augļa nogatavošanās fāze sākas pēc tam, kad beidzas sēklu nobriešana. Augļa audi paliek mīkstāki un garšas un aromātu veidojošo savienojumu un pigmentu uzkrāšanās notiek nogatavošanās fāzes laikā. Augļa kvalitāti nosaka dažādi attīstībā izmantotie signāli, kas atbild par vielmaiņas un strukturālām pārmaiņām nogatavošanās laikā. *Vaccinium* ģints ir plaši izplatīta visā pasaulē un tajā iekļautas daudzas ekonomiski svarīgas kultivētas un savvaļas sugas. To augļi anatomiski pieder pie neīstām ogām – daudzsēklu mīkstajiem augļiem. Ogas ir īpaši bagātas ar fenola savienojumiem, kam, kā zināms, piemīt antioksidantu īpašības. Šajā rakstā dots augļa attīstības raksturojums gan kultivētām, gan savvaļas *Vaccinium* sugām.

Key words: berries, fruit development, *Vaccinium.*

Introduction
Fruit development and ripening, typically preceded by successful flower pollination, represent one of the most complex and important developmental processes in plants. Functionally, the role of fruits is to cover the developing seeds and promote the dispersal of mature seeds by frugivore animals through the production of attractive colour, flavour and aroma compounds in addition to nutritional value. After fertilisation, the first phases of fruit development include the division and the expansion of the cells. The ripening phase is initiated after seed maturation has been completed. Tissue softening and accumulation of flavour compounds, aromatic volatiles and pigments occurs during the ripening phase (Brady 1987, Giovannoni 2001, 2004).
Fruits can be classified into climacteric and non-climacteric fruits according to the differences in the respiration rate and the production of the plant hormone ethylene during the ripening phase. According to the structure of the pericarp, fruits are classified as non-dehiscent (fleshy) or dehiscent (dry) fruits. Most fruits develop from a gynoecium that contains one or more carpels. In pseudocarpic fruits, organs other than the gynoecium (eg. receptacle bracts, floral tube, or the enlarged axis of the inflorescence) participate in the formation of the fruit (Gillaspy *et al.* 1993, Giovannoni 2004). The genus *Vaccinium* is widespread over the world with about 450 species of evergreen and deciduous woody plants varying from dwarf shrubs to trees. Most *Vaccinium* species originate from the cooler areas of the Northern hemisphere, although tropical species also exists. Well-known members of the genus are cultivated northern or southern highbush blueberries (*V. corymbosum* hybrids), lowbush (*V. angustifolium*) or rabbiteye blueberries (*V. ashei Reade*) and cranberries (*V. macrocarpon*), in addition to commercially utilized wild bilberries (*V. myrtillus*) and lingonberries (*V. vitis-idaea*). Other better known wild species are European cranberries (*V. oxycoccos*, *V. microcarpum*), odon (*V. uliginosum*) and numerous other especially American wild *Vaccinium* species. The *Vaccinium* species require soil with low pH and they grow most abundantly in heaths, bogs and acidic woodlands.
Pollination. Most northern highbush blueberries are self-pollinated, meaning that the pollen of the same individual plant can lead in successful fruit development. However, for many cultivars cross pollination produces higher fruit set and larger fruit. Southern highbush blueberries are only partially self-pollinated (Krebs and Hancock 1990). Hokanson and Hancock (2000) tested self-fertility in controlled hand self- and cross-pollinations with individual *V. corymbosum*, *V. angustifolium* and *V. myrtilloides* plants and detected that all three species showed a significant reduction in self fruit set and in the proportion of fertilized ovules that developed into mature seed in self compared to outcross fruit.

Rabbiteye and lowbush blueberries are also largely self-infertile and therefore cross pollination is required for the good fruit set and berry size. Bumble bees and wild bees pollinate blueberry flowers naturally. Cranberries are self-pollinated but insect or wind disturbance is needed for the pollen release (Rieger). Most wild *Vaccinium* species are cross pollinated by insects. Self-pollination is possible in bilberry and lingonberry but it reduces seed production (Nuortila *et al.* 2002). Fruit development. The fruit of the *Vaccinium* species is fleshy, round or oval by shape, they contain several to many seeds and belong in the category of berries. More strictly, the *Vaccinium* fruits are classified as false or epigynous berries, in which the fruit is formed from an inferior ovary, but the floral tube ripens along with the ovary. In true berries the entire ovary wall ripens into a soft pericarp (Fahn 1990). The fruits can grow clustered like blueberries and lingonberries or singly in branch or leaf axis like bilberries or cranberries.

Most *Vaccinium* species growing in the Northern hemisphere flower from May to June and produce berries from July to October. The fruit development of blueberries takes 45 to 90 days, and varies a lot between different species, cultivars, and growth locations. The growth of most blueberries, especially later ripening cultivars, has been found to exhibit a double sigmoid pattern (Godoy *et al.* 2008). Cranberry fruits mature in 60-120 days after fertilization, depending on the cultivar and weather. Among the cultivated blueberries highbush blueberries reach maturity faster (typically 45-75 days) compared to lowbush or rabbiteye blueberries (Rieger).

The development of bilberries from flower to ripe fruit lasts usually 55 to 70 days, varying between different years. The different phases of bilberry fruit development and ripening are presented in figure 1. Typically, the beginning of fruit development involves divisions and expansions of the cells (stages 2 – 3). During the ripening (stages 4 – 6) the accumulation of anthocyanins, and other secondary metabolites and sugars as well as the softening of the cell walls occur. Compared to the bilberry, lingonberry flowers and fruits ripen later during the season, approximately 78 – 84 days after full blossom (Gustavsson 2001).

Phenolic compounds. The berries of the *Vaccinium* species are known especially for their high content of phenolic compounds that are strong antioxidants and possess health beneficial properties. *Vaccinium* berries are among the best sources of anthocyanins and proanthocyanidins (Ovaskainen *et al.* 2008). Therefore, the accumulation of phenolic compounds has been one of the focus areas in the study on the fruit development of the *Vaccinium* species. The composition of flavonoids and other phenolic compounds in fruit development and ripening has been analysed in bilberries, cranberries, and blueberries (Jaakola *et al.* 2002, Vvedenskaya and Vorsa 2004, Castrejon *et al.* 2008, Celik *et al.* 2008). The overall profile of phenolic compounds at their different stages of development shares similarities between the examined *Vaccinium* species.

Figure 1. Fruit development and the ripening of bilberry from pollination to ripe fruit.

At the beginning of fruit development proanthocyanidins and flavonols are the main flavonols in *Vaccinium* fruits. At the onset of the ripening, the content of proanthocyanidins decreases at the same time anthocyanins begin to accumulate. A high content of proanthocyanidins at the early phases of fruit development has also been detected from fruits other than the *Vaccinium* species. Proanthocyanidins have been suggested to provide protection against fungal pathogens and the predation of unripe fruits (Harborne 1997).

The levels of flavonols are more constant over the period of fruit development, although especially qurcetin glycosides are found to be in slightly higher level at the beginning of the fruit development. In addition to quercetin glycosides, many *Vaccinium* berries contain myricetin glycosides (Määttä-Riihinen *et al.* 2004). In the bilberry, myricetin glycosides were found to accumulate during the ripening phase, along with anthocyanins (Jaakola *et al.* 2002). Of the phenolic compounds, in addition to flavonoids *Vaccinium* berries contain hydroxycinnamic acids. Blueberries contain high levels of caffeic acids (Määttä-Riihinen *et al.* 2004). The antioxidant activity during fruit development has been shown to be associated with the total phenolic content at the different developmental stages (Castrejon *et al.* 2008, Celic *et al.* 2008).

Other nutraceuticals. There is a lot of information on the nutritional value of *Vaccinium* berries, but it is mainly focused on ripe fruits. Only a limited number of studies report the contents of the nutritional compounds during fruit development. Cano-Merdano and Darnell (1997) analysed sugar accumulation during the development of rabbiteye blueberries. Glucose and fructose are the main sugars in *Vaccinium* fruits, whereas the contents of sucrose are low (Cano-Merdano and Darnell 1997, Viljakainen *et al.* 2002). According to studies of rabbiteye blueberries, the levels of sugars were at their lowest about 20 days after bloom, after which the contents increased up to ten fold. The maximum levels were reached between 60 and 90 days after bloom, reaching the maximum in the ripe fruits.

Celic *et al.* (2008) analysed the content of organic acids at four different maturity stages of cranberries. The most abundant organic acid in cranberries was citric acid (73 %) followed by malic and ascorbic acid. The overall concentration of citric and malic acid increased over the ripening, whereas the ascorbic acid concentration decreased. Regulation of fruit development. Recent discoveries have begun to reveal the developmental cues that are responsible for priming and initiating the ripening in fruit bearing species (Giovannoni 2004, 2007). The tomato (*Solanum lycopersicum*) has been the model system of choice due to its genetic resources and the knowledge that researchers have of its ripening physiology and biochemistry of ripening (Seymour *et al.*, 2008). However, key information has also been obtained from the model plant *Arabidopsis*, and to a lesser extent from strawberries (*Fragaria* spp).

The current state of the art is that a number of ripening related genes are known from these species including the *ripening inhibitor* (*rin*) and the *Colourless non-ripening* (*Cnr*) (Giovannoni, 2007). The gene at the tomato *ripening inhibitor* (*rin*) locus is a member of the MADS-box *SEPALLATA* (*SEP*) sub-family; *LeMADS-RIN* (Vrebalov *et al.*, 2002). MADS-box genes were previously associated with floral development, but *LeMADS-RIN* is necessary for ripening. The gene at the *Cnr* locus encodes an SBP-box transcription factor (Manning *et al.*, 2006), that is likely to interact with the promoters of the *SQUAMOSA* (*SQUA*) sub-family of MADS-box genes (Lännenpää *et al.*, 2004). These include *TDR4*, which shows enhanced expression during tomato fruit ripening (Eriksson *et al.*, 2004), but has yet to be assigned a function. This regulatory network appears to be conserved across fruit bearing species.

Nonetheless, links between the regulatory factors and the down stream effectors are poorly understood and thus far the studies are limited almost exclusively to the tomato, despite the diverse ripening behaviour of other important fruiting species.

Studies clarifying the molecular basis of fruit development in the *Vaccinium* species have, until recently, been limited and nothing is known about the genes controlling the fruit ripening in the *Vaccinium* species. Some structural genes encoding the key enzymes of the flavonoid biosynthetic pathway have been characterised from cranberry (Polaschock *et al.*, 2002) and bilberry fruits (Jaakola *et al.*, 2002). Just recently, two *SQUA* sub-family of MADS-box genes have been cloned from the bilberry (Jaakola *et al.*, unpublished). The other of the two cloned transcription factor genes was related by sequence homology with tomato *TDR4* and Arabidopsis *FRUITFULL* (*FUL*) genes. The functional analyses revealed a hitherto unsuspected link between the SQUA MADS-box gene and the production of secondary metabolites.

Conclusions

The genus *Vaccinium* includes several economically important berry species. Therefore, the study on the quality characteristics over the development and ripening of the fruits can open new commercially exploitable applications in the future. Understanding better the gene x environment

interaction during the ripening process is an important part of fruit quality research. Fruit development in various *Vaccinium* species shares several similarities, despite the different growth habits of the plants (e.g. highbush blueberry vs. cranberry). Even though there are differences in the phenolic profiles between the *Vaccinium* species, the trend in the accumulation of the compound groups is the same.

The development of new analytical methods has allowed for the accumulation of the new information in the entire area of plant science. However, most information to date has been gathered from the model species and the most economically important crop species. Along with new efficient sequencing technologies, we will soon have vastly more gene level information available for studying the gene x environment interaction in the various *Vaccinium* berries.

Acknowledgements

The two referees of the manuscript are thanked for the improvements in the paper.

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NORDIC BILBERRY PROJECT
ZIEMEĻVALSTU MELLEŅU PROJEKTS

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Abstract

Wild berries are a characteristic part of Northern nature and a particular speciality of Nordic countries. Wild berries are also a rich and valuable resource that has not yet been exploited in a satisfactory level. Approximately 90 – 95 % of the whole wild berry crop yield is left unpicked in the Nordic forests every year. The challenges of wild berry utilization are similar in Nordic countries - the logistics of berry picking including traceability, fragmented sector structure as well as the high share of unprocessed raw material in export. The Nordic project focusing on bilberry (*Vaccinium myrtillus*) "Bilberry: Towards functional food markets" (2007 – 2009) is a part of the New Nordic Food programme funded by the Nordic Innovation Centre. The programme aims to enhance cooperation and innovation among companies that utilize the natural resources of the Nordic countries. The aim of the project is to improve wild berry production and utilization in the Nordic and global market. To achieve this goal a network between the Nordic experts presenting the different fields of the wild berry sector has been established. The project has focused on marketing research, quality issues, biodiversity and the traceability of wild berries; especially the bilberry. The results of the marketing survey were published in November 2008. The aim of the survey was to generate an overall picture of the companies working with wild berries in Nordic countries and to gather information on the existence and willingness of the berry companies to cooperate in wild berry supply, logistics, marketing and research and development. According to the results, a general agreement for the need of increased cooperation at the Nordic level was highlighted.

Kopsavilkums
Savvaļas ogas ir Skandināvijas dabas raksturīga sastāvdaļa un īpaša Ziemeļvalstu dabas vērtība. Savvaļas ogas ir arī bagāts un vērtīgs resurss, kas vēl nav izmantots apmierinošā līmenī. Aptuveni 90 – 95 % no visas savvaļas ogu ražas Ziemeļvalstu mežos katru gadu paliek nenovāktas. Savvaļas ogu izmantošanas problēmas Skandināvijas valstīs ir līdzīgas - ogu savākšanas loģistika, ietverot izpŗti, sadrumstaloto sektora struktūru, kā arī lielu daļu neapstrādātās izejvielas eksportā. Ziemeļvalstu projekts koncentrējas uz mellenēm (*Vaccinium myrtillus*) „Mellenes: ceļā uz funkcionālās pārtikas tirgu" (2007 – 2009) un ir daļa no jaunās Ziemeļvalstu pārtikas programmas, ko finansē Ziemeļvalstu inovācijas centrs.
Programmas mērķis ir veicināt sadarbību un inovāciju starp uzņēmumiem, kuri izmanto dabas resursus Ziemeļvalstīs, kā arī uzlabot savvaļas ogu ražošanu un izmantošanu Ziemeļvalstīs un Pasaules tirgū. Lai sasniegtu šo mērķi, jākooperējas starp Ziemeļvalstu ekspertiem, kas nodarbojas ar dažādiem pētījumu virzieniem par savvaļas ogām. Projekts ir vērsts uz tirgus pētījumiem, kvalitātes jautājumiem, bioloģisko daudzveidību un savvaļas ogu, it sevišķi, melleņu izpēti.
Mārketinga aptaujas rezultāti tika publicēti 2008. gada novembrī. Aptaujas mērķis bija iegūt vispārēju priekšstatu par kompānijām, kas strādā ar savvaļas ogām Ziemeļvalstīs un apkopot informāciju par uzņēmumu gatavību sadarboties savvaļas ogu piegādē, loģistikā, tirdzniecībā, pētniecībā un attīstībā. Balstoties uz iegūtajiem rezultātiem, tika panākta vispārēja vienošanās par stiprākas kooperācijas nepieciešamību Ziemeļvalstu līmenī.

Key words: bilberry, quality, marketing survey, new nordic food

Introduction
Bilberry (*Vaccinium myrtillus* L.) belongs to the most important wild berries in northern Europe and is recognized for its bioactive properties (Lau *et al.* 2005, Canter & Ernst 2004). Wild berries are a valuable part of European nature and tradition. In the northern and eastern parts of Europe wild berries grow abundantly and in these areas the picking of wild berries and mushrooms is an important recreation for people. About half of the wild berries are picked for personal consumption and the remainder are used for commercial utilization. In these areas wild berries are a speciality, which could be utilised and marketed notably better. The average bilberry yield in Scandinavia has been estimated to be over 500 million kg per year, from which only 5 – 8 % is used (Salo 1995).
Wild berries are an excellent source for functional food that should demand a higher valuation and product development in Europe. Nowadays, the biggest part of wild berries picked commercially in North Europe is exported as frozen unprocessed raw material to East Asia or the Central-European food industry. China and Japan are the biggest buyers of European wild berries, a market of which is increasingly focused on health products. The wild berry industry in Europe is typically fairly small and fragmented. One problem is that the annual wild berry crop yields vary markedly in different areas and the yield estimates of the crop have been inaccurate with the present methods. In addition, to better utilise the valuable raw material more knowledge on the uniform quality of wild berries growing in different areas is needed. By characterising the attributes of the growth areas of the best wild berry crops, would provide a sustainable and natural ways to improve the future prospects of wild berry production in a changing climate. Moreover, sustainable methods for improving the logistics of wild berry picking would be needed. A Nordic project focusing on the bilberry (*Vaccinium myrtillus*) "Bilberry: Towards functional food markets" was initiated in 2007. The project is a part of the New Nordic Food programme funded by the Nordic Innovation Centre. The programme aims to enhance cooperation and innovation among companies that utilize the natural resources of the Nordic countries. The aim of the project is to improve wild berry production and utilization in the Nordic and global market. Three work packages including networking, marketing research and quality aspects have been the basis of the project to achieve the stated goal. The general scheme of the project is presented in figure 1.

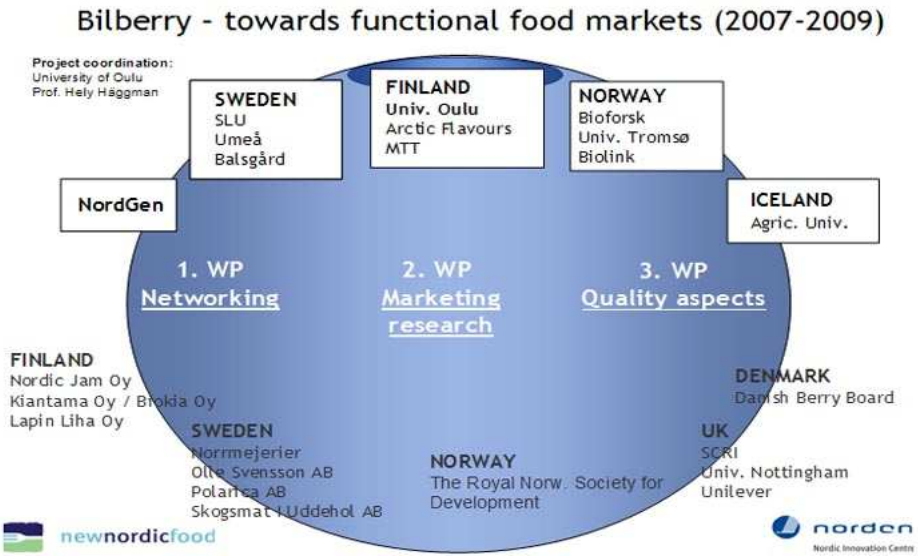

Figure 1. A general scheme of the Nordic bilberry project.

Networking. The bilberry project is co-ordinated by the University of Oulu, Finland, and the other participants are the Arctic Flavours Association and MTT Agrifood Research Jokioinen from Finland, the Nordic Gene Bank (NordGen), the Swedish University of Agricultural Sciences (SLU), Bioforsk, the University of Tromsø and Biolink from Norway and the Agricultural University of Iceland. There are also some companies and other institutions as network partners of the project. During the project, information gathered on companies dealing with wild berries in Nordic countries was listed.

The bilberry project organized the Nordic wild berry seminar at the University of Oulu, Finland on 6^{th}-7^{th} November 2008. In the seminar, there were altogether 50 participants from Finland, Sweden, Norway, UK, Canada and Japan. The participants were representatives of research institutes, wild berry companies and other related organizations. The two-day program consisted of presentations on health and quality issues, marketing and the product development of Nordic wild berries. A panel discussion with the title: "Wild berry production in the northern areas – Guidelines for the future" was held at the end of the seminar. In the discussions, increased co-operation between all interested parties in the wild berry sector was emphasised. The general consensus was that in the future, there would be a need for a joint organisation (e.g. Nordic Wild Berry Association) to maintain the established networking and productive discussions via the general meetings and mailing lists.

Marketing survey. The aim of the marketing survey was first of all to generate an overall picture of the rather heterogeneous and large group of Nordic companies working with wild berries – especially bilberries. Another aim was to gather information on how the companies feel about certain issues related to the wild berry sector, e.g. if companies have problems in gaining enough wild berries. A special emphasis was given to cooperative actions between the companies. The aim was to find out whether, and to what extent, the companies are willing to cooperate in order to reach some of their common goals and what are the areas of business that they consider worthwhile to cooperate in. The common goals include wild berry supply, logistics, marketing and research and development. The survey was carried out in 2007 – 2008. First task was to gather information from the Nordic companies dealing with wild berries. The eight page questionnaire was delivered to 1300 companies, 200 of which were Finnish, 750 Swedish and 350 Norwegian. The results were analysed, compiled and first published in the Nordic Wild Berry Seminar at Oulu, Finland in November 2008 and afterwards also as a printed report (Paassilta et. al., 2009). The results of the survey shown that Nordic wild berry companies are for increased co-operation in Nordic level concerning several common issues. For instance, creating a uniform traceability system for all Nordic countries in wild berry picking was supported. Most of companies also supported the development of common Nordic wild berry brand in the future.

Quality research. The most important issue for berries as a raw material for the functional food market is the quality characteristics of the fruit. Fruit quality is a consequence of proper fruit development, which is a complicated biochemical process and, to a great extent, genetically regulated. However, also environmental factors such as light conditions and temperature affect the ripening process, and the yearly fluctuations can influence the content of secondary metabolites in ripening fruits (Åkerstöm *et al.* 2009). In terms of bilberry production, more knowledge on the factors affecting the ripening process and quality of the fruit is needed. In the Nordic Bilberry project, quality research is going on in several areas. The effect of the growth conditions (day length and temperature) has been studied in a controlled experiment in a phytotrone using clonal material (Martinussen *et al.* unpublished). Additionally, bilberry samples from various altitudes have been collected as well as berry samples from trials in controlled environments. Also bilberries from different latitudes that have been growing in the same growth conditions for several years have been analysed (Åkerstrom *et al.* unpublished). Moreover, molecular level study on the regulation of bilberry fruit development has revealed new information on transcription factors that are necessary for fruit ripening and also about the accumulation of anthocyanins - the important pigments and antioxidants (Jaakola *et al.* unpublished).

In the project, bilberry clones have been collected from all Nordic countries. The samples are presently in the tissue culture, and they will be planted in the test field of the University of Oulu for further extend the clone collection for future research purposes. One task of the project was to optimise a method for bilberry diversity analysis. The retrotransposon based method has shown that genetic diversity exists between and among the bilberry populations from different regions and origins (Antonius *et al.* unpublished).

At present, a poor knowledge of the genome of the bilberry or other *Vaccinium* species is the limiting factor for many applications. A better knowledge of the bilberry genome could be utilised in determining the origins of small fruits for marketing purposes. Increasing berry imports and exports have demonstrated the need for new methods to confirm the origin of the raw material. Moreover, one major problem in the global wild berry markets is that the final products may also contain other berry or plant species than what is mentioned on the product label. In the Nordic bilberry project a bilberry fruit specific EST-library is under construction, with 454-sequencing technology (Roche Diagnostics). Moreover, a new DNA level method for authenticity analyses of wild berry species has been developed (Jaakola *et al.* unpublished).

Conclusions

The Nordic bilberry project has reached most of the results that were set at the beginning of the project. The project has shown that with co-operation and concerned and active participants it is easier to achieve the desired goals. The marketing survey among the companies dealing with wild berries gives a fresh overview on the prospects of the Nordic wild berry business. The scientific efforts in the project are shedding light on the gene x environment interaction related to the quality issues of the bilberry. These results are to some extent applicable to other wild berries also. Moreover, the networking and discussions between the interested participants of the whole wild berry sector have emphasised the need for increased international co-operation and created new ideas for future activities. However, the long term challenges in the wild berry sector still need additional brainstorming, research work, product development, customer surveys and action.

Acknowledgements

'The Bilberry – towards functional food markets' project is funded by Nordic Innovations Centre.

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DOMINANT PHYTOPHAGES OF EDIBLE HONEYSUCKLE (*LONICERA EDULIS* Turcz. ex Freyn) IN BELARUS AND THE EFFICIENCY OF BIOLOGICAL PREPARATIONS APPLICATION AGAINST THEM
DOMINĒJOŠIE ĒDAMĀ SAUSSERŽA (*LONICERA EDULIS* Turc. Ex Freyn) KAITĒKĻI BALTKRIEVIJĀ UN BIOPREPARĀTU EFEKTIVITĀTE TO KONTROLEI

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Abstract
The objective of our research was the study of the specific and structural diversity of phytophages in honeysuckle plantations and the generalization of optimization system of their phytosanitary condition. The phonological observations on the development of the host plant and phytophages were carried out in 2005 – 2008 in plantations of the Institute of fruit growing Minsk region. The pest records were kept every 10 days, starting with «buds breaking» on not less than 10 bushes of every variety. The experiments on the evaluation of the efficiency the biological preparations against the main honeysuckle phytophages were accomplished in 4 times repetition (3 bushes per repetition). The biological preparations against the dominant pest species were applied during the most vulnerable development periods for the noxious organisms.
It has been determined that the main pests both by occurrence and number in the honey suckle plantations of Belarus are honeysuckle – cereal aphid - *Rhopalomyzus lonicerae* Siebold, rose leafroller - *Archips rosana* L., bud codling moth - *Spilonota ocellana* Den. et Schiff. Sporodic damage is caused by spider mite - *Tetranychus urticae* Koch. and the European fruit lecanium - *Parthenolecanium corni corni* Bouch. Among three being studied (‘Goluboe vereteno’, ‘Vasilievskaya’ and ‘Lakomka’) the variety ‘Lakomka’ is less damaged by pest infestation. Also this variety was not damaged by the specialized honeysuckle pest – honeysuckle - cereal aphid.
It has been determined that the biological preparation baciturine, ps, titre 45 – 60 mlrd spores g^{-1} manufactured based on *Bacillus thuringiensis, var. darmstadiensis*, strain № 24 – 91 at the Institute of Microbiology National Academy of Sciences of Belarus and the Institute of plant protection at the rate of application 6 l ha^{-1} decreases the rose leafroller caterpillars number by about 42 – 55 %. The efficiency of the experimental batch of the biological preparation lecanicil application also produced in Belarus based on the fungus *Lecanicillium (Verticillium) lecanii* (Zimm.) Zare et W. strain BL-1 6 l ha^{-1} against honeysuckle-cereal aphid eventes a decrease of 22 – l42 %.

Kopsavilkums
Šo pētījumu mērķis bija izpētīt ēdamā sausserža kaitēkļu daudzveidību plantācijās un apkopot datus par to fitosanitārās situācijas optimizācijas iespējām. Saimniekauga un kaitēkļu fenoloģiskie novērojumi tika veikti no 2005. līdz 2008. gadam Augļkopības Institūta plantācijās Minskas apgabalā. Kaitēkļi tika uzskaitīti ik pēc 10 dienām, sākot no pumpuru plaukšanas fāzes ne mazāk kā 10 krūmiem no šķirnes. Biopreparātu efektivitātes pētījumi pret nozīmīgākajiem ēdamā sausserža kaitēkļiem tika veikti četros atkārtojumos pa 3 krūmiem katrā atkārtojumā. Biopreparāti tika pielietoti kaitīgā organisma visjutīgākajā attīstības fāzē.
Skaita un izplatības ziņā nozīmīgākie ēdamā sausserža kaitēkļi Baltkrievijā bija graudaugu laputs - *Rhopalomyzus lonicerae* Siebold, rožu lapu tinējs - *Archips rosana* L. un pumpuru kode - *Spilonota ocellana* Den. et Schiff. Atsevišķos gadījumos postīga bija arī tīklērce *Tetranychus urticae* Koch. un *Parthenolecanium corni corni* Bouch. Starp trīs pētītajām šķirnēm (‘Goluboe vereteno’, ‘Vasilievskaya’ un ‘Lakomka’), vismazāk kaitēkļu bojājumu bija šķirnei ‘Lakomka’. Šo šķirni nebojāja arī specifisks sausseržu kaitēklis – sausseržu-graudaugu laputs.

Tika noteikts, ka biopreparāts baciturīns (ps, titrs 45 – 60 mljr. sporu g^{-1}, ražots uz *Bacillus thuringiensis*, var. *darmstadiensis* celma Nr. 24 – 91 bāzes Baltkrievijas Nacionālās zinātņu akadēmijas Mikrobioloģijas institūtā un Augu aizsardzības institūtā) devā 6 l ha^{-1} samazina rožu lapu tinēja kāpuru skaitu par 42 – 55 %. Eksperimentālā preparāta lecanicila (ražots Baltkrievijā uz sēnes *Lecanicillium (Verticillium) lecanii* (Zimm.) Zare et W. celma BL-1 bāzes) pielietošana samazināja sausseržu-graudaugu laputu skaitu par 22 – 42 %.

Key words: entomocoenosis, phytophage, edible honeysuckle, biological preparations, efficiency

Introduction

The honeysuckle has appeared in amateur orchards of Belarus rather recently. In the eighties of the XX-th century, however, it quickly gained an increasing distribution as it is early ripening and frost-resistant and can grow on poor soils at minimum expense (Panteev A.V., 1997). Fructification starts 3 – 4 years after planting. In the conditions of Belarus the edible honeysuckle starts blossoming at the second or third decade of April and the flowers resist temperature decrease up to -8 ºC. The berries of early varieties start ripening at the end of May-beginning of June, 7 – 10 days earlier than garden strawberries. Honeysuckle berries are a pantry of vitamins and biologically active substances, have a pleasant taste similar to the blueberry and render curative and preventive properties.

The average edible honeysuckle productivity in the conditions of Belarus varies from 1.5 to 2.5 kg per bush, however, it is not always stable and also often low, which in many respects is determined by losses caused by noxious organism damages though until recently there was an opinion that dark blue or edible honeysuckle as well as the majority of ornamental honeysuckles practically were not damaged by noxious organisms and not infected by diseases (*http:luzhok.ru/encyclop/garden/ treesbrush/art489.html***)**. However, it is possible to say with confidence that the longer the bush is cultivated, more it suffers from these or other pests. V.P. Vasiliev (1975) points out that in the Ukraine the honeysuckle is damaged by 27 insect species, representatives of 7 orders and 1 mite species. Based on Z.S. Babenko's (1982) data in a taiga zone of western Siberia 37 honeysuckle pest insect species of the mainly leaf-eating kind are registered. In a non-chernozem zone of Russia the honeysuckle damage by the following pests are noted: rose tortrix moth (*Archips rosana* L.), black currant tortrix moth (*Pandemis ribeana* Hb.), honeysuckle plume moth (*Platyptilia calodactyla* Den. & Schiff.), honeysuckle aphid (*Semiaphis lonicerae* Shap.*),* willow scale (*Chionaspis salicis* L.) (Plekhanova M. N., 1990). In the middle zone of Russia the dangerous honeysuckle pests are: European fruit lecanium, willow scale, honeysuckle-cereal aphid, honeysuckle whitefly, rose, omnivorous and currant tortrix moths, honeysuckle hooktip moth, honeysuckle plume moth (Naumova L.V., 2002). In Latvia the following pests are registered for the honeysuckle: red spider mite – *Tetranychus urticae* Koch, honeysuckle apical aphid – *Semiaphis tataricae* Aizneberg., honeysuckle-spruce aphid – *Prociphilus xylostei* de Geer, honeysuckle-cereal leaf roller – *Rhopalomyzus lonicerae* Siebold, frost leafroller – *Exapate congelatella* Clerck, rose tortrix moth – *Archips rosana Linnaeus,* honeysuckle striped sawfly – *Zaraea fasciata* Linnaeus., honeysuckle miner – *Phytogromyza xylostei* Robineau-Desvoidy (Rupice A.A., 1981).

The research dealing with honeysuckle phytophages indicates the necessity of carrying out protective measures against pests under conditions of their mass development. There is information on the efficiency of preparations such as actellic, rogor, confidor, inta-vir, fufanon, decis against pest insects; against mites – the acaricides: omite, mavrik; against aphids – garlic, tobacco, pepper infusions (*http:flower.onego.ru/kustar/lonice_v.html*). As honeysuckle is an early ripening crop and it takes not more than two months from the beginning of bud breaking to berry maturation, it is necessary to spray against the phytophages only after the fruit harvest.

Till 2005 there were no purposeful researches on studying the specific and structural variety of edible honeysuckle phytophages, terms definition, expediency and the efficiency carrying out of protective measures against them.

In this connection the objective of the present research was the edible honeysuckle specific pest composition determination in Belarus, discovering the most widespread and harmful phytophage

species and studying the efficiency of the biological plant protection products available against them.

Materials and Methods

The stationary phytosanitary edible honeysuckle inspections, the experiences on studying the bioecological features and dynamics of pests development, an estimation of the phytophage harmfulness degree were carried out in the plantations of the Institute of Fruit Growing in Minsk on a total area of 2 hectares.

The estimate of the phytosanitary condition of the plantings was done using the general methods (Alekhin V.T. *et al*, 1988; Green N. *et al.*, 1996).

Records of the phytophage number were carried out every decade starting from the phenophase of 'buds breaking' not less than on 10 bushes of every variety. The leaf-eating phytophages number was determined by calculating per 2 m of branches taken in regular intervals from 4 bush sides. The aphids were recorded by calculating the colonies number per 100 leaves from each modeling bush. The mites number was determined by binocular viewing calculating both pest imago and larvae number per 100 leaves from each modeling bush. Scale and soft scale records were done by larvae and shields records per 2 m of branches from 4 bush sides. Honeysuckle plume moth records were determined by opening 100 berries from each registration bush, starting from the end of their growth period with 5 days periodicity.

The experiments concerning the efficiency of biological preparations against the main honeysuckle phytophages were done in 4 repetitions (3 bushes repetition). The biological preparations against the dominant pest species were applied during the most vulnerable for noxious organism development periods: against honeysuckle-cereal aphid – during the pest number increase period; rose leaf roller – II-instar larvae stage.

The systematization, generalization and statistical processing of the collected material was done based on dispersive, correlation and regression analysis methods (Zar H.J., 1996; Ulanova E.S. and Zabelin V.N., 1990).

Results and Discussion

As a result of the research it was determined that during the period from honeysuckle budding till the berries maturation the edible honeysuckle is damaged by leaf-eating butterflies (rose tortrix moth - *Archips rosana* L., bud moth - *Spilonota ocellana* Den. et Schiff., winter moth - *Operophthera brumata* L.), the number of which during the years of observations (2005 – 2008) varied from 2.1 (2007) to 4.5 (2008) caterpillars, on the average, per 2 m of branches (Table 1).

In all the years of the research among leaf-eating pests the greatest threat was represented by the rose tortrix moth. This pest caterpillar number varied from 1.4 to 3.9 per 2 m of branches. The honeysuckle bud moth in honeysuckle plantings also caused its annual damage, however, its number was much lower and did not exceed 1.2 caterpillars per record unit. Winter moth was observed in honeysuckle plantations only in 2005 and in 2007 and the caterpillar number did not increase 0.2 per 2 m of branches.

A complex of sucking pests in honeysuckle plantings are the following insect and mite species: honeysuckle-cereal aphid (*Rhopalomyzus lonicerae* Siebold), European fruit lecanium (*Parthenolecanium corni corni* Bouch.), red spider mite (*Tetranychus urticae* Koch.). The dominating role in the complex of the sucking phytophages belongs to the honeysuckle-cereal aphid. The pest eggs number for years of inspection fluctuated from 0.3 to 2.4 per 2 m of branches and the maximum phytophage number in the maturing berries was 6.6 – 8.9 colonies (1 colony up to 20 individuals) per 100 leaves (Table 1). The red spider mite number years of research except 2008, also was high and after harvest had reached 3.7 – 6.8 individuals per leaf. The European fruit lecanium was noticed in honeysuckle plantations only during the last two years of researches and the wintered larvae number during bud breaking was 3.0 – 5.9 individuals per 2 m of branches.

Thus, it has been determined that the main pests both by occurrence and number in honeysuckle plantings in Belarus are honeysuckle-cereal aphid - *Rhopalomyzus lonicerae* Siebold, rose tortrix leaf roller - *Archips rosana* L., bud moth - *Spilonota ocellana* Den. et Schiff. Sporadic damage is caused by the red spider mite - *Tetranychus urticae* Koch. and European fruit lecanium - *Parthenolecanium corni corni* Bouch.

Table 1. Specific composition and honeysuckle phytophage number (Institute of Fruit Growing, Minsk region, 2005 – 2008)

Phytophage species, record unit	2005	2006	2007	2008
Archips rosana , average larvae number per 2 m of branches	2.6	1.4	1.5	3.9
Spilonota ocellana, average number of larvae per 2 m of branches	1.1	1.2	0.4	0.6
Operophthera brumata , average number of larvae per 2 m of branches	0.1	-	0.2	-
Total number of leaf-eating butterflies	3.8	2.6	2.1	4.5
Rhopalomyzus lonicerae, colonies per 100 leaves	8.9	6.6	7.3	6.8
Tetranychus urticae,, average individuals number per leaf	6.8	4.4	3.7	-
Parthenolecanium corni corni , larvae number per 2 m of branches	-	-	5.9	3.0

Since 2006 research was started on the evaluation of the damageability by the dominant phytophages of honeysuckle cultivars regionalized in Belarus. The most spread cultivars: 'Lakomka', 'Goluboe vereteno' and 'Vasilievskaya' were under study. As a result of three years records and observations it has been determined that a variety 'Lakomka' is damaged by a complex of phytophages to a lesser degree than the varieties 'Vasilievskaya' and 'Goluboe vereteno' (Figure 1).

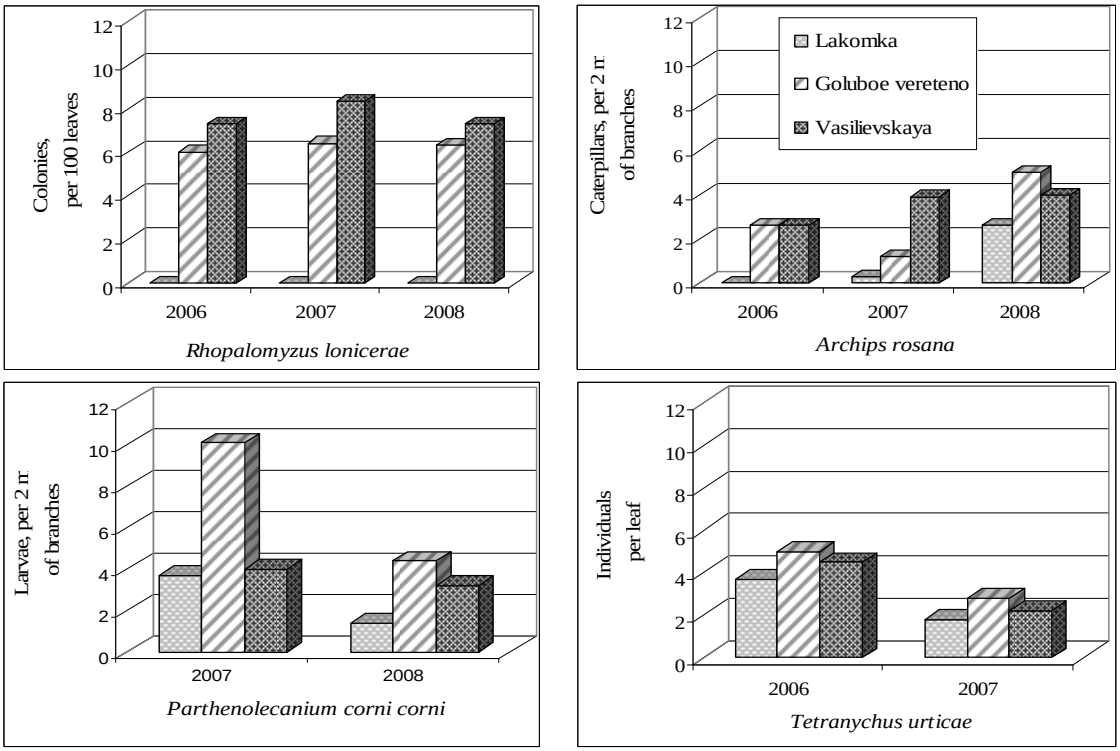

Figure 1. Regionalized edible honeysuckle cultivars colonization by main phytophages (Samokhvalovichi, Minsk region, 2006-2008)

On the cultivar 'Lakomka' for all years of inspections there was no honeythuckle-cereal aphid damage, while on the cultivars 'Vasilievskaya' and 'Goluboe vereteno' the pest number reached 6 – 8 colonies per 100 leaves. The rose tortrix moth number in cv. 'Lakomka' bushes for all years of inspection did not increase 2.6 caterpillars per 2 m of branches in comparison with the cultivars 'Vasilievskaya' and 'Goluboe vereteno' where there were 4 – 5 caterpillars. It was noticed that the European fruit lecanium and red spider mite also to a lesser degree colonized cv. Lakomka. There were no essential differences between the cultivars'Goluboe vereteno' and 'Vasilievskaya' by phytophages damage.

Thus, it has been determined that the cv. 'Lakomka' in the conditions of Belarus is the least damaged by main phytophages and not damaged by the honeythuckle-cereal aphid.

For the sake of extending the spectrum of preparations allowed for application on edible honeythuckle, in 2008 a series of experiments were started on studying the efficiency of biological plant protection products against sucking and leaf-eating phytophages. Studying the efficiency and possibility of biological preparation application on the crops where the chemical means of protection is limited and is authorized only after harvesting is rather actual and a perspective direction for research. For studying, the biological product baciturine, ps. is taken, titer 45 – 60 milliard viable spores g^{-1} (spore-crystal complex and exotoxin *Bacillus thuringiensis, var. darmstadiensis*, strain № 24 – 91), developed at the Microbiology Institute of the National Academy of Sciences of Belarus and at the Institute of Plant Protection. Also the antagonistic activity of the experimental sample of the biological preparation lecanicil developed in Belarus is based on a high-active strain of the fungus *Lecanicillium (Verticillium) lecanii* Zimm. Zare et W. Gams strain BL-1.

The spraying of bushes by a 1 % working solution of a biological product baciturine against rose tortrix moth caterpillars was carried out on April 30, at the end of honeysuckle blossoming, when 90 % of pest caterpillars had reached the II-instar larvae stage. The experimental sample of lecanicil was tested in a 1 % concentration against the honeysuckle-cereal aphid at the beginning of berry coloring the pest number increase. The records of the phytophage number were noted just before harvest and on the 3rd and 7th day after spraying. Repetition – 3 bushes taken at random by one block in a row.

Table 2. Efficiency of the biological preparation baciturine against the rose tortrix moth in edible honeythuckle (Cultivar-'Goluboe vereteno', p. Samokhvalovichi, Minsk r., 2008)

Variant	Number of caterpillars per 2 m of branches before treatment	Date of treatment, crop phenophase	Number of caterpillars per 2 m of branches after treatment		Biological efficiency, %	
			on the 3rd day	on the 7th day	on the 3rd day	on the 7th day
Baciturin, ps., titer 45-60 mlrd. spores g^{-1} (6 l ha^{-1})	4.3	30.04 end of honeythuckle blossoming	1.9	2.4	41.6	55.0
Control (without treatment)	4.5	-	4.4	4.3	-	-

As a result of the trials it was determined that the application of the biological preparation baciturine, ps., titer 45 – 60 mlrd. spores g^{-1} at the rate of preparation application and working solution use 600 l ha^{-1} caused a 41,6 % kill of rose tortrix moth on the 3rd day after treatment and 55 % on the 7th day after the product application (Table 2).

Table 3. Efficiency of the experimental sample of the biological preparation lecanicil against the honeythuckle-cereal aphid in edible honeythuckle (cv. 'Vasilievskaya', p. Samokhvalovichi, Minsk region, 2008)

Variant	Number of individuals per 100 leaves before treatment	Treatment date, crop phenophase	Number of individuals per 100 leaves after treatment		Biological efficiency, %	
			on the 3rd day	on the 7th day	on the 3rd day	on the 7th day
Lecanicil, 6 l ha^{-1}	132.8	2.06, start of honeythuckle berries coloring	126.1	120.2	22.3	41.8
Control (without treatment)	133.3	-	134.5	135.3	-	-

The application of an experimental sample of the preparation lecanicil based on the fungus *Lecanicillium lecanii* strain BL-1, in a 1 % concentration in the beginning of berry coloring against the honeythuckle-cereal aphid caused a pest number decrease on the 7th day after treatment of 41.8% (Table 3).

Conclusions

It was determined that the main pests both by occurrence and number in honeythuckle plantations in Belarus are the honeythuckle-cereal aphid - *Rhopalomyzus lonicerae* Siebold, the rose tortrix moth - *Archips rosana* L. and the bud moth - *Spilonota ocellana* Den. et Schiff. Considerably less damage is caused by – the red spider mite - *Tetranychus urticae* Koch. and the European fruit lecanium - *Parthenolecanium corni corni* Bouch.

It was revealed, that cv 'Lakomka' in the conditions of Belarus is not damaged by the honeythuckle-cereal aphid and to a lesser degree, in comparison with the cultivars 'Vasilievskaya' and 'Goluboe vereteno' is colonized by rose tortrix moth, the European fruit lecanium and the red spider mite.

The biological preparation baciturine, ps., titer 45 – 60 mlrd viable spores g^{-1} (spore-ctystal complex and the exotoxin *Bacillus thuringiensis, var. darmstadiensis*, strain № 24 – 91) at the rate of preparation application 6 l ha^{-1} and at the working solution rate of 600 l ha^{-1} has caused 41.6 % death rate of rose tortrix moth caterpillars on the 3rd day after treatment and 55 % - on the 7th day after preparation application. The efficiency of application of an experimental sample of the preparation lecanicil produced based on the fungus *Lecanicillium (Verticillium) lecanii* Zimm. Zare et W. Gams strain BL-1. 6 l ha^{-1} at the beginning of berry coloring against honeythuckle-cereal aphid was 41.8 % effective on the 7th day after treatment.

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CHEMICAL COMPOSITION OF HIGHBUSH BLUEBERRY CULTIVARS
KRŪMMELLEŅU ŠĶIRŅU ĶĪMISKAIS SASTĀVS

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Abstract
The breeding of blueberries has expanded so far that now the number of the highbush blueberry cultivars has reached several hundreds, and sixty of them are particularly widespread. The chemical composition and suitability for the processing of highbush blueberries has been researched quite extensively, mostly in the USA, but there are no such studies and data for blueberries grown in Latvia. Therefore the aim of this investigation was to compare the biochemical composition of different highbush blueberry cultivars grown in Latvia.
The experiments were done at the Faculty of Food Technology, Latvia University of Agriculture (LLU), Jelgava, and at the Latvia State Fruit Growing Institute, Dobele during the year 2008. The samples of highbush blueberry cultivars were collected from the blueberry collection of Institute of Agrobiotechnology (LLU). The contents of titratable acids, soluble solids, ascorbic acid, total phenols, and anthocyanins of frozen fruits of the highbush blueberry cultivars 'Northland', 'Spartan', 'Barkeley', Duke', 'Chippewa', 'Bluecrop', 'Jersey', 'Blueray', 'Chandler', and 'Bluejay' were analysed.
The cultivars 'Northland' and 'Chippewa' had the highest anthocyanin content (on average 297.59 and 288.83 mg 100 g^{-1}, respectively), the highest phenol content was detected in highbush blueberry cultivar 'Spartan' (on average 381.14 mg 100 g^{-1}), the highest content of ascorbic acid was in cultivar 'Bluejay' (on average 11.8 mg 100 g^{-1}), the highest titratable acids content in the berries of the cultivar 'Chandler' (1.35 %), and the cultivar with the highest soluble solids content was 'Duke' (on average 12.99 % soluble solids).

Kopsavilkums
Melleņu audzēšana jau ir izplatījusies tādā apmērā, ka uz doto brīdi krūmmelleņu šķirņu skaits jau ir sasniedzis vairākus simtus un sešdesmit no tām ir plaši izplatītas. Krūmmelleņu ķīmiskais sastāvs un piemērotība pārstrādei tiek intensīvi pētīta – visvairāk ASV, bet pagaidām nav tādu pētījumu un datu par Latvijā audzētajām krūmmellenēm. Pētījuma mērķis bija salīdzināt dažādu Latvijā audzēto krūmmelleņu šķirņu bioķīmisko sastāvu.
Eksperimenti tika veikti Latvijas Lauksaimniecības universitātes Pārtikas tehnoloģijas fakultātē un Latvijas Valsts Augļkopības institūtā 2008. gadā. Krūmmelleņu šķirņu paraugi tika ievākti no Agrobiotehnoloģijas institūta (LLU) kolekcijas. Šķirņu 'Northland', 'Spartan', 'Barkeley', Duke', 'Chippewa', 'Bluecrop', 'Jersey', 'Blueray', 'Chandler' un 'Bluejay' paraugiem tika analizētas titrējamās skābes, šķīstošā sausna, askorbīnskābe, kopējie fenoli un antociāni.
Šķirņu 'Northland' un 'Chippewa' paraugiem tika konstatēts augstākais antociānu saturs (vidēji 297.59 un 288.83 mg 100 g^{-1}). Augstākais kopējo fenolu saturs noteikts šķirnes 'Spartan' paraugam (vidēji 381.14 mg 100 g^{-1}), augstākais askorbīnskābes saturs bija škirnei 'Bluejay' (vidēji 11.8 mg 100 g^{-1}). Augstākais titrējamo skābju saturs bija šķirnes 'Chandler' (1.35 %) paraugam, un šķirne ar augstāko šķīstošās sausnas saturu bija 'Duke' (vidēji 12.99 % šķīstošās sausnas).

Key words: *Vaccinium corymbosym*, ascorbic acid, soluble solids, phenols, anthocyanins, titratable acids

Introduction
The highbush blueberries (family *Ericaea*, genus *Vaccinium corymbosym*) are a relatively novel shrub berry crop. In the early 1900's two enthusiasts - Elizabeth White and Dr. Frederick Coville - aspired to domesticate the wild blueberries. Their efforts and selection work by plant breeders and and pathologists has created nowadays plump, juicy, sweet and easy-to-pick cultivated blueberries.

The major growing areas of highbush blueberries are in the United States (in more than 38 states) and in some provinces in Canada; gradually they are being propagated all around the world - South America, Australia and New Zealand, and Europe (US Highbush Blueberry Council, 2009).
The popularity and demand of blueberries is growing increasingly due to the demand for their bioactive compounds (polyphenolics, pectic acids, ascorbic acid, carotenes and others) and their antioxidant activity (Sinelli *et.al.,* 2008).
Blueberries contain polyphenols in high quantities: the antocyanins (cyanidin, delphinidin, malvidin, peonidin, petunidin), flavonoids (cathechin and epicatechin, myricetin, quercetin and kaempferol), phenolic acids (ellagic acid, benzoic and cinnamic acid) and others. The content and composition of polyphenolics depends on the growing conditions, cultivating methods, maturity at harvest and other conditions (Giovanelli *et.al.* 2009; Giovanelli *et.al.* 2002; US Highbush Blueberry Council, 2009). Giovanella *et.al.* (2009) reported concentrations of total phenolics and total antocyanins, accordingly from 250 to 310 mg $100g^{-1}$ and from 92 to 126 mg 100 g^{-1}. These data are similar with those reported Prior *et.al.* (1998): total phenolics from 233 to 273 mg *100 g^{-1} and antocyanins from 62 to 157 mg 100 g^{-1}.
Antioxidants help to protect the body against free radicals caused damage, diseases and accelerated aging. The USDA Human Nutrition Research Center on Aging (Boston, MA) has stated that blueberries have the highest antioxidant activity among fruits. They used a test called ORAC (oxygen radical absorbance capacity), which determines the antioxidant capacity of foods, and showed that fresh blueberries the ORAC is 2400 per 100 grams and that is more than other fresh fruits and vegetables (for example, the blackberry ORAC is 2000 to 100 g^{-1}, strawberry - 1500 to 100 g^{-1}, apple 400 to 100 g^{-1} (Prior *et.al.,* 1998; Cao *et.al.,* 1998).
Beaudry (1992) has suggested highbush blueberries quality standards: >10 % soluble solids content (SSC), 0.3 – 1.3 % titratable acidity (TA), pH between 2.25 and 4.25 and SSC/TA ratio between 10 and 33.
Saftner *et. al.* (2008) explored the 11 most popular varieties of highbush blueberries (‘Chanticleer’, ‘Duke’, ‘Hannah’s Choice’, ‘Weymouth’, ‘Berkeley’, ‘Bluecrop’, ‘Bluegold’, ‘Coville’, ‘Elliott’ un ’Lateblue’) and concluded that their soluble solids content (SSC) differs from 10.6 – 13.2 %; varieties which SSC is the lowest are ‘Lateblue’, ‘Coville’ and ‘Duke’ (accordingly 10.6; 10.8 and 10.9), but highest are - ‘Chanticleer’ (13.0) and ‘Bluegold’ (13.2). The difference in titratable acidity (expressed as citric acid) among cultivars is large: ‘Coastal’ had only 0.35 % of the acid, whereas ‘Lateblue’ and ‘Elliott’ has a two times larger amount 1.22 – 1.27 %. Fruit pH ranged between 2.5 to 3.4 where the two last of mentioned varieties had a pH 2.5, but the ‘Bluegold’, ‘Bluecrop’, ‘Berkeley’, ‘Hannah’s Choice’ and ‘Chanticleer’ had the highest pH 3.1 – 3.4. All cultivars have these above mentioned Beaudry (1992) standards excepting three - ‘Coastal’, ‘Elliott’ and ‘Lateblue’ (Saftner *et.al.,* 2008).
Another autor (Giovanelli *et.al.,* 2009) studied the blueberries cultivars ‘Goldtraube’, ‘Patriot’, Bluecrop’ and ‘Darrow’. These varieties agree with Beaudry (1992) suggested standards in regard to pH which is 2.90 – 3.15, but the titratable acidity (expressed as citric acid) is higher – from 1.15 (‘Goldtraube’) to 1.47 % (‘Bluecrop’).
Due to their chemical content, blueberries are acknowledged as very healthy food and are sold not only fresh but also in jams, as blueberry juice and they can also be canned, frozen and dried, and producers are still looking for the new processing techniques.
The breeding of blueberries has expanded so far that now the number of the highbush blueberry cultivars has reached several hundreds, and sixty of them are particularly widespread. (Augļi un ogas Latvijā mūsdienu augļu dārzā, 2008).
The chemical composition and suitability for processing for highbush blueberries has been researched quite extensively, mostly in USA, but there are no similar studies and data of blueberries grown in Latvia.
Therefore the aim of this investigation was to compare the biochemical composition of different highbush blueberry cultivars grown in Latvia.

Materials and methods
The experiments were done at the Faculty of Food Technology, Latvia University of Agriculture (LLU), Jelgava, and at the Latvia State Fruit Growing Institute, Dobele. The samples of highbush

blueberry cultivars were collected from the blueberry collection (7 years old bushes) of the Institute of Agrobiotechnology (LLU), Jelgava.
The samples were analyzed after freezing. After harvesting the blueberries were sorted, frozen in the freezer PORKKA BF 710 at a temperature of -25±2 °C, then packaged and stored for one month in the freezer chamber VTK 201 U at a temperature of -20±2 °C.
The contents of titratable acids, soluble solids, ascorbic acid, the total phenols, and anthocyanins of frozen fruits of highbush blueberry cultivars 'Northland', 'Spartan', 'Barkeley', Duke', 'Chippewa', 'Bluecrop', 'Jersey', 'Blueray', 'Chandler', and 'Bluejay' were analysed.
The content of *ascorbic acid* was determined by titration with a 0.05-M iodine solution (Moor *et al.*, 2005). 25 g of berries were doused with a 100 ml of 6 % solution of oxalic acid and homogenized for 1 minute. Then the sample was filtered. 2 ml of 1 % solution of starch was added to10 ml of filtrate and the filtrate was titrated until a change of colour, which does not disappear during 30 seconds. The content of ascorbic acid mg per 100 g of berries was calculated from the following equation [1]:

$$C = 400 \cdot \frac{V_{sample}}{V_{standard}}, \qquad [1]$$

where V_{sample} – volume of the iodine solution titrated in a sample, ml;
$V_{standard}$ – volume of the iodine solution titrated in a standard solution, ml.

Total titratable acids were determined by titration with 0.1 N NaOH (ISO 750:1998) in fresh and frozen berries.
The contents of the soluble solids were determined by refractometer (ISO 2173:2003) in fresh and frozen berries.
Total phenol content was determined by the photometric method with Folin-Ciocalteau reagent (Singleton *et al.*, 1999). For analyses of phenols the Folin-Ciocalteau reagent and 4 ml 7.5 % sodium carbonate was used. After 30 minutes the samples were analyzed with a spectrophotometer at a wave length of 765 nm. As a control solution 1 ml water with 5 ml Folin-Ciocalteau reagent and 4 ml 7.5 % sodium carbonate solution was used. The content of phenols was calculated from formula [2]:

$$X = \frac{C}{a \cdot 10}, \qquad [2]$$

where C – content of phenols, mg 100 g^{-1};
a – the amount of analyzed sample, g.
The results of all analyses were recalculated to 100 g of dry weight.

Total anthocyanins were determined by the spectrophotometric method (Moor *et al.*, 2005). Initially 50 g of berries were homogenized. Then 20 g of this volume was doused with 40 g of ethanol and 1.5 M HCl solution (85:15 by volume) and homogenized for 1 minute. Then the sample was filtered, and light absorption at 535 nm was detected with a spectrophotometer. The sample was diluted until the absorption coefficient was between 0.6 and 0.8. The content mg per 100 g was calculated with the equation [3]:

$$C = \frac{A \cdot v \cdot d \cdot 1000}{980 \cdot m}; \qquad [3]$$

where A – absorption coefficient;
v – volume of the extraction (90);
d – dilution;
m – sample weight in g.

Results and Discussion
The total anthocyanins of highbush blueberry cultivars differed between 59 and 119 mg 100 g^{-1} of fresh weight (Figure 1).

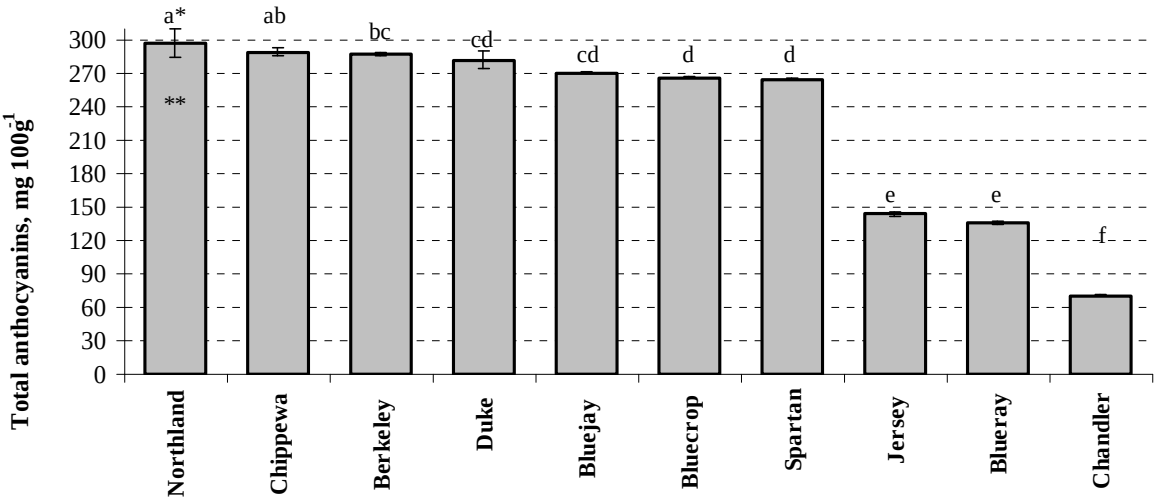

Figure 1. Total anthocyanin content in highbush blueberry cultivars.
**Values, marked with the same letter, are not significantly different at p=0.05.*
*** Bars corresponds the standard error of the mean of the cultivar.*

The cultivars 'Northland' and 'Chippewa' had the highest anthocyanin content (on average 297.59 and 288.83 mg 100 g^{-1}, respectively).
The total phenol content of highbush blueberries differed between 226 and 381 mg 100 g^{-1} of fresh weight (Figure 2). This is even higher than reported in the literature (Giovanella *et.al.,* 2009; Prior *et.al*, 1998).

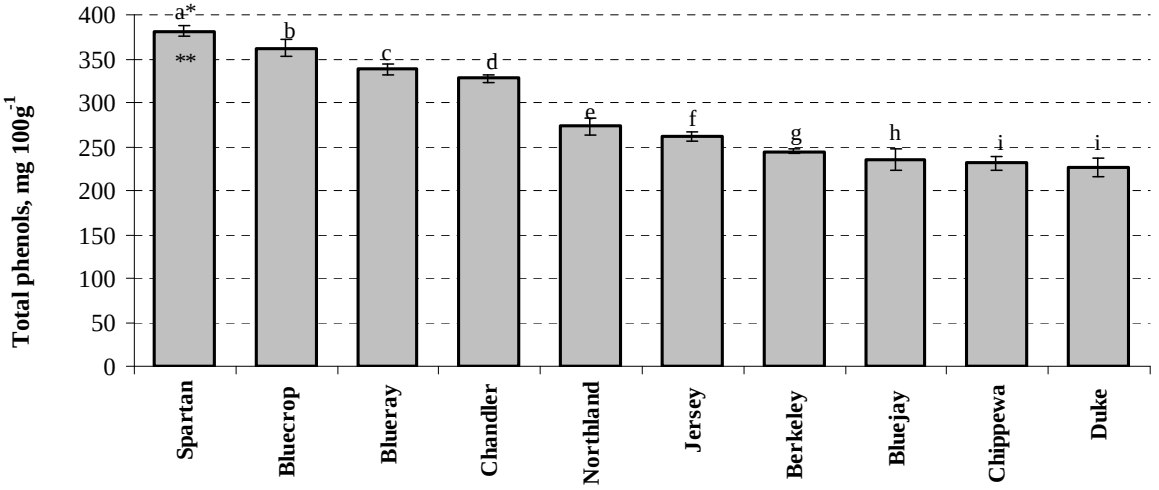

Figure 2. Total phenol content in highbush blueberry cultivars.
**Values, marked with the same letter, are not significantly different at p=0.05.*
*** Bars corresponds the standard error of the mean of the cultivar.*

The highest phenol content was measured in the highbush blueberry cultivar 'Spartan' (on average 381.14 mg 100 g^{-1}).
The ascorbic acid content in the highbush blueberry cultivars was low compared to the other berry crops (currants, strawberries, etc. (Augļi un ogas Latvijā mūsdienu augļu dārzā, 2008; Moor *et al.*, 2005): from 6.9 to 11.8 mg 100 g^{-1} (Figure 3).

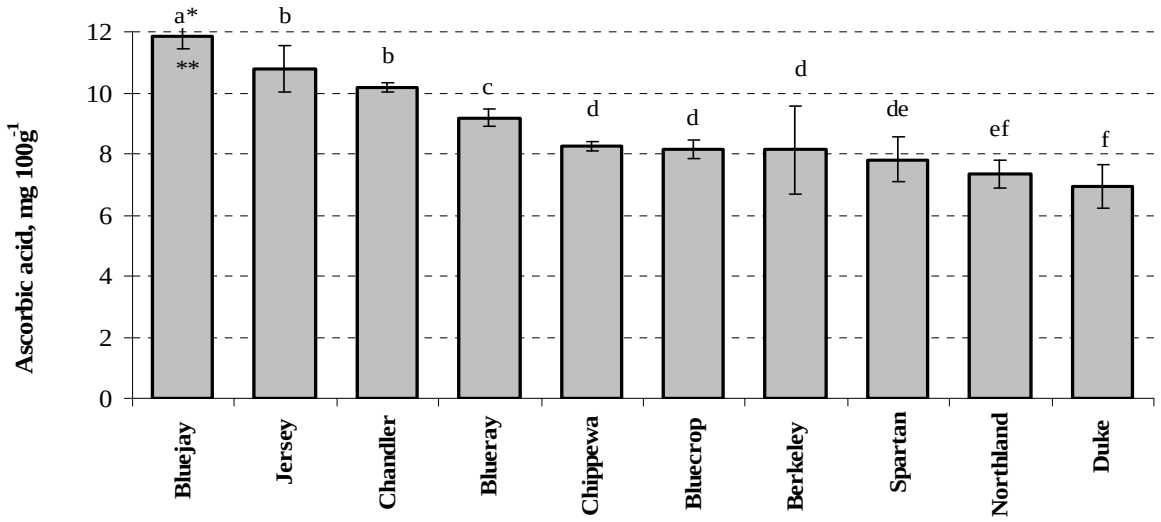

Figure 3. Ascorbic acid content in highbush blueberry cultivars.
**Values, marked with the same letter, are not significantly different at p=0.05.*
*** Bars corresponds the standard error of the mean of the cultivar.*

The cultivar 'Bluejay' had the highest content of ascorbic acid (in average 11.8 mg 100 g^{-1}).
The titratable acids content in highbush blueberries differed between 0.5 and 1.4 %, similarly to that reported in the literature (Beaudry, 1992; Saftner *et.al.,* 2008). There were high differences between the cultivars in titratable acid content (Figure 4).

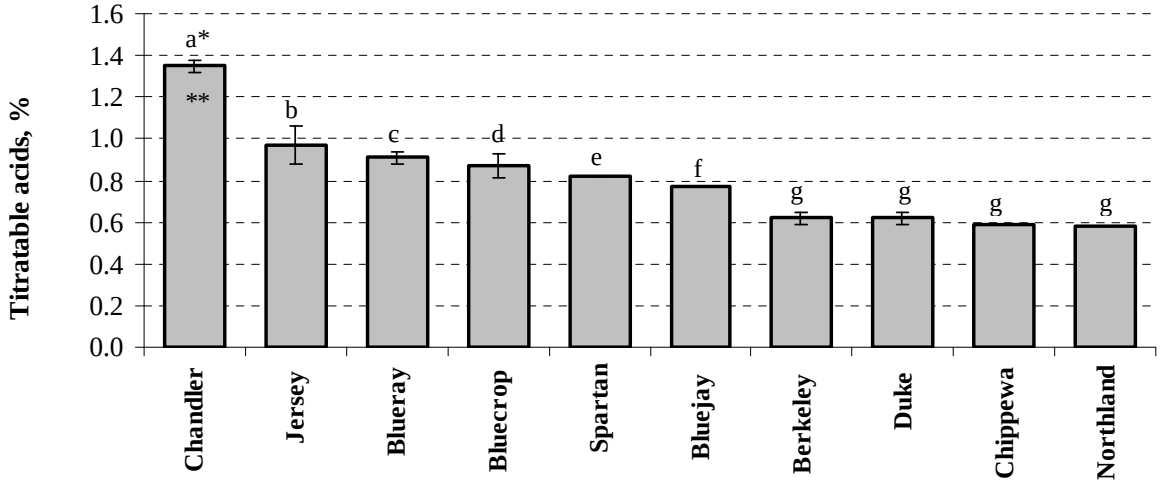

Figure 4. Titratable acid content in highbush blueberry cultivars.
**Values, marked with the same letter, are not significantly different at p=0.05.*
*** Bars corresponds the standard error of the mean of the cultivar.*

The titratable acid content in the berries of the cultivar 'Chandler' (1.35 %) were significantly higher than in all other evaluated cultivars. The titratable acids content in most of the cultivars did not exceed 1 % and is low compared to other berry cultivars. It is important for processing to find blueberry cultivars with the higher acidity therefore the cultivar 'Chandler' could be more suitable for the production of juice and other preserves.

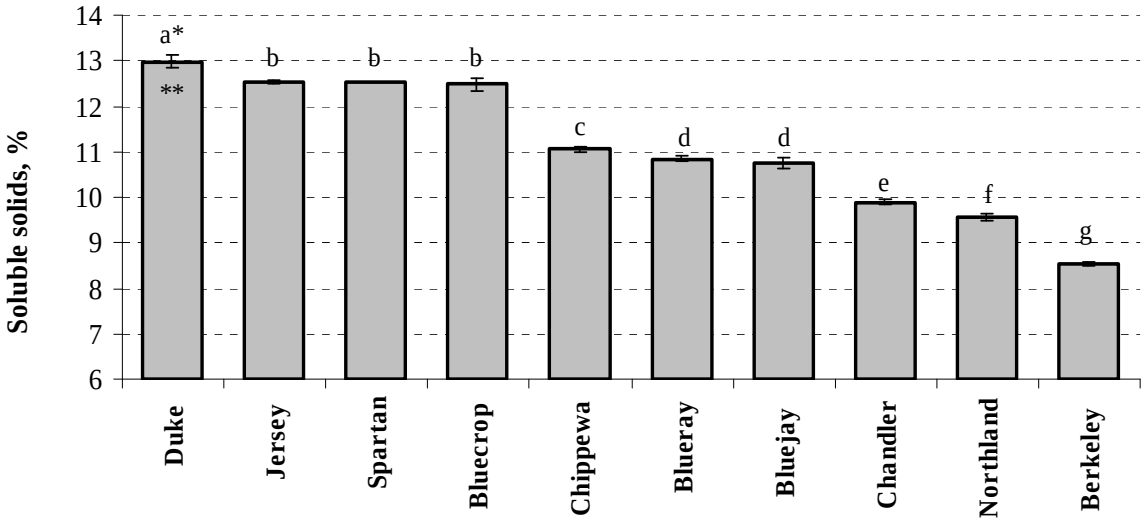

Figure 5. Soluble solids content in highbush blueberry cultivars.
**Values, marked with the same letter, are not significantly different at p=0.05.*
*** Bars corresponds the standard error of the mean of the cultivar.*

The soluble solids content in the evaluated highbush blueberry cultivars differed between 8.5 and 13 % (Figure 5). The cultivar 'Duke' had the highest soluble solids content (on average 12.99 % soluble solids). The soluble solids content in some blueberry cultivars grown in Latvia could be lower than in other growing regions due to more rainfall, less sunshine, and a colder climate. For example, the soluble solids content in the berries of the cultivar 'Berkeley' were only on average 8.5 %, which is significantly lower than that mentioned in the literature (Saftner *et. al.*, 2008).

Conclusions

The cultivars 'Northland' and 'Chippewa' had the highest anthocyanin content (on average 297.59 and 288.83 mg 100 g^{-1}, respectively), the highest phenol content was measured in the highbush blueberry cultivar 'Spartan' (on average 381.14 mg 100 g^{-1}), the highest content of ascorbic acid was in the cultivar 'Bluejay' (on average 11.8 mg 100 g^{-1}), the highest titratable acids content in the berries of cultivar 'Chandler' (1.35 %), and the cultivar with the highest soluble solids content was 'Duke' (in average 12.99 % soluble solids).

In total, the chemical composition of the evaluated highbush blueberry cultivars grown in Latvia were similar to the literature, but there was tendency that blueberries in Latvia had higher phenol content and lower soluble solids content compared to those grown in the other growing regions.

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BIOCHEMICAL COMPOSITION AND ANTIRADICAL ACTIVITY OF ROWANBERRY (*SORBUS L.*) CULTIVARS AND HYBRIDS WITH DIFFERENT *ROSACEAE* L. CULTIVARS

PĪLĀDŽU (*SORBUS* L.) ŠĶIRŅU UN TO HIBRĪDU AR CITIEM *ROSACEAE* L. AUGĻAUGIEM ANTIOKSIDATĪVĀ AKTIVITĀTE UN BIOĶĪMISKAIS SASTĀVS

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Abstract

Rowanberry (*Sorbus aucuparia* L.) is a common yellowish, wild berry that grows in the Northern part of Europe. Rowan has been described as an important source of flavonoids and their antioxidant activity affects reactive oxygen species and lipid peroxidation. The aim of this study was to evaluate the biochemical composition of 8 rowanberry cultivar fruits and the fruits of their hybrids with *Rosaceae* L. cultivars and to establish the correlation of their biochemical composition with antiradical activity. The experiments were done at the Faculty of Food Technology, Latvia University of Agriculture (LLU) and in the Latvia State Fruit Growing institute, Dobele. The content of ascorbic acid, the total phenols, anthocyanins, carotenoids, soluble solids, titrable acids, tannins and the antiradical activity of fresh and frozen rowanberry×hawthorn 'Granatnaya', rowanberry×chokeberry 'Likiornaya', rowanberry *(Sorbus aucuparia)* 'Rosina', rowanberry *(Sorbus aucuparia)* 'Zholtaya', wild rowanberry *(Sorbus aucuparia)*, rowanberry×pear 'Alaya Krupnaya', rowanberry *(Sorbus aucuparia)* 'Rosina Variegata', rowanberry *(Sorbus aucuparia)* 'Krasnaya Krupnaya' were analysed. There were no significant differences between the chemical composition of fresh and frozen rowanberry samples. The highest content of ascorbic acid was in fruits of the rowanberry 'Rosina', 'Rosina Variegata', 'Krasnaya Krupnaya', and 'Zholtaya' (49 – 53 mg 100 g^{-1}). The highest content of carotenoids were detected in rowanberry×hawthorn 'Granatnaya' (13.04 mg 100 g^{-1}), but the highest phenol content was detected in the rowanberry and chokeberry hybrid 'Likiornaya' (484.9 mg 100 g^{-1}). 'Likiornaya' showed also the highest antiradical activity (11.2 g of berries per 1g of DPPH radical).

Kopsavilkums

Pīlādži (*Sorbus aucuparia* L.) ir izplatīts savaļas augļaugs, kas aug Eiropas ziemeļu daļā. Pīlādži ir raksturoti kā nozīmīgs flavonoīdu avots un to antioksidatīvā aktivitāte ietekmē reaktīvo skābekli un lipīdu peroksidāciju. Pētījuma mērķis bija izvērtēt bioķīmisko sastāvu 8 pīlādžu šķirņu un to

hibrīdu ar citiem *Rosaceae* L. augļaugiem, noteikt korelāciju starp to antioksidatīvo aktivitāti un bioķīmisko sastāvu.
Eksperimenti tika veikti Latvijas Lauksaimniecības universitātes Pārtikas tehnoloģijas fakultātē un Latvijas Valsts Augļkopības institūtā 2008. gadā. Pīlādžu šķirņu un hibrīdu paraugi tika ievākti no Pūres Dārzkopības izmēģinājumu stacijas kolekcijas. Analizēts tika svaigu un saldētu pīlādža × vilkābeles 'Granatnaya', pīlādža × aronijas 'Likiornaya', pīlādža *(Sorbus aucuparia)* 'Rosina', pīlādža *(Sorbus aucuparia)* 'Zholtaya', savaļas pīlādža *(Sorbus aucuparia)*, pīlādža × bumbiera 'Alaya Krupnaya', pīlādža *(Sorbus aucuparia)* 'Rosina Variegata', pīlādža *(Sorbus aucuparia)* 'Krasnaya Krupnaya' paraugu askorbīnskābes, kopējo fenolu, karotinoīdu, šķīstošās sausnas, miecvielu saturs un antioksidatīvā aktivitāte.
Starp svaigiem un saldētiem pīlādžu un to hibrīdu paraugiem netika konstatētas būtiskas atšķirības. Augstākais askorbīnskābes saturs bija 'Rosina', 'Rosina Variegata', 'Krasnaya Krupnaya', un 'Zholtaya' paraugiem (49 – 53 mg 100 g^{-1}). Augstākais kopējo karotinoīdu saturs bija pīlādža × vilkābeles 'Granatnaya' augļiem (13.04 mg 100 g^{-1}), bet augstākais kopējo fenolu saturs bija pīlādža un aronijas hibrīdam 'Likiornaya' (484.9 mg 100 g^{-1}). 'Likiornaya' uzrādīja arī augstāko antioksidatīvo aktivitāti (11.2 g ogu uz 1 g of DFPH radikāļa).

Key words: ascorbic acid, phenols, antiradical activity (DPPH), frozen, fresh

Introduction

Rowanberry *(Sorbus aucuparia* L.*)* is a common yellowish, wild berry that grows in the northern part of Europe. They have been described as an important source of flavonoids and their antioxidant activity affects reactive oxygen species and lipid peroxidation (Gil-Izquierdo and Mellenthin, 2001).

The fruits of rowan (*Sorbus aucuparia* L.*)* have been traditionally used for jellies and jams, but their wider use as food ingredients has been less popular because of their bitter taste. The first sweet rowanberry clones were selected in the Sudety mountain area, in the current Czech Republic area in the 19th century. A breeding program for sweet rowanberries was started by Michurin in Russia at the beginning of 20th century, resulting in interesting hybrids of the rowanberry (*Sorbus aucuparia* L.*)* with the *Aronia, Malus, Mespilus*, or *Pyrus* species. Sweet rowanberries have been bred particularly for northern conditions and have shown excellent winter-hardiness in Russia and Finland. The taste of these berries is less astringent than that of wild rowanberries, and the berries are often larger. The total phenolic content can vary greatly among the sweet rowanberry cultivars ranging from 550 – 1014 mg 100 g^{-1} of fresh weight of berries. A high correlation between the antioxidant capacity and phenolic contents of sweet rowanberries was established (Hukkanen *et al.*, 2006). Including different types of berries other Finnish authors found that the phenolic composition data showed no remarkable correlation between antioxidant activity and total phenolics. A statistically significant correlation was observed between flavonol content and antioxidant activity, and between hydroxycinnamic acid and antioxidant activity (Kähkönen *et al.*, 2001).

Rowanberries contain also carotenoids, vitamin E, and vitamin C, which might also contribute to their antioxidant capacity. According to Piir and Niiberg (2003) carotenoid levels in sweet rowanberries are as high as those in carrots, and levels of vitamin C are close to those of strawberries, varying from 12-21 mg 100 g^{-1} ('Granatnaya') to 86 mg 100 g^{-1} ('Zholtaya') (Piir and Niiberg, 2003; Häkkinen *et al.*, 1999). Also higher vitamin C content is found in the rowanberry varieties (*S. aucuparia*) than in the hybrid cultivars.

The Pure Horticultural Research Centre has a large collection with sweet rowanberry cultivars and their hybrids with *Rosaceae* L. genus cultivars collected from Russia and other countries. But there is still little information about the biochemical composition and the nutritional value of the fruits of these cultivars.

Therefore the aim of this study was to evaluate the biochemical composition and nutritional value of 8 rowanberry cultivars and hybrids with other *Rosaceae* L. genus cultivars and to find correlations between of the biochemical composition and the antiradical activity.

Materials and methods

The experiments were done at the Faculty of Food Technology, Latvia University of Agriculture (LLU), Jelgava, and at the Latvia State Fruit Growing Institute, Dobele. The samples of rowan cultivars and their hybrids were collected from the Pure Horticultural Research Centre collection of genetic resources.

The samples were analyzed fresh and after freezing. After harvesting the rowanberries were sorted, frozen in a freezer PORKKA BF 710 at the temperature of -25±2 °C, then packaged and stored for one month in the freezer chamber VTK 201 U at a temperature of -20±2 °C.

Contents of titratable acids, soluble solids, ascorbic acid, total phenols, carotenoids, and the antiroxidant activity (DPPH) of the fresh and frozen fruits of wild rowanberry (*Sorbus aucuparia*), rowanberry×hawthorn 'Granatnaya', rowanberry×chokeberry 'Likiornaya', rowanberry (*Sorbus aucuparia*) 'Rosina', rowanberry (*Sorbus aucuparia*) 'Zholtaya', rowanberry×pear 'Alaya Krupnaya', rowanberry (*Sorbus aucuparia*) 'Rosina Variegata', and rowanberry (*Sorbus aucuparia*) 'Krasnaya Krupnaya' were analysed.

The content of the ascorbic acid was determined by titration with a 0.05-M iodine solution (Moor *et al.*, 2005). 25 g of berries were doused with 100 ml of 6 % solution of oxalic acid and homogenized for 1 minute. Then the sample was filtered. 2 ml of 1 % solution of starch was added to10 ml of filtrate and the filtrate was titrated until change of colour, which does not disappear during 30 seconds. The content of ascorbic acid mg per 100 g of berries was calculated from the following equation [1]:

$$C = 400 \cdot \frac{V_{sample}}{V_{s\,\tan dard}}, \qquad [1]$$

where V_{sample} – volume of the iodine solution titrated in a sample, ml;
$V_{standard}$ – volume of the iodine solution titrated in a standard solution, ml.

The total titrable acids were determined by titration with 0.1 N NaOH (ISO 750:1998) in fresh and frozen berries.

The contents of *soluble solids* were determined by a refractometer (ISO 2173:2003) in fresh and frozen berries.

The total phenol content was determined by the photometric method with a Folin-Ciocalteau reagent (Singleton *et al.*, 1999). For analyses of phenols the Folin-Ciocalteau reagent and 4 ml 7.5% sodium carbonate was used. After 30 minutes the samples were analyzed with a spectrophotometer at the wave length of 765 nm. As a control solution 1 ml water with 5 ml Folin-Ciocalteau reagent and a 4 ml 7.5 % sodium carbonate solution was used. The content of phenols is calculated from formula [2]:

$$X = \frac{C}{a \cdot 10}, \qquad [2]$$

where C – content of phenols, mg 100 g^{-1};
a – the amount of analyzed sample, g.

The results of all analyses were recalculated to 100 g of dry weight.

Carotenoids were analyzed by the spectrophotometric method at a wave length of 440 nm (Ермаков , 1987). One to two grams of homogenized berries were placed in a 100 ml conic retort and 20 ml 96 % ethanol was added. The sample was stirred by a magnetic stirrer for 15 min then 25 ml petrol ether was added and stirring was continued for one more hour. After 3 – 4 hours when both layers were completely separated, the top (yellow) layer was used for the further detection of carotenoids at a wave length of 440 nm. The carotene equivalent (KE) was found, using a graduation curve with $K_2Cr_2O_7$. The content of carotenoids (mg 100 g^{-1}) was calculated by equation 4:

$$X = \frac{0.208 \cdot 25 \cdot 100 \cdot KE}{36 \cdot a}, \qquad [4]$$

where 0,208 and 36 coefficients for the relationship between $K_2Cr_2O_7$ and carotenoids;

25 – dilution coefficient;
KE – carotene equivalent from the graduation curve;
a – sample weight, g.

The antiradical activity (ARA) of frozen berries was analyzed by the spectrophotometric method with the N,N-diphenil-N'-picrilhydrazil (DPPH) reagent at a wave length of 517 nm (Milauskas *et al.*, 2004). 50 ml of ethanol was added to 10 g of the homogenized sample; the glasses were closed by Parafilm and stirred for 2 hours for extraction. After 2 hours top clear layer was decanted, 50 ml ethanol added to the sample and the extraction was repeated for 2 hours. 2.9 ml $1 \cdot 10^{-4}$M DPPH was filled in the cuvette and 100 µl of extract added. The sample was stirred and placed in the dark for 30 min. Then the absorption was measured at 517 nm (spectrophotomter UV – 1650 PC). ARA was calculated by equation 5:

$$ARA = \frac{A_{DFPH} - A_{sample}}{A_{DFPH}} \cdot 100\%, \qquad [5]$$

where: A_{DPPH} – absorption of DPPH reactive;
A_{sample}- absorption of DPPH after addition of fruit extract.

Tannins. The total content of tannins was detected using the traditional method by titration with 0.1 n $KMnO_4$ (Шмыд, 1960). The content of tannins (x) was calculated from formula 6:

$$X = \frac{(v_1 - v_2) \cdot 0.04157 \cdot 100}{a}, \qquad [6]$$

where v_1- the amount of 0.1 n $KMnO_4$ used in the first titration, ml;
v_2 – the amount of 0.1 n $KMnO_4$ used in the second titration, ml;
a – the amount of 0.1 n $KMnO_4$ used for oxidizing of 10 ml of 0.1 oxalic acid, ml.

Results and Discussion

The evaluated components of chemical composition did not significantly (p=0.13-0.59) differ between the fresh and frozen samples that demonstarte the possibility of frozen rowanberry usage similar to the fresh ones.

The ascorbic acid content of rowanberry cultivars and their hybrids is similar to many other fruit species and did not exceed 53 mg 100 g^{-1}, which is similar to that mentioned in literature (Gil-Izquierdo Mellenthin., 2001). The highest content of ascorbic acid was detected in the rowanberry 'Rosina', 'Rosina Variegata', 'Krasnaya Krupnaya', and 'Zholtaya' (Figure 1). The wild rowanberry and hybrids with different *Rosaceae* L. genus cultivars had the significantly lower content of vitamin C.

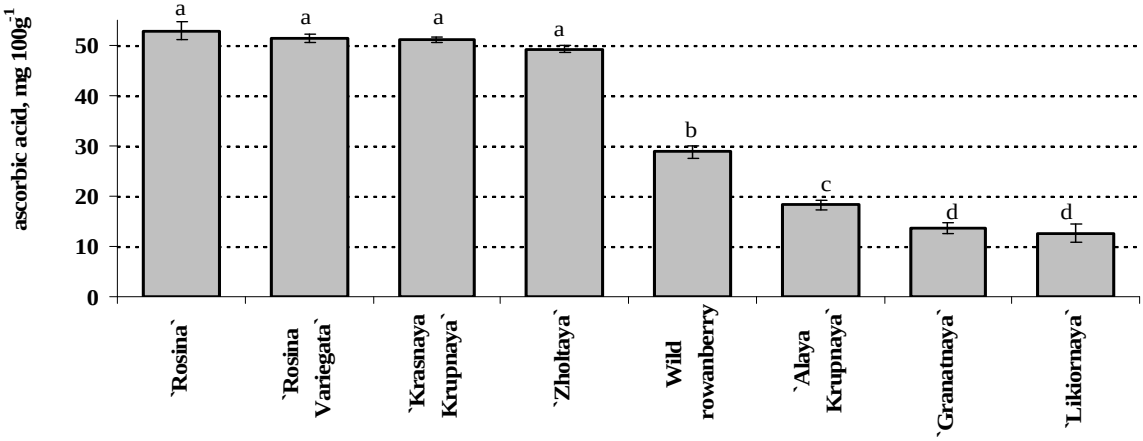

Figure 1. Ascorbic acid content in rowanberries and their hybrids.
**Values, marked with the same letter, are not significantly different at p=0.05*

The titrable acids content of the evaluated rowanberry cultivars and the hybrids was 1.6 – 2.8 % (Table 1). The highest acidity was found in the wild rowanberry, but the lowest titrable acids content were in the hybrids rowanberry×hawthorn 'Granatnaya', and rowanberry×chokeberry 'Likiornaya'.

Table 1. Biochemical composition of rowanberries and their hybrids

Cultivar	Titrable acids, %	Carotenoids, mg/100g	Tannins, %
Rowanberry×pear 'Alaya Krupnaya'	2,61 ± 0,10 ^{ab}	9,24 ± 0,16 ^{c}	0,33 ± 0,02 ^{b}
Rowanberry×hawthorn 'Granatnaya'	1,73 ± 0,04 ^{d}	13,04 ± 0,68 ^{a}	0,37 ± 0,01 ^{ab}
Rowanberry (*Sorbus aucuparia*) 'Krasnaya Krupnaya'	2,65 ± 0,12 ^{ab}	7,25 ± 0,24 ^{d}	0,13 ± 0,02 ^{c}
Rowanberry×chokeberry 'Likiornaya'	1,62 ± 0,02 ^{d}	10,48 ± 0,05 ^{b}	0,39 ± 0,02 ^{ab}
Rowanberry 'Rosina Variegata'	2,67 ± 0,02 ^{ab}	8,08 ± 0,14 ^{d}	-
Rowanberry (*Sorbus aucuparia*) 'Rosina'	2,48 ± 0,09 ^{b}	10,04 ± 0,27 ^{bc}	0,38 ± 0,00 ^{ab}
Wild rowanberry (*Sorbus aucuparia*)	2,80 ± 0,03 ^{a}	9,52 ± 0,31 ^{bc}	0,42 ± 0,02 ^{a}
Rowanberry (*Sorbus aucuparia*) 'Zholtaya'	2,09 ± 0,03 ^{c}	9,53 ± 0,36 ^{bc}	0,32 ± 0,04 ^{b}

The soluble solids content in rowanberries varied between 11 and 18 % (Figure 2). An interesting fact was that the highest soluble solid content was in the wild rowanberry (*Sorbus aucuparia*) (18.0 %, in average). All other rowanberry cultivars and hybrids had a significantly lower soluble solid content than the wild rowanberry and did not reach even 16 % though in literature the content of soluble solids in fruits of cultivars 'Rosina' and 'Zholtaya' were mentioned as being above 19 % (Hukkanen *et al.*, 2006). This means that the soluble solids of rowanberries can greatly fluctuate in different regions and during different years.

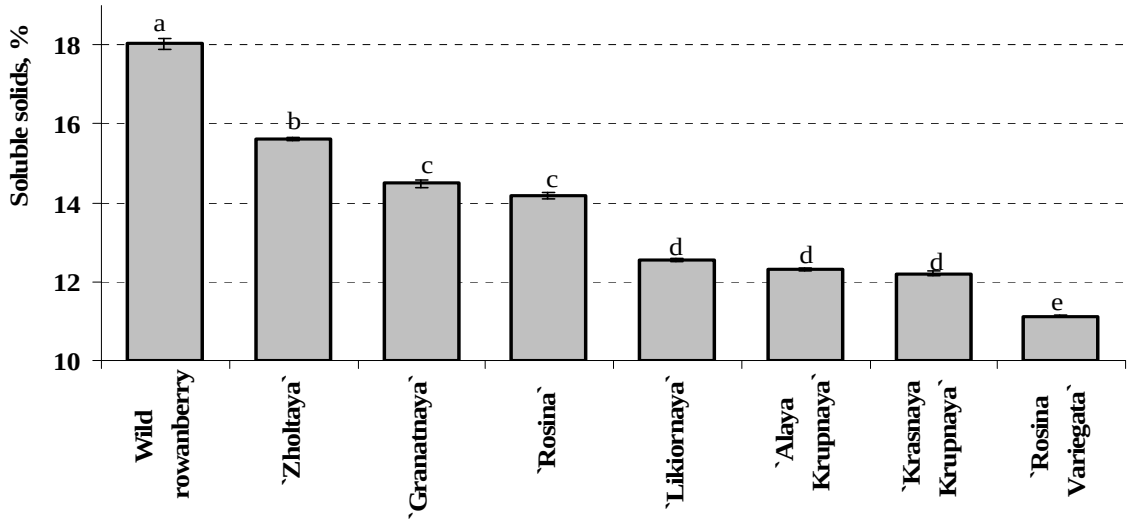

Figure 2. Content of soluble solids in rowanberries and their hybrids.
**Values, marked with the same letter, are not significantly different at p=0.05*

The total phenol content of rowanberries and their hybrids differed between 162 and 485 mg $100g^{-1}$ of fresh weight (Figure 3). It is lower than reported in literature (Hukkanen *et al.*, 2006). The highest phenol content was detected in the rowanberry and the chokeberry hybrid 'Likiornaya' (in average 484.9 mg 100 g^{-1}).

The differences in the *total carotenoid* content of eight rowanberry cultivars and hybrids were between 7 and 13 mg 100 g^{-1} of fresh berry weight (Table 1). The rowanberry and hawthorn hybrid 'Granatnaya' had the highest carotenoid content (13.04 mg 100 g^{-1}, in average).

The total tannin content of the evaluated samples was between 0.3 and 0.4 %, and only rowanberry cultivar 'Krasnaya Krupnaya' had a significantly lower content of total tannins (0.13 % on average) (Table 1). The wild rowanberry had the highest content of tannins (0.42 % in average) which explains the most astringent flavour of these berries compared to other rowanberry cultivars and hybrids.
The DPPH radical scavenging activity of the evaluated samples ranged from 2.5 to 11.2 g of berries per g of DPPH radical (Figure 3). The highest antiradical activity (DPPH) was found in the rowanberry and chokeberry hybrid 'Likiornaya' (11.2 g of berries per g of DPPH radical).
There was a significant (p=0.01) correlation between the antiradical activity and total phenolic content (r=0.960) (Figure 3).

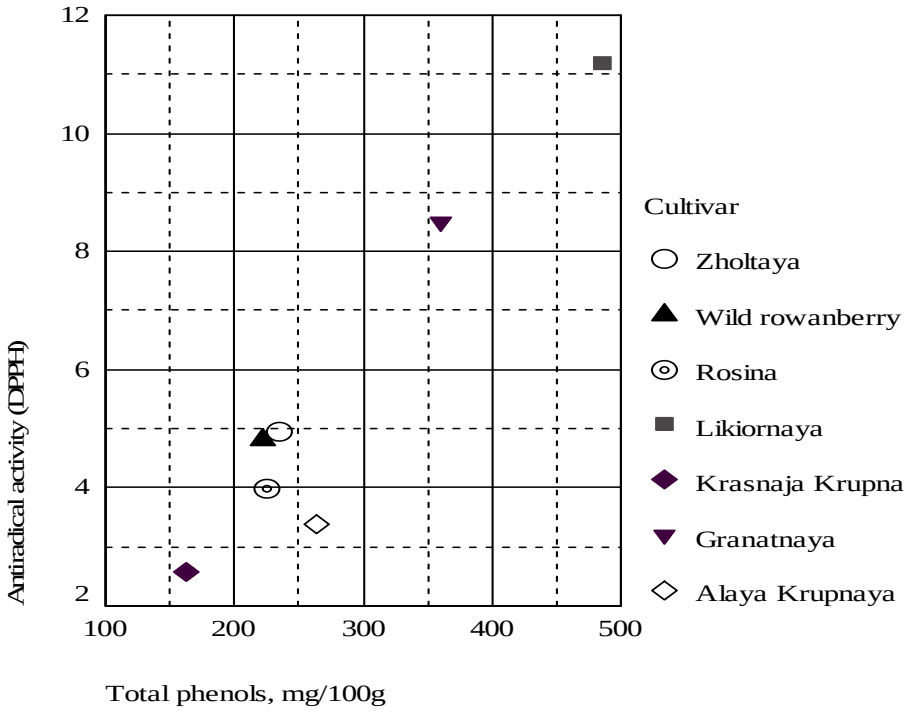

Figure 3. Pearson correlation plot between the antiradical activity (DPPH) and total phenolics of rowanberries and their hybrids.

The correlation between antiradical activity and phenolic content was found also in other investigations with berries (Hukkanen *et al.*, 2006; Gil *et.al.*, 2002). There was no statistically significant correlation between vitamin C content and antiradical capacity and also between carotenoids and antiradical capacity although these bioactive compounds are mentioned as strong antioxidants (Puupponen-Pimiä *et al.*, 2001; Sergio *et al.*, 1999; Sies and Stahl, 1995). This could be explained by the similar amounts of vitamin C and carotenoids in several rowanberry cultivars and hybrids while the phenolic content was different for the three better cultivars. It was also found that a significant negative correlation exists between the antiradical activity and the titrable acid content (p=0.008, r=-0.886). It is difficult to find any explanation for this correlation and it seems that it is specific only to the evaluated rowanberry cultivars.

Conclusions

There were no signifficant diferences between the chemical composition of the fresh and frozen rowanberry samples.
The highest content of ascorbic acid was found in rowanberries ‘Rosina’, 'Rosina Variegata', 'Krasnaya Krupnaya', and 'Zholtaya' (49 – 53 mg 100 g^{-1}). The highest content of carotenoids was detected in the rowanberry×hawthorn 'Granatnaya‘ (13.04 mg 100 g^{-1}), but the highest phenol content was detected in the rowanberry and chokeberry hybrid 'Likiornaya' (484.9 mg 100 g^{-1}). 'Likiornaya' also had the highest antiradical activity (11.2 g of berries per 1g of DPPH radical).

There was a significant correlation established only between the antioxidant activity and the total phenolic content (r=0.886).

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RESEARCH ON THE MINERAL COMPOSITION OF AMERICAN CRANBERRIES AND WILD CRANBERRIES IN LATVIA
AMERIKAS LIELOGU UN SAVVAĻAS DZĒRVEŅU OGU MINERĀLĀ SASTĀVA SALĪDZINOŠS IZVĒRTĒJUMS

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Abstract

Wild cranberry (*Vaccinium oxycoccus* L.*)* is one of the small fruit species of commercial importance in Latvia, traditionally used in folk-medicine and food. Typically, there are wide fluctuations in yield annually and between different parts of the country. The commercial cultivation of American cranberry (*Vaccinium macrocarpon* Ait.) was successfully started during last 15 years and today Latvia is the fourth major cranberries producing country. With the increase in consumption of cranberries, widely considered being as one of the healthiest foods, it becomes important to have detailed information on the nutritional content of cranberries. The aim of this study was to compare the contents of twelve biologically essential elements (N, P, K, Ca, Mg, S,

Fe, Mn, Zn, Cu, Mo, B) in berries of two *Vaccinium* species: *V. oxycoccus* and *V. macrocarpon.* Together 78 (leaf and berry) samples were collected from 4 main cranberry producing sites and 3 native bogs during autumn 2004.
The present study revealed significant differences in the mineral composition of *V. oxycoccus* and *V. macrocarpon.* Cultivated cranberry fruits had a higher content of P and Fe while wild cranberries showed higher levels of Ca, Mg Mn, Zn, Cu and B. Plant leaf analysis revealed organ-specific distribution of mineral elements in both cranberries studied. The present study shows that fruits of both *V. macrocarpon* and *V. oxycoccus* are a valuable source of the microelements: Mn, Fe, Cu, Mo, and B in human nutrition. Since the wild cranberry has a especially high concentrations of Mn, 100 g of fresh berries could supply 121% of the adult daily requirement.

Kopsavilkums
Savvaļas dzērvenes (*Vaccinium oxiccocus* L.) ir Latvijā augoša ogu suga, kuru tradicionāli izmanto pārtikā un tautas medicīnā. Raksturīgi, ka savvaļas dzērveņu ražas lielums ievērojami atšķiras gadu no gada, kā arī variē dažādās Latvijas vietās. Pēdējos 15 gados uzsākta Latvijā Amerikas lielogu dzērveņu (*Vaccinium macrocarpon* Ait.) kultivēšana un šobrīd Latvija ir ceturtā lielākā Amerikas lielogu dzērveņu ražojošā valsts pasaulē. Palielinoties dzērveņu, kā īpaši veselīga produkta, patēriņam ir svarīgi gūt pēc iespējas pilnīgāku priekšstatu par šo ogu minerālo sastāvu. Šī pētījuma mērķis bija salīdzināt divas *Vaccinium* ģints sugas *V. oxycoccus* un *V. macrocarpon* pēc 12 biogēno elementu (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) satura ogās. 2004 gada rudenī tika ievākti 78 lapu un ogu paraugi četrās ražojošās saimniecībās un trīs dabiskos purvos Latvijā.
Pētījumā tika konstatētas ievērojamas atšķirības savvaļas un Amerikas lielogu dzērveņu ogu minerālajās sastāvā. Kultivēto dzērveņu ogās bija augstāks P un Fe, bet savvaļas dzērvenēs augstāks Ca, Mg, Mn, Zn, Cu un B līmenis. Pētījums apstiprina, ka *V. oxicoccus* un *V. macrocarpon* ogas ir vērtīgs mikroelementu Mn, Fe, Cu, Mo un B avots cilvēka pārtikā. Jāatzīmē īpaši augsts mangāna saturs, 100 g svaigas ogas nodrošina 121 % no pieauguša cilvēka nepieciešamās Mn diennakts devas.

Key words: *Vaccinium oxycoccus* L., *Vaccinium macrocarpon* Ait., fruit mineral composition

Introduction
Wild cranberry (*Vaccinium oxycoccus* L.*)* is one of the small fruit species of commercial importance in Latvia, traditionally used in folk-medicine and food. Typically, there are wide fluctuations in yield annually and between different parts of the country (Ripa 1988; Yudina 1988). The yield of *V. oxycoccus* ranges from a few kilograms to 1000 kg ha^{-1} (from 5 to 100 berries m^2) (Cherkasov, 1988; Ripa, 1988; Yudina, 1988). *Vaccinium oxycoccus* usually is found on acid soils with humus type peat, dismoder or mor, and all the soils are poor in nutrients (Stewart and Nilsen, 1993; Schaminee *et. al*., 1995).
The commercial cultivation of the American cranberry (*Vaccinium macrocarpon* Ait.) was started only during last 15 years and is one of the youngest branches of agriculture in Latvia with a high potential in country's economical and ecological future. Today with more than 100 ha of commercial plantings Latvia is the fourth major cranberry producing country in the world. American cranberry producing plantings are mostly developed in high bog territories. *V. macrocarpon*, fruit indigenous to North America, are characterized as high yielding crop with significantly higher productivity (to 40 t ha^{-1}) in comparison with the wild cranberry (Karlsons and Osvalde, 2007).
Cranberries are valued for their fresh taste, high dietary and health values, as well as their great potential for being processed. Cranberry juice, sugared fruits, raisins, and other products have become increasingly popular in Latvia. A growing body of research suggests that the cranberry is a relatively unique fruit which accumulates one of the highest concentrations of phenolic compounds among fruit species with demonstrable human health benefits (Vinson *et al.*, 2001; Leahy *et al.*, 2002; Howell *et al.*, 2005). Cranberries are a good source of anthocyanins and their antioxidants, which plays a vital role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes (Konczak and Zhang, 2004). There are several reports focused on the effect of anthocyanins in cancer treatments (Castaneda-Ovando *et al.*, 2008; Lule and Xia, 2005;

Nichenametla *et al.*, 2006), human nutrition (Stintzing and Carle, 2004), and its biological activity (Kong et. al., 2003).

Increased consumption of fruits and vegetables can help replace foods high in saturated fats, sugar and salt and thus improve the intake of most micronutrients and dietary fibre (Ekholm *et al.*, 2007). Daily consumption of fresh fruits and vegetables (>400 $g \cdot d^{-1}$) is recommended to help prevent major non-communicable diseases such as cardiovascular diseases and certain cancers (WHO, 2003). Thus the chemical composition of *Vaccinium spp*. has important implications for human health. Many minerals are essential for normal metabolic functions and are required components in a balanced diet (Grusak and DellaPenna, 1999).

The mineral element contents of plants are known to be affected by the species and cultivar of the plant, soil conditions, weather conditions, the use of fertilizers and the state of the plants maturity at harvest (Pietola and Salo, 2000; Bálint *et al.*, 2001).

The aim of this study was to compare the contents of twelve biologically essential elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) in berries of two *Vaccinium* species: *V. oxycoccus* and *V. macrocarpon.*

Materials and methods

The study was carried out on the wild cranberry (*V. oxycoccus*) and the American cranberry (*V. macrocarpon*) crops in different regions of Latvia. Together 78 (leaf and berry) samples were collected from 4 main cranberry producing sites (Aluksne, Talsu, Madonas and Riga district) and 3 native bogs (Riga, Jelgava and Saldus district) during autumn 2004.

For each leaf sample 200 current season upright tips and for the berry sample about 400g of cranberry berries were collected from locations representative of the planting. The leaf and berry material was oven-dried at 60 ^{o} C and ground. Then the samples were dry-ashed in concentrated HNO_3 vapours and re-dissolved in HCl solution (HCl - distilled water mixture 3:100) (Rinkis *et al*., 1987).

Concentrations of 12 biogenous elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) were determined in all berry and leaf samples. The levels of Ca, Mg, Fe, Cu, Zn, and Mn were estimated by atomic absorption spectrophotometer (Perkin Elmer AAnalyst 700, acetylene-air flame)), those of N, P, Mo, B by colorimetry, S by turbidimetry, and K by flame photometer (Jenwey PFP7, air-propane butane flame).

The mineral element content in the berries was expressed as $mg \cdot 100g^{-1}$ fresh fruit. All chemical analyses were done in the Laboratory of plant mineral nutrition of the Institute of Biology, University of Latvia. The levels of statistical significance were determined with MS Excel 2003. T-test "Two-Sample Assuming Unequal Variances" ($p<0.05$) was used to compare the mean element concentrations in *V. oxycoccus* and *V. macrocarpon* fruits and leaves.

Results and Discussion

To characterize the mineral content of cultivated and wild cranberries, the levels of 12 biologically essential elements were estimated. Mean macro- and micronutrient concentrations, as well as the concentration range are shown in Table 1.

A comparison of two species studied showed similar concentrations for the macronutrients N, K and S in the cranberries. Statistically significant differences ($p < 0.05$) were noted for Ca, Mg and P. The data indicated that nitrogen and potassium were the major mineral constituents in the both cranberry species tested. The richest source of Ca and Mg (on average, 13.1 and 7.8 $mg \cdot 100g^{-1}$ fresh fruit) in this study was *V. oxycoccos*, while highest P (9.1 mg 100 g^{-1} fresh fruit) contents were found in *V. macrocarpon* fruits.

Table 1. Nutrient concentrations in cranberry berry samples from producing plantings (Aluksne, Talsu, Madonas and Riga district) and woodlands (Riga, Jelgava and Saldus district) in Latvia.

Element	*V.macrocarpon*		*V. oxycoccos*	
	Range	Mean ± SE	Range	Mean ± SE
	Macroelements (mg·100g^{-1} fresh berries)			
N	10.4 – 65.0	40.3 ± 3.38 a[1]	13.0 – 78.0	35.1 ± 9.36 a
P	6.5 – 11.7	9.1 ± 0.26 a	6.5 – 7.8	6.5 ± 0.13 b
K	52.0 – 98.8	79.3 ± 2.08 a	78.0 – 93.6	81.9 ± 1.82 a
Ca	7.8 – 14.3	10.4 ± 0.39 a	9.1 – 18.2	13.0 ± 1.30 b
Mg	5.2 – 9.1	6.5 ± 0.13 a	7.8 – 9.1	7.8 ± 0.13 b
S	5.2 – 14.3	9.1 ± 0.39 a	6.5 – 18.2	11.7 ± 1.43 a
	Microelements (mg·100g^{-1} fresh berries)			
Fe	0.22 – 1.17	0.51 ± 0.049 a	0.33 – 0.42	0.36 ± 0.010 b
Mn	0.06 – 0.57	0,21 ± 0.005 a	2.18 – 3.95	2.78 ± 0.194 b
Zn	0.07 – 0.17	0,12 ± 0.005 a	0.14 – 0.19	0.16 ± 0.007 b
Cu	0.04 – 0.08	0.05 ± 0.002 a	0.06 – 0.08	0.07 ± 0.002b
Mo	0.01 – 0.02	0.01 ± 0.001 a	0.01 – 0.02	0.01 ± 0.001 a
B	0.03 – 0.12	0.07 ± 0.005 a	0.10 – 0.17	0.13 ± 0.008 b

[1]Means with different letters in a row were significantly different (t-Test, $p < 0.05$)

Statistically significant differences ($p<0.05$) were found in the micronutrients (except Mo) composition of wild and cultivated cranberries.

The highest mean concentration of Fe (0.51 mg 100 g^{-1} fresh fruit) were found in the American cranberry, while the highest Mn, Zn, Cu and B (on average, 2.78, 0.16, 0.07 and 0.13 mg 100 g^{-1} fresh fruit, respectively) concentrations were found in the wild cranberry. Plant leaf analysis revealed organ-specific distribution of mineral elements in both cranberries studied (Table 2). Statistically significant differences were found between *V. macrocarpon* and *V. oxycoccus* for P, K, Ca, Mg, S, Fe, Mn, Zn and B results in leaf samples.

Table 2. Nutrient concentrations in cranberry leaf samples from producing plantings (Aluksne, Talsu, Madonas and Riga district) and woodlands (Riga, Jelgava and Saldus district) in Latvia.

Element	*V. macrocarpon*		*V. oxycoccos*	
	Range	Mean ± SE	Range	Mean ± SE
	Macroelements (% dry weight)			
N	0.52 – 0.78	0.66 ± 0.013 a	0.54 – 0.78	0.65 ± 0.032 a
P	0.06 – 0.27	0.12 ± 0.010 a	0.04 – 0.08	0.06 ± 0.005 b
K	0.29 – 0.80	0.56 ± 0.024 a	0.30 – 0.44	0.35 ± 0.013 b
Ca	0.64 – 1.45	0.84 ± 0.033 a	0.50 – 0.75	0.63 ±0.029 b
Mg	0.15 – 0.29	0.22 ± 0.007 a	0.09 – 0.14	0.12 ±0.006 b
S	0.06 – 0.17	0.11 ± 0.006 a	0.07 – 0.11	0.09 ± 0.004 b
	Microelements (mg·kg^{-1} dry weight)			
Fe	25.00 – 132.00	63.10 ± 5.28 a	36.00 – 360.00	137.44 ± 34.456 b
Mn	86.00 – 920.00	282.87 ± 45.60a	820.00 – 2860.00	1583.00 ± 259.850b
Zn	15.00 – 39.00	26.00 ± 1.13 a	28.00 – 50.00	37.67 ± 2.321 b
Cu	2.00 – 21.90	7.89 ± 1.07 a	5.60 – 7.60	6.52 ± 0.194 a
Mo	0.20 – 0.68	0.39 ± 0.03 a	0.25 – 0.50	0.34 ± 0.029 a
B	13.00 – 60.00	35.07 ± 2.16 a	18.00 – 34.00	21.78 ±1.623 b

[1]Means with different letters in a row were significantly different (t-Test, $p < 0.05$)

The contribution of *V. macrocarpon* and *V. oxycoccus* fruits as a dietary source of mineral elements was estimated from our study results. The potential contribution of 100 g of cultivated and wild cranberries to the Recommended Dietary Allowances (RDA) (USDA RDA chart, 2004) for mineral elements is presented in Table 3.

Table 3. Contribution of 100 g of cranberries to the Recommended Dietary Allowance (RDA) for adults per day.

Element	RDA*, mg	Element concentration mg 100 g^{-1} g fresh fruit		% of RDA supplied by 100 g cranberries	
		V.macrocarpon	*V.oxycoccos*	*V.macrocarpon*	*V. oxycoccos*
P	700	9.1	6.5	1.32	0.97
K	2500	79.3	81.9	3.16	3.28
Ca	1000	10.4	13.0	1.07	1.31
Mg	420	6.5	7.8	1.64	1.93
S	850	9.1	11.7	1.06	1.39
Fe	8	0.51	0.36	6.33	4.46
Mn	2.3	0.21	2.78	9.26	120.96
Zn	11	0.12	0.16	1.11	1.49
Cu	0.9	0.05	0.07	6.00	7.67
Mo	0.045	0.01	0.01	8.89	8.89
B	1.5	0.07	0.13	4.73	8.73

* USDA RDA chart (2004)

Wild and cultivated cranberries supply 9.26 % and even 120.96 %, respectively, of the adult daily requirement for Mn. The content of Fe, Cu, Mo, and B in 100 g fresh fruits of both cranberries studied also contributes from 4.46 % to 8.89 % of the daily micronutrient requirement. From macronutrients only K in *V. macrocarpon* and *V. oxycoccus* fruits was found in appreciable amounts (3.16 and 3.28 %, respectively of the RDA). One hundred grams of fresh wild and cultivated cranberries may supply also a few percent of RDA for P, Ca, Mg, S and Zn.

The relationship between food and health becomes increasingly significant as consumers now demand healthy, tasty and natural foods that have been grown in uncontaminated environments. Numerous studies have shown that among horticultural crops fruits are an important source of dietary nutrients, especially with respect to minerals (Grusak and DellaPenna, 1999). Among berry fruits, cranberries are considered to be not only an excellent source of phenolic compounds and vitamins, but also as valuable source of mineral nutrients (Ekholm *et. al*., 2007).

Our research revealed statistically significant differences between *V. macrocarpon* and *V. oxycoccus* results for P, Ca, Mg, Fe, Mn, Zn, Cu and B in fruit samples and P, K, Ca, Mg, S, Fe, Mn, Zn and B in leaf samples. Nitrogen is the controlling element for American cranberry nutrition and adequate fertilization, in general, is used to maintain renewal growth, crop production, and flower bud development for the next crop (DeMoranville, 1997). However surprisingly similar N concentrations were found in the cultivated American cranberry and wild cranberry leaf samples. It should be stressed that *V. oxycoccos* growing medium – sphagnum peat are especially N poor (Stewart and Nilsen 1993). Our research revealed a considerably higher content of Mn in *V. oxycoccus* leaves (1583.0 mg kg^{-1}) in comparison to American cranberry leaves (282.9 mg kg^{-1}). This phenomenon could be explained by the different pH of the growing substrata for both species studied. Significantly lower pH and consequentially higher availability of Mn in natural high bogs was found in our previous studies (Karlsons and Osvalde, 2007). Both cranberry fruit Mn contents supported these differences.

Cultivated cranberry fruits had higher content of P and Fe while wild cranberry fruits showed the highest levels of Ca, Mg, Mn, Zn, Cu and B. The sequences with regard to the content of macro- and microelements in *V. macrocarpon* fruits were as K > N > Ca > P = S > Mg and Fe > Mn > Zn

> B > Cu > Mo, respectively. The order of macro- and micronutrient concentrations in *V. oxycoccos* was N > K > Ca = S > Mg > P and Mn > Fe > Zn > B > Cu > Mo.

In general, the fruit mineral nutrient concentrations found in the studied species in Latvia were similar or considerably higher (Ca, Mg, Fe, Zn and Cu) than values reported for the American cranberry (USDA, 2008). Reported mean values for Fe content (0.25 $mg \cdot 100g^{-1}$ fresh fruit) in *V. macrocarpon* fruits (USDA, 2008) were almost twice lower than our Fe results for American cranberries. To the author's knowledge, there is little comparable data in the literature which show the detailed mineral content of *V. oxycoccus*. However the macronutrient content in wild cranberries analyzed was lower (except Ca), but Mn concentrations significantly higher than the reported values for *V. oxycoccos* in Finland (Ekholm *et. al*., 2007)

The nutritional significance of fruits as a dietary source of minerals is related to the contribution they make to the Recommended Dietary Allowance (RDA). The present study shows that fruits of both *V. macrocarpon* and *V. oxycoccus* are a ood sources of Mn (9.26 % and 120.96 % of the recommended daily dose, accordingly) in human nutrition. The content of Fe, Cu, Mo, and B in 100 g of fresh fruits of both cranberries studied also contributes from 4.46 % to 8.89 % of the daily micronutrient requirement.

All macronutrients, except K, in both species of cranberries were stated in relatively low concentrations. Only K in *V. oxycoccus* berries was found in appreciable amounts (3.28 % of the RDA). One hundred grams of fresh wild and cultivated cranberries may supply also a few percent of the RDA for P, Ca, Mg, S and Zn. The availability of Ca in the body to great extent depends on the calcium to phosphorous ratio. The recommended optimal Ca : P ratio in the diets is 1.0 to 1.3 (Calvo & Park, 1996). In our study such a Ca : P ratio was characteristic only for American cranberries.

Conclusions

The present study revealed significant differences in the mineral composition of *V. oxycoccus* and *V. macrocarpon.* Cultivated cranberry fruits had higher content of P and Fe while wild cranberries had higher levels of Ca, Mg Mn, Zn, Cu and B. Plant leaf analysis revealed organ-specific distribution of mineral elements in both species of studied cranberries.

The present study shows that fruits of both *V. macrocarpon* and *V. oxycoccus* are a valuable source of microelements: Fe, Cu, Mo, B and especially Mn, in human nutrition.

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CLIMATIC EFFECTS ON THE PRODUCTION AND QUALITY OF BILBERRIES (*VACCINIUM MYRTILLUS*)
KLIMATA IETEKME UZ MELLEŅU (*VACCINIUM MYRTILLUS*) RAŽU UN KVALITĀTI

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Abstract

The bilberry (*Vaccinium myrtillus*), also called European blueberry, is one of the most significant wild berries in the Nordic countries. The berries are recognized for their bioactive properties and distinctive aroma and flavour. The effects of climate on the production and the quality of bilberries have been studied in a controlled experiment in a phytotrone using clonal material. In the experiment individual plants from two Northern clones and two Southern clones of bilberry were grown at 12° and 18 °C. At each temperature 2 different light treatments were tested; 1) 24 hour (h) natural light (long day) and 2) 12 h natural light (short day). All plants were kept outside during flowering to ensure pollination by insects. Berries were sampled when ripe, weighed and stored at -80°C for later analysis.

Kopsavilkums

Mellenes (*Vaccinium myrtillus*), sauktas arī Eiropas mellenes, ir ļoti nozīmīgas savvaļas ogas Ziemeļu valstīs. Ogas ir atzītas to bioloģiski aktīvo vielu satura un īpašās smažas un garšas dēļ. Klimata ietekme uz ražu un tās kvalitāti tika pētīta kontrolējamos eksperimenta apstākļos,

izmantojot klonu materiālu. Atsevišķi augi no diviem Ziemeļu melleņu kloniem un diviem Dienvidu melleņu kloniem tika audzēti 12° un 18 °C temperatūrā. Katras temperatūras apstākļiem tika izmantoti divu veidu apgaismojumi: 1) 24 stundas dabīgs apgaismojums (garā diena) un 2) 12 stundas dabīgs apgaismojums (īsā diena). Lai nodrošinātu apputeksnēšanos ar kukaiņu palīdzību, visi augi ziedēšanas laikā tika turēti āra apstākļos. Ogu nogatavošanās laikā tika ievākti ogu paraugi, nosvērti un uzglabāti – 80 °C vēlākām analīzēm.

Key words: bilberry, clonal differences, European blueberry, quality, temperature effects

Introduction

The bilberry (*Vaccinium myrtillus* L.), also called the European blueberry, is a long-lived ericaceous dwarf shrub that grows wild in Europe and Asia, and most abundantly in the northern and eastern parts of Europe. The berries are recognized for their distinctive aroma and flavor and their high concentrations of phenolic compounds such as anthocyanins (Lätti *et al.*, 2008). Obvious differences between the European blueberry and other blueberry species like *V. angustifolium*, *V. corymbosum* (highbush), *V. ashei* Reade (rabbiteye) and *V. myrtilloides* Minchx (sour-top lowbush) are the blackish-blue colour of the flesh compared to the whitish fruit flesh of the others. Results from Giovanelli and Buratti (2009) show that total phenolics and total anthocyanin concentrations were, respectively, two fold and three fold higher in *V. myrtillus* than in *V. corymbosum*. The bilberries also had a higher anthocyanin-to-total phenolic ratio. In a study from Finland (Lätti *et al.*, 2008) it was found that the berries of the southern regions had a significantly lower content of total anthocyanins compared with the central and northern regions. The aim of this study was to study the role of the genotype and the environment (temperature and daylength) under controlled conditions in a phytotrone on the contents of carbohydrate, acids and phenols in the bilberry. Such information is important for the development of the European blueberry as a commercial crop.

Material and Methods

Material and experimental design. Individual plants of four clones derived from the southern (S1 and S2) and northern (N1 and N2) part of Finland were grown in phytotron in Tromsø, Norway at 12° and 18 °C. At each temperature 2 different light treatments were tested; 1) 24 hour (h) natural light (long day) and 2) 12 h natural light (short day). All plants were kept outside during flowering to ensure pollination by insects. Berries were sampled when ripe, weighed and stored at -80°C for later.

Bilberry Extraction Procedure. Frozen bilberries (3-6 berries) were sliced with a scalpel, and 320 mg f.w. of each sample was transferred to a round-bottom shaped microtube (2 ml). Pre-cooled (-20°C) methanol (400 µl) containing ribitol as the internal standard (25 µg/ ml), was added to each tube and vortexed for 5 seconds (s). Sample tubes were treated for 1 hour (h) at 60°C in an ultrasonic bath, and cooled down to room temperature before the next step. 200µl chloroform was added, and tubes vortexed for 5 s to remove the lipids. Additional 400 µl H_2O were added and tubes vortexed for 10 s. Samples were centrifuged at 13,000 rpm and 4°C for 10 min. An aliquot of 300 µl from the clear supernatant was transferred into a V-shaped 1.5 ml microtube for GC-MS analysis. Yet another 300 µl aliquot was stored in a 1.5 ml microtube at -20 °C for later phenol analyses. The GC-MS samples were dried in a speed vac overnight without heating and stored at -80°C prior to further processing. For the derivatization of extracted sugars, acids and secondary metabolites, sample residues were re-dissolved in 80 µl of 20 mg ml^{-1} methoxyamine hydrochloride in pyridine, and treated at 30°C for 90 min in an incubator (200 rpm). Finally, samples were treated with 80 µl of MSTFA (N-Methyl-N-(trimethylsilyl)trifluoroacetamide) at 37°C for 30 min in an incubator (200 rpm). Samples were transferred to 1.5 ml autosampler vials with glass inserts, and stored at -20 °C prior to GC-MS analysis.

Gas Chromatography–Mass Spectrometry analysis (GC-MS). An Agilent 6890/5975 GC-MS was used for all analyses. Sample volumes of 1 µl were injected with a split ratio of 25:1. GC separations were carried out on a HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The injection temperature was 230 °C, and the interface was set to 250 °C. The carrier gas was helium at a constant flow rate of 1 ml min^{-1} The GC temperature program was held

isothermically at 70°C for 5 min, ramped from 70 °C to 310 °C at a rate of 5 °C min, and finally held at 310 °C for 7 min (analysis time: 60 min). The MS source was adjusted to 230 °C and a mass range of *m/z* 50-700 was recorded. All mass spectra were acquired in EI mode.
Chromatogram visualization and peak integration was carried out using the Agilent ChemStation software. For mass spectra evaluation and peak identification, the AMDIS software (v. 2.64) was used in combination with the following mass spectral libraries: NIST05 database and a target TMS database (Max-Planck Insitute for Molecular Plant Physiology, Golm, Germany). All compounds were quantified based on the internal standard ribitol and expressed as mg/100 g f.w.
Total Phenolics. The analysis of total phenolics content was based on a modified Folin-Ciocalteu method (Ainsworth and Gillespie, 2007). Berry extracts (see *Bilberry Extraction Procedure*) were diluted 1:40 before incubation at ambient temperature for 2 h. Samples (200 µl) were transferred to a clear 96-well microplate, and the absorption was measured at 750 nm on a plate reader (Labsystems Multiskan MS). Total phenolics were expressed as mg gallic acid equivalents (GAE) 100 g f.w. of berries.
Statistics. Statistics were done using Mintitab software. At each treatment there were two individual plants of each clone.

Results and Discussion
At the lowest temperature the two Northern clones produced berries earlier than the two Southern clones (Figure 1) and Southern clones produced far more berries during short days than during long days. These results indicate that the Northern clones are able to grow rapidly at reasonably low temperatures when given 24 hour of light, while the Southern clones are adapted to shorter days and are not able to use the 24 h light efficiently. At 18 °C all clones produced berries as early as 28 days after pollination (Figure 2). The Northern clones produced unexpectedly more berries than the Southern clones during both the long day and the short day treatment at 18 °C.
Berries produced at 12 °C had a significantly higher % of sugars (sucrose, fructose and glucose), phenolic acids and total phenols than berries produced at 18 °C (Table 1). The increased amount of total phenols found at low temperatures contradicts the findings of Rieger *et al.* (2008) where the level of anthocyanins in bilberries decreased in amounts while the antioxidant level increased with rising altitudes. The study of Rieger *et al.* (2008) was, however, only focused on anthocyanins and was not performed under controlled conditions. Howard *et al.* (2003) analyzed five commercial cultivars and 13 breeding selections of *V. corymbosum* L. grown at the same location for total phenolics over two growing seasons and found that the variation between genotypes was much greater than that observed between growing seasons.
However, several genotypes varied significantly over the two growing seasons indicating that environmental growing conditions can impact the amount of phenolics in blueberries.

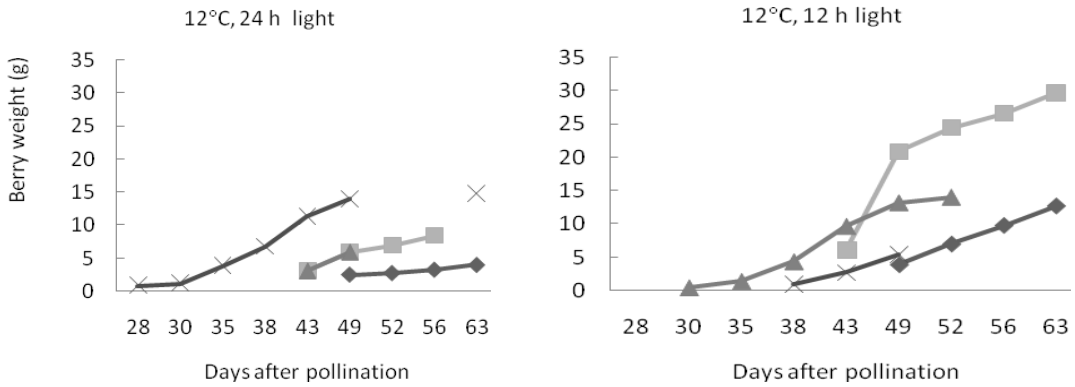

Figure 1. Accumulated production of berries at 12°C.
♦ clone S1, ■ clone S2, ▲ clone N1, × clone N2

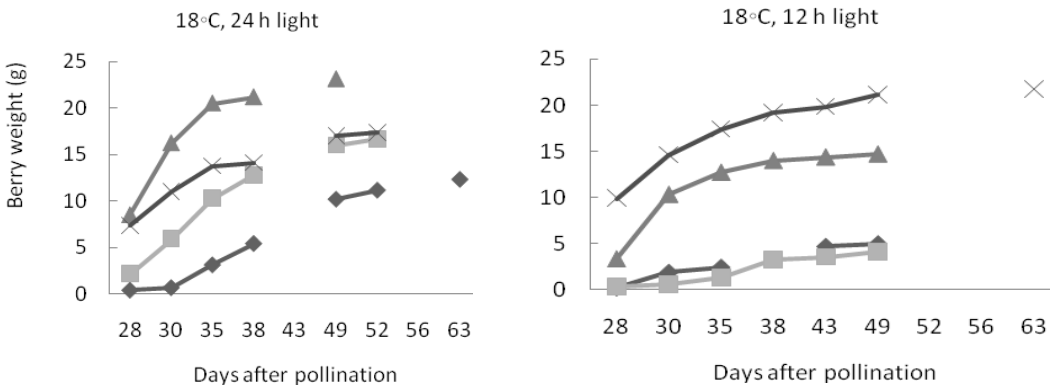

Figure 2. Accumulated production of berries at 18°C.
♦ clone S1, ■ clone S2, ▲ clone N1, × clone N2

Studies on raspberries (Anttonen and Karjalainen, 2005) also indicate that growing conditions (light, temperature and soil condition) affect the phenolic content in northern latitudes; however, the variation between genotypes was considerable.

Table 1. Analysis of % sugars, % acids and total phenols in bilberries grown at 12° and 18°C.

Temp.°C	% Sugars	% Acids	Total phenols mg/100 g
12	13.1	3.7	583.8
18	9.2	2.9	556.8

(The results are presented as the mean for all clones and all treatments at the given temperature.)

Conclusions

The results show that the clones of bilberries originating from northern areas are better adapted to low temperatures and long days (24 h light) than clones originating from southern areas. Breeding should therefore aim both for the selection of material for northern conditions and material for southern conditions. Low temperatures lead to a significantly higher content of sugars, acids and total phenols. Berries from the northern areas possess the added value of sweetness and a higher content of the health beneficial phenols. In conclusion, breeding material should be selected based on the evaluation of results achieved from the health benefit compounds of bilberry clones. Moreover, the environmental effects on berry quality will open the door for breeding specific clones for specific regions.

Acknowledgements

The study was financed by Norden Nordic Innovation Centre, program “New Nordic Food” and the Norwegian Research Council, program “Matprogrammet”.

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IN VITRO PROPAGATION OF SEVERAL *VACCINIUM CORYMBOSUM* L. AND *VACCINIUM VITIS-IDAEA* L. CULTIVARS
VAIRĀKU *VACCINIUM CORYMBOSUM* L. UN *VACCINIUM VITIS-IDAEA* L. ŠĶIRŅU *IN VITRO* PAVAIROŠANA

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Abstract

In the paper the efficient in vitro regeneration and propagation systems in several *Vaccinium corymbosum* L. and *Vaccinium vitis-idaea* L. cultivars are described. It was found that shoot regeneration ability is highly dependent on the cultivar and cytokinin applied. Zeatin showed itself to be efficient for axillary and adventitious shoot induction in both species. A zeatin concentration of 2 mg l^{-1} was the best for axillary shoot regeneration in *V. corymbosum*, while zeatin in a concentration of 0.75 - 1.5 mg l^{-1} was suitable for *V. vitis-idaea* cultivars. Multiple adventitious shoot formation on leaf explants of *V. corymbosum* occurred on the medium with 0.5 mg l^{-1} zeatin. In *V. vitis-idaea* indirect shoot regeneration was observed in cv. ´Red Pearl´ after transfer of the calli on the medium with 0.5 mg l^{-1} zeatin. For long-term proliferation of in vitro regenerated axillary or adventitious shoots an AN medium with 0.5 mg l^{-1} zeatin was successfully used. Satisfactory rooting of isolated microshoots was achieved in vitro on an AN medium supplemented with 0.8 mg l^{-1} IBA and 0.8 g l^{-1} charcoal.

Kopsavilkums

Šajā rakstā ir apskatīta efektīva *in vitro* reģenerācijas un pavairošanas sistēma vairākām *Vaccinium corymbosum* L. un *Vaccinium vitis-idaea* L. šķirnēm. Dzinumu reģenerācijas spēja ir atkarīga no šķirnes un apstrādes ar citokinīniem. Zeatīns bija efektīvs, lai veicinātu sānu un adventīvo dzinumu augšanas veidošanu. *V. corymbosum* sāndzinumu veidošanai piemērotākā zeatīna koncentrācija bija 2 mg l^{-1}, bet *V. vitis-idaea* šķirnēm piemērotāka bija 0.75 - 1.5 mg l^{-1} zeatīna koncentrācija. Barotnē ar 0.5 mg l^{-1} zeatīnu novērota masveidīga adventīvo dzinumu veidošanās *V. corymbosum* lapu eksplantiem. Netieša dzinumu reģenerācija tika novērota *V. vitis-idaea* šķirnei 'Red Pearl' pēc kallusa pārnešanas uz barotni ar 0.5 mg l^{-1} zeatīnu. Zeatīns 0.5 mg l^{-1} koncentrācijā tika sekmīgi pielietots AN vidē, lai nodrošinātu ilgstošu *in vitro* reģenerēto sānu un adventīvo dzinumu proliferāciju. Apmierinoša izolēto mikrodzinumu apsakņošanās tika sasniegta AN vidē, kas papildināta ar 0.8 mg l^{-1} indolilsviestskābes un 0.8 g l^{-1} kokogļu.

Key words: *Vaccinium corymbosum* L., *Vaccinium vitis-idaea* L., axillary shoot and adventitious shoot regeneration

Abbreviations: AN – Anderson medium; IBA – indole-3-butyric acid; TDZ - thidiazuron (N-phenyl-N1-1,2,3,-thiadiazol-5-ylurea); 2-iP (N6-[2-Isopentenyl]adenine)

Introduction

The *Vaccinium corymbosum* L. and *Vacinium vitis-idaea* L. represent commercially important and biologically valuable small fruits (Song and Sing, 2004) desirable for cultivation in various parts of the world. Production of high quality plants in large amounts is needed for commercial plantation establishment. In this respect, in vitro plant regeneration from dormant buds and through adventitious organogenesis enables effective mass production of plant material and to the create necessary conditions for the application of genetic engineering. Several papers have described micropropagation methods for these economically important species (Shibli and Smith, 1996; Jaakola *et al.*, 2001; Debnath and McRae, 2002; Ostrolucká *et al.*, 2002; Cao *et al.*, 2003;

Ondrušková *et al.*, 2003; Petri and Burgos, 2005; Gajdošová *et al.*, 2006; Gajdošová *et al.*, 2007). However, the results show that the different cultivars within the same species can differ in their requirements for the optimal growth regulator concentrations therefore it is important to get exact information on culture conditions for different cultivars.
In this paper micropropagation by axillary branching and adventitious shoot regeneration is described in a wide range of *Vaccinium corymbosum* L. and *Vaccinium vitis-idaea* L. cultivars.

Material and methods
As an initial plant material for axillary shoot formation stem cuttings with dormant buds were collected from the selected cultivars of mature plants during February and the beginning of March: *Vaccinium corymbosum* L. - cvs. ´Berkeley', ´Bluecrop´, ´Blueray´, ´Duke´, ´Brigitta´ and *Vaccinium vitis-idaea* L. - cvs. ´Red Pearl´ and ´Koralle´. Nodal segments with buds were washed under running water for 1 h and sterilised 2 min in 70 % ethanol, 6 min in a 0.1 % solution of mercuric chloride with 3 drops of Tween, followed by washing 3x15 min in sterile distilled water. For shoot regeneration dormant apical and axillary buds were used, from which the upper scales were removed after sterilisation. Anderson's Rhododendron medium - AN (Anderson, 1980) with 30 g l^{-1} sucrose, 8 g l^{-1} Phyto agar, pH adjusted to 4.8 – 5.0 and supplemented with different cytokinin concentrations was used for cultivation. Cultures were maintained in the growth chamber at 23 ± 2 ^{o}C under 16/8 light and dark photoperiod and a light intensity of 50 μmol m^{-2} s^{-1} provided by white fluorescent lamps. In *V. corymbosum* cvs. 0.5 and 2.0 mg l^{-1}zeatin and in *V. vitis-idaea* L. cvs. 0.25 – 2.0 mg l^{-1} zeatin and 2.5 - 20.0 mg.l^{-1} 2-iP, were tested for axillary shoot regeneration. The influence of cytokinins, zeatin and 2-iP, on shoot regeneration ability was evaluated after 5 weeks. Data evaluation was performed using Statgraphic PLUS 5 for Windows.
For adventitious shoot regeneration leaves from the in vitro plants of *V. corymbosum* L. - cvs. ´Berkeley´, ´Bluecrop´ and ´Brigitta´ with cut margins were cultivated horizontally with adaxial surface on an AN medium with 30 g l^{-1} sucrose, 8 g l^{-1} Phyto agar, pH adjusted to 4.8-5.0, supplemented with 0.5 mg l^{-1} zeatin and 2.2 mg l^{-1} TDZ. Their regeneration ability based on shoot proliferation was evaluated in three subcultures, during a five week period. In *V. vitis-idaea* L. - cvs. ´Red Pearl´ and ´Koralle´ as primary explants stem cuttings and leaves from in vitro plants were used for adventitious shoot induction. For each experiment 30 explants were cultivated (5 explants/culture dish x 6 culture dishes). AN medium with zeatin in concentrations 2.2 and 4.4 mg.l^{-1} or thidiazuron in concentrations 1.1 mg l^{-1}, 2.2 mg l^{-1} and 3.3 mg l^{-1} was used the for induction of adventitious organogenesis. After 5 weeks of cultivation the explants were transferred into an AN medium with 0.5 mg.l^{-1} zeatin for shoot multiplication. The percentages of explants regenerating shoots or inducing callus and the number of regenerated shoots per explant after 3 subcultures were recorded. The long-term shoot proliferation was performed on an AN medium with 0.5 mg l^{-1} zeatin. For the microshoot rooting an AN medium supplemented with 0.8 mg l^{-1} IBA and 0.8 g l^{-1} charcoal was tested.

Results and Discussion
The results of the experiments on shoot regeneration from dormant apical and axillary buds in *V. corymbosum* L. showed that shoot regeneration ability is highly dependent on cultivar and cytokinin concentrations. Zeatin in a concentration of 2 mg l^{-1} was proved to be more effective for shoot regeneration in comparison with 0.5 mg l^{-1} zeatin (Figure 1).
The statistically significant differences in the number of shoots per explant were obtained on a medium with 2 mg l^{-1} zeatin in tested cultivars with the highest regeneration ability in cvs. 'Brigitta' and 'Blueray'.

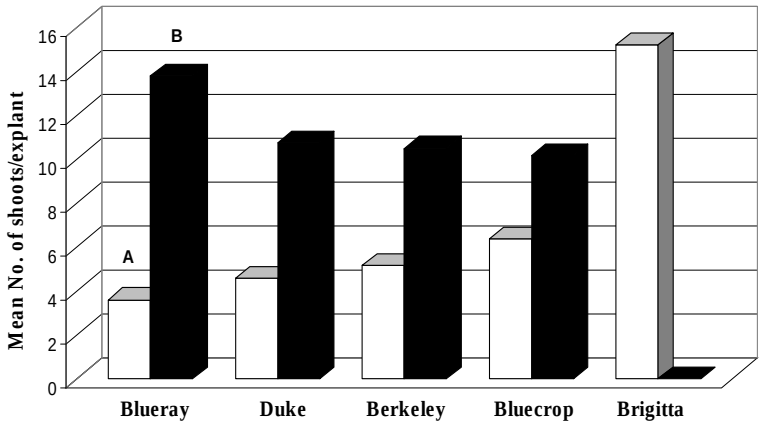

Figure 1. Effect of zeatin (A - 0.5 mg l^{-1}; B - 2.0 mg l^{-1}) on *V. corymbosum* L. shoot regeneration.

The positive influence of zeatin on multiple shoot induction from apical and axillary buds was confirmed also in *V. vitis-idaea* cvs. Statistically significant differences in the number of shoots per explant were obtained in cv. 'Koralle' with the highest shoot formation on the zeatin concentration of 0.75 mg l^{-1}. The differences between the control and the variants with different concentrations of 2-iP were not statistically significant (Table 1.).

Table 1. Multiple range analysis for shoot number/explant at different zeatin and 2-iP concentrations in *Vaccinium vitis-idaea* L. - cvs. 'Koralle' and 'Red Pearl'

Zeatin (mg l^{-1})	Mean number of shoots/explant		2-iP (mg l^{-1})	Mean number of shoots/explant	
	'Koralle'	'Red Pearl'		'Koralle'	'Red Pearl'
0.00	1.24 a	0.99 a	0.00	1.24 a	0.99 a
0.25	3.00 ab	3.93 b	2.50	1.25 a	1.82 ab
0.50	3.84 bc	5.02 bc	5.00	1.60 a	3.44 bcd
0.75	5.19 c	5.55 bc	10.00	1.42 a	2.84 abc
1.00	4.76 bc	6.98 c	15.00	1.27 a	6.66 e
1.50	3.69 abc	6.90 c	17.50	1.16 a	5.47 de
2.00	3.13 abc	6.20 bc	20.00	1.09 a	4.68 cde

In cv. 'Red Pearl' the both tested cytokinins were effective in shoot regeneration. On the media with zeatin the highest shoot regeneration was obtained at the zeatin concentration 1.0 – 1.5 mg l^{-1}. The most effective 2iP concentration was 15 mg l^{-1} (Table 1.). Regeneration ability was different for each cultivar. The shoot proliferation per explant was higher in ´Red Pearl´ in comparison with cv. ´Koralle´ for both tested cytokinins.

For induction of adventitious organogenesis on the leaf explants of *V. corymbosum* L. 0.5 mg l^{-1} zeatin and 2.2 mg l^{-1} TDZ were used. On the media with TDZ callus formation was observed, but no adventitious buds were visible during the 5 weeks of cultivation. Shoot formation occurred only after their transfer on medium with 0.5 mg.l^{-1} zeatin, which confirms the positive zeatin influence on shoot regeneration. On the other hand, multiple adventitious shoots were formed after 5 weeks of cultivation on the leaf explants initially cultivated on an AN medium with 0.5 mg.l^{-1} zeatin, produced via direct regeneration. After the 3rd subculture on the same medium a high number of vigorous shoots with good elongation growth was obtained per explant in the tested cultivars (Table 2.).

Table 2. Adventitious shoot regeneration from leaf tissue in *Vaccinium corymbosum* L. with 0.5 mg l^{-1} zeatin

Cultivar	% of leaves regenerating shoots	Total number of adventitious shoots			Mean no. of shoots/explant
		I. subcultivation	II. subcultivation	III. subcultivation	
'Berkeley'	35	110.0	237.14	362.0	18.1
'Bluecrop'	10	13.3	50.33	133.5	6.68
'Brigitta'	40	129.37	257.50	380.0	19.0

The regeneration ability of the cultivars was different. The highest in vitro shoot regeneration was obtained in cv. ´Brigitta´ (19.0), which is in correlation with regeneration achieved via culture of apical and axillary buds (Figure 1.).

In the *V. vitis-idaea* L. an AN medium with zeatin 2.2 mg l^{-1} and 4.4 mg l^{-1} or thidiazuron 1.1 mg l^{-1}, 2.2 mg l^{-1} and 3.3 mg l^{-1} was used for the induction of adventitious organogenesis on the stem and leaf explants of in vitro plants. On the media with TDZ callus formation was observed, but even after transfer on medium with zeatin no shoot formation occurred and calli necrotized. In contrast to *V. corymbosum* L. no direct regeneration of adventitious buds was recorded in *V. vitis-idaea* L. on media with zeatin. The callus formation was observed on leaf and stem explants of both tested cvs. ´Red Pearl´ and ´Koralle´, with small differences in the percentage of callus formation between tested zeatin concentrations (2.2 and 4.4 mg l^{-1}). However, the percentage of callus formation was markedly higher from stem tissue in comparison with leaf tissue. After the transfer of callus on an AN medium with 0.5 mg l^{-1} zeatin adventitious buds developed on the callus surfaces on both leaf and stem explants only in cv. ´Red Pearl´. No bud induction and shoot regeneration occurred from callus in cv. 'Koralle'. After the 3rd subculture the number of adventitious shoots in 'Red Pearl' regenerated from the leaf originated callus was significantly higher than from the stem callus. The total shoot number reached 1592 shoots/explant induced from the leaf and 266 from the stem explants (Table 3).

Table 3. Indirect regeneration of adventitious shoots in *Vaccinium vitis-idaea* L. on AN medium with zeatin

Cultivars	Primary explant	Shoot regeneration via callus phase			Total mean number of shoots/explant
		2.2 mg l^{-1} zeatin (A) callus %	4.4mg l^{-1} zeatin (B) callus %	0.5 mg l^{-1} zeatin A + B total number of shoots	
'Red Pearl'	Leaves	20.0	23.3	1592	53.07
	Stems	83.3	83.3	266	44.33
'Koralle'	Leaves	13.3	6.6	0	0
	Stems	16.6	16.6	0	0

Our results, as well as the results of other researchers, confirmed that zeatin is the best for axillary and adventitious shoot regeneration and proliferation in *Vaccinium* spp. (Chandler and Draper, 1986; Rowland and Ogden, 1992; Marcotrigiano *et al.*, 1996; Cao and Hammerschlag, 2000). Unlike who results of other researchers (Reed and Abdelnour-Esquivel, 1991; Debnath and McRae, 2002; Meiners *et al.*, 2007), the used higher zeatin concentrations (4.0 mg l^{-1} and more), in our experiments the zeatin in the lower concentrations was successfully applied for axillary (0.75 – 2.0 mg l^{-1}; Figure 2.) and adventitious (0.5 mg l^{-1}) shoot regeneration in *V. corymbosum* and *V. vitis idaea*, which is an important factor reducing the micropropagation cost, as well as minimizing undesirable somaclonal variation, as it was confirmed in the previous paper of Ostrolucká *et al.* (2007).

For long-term proliferation of in vitro regenerated axillary or adventitious shoots an AN medium with 0.5 mg l^{-1} zeatin can be successfully used in both *Vaccinium* species, on which formation of vigorous multiple shoot cultures was observed (Figure 3). The differences in intensity of shoot proliferation on an AN culture medium with the zeatin content 0.5 mg l^{-1} confirmed dependence of shoot formation and multiplication on the regeneration ability of the cultivars. Similar correlations were found also by other authors (Popowich and Filipenya, 1997).

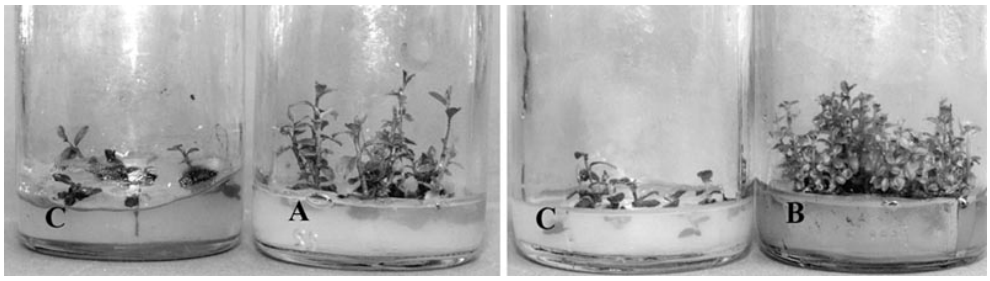

Figure 2. Axillary shoot regeneration in *V. vitis-idaea* cv.'Koralle' on AN medium with 0.75 mg l^{-1} zeatin (A) and cv.'Red Pearl' on AN medium with 15 mg l^{-1} 2-iP (B), control (C).

Satisfactory rooting of isolated microshoots in *V. corymbosum* L. and *V. vitis-idaea* L. was achieved on an AN medium supplemented with 0.8 mg l^{-1} IBA and 0.8 g l^{-1} charcoal. In *V. corymbosum* the percentage of rooting was 95 %, 90 % and 80 % in cv. 'Berkeley', 'Bluecrop' and 'Brigitta', respectively. In *V. vitis-idaea* the rooting percentage reached 60 % and 40 % in 'Read Pearl' and 'Koralle', respectively. Rooted plantlets transplanted into peat substrate were subsequently acclimatized under greenhouse conditions without any special light treatment, with regular irrigation, in containers at the beginning (2 weeks) covered by lid, later opened. Plantlets in length 80 – 100 mm (after 2 months) were replanted into bigger containers (120 mm in diameter) and placed under open-air conditions. Transfer of regenerants from in vitro to *ex vitro* conditions and their acclimatization was successful, as almost 80-90 % of transferred plants survived.

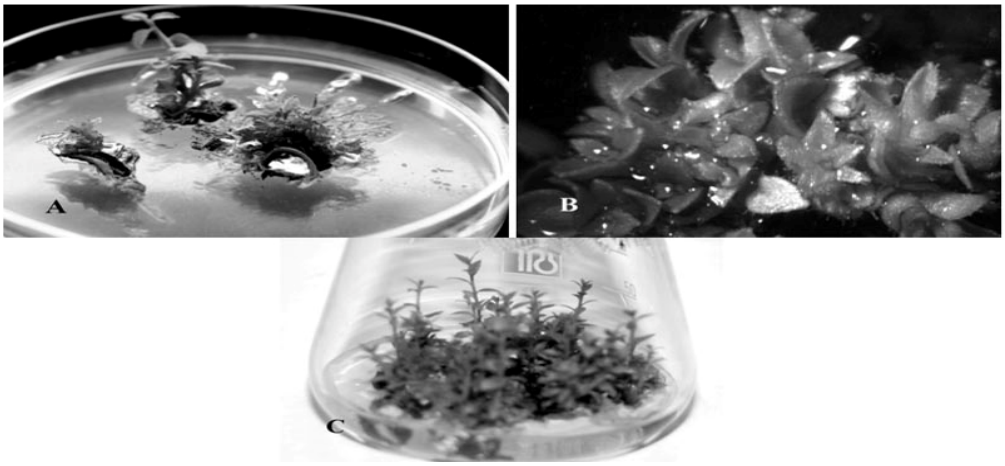

Figure 3. Adventitious shoot proliferation in *V. vitis-idaea* cv.'Red Pearl'derived from leaf tissue cultivated on AN medium with 0.5 mg l^{-1}zeatin (A, B). High shoot proliferation in *V. corymbosum* cv. 'Bluecrop' on AN medium with 0.5 mg l^{-1}zeatin (C).

In conclusion we can state, that efficient cloning protocols were developed for the several selected blueberry and lingonberry cultivars, which enable large-scale propagation of high quality, true to type plants for economic and growing needs.

Acknowledgements
The work was supported by Slovak Grant Agency VEGA, project no. 2/0004/08 and MVTS-COST 863 Action.

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COMPARISON STUDY OF CULTIVATED HIGHBUSH AND WILD BLUEBERRY NUTRIENT STATUS IN PRODUCING PLANTINGS AND WOODLANDS, LATVIA
KRŪMMELLEŅU UN SAVVAĻAS MELLEŅU MINERĀLĀS BAROŠANĀS NODROŠINĀJUMA LĪMENIS RAŽOJOŠĀS SAIMNIECĪBĀS UN LATVIJAS MEŽOS

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Abstract
Highbush blueberries (*Vaccinium corymbosum* L.) grown on more than 170 ha have become an important horticultural commodity in Latvia with a high market demand. To realize the full potential of this modern high yielding crop, balanced plant nutrition is vitally important to ensure adequate growth and fruit production. The main aim of this study was to find out the actual mineral nutrition status of cultivated highbush and wild blueberries (*Vaccinium myrtillus* L.) in Latvia as well as to evaluate the peculiarities of blueberry mineral nutrition in producing plantings and native woodlands. About 100 (soil and plant) samples were collected from different blueberry producing sites and 5 woodlands during the summer of 2008. Plant leaf analysis and soil testing were used to

evaluate the blueberries supply with all of the biogenous elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B). Our results suggest that only about 40 % of the plant leaf nutrient indices and soil tests were in the optimal range. Insufficient levels of N, P, Ca, Mo, B and increased concentrations of Mn in highbush blueberry leaves were the main problems. In spite of the high heterogeneity of the element concentrations in soils, deficiency of N, Zn, Mo, B was found in the vast majority of samples analyzed. Although forest soils were characterized as an acid nutrient poor environment, wild blueberries showed especially high efficiency of K, Ca, Mg, Fe, and Mn accumulation in their leaves. The present investigation forms the basis for the development of scientific knowledge based blueberry fertilization practices in Latvia

Kopsavilkums

Pēdējos gados Latvijā strauji attīstās jaunas netradicionālas ogu kultūras - augstkrūmmu melleņu (*Vaccinium corymbosum*) audzēšana. Krūmmelleņu stādījumu kopplatība Latvijā 2008. gadā jau sasniedza 170 ha. Darba mērķis bija noskaidrot un salīdzinoši izvērtēt krūmmelleņu un savvaļas melleņu (*Vaccinium myrtillus*) minerālās barošanās stāvokli Latvijā, kā arī novērtēt minerālās barošanās īpatnības ražojošu saimniecību un meža augsnēs. 2008. gadā no piecām Latvijas lielākajām krūmmelleņu saimniecībām un piecām dabisko mežu audzēm ievāca 100 (augsnes un lapu) paraugus. Noteica 12 augiem nepieciešamos barības elementus (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B), augsnes pH un kopējo sāļu koncentrāciju (EC). No visiem iegūtajiem augsnes un lapu analīžu rezultātiem tikai 40 % rādītāju bija optimālā līmenī. Krūmmelleņu lapās kopumā raksturīgs – N, P, Ca, Mo un B deficīts, kā arī paaugstinātas Mn koncentrācijas. Neskatoties uz elementu koncentrāciju augsto heterogenitāti, N, Zn, Mo un B deficītu konstatēja vairākumam izanalizēto augšņu paraugu. Lai arī meža augsnes raksturojas ar īpaši skābu un barības elementiem nabadzīgu vidi, savvaļas mellenes uzrāda augstu K, Ca, Mg, Fe, Mn un Zn uzņemšanas efektivitāti. Pētījumā gūtās atziņas papildina zināšanas un izpratni par krūmmelleņu mēslošanas likumsakarībām Latvijā.

Key words: *Vaccinium corymbosum* L., *Vaccinium myrtillus* L., mineral nutrition, plant analysis, soil testing

Introduction

The wild blueberry (*Vaccinium myrtillus* L.) is one of the most popular wild-harvested fruit in Latvia, traditionally used fresh and processed as a health food. The leaves and fruits of the wild blueberry are dried for teas and used in folk-medicine. Unfortunately berry production of the wild blueberry fluctuates annually with weather conditions – spring frosts and summer droughts can greatly decrease yields (Kuchko, 1988). Latvia, where wild blueberries have been picked for centuries, is now replacing them with cultivated North American highbush varieties.

The commercial cultivation of the highbush blueberry (*Vaccinium corymbosum* L.) in Latvia was successfully started during last 10 years. Highbush blueberries grown on more than 170 ha have become an important horticultural commodity in Latvia with high market demand. Cultivated blueberry fruits and juice have become increasingly popular due to their excellent taste, high dietary and health values. Among berry fruits, blueberries are considered to be not only an excellent source of phenolic compounds and vitamins, but also a valuable source of minerals and dietary fibre (Ehlenfeldt and Prior, 2001; Trehane, 2004; USDA National Nutrient Database for Standard Reference, 2006).

Many external factors are important in producing high blueberry yields including soil type, light, temperature, water availability and quality, as well as management practices. Highbush blueberries evolved in low pH (optimum between pH 4.3 and 4.8) soils that were poor in nutrients and rich in organic matter are adapted specifically to these soil conditions and are generally regarded as very sensitive to excessive fertility (Smolarz and Mercik, 1989; Hanson and Hancock, 1996; Hanson, 2006). Although the fertilization requirements of cultivated blueberries are relatively smaller than for other berry crops, investigations indicate that balanced and precise fertilizer applications can improve the nutrient status, growth, development and yield of blueberries (Percival *et al.*, 2003).

While the nutrient status of highbush blueberries in the United States and Canada (the main blueberry production countries in the world) has been studied in considerable detail (Eck, 1988; Strik *et al.*, 1993; Hart *et al.*, 2006), investigations on optimal cultivation technologies of the blueberry crop in Latvia are in their very beginning stages. At present high bush blueberries in Latvia are cultivated on light, acid mineral soils rich in organic matter as well as on high bogs after peat extraction. As highbush blueberries are grown in many non-native regions all over the world (Strik, 2005) and often on soils that have been differently modified by acidification, liming or organic matter incorporation, direct application of nutrient recommendations and management practices from North America are limited in their practicality. Therefore intensive scientific research on soil, climatic requirements and the mineral nutrition regime of highbush blueberries as well as wild blueberries in Latvia are very important.

The main aim of this study was to find out the actual mineral nutrition status of the cultivated highbush (*Vaccinium corymbosum* L.) and wild blueberries (*Vaccinium myrtillus* L.) in Latvia as well as to evaluate the peculiarities of blueberry mineral nutrition in producing plantings and native woodlands.

Materials and methods

About 100 (soil and plant) samples were collected from different blueberry producing sites (Aluksne, Jelgava, Riga, Valmiera and other districts) and 5 woodlands (Cesis, Saldus, Tukums, Valka districts and forest territory of Jurmala city) in Latvia during summer 2008.

The soil samples were taken with a soil probe to a depth of 20 cm. For each sample, five to eight subsamples were obtained and thoroughly mixed to form one sample. The soil samples were air-dried; plant roots and all particles, mineral and organic with a diameter larger than 2 mm were removed by sieving through a 2-mm sieve. To determine the plant available amounts of 12 biogenous elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) the soil samples were extracted with 1 *M* HCl (soil - extractant volume ratio 1:5) (Rinkis *et al*., 1987).

For each highbush blueberry plant sample 50-100 of the most recently fully expanded leaves from current season shoots were collected from locations representative of the planting. Wild blueberry leaf materials were collected at each site as a composite sample from an area of about 10x10 m. The plant material was dried at 60 $^{\circ}$C and ground. Then the plant samples were dry-ashed with HNO_3 vapours and re-dissolved in HCl solution (HCl - distilled water mixture 3:100) (Rinkis *et al*., 1987).

Concentrations of 12 biogenous elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) were determined in all soil and leaf samples. The levels of Ca, Mg, Fe, Cu, Zn, and Mn were estimated by an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 700, acetylene-air flame), those of N, P, Mo, B by colorimetry, S by turbidimetry, and K by flame photometer (Jenwey PFP7, air-propane butane flame). Soil pH was determined in 1 *M* KCl (soil - extractant volume ratio 1:2.5) potentiometrically by pHmeter Sartorius PB-20 (Rinkis *et al*., 1987). Soil electrical conductivity (EC, mS cm^{-1}) was determined in distilled water extract (soil - distilled water volume ratio 1:5) by the conductometer Hanna EC 215. Analytical replication was performed at least three times. The levels of statistical significance were determined using MS Excel 2003. T-test "Two-Sample Assuming Unequal Variances" ($p<0.05$) was used to compare mean element concentrations between species.

Results

To determine the mineral nutrition status of the highbush blueberry and wild blueberry the levels of 12 biogenous elements as well as pH and EC were estimated in the blueberry soil samples. Mean macro- and micronutrient concentrations, the concentration range as well as soil standards developed by Dr. Nollendorfs (2004) for highbush blueberries in Latvia are shown in Table 1.

Our research revealed an especially high heterogeneity of plant available nutrient concentrations in highbush blueberry soils. The highest concentration diapason was found for S, Fe and Mn (max/min: 117.9, 406.3, 1850.0, respectively), but the lowest for the macronutrients N, K (max/min: 16.3 and 26.3) and the micronutrients Zn, Mo and B (max/min: 14.8, 14.0, 14.0,

respectively). It should be stressed that almost all the elements concentration range, with exception of Ca and Mo, in woodland soils was narrower.

Table 1. Nutrient concentrations in blueberry soil samples from producing plantings and woodlands in Latvia, 2008.

Ele-ment	Concentrations in 1*M* HCl extraction, mg l^{-1}				
	V. corymbosum L.		*V. myrtillus* L.		optimal in highbush blueberry soils
	range	mean ± SE	range	mean ± SE	
N	15-245	70.59±8.30a[1]	13-32	22.87±1.61b	120 - 150
P	34-1908	334.92±56.17a	27-47	35.27±1.43b	150 - 180
K	19-500	150.43±18.48a	30-130	70.07±6.27b	100 - 140
Ca	288-16900	2388.46±572.16a	7-1400	588.73±84.57b	700 - 1200
Mg	100-3875	383.92±101.51a	22-350	109.33±18.78b	120 - 250
S	7.0-825	79.54±26.12a	7-11	8.67±0.33b	30 - 50
Fe	24-9750	1115.62±275.72a	90-380	177.93±23.87b	600 - 1500
Mn	0.10-185.00	47.31±7.87a	0.70-24.00	10.19±2.42b	15 - 25
Zn	1.35-20.00	5.77±0.69a	1.60-12.50	5.77±0.72a	8 - 20
Cu	0.45-17.50	3.61±0.57a	0.10-2.00	0.51±0.13b	2.5 – 4.0
Mo	0.01-0.14	0.04±0.005a	0.01-0.41	0.06±0.03a	0.06 – 0.20
B	0.10-1.40	0.48±0.06a	0.10-1.00	0.42±0.08a	0.6 – 1.2
pH_{KCl}	2.76-7.20	4.30±0.16a	2.59-4.01	3.06±0.10b	4.2 - 4.8
EC, mS cm^{-1}	0.15-5.39	1.05±0.19a	0.20-0.37	0.28±0.01b	0.8 – 1.2

[1]Means with different letters in a row were significantly different (t-Test, $p < 0.05$)

Significantly ($p<0.05$) higher levels of the macronutrients N, P, K, Ca, Mg and S were found in highbush blueberry soils in comparison with the nutrient poor forest soils of the wild blueberry. A comparison of the two species studied showed statistically similar mean concentrations for the microelements Zn, Mo and B, while the Fe, Mn, and Cu content were higher in the cultivated blueberry soils. Corresponding to macronutrient levels in the growing medium, wild blueberry soils had a 3.8 times lower mean EC than highbush blueberry soils. In addition, wild blueberry soils could be characterized as a particularly acid growing environment (mean pH_{KCl} 3.06).

Information obtained on mean nutrient concentrations in blueberry plant samples, concentration range as well as highbush blueberry tissue standards (Nollendorfs, 2004) are shown in Table 2. In accordance with wide dispersion of plant available mineral element concentrations in cultivated blueberry soils, the highest concentration variance for almost all nutrients (except Mg) was stated in highbush blueberry leaf samples. Particularly high concentration range was noted for Cu (0.8-362.0 mg kg^{-1}, Cu_{max}/Cu_{min}= 452.5).

Statistically significant differences ($p<0.05$) were found in the macronutrient composition of wild and cultivated blueberry leaves. The highest mean concentrations of N, P and S (1.36, 0.16 and 0.16 mg kg^{-1}, respectively) were found in the highbush blueberry, while the highest K, Ca and Mg (on average, 0.77, 0.84 and 0.84 mg kg^{-1}, respectively) concentrations were found in wild blueberry plant samples. A comparison of the two species studied showed similar concentrations for the micronutrients Fe, Zn and B in blueberry leaves. The chemical analysis of plant samples revealed significantly higher mean levels of Cu and Mo in highbush blueberry leaves. Especially high mean

concentration of Mn (814.4 mg kg^{-1}) was found in wild blueberry leaves, which exceeded the value for cultivated blueberries 4.3 times.

Table 2. Mineral element concentrations in *V. corymbosum* L. and *V. myrtillus* L. leaf samples in Latvia, 2008

Element	Concentrations in dried tissue				optimal levels in highbush blueberry leaves
	V. corymbosum L.		*V. myrtillus* L.		
	range	mean ± SE	range	mean ± SE	
			Macronutrients (%)		
N	0.63-4.6	1.36±0.13a[1]	0.80 – 1.5	1.08 ± 0.05b	1,70 – 2,00
P	0.07-0.33	0.16±0.01a	0.08 – 0.17	0.12 ± 0.01b	0,20 – 0,30
K	0.36-1.70	0.62±0.04a	0.64 – 1.0	0.77 ± 0.03b	0,45 - 0,70
Ca	0.23-0.80	0.40±0.02a	0.56 – 1.12	0.84 ± 0.05b	0,50 - 0,80
Mg	0.12-0.31	0.18±0.01a	0.17 – 0.54	0.32 ± 0.03b	0,15 - 0,30
S	0.08-0.46	0.18±0.01a	0.12 – 0.18	0.15 ± 0.01b	0,15 - 0,25
			Micronutrients (mg kg^{-1})		
Fe	26.0-362.0	76.4±11.2a	54.0 – 128.0	81.87 ± 6.76a	80 - 150
Mn	24.00-880.00	189±29.4a	130.0 – 1720.0	814.40 ± 146.42b	40 - 100
Zn	8.8-76.0	19.3±2.4a	14.0 – 26.0	18.60 ± 0.96a	20 - 60
Cu	0.8-362.0	19.8±11.0a	3.6 – 6.4	4.97 ± 0.24b	8 - 12
Mo	0.10-2.49	0.46±0.09a	0.2 – 0.3	0.23 ± 0.01b	1 - 5
B	7.0-58.0	24.1±2.2a	15.0 –30.0	22.73 ± 1.21a	30 - 60

[1]Means with different letters in a row were significantly different (t-Test, $p < 0.05$)

The results obtained on the percentage of highbush blueberry plant and soil samples in low, optimum and excessive nutrient supply levels are shown in Figure 1. Almost 60 % of the plant samples were completely sufficient in macronutrients. The worse situation was found for the micronutrient supply – more than 55 % of indices were in the deficient range, optimal supply was provided only for 31 % of leaf samples. In general, equal distribution (39 %) between all indices in low and optimal levels was found for highbush blueberry soil samples. Unlike blueberry leaves, a better situation was found for the micronutrient supply in cultivated blueberry soils.

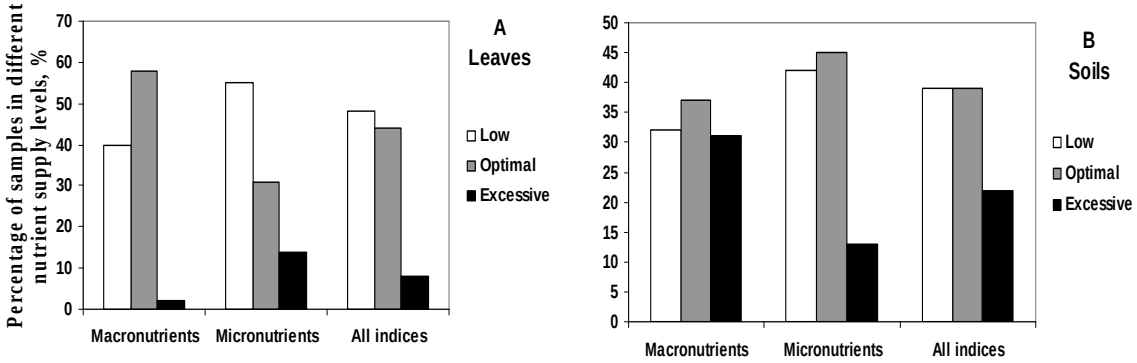

Figure 1. Distribution of highbush blueberry leaf (A) and soil (B) samples in different nutrient supply levels in Latvia, 2008

Discussion

Increased consumer demand for blueberries is being driven by the health benefits associated with functional food characteristics. Wild blueberry have historically been used to help improve eyesight, treat diarrhoea, improve blood sugar levels in diabetics, strengthen blood vessels and improve circulation (Trehane, 2004). The powerful antioxidant properties of the highbush

blueberry appear to play a conclusive role in the prevention and delay of certain diseases such as cancer, cardiovascular issues, and the aging process (Ehlenfeldt and Prior, 2001).

To realize the full potential of modern high yielding crop, balanced plant nutrition is vitally important to ensure adequate growth and fruit production. A deficiency in any of the essential nutrients will disrupt either the vegetative or reproductive growth cycles in plants (Marshner, 1995; Fuqua et al, 2005).

The results obtained on the nutrition status of the highbush blueberry revealed the main problems in plants supply with essential mineral elements in Latvia. In general, our results suggest that only about 40 % of plant tissue nutrient indices were within the sufficient range. Mean macronutrient concentrations, with the exception of low N and slightly decreased P and Ca could be characterized as optimal. Nitrogen is the controlling element in blueberry nutrition and adequate fertilization is necessary to maintain renewal growth, crop production, and flower bud development (Strik and Hart, 1997). Our study suggests that optimal N levels were provided by only 20 % of blueberry leaf samples in Latvia. It can be mentioned that deficiencies of N are the most frequently encountered problems in growing blueberries in North America also (Pritts, 2000; Fuqua et al 2005).

The main tendencies in mean micronutrient supply were also detected – deficiency of Mo, slightly decreased B, optimal levels of Fe and Zn, as well as increased concentrations of Mn and Cu in the plant tissue. While Mn concentrations in highbush blueberry leaves were rather high, in most of cases they could not be considered as toxic, with the exception of the highest concentrations: 450 mg kg^{-1} to 880 mg kg^{-1}. As the cultivation environment influences the chemical composition of plant leaves, our study suggests that the increased Mn concentrations in highbush blueberries reflect high Mn availability in low-pH soils, excessive soil Mn, and sometimes the mistaken application of fertilizers containing Mn. According to recommendations based on the first studies on blueberry nutrition in Latvia the use of the foliar micronutrient (Cu, Zn, B, Mo) fertilization was started during recent years to correct the specific deficiencies stated in the tissue tests. Consequently, excessive Cu concentrations could be caused by the adhesion properties of foliar fertilizers and the use of fungicides.

Our results revealed statistically significant differences between wild blueberry and highbush blueberriy results for N, P, K, Ca, Mg, S, Mn, Cu, Mo in leaf samples. Cultivated highbush blueberry leaves had higher mean concentrations of N, P, S, Cu, and Mo while wild blueberry leaves showed higher levels of K, Ca, Mg, and Mn. It is interesting that there were no significant differences in leaf Fe contents between wild blueberry and highbush blueberry growing in cultivated and forest soils with completely different Fe concentrations: 1115.6 mg Fe l^{-1} and 177.9 mg Fe l^{-1}, respectively. Although wild blueberry leaves were comparatively high in Mn content (on average, 814.4 mg kg^{-1}), reported mean values for Mn (1900 mg kg^{-1}) in wild blueberry leaves from Northern Europe (Reumann *et al.*, 2002) and Poland - 2758 mg kg^{-1} (Kozanecka *et al.*, 2002) were twice to three times higher than our Mn results for wild blueberries in Latvia.

Our previous studies suggest that, in general, blueberry fruit chemical analysis confirmed mineral element differences in wild blueberry and highbush blueberry leaves. A comparison of the two species studied showed that the highest mean K, Ca, and Mn concentrations were also characteristic for wild blueberry fruits (Pormale et al, 2009).

Several investigations have shown that leaf analysis provides a picture of the nutrient status of a crop at a particular point in time resulting from all factors affecting plant growth and is an excellent method to be used on established plantings (Strik *et al.*, 1993; Marschner, 1995). But this diagnostic method cannot detect the character of the nutrient deficiencies or toxicities and the soil content of the nutrients. Therefore soil testing is very important in determining the ability of the soil to supply the nutrients needed for optimum plant growth.

Although a particularly broad range of element concentrations in highbush blueberry soil samples was found, in general, soil tests revealed nutrient deficiencies in the cultivated blueberries. Deficiencies of N, Zn, Mo, and B as well as increased Mn levels were the main problems found. Our results suggest that only 39 % of all soil indices in blueberry producing plantings in Latvia were in the optimal range. It should be noted that the lowest macro- and microelement concentration levels in cultivated blueberry soils are highly comparable with element concentrations in nutrient poor forest soils, thus indicating insufficient fertilization. This could

seriously limit the highbush blueberry yield in Latvia. Although mean levels of soil $pH/_{KCl}$ and EC meet requirements of soil standards, the samples with marginal values did not correspond to the optimum for highbush blueberry growth and development.

The results obtained on nutrient concentrations in wild blueberry soils revealed significantly lower N, P, K, Ca, Mg, S, Fe, Mn and Cu levels compared with highbush blueberry soils. Particularly low concentrations were found for N and S in the woodland soils. This is not surprisingly because nutrients in anion form are more leachable from light acid forest soils, as well as during the last 20 years industrial emissions have been seriously decreased in Latvia (Ļulko *et al*., 2008).

Although both wild blueberries and highbush blueberries can be classified as typical calcifuges, since they thrive in nutrient poor soils with low pH (Korcak, 1989), wild blueberries showed an especially high efficiency rate of K, Ca, Mg, Fe, Mn, accumulation in their leaves. This phenomenon can be considered as a potential wild plant physiological adaptation mechanism to infertile soils.

Conclusions

The results obtained on the nutrition status of the highbush blueberry revealed the main problems in plant supply with essential mineral elements in Latvia. Insufficient levels of N, P, Ca, Mo, B and increased concentrations of Mn in highbush blueberry leaves were found to be the main problems. In spite of the high heterogeneity of the element concentrations in soils, the deficiency of N, Zn, Mo, B was found in a vast majority of samples analyzed. In general, our results suggest that only about 40 % of plant leaf nutrient indices and soil tests were in the optimal range. Although forest soils were characterized as acidic and a nutrient poor environment, wild blueberries showed an especially high efficiency of K, Ca, Mg, Fe, Mn accumulation in their leaves. The present investigation forms the basis for the development of ongoing scientific research and knowledge about blueberry fertilization practices in Latvia.

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EXAMINATION OF CORNEL (*CORNUS MAS* L.) FRUITS IN BORSOD-ABAUJ-ZEMPLEN COUNTY (HUNGARY)
KIZILA (*CORNUS MAS* L.) AUGĻU IZVĒRTĒŠANA BORSOD-ABAUJ-ZEMPLEN APGABALĀ (UNGĀRIJA)

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Abstract
The domestication of cornel has already begun in the neighbour countries by the selection of valuable individuals. The spontaneously growing North-Hungarian specimens could be a good material for similar selection and domestication because foreign cultivars may not adapt to the dry climate of Hungary. Fruit collectors are making from cornel berries delicious palinka (a kind of spirit) and jam. The needs of the processing industry and the market is larger than the gathering can provide, therefore the culturing of new cultivars would be favorable. Our purpose was to show there are several valuable individuals in the Hungarian cornel population, which have outstanding qualities. We observed individuals growing in a natural habitat in 2006, measured the weight of fruit, seeds, and the parameters of fruit length and width. The 19 samples were collected in the Aggtelek Karsts and the southern slopes of Bükk hill. The measured fruits weighed between the 1.2 – 2.85gramm, the seeds were 0.27 – 0.41gramm, so the calculated flesh ratios were average 72.93 – 86.66 %. The average lengths of fruits were between 15.43mm and 20.02 mm, and the average widths of them were between 10.41 – 14.67 mm. Even in a small sample there can be found valuable fruit types for further examination, although it is necessary to observe a wider range of samples to find a good cultivar which is adapted to the local climate.

Kopsavilkums
Kizila kultivēšana kaimiņu valstīs ir sākusies ar vērtīgāko indivīdu atlasi. Ziemeļungārijā spontāni augošie eksemplāri varētu būt labs materiāls līdzīgai atlasei un kultivēšanai, jo ievestās šķirnes varētu nebūt piemērotas sausajam Ungārijas klimatam. Augļu vācēji no kizila ogām gatavo garšīgu "palinku"(alkoholiskā dzēriena veids) un ievārījumu. Pārstrādes rūpniecības vajadzības un tirgus ir lielāks kā ogu vācēji var nodrošināt, tādēļ jaunu šķirņu kultivēšana varētu būt izdevīga. Mūsu nolūks bija parādīt dažas vērtīgas ungāru kizila populācijas ar izcilu kvalitāti. 2006. gadā mēs novērojām eksemplāru augšanu dabiskā vidē, nosakot augļu un sēklu svaru un augļu garuma un platuma parametrus. Aggtelek Karsts un Bükk kalna nogāzēs tika savākti 19 paraugi. Augļu masa bija starp 1.2 – 2.85 g, sēklu masa 0.27 – 0.41 g, aprēķinātā augļa mīkstuma masas attiecība pret sēklu masu bija vidēji 72.93 – 86.66 %. Vidējais augļa garums bija starp 15.43 un 20.02 mm un vidējais platums bija 10.41 – 14.67 mm. Pat mazā paraugā varēja atrast vērtīgus augļu veidus

tālākai pārbaudei, tomēr ir nepieciešams novērot daudz vairāk paraugu, lai atrastu labu šķirni, kura ir piemērota vietējiem klimatiskajiem apstākļiem.

Key words: cornel, fruit parameters, flesh ratio

Introduction

The cornel (European cornel, cornelian cherry, *Cornus mas* L.) is a medium to large deciduous shrub or small tree growing to 5 – 12 m tall. The fruit is an oblong red drupe containing a single seed. The fruit is edible, it has an acidic flavour which is best described as a mixture of cranberry and sour cherry; it is mainly used for making jam, makes an excellent sauce and also can be eaten dried. The cornel is a relatively new species in the circle of the fruit breeders and growers. In those countries, where cornel has native populations with high variability and valuable genetic sources, with the mapping and selection of precious specimens, the cultivar breeding has already begun (Priszter, 1990). In the Ukraine, Bulgaria, Slovakia, Austria, France, Germany, Poland and Turkey a systematic collecting, selecting and breeding program has started in the last years (Klimenko, 2004). In Bulgaria, Poland, and Romania there are several selected cultivars and in Slovakia even breeded cultivars are available as for instance 'Santana', 'Titus,' 'Ovidus', etc... (Pirc, 1992; Porpaczy, 1997; Sipos, 2002; Zeitlhöfer, 2002; Porpáczy, 2004; Klimenko, 2004). In the Kiev Botanical Garden were bred several cultivars which became popular in the USA named 'Pioneer', 'Elegant', 'Red-star' (Zeitlhöfler, 2002). According to Reich's (2004) study, the cultivars bred by Svetlana Klimenko are very delicious ('Elegant', 'Pioneer', 'Red Star', 'Siretski', 'Vavilov'). The main cultivars of the Russian cornel plantations are 'Ispolinskij', 'Karazogal', 'Kyrymzy-zogal', 'Gjul-zogal' and since 1990, two Danish cultivars were introduced 'Macrocarpa' and ' Cormas' (Zeitlhöfer, 2002). In Hungary between 1972 and 1981 cornel collecting and processing was on top (Szepesi, 1983), but in the near future, large-scale production is expected with the promotion of cultivar evaluation and wider cultivation, because cornel grows well in relatively dry soils and is easy to harvest by shaking (Sipos, 2002). However, cornel can be collected but is also valuable for bio production, because they are not damaged by dangerous pests. 50 years ago, Nyékes (1953) described a Hungarian form of *Cornus mas* called *forma csaszloiensis*. It has scarlet red drupes, weight is about 2.7 – 4 g, and the size is about 21 – 27 x 12 – 17 mm. There is only one Hungarian cornel cultivar in ornamental use, called 'Autumn Fire'. Its fruits are pear shaped and 4 – 5 g heavy (Priszter, 1990). Priszter (1962) described 11 types of shape and size of the cornel fruits. The most well spread types are the *forma mas*, the *f. macrocarpa*, and the *f. sphaerocarpa*. Szepesi (1983) pressed for plantations of wild trees, but because of the modified lifestyle and customs, the collecting does not ensure enough fruits for industry. Plantations could be established on such areas, where wood production is not economic. Generally cornel and the other (so-called) forest fruits are pioneer plants. These could be a kind of „fruit bearing forests" near human settlements with a double interest: 1. providing a concentrated fruit collecting area; and 2. using the soil and environment protecting function of the shrubs. Cornel is prolific but teems unevenly. To turn productive it takes 5 – 6 years, then bears generally (30 kg plant^{-1}) 7 – 11 t ha^{-1}. The fruits can be harvested by hand or shakers for tart cherry. (Porpáczy, 1999; Gilbert and Lorraine, 2003). Propagation is difficult by seeds; they need cold stratification (Priszter, 1990). However, seedlings are different from mother plants, for the preservation of the attributes of the mother plant it is better to propagate by suckers, division, cuttings or grafting (Priszter, 1990; Pirc, 1992; Schmidt *et al.*, 1996). Fruit collectors are making from cornel berries delicious palinka (a kind of spirit) and jam. The needs of the processing industry and the market is larger than the collecting can provide, therefore the culturing of new cultivars would be advisable (Makai and Balázs, 2002). Our purpose in this study was to show that there are several valuable and variable individuals in the Hungarian cornel population, which have outstanding qualities. This might be the first step for further examination and probably the start of the breeding work.

Materials and Methods

We examined the drupes of wild cornel genotypes, collecting samples from the area of our hometown (the Karsts of Aggtelek and the Bükk-hill southern slopes). In both area the collecting and the use of the fruits in daily cuisine is remarkable. Our study is useful for the evaluation of

each type, and for comparison samples to each other, but not suitable to give a general synopsis of cornel populations in the observed area, because the number of samples was very small. The location of the sampled bushes are far from each other (a few 10 km-s) and the local climatic factors in some cases were quite different for characterization of the locus, but the average of all sample is eligible for comparing to the data of studies in the surrounding countries. The sample name is the name of the town near the sampling place. At each place we took sample from more than one bush, because the fruit size and colour is very various even on the plants that are growing at the same location, these were marked with a number of the order in the sample name. The collection places were near to the following 7 towns, (the number of observed bushes is written in brackets): Szögliget (3), Szinpetri (2), Varbóc (3), Tornaszentandrás (2), Kács (2), Kisgyőr (4), Felsőtárkány (3); all together there are 19 cornel bushes. The samples were harvested between 9th and 24th of September in 2006. Only the fully ripe, easily separable, healthy, good quality fruits were collected. In the measurement only 20 pieces of randomly selected fruits were examined. Each of the 20 fruits was measured one by one within 24hrs of the harvest. The measured attributes were the followings: weight of fruit in grams, weight of seed (g), length and width of fruit (millimetres). Then the next attributes were calculated: flesh weight (g), proportion of flesh (percentage) and profile index. For weight measuring, we used an OHAUS EXPLORER balance to two places of decimals. The width and length were measured by calliper square (1/20) to one place of decimals. The flesh weight was calculated with this formula: fruit weight minus seed weight equal to flesh weight. The flesh ratio (%) equal to ((fruit weight minus seed weight) divided by fruit weight) multiplied by 100 and the profile index is equal to the fruit length devided by the fruit width. For data registration Microsoft Excel was used, analysis of one-factor variance and analysis of regression was used for the evaluation. The significant difference was calculated at 5% of error.

Results and Discussion

Table 1 shows all the measured and calculated data of samples. The mean of the weight of all samples is 1.95g, comparing to it, 10 samples are not significantly different, 4 samples are positively significant. The Szinpetri 2 sample has an outstandingly high value (2.85 g) in a positive direction. The Kács 2 sample shows the lowest average weight (1.2 g), being the negative significance peak. According to the data of references, the natural cornel populations have a fruit weight between 1.5 – 4.12 g (Demir *et al.*, 2003), 1.46 – 3.81 g (Ercisli *et al.*, 2006), 0.55 – 3.44 g (Brindza *et al.*, 2007). Priszter (1990) found that *forma mas* type has a weight of about 1.4 g. Karadeniz (2002) reported that the fruit weight of selected types was 3.08 – 3.71 g. In another study Pirlak *et al.* (2003) measured 2.9 – 5.2 g. Our data comparing the references is similar, but there was no outstandingly heavy weight. The order of the weight of the seed does not follow the order of the fruit weight; the correlation between them is not significant (Figure 1).

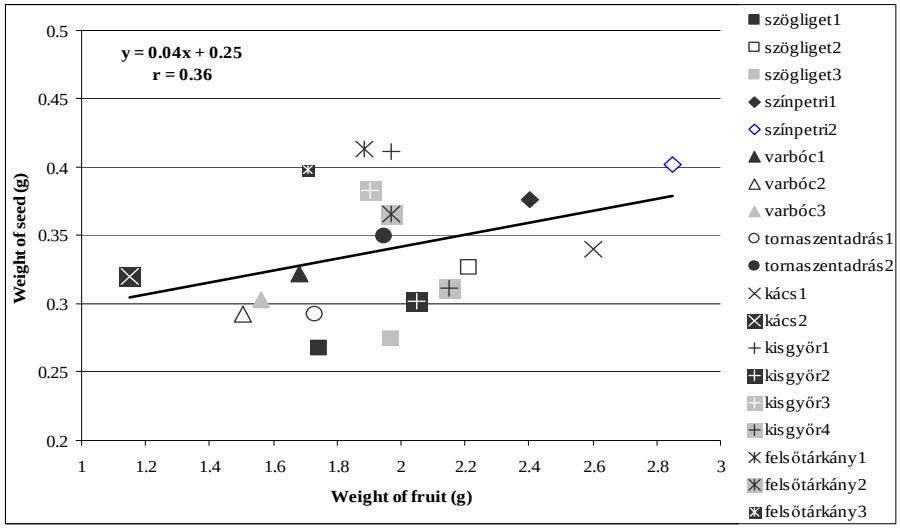

Figure 1. Correlation of fruit weight and seed weight of cornel samples

Felsőtárkány 1 has the heaviest seed: 0.41 g, but it has only 1.89 g fruit weight. The Kisgyőr 1, Szinpetri 2, Felsőtárkány 3, Kisgyőr 3 and Szinpetri 1 samples have similar values, but the Szinpetri 1 and Szinpetri 2 samples have the fruit weigh on the 1st and 3rd place. The lightest seed belonged to the Szögliget 1 sample (0.27 g) and the Varbóc 3, Kisgyőr 2, Varbóc 2, Tornaszentandrás 1 and Szögliget 3 are significantly not different. Priszter (1990) described the typic (f. *mas*) cornel seed weight about 0.2 g. Demir *et al.* (2003) examined 6 types and found the seed weight between 0.14 – 0.37 g; and Brindza *et al.* (2007) reported 0.11 – 0.55 g seed weight of cornel. We measured the cornel seed weight between 0.27 – 0.41 g similar to the references. The results of flesh weight measurement gave nearly the same order of samples as the fruit weight did. Between the two attributes there is a significant correlation illustrated in figure 2. The mean of the flesh weight of samples is 1.61 g; the means of 9 samples are not significantly different from this value. Five-five samples are positively and negatively different from this value in a significant way. The heaviest flesh weight belonged to Szinpetri 2 sample (2.45 g) and Kács 1 sample is similar to it. The Kács 2 sample had the lightest flesh weight 0.88 g, significantly different from all samples. The mean of flesh proportion of the 19 samples was 81.93 %, but the values of each sample were scattered on a wide range from a 72.93 % to 86.66 %. The data, found in the references, are also varied on similar wide range as Ercisli *et al.* (2006) recorded in natural populations 79 – 88 % and Brindza *et al.* (2007) reported between 80 and 87 %. Our 18 samples were similar to these results, but Kács 2 with 72.93 % flesh ratio was remarkably low. This could be due to the flesh stacked inseparably to the seed. The highest flesh ratio belonged to the Kács 1 sample. This fact proves that cornels even growing on the same locus can show great variability.

Table 1. The results of measurement combined with the results of ANOVA

Name of samples	Fruit weight (g)	Seed weight (g)	Flesh weight (g)	Proportion of flesh (%)	Fruit length (mm)	Fruit width (mm)	Profile index
Felsőtárkány 1	1.89^{fgh}	0.41^{a}	1.47^{ghi}	78.05^{jk}	16.31^{ef}	13.64^{b}	1.2
Felsőtárkány 2	1.97^{ef}	0.37^{bcde}	1.6^{efg}	81.4^{fg}	18.64^{bc}	13.01^{cde}	1.43
Felsőtárkány 3	1.71^{hij}	0.4^{ab}	1.31^{ijk}	76.59^{k}	18.47^{c}	11.9^{i}	1.55
Kács 1	2.6^{b}	0.34^{defg}	2.26^{a}	86.66^{a}	19.37^{ab}	14.67^{a}	1.32
Kács2	1.15^{k}	0.32^{fgh}	0.83^{l}	63.95^{l}	16.81^{de}	10.41^{j}	1.62
Kisgyőr 1	1.97^{ef}	0.41^{a}	1.56f^{g}	78.86^{ij}	20.02^{a}	12.34^{fghi}	1.62
Kisgyőr 2	2.05^{def}	0.3^{ghij}	1.75^{cde}	85.18^{abc}	16.93^{de}	13.57^{bc}	1.25
Kisgyőr 3	1.9^{fgh}	0.38^{abc}	1.52^{fgh}	79.71^{hi}	18.36^{c}	12.6^{efg}	1.46
Kisgyőr 4	2.15^{de}	0.31^{fghi}	1.84^{bcd}	85.46^{abc}	18.57^{c}	13.59^{b}	1.37
Szinpetri 1	2.4^{bc}	0.38^{abcd}	2.03^{b}	84.35^{cd}	18.64^{bc}	13.58^{b}	1.38
Szinpetri 2	2.85^{a}	0.4^{ab}	2.45^{a}	85.82^{abc}	19.85^{a}	14.58^{a}	1.37
Szögliget 1	1.74^{ghi}	0.27^{j}	1.48^{ghi}	84.41^{cd}	15.54^{g}	12.49^{efgh}	1.24
Szögliget 2	2.21^{cd}	0.33^{efgh}	1.89^{bc}	85.13^{bc}	17.38^{d}	12.66^{efg}	1.38
Szögliget 3	1.97^{ef}	0.27^{ij}	1.69^{def}	86.04^{ab}	17.24^{d}	13.27^{bcd}	1.3
Tornaszent-andrás 1	1.73^{gh}i	0.29^{hij}	1.44^{ghij}	83.11^{de}	17^{de}	12.57^{efg}	1.35
Tornaszent-andrás 2	1.95^{efg}	0.35^{cdef}	1.6^{efg}	81.73^{ef}	18.32^{c}	12.83^{def}	1.43
Varbóc 1	1.68^{hij}	0.32^{fgh}	1.36^{hijk}	80.68^{fgh}	15.72^{fg}	12.1^{ghi}	1.3
Varbóc 2	1.5^{j}	0.29^{hij}	1.21^{k}	80.37^{fgh}	15.43^{g}	11.98^{hi}	1.29
Varbóc 3	1.56^{ij}	0.3^{ghij}	1.26^{jk}	80.17^{ghi}	17.08^{d}	10.65^{j}	1.61
mean	1.95	0.34	1.61	81.93	17.67	12.76	1.4
p-value	5.5E-29	2.9E-28	2.2E-33	2.6E-56	8.9E-18	2.1E-12	3.6E-48
SD5%	0.22	0.04	0.19	1.5	0.75	0.56	0.04

The mean of the fruit length of all samples was 17.67 mm. Seven samples were not significantly different, seven samples were different in a positive direction and five were negative compared to

the mean. Kisgyőr 1 sample has the longest fruits average of 20.02 mm, Szinpetri 2 and Kács 1 samples were similar to it. The shortest fruits belonged to the Varbóc 2 samples (15.43 mm).

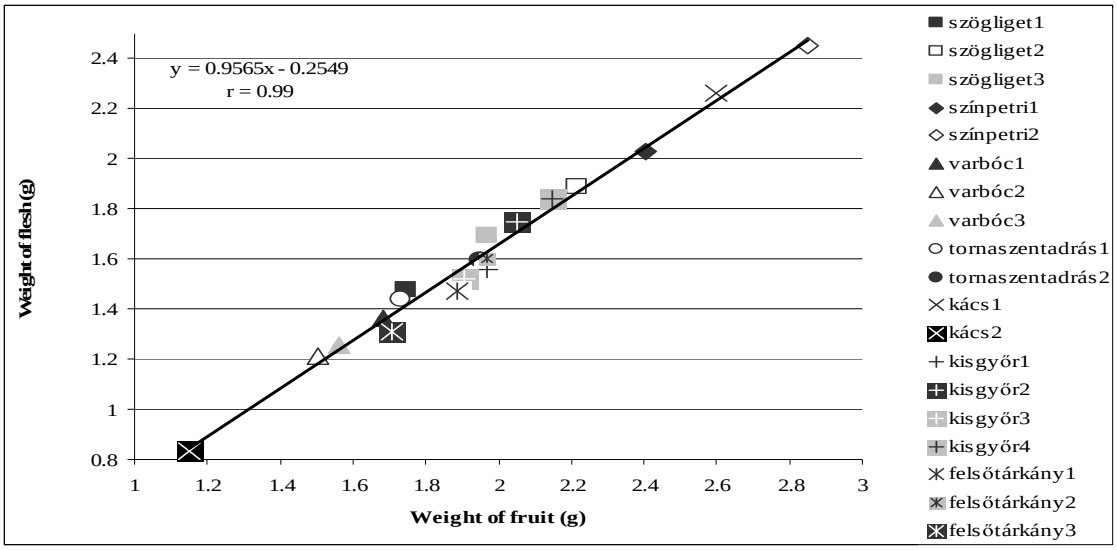

Figure 2. Correlation of fruit weight and flesh weight of cornel samples

Demir *et al.* (2003) examined 6 types and found the fruit length between 15.95 – 20.77 mm. Brindza *et al.* (2007) reported 12.55 – 19.55 mm fruit length of cornel. Our results were similar to the references. The results of fruit weights gave a mean of 12.76 mm, eight samples were not significantly different from it. Six samples were significantly different in a positive direction – five of them had the highest fruit weight also – and five samples were in negative significances accompanied with the lowest fruit weight ranking. Kács 1 sample was 14.67 mm wide, and Kács 2 was 10.41 mm. Our data was very similar to data mentioned in the references: 10.91 – 16.4 mm (Demir *et al.*, 2003) and 7.43 – 15.22 mm (Brindza *et al.*, 2007).

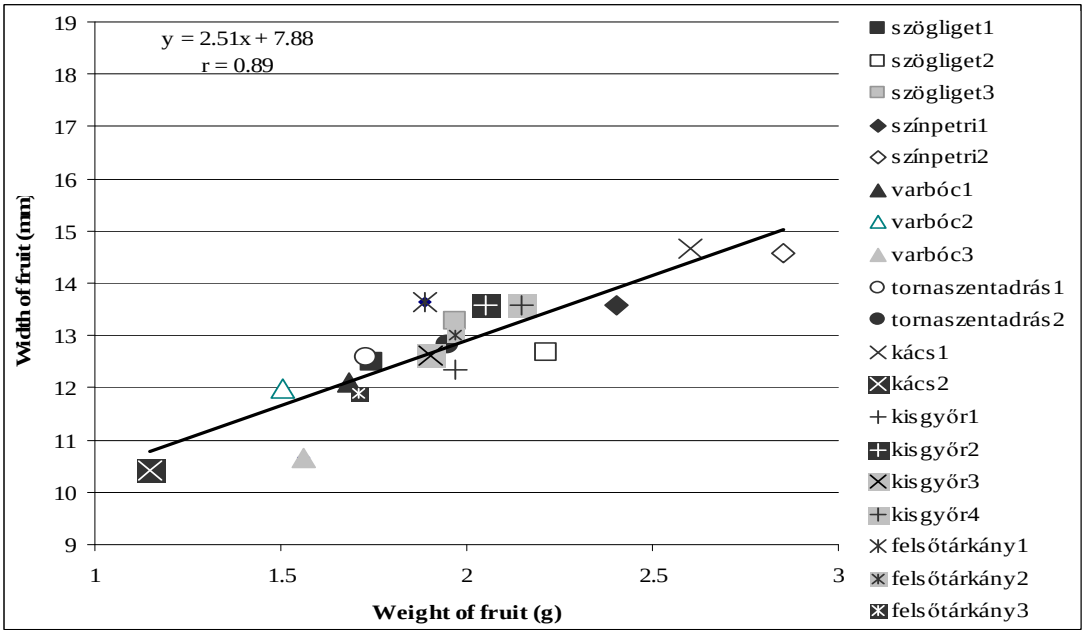

Figure 3. Correlation of fruit weight and fruit width of cornel samples

The correlation of fruit weight and fruit width is strong, illustrated in Figure 3. The width of fruit is growing together with the fruit weight, but the length of the fruit does not follow this tendency.

Table 2. Groups of cornel samples and their attributes

Cornus mas forma	Sample's name	Profile index	Shape, colour and weight of fruits
forma mas (basic type)	Felsőtárkány 2, 3	1.43; 1.55	red, cylindrical-elliptic
	Kács 2	1.62	less than 2g
	Szögliget 3	1.3	
	Tornaszentandrás 1, 2	1.35; 1.43	
	Varbóc 1, 2, 3	1.3; 1.29; 1.61	
	Kisgyőr 1	1.62	
f. mas or costata	Kisgyőr 3	1.46	striate, ovoid
forma macrocarpa	Kács 1	1.32	dark red, elliptic,
	Kisgyőr 4	1.37	heavier than 2 g
	Szinpetri 1, 2	1.38; 1.37	
	Szögliget 2	1.38	
forma sphaerocarpa	Felsőtárkány 1	1.2	dark red, roundish
	Kisgyőr 2	1.25	and nearly 2g
	Szögliget 1	1.24	

The length and the width of fruits show a great variance even among the fruits of one sample, but the calculated profile index is a reliable number to compare the samples and describe the shape of fruits. Using the measured length, width, weight and the calculated profile index data, the samples can be divided in groups of fruit forms. The base of the fruit form groups were the works of Priszter (1962) and Fintha and Szabó (2005). We classified the samples in the following groups showed in table 2.

Conclusions

The results of our experiments correspond to the data of similar studies in neighbour countries. Observing a small number of specimens, we found valuable types for further investigation like Szinpetri 2 and Kács 1 with heavy fruits and outstanding flesh proportion, and Kisgyőr 1 and 3 with dark red colour and easy to core attributes. The possibility of finding valuable specimens of course is higher when the sample amount is larger, and this increases the chance of finding and selecting several well-adapted local cultivars.

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RABBITEYE BLUEBERRY, AMERICAN CRANBERRY AND LINGONBERRY BREEDING IN LATVIA
EŠA ZILEŅU, AMERIKAS DZĒRVEŅU UN BRŪKLEŅU SELEKCIJA LATVIJĀ

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Abstract
The purpose of the breeding work was to create adapted to the climatic conditions of Latvia and disease resistant cultivars of rabbiteye blueberry (*Vaccinium ashei* Reade), American cranberry (*Vaccinium macrocarpon* Ait.) and lingonberry (*Vaccinium vitis-idaea* L.). The main task was the breeding of cultivars with large berries, high productivity and different ripening time. Breeding research has been carried out in National Botanic Garden of Latvia since 1980. In the work, classic plant breeding, interspecific breeding and polyploidy breeding have been employed, and wild lingonberry clones were used. The rabbiteye blueberry cultivar 'Salaspils Izturīgā' was selected from seedlings of open pollination of the cultivar 'Tifblue' in 1993 and the cultivar 'Lielogu' was selected from the hybrid ('Delite' x 'Woodart') seedlings in 1995. From the hybrid (cranberry cultivar 'Franklin' x lingonberry) seedlings were selected the cultivar 'Dižbrūklene' in 1997, the cultivar 'Salaspils Agrās' in 1996 and the cultivar 'Tīna' in 2006. The lingonberry cultivar 'Salaspils Ražīgā' origin of the wild lingonberry clone was selected as cultivar in 1993; the cultivar 'Rubīna Lāse' was selected in 1988 from open pollination seedlings of the cultivar 'Salaspils Ražīgā', but the cultivar 'Jūlija' was selected in 1995 from open pollination seedlings of the clone 'Krasnojarska'.

Kopsavilkums
Selekcijas mērķis bija radīt Eša zileņu *Vaccinium ashei* Reade, Americas lielogu dzērveņu (*Vaccinium macrocarpon* Ait.) un brūkleņu (*Vaccinium vitis-idaea* L.) šķirnes, kas būtu piemērotas Latvijas klimatiskajiem apstākļiem un slimību izturīgas. Galvenie uzdevumi bija izveidot šķirnes ar lielām ogām, ražīgas un ar dažādiem ienākšanās laikiem. Selekcija tika veikta Nacionālajā Botāniskajā dārzā Latvijā kopš 1980. gada. Darbā tika izmantota klasiskā selekcija, starpsugu selekcija un poliploīdija, kā arī izmantoti savvaļas brūkleņu kloni. Eša zilenes šķirne 'Salaspils Izturīgā' tika izveidota no brīvas apputes 'Tifblue' sēklaudžiem 1993. gadā, bet šķirne 'Lielogu' tika atlasīta no hibrīda ('Delite' x 'Woodart') sēklaudžiem 1995. gadā. No hibrīda (dzērveņu šķirne 'Franklin' x brūklene) sēklaudžiem tika atlasīta šķirne 'Dižbrūklene' (1997.), 'Salaspils Agrās' (1996.) un 'Tīna' (2006.). Brūkleņu šķirne 'Salaspils Ražīgā' tika atlasīta no savvaļas klona 1993. gadā. Šķirne 'Rubīna Lāse' tika atlasīta 1988. gadā no šķirnes 'Salaspils Ražīgā' brīvas apputes

sēklaudžiem, bet šķirne 'Jūlija' tika atlasīta 1958. gadā no klona 'Krasnojarska' brīvas apputes sēklaudžiem.

Key words: clones, clusters, hybrid, phenological phases, pollination, resistance, upright, vine, yield.

Introduction

The abundant yields and medicinal properties of the berries of rabbiteye blueberries, American cranberries and lingonberries contribute to the popularity of these cultures. The fresh berries of rabbiteye blueberries contain 16.6 g kg^{-1} of soluble dry matter, 7.8 g kg^{-1} of glucose and fructose, to 1.4 g kg^{-1} of titrable acid, 0.001 g kg^{-1} amino acid. The rabbiteye blueberry berries are a valuable source of physiologically active and mineral substances (Ripa 1992, Ripa 1998). American cranberries are characterized by a considerable amount of biologically active substances. The content of anthocyans in fresh berries constitutes 0.604 – 0.186 g kg^{-1} and catechines 0.152 – 0.126 g kg^{-1}, and vitamin C 0.062 – 0.068 g kg^{-1}. The fresh berries contain 10.3 – 11.6 g kg^{-1} of soluble dry matter, 7.2 – 8.2 g kg^{-1} glucose and fructose, to 3.0 g kg^{-1} of titrable acid. The berries are a valuable source of pectin and mineral substances (Ripa 1980, Ripa 1996). The berries of the lingonberry contain 17.7 g kg^{-1} of soluble dry matter, 10.37 g kg^{-1} of glucose and fructose, to 2.11 g kg^{-1} of titrable acid. The lingonberry berries are a valuable source of physiologically active substances and mineral substances (Ripa 1981, Ripa *et al.* 1992).

The purpose of the breeding work was to create adapted to climatic conditions of Latvia and disease resistant cultivars of the rabbiteye blueberry (*Vaccinium ashei* Reade), the American cranberry (*Vaccinium macrocarpon* Ait.) and the lingonberry (*Vaccinium vitis-idaea* L.). The main task was the breeding of cultivars with large berries, high productivity and different ripening times.

Materials and methods

In 1983 the ripe berries of the following open pollination clones of lingonberries were gathered: 'Salaspils Ražīgā', 'Krasnojarska', and in 1986 the ripe berries of the following open pollination cultivars of rabbiteye blueberries were gathered: 'Delite', 'Tifblue'. In 1988 – 1989 the self-pollination and cross-pollination of rabbiteye blueberry cultivars was carried out. During cross-pollination the cultivar 'Woodart' was used as the pollinator. In 1987 – 1990 the cross-pollination of cranberry cultivar 'Franklin' with pollen of lingonberry clones was carried out. The lingonberry clones from the wild were distributed in 1980.

During cross-pollination the flower buds were isolated by gauze sacs. In each cultivar 30 blooms were isolated. The blooms were pollinated two days after their opening. Pollen was collected from unfolded blooms. The germination capacity of the pollen was determined in a medium consisting of 1.0 g kg^{-1} agar and 15.0 g kg^{-1} saccharose. The berries obtained from open-pollination and cross-pollination were stored in a fridge and stratified for six months at +4 °C. The seeds of hybrid (cranberry cultivar 'Franklin' x lingonberry), before sown, were soaked for 24 hours in a 0.5 g kg^{-1} solution of colchicine. The seeds were sown in acid peat (pH 3.5 – 4.5) 2 – 3 mm deep and covered with sand. In the seedling boxes the peat was constantly kept humid at an air temperature of 22 °C. A month after shoot emergence the seedlings of rabbiteye blueberries, lingonberries and hybrids (cranberry x lingonberry) were planted in acid peat at a distance of 5 x 10 cm and later replanted in a permanent place at a distance of 2 x 1 m.

The soil for planting the rabbiteye blueberry seedlings of open- and cross-pollination was sandy clay, soil reaction neutral to slightly acid (pH KCl – 6.1). The soil had a high level of organic matter (11.3 g kg^{-1}), and total nitrogen (0.3 g kg^{-1}) the level easily absorbed potassium was medium (0.01 g kg^{-1}) and that of phosphorus was high (0.024 g kg^{-1} soil), the level of replaceable calcium was 0.279 g kg^{-1} soil. The level of trace elements (copper, boron, iron) in the soil was sufficient, i.e. 0.0003, 0.0001 and 0.0040 g kg^{-1} soil, respectively. Since 1992 each spring (in May) supplementary fertilizers have been cultivated around the seedlings superphosphate 70.0 – 80.0 kg ha^{-1}, potassium sulphate 20.0 – 30.0 kg ha^{-1}. The soil for planting the lingonberry and hybrid (cranberry x lingonberry) seedlings was 20 – 30 cm thick acid sphagnum peat (pH KCl 2.3 – 2.6). The peat had a high level of organic matter (87.5 – 94.9 g kg^{-1}), and total nitrogen (1.1–1.5 g kg^{-1}), the level of easily absorbed potassium was high (0.060–0.148 g kg^{-1} soil) and that of phosphorus

was medium (0.014 – 0.017 g kg^{-1} soil), the level of replaceable calcium was 0.089 – 0.290 g kg^{-1} soil. The level of trace elements (copper, boron, iron) in the peat was sufficient, i.e. 0.0003, 0.0004 and 0.0016-0.0080 g kg^{-1} substrate, respectively. The lingonberry and hybrid (cranberry x lingonberry) plants had been grown using organic methods.
During the growing period the following qualities were determined: growth rhythm (phenological phases), the colour, shape, and quality of the berries, the yield of berries, the weight of berries, the frost and disease resistance of plants.

Results and Discussion
The rabbiteye blueberry cultivar 'Salaspils Izturīgā' was selected from seedlings of the open pollination of the cultivar 'Tifblue' in 1993.
'Salaspils Izturīgā'. Shrub: upright, vigorous, the ten years old shrub is about 1.5 m high and 1.4 m wide, open spreading, tolerates -36 °C, productive, the average yield of berries in 14 years was 3.06 kg per shrub. The leaves are small (the length 5.5 – 6.1 cm, width 2.4 – 2.7 cm), bright green, elliptic. The raceme is loose cluster, in the cluster are 7 – 37 flowers. The flowers are small, white with medium anthocyans paint. The berries are medium (the length 1.0 – 1.3 cm, diameter 1.3-1.6 cm, the weight of one berry 1.2 – 1.8 g), dark blue, good quality, round-oblate, and ripens about 22 July.


Figure 1. Rabbiteye blueberry cultivar 'Salaspils Izturīgā'

The rabbiteye blueberry cultivar 'Lielogu' was selected from hybrid ('Delite' x 'Woodart') seedlings in 1995.
'Lielogu'. Shrub: upright, vigorous, the 10 years old shrub is 1.8 m high and 1.75 m wide, very productive, the average yield of berries in 9 years was 6.05 kg per shrub, tolerates -34 °C. The leaves are medium (the length 6.6 – 7.1 cm, width 3.2 – 3.6 cm), bright green, elliptic. The flowers are medium size, white with medium anthocyans paint. The raceme is loose cluster, in the cluster are till 39 berries. The berries are large (the length 1.0 – 1.2 cm, diameter 1.5 – 1.6 cm, the weight of one berry 1.7 – 2.0 g.), aromatic, light blue, good quality, ripens about 20 July.
The vegetation period for the rabbiteye blueberry cultivars 'Salaspils Izturīgā' and 'Lielogu' started depending on the year's climatic conditions from April 14th to 28th, when the average air daily temperature was above 4.7 °C, bud swelling continued a week, after that the buds began to dehisce and green leaves appeared. In the beginning of May, when the average air daily temperature is above 10 °C and the positive temperature sum is 126 – 220 °C, the flower buds begin to swell. Depending on the vegetation period and the average air daily temperature the rabbiteye blueberry cultivars 'Salaspils Izturīgā', 'Lielogu' begin to flower from the 17th to 23rd of May and lasts 22 – 27 days. During the period of flowering the sum of positive temperatures is 220 – 550 °C. The first berries of the cultivars 'Salaspils Izturīgā' and 'Lielogu' ripen from 20th to 22nd July.

From the hybrid (cranberry cultivar 'Franklin' x lingonberry) seedlings the cultivars 'Salaspils Agrās' (in 1996), 'Dižbrūklene' (in 1997), and 'Tīna' (in 2006) were selected.
'Salaspils Agrās'. This cultivar produces large to medium berries (the length 1.5 – 1.8 cm, the diameter 1.4 – 1.8 cm, the weight of one berry – 1.2 – 2.0 g). The berries are round, dark red, ripen very early. Fine textures vines produce short uprights that are capable of good production, the average yield of berries was in 6 years 3650 kg ha^{-1}. The elliptical leaves are medium large (the length – 9 – 12 mm, the width – 3 – 5 mm), bright green. The buds of the top of uprights are vegetative-generative. The raceme is intercalary cluster, in the cluster are 1 – 9 flowers. The flowers are light red. The berries cluster is loose, in the cluster are 1 – 5 berries.
'Dižbrūklene'. The berries are exceptionally large (the length – 1.8 – 2.1 cm, the diameter – 1.4 – 2.0 cm, the weight of one berry – 1.7 – 2.4 g) blackish red, round to oval. The berries ripen very early. This productive cultivar produces coarse vines with medium high uprights, the average yield of berries in 6 years was 2810 kg ha^{-1}. The elliptical leaves are medium large (the length 10 – 11 mm, the width – 4 – 5 mm), bright green. The buds on the top of uprights are vegetative – generative. The raceme is intercalary cluster, in the cluster are 1 – 8 flowers. The flowers are light red. The berries cluster is loose; in the cluster are 1 – 6 berries.
'Tīna'. This cultivar produces red, round exceptionally large berries (the length – 1.6 – 1.8 cm, the diameter – 1.3 – 1.6 cm, the weight of one berry – 1.4 – 2.2 g). The berries ripen early. The moderately vigorous vines produce short uprights that have consistently large yield, in 6 years the average yield of berries was 4530 kg ha^{-1}. The elliptical leaves are medium large (the length – 10-11 mm, the width – 4 – 5 mm), bright green. The buds on the top of uprights are vegetative-generative. The raceme is intercalary cluster, in the cluster are 1 – 8 flowers. The flowers are light red. The berries cluster is loose; in the cluster are 1 – 6 berries.

Figure 2. Hybrid cranberry x lingonberry 'Tīna'

The vegetation period of the hybrid cultivars 'Salaspils Agrās', 'Dižbrūklene', and 'Tīna' started from 28-th April to 18-th May, when the average daily air temperature was 4.4 °C. The growth of shoots began from 11-th to 28-th of May. Buds forming the uprights were the first ones to grow. After opening of the buds the first thirty five days the uprights growth was intensive. During that time their length reached 3.0-7.0 cm.
At the end of May, when the average daily air temperature was over 10 °C and sum of positive temperatures exceeded 412 °C, the buds of the uprights began to swell and the flower buds emerged. Hybrid cultivars under trial began to flower from 14th June to 6th July and continued for 30 – 35 days. The flowering period of each flower lasted for 15 – 17 days.
The ripening of the berries depends on cultivar properties and the average daily air temperature during the vegetation period. The first berries of the very early hybrid cultivars 'Salaspils Agrās' and 'Dižbrūklene' ripened from September 5th to 11th, and of the early hybrid cultivar 'Tīna' – from September 8th to 15th.
The lingonberry cultivar 'Salaspils Ražīgā' originated from a clone found in Pļavu forestry (Talsi region), selected as cultivar in 1993 (Audriņa, 1996).

‘Salaspils Ražīgā’. Vigorously growing, upright, compact shrub (mature plant height 16 – 35 cm), with moderate plant spread (rhizome production). Leaves are dark green, medium size (length 1.6 – 2.2 cm, width 0.7 – 1.1 cm).
This cultivar has only one pronounced blooming period (middle of May – beginning of June), the second blossoming is sparse. Flowers are set in medium size clusters (2 – 12 flowers) or alone. Flowers are white or white with anthocyan paint. Berries ripen in the end of August – beginning of September. Fruit clusters are medium size (2 – 10 berries). Berries are deep red, roundish, medium size (0.21 – 0.31 g), length 0.7 – 1.1 cm, diameter 0.7 – 1.0 cm. The cultivar is productive – fruit yield of mature plants – 90 – 180 g per plant. The cultivar is winter hardy and showed relatively high resistance to “little leaf disease”.

Figure 3. Lingonberry cultivar ‘Salaspils Ražīgā’

The lingonberry cultivar ‘Rubīna Lāse’ was selected in 1988 from open pollination seedlings of ‘Salaspils Ražīga’.
‘Rubīna Lāse’. It is vigorous, upright, compact shrub with moderate plant spread. Mature plant height 20 – 30 cm. Leaves are light green, medium size (length 1.8 – 2.6 cm, width 0.8 – 1.4 cm). This cultivar has the ability to bloom twice in a season, but the second blossoming is sparse. More intensive second blossoming was observed in the years when the first blooms were damaged by frosts. The average first blossoming period is in the middle of May – beginning of June, but the second – end of July – beginning of August. Flowers are set in medium size clusters or alone. Flowers are white with anthocyan paint, larger then flowers of other cultivars. The first berry crop ripens in the beginning to middle of August, but the second crop – in the end of September – beginning of October. Fruit clusters are medium size (2 – 9 berries). Berries are very deep red, drop-like, medium size (0.30 – 0.42 g), length 1.0 – 1.3 cm, and diameter 0.8 – 1.0 cm. Berries yield 35 – 200 g per plant. The characteristic distinctive feature of ‘Rubīna Lāse’ from other lingonberry cultivars is their relatively light colour of leaves contrasting with very dark berries, and an unusual crop-like form of berries. The cultivar is winter hardy and showed high resistance to “little leaf disease” (Audriņa, 2004).
The lingonberry cultivar ‘Jūlija’ was selected in 1995 from open pollination seedlings of the clone ‘Krasnojarska’.
‘Jūlija’. It has compact, 13 – 20 cm high shrub with moderate plant spread. Leaves are dark green, medium size (length 1.7 – 2.1 cm, width 0.6 – 1.2 cm). The cultivar has only one pronounced blossoming period (middle of may-beginning of June). Flowers are set in medium size clusters (2 – 13 flowers) or alone. Flowers are white with anthocyan paint. ‘Jūlija’ is an early ripening cultivar – in the end of July to beginning of August. Berries are dark red, roundish, medium size (0.20-0.39 g), length 0.6 – 1.0 cm, and diameter 0.7 – 1.0 cm. Fruit yield 21 – 92 g per plant. This cultivar also is winter hardy, and showed high resistance to “little leaf disease’ (Audriņa, 2004).

Conclusions

The highly productive and cold resistant cultivars of rabbiteye blueberries 'Salaspils Izturīgā' and 'Lielogu' were selected.

The highly productive hybrid (cranberry cultivar 'Franklin' x lingonberry) cultivars 'Salaspils Agrās', 'Dižbrūklene', and 'Tīna' were selected. These very early and early ripening cultivars have large and very large berries.

The highly productive and disease resistant cultivars of lingonberry 'Salaspils Ražīgā', 'Rubīna Lāse' and 'Jūlija' were selected.

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VOLATILE PROFILES OF EUROPEAN BLUEBERRY: FEW MAJOR PLAYERS, BUT COMPLEX AROMA PATTERNS
EIROPAS MELLEŅU AROMĀTS: DAŽAS GALVENĀS SASTĀVDAĻAS, BET DAUDZVEIDĪGS KOPĒJAIS AROMĀTA SASTĀVS

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Abstract

As part of a project on cultivation and industrial exploitation of European Blueberry (*Vaccinium myrtillus*) started in 2008, berries from different wild populations from South, Mid and North Norway were investigated. One aspect of fruit quality analyses was to identify and describe blueberry aroma profiles. Volatiles were extracted by headspace solid-phase microextraction (HS-SPME) and analysed by gas chromatography/ mass spectrometry (GC/MS). A total of 132 potential aroma volatiles could be detected, of which 99 structures were identified based on MS database search and retention indices, also comprising aroma impact compounds not being described in blueberries earlier. Detected aliphatic and aromatic structures belonged to different chemical groups such as alkanes, acids, alcohols, aldehydes, esters, ketones and mono- and sesquiterpenes. Ten major compounds (mostly C_4-, C_6- and C_9-structures) accounted for averagely 65–75 % relative amount of all detected peaks. However, HS-SPME analyses revealed complex volatile profiles including terpenes (23 compounds, e.g. *p*-cymene, 1,8-cineole, linalool) and aromatic structures (10 compounds, e.g. benzaldehyde, ethyl benzoate, 2-phenylethyl acetate, benzyl benzoate), which contribute to the characteristic and flavourful blueberry aroma.

Kopsavilkums
Eiropas melleņu (*Vaccinium myrtillus)* ogas no dienvidu, vidus un ziemeļu Norvēģijas savvaļas audzēm tika pētītas kā daļa no 2008. gadā uzsāktā projekta par šo ogu kultivēšanu un ekspluatēšanu. Daļa no augļu kvalitātes analīžu uzdevumiem bija identificēt un aprakstīt melleņu aromātu. Gaistošās vielas tika izdalītas ar HS-SPME metodi un analizētas ar gāzu hromatogrāfijas – masu spektrometrijas (GC/MS) metodi. Kopumā tika noteiktas 132 aromātu veidojošas gaistošās vielas, no kurām 99 sastāvdaļu struktūras tika identificētas, balstoties uz spektra datubāzes meklējumiem un izdalīšanās laiku, tai skaitā atrastas sastāvdaļas, kas iepriekš mellenēs nav aprakstītas. Noteiktās alifātiskās un aromātiskās struktūras vielas piederēja dažādām ķīmiskajām grupām ,tādām kā alkāni, skābes, spirti, aldehīdi, ēteri, ketoni, terpēni. Desmit galvenās sastāvdaļas (galvenokārt C_4-, C_6- un C_9- struktūras) sastādīja, vidēji 65 – 75 % no relatīvā visu noteikto sastāvdaļu daudzuma. Tomēr HS-SPME analīze uzrādīja ļoti daudzveidīgu gaistošo vielu sastāvu, ieskaitot terpēnus (23 sastāvdaļas, piemēram, p-cimēns, 1,8-cineols, linalols) un aromātiskas struktūras (10 sastāvdaļas, piemēram, benzaldehīds, etilbenzoāts, 2-feniletilacetāts, benzilbenzoāts), kas nosaka raksturīgo un bagātīgo melleņu aromātu.

Key words: *Vaccinium myrtillus* L., headspace (HS), solid-phase microextraction (SPME), gas chromatography/mass spectrometry (GC/MS), aroma, quality.

Introduction
European blueberry (*Vaccinium myrtillus* L.), also called bilberry, is a perennial dwarf shrub in the Ericaceae family, being native to northern and eastern parts of Europe and Asia. Compared to other species in the same genus e.g. highbush blueberry (*V. corymbosum* L.), the wild blueberries produce fruits with a higher content of desirable polyphenols and other health-beneficial compounds (Giovanelli and Buratti, 2009). Furthermore, the characteristic and pleasant flavour of berries from wild-growing plants is very complex compared to cultivated highbush blueberries (Parliment and Kolor, 1975; Hirvi and Honkanen, 1983a) and rabbiteye blueberries (Horvat *et al.*, 1996). Already in 1969, Von Sydow and Anjou published results about the vast variety of 109 aroma volatiles found in *V. myrtillus*, and described 19 aliphatic alcohols, 24 aliphatic aldehydes and ketones, 26 terpene derivatives, 24 aromatic compounds, and 16 other chemical structures. Berry samples from putative progenitor species of cultivated highbush, rabbiteye and lowbush blueberries have been shown to contain many of the same aroma volatiles (Baloga *et al.*, 1995), and later reports underscored the complexity of aroma patterns also of cultivated *Vaccinium* species through the identification of new potential key aroma volatiles such as sulphur-compounds (Hanoglu and Pucarelli, 2007) and other chemical structures (Di Cesare *et al.*, 1999). Major goals of our preliminary study on aroma volatile composition of berrries from wildgrowing *V. myrtillus* plants in Norway were the (1) Identification of aroma-impact compounds, (2) Influence of maturation stage on aroma patterns, and (3) Potential effect of location on berry aroma , in order to characterize the significance of different factors affecting the flavour properties of blueberries.

Materials and Methods
Plant Material. Blueberry samples from different wild populations from South, Mid and North Norway where harvested at maturation stage in August-September 2008 and stored at -20°C prior to extraction and analysis.
Aroma Volatile Analysis. Frozen berries were cut into halves and a total of 3 g from single halves of 10 – 15 fruits were placed in a 15 ml headspace vial (Supelco Inc.). After adding 5 ml H_2O and 1 g NaCl, the vial was closed with a screw cap with Teflon-coated septum, and the sample was constantly agitated on a magnetic stirrer during extraction (45 min). Headspace solid-phase microextraction (HS-SPME) was applied for isolation and concentration of volatile aroma compounds by using a manual SPME holder (Supelco Inc.) with a PDMS/DVB-coated 65 µm fibre exposed to the atmosphere in the sample vial (Rohloff, 2004). HS-SPME sampled volatiles were desorbed in the the injection port of a gas chromatograph (GC) for 3 min. Aroma volatiles were analysed using a VARIAN Star 3400 CX GC coupled with a Saturn 3 mass spectrometer (GC/MS). GC separations were carried out on a HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Injection temperature was 220 °C, and the interface was set to 220 °C. The

carrier gas was helium, the GC temperature program was held isothermically at 40°C for 1 min (splitless injection mode), ramped from 40° to 220 °C at a rate of 4.5 °C min, and finally held at 220 °C for 4 min (analysis time: 45 min). The MS source was adjusted to 220 °C and a mass range of *m/z* 40 – 300 was recorded. Compounds were tentatively identified based on mass spectral database search using the NIST05 MS Database, a customized in-house fruit flavour MS database, and retention indices from literature.
Statistic Analysis. GC/MS data (TIC) were analysed using AMDIS deconvolution software (v. 2.64). The raw data sets of 32 samples were aligned using the on-line service 'SpectConnect' at Massachusetts Institute of Technology (MIT, 2009). Chemometric Principal Component Analysis (PCA) was carried out with the statistical software Minitab (v. 15.1.30).

Results and Discussion
The application of HS-SPME coupled with GC/MS revealed 132 volatile aroma compounds in 32 different samples of blueberries harvested at different loactions in Norway. A total of 99 compounds could be tentatively identified using mass spectral database search. The most abundant compounds were related to the group of C_6-structures or so-called 'green leaf volatiles' (Figure 1). In addition, impact-aroma compounds belonging to the chemical group of monoterpenes, could also be extracted from all samples, e.g. 1,8-cineole, linalool, and linalyl acetate.

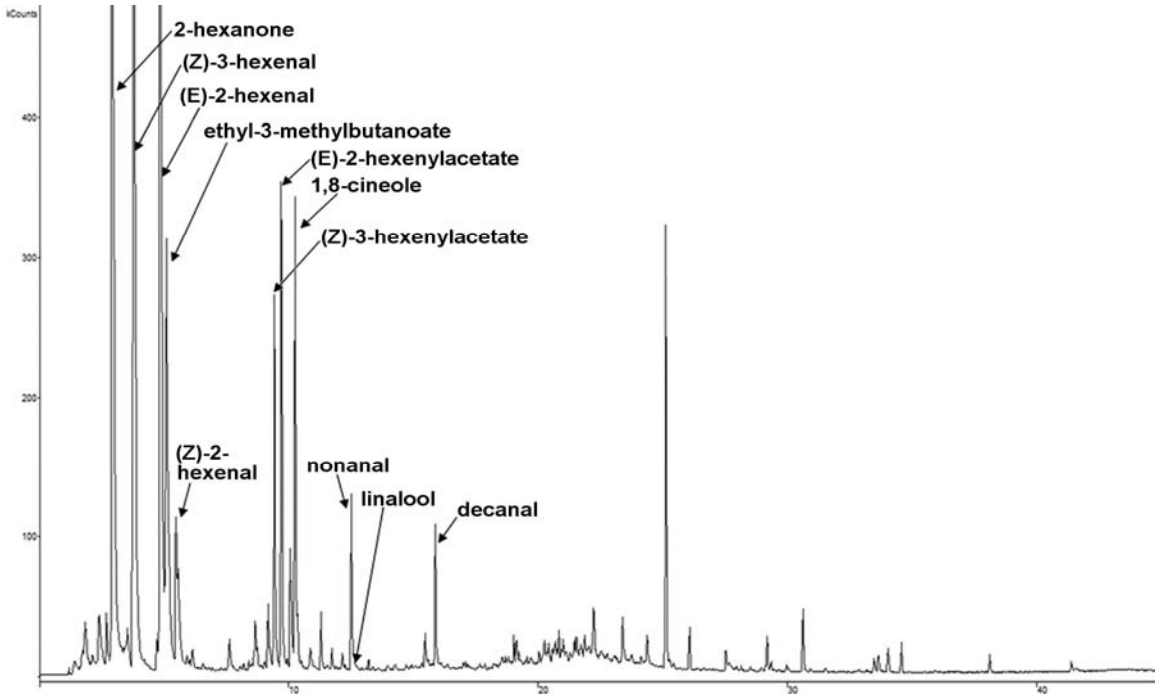

Figure 1. GC/MS chromatogram of a headspace-SPME profile of blueberry aroma volatiles. Important compounds such as C_6-structures or 'green leaf volatiles' (hexyl- and hexenyl-derivatives), terpenes (1,8-cineole, linalool) and aldehydes (nonanal, decanal) are indicated in the figure.

Based on chemometric PCA of all data sets from GC/MS analyses, blueberry samples could be grouped into different clusters depending on from which location the blueberries were harvested (Figure 2). Samples from South Norway clustered in an own group, while the samples from Mid- and North-Norway were overlapping. Regarding differences in the maturation stage, not-fully ripened blueberries were clearly separated from fully-ripened fruits, thus indicating significant differences in their aroma profiles.

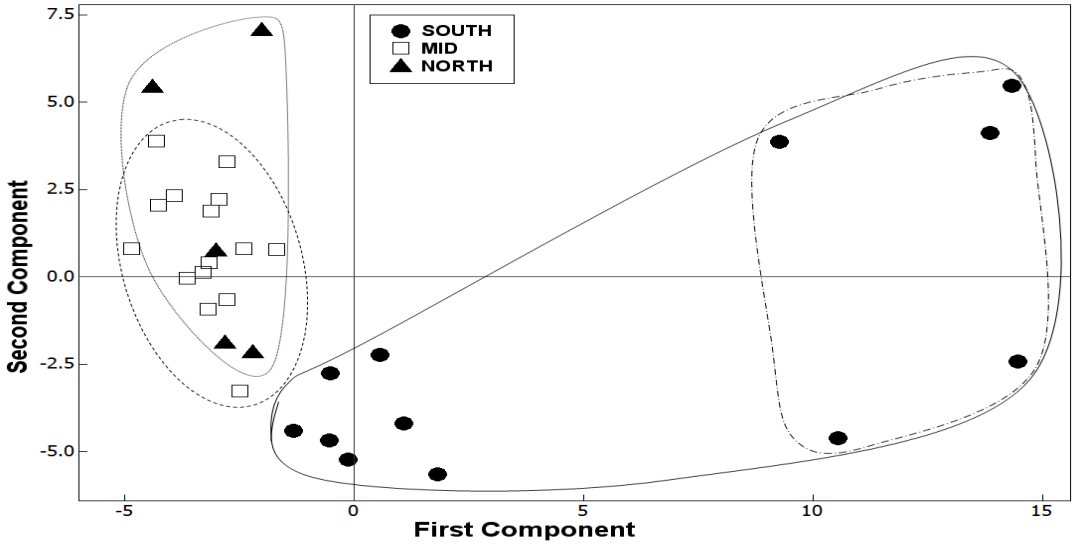

Figure 2. Chemometric PCA of 32 blueberry samples from different locations in Norway. Five berry samples from South Norway clearly clusterd in an own group (right side of the figure with dash-dot line); these were not fully-ripened fruits in contrast to all other berry samples harvested at full maturation stage.

All identified aroma volatiles are presented in Table 1, showing the vast diversity of chemical structures found in berry samples from wild-growing *Vaccinium myrtillus*.

Table 1. Identified aroma volatiles in 32 blueberry samples from Norwegian *Vaccinium myrtillus* populations.

Aliphatic Esters	nonanol
ethyl acetate	2-butyl 1-octanol
ethyl 2-methylpropanoate	2-ethyl-1-decanol
methyl 3-methylbutanoate	hexyl octanol
ethyl 2-methylbutanoate	**Aliphatic Ketones**
ethyl 3-methylbutanoate	2-hexanone
3-methylbutyl acetate	4-hepten-2-one
methyl 2-hydroxy-3-methylbutanoate	4-methyl 2-heptanone
3-methyl-2-butenylacetate	4-octen-3-one
pentyl acetate	6-methyl-5-hepten-2-one
methyl hexanoate	2-dodecanone
ethyl 3-hydroxy-3-methylbutanoate	geranyl acetone
ethyl 2-hydroxy-3-methylbutanoate	β-ionone
ethyl hexanoate	**Aliphatic Acids**
(Z)-3-hexenyl acetate	nonanoic acid
hexyl acetate	decanoic acid
(E)-2-hexenyl acetate	undecanoic acid
(Z)-2-hexenyl acetate	hexadecanoic acid
(E,E)-2,4-hexadienyl acetate	**Monoterpenes and Sesquiterpenes***
hexyl propanoate	cumene
2-ethylhexanoate	tricyclene
(Z)-3-hexenyl butanoate	α-pinene
(E)-2-hexenyl butanoate	sabinene
ethyl octanoate	β-myrcene
(Z)-3-hexenyl 3-methylbutanoate	α-terpinene
hexyl-3-methylbutanoate	p-cymene
hexyl-2-methylbutanoate	1,8-cineole
octyl 2-methylpropanoate	limonene
hexyl hexanoate	(Z)-ocimene
isopropyl myristate	(E)-ocimene
Aliphatic Aldehydes	γ-terpinene

2-methyl butanal	terpinolene
(Z)-3-hexenal	linalool
hexanal	citronellal
(E)-2-hexenal	α-terpineol
heptanal	cyclocitral
(Z)-2-heptenal	linalyl acetate
2,4-heptadien-1-al	geranial
octanal	bornyl acetate
(E)-2-octenal	(E)-anethole
nonanal	β-caryophyllene*
(E,Z)-2,6-nonadienal	(E,E)-farnesyl acetate*
(E)-2-nonenal	**Aromatics**
decanal	toluene
dodecanal	styrene
tetradecanal	benzaldehyde
Aliphatic Alcohols	propyl benzene
2,4-hexadien-1-ol	acetophenone
(Z)-3-hexenol	5-ethyl-m-xylene
(E)-2-hexenol	p-methyl benzaldehyde
hexanol	ethyl benzoate
1-octen-3-ol	2-phenylethyl acetate
octanol	benzyl benzoate

Major chemical groups being represented comprise a total of 66 aliphatic hydrocarbons (29 esters, 15 aldehydes, 10 alcohols, 8 ketones, 4 acids), 23 terpenes (21 mono- and 2 sesquiterpenes), and 10 aromatic structures. The abundance of esterified aroma volatiles contribute to the overall fruity flavour of the European blueberry, though also many of the other reported aliphatic structures have similar aroma properties. Except for 1,8-cineole (mint-spicy note), most of the identified terpenes have to be considered as minor constituents. However, due to their low olfactory threshold, characteristic flavour notes are added to blueberry aroma such as α-terpinene, p-cymene, limonene, geranial (citrus-lemon-like), β-myrcene, (Z)- and (E)-ocimene, γ-terpinene, terpinolene (herb-spicy notes) and the aroma-impact compound linalool with its acetate (flowery-fruity notes). In addition, many of the detected aromatic structures have also strong aroma potential and supplement the characteristic blueberry aroma with their spicy, flowery, and fruity notes. In general, the presented results underscore the suitabilty and sensitivity of HS-SPME for the fast and reliable description of aroma volatiles from plant samples (Rohloff, 1999, 2002; Rohloff *et al.* 2004), also with regard to the detection of low-abundance compounds (Rohloff, 2004; Rohloff and Bones, 2005). Aroma volatile patterns found in our study, are in accordance with earlier results from *V. myrtillus* (Von Sydow and Anjou, 1969). Furthermore, many of the described structures have also been reported from other cultivated *Vaccinium* species (Parliment and Kolor, 1975; Hirvi and Honkanen, 1983a,b; Baloga i, 1995; Horvat *et al.*, 1996; Di Cesare *et al.*, 1999; Polashock *et al.*, 2007; Hanoglu and Pucarelli, 2007).

Conclusions

Results from our study show the complexity but also homogeneity of aroma compounds being detected in blueberries (*Vaccinium myrtillus*) from Norwegian populations, not least because almost 90 % of the identified structures were found in all samples. However, aroma patterns differed with regard to location and maturation stage, and underline the significance of both environmental, genetical and ontogenetical factors and thus, potential effects on blueberry aroma and quality. These questions will be further adressesd in our blueberry project as a continuation of the preliminary study.

Acknowledgements

Financial funding from the Research Council of Norway (RCN) through grant no. 184797 is greatly acknowledged.

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VARIABILITY OF THE STRUCTURE OF THE BIOCHEMICAL COMPOSITION OF THE FRUITS OF THE HIGHBUSH BLUEBERRY
AUGSTKRŪMU MELLEŅU OGU BIOĶĪMISKĀ SASTĀVA MAINĪGUMS

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Abstract

In this article the values of the coefficients of variation of 30 indicators of the biochemical composition of the fruits of the blueberry in a three-year cycle of supervision are presented. Signs

with resistance against the complex influence of meteorological factors based on the level of the variability of traits are designated. On the basis of the comparative analysis of the averaged variation coefficients calculated for a varietal row of *Vaccinium corymbosum* L. during a long-term cycle of observation it has been established that the parameters of accumulation of solids, fructose, total soluble sugars, flavonols, total bioflavonols, benzoic acid, nitrogen, potassium, calcium and magnesium in fruits can be characterized by the smallest degree of genotypic variability and, consequently, of intervarietal distinctions, whereas the content of free organic acids, anthocyanins proper and the values of a sugar-acid index in fruits are characterized by the highest degree of genotypic variability.

Kopsavilkums
Šajā rakstā ātspoguļoti 30 melleņu ogu bioķīmiskā sastāva indikatoru variācijas koeficienti trīs gadu izmēģinājumu ciklā. Salīdzinošai analīzei ir aprēķināts vidējais variācijas koeficients *Vaccinium corymbosum* L. šķirnu rindai ilgtermiņā ir novērots, ka sausnes, fruktozes, kopējo šķīstošo cukuru, flavonolu, kopējo bioflavonolu, benzoskābes, slāpekļa, kālija, kalcija un magnija uzkrāšanās ogās var raksturot ar mazāku genotipisko mainību un, tātad arī mazākām atšķirībām starp šķirnēm. Turpretim brīvo organisko skābju saturu, antociānu saturu un cukuru-skābju attiecības indeksu raksturo augstāka genotipiskā mainība.

Key words: *Vaccinium corymbosum* L., cultivars, biochemical composition, fruits

Introduction
One major aspect of the introductory research examining berry plant cultivars is an integrated assessment of the biochemical structure of fruits which can prove that the latter contain a wide spectrum of wholesome substances. At the same time it is well-known that the quantity of these substances in a fruit is cultivar dependant. In recent years the collection stock of the Central Botanical Garden of Belarus' NAS has been replenished with some new taxons of *Vaccinium corymbosum* L. that have different ripening times under the weather conditions in Belarus. Among them are such early-ripening varieties as Bluetta, Northblue, Weymouth, Duke, Reka, Earliblue, Spartan, Puru, Nui; such mid-ripening varieties as Bluecrop, Northland, Patriot, Toro, Jersey and such late-ripening varieties as Elizabeth and Coville. This replenishment gives additional opportunities to expand the assortment of the varieties offered for regionalization and selection which is done by choosing the most promising ones according not only to their crop and bioproductional characteristics, but also to the nutritive and vitamin value of berries that depends on their biochemical composition.
At the same time it seems fair to suppose that there exist some distinctions among the cultivars according to the genotypic variability of the parameters that influence the accumulation of certain compounds in fruits. The distinctions can indirectly specify the degree of the genetic determinancy of the parameters and make it possible to bring out the characteristics that are more or less stable when breeding new cultivars.

Materials and methods
In 2006 – 2008 research was conducted into the biochemical composition of the above-listed cultivars of *Vaccinium corymbosum* L. examining a wide spectrum of indicators relating to different classes of active substances. Fresh averaged samples of vegetable material were taken to determine the content of: solid matter – in accordance with GOST (State Standard) 8756.2 – 82 (1982); ascorbic acid (vitamin C) – using the standard indophenol method (Ermakov, etc., 1987); titratable acids (total acidity) – using the volumetric method (Ermakov, etc., 1987). Dried at 65 degrees Celsius averaged samples of fruits were used to determine the content of such chemical elements as nitrogen, phosphorus, potassium using the method of K.P. Fomenko and N.N. Nesterov (1971); calcium and magnesium – using the complexometric method (Ermakov, etc., 1987); glucose, fructose, sucrose – using the resorcinol and aniline-phthalate methods of paper chromatography of I.G. Zavadsky and others (1962); pectins (water-soluble pectin and protopectin) – using the carbazolic method (Ermakov, etc., 1987); the sums of anthocyanic pigments – using the method of T. Swain, W. E. Hillis (1952) constructing a calibration curve on cyaniding crystal

obtained from fruits of black chokeberry and cleared using the technique of J.G. Skorikova and E.A. Shaftan (1968) and employing S.S. Tanchev's formula evaluation (1980); anthocyanins – using the method of L.O. Shnaidman and V.S. Afanasyeva (1965); the flavonol sums – according to the method of L.Sarapuu and H.Miydla (1971); the catechine sums – according to the photometric method with the use of a vanillin reagent (Zaprometov, 1964); phenol-carboxylic acids (in terms of chlorogenic acid) – according to the method of descending chromatography on paper (Mzhavanadze, etc., 1971); tannins – according to the titrimetric method of Levental (State Pharmacopoeia of the USSR, 1987); lignins – using the modified method of Klason (Ermakov, etc., 1987); benzoic acid – using the method of M.I. Kalebin and A.A. Kolesnik (1949); fatty oils – using the method of V.A. Sapunov and I.I. Fedunyak (1958); triterpenic acids (in terms of ursolic acid) – using the method of A.V. Simonyan and others (1972). All analytic determinations have been carried out in triplicate. The data has been statistically processed using the computer program Excel.

When estimating the genotypic variability of the parameters that influence the accumulation of the above-mentioned compounds we were guided by the coefficients of variation (V) of the characteristics under study within the range of *Vaccinium corymbosum* L. taxons that were examined during a long-term cycle of observation. The values of variation coefficients indicate that they depend on a cultivar, i.e. the higher the variation coefficient is, the stronger this dependence is and, therefore, the level of genetic determinacy of the characteristic goes down, and vice versa.

According to S.N. Sennov and V.F. Kovyazina (1990), series variability for biological objects is regarded low if it is equal to 11-30 % and is considered high if it exceeds 31 %. When examining the data presented in this paper, we should take into account the active reaction of an alien crop to the breeding process. This reaction allows the crop to resist the breeding process to a certain extent as well as to regulate the biochemical composition of generative organs within genetically determined variation ranges of each characteristic. It allows one to narrow down the above mentioned limits of small series variability for the indicators under study to 15 %. Accordingly, its average range will be characterized by the level of variability within 16 – 30 %, while the maximum one is over 31 %.

Results and Discussion

The biochemical screening of the three groups of *Vaccinium corymbosum* L. cultivars characterized by different terms of ripening made it possible to identify the taxons with the highest and the lowest content of wholesome substances in fruits. These substances refer to different classes of chemical compounds. At the same time, there have been discovered some essential intervarietal distinctions concerning the accumulation of certain compounds in the dry weight of fruits, which is confirmed by the wide range of changes of their quantitative characteristics that during a three-year cycle of observations made up (Table 1): for titratable acids – 1.5 – 11.1 %; for ascorbic acid – 296-941.0 mg% (mg of ascorbic acid in 100 g of dry weight of sample); phenol-carboxylic acids – 543-1189 mg%; benzoic acid – 0.82 – 1.59 %; soluble sugars – 10.9 – 29.2 %, including glucose – 3.2 – 7.1 %, fructose – 5.2 – 19.3 %; sucrose – 0.4 – 4.5 %; pectins – 3.6 – 7.9 %, including hydropectin – 1.3 – 3.2 %, protopectin – 1.8 – 5.2 %; for the total content of bioflavonols – 1935 – 3110 mg %, including anthocyanic pigments – 9 – 58 mg %, catechines – 383 – 1404 mg %, flavonols – 1471 – 2251 mg %; tannins – 0.9 – 2.5 %; fat oils – 1.6 – 5.5 %; major mineral elements: N – 0.57 – 1.34 %, P – 0.10 – 0.24 %, K – 0.44 – 0.99 %, Ca – 0.26 – 0.45 %, Mg – 0.07 – 0.12 %. The content of solid in the fresh weight of fruits changed from 10.9 % to 17.6 %.

The analysis of the information presented in the table has revealed among the given taxons of *Vaccinium corymbosum* L quite a wide range of coefficients showing variation in the quantitative indicators of the biochemical composition of fruits both during individual years and during the whole period of observation. That indicated different levels of their dependence on the genotype of a plant. This fact enabled us to identify the characteristics possessing the highest and the lowest degree of varietal distinctions and to order them according to the decrease in genetic determinancy. The majority of the parameters showing accumulation of reactants in blueberries within the period of observations were characterized mainly by an average variability (V = 15 – 30 %). A considerable part of the indicators were notable for a low (V <15 %) level of variability in a varietal series, testifying to their weak dependence on genotype, and only some of the

characteristics under study have shown a high level (V > 30 %) of variability indicating the highest degree of varietal distinctions.

Table 1. Averaged quantity indicators of the biochemical structure of fruits (dry weight) and variation coefficients in a long-term cycle of supervision for a varietal row of *Vaccinium corymbosum* L.

Index	2006		2007		2008		Period average V, %
	mean	V, %	mean	V, %	mean	V, %	
Soluble solids, % of fresh fruit	13.9	12.8	13.9	10.1	14.1	12.1	11.7
Free organic acids, %	5.7	36.4	3.8	45.7	6.7	39.1	40.4
Ascorbic acid, mg %	601.2	20.3	426.6	26.3	604.8	30.3	25.6
Glucose, %	5.34	16.4	4.49	15.8	4.96	27.5	19.9
Fructose, %	18.74	2.9	14.22	3.9	7.26	22.3	9.7
Sucrose, %	3.19	19.8	2.08	20.2	0.56	22.1	20.7
Sum of soluble sugars, %	27.25	4.1	20.79	5.1	12.79	6.8	5.3
Fructose/ Glucose	3.6	19.2	3.2	16.5	1.7	46.0	27.2
Monose/Disaccharide	7.9	19.4	9.4	19.8	22.7	21.5	20.2
Sugar-acid index	5.4	31.2	6.5	39.5	2.5	75.9	48.9
Hydropectin, %	1.98	24.3	2.18	23.8	2.37	18.0	22.0
Protopectin, %	3.45	22.4	2.60	19.8	3.35	21.0	21.1
Sum of pectins, %	5.43	16.5	4.77	19.2	5.71	17.8	17.8
Protopectin/Hydropectin	1.8	33.8	1.2	22.9	1.4	17.1	24.6
Anthocyanins, mg % *	7.6	45.1	2.0	91.5	17.1	35.5	57.4
Leucoanthocyanins, mg%	17.4	23.2	12.1	20.6	24.1	29.1	24.3
Sum of anthocyanic pigments, mg %	25.0	19.7	14.1	21.5	41.2	27.1	22.8
Catechines, mg %	984.3	20.2	923.4	27.6	570.1	15.4	21.1
Flavonols, mg %	1766.6	5.5	1626.0	11.0	1890.6	12.2	9.6
Flavonols/Catechines	1.9	27.1	1.9	36.3	3.4	20.9	28.1
Sum of bioflavonols, mg %	2776.0	6.9	2563.5	11.7	2501.8	9.2	9.3
Phenol-carboxylic acids, mg %	781.4	18.0	800.3	24.4	787.3	24.3	22.2
Benzoic acid, %	1.15	6.1	1.11	18.2	1.18	14.3	12.9
Tannins, %	1.21	19.5	1.72	23.2	1.83	16.3	19.7
Fat oils, %	3.61	29.9	3.17	13.0	3.25	21.9	21.6
Nitrogen, %	0.76	15.1	0.91	16.2	1.10	10.3	13.9
Phosphorus, %	0.17	24.0	0.14	11.5	0.14	13.0	16.2
Potassium, %	0.53	10.0	0.57	8.7	0.76	11.3	10.0
Calcium, %	0.31	10.0	0.42	4.9	0.40	4.9	6.6
Magnesium, %	0.09	11.9	0.08	8.8	0.11	6.8	9.2

* mg% - mg of substance in 100 g of dry weight of sample.

The variability level of a particular characteristic only in some cases remained stable throughout the whole 3-year period of observation. For example, such stability of a low variability level was typical for the parameters of the accumulation of solid, flavonols, potassium, calcium, magnesium, the total amount of sugars and the sum of bioflavonols in fruits whereas the parameters of the accumulation of fructose, benzoic acid, fat oils, nitrogen and phosphorus in fruits were notable for low variability only during one or two seasons. Similarly, the high level of genotypic variability during the whole period of observation was typical for the parameters of the accumulation of free organic acids, anthocyanins and the values of a sugar-acid index whereas for the ascorbic acid content it has been notable only in 2008. Moreover, even within a particular area of variability of the characteristics analyzed there have been identified some essential interseasonal distinctions. This fact unequivocally indicates a dependence of the genotypic variability of quantity indicators of biochemical composition of *Vaccinium corymbosum* L. fruits on the hydrothermal mode of its formation period.

In our opinion, the best estimate about the degree of variability of the indicators in the varietal row can be given by the averaged values of variation coefficients calculated within a 3-year period of observation. In this case the characteristics under study can be provisionally divided into 3 groups in an increasing order of genotypic variability, and, hence, intensification of varietal distinctions:

1 – with small variability (V = 5.3 – 13.9 %) – the content of solid, fructose, total of soluble sugars, flavonols, total bioflavonols, benzoic acid, nitrogen, potassium, calcium and magnesium in fruits;

2 – with average variability (V = 16.2 – 25.6 %) – the content of phosphorus, ascorbic acid, glucose, sucrose, hydropectin, protopectin, leukoanthocyanins, catechines, phenol-carboxylic acids, tannins and fat oils in fruits;

3 – with high variability (V = 40.4 – 57.4 %) – the content of free organic acids, anthocyanins proper and values of a sugar-acid index in fruits.

Conclusion

The biochemical screening of the fruits of 16 representatives within three groups of introduced blueberry cultivars with different terms of ripening (early-ripening, mid-ripening and late-ripening) according to 30 indicators specifying the content of some organic acids, carbohydrates, bioflavonols, terpenoids and chemical elements under vegetative seasons of 2006 – 2008 made it possible to reveal the essential intervarietal distinctions in the accumulation of certain compounds in the dry weight of fruits, confirmed by a wide range of changes of their quantitative characteristics. On the basis of the comparative analysis of the averaged variation coefficients calculated for a varietal row of *Vaccinium corymbosum* L. during a long-term cycle of observation it has been established that the parameters of accumulation of solids, fructose, total soluble sugars, flavonols, total bioflavonols, benzoic acid, nitrogen, potassium, calcium and magnesium in fruits can be characterized by the smallest degree of genotypic variability and, consequently, of intervarietal distinctions, whereas the content of free organic acids, anthocyanins proper and the values of a sugar-acid index in fruits are characterized by the highest degree of genotypic variability.

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MICROPROPAGATION OF HIGHBUSH BLUEBERRY CULTIVARS
AUGSTKRŪMU MELLEŅU ŠĶIRŅU MIKROPAVAIROŠANA

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Abstract
The aim of this study was to determine an efficient micropropagation system for the highbush blueberry cultivars 'Spartan', 'Bluecrop' and 'Berkeley'. The shoot tips of the selected three genotypes were successfully established *in vitro* using mercuric chloride in a concentration of 0.15 % as a sterilization solution. Anderson's rhododendron medium (AN), half-strength Murashige and Skoog medium (half-MS) and McCown woody plant medium (WPM) containing cytokinin zeatin in concentrations 0.5, 1 or 2 mg l^{-1} were tested. Multiplication rates varied depending on the cultivar, medium and concentration of the zeatin. The highest multiplication 4.8 ± 0.2 was noted for 'Berkeley' on WPM medium with zeatin (2 mg l^{-1}). Out of three media tested, the WPM medium was found to be more effective than the AN medium and half-MS medium for shoot multiplication. The *in vitro* rooting on the WPM medium is also reported.

Kopsavilkums
Pētījuma mērķis bija noteikt augstkrūmu melleņu šķirņu 'Spartan', 'Bluecrop' un 'Berkeley' efektīvāko mikropavairošanas sistēmu. Izvēlēto trīs genotipu dzinumu gali tika veiksmīgi ievietoti *in vitro* izmantojot 0.15% koncentrācijas dzīvsudraba hlorīdu kā sterilizācijas šķīdumu. Tika pārbaudīta Andersona rododendra barotne (AN), pus-stiprā Murashige un Skoog barotne (pus-MS) un McCown koksnaino augu barotne (WPM), kas satur citokinīna zeatīnu 0.5, 1 vai 2 mg l^{-1} koncentrācijā. Pavairošanas ātrums bija atkarīgs no šķirnes, barotnes un zeatīna koncentrācijas. Augstākais pavairošanas koeficients 4.8 ± 0.2 bija vērojams šķirnei 'Berkeley' WPM barotnē ar zeatīnu (2 mg l^{-1}). No trim pārbaudītājām barotnēm dzinumu pavairošanai WPM barotne bija efektīvāka par AN barotni un pus-MS barotni. WPM barotrnē tika novērota arī dzinumu *in vitro* apsakņošanās.

Key words: explant, *in vitro*, zeatin, multiplication, rooting

Introduction
The highbush blueberry (*Vaccinium corymbosum* L.), native to North America, is a commercially important fruit crop (Zmarlicki, 2006). Although plants of the *Vaccinium* genus have not been cultivated on a large scale in the Czech Republic, there is potential for commercial highbush blueberry production in some mountain regions. A number of research programs continue to find suitable high yielding highbush blueberry cultivars with superior berry qualities for commercial growing in the rural countryside of the Czech Republic (Paprstein *et al.*, 2006; Paprstein and Ludvikova, 2006). In recent years, the blueberry has received considerable attention for its nutritional quality and health benefits (Howell, 2009).
If suitable cultivars are to receive wide distribution rapid propagation techniques will be essential. Highbush blueberry can be propagated vegetatively by multiple-node softwood or hardwood cuttings. This method, although generally successful, is slow and labor intensive. Success with

cuttings also varies markedly with the individual genotype, age of the stock plant and the vegetation period (Kosina and Sedlak, 2006).
The micropropagation can potentially multiply selected cultivars more rapidly than traditional nursery methods utilizing softwood or hardwood cuttings. Over the last three decades, *in vitro* culture propagation methods on various basal media using axillary bud proliferation and adventitious shoot regeneration has been achieved with varying success (Reed and Abdelnour, 1991; Noe *et al.*, 1998; Gajdosova *et al.*, 2006; Li *et al.*, 2006). Unfortunately, these results are not broadly applicable, because the effectiveness of the medium and morphogenesis of *Vaccinium in vitro* plants seems to be highly dependent on plant growth regulators and the media used for the culture, and this dependence is genotype specific (Ostrolucka *et al.*, 2004; Debnath, 2007). Moreover, the response of individual genotypes can vary during the whole cycle of micropropagation (Mehri-Kamoun *et al.*, 2004). The suitability and genetic stability of blueberry plants micropropagated in tissue culture have also been discussed for a long time (El-Shiekh *et al.*, 1996; Smolarz and Chlebowska, 1997).
As a part of the project to introduce highbush blueberry culture to the Czech Republic, studies were conducted to investigate new genetic resources and their possible multiplication by *in vitro* culture (Paprstein *et al.*, 2005). The objective of this study was to compare various basal media with different zeatin concentrations for shoot proliferation and to determine the best one for micropropagation of three highbush blueberry cultivars.

Materials and Methods
For the *in vitro* culture establishment, twenty actively growing shoot tips (5 to 15 mm in length) were cut from shoots of three blueberry cultivars ('Spartan', 'Bluecrop' and 'Berkeley') sprouting in laboratory conditions. The donor shoots were removed from mature shrubs growing in field germplasm collection of RBIP Holovousy in March. After removal of most of the leaves, the initial explants were dipped in a 0.15 % solution of $HgCl_2$ with a wetting agent added (0.05 % Tween-20) for 1 min. This was carried out under sterile conditions under a laminar flow hood. Following sterilization, the tips were rinsed in sterile distilled water and cultured in 200 ml glass culture flasks (seven shoots per flask), each with 35 ml of WPM (woody plant medium) according to Lloyd and McCown (1981). The initial WPM medium was with 1 mg l^{-1} zeatin. Culture vessels were glass bottles capped with clear permeable polypropylene caps. The contamination rate, the survival and development of shoots from excised shoot tips were analyzed after sterilization. Uncontaminated shoots established on WPM medium were transferred after one month to a fresh proliferation medium. All shoot cultures were serially subcultured for at least 4 months on a WPM medium supplemented with 2.0 mg l^{-1} zeatin. This provided a stock collection of shoots for proliferation studies.
All initiation and multiplication media contained 7.0 g l^{-1} Difco agar. The pH of the media was adjusted to 5.2 before autoclaving at 120 °C at 100 kPa for 15 minutes. Cultures were grown in rooms under cool-white fluorescent tubular lamps at 60 $\mu mol.m^{-2}.s^{-1}$ (16-hour photoperiod) at 22 ± 1 °C.
For the multiplication phase, three basal nutrient media WPM, AN (Anderson's rhododendron medium) according to Anderson (1980) and modified MS (Murashige and Skoog, 1962) medium containing half macro and micronutrients (half-MS) were tested. The shoot tip cultures were multiplied by removing several elongating shoots from the basal mass and subculturing the shoots on a fresh medium. To induce new shoots, basal nutrient media included three concentrations 0.5, 1 or 2 mg l^{-1} of the cytokinin zeatin. The zeatin was filter sterilized (25 mm, Acrodisc Syringe Filter 0.2 μm, Pall Gelman, USA) and added to multiplication media after autoclaving. Uniform single shoot tips (5 to 10 mm in length) excised from apical parts of established proliferating cultures were used in all multiplication experiments. The morphological appearance of the shoots (primarily callus formation, hyperhydricity etc.) was also noted.
The multiplication rate was defined as the number of newly formed shoots (>10 mm) per initial shoot tip after four weeks of culture. The shoot formation was recorded between the fifth and fifteenth subculture. In all experiments 25 shoot tips were used. Each experiment was repeated four times. Data from four independent experiments were pooled and expressed as the mean. To evaluate the accuracy of the estimate of the mean of population, treatment means were compared

with the standard error (SE) of the mean as a measure of variance. For three genotypes ('Spartan', 'Bluecrop', 'Berkeley'), shoots (10 to 20 mm in length) derived from the best proliferation medium, were excised and rooted on WPM medium with 1 mg l^{-1} IBA. Although several media were evaluated for the induction of roots, only results from medium that showed maximal root induction are presented in this report. Culture conditions during root initiation and root growth were the same as during shoot culture. A hundred microcuttings were used for this treatment. The number of rooted *in vitro* plants was recorded five weeks after transfer to rooting medium. The treatment means were compared with the standard error (SE) of the mean. Shoots with roots were rinsed in water to remove remnants of the medium and then transferred to Jiffy 7 peat pellets (AS Jiffy Products, Norway) soaked with water. The shoots were misted with water to prevent wilting during transplanting. The Jiffy 7 pellets with rooted plants were placed on a greenhouse bench equipped with transparent plastic covers (100 % air humidity) under the standard greenhouse condition. The plants were gradually acclimated by opening the covers over fourteen days.

Results and Discussion

The numbers of uncontaminated explants that survived and developed into shoots are shown in Table 1. Selected three genotypes were successfully established *in vitro* using mercuric chloride in a concentration of 0.15 % as a sterilization solution. Bacterial and fungal contamination was infrequent. Of the 60 shoot tips taken only one explant of 'Spartan', one explant of 'Berkeley' and two explants of 'Bluecrop' were visibly contaminated with micro-organisms. These explants were later discarded. The use of mercuric chloride had a direct beneficial effect and overcame the contamination from the microflora of the field germplasm collections of blueberry. On the other hand, the toxicity to tissues caused by mercuric chloride was high. In the case of the cultivars 'Spartan' and 'Bluecrop', about 50 % of initial uncontaminated explants did not develop shoots and turned brown. However, the remaining uncontaminated initial explants of these two cultivars had a greenish color and produced shoots. Debnath and McRae (2001) reported that although regeneration from primary explants is a first necessary step in any micropropagation of *Vaccinium* genus, the regeneration frequency has no effect on the further success of the micropropagation program. Many shoots could be obtained from a few clean shoots regenerated from the primary explant.

Table 1. Surface sterilization of highbush blueberry cultivars by 0.15 % mercuric chloride

Cultivars	Explants contaminated		Explants died without contamination		Established explants which developed shoots	
	Number	(%)	Number	(%)	Number	(%)
Spartan	1	5	11	55	8	40
Bluecrop	2	10	10	50	8	40
Berkeley	1	5	5	25	14	70

After 5 months in the culture, all surviving explants showed active and uniform shoot growth and multiplication. Dividing and subculturing the basal shoot mass did not cause tissue breakdown or exudation. The results of the multiplication of highbush blueberry cultivars are shown in Tables 2 - 4. The number of newly formed shoots varied with the cultivar, the medium tested and the concentration of zeatin. Across all experiments, the highest multiplication rate (4.8) was obtained for 'Berkeley' on WPM medium with the highest concentration 2 mg l^{-1} of zeatin. On three tested media, 'Berkeley' was the cultivar with the highest ability to produce vigorous multiple shoot cultures. On the contrary, for the cultivar 'Spartan', neither of the three tested media containing different concentrations of zeatin promoted markedly *in vitro* shoot formation and the number of newly formed shoots was thus very low (from 1.3 to 1.8). Within the same range of zeatin concentration, the three genotypes gave higher multiplication rates on the WPM medium. The woody plant medium (WPM) was found to be more effective than an AN and a half-MS medium for the initiation of new shoots in our study. The lowest multiplication rates were noted for 'Bluecrop' and 'Berkeley' on a half-MS medium. The lowest multiplication rates for 'Spartan' were noted on the AN medium. Short shoots (shorter than 10 mm) were frequently observed in the case of the cultivar 'Bluecrop' on half-MS medium with all concentrations of zeatin tested. The

shoots shorter than 10 mm were not counted for our multiplication studies. These small shoots did not elongate and were difficult to use directly in further procedures. The half-MS medium proved to be less suitable for the multiplication of three highbush blueberry cultivars.

Table 2. Multiplication rates for highbush blueberry cultivars on WPM medium with zeatin

Zeatin (mg l^{-1})	Cultivar		
	Spartan	Bluecrop	Berkeley
0,5	1.4 ± 0.1	1.3 ± 0.1	3.0 ± 0.1
1	1.8 ± 0.1	1.5 ± 0.1	4.0 ± 0.2
2	1.7 ± 0.1	2.0 ± 0.1	4.8 ± 0.2

Table 3. Multiplication rates for highbush blueberry cultivars on AN medium with zeatin

Zeatin (mg l^{-1})	Cultivar		
	Spartan	Bluecrop	Berkeley
0.5	1.4 ± 0.1	1.2 ± 0.0	1.4 ± 0.0
1	1.3 ± 0.1	1.2 ± 0.1	2.2 ± 0.1
2	1.3 ± 0.1	1.9 ± 0.1	2.3 ± 0.1

Table 4. Multiplication rates for highbush blueberry cultivars on half MS medium with zeatin

Zeatin (mg l^{-1})	Cultivar		
	Spartan	Bluecrop	Berkeley
0.5	1.5 ± 0.1	1.2 ± 0.0	1.2 ± 0.1
1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
2	1.5 ± 0.1	1.5 ± 0.1	1.6 ± 0.1

In the case of the cultivars ‘Bluecrop’ and ‘Berkeley’, the increasing zeatin concentration in the tested media also increased the shoot multiplication without excessive callus formation. Zeatin proved its ability to stimulate adventitious shoot development in *Vaccinium in vitro* culture. The highest multiplication rates were always noted on media with the highest concentration of zeatin (2 mg l^{-1}). The zeatin level 2 mg l^{-1} can be recommended for the multiplication of the cultivars ‘Bluecrop’ and ‘Berkeley’. Earlier reports indicated that zeatin was an important plant hormone for efficient multiplication and growth in *Vaccinium* micropropagation (Reed and Abdelnour, 1991; Debnath and McRae, 2001; Ostrolucka *et al.*, 2004; Jiang *et al.*, 2009). According to Reed and Abdelnour (1991), the cultivation medium with relatively high levels of zeatin (4 mg l^{-1}) promoted a significantly higher initiation of axillary shoots in eight of twelve *Vaccinium corymbosum* genotypes than on the control medium. On the contrary, Gajdosova *et al.* (2006) pointed out the effectiveness of zeatin in low concentration (0.5 mg l^{-1}) for inducing multiple shoot development in meristem cultures of *Vaccinium* sp. Zeatin concentrations of 2 mg l^{-1} and higher promoted callus formation and suppressed shoot regeneration in Gajdosova’s experiments, which is contradictory to our findings. In our experiments on all media, any physiological disorders or morphological abnormalities such as excessive callus formation or the production of abnormally narrow leaves were not observed during the *in vitro* shoot proliferation stage. For the cultivar ‘Spartan’ the highest multiplication rate 1.8 was noted on media with the zeatin concentration of 1 mg l^{-1}. However this multiplication rate (1.8) achieved on a WPM medium with 1 mg l^{-1} of zeatin can be sufficient only for *in vitro* culture establishment and maintenance, but is not satisfactory for larger scale *in vitro* shoot production. Future research and testing of other media and plant growth regulators is needed in the case of ‘Spartan’.

The results of rooting are summarized in Table 5. There was considerable variation in the rooting percentage of used blueberry cultivars. WPM medium with a high concentration of IBA (1 mg l^{-1}) was effective for root induction in the case of cultivars ‘Berkeley’ and ‘Bluecrop’. Root initiation started within two weeks. The percent of rooting was 70 % for the cultivar ‘Berkeley’ and 61% for ‘Bluecrop’. However, the same treatment yielded considerably fewer rooted plants (9 %) in the case of ‘Spartan’. On an average, IBA promoted development of two to six good quality roots per shoot without callusing at the basal portion of shoots. Roots originated directly from the base of the main shoot. High survival (more than 80 %) was obtained after acclimatization of rooted plants in *ex vitro* conditions. These plants showed normal growth and developmental characteristics,

compared to conventionally grown plants. Ostrolucka *et al.* (2007) reported 80 % rooting and 80 – 90 % survival after transfer to *ex vitro* conditions for *V. corymbosum* genotypes with both *in vitro* and *ex vitro* rooting using 0.8 mg l^{-1} IBA for root induction.

Table 5. Rooting of highbush blueberry on WPM medium with 1 mg l^{-1} IBA

Cultivar	Rooting shoots (%)	Root number per shoot ± SE
Spartan	9	6.0 ± 0.6
Bluecrop	61	2.3 ± 0.1
Berkeley	70	2.6 ± 0.1

Conclusions

Micropropagation techniques described in this paper increased multiplication mainly in the highbush blueberry cultivar 'Berkeley' on WPM medium. A rapid *in vitro* shoot multiplication procedure could have a crucial impact on our ability to rapidly proliferate and maintain desirable highbush blueberry cultivars, while at the same time allowing for initial plant material availability throughout the year. By using a zeatin supplemented WPM medium for shoot initiation and proliferation, thousands of plants a year could be produced from a single initial shoot. In comparison, conventional nursery techniques using multiple-node softwood or hardwood cuttings produce only a few plants annually. However, some cultivars of highbush blueberry would still require further research to optimize the proliferation media.

Acknowledgements: This work was realized in the framework of project QH82232 from National Agency for Agricultural Research (MZe CR).

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INFLUENCE OF DRYING TECHNOLOGY ON THE QUALITY OF DRIED CANDIED *CHAENOMELES JAPONICA* DURING STORAGE
KALTĒŠANAS TEHNOLOĢIJU IETEKME UZ *CHAENOMELES JAPONICA* SUKĀŽU KVALITĀTI UZGLABĀŠANAS LAIKĀ

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Abstract
In the Baltic region, the development of Japanese quince as a fruit crop started in Latvia in 1951. The fruits of *Chaenomeles japonica* are very firm, acidic, with too low sugar content to be consumed fresh, but they are useful for processing and valuable because of the high content of organic acids, vitamin C, phenolic compounds and fresh aroma. The interest for growing Japanese quince in Latvia reached a peak in 1993, but the processing was not solved. Only juice and puree were produced. Therefore a new Japanese quince processing technology was worked out and patented (RL patent Nr. LV 12779 B) at Dobele Horticultural Plant Breeding Experimental Station (presently Latvia State Institute of Fruit-Growing) and Latvia University of Agriculture in 2002.
In order to enlarge the product spectrum made from Japanese quince, investigations were conducted at the Latvia State Institute of Fruit-Growing during the 2007. The aim of the present work was to characterize the influence of the drying technology on the quality of dried candied Japanese quince during storage for six months. Two different drying technologies were used: with forced air circulation and vacuum-microwave. The sweet dried Japanese quince products were tested for content of vitamin C, phenolic compounds and changes of colour by using the CIE L*a*b* colour system.
The obtained data showed that the content of vitamin C decreased on average by 40 %, but the content of phenolic compounds by 17 % in the product. The over colour of the product changed after two months of storage.

Kopsavilkums
Baltijas reģionā krūmcidoniju selekcija uzsākta Latvijā kopš 1951. gada. *Chaenomeles japonica* augļi ir cieti, skābi, ar pārāk mazu cukura daudzumu, lai tos varētu patērēt svaigā veidā, bet, pateicoties augstajam organisko skābju, C vitamīna, fenolu savienojumu saturam un patīkamā aromāta dēļ, tie ir noderīgi pārstrādei. Interese par Japānas krūmcidoniju audzēšanu Latvijā virsotni sasniedza 1993. gadā, bet netika atrisināta augļu pārstrāde. Galvenokārt tika ražota sula un biezenis. Tādēļ Dobeles Dārzkopības selekcijas un izmēģinājumu stacijā (šobrīd Latvijas Valsts augļkopības institūtā) un Latvijas Lauksaimniecības universitātē 2002. gadā tika izstrādāta un

patentēta (LR patents Nr. LV 12779 B) jauna krūmcidoniju pārstrādes tehnoloģija. Pētījums tika veikts Latvijas Valsts augļkopības institūtā 2007. gadā, lai paplašinātu krūmcidoniju pārstrādes produktu piedāvājumu. Pētījuma mērķis bija novērtēt kaltēšanas tehnoloģiju ietekmi uz krūmcidoniju sukāžu kvalitāti sešu mēnešu uzglabāšanas laikā. Pārbaudītas divas atšķirīgas kaltēšanas tehnoloģijas: piespiedu gaisa cirkulācija un kaltēšana vakuma mikroviļņu iekārtā. Krūmcidoniju sukādēm tika noteikts C vitamīna un polifenolu daudzums, kā arī krāsas izmaiņas CIE L*a*b* krāsu sistēmā. Iegūtie rezultāti rāda, ka pēc 6 mēnešu uzglabāšanas C vitamīna saturs produktā samazinājās vidēji par 40 %, bet polifenolu saturs – par 17 %. Uzglabāšanas laikā produkta krāsa sāk būtiski izmainīties pēc diviem uzglabāšanas mēnešiem.

Key words*: Chaenomeles japonica*, products, technology.

Introduction

The fruits of *Chaenomeles japonica* are used in the food industry for processing because of their high content of biologically active compounds: organic acids, vitamin C, phenolic compounds, pectin and aroma components (Lesinska *et al.*, 2006; Ruisa, 1996). Vitamin C content in fruits differs from 41.2 to 105.8 mg 100 g^{-1}, phenolic compounds range from 523.9 to 1271.7 mg 100 g^{-1} (Krasnova *et al.*, 2007). The juice of Japanese quince can be a useful ingredient for the food industry due to its flavour and high acidity. Another property of great interest is its presumed high antioxidant capacity due to the content of vitamin C and phenolic compounds. The content of vitamin C is 45 to 109 mg 100 ml^{-1} and phenolic compounds in juice of the *Chaenomeles japonica* was 210 – 592 mg 100 ml^{-1} (Hellin *et al.*, 2003). However, juice and puree from the fruits are not demanded in the market.

To get healthy products from *Chaenomeles japonica* fruits with better market demand, a new processing technology was developed at Dobele in cooperation with the Faculty of Food Technology of Latvia Agricultural University. As a result two products were obtained – sweet dried candies and syrup. The technology was patented in 2002 (RL patent Nr. LV 12779 B). The way of obtaining dried candies is based on cell destruction (mechanically or using low temperatures).

Various methods can be used for drying: convective drying (COD), vacuum-microwave drying (VMD) and others. Drying in vacuum-microwave influences the quality of product slightly, however, it facilitates production on the industry scale.

Small fruit and vegetable companies prefer convective drying, yet increased temperatures are used very often to get faster results, which in turn reduce the nutritive value of the product (Diaz-Moroto *et al.*, 2002). Vacuum-microwave drying of food is becomming more and more popular due to its advantages and simple usage. By this method microwaves penetrate the interior of the material causing water to boil at a relatively low temperature. This creates a high vapour pressure in the centre of the material, allowing rapid transport of moisture out of the product (Sham *et al.*, 2001). For example, the quality of herbs: parsley and oregano (colour, content of volatile oil, aroma) was higher after drying in VM if compared to CO drying (Boehm *et al.*, 2002). In addition the quality of Mexican oregano (*Lippia berlandieri Schauer*) was similar to the results obtained by process of sublimation, which can be characterized as one of the more cautious drying method (Jaloszinsky *et al.*, 2008; Yousif *et al.*, 2000). Similar results were obtained by testing samples of strawberries and carrots – in the process of VM drying the content of vitamin C and phenolic compounds keeps better and antiradical activity is higher compared to drying in free air admission (Wojdylo *et al.*, 2009; Böhm *et al.*, 2006**).**

The aim of the present work was to characterize the influence of the drying technology on the quality of dried candied Japanese quince during storage for six months.

Material and methods

The experiment was carried out at the Experimental Processing Laboratory of Latvia State Institute of Fruit-Growing 2007. The object of the study – sweet dried quince candies were made corresponding to the patented technology (RL patent Nr. LV 12779 B). Sugar was used as a sweetener, preservatives and colours were not used in the production. The vegetable chopper

„Metos RG-350" (http://www.metos.fi/); was used for cutting up Japanese quince fruits; size of pieces – 13x13x4.5 mm. Two drying methods were used:

- Convective drying (COD) – using equipment „ORAKAS" (www.lkv.com/marlemi) on sieves with forced air circulation at +60 ºC; duration of drying on average 8 hours, until the moisture of the product reaches 32±3%.
- Vacuum-microwave drying (VMD) - using the equipment „Musson-1" (www.ingredient.spb.ru) by an individual program; moisture of the product: 32±3%; duration of drying depends on the initial moisture content. Duration of drying on average for 12 kg was 155 minutes.

Ready sweet dried candies were packed in sealed polypropylene (PP) bags (thickness 25 μm), inserted in paper bags. The mass of the product in each bag was 100 ± 5.0 g. Packed candies were stored at a room temperature of 20±2 °C; relative air humidity ~60±2%.

The duration of the study was 6 months. Three separate replications of product preparation were made. The analyses of the product were carried out before packaging (0 days) and two times per month. At each time of testing, three identical packages were analyzed.

The following properties of dried sweetened quince candies were determined:

- Vitamin C – the content of ascorbic acid (vitamin C) (mg 100 g^{-1}) was determined by the HPLC method LVS EN 14130:2003.
- Phenolic compounds - the total content of phenols (mg 100 g^{-1}) was determined by the method of spectrometry, by spectrometer UV-1650-PC at a wave length 765 nm (Singleton *et al.*, 1999).
- Colour changes - measured during the storage in CIE L*a*b* colour system using Colour Tec PCM/PSM device. The product was placed in a glass container (diameter 30 mm); the changes in colour were determined for three packages; the measurements were repeated for 15 series.

The data were statistically evaluated using SPSS (version 11.5) for Windows and MS Excel variance analysis, significance level at $P < 0.05$.

Results and Discussion

The colour of the product is one of the important organoleptic parameters for consumers. Sharp changes in the colour of dried candied quince were observed during storage. These changes occur as a result of the destruction of carotenoids, vitamins and other substances and the action of enzymes. The changes influence the colour forming component L*, a* and b* values of CIE L* a* b colour system (MacDougall, 2002).

Greater colour intensity L* (0=black, 100=white) at the beginning of the study was observed for the product dried in the microwave vacuum equipment (value 72.4). However, the product dried in forced air circulation was darker (L* value 66.9). The colour of the product changed during storage – colour intensity value L* and b* (+ yellow, - blue) reduced during first 2 months, but a* (+ red, - green) value increased for both product types. Statistically significant differences depending on the drying method were ascertained between the colour intensity values L* and b* of the product ($p=0.004$; $p=0.009$ respectively), but a* value did not differ significantly ($p=0.86$).

The total changes of the colour component of the product during storage characterize the sum of differences – total difference ΔE^*, formed by L*, a* and b* values. The colour changes of the product were observed on average after 2 months of storage – products became darker independent of the drying method. Calculating the total colour difference ΔE^* after 48 days of storage one can conclude that differences do exist but are hard to evaluate organoleptically (Figure 1). However, the total colour differences of the product were significant at the end of the study - the value for COD product was 4.75, but for VMD 10.25.

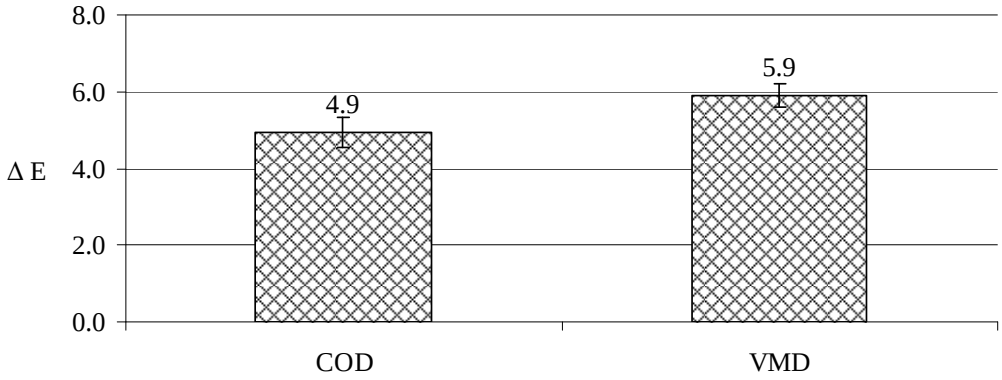

Figure 1. The colour difference ΔE of dried candied quince product, after 48 days of storage (COD - convective drying, VMD - vacuum-microwave drying)

Since the drying temperature for both drying methods did not exceed the 60 °C necessary for the Maillard reaction (between amino acids and reducing sugars), there is a reason to assume that the action of enzymes could cause the browning of Japanese quince candies during their storage.

Plant enzymes like oxireductases (polyphenoloxidases, lipoxidases and peroxidases) enables the oxidation and reduction processes of biologically important substances. Enzymatic browning of plant material is caused mainly by polyphenoloxidases. They are released from membranes because of damage occurring to fresh fruits and come into contact with plant phenolic compounds. Polyphenoloxidases dehydrogenate phenolic compounds form into unstable chinons, therefore causing the formation of melanin (black, brown or red polymer colour). Peroxidases found in plant products in turn transfer hydrogen peroxide, oxidizing hydrogen donors. Peroxidases become inactive after heating. However, it has been found frequently that inactivated peroxidase after some time can become active (Baltess, 1998).

Phenolic compounds are a large group of organic substances determining fruit taste and aroma. However, the aroma in the skin, flesh and juice of Japanese quince fruits constitutes an essence which is a solution of aromatic components in water and not an essential oil. These substances are susceptible to changes of temperature, so after freezing fruits loose their aroma. Taste of the obtained product – sweet dried candies is good after adding sugar and drying at high temperature.

The total content of the phenolic compounds of both dried products at the beginning of the investigation was similar: for the product dried in forced air circulation (COD) it was 467.2 mg 100 g^{-1}, but 481.5 mg 100 g^{-1} after drying by vacuum-microwave (VMD) equipment (Figure 2). The content of phenolic compounds was reduced on average by 9 % in products obtained in COD and by 4.7 % if obtained in VMD, after 2 months of storage. But at the end of the study the total content of phenolic compounds reduced on average by 17 % apart from the drying method.

Significant differences (p=0.037) between the quince products obtained by the two drying methods were ascertained during storage, the higher total content of phenolic compounds remained in VMD product method.

Several investigations on the biochemical composition of fruits, berries and herbs present evidence that the VMD method is the better way to preserve the phenolic compounds compared to the COD method.

Research by Mejia-Meza (2008) at Washington State University on dried bilberries (*Vaccinium corymbosum L.*) indicates that the total content of phenolic compounds is maintained the best by drying at low temperatures (sublimation) followed by the VMD method and after that the COD method. Similar results were obtained at Jena and Dresden Universities investigating different VM drying regimes and vacuum-microwave drying combined with convective pre- and post-drying to improve the quality of dried strawberries (Böhm, 2006). Convective drying and VM drying decreased the content of ascorbic acid to approximately 40 % of the initial value, phenolic compounds to approximately 35 % in dried strawberries. Jaloszynski *et al.* (2008) found that the content of phenolic compounds were reduced sharply by using CO drying for herbs at 60 – 70 °C.

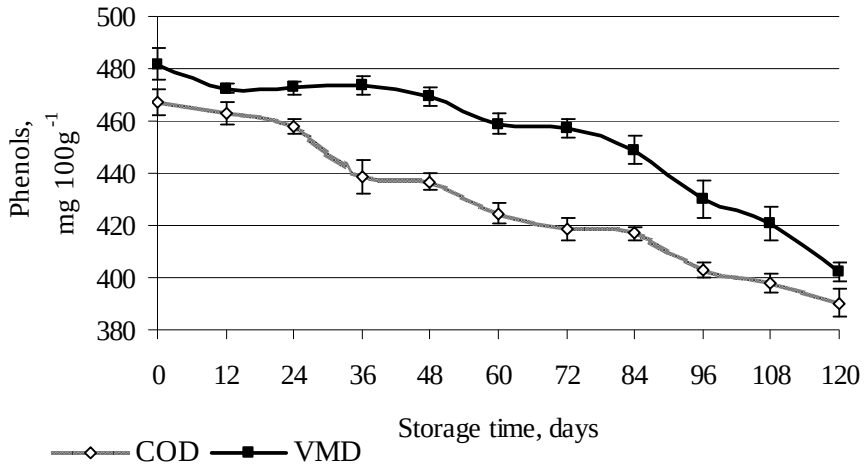

Figure 2. Changes of total content of phenolic compounds during storage (COD - convective drying, VMD - vacuum-microwave drying)

The content of vitamin C in the products was on average 51 mg 100 g^{-1} at the beginning of the investigation – immediately after drying (Figure 3). A sharp reduction of vitamin C was observed after 24 days of storage. After 2 months of storage the content of vitamin C was reduced by 29.5 % using the COD method and by 33 % using the VMD, but by 37.9 % and 40.2 % respectively at the end of the investigation. Drying methods used in this study did not influence the content of vitamin C (p=0.71).

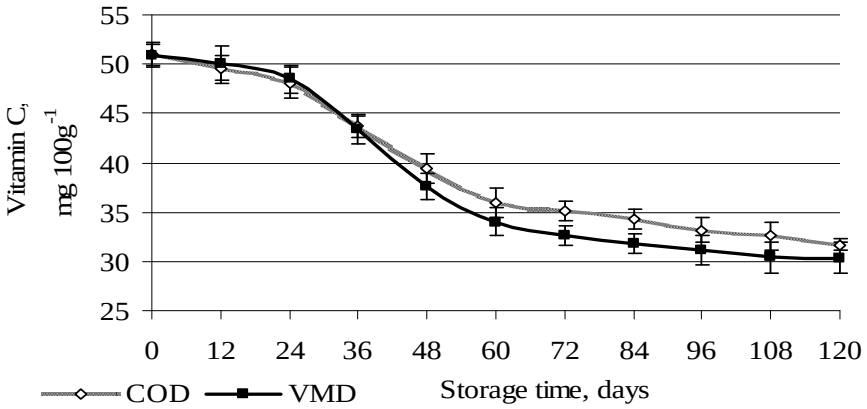

Figure 3. Changes of the content of vitamin C during storage (COD - convective drying, VMD - vacuum-microwave drying)

Due to the high content of total phenolic compounds in fruits vitamin C was retained during the processing. Several researchers emphasized that drying by the vacuum-microwave method the vitamin C in the product was preserved better than by the CO or spraying methods. Tein *et al.* (1998) compared the effect of different drying methods on the quality of dried carrot slices and established fact that a higher content of vitamin C and carotene was found in the product dried by the VMD method compared to the COD method. The content of vitamin C was influenced not only by the drying method, but also by the microwave power intensity. Nurul Asyikin *et al.* (2007) studied the content of vitamin C in the dried fruits of papaya depending on the drying temperature and concluded that lower microwave intensity helped to keep a higher content of vitamin C in the product.

Conclusions

The drying methods used in this study influenced the quality of the dried candied Japanese quince. A higher content of total phenolic compounds remained in the product dried by the vacuum-

microwave method. The drying method did not influence the content of vitamin C in the product (p=0.71). The colour of the product started to change after two months of storage.
For the industrial production of sweet dried candies it is recommended to use the vacuum-microwave drying method, and the optimal storage time of the product without significant losses of quality is two months.

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SHORT INFORMATION ABOUT THE HISTORY OF THE COMMERCIAL CULTIVATION HIGHBUSH BLUEBERRY IN POLAND
ĪSA INFORMĀCIJA PAR AUGSTKRŪMU KRŪMMELLEŅU KOMERCIĀLAS AUDZĒŠANAS VĒSTURI POLIJĀ

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Abstract
The first notes concerning highbush blueberry cultivation in Poland appeared about the year 1935 – 1939. The results obtained at that time were not positive. Also the first trials with the cultivation of this plants soon after the second world war were negative. The problem was in choosing improper soil for the blueberry. The further experiments concerning the quality of soil and mineral fertilization brought interesting results and caused an increase of interest in blueberry cultivation on large scale. The experimental field in Skierniewice, belonging to the Warsaw Agriculture University has plots with different long-term soil mineral fertilization and different pH status since 1923. On those plots the first methodical experiment with the highbush blueberry was begun in 1976 on drained soil with 1.3 % of humus. This experiment brought very interesting and important results. It explained some basic problems, first of all the meaning of pH value and fertilization of soil for blueberry cultivation. It appeared that blueberry did crop well when soil pH, determined in KCl is between 3.5 – 4.0. The soil should be rather light and level of ground water not higher than 40 – 50 cm below the surface. It was also found that blueberry reacts badly for lack of nitrogen and to a lesser degree, for deficiency of P and K. The growth and yield of blueberry plants on single plots was differentiated and depended mainly on soil pH and nitrogen fertilization. The plants of cultivars Bluecrop and Jersey still grow well, though they are 32 years old. Now the rejuvenation pruning research is being done on them.

Kopsavilkums
Pirmās ziņas par augstkrūmu krūmmelleņu audzēšanu Polijā ir atrodamas laikā no 1935. – 1939. gadam. Tomēr tā laika pētījumu rezultāti nebija pozitīvi. Arī pirmie izmēģinājumi par šo kultūru audzēšanu, neilgi pēc otrā pasaules kara, bija negatīvi. Problēma bija saistīta ar nepiemērotu augsnes izvēli krūmmellenēm. Turpmākie eksperimenti par augsnes kvalitāti un augu minerālo barošanos deva interesantus rezultātus un radīja interesi par krūmmelleņu audzēšanu lielākos apjomos. Varšavas Lauksaimniecības universitātes Eksperimentālajā laukā Skiernevicē kopš 1923. gada ir ierīkoti ilgstoši izmēģinājumi ar dažādiem augu mēslošanas un pH līmeņiem. Šajos pētījumos pirmie eksperimenti ar krūmmellenēm tika sākti 1976. gadā drenētā augsnē ar 1.3 % humusa saturu. Šis eksperiments deva interesantus un nozīmīgus rezultātus. Tie izskaidroja dažas pamatproblēmas, pirmkārt par augsnes pH saturu un mēslošanu. Izrādījās, ka krūmmellenes labi ražoja, kad augsnes pH_{KCL} izvilkumā bija robežās no 3.5 – 4.0. Augsnei jābūt vieglai un gruntsūdenim jābūt ne augstākam par 40 – 50 cm. Tika arī konstatēts, ka krūmmellenes slikti reaģē uz slāpekļa un, mazākā pakāpē, uz fosfora un kālija trūkumu. Krūmmelleņu augšanas un ražības atšķirības noteica, galvenokārt, augsnes pH un mēslošana ar slāpekli. Šķirnes 'Bluecrop' un 'Jersey' joprojām aug normāli, lai arī to vecums jau ir 32 gadi. Pašreiz šajos lauciņos turpinās pētījumi par atjaunojošo apgriešanu.

Key words: *Vaccinium corymbosum*, fertilization, soil pH, cultivar evaluation, pruning, yield.

Introduction
The highbush blueberry (*Vaccinium corymbosum* L.) grows in the wild in the North – East part of USA. The bushes grow up to 3 m high, are relatively frost resistant can stand the temperature up to -35 ^{o}C. The bushes can be found in forest, on high peat soil, as well as on light, acid mineral soil. The roots grow close under the soil surface, most of them are very thin, only some of them are thick enough to support the plant. The blueberries are very fruitful, the fruits are relatively large, tasty, mainly for fresh eating but also for processing.

At present the highbush blueberry is being cultivated in many countries of the world. Also in Poland, since many years the people have been interested in growing them, however the plants did not grow well in the field. In the sixties of past century there was an idea to cultivate the plants in concrete rings or in deep holes, covered with plastic and filled with acid peat or with the forest soil mixed with acid peat. In such conditions the plants grew very well and gave a good yield. The observation of those plants convinced us that the blueberries can be cultivated in our climatic and soil condition. However the method of growing plants in concrete rings or deep hols could not be recommended for commercial cultivation from economical reasons. We started to look for the reasons, why in Poland the blueberries do not grow well in the open field, like it is in the USA and many other countries.
In seventies of past century some experiments were started to explain this phenomenon.

Materials and Methods
In 1976 one of the first experiments was set on the Experimental Field belonging to the Dept of Agricultural Chemistry of Warsaw Agricultural College (SGGW) in Skierniewice. The plots of this Experimental Field are being characterised by constant fertilization, since 1923, with the some mineral compounds. (0-control, CaNPK, NPK, PK, PN and KN). No organic fertilization is being used. The experiment with blueberries was set on plots with mineral fertilizers and characterised by low pH of soil. On those plots the nitrogen is being used only as ammonium sulphate.
The soil on whole Experimental Field was drained, and belongs to the IV class according to Polish nomenclature. The same way of fertilization influences on the content of single compounds in the soil, and differentiates its pH.

Results and Discusion
By planting the blueberry on such plots, it was possible to conclude after some years, what is the influence of single compounds on the growth and cropping of two blueberry cultivars – Bluecrop and Jersey, used in this experiment (Table 1.)

Table 1. Soil pH (KCl) and concentration of the nutrients (mg 100 g^{-1} soil)

Treatments	pH	P	K	Ca	Mg
0	4.4	2.7	5.6	29.0	3.2
Ca NPK	4.5	11.2	17.8	34.0	3.4
NPK	**3,6**	10.8	13.5	14.1	2.1
PK	4.5	11.9	20.2	25.0	2.6
PN	3.5	10.6	**4.7**	15.2	2.0
KN	3.7	**3.3**	13.7	13.8	2.1

- After 60 years of treatment

The main aim of this experiment was also to find out what are the possibilities of growing blueberries not only on light sandy soils and but also on more fertile soil of low pH 3,5 – 4,0 (Table 2.) The method of determination of soil pH in USA and in Poland are different and this caused great misunderstanding in choosing the right soil for blueberry cultivation in our country. In Polish chemical stations pH is determined in KCl what means, that most suitable for blueberries soil with pH 3,5 – 4,0 (this value according to American method of pH determination would be pH 4.0 – 5.0). The Polish IV class soil (the type which is dominated in Poland) might be suitable for blueberry, in case that the other physical conditions required by this plant are proper. Those types of soil are more fertile, do not require so much of irrigation in comparison to light sandy soil.

Table 2. Average yield of two cultivars of highbush blueberry (kg/plant) (1988 – 1991).

Treatments	Bluecrop	Jersey
0	0.94 a	0.68 a
Ca NPK	0.90 a	1.34 b
NPK	**3.47 c**	**2.38 c**
PK	1.26 a	0.76 a
PN	2.09b	1.53b
KN	2.99 c	1. 67 b

Evaluation of cultivars. After the resolving of the soil and its pH problem, the next experiments were devoted to the evaluation of blueberry cultivars, their economical value and suitability for growing in Polish condition. In 1978 was performed the first experiment in Pomological Orchard of our Institute to compare the value of different blueberry cultivars. The plants were not irrigated but some of them gave relatively good crop. On the basis of this experiment the following cultivars were selected as the most suitable for cultivation in our condition: Weymouth, Earliblue, Concord, Bluecrop, Jersey, Herbert, Darrow (Table 3).

Table 3. Yield of highbush blueberry cultivars in kg/plant and mean weight of 100 berries in g from years 1984 – 1986 and 1988 – 1990

Cultivars	1980 - 83	1984 - 86	1988 - 90	1993 - 94	Total from 12 years	Wheigh of fruits (g)
Earliblue	1.57 cd	2.40 abc	1.38 a	1.64 ab	6.99 a	109
Weymouth	1.65 d	3.36 e	2.68 def	5.06 f	12.75 e	100
Collins	1.01 a	2.56 bcd	2.08 bcd	1.18 a	6.83 a	131
Bluecrop	1.42 bcd	2.68 cd	3.29 f	3.21 de	10.60 d	140
Concord	1.38 bcd	2.43 bcd	2.86 ef	3.39 e	10.06 cd	83
Ivanhoe	1.64 d	2.32 abc	2.51 cde	1.28 ab	7.75 ab	133
Jersey	1.60 cd	2.00 ab	1.86 abc	1.99 bc bc	7.45 a	83
Herbert	1.63 d	1.78 a	1.78 ab	3.42 e	8.61 abc	144
Darrow	1.15 ab	2.11 abc	2.09 bcd	2.63 cd	7.98 ab	197
Coville	1.34 bc	2.73 cd	2.29 b-e	3.19 de	9.55 bcd	131
Lateblue	1.91 e	2.66 cd	2.45 b-e	3.72 e	10.74 d	129
13-16-A	2.05 e.	3.05 de	2.87 ef	4.06 f	12.93 e	76

* It is the first experiment with cultivar evaluation in Poland

The evaluation of newly bred cultivars are still being conducted in Skierniewice, some of them: Spartan, Duke, Bluegold, Nelson, Toro, Reka, Puru, Nui, Denise Blue, Brigitta, Bonifacy (obtained by dr. Pliszka) have been introduced for cultivation.
At present the following newest cultivars are tested: Aurora, Draper, Liberty, Chandler, Bonus and few others. The suitability of cultivars: Rubel, Hard Blue are being studied for their suitability for mechanical harvesting and processing of fruits.
Some other experiments concerning pruning methods of different age plants, nitrogen fertilization, weeds control, irrigation and fertigation are being conducted at present (Table 4).

Table 4. Influence of pruning on the yield of blueberry Bluecrop cv

Pruning	Yield kg/plant					
	1993 r.	1994 r.	1995 r.	1996 r.	1997 r.	Sum 1993 – 1997
Control	3,24	3,48	3,51	1,66	1,72	13,61
Very strong	3,14	4,07	5,40	2,97	4,09	**19,67**
Medium	3,26	4,68	5,17	2,44	3,27	18,82
Light	2,98	3,53	3,46	1,28	1,76	13,01

Conclusion

The blueberry can be cultivated on different types of soil, more or les fertile but characterized by good physical condition and the proper pH levels.
The pH of soil, the determined in KCl, should be 3.5 – 4.0.
The blueberry reacts very strongly for lack of nitrogen in soil, but the doses per ha of this compound should not be higher than 100 kg N
The blueberry is less sensitive to the deficiency of potassium and phosphorous than for nitrogen.
The cultivars, which can be recommended for cultivation are: Earliblue, Spartan, Duke, Bluecrop, Nelson.
The recent results suggest that also Toro, Bluegold, Brigitta, Draper, Liberty can be recommend.

The plants should be pruned since they start cropping. The rejuvenesce pruning should be done when the plantation is about 15 year old.
It is recommended to use in rows organic substances like sawdust from coniferous trees, acid peat, bark and stow.
It is also possible to spread in rows the black plastic.

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CLOUDBERRY BREEDING IN NORWAY
LĀCEŅU SELEKCIJA NORVĒĢIJĀ

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Abstract
Two female and two male cultivars have previously been released as a result of clone evaluation at Bioforsk Nord Holt. The selection criteria were the number of pistils or stamens per flower, the number of flowers and the number of shoots per m^2. Currently a new group of clones are being evaluated with the aim of finding new cultivars for release. The preliminary results on flowering and berry production show a strong variance in these traits. Over three years of registration, the number of produced flowers varied from under 100 for the weakest clone to more than 1000 for the best clone. The number of produced berries varied from 9 for the weakest clone to 242 for the best clone. The three clones that produced the most flowers all had very low berry production and deviated from the rest of the clones by having high numbers of flowers per harvested berry. New selection criteria will be considered before selection of new cultivars for release. In addition to the production traits, new selection criteria will most likely include berry contents, such as the levels of antioxidants.

Kopsavilkums
Klonu izvērtēšanas rezultātā Bioforsk Nord Holt tika izveidotas divas sievišķās un divas vīrišķās lāceņu šķirnes. Izlases kritēriji bija drīksnu vai putekšņnīcu skaits ziedā, ziedu un dzinumu skaits uz kvadrātmetru. Pašlaik tiek izvērtēta jauna klonu grupa, lai iegūtu jaunas šķirnes. Iepriekšējie rezultāti parāda lielas atšķirības starp klonu ziedēšanu un ogu ražošanu. Trīs gadu laikā ziedu skaits uz m^2 svārstījās no mazāk nekā 100 sliktākajam klonam līdz vairāk nekā 1000 labākajam klonam, bet izveidoto ogu skaits – no 9 līdz 242. Trīs kloni, kuriem novēroja visbagātīgāko ziedēšanu, veidoja ļoti maz ogu un atšķīrās no pārējiem ar lielu ziedu skaitu, attiecībā pret izveidoto ogu skaitu. Pirms jaunu šķirņu izdalīšanas tiks izstrādāti jauni izlases kritēriji – neskaitot ražošanas īpatnības, tiks iekļauts arī ogu sastāvs, piemēram, antioksidantu saturs.

Key words: cloudberry, clone evaluation, selection criteria

Introduction
Two female (‘Fjellgull’ and ‘Fjordgull’) and two male (‘Apollen’ and ‘Apolto’) cultivars of cloudberry (*Rubus chamaemorus*) have previously been released as a result of clone evaluation at the Norwegian Institute for Agricultural and Environmental Research. The evaluation consisted of comparisons of different clones under controlled environmental conditions. Selection criteria were

the number of pistils (female clones) or stamens (male clones) per flower, the number of flowers and the number of shoots per m^2 (Rapp, 1991). Currently a number of clones collected from different wild populations are being evaluated at Bioforsk Nord Holt. The group consists of 11 female and 1 male genotype collected around 1995. The aim of these evaluations is to find new genotypes suited for cultivar release. Preliminary results from these evaluations are presented here, focusing on the female clones for the parameters of the number of flowers and numbers of berries.

Materials and methods

The evaluations were performed at Bioforsk Nord Holt in Tromsø (latitude 69º40', altitude 30 meters above sea level). The different clones were planted on peat in open benches outdoors. The different clones were planted in separated squares that contained 16 plants of the clone, and every clone was represented in two different squares at random positions on the bench. The bench was established in 2003, the square size within the bench was 0,8 m^2. The squares were separated to avoid rhizomes propagation between the squares.

Table 1. Description of the evaluated clones.

Number	Origin	Sex	Latitude	Altitude
002	England	Female	54º30'	.
102	Aust Agder	Female	58º30'	50
104	Aust Agder	Female	58º30'	350
105	Aust Agder	Male	58º30'	650
106	Aust Agder	Female	58º30'	650
202	Hedmark	Female	62º30'	50
206	Hedmark	Female	62º30'	650
208	Hedmark	Female	62º30'	950
304	Nordland	Female	66º30'	350
306	Nordland	Female	66º30'	650
404	Finnmark	Female	70º30'	350
602	Svalbard	Female	78º38'	80

Table 1 gives a brief description of the evaluated clones. Ten clones were collected from four different Norwegian counties; two in Northern Norway (Finnmark and Nordland), and two in Southern Norway (Aust Agder and Hedmark). In addition, one clone was collected in England and one at Svalbard. The clones were collected at altitudes ranging from 50 to 950 meters above sea level. One of the clones was male. In addition, 5 male clones were located on nearby benches, thus 6 different male clones were available for the pollination of the evaluated female clones.

The first berries were produced in 2005, and from 2006 flowering and berry production have been registered. Number of flowers are registered once every season, thus both number of flowers, number of buds and number of withered flowers are registered and added up to a measure of total number of flowers. Berries are harvested at maturity and are registered several times every season. Registrations are made for number of drupes and berry weight.

Results and Discussion

Figure 1 shows the number of flowers produced by the different clones. There are large differences in the total number of flowers over the three years, with clone 106 being the best with just over 1000 flowers registered and clone 202 the poorest with fewer than 100 registered flowers.

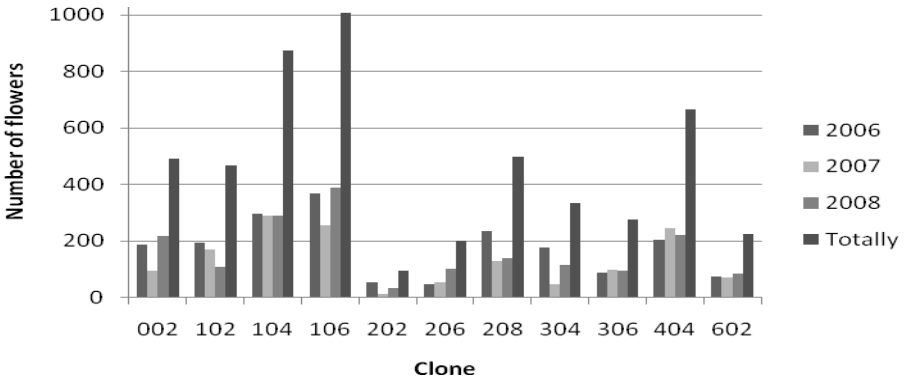

Figure 1. Number of flowers produced by the different clones
in the years 2006, 2007 and 2008.

Figure 2 shows the berry production of the clones. The results are surprising based on flower production as the clones that produced the most flowers would be expected to produce most berries. In this case, the three clones that produced most flowers have a very low production of berries. The three clones that produced the most berries, clones 002, 102 and 208, all had intermediate flower production.

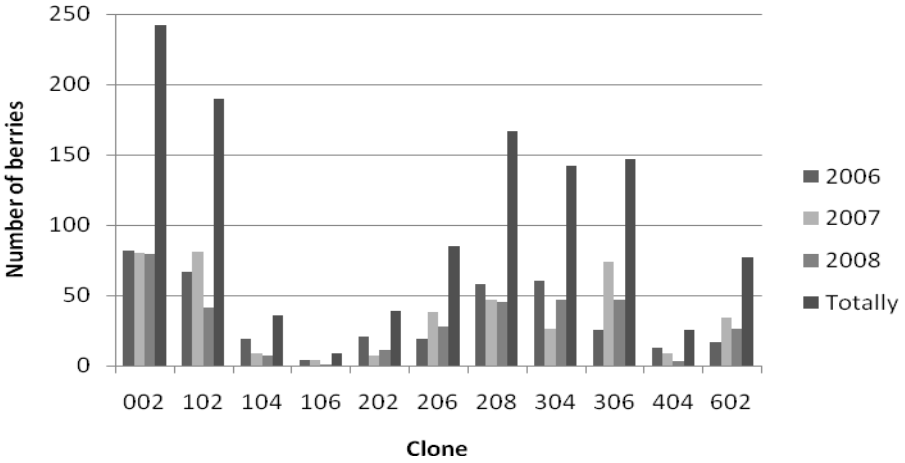

Figure 2. Number of harvested berries produced by the different clones
in the years 2006, 2007 and 2008.

Table 2 combines the results for the number of flowers and the number of berries and shows the number of registred flowers per harvested berry. In general, most clones show similar results, ranging from 1,88 to 2,90 flowers per berry over the three years. Three clones deviate from this. Clones 104, 106 and 404. Again, these are the clones that produced the most flowers and less berries.

Based on the registrations on flowering, the clones with high numbers of flowers per produced berry do not seem to be neither particularly late nor early flowerers and it also seems that there were male flowers available at the time of flowering. Thus, deviating flowering times do not explain the small amount of berries produced from the abundance of flowers, but as flowering is registered only once per year it still may be that some tendencies that the registered data are not showing up.

In this case there were 6 different male clones available for pollination. It may be that the female clones with low berry production combine poorly with the available male clones, which might explain the low production.

Table 2. Number of flowers per harvested berry for the different clones in the years 2006, 2007 and 2008.

Clone	2006	2007	2008	Totally
002	2,26	1,16	2,72	2,04
102	2,88	2,07	2,56	2,45
104	15,63	32,11	41,29	24,31
106	92,00	63,25	389,00	112,22
202	2,52	1,57	2,73	2,41
206	2,37	1,39	3,61	2,34
208	4,05	2,68	3,02	2,98
304	2,93	1,69	2,38	2,34
306	3,48	1,30	1,98	1,88
404	15,54	27,11	73,33	26,64
602	4,29	2,03	3,12	2,90

The northern clones might be expected to be best adapted to the environmental conditions in Tromsø. However, the clones with the highest berry production all had a southern origin. At the same time other southern clones produced few berries, so there were no obvious tendencies concerning origin when berry production was considered. For flower production, the three clones from Aust Agder (102, 104 and 106) all produced above average, while the clones from Nordland and Hedmark produced at different levels. In total, origin did not explain the variations in production.

Based on these evaluations, selection based on the number of flowers and the number of pistils may theoretically entail a risk of selecting genotypes with high potential, but low ability for production under certain environmental conditions. Thus, there may be genotype-environment interactions involved, such that specific genotypes are adapted to specific environmental conditions. More knowledge about the genetic and environmental basis for berry production is needed to find the optimal selection criteria. The genotypes evaluated here are also planted at different natural sites in Norway. Registrations from these sites may provide additional information about the different genotypes and environmental factors influencing berry production.

Further evaluations will be done before the selection of new cultivars for release. In addition to production traits, the new selection criteria most likely will include berry contents, such as the level of antioxidants.

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FUNGAL DISEASES OF *VACCINIUM MACROCARPON* IN LATVIA
VACCINIUM MACROCARPON SLIMĪBAS LATVIJĀ

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Abstract

The American cranberry (*Vaccinium macrocarpon* Ait.) has been known for fifteen years, but fungal diseases have been investigated only last years in Latvia. Upright dieback and berries rot were observed several years ago, but growers did not know the causal agents of these symptoms. The aim of the study was to detect the causal agents of cranberry diseases in Latvia. Samples of upright dieback (in summer) and berries (during the harvesting) from different regions of Latvia were collected for causal agent detection. *Botrytis cinerea, Fusiccocum putrefaciens, Phomopsis vaccinii, Pestalotia vaccinii, Discosia artocrea, Physalospora vaccinii* were detected from upright

dieback. *Botrytis cinerea, Allantophomopsis cytisporea, Fusiccocum putrefaciens, Phomopsis vaccinii, Coleophoma empetri, Phyllosticta elongata, Physalospora vaccinii, Pestalotia vaccinii, Gloeosporium minus* and *Discosia artocreas* were detected from rotted berries. In the future *Fusiccocum putrefaciens* and *Phomopsis vaccinii* could become the most harmful fungi in the cranberry plantations, because it is difficult to control them.

Kopsavilkums
Lielogu dzērvenes (*Vaccinium macrocarpon* Ait.) Latvijā jau ir zināmas vairāk kā piecpadsmit gadus, bet to slimības pētītas tikai pēdējos gados. Lai gan audzētāji dzinumu atmiršanu un ogu puves pazīmes bija novērojuši jau iepriekš, tomēr neviens īsti nezināja, kas ierosina šīs slimības. Lai noteiktu slimību ierosinātājus, no lielogu dzērveņu stādījumiem dažādos audzēšanas rajonos Latvijā vasarā tika ievākti vertikālo dzinumu atmiršanas paraugi, bet ogas - ražas vākšanas laikā. No vertikāliem atmirušiem dzinumiem tika noteiktas sekojošas slimības: *Botrytis cinerea, Fusiccocum putrefaciens, Phomopsis vaccinii, Pestalotia vaccinii, Discosia artocrea, Physalospora vaccinii.* No puves bojātām ogām tika noteikti: *Botrytis cinerea, Allantophomopsis cytisporea, Fusiccocum putrefaciens, Phomopsis vaccinii, Coleophoma empetri, Phyllosticta elongata, Physalospora vaccinii, Pestalotia vaccinii* and *Discosia artocreas.* Turpmāk nopietnus bojājumus varētu izraisīt *Fusiccocum putrefaciens* un *Phomopsis vaccinii* izplatība dzērveņu stādījumos, jo šo ierosināto slimību ierobežošana ir sarežģīta.

Key words: cranberry diseases, upright dieback, berries rot, causal agent.

Introduction
The American cranberry (*Vaccinium macrocarpon*) is a perspective and marketable culture in the market of Latvia. The climate and peat bogs are similar to the cranberry growing areas in North America (Ripa, 1996). Fungal diseases are one of the most important problems, because they reduce and damage the quality of the harvest in America. The cranberry is a well known cultivated fruit crop for fifteen years in Latvia as well, and some investigations of cranberry diseases started in 2004, but significant studies - in 2006. Mainly uprights dieback, blossom blight and berry rot were caused by fungi in North America and in Latvia as well. Detection of cranberry diseases is important to make control options in future.
The study aim was to detect the causal agents of cranberry diseases in Latvia.

Materials and methods
Eight cranberry plantations (Jelgava, Talsi, Riga, Kuldiga, Liepaja, Aluksne, Cesis and Gulbene districts) in 2007 were inspected during the flowering and harvesting time. From cranberry plantations in different regions in Latvia were taken samples of upright dieback, blossoms, ovaries in summer, but berries were taken at harvest time.
Samples of upright dieback, blossoms and ovaries blight were put in a moisture camera (wet filter paper in Petri dishes) and kept at room temperature (20 – 25 ^{o}C) in sunlight.
At harvest from each farm 200 sound berries were collected randomly along a diagonal through the plantation; in total 1200 berries from six plantations. Berries were kept in plastic bags in refrigerated storage at +5 ^{o}C for up to 4 months. At the end of each month (December – March) the berries were counted and the rotted berries were placed with the cut surface down on potato-dextrose agar for causal agent of storage rot detection
The samples of cranberry diseases before being placed on PDA were surface-disinfested in 70 % alcohol and then rinsed in sterile water twice and pieces of samples put on PDA. The growing fungal colonies were transferred on PDA and pure cultures were incubated at room temperature 20 – 25 ^{o}C for 3 to 4 weeks. Fungi were identified directly on the isolation plates by comparing the morphological characteristics of the spores and spore bearing structures with descriptions in the literature (Caruso *et al.,* 1995; Kačergius *et al.,* 2004; Горленко *et al.,* 1996). Morphological characteristics of discovered fungi were fixed using microscope OLYMPUS CX31, magnifier MEIJI EMZ and camera SONY DSC – H2. Liga Vilka took the photos for fungi identification and collected them for the archive database of cranberry diseases in Latvia.

Results

First time upright dieback in Latvia was observed in 2004. In the last three years the incidence level of upright dieback was only 1-3 % (from 100 uprights). In the beginning of the summer uprights of the previous year usually were dark brown or red-brown, but young uprights - bronzing brown, with top slope and died. These symptoms could be caused by non parasitic diseases (sun, drought or rain, fertilization problems, etc.) or by fungi.

In Latvia from upright dieback 6 causal agents were detected (Table 1), and mainly they caused blossom and ovaries blight and berries rot. From berries damaged by rot 9 causal agents were detected (Table 1).

Table 1. Detected causal agents of cranberry diseases in Latvia, 2007 – 2008

Causal agents from upright dieback	Causal agents from berries rot
Botrytis cinerea Pers.:Fr.	*Botrytis cinerea* Pers.:Fr.
Fusicoccum putrefaciens Shear vaccinii Groves)	*Fusicoccum putrefaciens* Shear vaccinii Groves)
Phomopsis vaccinii Shear in Shear, N. Stevens, & H. Bain	*Phomopsis vaccinii* Shear in Shear, N. Stevens, & H. Bain
Discosia artocreas (Tode) Fr.	*Discosia artocreas* (Tode) Fr.
Pestalotia vaccinii (Shear) Guba	*Pestalotia vaccinii* (Shear) Guba
Physalospora vaccinii (Shear) Arx & E. Müller	*Physalospora vaccinii* (Shear) Arx & E. Müller
	Phyllosticta elongata G. J. Weideman in G. J. Weideman, D. M. Boone, & Burdsall
	Coleophoma empetri (Rostr.) Petr.
	Allantophomopsis cytisporea (Fr.: Fr.) Petrak

Causal agents survive and reproduce during the vegetation season on berries or other plants parts, increasing the incidence level of the diseases in the next year.

Botrytis cinerea caused upright dieback, blossom and ovaries blight and yellow rot in Latvia. Flowers and ovaries were yellowish brown and later became dark brown. Upright dieback was bronzing brown, the end of the top sloped. Berry rot was yellow or yellowish brown. Yellow rot mostly appeared in the field, few of berries were affected during storage. Yellow rot can be easily confused with end rot caused by *Fusicoccum putrefaciens.* The fungus grew rapidly at 20 – 24 ^{o}C on the PDA. At first the colonies were white, with loose aerial mycelium. Later the mycelium became pale gray-brown. On the surface after 10 days white sclerotia appeared, after maturation they turned black. From black sclerotia developed conidiophores and on the top ovate or elliptical, green-grey conidia appeared. In the moisture camera on upright diebacks, blossoms and ovaries conidia appeared as well. According to the symptoms of cranberry disease and fungus peculiarities in the moisture camera and pure culture, upright dieback, blossom and ovaries blight and yellow rot was caused by *Botrytis cinerea* Pers.:Fr. The causal agent of the disease was identified based on symptoms and the morphological characteristics as described by Горленко *et al*., 1996 and Caruso F. L., 1995.

Fusicoccum putrefaciens caused upright dieback, blossom and ovary blight and end rot in Latvia. Uprights, blossoms and ovaries turned brown and died. Some damaged berries of end rot were observed in the field, but mostly berry rot appeared in storage. Berries, damaged in field, were soft, wet, pale yellow, but those damaged during storage turned pale rosy or yellowish brown. Damage from rot on berries mostly appeared at the calyx; probably berries were infected by fungus during blossoming. Later in storage life the rotted berries shrunk. Upright dieback and end rot caused by *Fusicoccum putrefaciens* were the widely distributed cranberry diseases in Latvia. The fungus grew rapidly on PDA at 20 – 24 ^{o}C. Aerial mycelium was fluffy, compact, grey-yellow or olive-yellow. Pycnidia under mycelium matured, and on the surface appeared a pale orange cream spore mass. Separately conidia were hyaline, elliptic to fusiform, with aseptate or pseudosaptate, measurement on average 2.0 x 8.8 µm (1.5 – 3 x 6-11µm) (Figure 1).

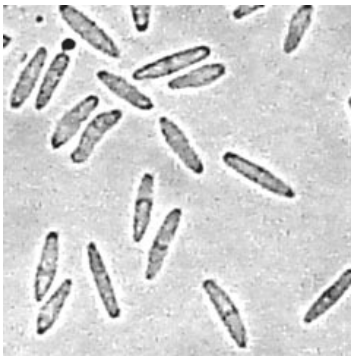

Figure 1. Conidia of *Fusicoccum putrefaciens* on PDA (400x).

According to symptoms upright dieback, blossom and ovary blight and end rot were caused by *Fusicoccum putrefaciens* Shear. The teleomorph stage caused by *Godronia cassandrae* Peck f. vaccinii Groves was not found in Latvia. The causal agent of the disease was identified based on symptoms and the morphological characteristics described by Горленко *et al*., 1996 and Caruso F. L., 1995.

Phomopsis vaccinii from upright dieback, blossom and ovary blight and viscid rot samples was detected. Uprights, blossoms and ovaries turned brown and died, but viscid rot was off-color, slightly mottled or yellowish brown and firm but wet inside with a viscous, sticky substance. Viscid rot was common in the field and during the first months of storage. Colonies on the PDA grew up to 15 mm per day; white, circular, and near to centre dark rings were produced. The aerial mycelium was not compact, was grayish white, and toward the centre a wall was produced. The pycnidia mostly set on the wall. They were 1 – 4 mm in diameter, partly embedded, leathery, pale grey and then turned black (Figure 2). From maturity pycnidia emitted a yellow creamy spore mass in the moisture camera on uprights dieback and the pure culture. *P. vaccinii* had two types of spores (Figure 3). Alfa conidia were hyaline, one-celled, ellipsoid, with two oil globules at both ends and measured 7.8 x 3.1µm (4.3 – 9.8 x 2.0 – 4.4 µm). Beta conidia were unicellular, hyaline, filiform – hook-shaped at the end, and measured 18.6 x 0.8 µm (13.4 – 22.1 x 0.3 – 1.2 µm).

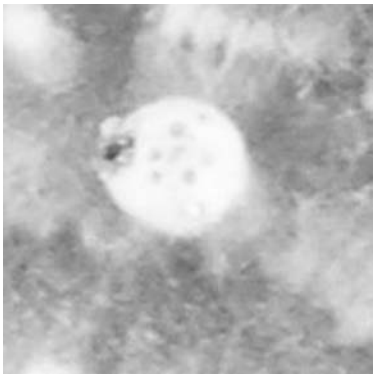

Fig. 2. Pycnidia of *P.vaccinii* on PDA (10 x).

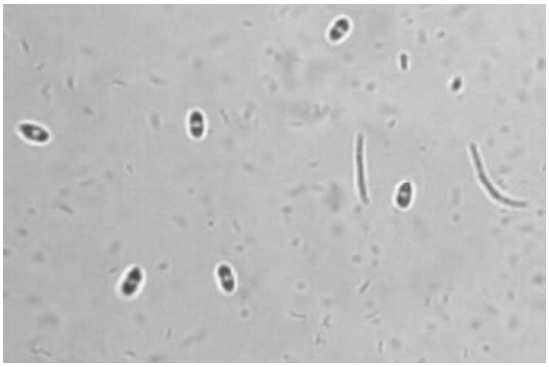

Fig. 3. α and β conidia of *P. vaccinii* on PDA (400 x).

According to the symptoms of cranberry disease and fungus anamorph morphological peculiarities in the moisture camera and pure culture, upright dieback, blossom and ovary blight and viscid rot were caused by *Phomopsis vaccinii* Shear in Shear, N. Stevens, & H. Bain. The teleomorph stage, which is caused by *Diaporthe vaccinii* Shear in Shear, N. Stevens, & H. Bain., was not detected in Latvia. The causal agent of the disease was identified based on symptoms and morphological characteristics as described by EPPO, 1997 and Kačergius *et al*., 2004.

Discosia artocreas from upright dieback, blossom blight and berry rot was detected only in few samples. Young uprights were bronze, brown with end of the top sloped and the uprights of last year were dark brown. Blossoms damaged by disease were brown, but discosia fruit rot was with yellowish brown spots. *Discosia artocreas* mostly was detected from upright dieback. The fungus

grew rapidly on PDA at 20 – 24 ^{o}C. Aerial mycelium was low, compact, leathery and pale gray. Mycelium produced paler irregular rings. Colonies were dark pale. In the moisture camera on uprights dieback and pure culture appeared pyriform, dark grey pycnidia. From maturity pycnidia emitted a yellowish white, creamy spore mass. Separately conidia were hyaline or pale grey, oblong, measured 3.2 x 14.2 µm (2-4 x 12-17 µm); 2 – 3 septates, end of tops had two long appendages (Figure 4). In the pure culture septates and appendages of conidia hardly were observed.

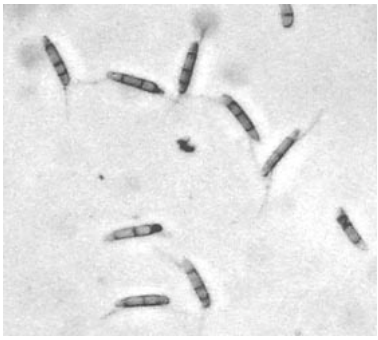

Figure 4. Conidia of *Discosia artocreas* in moisture camera and PDA (400 x).

According to the symptoms of cranberry disease and fungus morphological peculiarities in moisture camera and pure culture was caused by *Discosia artocreas* (Tode) Fr. The teleomorph stage of the fungus caused by *Gnomonia setae* was not detected. The causal agent of the disease was identified based on symptoms and morphological characteristics as described by Горленко *et al*., 1996.

Pestalotia vaccinii caused upright dieback, blossom blight and pestalotia fruit rot in Latvia. Uprights were bronze, with end of the top sloped. They were spread only in several cranberry plantations and the incidence level was not high. In storage on some berries appeared yellow-brown, circular, slightly sunken rot spots with darker, concentric rings. Fungus grew rapidly on the PDA, at 20 – 24 ^{o}C. The aerial mycelium was fluffy, at the centre lemon-white, up to margins white appeared. Colonies were lemon-yellow. In the culture through mycelium appeared black, watery, and spore mass was scroll-shaped (Figure 5). In the moisture camera on uprights dieback acervuli matured also. The conidia were elongated fusoid, straight or slightly incurved and measured 5.8 x 27.5 (4.7 - 6.8 x 22 - 32 µm). The conidia had five-cells, the apical and basal cells were hyaline, but inside three cells were green-brown (Figure 6). The conidia had appendages at both ends. The end of the basal cell had on average a 13.7 µm (9.5 – 18 µm) long appendage, but the end of the apical cell had 3 – 4 on average 23.9 µm (16 – 33 µm) long moustached appendages. In the culture appeared hyaline, ellipsoid, curved microconidia, in diameter 2.0 x 6.3 µm (1.3 - 2.7 x 4.5 – 7.8 µm).

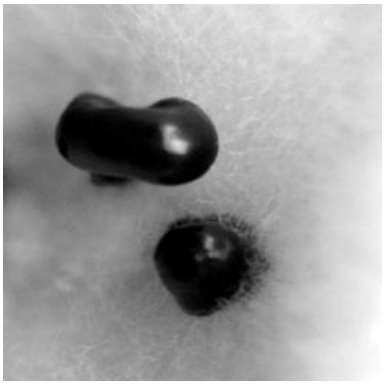

Figure 5. Spore mass of *P. vaccinii* on PDA.

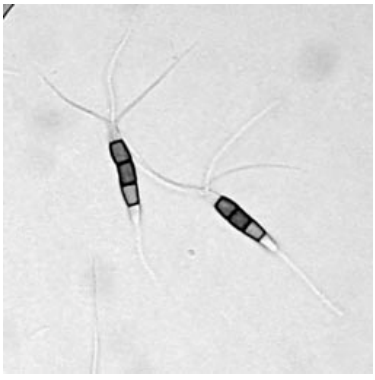

Figure 6. Conidia of *P. vaccinii* on PDA(400x).

According to the symptoms of cranberry disease and fungus morphological peculiarities in the moisture camera and the pure culture was caused by *Pestalotia vaccinii* (Shear) Guba. The causal

agent of the disease was identified based on symptoms and morphological characteristics described by Горленко *et al*., 1996.

Physalospora vaccinii from upright dieback and blotch rot was detected. Uprights from last year were dark brown or red-brown and they were collected only from some cranberry plantations. On berries pale rosy, circular, flattened or sunken spots were observed. Gradually the berries became dried and shriveled. Only after three or more months in storage blotch rot was observed. Rot damage on berries mostly appeared at the calyx, probably berries were infected by fungus during blossoming. The fungus had two different strains. On the PDA the white colony type produced poor, low, yellowish white mycelium, which was most common in Latvia. The dark colony produced poor, low, brownish grey or green-grey mycelium. In the pure culture both strains after two weeks at 20 – 24 ^{o}C abundantly produced perithecia, but ascospores matured only after 5 weeks. The perithecia of the dark strain were slightly smaller than the perithecia of white strain (Figure 7). They were globose to pyriform, dark brown, at the end of ostiole and had black spines. White stain on average was 199.2 x 42.1 µm (133–251 x 19.6 – 64.1 µm) large, hyaline, fusoid with eight spores asci produced. Ascospores of white strain were acuminated obovoid, pale yellowish brown, with a punctate surface (Figure 7) and measured 43.5 x 17.4 µm (33.8 – 53.8 x 12.3 – 24.9 µm). When ascospores were not mature they were hyaline. Ascospores of the dark strain were slightly smaller (26.5 – 33.2 x 12.3 – 18.6 µm), broadly obovoid with blunt ends. Both of strains had a lot of large paraphyses (Figure 8).

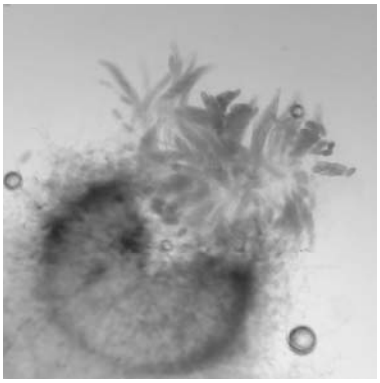

Figure 7. Asci and perithecia of *P. vaccinii* paraphyses of *P. vaccinii on* white strain on PDA(100 x).

Figure 8. Asci, ascospores and PDA (400 x).

According to symptoms of cranberry disease and fungus teleomorph morphological peculiarities in pure culture, blotch rot and upright dieback were caused by *Physalospora vaccinii* (Shear) Arx & E. Müller. *P. vaccinii* has no anamorph stage known in the world. The causal agent of the disease was identified based on symptoms and morphological characteristics as described by Caruso F. L., 1995 and Oudeman 1998.

Phyllosticta elongata caused fruit rot only in storage. At first on the berries appeared small, light-colored spots then they developed soft, watery rot. In the centre of the rot spots dark red rings appeared. Fungus on the PDA grew rapidly, produced dark, ranges in color from blue-gray to green-grey and thick colonies. The aerial mycelium was floury, pale blue-grey. In the culture after few days at 20 – 24 ^{o}C there appeared globose and black and a lot of pycnidia. From maturity pycnidia emitted a pale grey spore mass. The conidia were hyaline, single-celled, obovate to oblong and measured 13.5 x 5.6 µm (10.1 – 16.4 x 3.9 – 7.3 µm) (Figure 9). At the end the conidia had a mucilaginous, long appendage. When conidia were flown off, the pycnidia turned black.

Figure 9. Conidia of *Physalospora vaccinii* on PDA (400 x).

According to symptoms of cranberry rot and fungus morphological peculiarities in pure culture, fruit rot was caused by *Phyllosticta elongata* G. J. Weideman in G. J. Weideman, D. M. Boone, & Burdsall. The teleomorph stage *Botryosphaeria vaccinii* (Shear) Barr) in the laboratory was not detected. The causal agent of the disease was identified based on symptoms and morphological characteristics described by Caruso F. L., 1995 and Weidemann 1983.

Coleophoma empetri from ripe rot in storage was detected. Berries were off-colored, soft, watery inside, and squirted fluid when squeezed. Symptoms of rot were similarly to end rot caused by *Fusicoccum putrefaciens.* Ripe rot is common in Latvia. Fungus on the PDA produced a dark, thick, colony, but started it with a whitish color. Aerial mycelium was low, fluffy and dark grey. In the culture appeared dark grey – brown or black, globose at first, but then turned into a disc shape with fluffy walls. Pycnidia formed in a ring near the outer edge of the colony by group or scatter. The conidia were hyaline, straight, uniformly cylindrical, and slightly punctuated and measured 3.0 x 14.8 μm (2.6 – 3.4 x 12.2 – 17.08 μm).

Figure 10. Pycnidia of *Coleophoma empetri* on PDA (10x).

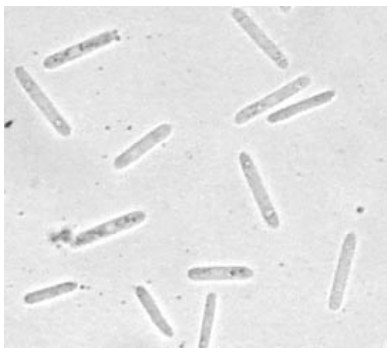

Figure 11. Conidia of *C. empetri* on PDA (400 x).

According to the symptoms of cranberry rot and fungus morphological peculiarities in pure culture, ripe rot were caused by *Coleophoma empetri* (Rostr.) Petr. Teleomorph stage was not detected yet. The causal agent of the disease was identified based on symptoms and morphological characteristics described by Caruso F. L., 1995.

Allantophomopsis cytisporea caused black rot in the field, but mostly during the first months in storage. At first damage appeared like pale brown spots, afterwards berries became uniform black or dark grey. Their mass was firm and dry, but gradually the berries became dried and shriveled. The fungus grew rapidly on potato-dextrose agar at 20 – 24 ^{o}C. The colonies were dark green-gray and produced poor, low aerial mycelium. In the moisture camera on berries and the pure culture appeared globose to pyriform, dark grey pycnidia (Figure 12). From maturity pycnidia emitted a black, little creamy spore mass. The conidia were hyaline, unicellular, allantoid to lunate, binucleate and measured 7.6 x 2.7 μm (6.6 – 8.6 x 2.2 – 3.8 μm), end of top mucoid appendages were hardly observed (Figure 13).

Figure 12. Pycnidia of *Allantophomopsis cytisporea* on PDA(10x).

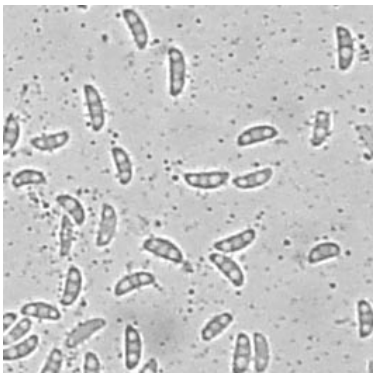

Figure 13. Conidia of *A. cytisporea* on PDA (400x).

According to symptoms of cranberry rot and fungus morphological peculiarities moisture camera and pure culture, black rot were caused by *Allantophomopsis cytisporea* (Fr.: Fr.) Petrak. The causal agent of the disease was identified based on symptoms and morphological characteristics described by Caruso F. L., 1995 and Горленко *et al*., 1996.

Discussion

Conclusions after two years (2007, 2008) investigations: in Latvia are distributed the same causal agents of cranberry diseases as in North America. According to the literature in the USA and Canada *Colletotrichum acutatum, Pestalotia vaccinii, Phyllosticta vaccinii, Physalospora vaccinii, Phomopsis vaccinii* and *Coleophoma empetri* are the most common fungi isolated from sound fruit in storage (Olatinwo *et al.* 2003; Oudemans *et al.*, 1998). The fungus *Fusicoccum putrefaciens* was recovered in less than 1 % of the total isolations, it is a minor fungal pathogen in the USA according to investigations carried out in Michigan (1999 – 2001) and New Jersey (1994 – 1996) (Olatinwo *et al.* 2003; Stiles *et al.*, 1999). Shear C. L. and Bain H. F. investigated the life cycle of fungus in cranberry plantations and established that *Fusicoccum putrefaciens* grows well at low temperatures and in some seasons causes significant losses (Stiles *et al.* 1999). End rot was mostly spread in Latvia (2007). The weather conditions are suitable for cranberry growing in Latvia. Upright dieback caused by *Phomopsis vaccinii* are widely distributed in the USA. In Latvia the incidence level of *Phomopsis vaccinii* is still low, but in the future it could be an economically important disease in Latvia as well.

Probably causal agents with seedling material have been imported to Latvia. American cranberries mostly propagate using uprights. It is the most common method in all the world. Next year after over wintering fungi can develop and infect blossoms, ovaries, uprights and berries again. In the future a system should be developed how to control spread of cranberry diseases in plantations.

Cranberry growers still do not know all specific agro technical methods of cranberry growing. Damage to cranberries can be caused also by non-parasitic diseases like drought and wrong fertilization.

Conclusion

Upright dieback of cranberry was caused by several agents. In the Latvian Plant Protection Research Centre laboratory 6 fungi from upright dieback and 9 causal agents from fruit rot were detected. Mainly the fruit rot symptoms were similar; therefore precise detection of the causal agent is so important.

In the future causal agents *Fusicoccum putrefaciens* and *Phomopsis vaccinii* could be dangerous, because they cause upright dieback, blossom blight and fruit rot and their control could be difficult in the future.

In the future research into to cranberry diseases should be continued.

Acknowledgements

This project was supported by the Ministry of Agriculture of Latvia (Contract No. 43/2007).

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STORAGE ROTS OF *VACCINIUM MACROCARPON* SPREAD AND DEVELOPMENT IN LATVIA
VACCINIUM MACROCARPON OGU PUVES IZPLATĪBA LATVIJĀ

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Abstract
American cranberries (*Vaccinium macrocarpon* Ait.) have been cultivated for ten years in Latvia and their plantations have a tendency to enlarge every year. Latvian cranberry growers realize mainly fresh berries, because it is difficult to keep quality during prolonged storage. One of the main why quality is reduced is berry rot caused by different fungi. Berries from six cranberry plantations in different regions in Latvia for rot detection were taken at harvest time.
Different berry rot appeared on 61 % of all berries in storage. In the berries *Botrytis cinerea* (yellow rot), *Allantophomopsis cytisporea* (black rot), *Fusiccocum putrefaciens* (end rot), *Phyllosticta elongata* (Botryosphaeria fruit rot), *Physalopora vaccinii* (blotch rot), *Phomopsis vaccinii* (viscid rot), *Pestalotia vaccinii* (Pestalotia fruit rot), *Coleophoma empetri* (ripe rot), *Discosia artocreas* (Discosia fruit rot) were detected. Causal agents of berry rot had different dynamics of development. These fungi had different incubation periods. This is important information for the growers that helps to determine the real time for realization, before rotting is started. After four months of storage, the amount of causal agents was different from each plantation area.
In further investigations it is necessary to establish conditions influencing the development of causal agents.

Kopsavilkums
Lielogu dzērvenes (*Vaccinium macrocarpon* Ait.) Latvijā ir zināmas jau piecpadsmit gadus un to platības turpina palielināties. Latvijas lielogu dzērveņu audzētāji saražoto produkciju realizē svaigā veidā, pārstrādā un, ja iespējams, sasaldē, jo galvenokārt puve uz ogām parādās jau līdz decembrim. Ražas laikā 2007. gadā, lai noteiktu ogu puves ierosinātājus, tika ievāktas ogas no sešām dažādām lielogu dzērveņu audzēšanas vietām Latvjā.
Uzglabāšanas laikā 61 % ogu bija puves bojātas. No puves bojātām ogām, galvenokārt, tika konstatētas: *Allantophomopsis cytisporea* (ogu melnā puve), *Fusiccocum putrefaciens* (ogu galotnes puve), *Phyllosticta elongata* (Botryosphaeria ierosinātā ogu puve), *Physalopora vaccinii* (ogu gaišā puve), *Phomopsis vaccinii* (viskozā ogu puve), *Coleophoma empetri* (gatavo ogu puve). Ogu puves ierosinātājiem glabāšanas laikā ir atšķirīga attīstības dinamika, tas nozīmē, ka sēnēm ir

dažādi inkubācijas periodi. Šī informācija būtu nozīmīga lielogu dzērveņu audzētājiem, jo tā varētu palīdzēt realizēt produkciju pirms puves parādīšanās. Nosakot puves ierosinātājus glabāšanas laikā, varēja secināt, ka dažādos Latvijas audzēšanas rajonos to izplatība ir atšķirīga.
Turpmāk būtu vēl jāveic pētījumi par ogu puves ierosinātāju izplatības veicinošiem iemesliem Latvijā.

Key words: cranberry, storage rot, dynamics

Introduction
Cranberries are a well known crop in world over 200 years, but for only 10 years in Latvia. Cranberry plantations in Latvia are up to 100 ha. Growers are interested to know how to keep fresh berries in good condition during the storage time and what are the reasons for the yield losses. Storage rot fungi are those species that reduce fruit quality during storage.
According to literature in the USA and Canada *Colletotrichum acutatum, Pestalotia vaccinii, Phyllosticta vaccinii, Physalospora vaccinii, Phomopsis vaccinii* and *Coleophoma empetri* are the most common fungi of sound fruit in storage (Olatinwo *et al.* 2003; Oudemans *et al.*, 1999; Stiles *et al.*, 1999). The fungus *Fusicoccum putrefaciens* was recovered in less than 1% of the total isolations. It is the minor fungal pathogen in the USA according to investigations carried out in Michigan (1999 – 2001) and New Jersey (1994 – 1996) (Olatinwo *et al.* 2003; Stiles *et al.*, 1999).
Investigations on the fungal diseases of American cranberries have been carried out since 2004 in Latvia, but storage rotting has not been investigated until 2007.

Materials and methods
Six cranberry plantations (Talsi, Riga, Kuldiga, Liepaja, Aluksne, and Cesis districts) were inspected during harvesting time in 2007. From each cranberry plantation in different regions in Latvia (Figure 1) 200 sound berries (in total 1200 berries) were taken at harvest for causal agent detection. The cultivar 'Stevens' was used for the observations.

Figure 1. Inspected cranberry plantations in Latvia.

Berries were held in cold camera (+5 °C, RH – 50 %) in plastic boxes until March for causal agents of storage rot detection. Berries damaged by rotting at the end of each month for four months were counted. The causal agents of berry rot were isolated only in a pure culture, using potato dextrose agar (PDA). Plates were incubated at room temperature (20 – 25 °C) for 3 to 4 weeks. Fungi were identified directly on the isolation plates by comparing the morphological characteristics of the spores and spore bearing structures with descriptions in the literature (Caruso *et al.*, 1995; Kačergius *et al.*, 2004; Горленко *et al.*, 1996). The discovered fungi were identified in the laboratory of Latvian Plant Protection Research Centre. All calculations were performed using Microsoft Excel 2003.

Results und Discussion

Berries were taken from the field at the end of October or in the beginning of November. For the first time berries were evaluated at the end of December and on average 12% of all berries were rotted. The assessment of rotted berries was continued for the next three months until the end of March. Spread of berry rot made equal progress during all the storage time (Figure 2).

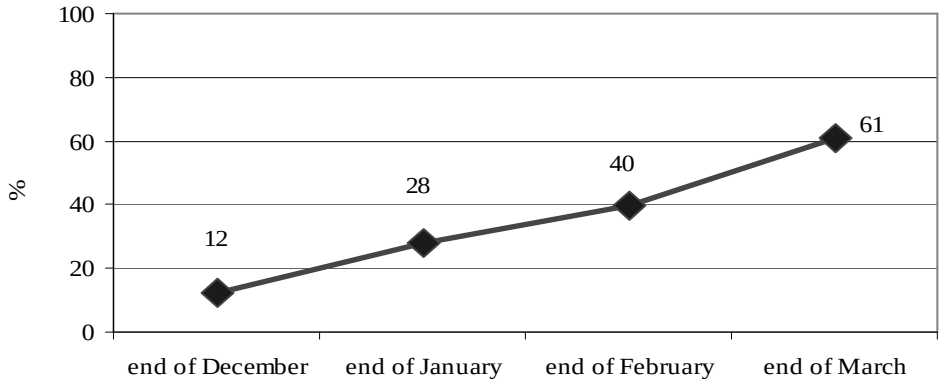

Figure 2. Dynamics of fruit rot during storage, %.

At the end of March berry rot was on average 61 % of all collected berries from the inspected cranberry plantations in Latvia. This means, berries should be very quickly realized, before they are damaged by storage rot. It is very difficult to store fresh cranberry in cold cameras without freezing.

All rotted berries at the end of each month were sorted and put on the pure culture (used PDA) for causal agent detection.

The spread of berry rot from different growing regions in Latvia where berries were collected differed. Most of all of the rotted berries – 74 % were in the Riga district, but only 52 % of the damaged berries were in Talsi district (Figure 3). Probably weather conditions and plantation age had a significant influence on the spread of the berry rot. The cranberry plantation in the Talsi district was younger and the climate was optimal in comparison with the plantation in Riga district.

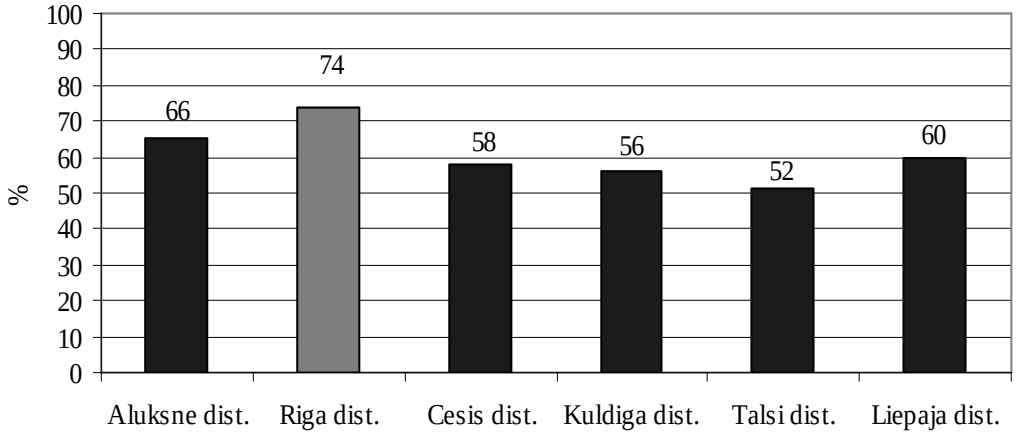

Figure 3. Berry rot spread from different cranberry growing districts during storage, %.

Climate has a strong influence on berry rot caused by different fungi. The winter 2006 was the warmest of the last 84 years in Latvia. In November, December and January the air temperature exceeded +10 – 12 °C and was 7 – 13 °C above normal. Usually such temperatures are found in September or May. February was the coldest month. In the Aluksne district the air temperature fell

to – 30°C and the snow layer was very thin, in some places it did not exist at all. March was very warm in Latvia (the warmest of the last 100 years). The air temperature was +17 – 19 °C, but at the end of month snow in the Aluksne district was observed. After such changeable weather conditions during the winter uprights were damaged very hard in the Aluksne district in comparison with other inspected cranberry plantations in Latvia. In April there was very little rainfall in the Liepaja and Riga districts. May was very hot (25 – 30 °C) and dry in the Riga district, but in other cranberry plantations was rich rainfall. In June the temperature throughout Latvia was very high up to 5 – 6 °C over the average standard and significant rain was observed in the Liepaja, Kuldiga and Talsi districts, but in the Riga and Aluksne districts first rainfall was only June 14th and 17th. Although all inspected cranberry plantations had irrigation systems, probably drought affected growth of the upright and development of the blossoms. During the cranberry flowering time in the Liepaja district it was very hot 27 – 30 °C and wet. The rainfall exceeded three times norm of the month, rainfall was in the Kuldiga and Cesis districts as well. These conditions were favorable for parasitic diseases, including flower blight and berry rot in the field and in storage. Berry formation was affected by heat in August in the Cesis and Aluksne districts, but beneficial conditions for the cranberry growth were in the Talsi and Kuldiga districts. There was rainfall in September in the Talsi, Kuldiga and Riga districts, but in the Aluksne was very dry and hot up to 6 – 7 °C over the standard norm. High rainfall amount could increase infection by fungi for berry rot development in the Riga, Kuldiga and Talsi districts. (www.meteo.lv)

From rotted berries in storage fungi *Fusiccocum putrefaciens* (end rot), *Physalopora vaccinii* (blotch rot), *Phyllosticta elongata* (botryosphaeria fruit rot), *Allantophomopsis cytisporea* (black rot), *Phomopsis vaccinii* (viscid rot), *Coleophoma empetri* (ripe rot), *Botrytis cinerea* (yellow rot), *Discosia artocreas* (discosia fruit rot), *Pestalotia vaccinii* (pestalotia fruit rot) were detected.

From inspected cranberry plantations in storage end rot caused by *Fusicoccum putrefaciens* was the most widespread (Figure 4). End rot developed very quickly during first months (December – January) in storage, and further incidence of berry rot decreased. The ripe rot (causal agent *Coleophoma empetri*) mostly developed during the first months as well, but botryosphaeria fruit rot caused by *Phyllosticta elongata* and blotch rot caused by *Physalopora vaccinii* mostly appeared in March. Causal agents of berry rot had different incubation periods. It is important for cranberry growers, if the end rot, which is economically the most important disease, was not spread during the previously year, fresh berries could be stored until January, other berry rots did not produce important material loses.

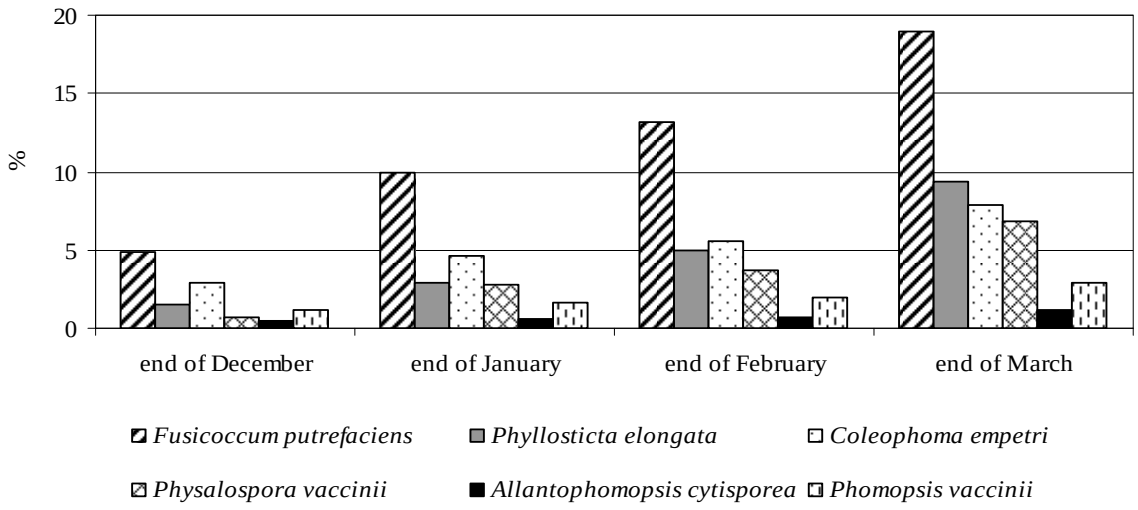

Figure 4. Causal agents of berry rot spread during storage, %.

In literature Caruso eds. and Горленко wrote, the black rot (causal agent *Allantophomopsis cytisporea*) is common in the field or during the first months in storage (Caruso *et al.*, 1995; Горленко *et al.*, 1996) but in Latvia, black rot was common till the end of March.
The incidence level of berry rot was different in each inspected cranberry plantation. End rot (*Fusicoccum putrefaciens)* was common in the Aluksne, Kuldiga and Cesis districts, but in the Riga and Liepaja districts pathogen was spread very infrequent (Fig. 5). In the cranberry plantation of the Riga district ripe rot (causal agent *Coleophoma empetri*) was widely spread and in Liepaja district botryosphaeria fruit rot (*Phyllosticta elongata*) and viscid rot (*Phomopsis vaccinii*) which is a quarantine organism were found. *Phomopsis vaccinii* was identified in Lithuania in 2002 as well (Kačergius *et al.*, 2004). Blotch rot caused by *Physalospora vaccinii* in the cranberry plantation located in the Talsi district was widely spread, but this rot in the Liepaja district was not observed. In the cranberry plantations were common 5 - 6 causal agents of berry rot, but from samples taken in the cranberry plantation in the Cesis district, developed only three rots - end rot, botryosphaeria fruit rot and blotch rot.

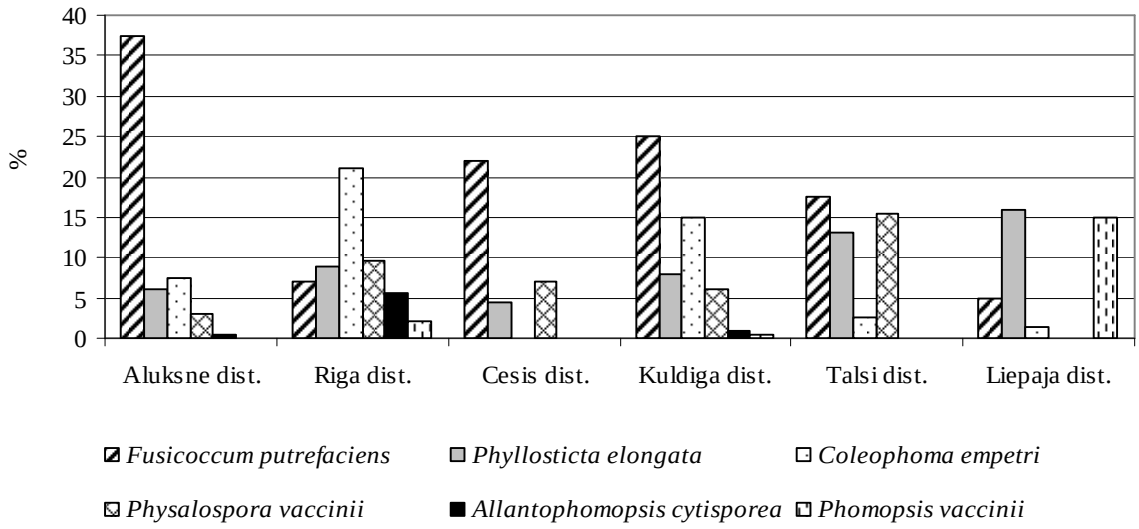

Figure 5. Causal agents of berry rot spread in different regions in Latvia during storage, %.

Conclusion

From 6 inspected cranberry plantations in Latvia six causal agents of berry rot were detected in the storage berries. The end rot caused by *Fusicoccum putrefaciens* was the most widespread.
The incidence level of berry rot in all cranberry plantations was not similar; therefore in future investigations should focus on what promotes the fungi in each cranberry plantation.

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EXPERIMENTAL AND RESEARCH WORKS ON SOME OF *VACCINIUM* TAXA IN BELARUS
DAŽU *VACCINIUM* ĢINTS SUGU EKSPERIMENTĀLIE UN IZPĒTES DARBI BALTKRIEVIJĀ

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Abstract
Carrying out of the experimental and research works on the study of plants of genus *Vaccinium* in Belarus can be divided into several stages. The first stage is the study of biological and resource potential of wild-growing berry plants: cranberry (*V. oxycoccus* L), bog blueberries (*V. uliginosum* L.), lingonberry (*V. vitis-idaea* L.), bilberries (*V. myrtillus* L.).
The second stage is the introduction of the cultivars of large cranberry (*V. macrocarpon* Ait.), of highbush blueberry (*V. corymbosum* L.), of lingonberry under the conditions of Belarus to built the first experimental and industrial plantations.
The third stage is the preparation of scientific manpower, the formation of scientific school, the development of production schedules of the estate berry culture.
The fourth stage is the dilating of the assortment of *Vaccinium* taxa used in the culture and the augmentation of the areas of plantings under berrylike cultures.

Kopsavilkums
Eksperimentālos un izpētes darbus par *Vaccinium* ģints augiem Baltkrievijā var iedalīt vairākos posmos. Pirmais posms ir bioloģiskie pētījumi, kur tiek apsekoti potenciālie savvaļā sastopamie ogaugļaugi: dzērvenes (*V. oxycoccus* L), zilenes (*V. uliginosum* L.), brūklenes (*V. vitis-idaea* L.) un mellenes (*V. myrtillus* L.).
Otrais posms ir Baltkrievijas audzēšanas apstākļiem piemērotu lielogu dzērveņu šķirņu (*V. macrocarpon* Ait.), krūmmelleņu (*V. corymbosum* L.) un brūkleņu ieviešana. Tiek ierīkoti eksperimentālie un rūpnieciskie stādījumi.
Trešais posms – tiek sagatavots zinātniskais personāls, veidojot zinātniskās skolas, izstrādājot ražošanas tehnoloģijas katrai ogaugļu sugai.
Ceturtais posms ir *Vaccinium* ģints augu sortimenta un izmantoto augu stādījumu paplašināšana.

Key words: berry plants, natural resources, Belarus, *Vaccinium*

Introduction
There are 5 kinds from berry plants of *Vaccinium* L. genus, such as *V. oxycoccus* L., *V. microcarpon* Ait., *V. uliginosum* L., *V. myrtillus* L., *V. vitis idaea* L. in natural flora of Belarus. All of them are rich with biologically active substances, possessing by capillary tonic action, by anti-inflammatory, bactericidal, hematopoietic and antitumoral kinds of action. Berries of a cranberry, a bilberry, a blueberry, a cowberry differ also by high level of anti-oxidative activity. Berries promote the deducing of radioactive nuclides from an organism and in certain degree make positive impact on strengthening of immune system of the person.
Requirements of a national economy for wood berry plants are only partially satisfied at the expense of operation of natural resources which feel now the powerful man-caused and anthropogenic influence. There is a necessity for working out of actions for protection, rational use and intensive reproduction of wood berry plants.
The first stage of rational use of plants of *Vaccinium* L. genus in Belarus is the inventory of wild-growing bush and it is basically solved. So, according to researches of the Belarus research institute of forestry with the use of forest regulation materials, it is established that the areas occupied under a cranberry in republic are made by 84514 hectares, and average long-term productivity is 260 kg per hectare (Sautin, etc., 1975). On the data of Ministries of statistics and the analysis of the Republic of Belarus, the possible purchases of a cranberry at average productivity make 8.7 thousand tons taking into account losses. V.I. Parfyonov etc. (1996) cite a little different data that

the total area under a cranberry makes 552.2 thousand hectares, bacciferous is 364.5 thousand hectares; the general biological stock of berry plants is estimated in 198.9 thousand tons, at average productivity of 364.5 kg per hectare.

Researches on studying of distribution of a bog bilberry bush and the account of their resources have been conducted also. So, according to V.E. Volchkov etc. (1982), in Belarus blueberry plantations occupy the space of 11.8 thousand hectares with a biological stock of berries of 9.7 thousand tons. V.I. Sautin (1980) notices that blueberry resources on all territory of Republic of Belarus make approximately about 10 thousand tons, productivity is about 20 – 235 kg per hectare, depending on ecotope. Possible purchases of a blueberry in woods of Belarus at average productivity of 120 kg per hectare can make about 1.7 thousand tons taking into account losses (Karas, 1983).

Despite on considerable resources of wild-growing berry plants of *Vacciniaceae* family, volumes of industrial purchases of berries tend to decrease. They do not satisfy of growing requirements of the population for them, as well as they do not satisfy of valuable raw materials in pharmaceutical and the food-processing industry. So, mid-annual preparations of a cranberry in 1961-1965 made about 9 thousand tons, in 1966 – 1970 – 5.8 thousand tons, in 1971 – 1975 – 2.3 thousand tons, and in 1980 – 1985 – did not exceed 1.5 thousand tons (Marsh cranberry, 1987). Last years, owing to the radionuclide pollution of the large territories caused by failure on the Chernobyl atomic power station, volumes of berries purchases have considerably decreased, especially in southern and central areas of the Republic, and now make no more than 1 thousand tons (Environment, 2000).

Industrial purchases of a blueberry last 25 years are not spent at all. It is connected with many reasons, mainly, with reasons of anthropogenic character. The general tendency to decrease in resources of natural berry-field is revealed as because of drying land improvement and of working out of peatbogs, and as a result of forest fires and deforestation (including clearings of care by wood) in the period of vegetation of berry-fields. Besides, the biological efficiency of wild-growing berry plants rather low and considerably fluctuates on years that also causes decrease in volumes of their purchases (Evtuhova, 1991).

For today the maximum resources of fruit-berry plants are located on territory of Minsk (28 %) and Gomel (26 %) regions. The greatest stocks as a whole on the Republic are marked a bilberry and a cranberry (about 33.0 thousand tons or 66 % and 11.2 thousand tons or 22.5 %, accordingly). By the least stocks are marked a mountain ash (1.1 thousand tons, or 2.2 %) and a blueberry (1.3 thousand tons, or 2.6 % from a biological stock of all principal kinds).

On purchases volumes on the first place there are a bilberry (up to 5 thousand tons annually) and a cranberry (about 1 thousand tons). The cowberry last years is prepared by the population basically for own needs; blueberry preparations are rather insignificant.

A principal cause of low level of use of natural stocks of berries is imperfection of technologies and systems of the organization of their purchases. Purchases of wild-growing berries are carried out till now by their purchase at local population that always bears in itself the elements of chance. To provide the greatest efficiency of the organized gathering of berries, the service of the account of resources and the forecast of terms as well as a crop of berry production is necessary.

Thus, all-round studying of biology of wild-growing berry-fields, their distribution, efficiency and stocks has allowed to develop scientific bases of rational use, protection, reproduction, introduction, breeding, technologies of cultivation, storage and production processing. Became obvious that stocks of berry-fields should increase not only at the expense of protection, rational and careful use of natural thickets, but also by the organization of industrial culture of berry plants, including a cranberry and a blueberry.

Rational use of berry plants can be conducted in three directions: 1 – gathering of berries in the most productive grounds; 2 – the same, but with application of a complex agrotechnical and forestry actions; 3 – introduction of useful berry plants in culture by crops and landing, both in a natural area, and in new areas for the species.

The elementary way of increase of productivity of wild-growing berry-fields is regular care of natural bush. In the Republic were created the semi-cultures of a cranberry and works on its restoration in a natural cover of bogs were conducted.

Actions for creation of semi-cultures are developed for increase of biological efficiency of natural bush of a blueberry with annual affordability of 22 rubbles per hectare (in the prices of the end of

1980th) (Grimashevich, 1986). The average increase of productivity thus makes 180 kg per hectare. In necessity of carrying out of actions for increase of efficiency of natural bush specified by E.I. Proskurjakov (1937) in due time. However the creation of semi-cultures does not answer in the full measure to problems of a forestry intensification. It is because difficult to mechanize processes of cultivation of a cranberry and a blueberry (application of fertilizers, harvesting, rejuvenescence of bushes etc.) under natural conditions therefore the expected effect is not always reached.

The beginning of introductional works in the Republic is connected with occurrence in the seventies of XX century of the generalizing data about achievements of foreign countries. Since the same time in the scientific institutions of Belarus it was studied the possibility of marsh cranberry cultivation (*Vaccinium macrocarpon* Ait). Long-term ecological and biological researches had been proved advantage of cultivation in culture of the North American alien cultivars in comparison with a European cranberry.

The first research-and-production plantation on the area of 10 hectares has been put in 1980-1983 of "Glavpolesyevodstroy" of MWE of the USSR in Gantsevichi district of the Brest region (nowadays it is the experimental base of CBG of NAS of Belarus). Small skilled plantations of a marsh cranberry (of 2-3 hectares) are created at the same years in skilled timber enterprises of Institute of wood of NAS of Belarus. Successful cultivation of a marsh cranberry on skilled plantations was the concrete proof of high prospects of industrial culture of this species.

On the basis of theoretical and practical elaborations in 1986 by the Council of Ministers of Belarus it was accepted Decision "About the organization in the Belarus Soviet Socialist Republic of marsh cranberry manufacture" therefore the first industrial plantation of a North American cranberry has been created in Pinsk district of the Brest region on the area about 60 hectares by concern "Polesyevodstroy" in 1985 – 1990. By this time the first domestic technologies of industrial cultivation of marsh cranberry is accepted for reception of a planting stocks and on berry production. In effect, each of technologies represents the complex of actions directed on reception of the maximum productivity of plants. The basic operations on cultivation of a cranberry with maintenance of conditions of probably full use of fertility of soils, their optimization of water-air and nutritious modes, with application of reliable measures of protection from extreme biotic and abiotic factors, all of these operations are working out.

The successful decision of many put questions has created a necessary basis for adjustment of wide industrial production of a marsh cranberry. By this time in the Republic functions about 70 hectares of skilled and skilled-industrial plantations where 11 perspective varieties of the American breeding are tested. Average productivity of berries has made 12-15 tons per hectare over the last 5 years depending on a variety and a growth place.

The considerable attention in the works devoted to a marsh cranberry has been given for research of biochemical structure of its berries in connection with the optimization of a mode of a mineral food, of definition of optimum terms of berries harvesting for a putting on storage and for an establishment of optimum modes of the storage.

In 1980 there were begun tests of 11 varieties of high-bush blueberry (*Vaccinium corymbosum* L.) of American breeding, received of the Main Botanical Garden of Academy of sciences of the USSR (nowadays MBG of the Russian Academy of Sciences). The culture of this alien crop has proved to be as perspective under the conditions of Belarus, and collection replenishment by new varieties of early term of maturing has allowed to advance it in more northern areas of Belarus. Economic advisability of cultivation *Vaccinium corymbosum* L. as crop made active the works on creation of plantations in economy with a various pattern of ownership. Today more than 50 hectares of farmlands in the Republic of Belarus have been taken away under skilled landings of this North American alien crop.

The bog bilberry, unlike of high-bush blueberry, neither in industrial crop abroad, nor in all territory of the CIS countries is not cultivated. At the same time for areas with the short vegetative period, with insufficient quantity of heat in the summer and with the severe winter it is enough perspective species for introduction. Besides, this species is characterized by wide ecological amplitude (it grows on sphagnous bogs, in boggy woods, on sandy and stony soils), by high winter hardiness, by stability to fungus pathogens and by the expressed polymorphism (habitus of plants, the form of berries and other features).

Skilled works on development of the bog bilberry culture are realized from the end of 70th years at Institute of wood of NAS of Belarus. By this time the working out of technology of plantation cultivation of a bog bilberry on peat-bog soils has been already almost complete. The results received under skilled conditions testify to high profitability of the cultivation of the species mentioned above. Without considerable expenses on care of plants it is possible to receive annually about 5 and more tons of berries from 1 hectare of plantations (Evtuhova, 1991). However now there are only skilled landings of a bog bilberry, created by Institute of wood of NAS of Belarus in the Gomel and Vitebsk regions (it is near 1.5 hectares).

The multi-plan researches on a cowberry are conducted in the Republic. In various agro-climatic zones of Belarus on the basis of studying of a shaped variety it is created the gene pool representing the necessary base for practical breeding. Approbation as the crop (cultivation) is spent basically for the purpose of revealing of the most valuable forms and development of the general strategy of agro-technical actions. On a plot of sorts trial there are tested such Swedish cultivars as 'Sussi', 'Sanna'; such German cultivars as 'Ammerland', 'Erntedank', 'Erntecrone', 'Erntesegen'; such Polish cultivar as 'Masovia'; such Dutch cultivars as 'Koralle', 'Red Pearl'; and Russian cultivar 'Rubin'.

Positive results of introduction and prospects of intensive development in our republic of culture of wood berry plants put forward the variety of problems on research of ways and of control facilities by its efficiency. A special urgency thus find researches of physiological features of development of plants, and knowledge of character of their changes at a various combination of natural and anthropogenic influences. It has allowed during the period of 1985 throw 2000 to prepare highly skilled experts in area of natural resource, of introduction and rational use of berry plants of *Vaccinium* L. species. The 9 dissertations for a candidate of science's degree of biological and agricultural sciences were prepared and protected this time; 1 thesis for a Doctor's degree. The next 7 years there were the 2 more dissertations for a Doctor's degree of biological sciences and 1 dissertation for a candidate of science's degree. It allows to tell us about the formation of the Belarus school in area of the unconventional berry plants cultivation which work results are widely used so in the country and as far behind abroad.

Last years the collection fund of Central botanical garden of NAS of Belarus has replenished with new cultivars and forms of marsh cranberry, of European cranberry, of high-bush blueberry, of low-bush blueberry and of their inter-specific hybrids. It also has replenished with new varieties and forms of a cowberries of American, Canadian and Russian breeding. It gives additional possibilities for expansion of assortment of the cultivars and forms offered for division into districts and for breeding, on the basis of revealing of the most perspective of them by results of a complex estimation not only of crop production and of bio-productive parameters, but also of nutritious and vitamin value of berries.

At the same time, it is necessary to use the more rationally of earlier received own results, of the advanced foreign experience and of qualification of the prepared experts for more active works on creation of industrial plantations of non-traditional berry plants of *Vaccinium* L. species under soil-environmental conditions of Belarus. At the introduction of skilled plants as crops it is also important that they can be grown up on invaluable in the agricultural relation areas such as the developed peat deposits, the boggy earths, sandy uncultivated plots and other kinds of uncomfortable areas. Thus, wide cultivation of berry plants on the developed peat-bogs can not only help with the decision of a problem of providing of the population by berries, but also promote rational use of the "waste" areas in such plough-land deficiency region as Belarus.

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PLANT MORPHOLOGY AND RAPD MARKER CHARACTERIZATIONS OF *VACCINIUM OXYCOCCUS* LITHUANIAN POPULATIONS
VACCINIUM OXYCOCCUS LIETUVAS AUGU POPULĀCIJU MORFOLOĢIJA UN RAKSTUROJUMS AR RAPD MARĶIERIEM

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Abstract
Plant morphogenesis is a useful tool for physiological, biochemical, and molecular studies. The diversity in plant form is produced mainly because different parts of the plant grow at different rates. Furthermore, the growth of an individual structure is different in various dimensions. Such differential growth rates are very well determined by genetic factors.
Understanding the relationships among wild cranberry morphologic and genetic characteristics may provide insights for the better utilization of germplasm. The objectives of this research were to determine the relationships between the genotype and the development of morphologic features. Eleven morphologic characteristics (vegetative stage, flowering time, ripening start and ending, mass, volume and size of berry, berry form, leaf shape, steepness of upright) of 56 morphologically diverse genotypes from the 4 Lithuanian bogs were analyzed using random amplified polymorphic DNA (RAPD) classifications. To compare wild cranberry (*Vaccinium oxycoccus*) morphologic characteristic 213 RAPD bands were used. Comparing wild cranberry genetic and morphologic features there were found some significant variations.

Kopsavilkums
Augu morfoģenēzes analīze ir noderīga metode fizioloģijas, bioķīmijas un molekulārajās studijās. Augu formu dažādība rodas galvenokārt tāpēc, ka dažādas auga daļas aug dažādos ātrumos. Turklāt, atsevišķas struktūras augšana var notikt vairākās dimensijās. Šīs augšanas atšķiribas tiek galvenokārt noteiktas ģenētiski.
Sakarības izzināšana starp savvaļas dzērveņu morfoloģisko un ģenētisko raksturojumu var ļaut labāk izmantot dzērveņu genofondu. Sī pētījuma mērķis bija noteikt sakarības starp genotipu un morfoloģisko pazīmju attīstību. Kopumā tika analizētas 56 morfoloģiski atšķirīgi genotipi no četriem Lietuvas purviem. Izmantojot klasifikāciju pēc RAPD marķieriem, tika pētītas vienpadsmit morfoloģiskās pazīmes (veģetatīvās fazes ilgums, ziedēšanas laiks, nogatavošanās sākums un beigas, ogas masa, tilpums un lielums, ogu un lapu forma, dzinumu stāvums). Savvaļas dzērveņu (*Vaccinium oxycoccus*) morfoloģisko atšķirību raksturošanai tika izmanotas 213 RAPD līnijas.

Tika atrastas dažas būtiskas atsķirības starp savvaļas dzērveņu ģenētisko un morfoloģisko raksturojumu.

Key words: genetic and morphological correlation; morphological characteristics; RAPD characterization

Introduction.

The berries of wild cranberry *(Vaccinium oxycoccos* L.) are very valuable because they possess a large content of biologically active substances (flavonoids, acids, vitamins), that may help protect against heart disease, the ability of blood vessels to relax - in subjects with high blood cholesterol and atherosclerosis (Vorsa *et al*., 2002; Wang *et al*., 1996). Antioxidants found in this berries help to neutralize harmful free radicals that are thought to be linked to most chronic diseases including cancer, diabetes and heart disease (Budriuniene, 1998; Vedenskaja and Vorsa, 2004).

Multiple characteristics provide good support for the recognition of species and the determination of relationships among taxa. The strength of support for a lineage is based on the assumption that multiple characteristics have been acquired independently of each other. However, many suites of characteristics, such as the complex flowers of *Asclepiadaceae* and *Orchidaceae*, may be functionally integrated with each other and are probably not acquired independently. Others, such as the overall size of different organs, may have a common genetic and developmental basis in addition to functional integration (McLellan, 2005).

Genetic basis play important role in evolution of plant morphology (Davis, 2001; Conner, 2002; Ungerer *et al.,* 2002). Two possible mechanisms for these correlations are the association of more than one phenotypic characteristic with a single genotype, and linkage, when independent genes that determine different traits are inherited together because they are located near each other in the genome (McLellan, 2005).

The purpose of this paper is to examine correlations between eighteen different morphological traits among four populations of the *V. oxycoccus*. Also to compare the morphological and RAPD marker classifications of 56 morphologically diverse genotypes, and to determine the relationship of their RAPD and morphologic features.

Material and methods.

Morphologic features analysis. Fifty six cranberry cuttings with clearly differing vegetative indications (color, size, shape of berry and productivity) (Daubaras et all., 2004) were collected from 4 populations in Lithuania bogs (Čepkeliai (54^{o}00’-54^{o}03’N, 24o25’-24^{o}35’E), Žuvintas (54^{o}23’- 54^{o}30’N, 23^{o}25’-23^{o}40’E), Kamanos (56^{o}15’-56^{o}20’N, 21^{o}35’-22^{o}45’E) and Aukštatija(55 ^{o}40’ - 55^{o}00’N, 25 ^{o}80’ – 26 ^{o}25’)) during 1995–1999. The cuttings were arranged in the Kaunas Botanical Garden collection into special peat (pH 4.0-5.0) beds. These clones were selected for further evaluation *ex situ*. Eleven morphological properties of shoots, leaves and berries for the morphological characterization of the clones were used along the descriptor list for cranberries (Budriūnienė, 1998). Depending on the size berries were classified in to 5 groups: very small (<0.3 g); small (0.3 - 0.5 g); medium (0.6-1.0g); large (1.1-1.5 g); very large (>1.5 g). Flowering shoots were measured at the end of growth. The main phenological phases of the investigated clones were recorded also. During four years (2001 - 2004) clones were observed and evaluated according to the phenological parameters (stages) as: vegetative stage, beginning of blossoming, beginning of berry ripening, and end of berry ripening. The length of generative shoots, volume of the berries, leaf length, leaf width, and berry weight was also measured. These morphological data were used for statistical analysis.

DNA analysis. DNA extraction and RAPD analysis was done as it is described previously (Areškevičiūtė et all, 2006). Nine (OPA-1, OPA-4, OPA-5, OPA-9, OPA-10, OPB-11, ROTH-6, ROTH-8, ROTH-9) 10 base pair length primers of random sequence (Fermentas, Lietuva; Roth, Germany) were used. DNA amplification was performed in a thermocycler (Mastercycler, Eppendorf, Germany) under the following conditions: initial denaturation for 4 min at 94 °C, 44 cycles of denaturation for 1 min at 94 °C, primers annealing for 1 min at 35 °C, extension for 2 min at 72 °C followed by a final extension for 5 min at 72 °C.

Calculation of the observed number of alleles, Nei's (Peer et all., 1994; Nei, 1973) gene diversity (H), Shannon's Information Index (I), total gene diversity (Ht), gene diversity within populations (Hs), gene diversity among populations (Gst = (Ht–Hs) / Ht), gene flow (Nm = 0.5 (1–Gst) / Gst) and generation of a Nei's genetic distance based dendrogram were carried out with POPGENE V 1.31 software (Yeh and Yang 1999). Principal coordinates analysis were performed with the GenAlEx 6 program (Peakala and Smouse 2006).
Statistical Analyses. The test for the significance of each 213 RAPD product band was done using the Pearson Chi-Square ($p \geq 0.05$) (Kish, 1987).To find out relatedness between RAPD fragments and phenological stages we searched for correlations. Stepwise multiple correlations (two-tailed significance level 0.05) were used to determine which combinations of genetic variables were associated with morphologic traits were carried out with SPSS 13.0 for Widows software.

Results and Discussion
In our study, RAPD markers proved to be a powerful method for the detection of spatial genetic variation. Based on the literature (Nei and Li, 1979).we have chosen nine 6 OPA and 3 ROTH primers. With nine primers, we obtained 213 fragments and could differentiate the 56 *V. oxycoccus* clones, reflecting a rich allelic diversity among the populations.
The size of the amplified fragments ranged from 80 to 2750 bp, and all loci were polymorphic. The number of bands per primer ranged from 13 (RAPD OPB-11) to 30 (RAPD ROTH-180-09). The polymorphism level for populations was as follows: Čepkeliai 52.11%, Žuvintas 37.09 %, Aukštaitija 43.19% and in Kamanos population 36.62 %.
To estimate genetic variation between populations, the values of Shannon's Information Index (I), Nei'sgene diversity (H) and the observed number of allelesper locus (Na), the number and percentage of polymorphic loci were calculated (Table 1). For the total sample, Shannon's Information Index was 0.2 and Nei's gene diversity 0.10. The observed number of alleles per locus ranged from 0.1 in Čepkeliai population to 1.4 in Kamanos and Žuvintas population. The estimated total proportion of diversity among populations (G_{ST}) and gene flow (Nm) were 0.15 and 2.9 respectively.
Shannon's index estimates of intraspecific genetic diversity within *V. oxycoccus* were higher than in other plant species (Jogaitė et all, 2006). Shannon's information index has general applications in ecology and is relatively insensitive to the skewing effects caused by the inability to detect heterozygous loci (Dawson, 1995).

Table 1. Values of genetic diversity indicated in Lithuanian populations of *V. oxycoccus.*

Population	Shanon's Information Index (I)	Nei's gene diversity (H)	Observed mean number of alleles (Na)	The number of polymorphic loci	The percentage of polymorphic loci %(P)
Čepkeliai	0.225	0.142	1.5211	111	52.11
Žuvintas	0.120	0.071	1.3709	79	37.09
Kamanos	0.086	0.045	1.3662	78	36.62
Aukštaitija	0.189	0.120	1.4319	92	43.19
Total	0.195	0.102	1.9953	213	99.53

Owing to this life history trait on genetic diversity, a low genetic diversity within but a high diversity among populations is expected (Kreher, 2000). Plants with highest genetic diversity within and among populations can better adapt to different environmental conditions.
Analyzing molecular variance of *Vaccinium* and other plant species were observed that biggest part of molecular variance were within populations (Stewart and Excoffier, 1996; Jordano and Godoy 2000; Jürgens et all., 2007). Average molecular variance within *Vaccinium* species populations were 87.7% and within populations were 27.7 %. Highest molecular variance was detected within american cranberry (*V. macrocarpon*) populations (more than 91 %) (Stewart and Excoffier, 1996). In *V. ulingosum* – 90.3 %(Kreher et all., 2000) in *V. myrtillus* – 86.19 %(Albert et all., 2004, Garkava-Gustavson et all., 2005).Thus, our study revealed a comparably low DNA polymorphism level in *Vaccinium oxycoccus* populations (Table 1). RAPDs indicate that Lithuanian *V. oxycoccus*

appears to maintain a quite low level of the genotypic variance among populations (25 %). and within (75 %) Lithuanian *V. oxycoccus* populations was found compared to American *V. macrocarpon* and the other *Vaccinium* species.

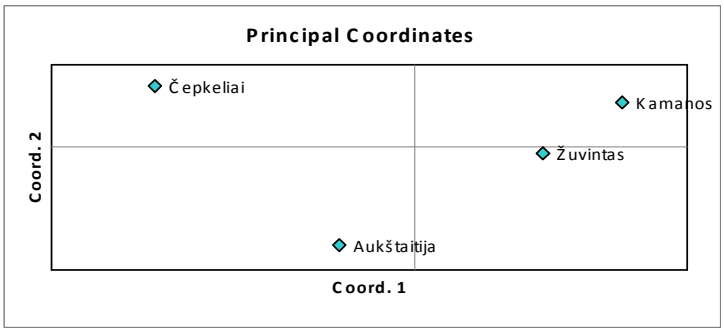

Figure 1. Distribution of *V. oxycoccus* populations in principal coordinates analysis.

To estimate the relationship between *V. oxycoccus* populations, Nei's genetic distance between pairs of populations was calculated. To generate graphs principal coordinate analysis (PCA) was used. Graphs show genetic distances among samples and populations. The PCA analyses have revealed that Čepkeliai populations of *V.oxycoccus* had homogenous genotype and all samples are in one lineage. Kamanos, Žuvintas and Aukštaitija populations are found in one site and have mixed genotypes. (Figure 2). The resulting different and mixed lineages can confirm the prediction that these three Lithuanian *V. oxycoccus* populations were derived from one population before glaciation and the Čepkeliai population differed from them. This prediction also confirms and PCA analysis (Figure 1). Postglacial decolonization one more factor that could have influenced these differences is because the populations Čepkeliai, Žuvintas, Kamanos and Aukštatija (Webb and Bartelein, 1992). According to Lithuanian deglaciation periods these four populations genetically separated into different lineages this can confirm glaciation stages in Lithuania (Figure 1). The second factor explaining our results is wide river Nemunas separating Čepkeliai from other evaluated bogs.

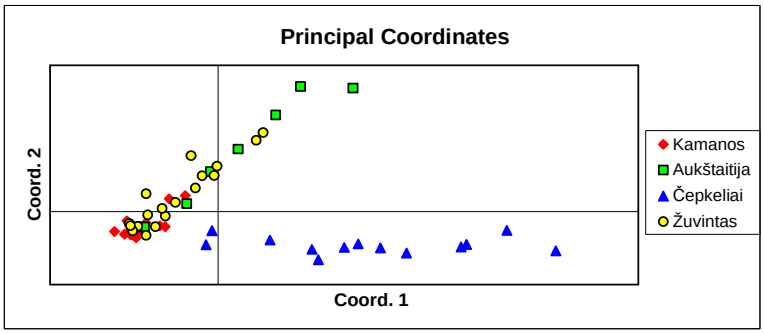

Figure 2. Distribution of *V. oxycoccus* populations by clones in principal coordinates analysis. Different population clones are marked by different marker.

Using statistic analysis 119 out of 213 RAPD band products were identified as significantly contributing to the genetic and morphologic classification (OPA-1,125, 275, 400, 450, 500, 550, 625, 650, 700, 750, 850, 1131, 1200, 12800, 1300, 1750 bp. OPA-4 225, 550, 600, 810, 1040, 1150, 1550 bp. OPA-5 280, 350, 375, 400, 440, 500, 550, 590, 600, 700, 750, 800, 900, 1031, 1040, 1230, 2000 bp. OPA-9 320, 350, 390, 400, 465, 550, 630, 650, 680, 710, 750, 850, 1234, 1500, 2900 bp. OPA-10 290, 325, 375, 400, 450, 500, 550, 690, 700, 750, 800, 850, 900, 690, 1031, 1150, 1200, 1234, 1550, 2000 bp. OPB-11 300, 400, 650, 700, 750, 850, 1031, 1131, 1200, 1700, 1900 bp. ROTH-6 175, 420, 460, 500, 520, 600, 700, 820, 1031, 1150 bp. ROTH-8 440, 600, 950 bp. ROTH-9 110, 190, 200, 250, 300, 310, 320, 350, 420, 440, 450, 500, 580, 650, 680, 700, 850, 900, 1031, 1350, 1750, 2400 bp.). Some significant molecular fragments and morphologic data correlations were also found (Table 2).

Table 2. Calculated significant fragments and phenological parameters correlations per primer in Lithuanian populations of *V. oxycoccus.*

Morphologic characteristics	roth 9	opa 1	opa 4	opa 5	opa 9	opa 10	opb 11	roth 6	roth 8	total
Vegetative stage	4*	5	1	4	3	3	6	3	1	31
beginning of blossom	3	4	-**	1	2	3	5	2	1	21
beginning of berries ripening	1	1	-	-	1	2	-	-	-	6
end of berries ripening	1	-	1	1	1	-	1	-	1	6
length of generative uprights	2	2	-	-	1	-	1	-	-	6
volume of berry	3	-	-	1	-	-	1	-	-	5
leaf length	2	1	1	2	1	1	1	1	1	11
leaf width	4	3	1	3	-	3	3	-	-	17
berry mass	5	-	1	2	1	4	-	1	-	14

* Numbers inside table shows how many correlations were find with significant fragments and morphologic feature per primer.

** Trace shows that there were no correlation.

According to correlations between phenological parameters and significant fragments per primer, we can see that biggest correlations were with vegetative stage and beginning of flowering. Low correlations were with the beginning of berry ripening, the end of berry ripening, length of generative shoots, and the capacity of the berries.

Conclusions

According to correlations between morphologic features and primers fragments we can see that the biggest correlations were with vegetative stage and the beginning of flowering. Low correlations were between the beginning of berry ripening, the end of berry ripening, length of generative shoots, and the volume of the berries.

The resulting different and mixed lineages can confirm the prediction that these three Lithuanian *V. oxycoccus* (Žuvintas, Kamanos and Aukštaitija) populations belonged to one population before glaciations and the Čepkeliai population differed from them. This could be because of the influence of the river Nemunas and of postglacial decolonization

Acknoweledgments

This work was partially supported by the Lithuanian Ministry of Education and Science programme and the Lithuanian state science and studies foundation.

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Agronomijas Vēstis. – Nr. 12.
Latvian Journal of Agronomy No. 12.
Jelgava, 2009
Latvijas Lauksaimniecības universitāte
Latvijas Lauksaimniecības un meža zinātņu akadēmija
Parakstīts iespiešanai: 2009. gada 27. novembris
Tirāža 150 eks.

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