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Special Issue

Celebrating Polli 100

Dear authors, colleagues and all the friends of horticulture,

The current Special Issue of Agronomy Research is dedicated to the anniversary of 100 years of horticultural education in Polli as a location. Therefore it is also appropriate to share with you some facts from the history of horticulture in Polli during the past century.

The history started with Polli School of Agriculture (1920–1944) and the special class of horticulture (1929), which was established next to the Polli manor (1694–1920) administered by the von Stryk family. The affiliations changed many times during 1935–1992 due to many turns in course of time. Later, Polli was as a state holding under the Ministry of Agriculture, named as the Polli Experimental Station (1992–1995). The main building of the institution was built in 1974, and the construction of the fruit (apple) storage facility ended in 1982. Now coming more close to the present day, during 1995–2005, we held an affiliation of Polli Institute of Horticulture and since then we have been the sub-unit of the former Estonian University of Agriculture. Currently, since the year 2005, we carry a name of Polli Horticultural Research Centre and belong to the Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences. The renovation of fruit storage and primary processing facilities ended in 2009, and our main building had new outlook, modern laboratories and high technological capacity in 2014 with the establishment of Competence Centre for Knowledge-Based Health Goods and Natural Products (PlantValor).

Since 1945, the research and activities have been focused mainly on preserving genetic resources of locally grown fruit and berry cultivars, but breeding the new ones as well. There are 255 Estonian fruit and berry cultivars, from which 104 are bred in Polli. The collections of genetic resources of fruits and berries include over 1,100 accessions on more than 60 ha of experimental orchards. In addition, research and practical knowledge on cultivation technologies suitable for the local producers are provided continuously. The most recently established competence centre PlantValor acts mainly as the base of research and development of health goods and natural products using modern, high-technology methods, including extraction of bioactive ingredients of plant origin that can be used in functional foods, eco-cosmetics, household chemicals, pharmaceuticals etc.

Furthermore, the international cooperation and collaboration has been always an important factor in the development of horticultural research and activities in Polli and in Estonia generally. The most intense connections have been with neighbouring countries, Latvia, Lithuania, Finland, Russia, but countries with high level horticultural production and advanced research such as Poland, Germany, Belarus and many others as well. This book of scientific articles represents also the continuous international collaboration related to fruit breeding, cultivar trials and research in the field of horticulture.

We express our sincere gratitude to all the people involved in the compilation of this Special Issue of Polli 100. Hopefully we will meet you all at the thematic conference of ‘Sustainable fruit and berry cultivation’ that will be held in September 2021 in order to celebrate the anniversary of 100 years of horticultural education in Polli.

Sincerely,
Reelika Rätsep
Researcher
Polli Horticultural Research Centre

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Preliminary results of rootstock evaluation for Estonian sweet cherry cultivar 'Anu'

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Abstract. Mahaleb cherry *(Prunus mahaleb* L.) is the most widely used rootstock for sweet cherries in Latvia, however it has several disadvantages - strong vigour of grafted trees and an intolerance to heavy, waterlogged soils. The aim of the study was to test the suitability of rootstocks of different origins for winter-hard sweet cherry cultivar in Latvian climate. The trial was established in the spring of 2014 at the Institute of Horticulture (LatHort) to test four clonal rootstocks: 'PiKu 1', PHL-A', 'GiSelA 5', 'VSL-1', and generative rootstock *P. mahaleb* (control) grafted with cultivar 'Anu' (Estonian breeding). Cherries were planted at 5×3.5 m in a random block design in three replications with three trees per plot. Tree height, annual growth of shoots, the viability of trees after wintering period and the intensity of flowering and production were evaluated in 2016–2019. Sweet cherry cultivar 'Anu' had the best overall winter-hardiness in the combinations with rootstocks 'GiSelA 5' and *P. mahaleb*. The highest intensity of flowering and production were observed in trees grafted on 'GiSelA 5'. Trees on 'VSL-1' had the highest decease rate and the lowest winter hardiness.

Key words: *P. avium, P. mahaleb*, 'GiSelA 5', winter-hardiness, flowering.

INTRODUCTION

Sweet cherries are popular summer fruits in Europe (Hajagos et al., 2012), and their production has stable or increasing trend in most of cherry growing countries (Bujdosó & Hrotkó, 2017). The suitability of cultivar-rootstock combinations to local climate, soil and growing technologies is one of key factors in sweet cherry production (Pal et al., 2017). In Latvia, the total area of sweet cherry plantations is gradually increasing mostly using generatively propagated mahaleb cherry (*Prunus mahaleb* L.) as winter-hard rootstock. However, it has several disadvantages such as strong vigour of grafted trees and an intolerance to heavy, waterlogged soils (Lanauskas et al., 2012).

The cultivation of cherry rootstock 'GiSelA 5' (syn. 'Gi 148/2'), originated in Germany, spreads throughout the world rapidly due to good compatibility with cherry cultivars, reduced vigour and high productivity of grafted trees (Franken-Bembenek, 2005). However, trees on the rootstock 'GiSelA 5' tend to overproduce, therefore, fruit thinning might be needed for highly productive cultivars (Andersen et al., 1999). Cherries grafted on 'GiSelA 5' are more suitable for growing in irrigated orchards (Fajt et al., 2014). In Latvia, 'GiSelA 5' was tested for sweet cherry cultivars 'Iput' (originated in Russia) and 'Krupnoplodnaya' (originated in Ukraine), and it was found as promising rootstock in the plantations with adequate tree densities and growing technologies (Rubauskis et al., 2014).

The rootstock ‘PHL-A’ (syn. ‘PHL 84’) is found as the most winter-hardy of the series PHL, created in Czech Republic, however it is not resistant to drought (Blažková, 2004). ‘PHL-A’ is recommended as reliable rootstock for modern sweet cherry orchards in Czech Republic climatic conditions (Blažková & Hlušičková, 2007).

The rootstock ‘VSL-1’ was originated in Russia, Krymsk Experimental Breeding Station by crossing of BC-2 (*P. fruticosa* Pall.) and L-2 (*P. lannesiana* Wils.), it is tolerant to drought, cold and cherry leaf spot, and has pronounced dwarfing effect (Kolesnikova, 2003; Eremin, 2008). The rootstock ‘PiKu 1’ (syn. ‘PiKu 4.20’) was characterized as the most winter-hardy and resistant to unfavourable growing conditions of PiKu series rootstocks, created in Germany (Fajt et al., 2014). It showed good vitality and high productivity in non-irrigated orchards (Balmer, 2008).

Generally, climate in Latvia is classified as temperate, amount of precipitation compensates or exceeds evaporation and the winter-hardiness of woody plants mainly corresponds to the zone 5–6 (Gloning et al., 2013). It should be appropriate for above mentioned rootstocks in the terms of temperature and humidity. However, the closeness of Baltic Sea and diverse impact of continental and maritime air-masses cause considerable fluctuations of temperature during winter and uneven distribution of precipitation, which changes year-to-year. There is a lack of the information about rootstock effect on tree growth, precocity and winter-hardiness for sweet cherry cultivars originated in Northern Europe and grown in the climate influenced by Baltic Sea. The aim of the study was to test the suitability of vigour-reducing rootstocks of different origins (‘PiKu 1’, ‘PHL-A’, ‘GiSelA 5’, ‘VSL 1’) vs. control rootstock *P. mahaleb* for winter-hard sweet cherry cultivar ‘Anu’ in Latvian climate.

MATERIALS AND METHODS

The trial was established in the spring of 2014 at the Institute of Horticulture (LatHort) in Pūre, Latvia, where four clonal rootstocks: ‘PiKu 1’, PHL-A’, ‘GiSelA 5’, ‘VSL 1’, and generative rootstock *P. mahaleb* (control) were included. Sweet cherry cultivar ‘Anu’ (Estonian breeding) was grafted on all rootstocks. Cherries were planted at a distance 5×3.5 m in three replications with three trees per plot. The orchard was not equipped for irrigation. The grass was regularly mowed in the inter-rows and herbicides were applied to control weeds around the trees in rows. Pests, and diseases were controlled according to integrated plant protection management. Compound fertilizer, containing 8% of N, 11% of P_2O_5, 23% of K_2O, was given yearly at the rate 25 g m^{-2}. The trees were trained to free-standing central leader. The lateral branches were grown in horizontal position, the semi-upright shoots were bended and the steepest shoots were cut off.

Tree height, growth of annual shoots, the viability of trees after wintering period and the intensity of flowering and production were evaluated from 2016 to 2019. Tree viability after wintering was scored on following scale: 0 – tree is completely dead, 1 – tree has lost ability to grow, 2 – above-ground part is completely damaged, but new shoots are developed, 3 – two and three years old branches and trunks are damaged, 4 – one year shoots are damaged, 5 – tree is in excellent condition. Flowering and production intensity were evaluated according to the scale from 0 to 5, where 0 – no flowers nor fruits developed, 1 – few, scattered flowers or fruits are seen, 2, 3 and 4–abundant flowering and at least several fruits have developed for about 25%, 50% and 75% of clusters on fruiting branches, respectively, 5 – all fruiting branches are abundantly flowering and several fruits have developed in every cluster.

The growth of annual shoots was determined in the autumn, after leaf fall - the length of 5 typical shoots were measured for each tree. Number of dead trees was registered at the end of investigation - in 2019.

Meteorological data were obtained from the automatic meteorological station 'Luft' registering meteorological conditions each 10 minutes. The hydrothermal coefficient (the ratio of between precipitation sum and air temperature sum) was calculated for the period of active vegetation with mean diurnal temperature ≥ 10 °C. The data are shown in the Table 1.

Table 1. Precipitation sum (mm), air temperature sum (°C) and hydrothermal coefficient (HTC) during the periods of active vegetation (with mean diurnal temperature ≥ 10 °C) in 2016–2019

Month	2016		2017		2018		2019	
	mm	°C	mm	°C	mm	°C	mm	°C
May	51.7	41.2	11.8	25.8	9.2	50.2	30.7	26.7
June	38	51.1	45	45.0	18.1	50.6	48.3	54.8
July	75.2	55.0	20.2	50.1	42.6	63.4	117.3	47.1
August	69.6	49.7	10.7	51.1	58.6	57.2	21.1	50.4
September	18.4	40.3	173.9	38.2	32.3	44.8	50.7	36.3
Total	252.9	237.2	263.2	218.2	160.8	266.2	268.1	27.3
HTC	1.06		1.19		0.60		1.30	

During first growing years - in 2014 and 2015, the hydrothermal coefficient calculated for the periods of active vegetation was 1.5 and 0.8, respectively.

Minimal and average air temperatures during winter and spring periods are presented in the Table 2.

Table 2. Minimal and average air temperature during the winter and spring periods of 2015/2016–2018/2019 (°C)

Month	2015/2016		2016/2017		2017/2018		2018/2019	
	min	average	min	average	min	average	min	average
November	-7.5	4.8	-12.9	1.3	-2.8	3.6	-12.9	1.3
December	-18.4	2.9	-13.8	1.6	-3.3	1.8	-13.8	1.6
January	-22.4	-6.6	-24.6	-2.2	-12.7	-1.5	-11.9	-2.5
February	-5.8	1.6	-15.9	-1.6	-24.9	-6.7	-7.5	-0.6
March	-9.3	1.6	-4.8	2.5	-20.7	-2.1	-9.1	0.8
April	-5.5	6.2	-8.2	4.6	-4.9	7.7	-7.9	9.4
May	0.9	13.7	-5.1	11.2	9	16.7	-3.1	14.3

In the first winter period (2014/2015), the lowest air temperature was -14.8 °C in January.

Statistical analysis of results was performed by analysis of variance in Microsoft Excel with Fischer's test to detect the differences between the means of sample sets at significance level of 0.05. The least significant difference (*LSD*) was calculated for post-hoc analysis.

RESULTS AND DISCUSSION

Tree height of the cultivar 'Anu' differed considerably under the influence of rootstocks ($p < 0.001$). The trees on the rootstock *P. mahaleb* were the highest in all years of evaluation reaching 2.9 m in 6th growing year (Table 3). The growth of the trees on 'GiSelA 5' was similar - the average tree height reached 2.7 m, which was for 6% lower than for those grafted on *P. mahaleb*. Generally, the rootstock 'GiSelA 5' can reduce the height of the tree by 20–40% comparing to vigorous rootstock Mazzard F12/1 (Sansavini & Lugli, 2014). However, strong vegetative growth of juvenile trees on 'GiSelA 5' in first growing years and delayed growth of mature trees have been observed also in other studies and the beginning of maturity stage seems depending on environment and cultivar (cv.). In the study performed at Washington State University, trunk growth of the trees on 'GiSelA 5' and Mazzard did not differ until growing year 4, while significant differences were detected in growing year 7 (cv. 'Bing') (Whiting et al., 2005). In Poland, significantly reduced tree growth under impact of rootstock 'GiSelA 5' was observed for 5 years old trees (cv. 'Vanda') (Grzyb et al., 2008), while in Portugal - for 2 years old trees (cv. 'Sweetheart' and 'Skeena') (Santos et al., 2005).

Table 3. Influence of rootstock on tree height (m), growth increase (%) and annual shoot length (cm)

Rootstock	Tree height (m)			Growth increase 2017–2019 (%)	Annual shoot length (cm)		
	2017	2018	2019		2017	2018	2019
PHL-A	1.2	1.4	1.6	34.6	27.6	20.6	11.6
PiKu 1	1.9	2.0	2.2	17.7	27.9	17.4	12.2
GiSelA 5	2.3	2.5	2.7	14.0	27.6	18.2	11.4
VSL 1	1.1	1.2	1.3	19.3	24.4	18.3	13.8
P. mahaleb	2.4	2.7	2.9	18.5	30.9	18.1	14.0
$LSD_{0.05}$	0.59	0.52	0.84		9.26	4.98	4.99

Rootstocks 'VSL 1' and 'PHL-A' significantly reduced tree height comparing to *P. mahaleb*. During first three growing years, trees on the rootstock 'PHL-A' had no increase in the height and their annual shoot growth was close to zero. They began to grow faster only in the growing year 4 (in 2017). Over three-year period (2017–2019), the trees on rootstock 'PHL-A' had the highest relative increase of tree height (increase by 34%).

Rootstock 'PiKu 1' showed the tendency to decrease tree height. However, the differences of the height between the trees grafted on 'PiKu 1' and *P. mahaleb* did not exceed *LSD* in two of the three evaluation years.

The effect of the rootstock on annual shoot growth was less discovered. Generally, the annual shoot length tend to be shorter for the trees grafted on clonal rootstocks comparing to those grafted on *P. mahaleb*. During growing years 3 to 6, annual shoot length decreased gradually for all grafting combinations wich could be explained by the completion of juvenile phase of trees.

Assessing tree viability and winter damages, significant differences were observed between rootstocks ($p < 0.001$). The trees on the rootstocks *P. mahaleb* and 'GiSelA 5' were in the best condition (Fig. 1). Only few trunk damages (bark splitting) and slight damages of shoots were observed for the trees on these rootstocks. There were no dead trees on the rootstock 'GiSelA 5' and small amount of dead trees (16.7%) on the rootstock *P. mahaleb*.

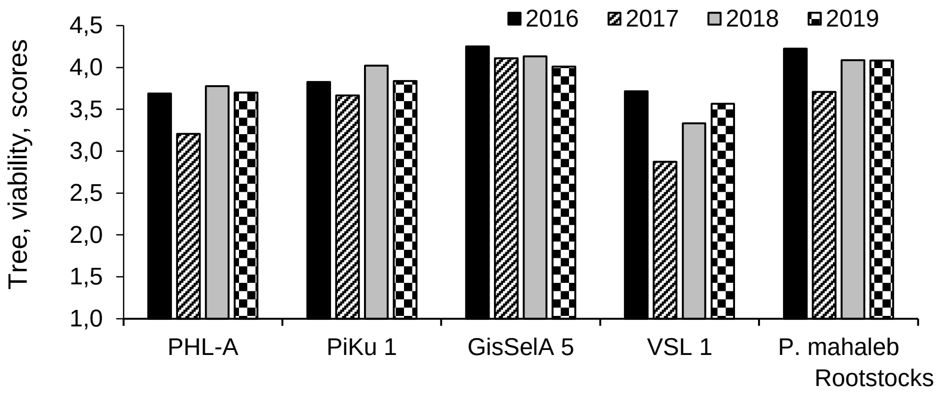

Figure 1. Influence of rootstocks on tree viability after wintering.

Tree viability decreased significantly under the influence of the rootstock 'VSL 1'. Trees on the 'VSL 1' had the highest level of winter damages on trunks and shoots among the evaluated rootstocks during all evaluation years. Trees showed essential splitting of bark and gumming.

In 2019, the highest amount of dead trees (33% of the total) for cultivar 'Anu' was recorded on the rootstock 'VSL 1' (Fig. 2), which could be an indicator of incompatibility of this rootstock with the cultivar, although the compatibility of the rootstock with other sweet cherry cultivars previously was assessed as good (Eremina, 2017). 'VSL 1' rootstock was originated in continental climate and it might be unsuitable for winters with sharp temperature fluctuations.

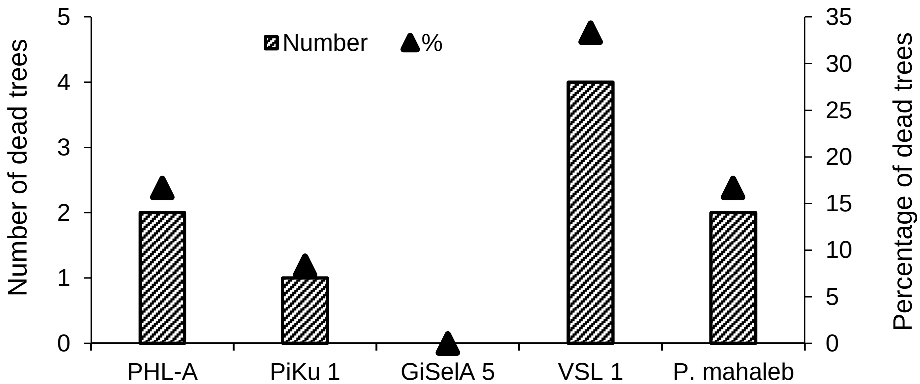

Figure 2. Influence of rootstocks on the amount of dead trees.

The damage of annual shoots with medium level was observed for the trees grafted on 'PHL-A' and 'PiKu 1'. The amount of dead trees did not differ from control. Trees on rootstocks 'PHL-A' and 'PiKu 1' died more in the first years after planting, when minimal air temperature dropped below 20 °C in winter: -22.4 °C in 2016, -24.6 °C in 2017 and -24.9 °C in 2018. Tree death could also be influenced by prolonged drought periods in 2015 and in 2018.

Trees on the rootstocks ‘GiSelA 5’ and *P. mahaleb* had high flowering intensity during 2017 to 2019 without significant differences observed between them (Table 4). The effect of other rootstocks on flowering intensity was rather inconsistent and depended on the year.

In the spring of 2017 and 2019, the weather conditions were unfavourable for fruit development. During flowering time, the lowest air temperature, recorded at the meteorological station in Pūre, was -5.1 °C in 2017 - at the beginning of flowering, and -3.6 °C in 2019 - at full bloom. In 2017, which was the first production year, the yield due to spring frosts was reduced. In 2019, trees bloomed well on all rootstocks, however spring frost destroyed cherry yield completely.

Table 4. Intensity of flowering and productivity (scores 0–5 with 5 being the best)

Rootstock	Intensity of flowering				Productivity	
	2017	2018	2019	average	2017	2018
PHL-A	1.1	1.2	4.0	2.1	0.3	0.6
PiKu 1	2.6	3.3	4.3	3.4	2.3	2.1
GiSelA 5	4.1	4.6	4.5	4.4	2.6	4.3
VSL 1	3.2	0.2	3.7	2.4	0.5	0.1
P. mahaleb	3.4	3.8	4.5	3.9	2.8	3.6
$LSD_{0.05}$	1.9	1.7	0.3	1.3	1.9	1.2

The productivity of the trees on the rootstocks ‘GiSelA 5’ and *P. mahaleb* was higher than of those on other clonal rootstocks both in 2017 and 2018. The cultivar ‘Anu’ grafted on *P. mahaleb* started to bear fruits in growing year 4 showing the precocity similar to the trees on ‘GiSelA 5’. It could indicate advanced development of flower buds and high resistance in winter and spring frosts of sweet cherry cultivar ‘Anu’ under the influence of rootstocks ‘GiSelA 5’ and *P. mahaleb* in Latvia.

Our results for the rootstock ‘Gisela 5’ were consistent to other studies in northwest Croatia and in Romania, showing high productivity and yield intensity of trees grafted on that rootstock in the first cropping years (Biško et al., 2017; Pal et al., 2017).

In contrary, the effect of the rootstock PHL-A’ on productivity of grafted trees in Latvia conditions completely differed from the results obtained in Poland (Rozpara et al., 2004). In our study, prolonged drought in the summer of 2018 could be one of the reason of low productivity for the trees on rootstock PHL-A.

CONCLUSIONS

Sweet cherry cultivar ‘Anu’ in the combinations with rootstocks ‘GiSelA 5’ and *P. mahaleb* had the best overall winter-hardiness and the most vigorous growth of trees at least until the growing year 6.

Trees on rootstocks ‘GiSelA 5’, ’PiKu 1’ and *P. mahaleb* had the highest flowering intensity among rootstocks evaluated.

The productivity of cultivar ‘Anu’ during first yielding years decreased under the influence of rootstocks ‘VSL 1’ and ‘PHL-A’.

The cultivar ‘Anu’ had the lowest tree viability and highest level of winter-damages on the rootstock ‘VSL 1’.

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Evaluation of Estonian apple cultivars and hybrids in Latvia

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Abstract. Estonian apples have always been popular in Latvia. At present, 'Tiina' is widely grown commercially as well as in home gardens, and 'Liivika' is promising for organic and home orchards. A number of new Estonian apple cultivars and hybrids have been screened in 1990–2020. Several new selections by breeder Kalju Kask (Polli) are included in field trials at Institute of Horticulture - 'Aule', 'Kastar' and KK 201-2 ('Karlote') since 2011, 'Kersti' since 2014, KK 5-16 ('Kelin') with scab resistance gene *Rvi6* and KK 2812 since 2015. Their trees were planted on dwarfing rootstock B.9 as one-year-old whips at distances 1.5×4 m, in 3 to 5 replications with 2 or 1 trees. Commercial cultivars 'Auksis', 'Antei' and 'Zarya Alatau' were used as controls. The highest productivity had 'Aule' and 'Kastar', the best fruit quality - 'Aule' and 'Kelin'. 'Aule' has been highly esteemed also by some Latvian farmers. Fruits of 'Kelin' had the best storage, which is crucial for a cultivar's commercial success in Latvia. On the other side, 'Kersti' proved to be unsuitable for Latvian conditions, having very strong tree vigour and low yields. 'Kastar' showed a high tendency to fruit cracking at calyx, while KK 201-2 and KK 2812 had irregular or low yields. Of newer acquisitions, scab resistant (gene *Rvi6*) 'Virve' and KK 4-11 show good preliminary results and have been propagated for trials on dwarfing rootstocks. Productivity, tree characteristics, fruit quality traits and taste panel evaluation of Estonian apples in Latvia are discussed.

Key words: *Malus* x *domestica*, productivity, fruit quality, storage, scab resistance.

INTRODUCTION

Estonian apples have been long-time favourites in Latvia because of their winter-hardiness and good fruit quality. Old cultivars 'Suislepp', 'Tallinna Pirnõun' (syn. 'Revaler Birnapfel') and 'Treboux Sämling' (syn. 'Pärnu Tuviõun') have not lost their popularity in home gardens since 19th century. In 1980ties, 'Põltsamaa Taliõun', 'Sidrunkollane Taliõun', 'Talvenauding', 'Tellissaare' and 'Tiina' were recommended for wider planting (Gronskis & Ūdris, 1988). At present, 'Tellissaare' and 'Tiina' are grown commercially, although their share in orchard area is only 1.6% and 1.3%, respectively (Kaufmane et al., 2017), in new plantings 'Tiina' is more popular. 'Sidrunkollane' and 'Talvenauding' are not propagated anymore, although 'Talvenauding' is recommended as a hardy frame-builder, tolerant to canker (*Neonectria ditissima*). Fruits of 'Tellissaare' are used for processing into purée, 'Talvenauding' - mostly for juice. 'Sügisdessert' is sold by nurseries for planting in home gardens. Crab apple 'Kuku' is popular in the home gardens, for eating fresh and processing, and promising for cider production (Krasnova et al., 2013).

From newer cultivars, ‘Liivika’ is promising for organic and home orchards and shows rather good storage potential, although prone to flesh browning with incorrect harvest time (Juhnevica-Radenkova et al., 2016). It has been already tested in an earlier trial, planted in 2008 on rootstock B.9, and was not included in the current study. ‘Liivika’ is promising for organic growing and home gardens in Latvia (Ikase, 2015).

The climate of Latvia requires winter-hardy cultivars, so Estonian apples present significant interest to Latvian growers. On the other side, good storage is a critical demand for commercial growing of a cultivar in Latvia, as the supermarkets do not welcome early apples. The most widely grown commercial cultivar in Latvia ‘Auksis’ with 200.8 thousand trees (Kaufmane et al., 2017) which is imported in mass also from Lithuania. ‘Auksis’ is a favourite because of very good eating quality which remains stable from September till March in common storage. It is suited also for ULO and 1-MCP storage (Juhnevica-Radenkova et al., 2016). Its trees are easy in training, productive and relatively regularly bearing. Main drawbacks of ‘Auksis’ are easy wind drop and medium resistance to scab. Cultivars without these drawbacks may replace ‘Auksis’ if they have equal fruit quality. Apples with shorter storage than ‘Auksis’ have limited use, especially if they mature in the same season and are not superior to it in quality.

The geographical location difference in south-north direction may have both positive and negative effect on productivity and fruit quality parameters like size, colour and biochemical composition (Kviklys et al., 2013; Viškelis et al., 2019). As the summers in Latvia are warmer compared to Estonia, many Estonian apple cultivars there ripen earlier and store for a short time, and may have lower acid and higher sugar content than in their home country. For example, cultivar ‘Krista’, which is promising in Estonia (Kivistik, 2014), in Latvia tastes bland. Infection of apple mildew (*Podosphaera leucotricha*) is more severe, too. In recent years, growing of ‘Tiina’ in southern Latvia has become problematic because of high mildew infection, killing new growth.

Apple breeding work in Estonia has been intense recently, resulting in many new selections, mostly by Dr. Kalju Kask (Kask et al., 2010; Kask, 2010). There has been also cooperation in breeding with Latvia - in 1997 hybrid seeds were shared with K. Kask at Polli, from 12 populations with scab resistance gene *Vf (Rvi6)*, obtained at our Institute. Elite hybrids were selected from crosses ‘Lobo’ x ‘Remo’, ‘Merrigold’ x ‘Stars’, open pollinated seeds of ‘Florina’, ‘Remo’, ‘Imrus’, ‘Siostra Liberty’ and hybrid BM41497. Scab (*Venturia inaequalis*) resistant cultivars ‘Kalju’ and ‘Virve’ have been registered in Estonia (Univer, 2019). This gives promise for finding new cultivars suitable for growing in Latvia. Yet these cultivars performance in Latvian climate must be tested.

The aim of the research was to evaluate the productivity and fruit quality of new Estonian apple cultivars and hybrids with good storage potential.

MATERIAL AND METHODS

The trial site is located at the Institute of Horticulture, Dobele, in Zemgale region, southern Latvia (56°37´N 23°16´E). The soil of the orchard site is sandy loam, sod carbonate gleyic, with organic matter 2.3%. Soil pH_{KCl} is 6.7, content of phosphorus (P_2O_5) 207 mg kg^{-1}, potassium (K_2O) 255 mg kg^{-1}, magnesium (Mg) 230 mg kg^{-1}.

The climate of the region is among the warmest in Latvia, but rather unstable (Table 1). The summer temperatures exceeding 30 °C may cause fast over-ripening and flesh browning of apple cultivars originated in Northern Europe. This has become more frequent recently (years 2018 and 2019). In this climate, mildew infection on apple-trees is higher than in the rest of Latvia. Spring frosts during apple flowering are uncommon, but in 2019 frost to -3 °C caused significant fruit damages.

Table 1. Long-term climate and apple-tree phenology of the trial site

Length of vegetation season (t° above 5 °C)	198 days
Sum of temperatures over 10 °C	2,000–2,100 °C
Average annual precipitation	581 mm
Extreme summer temperatures	> 3 °C
Extreme winter temperatures	< -30 °C (every 5–10 years)
Apple flowering	Beginning to end of May (depending on year)
Apple harvest	End of July to 1st decade of October (first autumn frosts)

The testing of Estonian apple cultivars and hybrids were done in two stages. It included preliminary trials and second stage trials of the most promising hybrids.

Preliminary trials

A number of new Estonian apple cultivars and hybrids have been screened at the Latvian Institute of Horticulture in 1990–2020. Most were selections of Dr. Kalju Kask, including such recognized cultivars or candidate cultivars as 'Aule', 'Els', 'Kaari', 'Kaimo', 'Kallika', 'Karamba', 'Kastar', 'Katre', 'Kersti', 'Kikitriinu', 'Krista', 'Liivika', scab resistant KK 5-16 ('Kelin'), 'Virve', KK 4-11 with gene *Vf (Rvi6)*, crabapples 'Kuku', 'Ritika', 'Ruti' and other selections, in total 53. Preliminary testing included also new cultivars of Uno Kivistik ('Reuno', 'Tiit') and Asta Kask ('Kasper', 'Koonik').

For preliminary testing, selections were planted in different years (from 1990 till 2016) on rootstocks B.9, M26 or MM106. The number of trees varied from 2 to 5. Evaluation was done at least for 3 years. The tree health, flowering and productivity were evaluated in points (0–9, where 0 – none, 9 – maximum value). Fruits were stored in common storage at 2 ± 1 °C to determine storage length. Fruits at eating maturity were evaluated at least twice by a taste panel. Tree growth and production habit were evaluated visually. After preliminary testing, the most promising cultivars and hybrids - 'Aule', 'Kastar', KK 201-2 ('Karlote') 'Kersti', KK 5-16 ('Kelin') and KK 2812 were selected for second stage trials at our Institute.

Second stage trials

Three trials were planted in 2011, 2014 and 2015.The first trial was established in 2011 with apple cultivars 'Aule', 'Kastar' and KK 201-2 ('Karlote') and control 'Auksis', trees were planted in 3 or 4 replications of 1 tree. The second trial was planted in 2014 with cultivar 'Kersti' and control 'Zarya Alatau', in 3 replications with 2 trees each. The third trial was planted in 2015 with KK 5-16 ('Kelin') and KK 2812, using as control 'Antei', in 5 replications with 2 trees each.

In all second stage trials, the trees were planted on dwarfing rootstock B.9 as one-year-old whips at distances 1.5×4 m. Trees were staked and trained as slender spindle.

Grass was sown in alleyways and regularly mown, keeping tree strips clean with herbicides. Standard spraying (fungicides and pesticides) and fertilizing were used, according to the standard of integrated growing (MK regulation Nr. 1056, 2009). The fruits were thinned by hand.

The following parameters were evaluated in the second stage trials annually:

- Tree general health in spring and summer (points 0–9, where 0 - tree perished, 9 – excellent tree health),
- Flowering and yielding intensity (points 0–9, where 0 - none, 9 - abundant),
- Scab and mildew damages (points 0–9, where 0 – no visible infection, 9 - > 90% infection, almost all tree damaged),
- Full bloom and harvest dates,
- Yield amount from each tree (kg),
- At harvest for a 5–6 kg sample from each tree: average fruit mass (g), amount on non-standard fruits - undersize or damaged (%), type of damage,
- In storage (common storage at 2 ± 1 °C) for a sample of 30–50 fruits, every 2 weeks: amount of damaged fruits (%), type of damage (disease, physiological), date of latest storage (end of storage - over 25% damaged or overripe fruits).

Harvest maturity was determined by starch-iodine test. For select cultivars optimal harvest maturity was determined by calculating Streif index (SI) (Streif, 1996):

$$SI = PE : (RE \times SV)$$

where PE – flesh firmness by penetrometer (kg cm^{-2}); RE – soluble solid content by refractometer (°Brix); SV – starch-iodine coloration index of flesh (points 1–10, where 1–100% iodine coloration, 10 – no coloration).

For determination of Streif Index, a sample of 10 fruits was collected at eye level from 4–5 trees, from both sides of a row. It was done by spraying cut fruit surface with potassium iodide (KI) Lugol solution (10 g KI + 3 g sublimated iodine per 1 litre of solution). Flesh firmness was measured with penetrometer FT 327 (10 mm head) at 2 opposite sides of each fruit. Soluble solids in juice were measured with Atago refractometer PAL-1.

Taste panels were carried out since 2009 and included 10–12 tasters, evaluating the following parameters: fruit look, taste, aroma, juiciness, firmness. For each tasting 5–6 fruits of about 10 cultivars were taken at eating maturity. Whole fruits were evaluated visually then cut into slices for tasting (unpeeled). A 5-point scale was used, where 1 – very poor, 2 – poor, 3 – medium, 4 – good, 5 – very good. Taste panel data were processed mathematically, finding averages and standard error *(Sx)* for each tasting.

Alternance index

For the trial planted in 2011, starting from 3rd cropping year alternance index (AI) was calculated, to characterise regularity of yielding (in kg per tree) between each 2 years (Monselise & Goldschmidt, 1982):

$$AI = (Yield_{year\ 2} - Yield_{year\ 1}) : (Yield_{year\ 2} + Yield_{year\ 1})$$

Tree vigour

In 2019, trunk diameter at 20 cm height was measured in trials, and trunk cross section area was calculated, to determine tree vigour:

$$TCSA = \pi\ (d : 2)^2$$

where TCSA – trunk cross section area (cm^2); d – trunk diameter (cm).

Data were processed mathematically using SPSS (IBM Statistics 25), Tukey *HSD* and LSD_{05} criteria. Differences were considered to be significant for *p-value* < 0.05.

RESULTS

Results of preliminary trials

As the number of cultivars and hybrids in preliminary testing was over 50, only a short summary of the results is given here. All new Estonian cultivars and selections in preliminary testing showed good tree health and tolerance to scab. No significant scab damages were observed with 5–6 annual fungicide sprayings applied in the framework of the integrated growing system. However, several of them were susceptible to mildew in the conditions of southern Latvia, especially ‘Tiit’.

Most cultivars and hybrids had attractive fruits with sweet taste, but ripened in autumn, at the peak of apple season, and could be stored 1–2 months. Some became mealy or developed water-core in hot summers when temperatures exceeded 30 °C, especially sweet dessert apples ‘Els’, ‘Kallika’, Kata 3 (‘Madli’) and KK 25-1-20 (‘Tiiu’). On the other side, ’Kaisa’, ’Katre’ and ‘Krista’ stored well and had large attractive fruits but lacked in flavour. Cultivars ‘Kaimo’, ’Kanni’, ‘Koonik’ ripening in August had good productivity, but short storage and tasted mediocre.

Number of cultivars and hybrids had tree habit difficult in training, with lots of bare wood, like Kata 1, ‘Katre’, ‘Kirki’, ‘Madli’, KK 281-1. Very vigorous trees with poor productivity had ‘Kikitriinu’, although with attractive and tasty fruits.

Performance of the most interesting cultivars and hybrids from preliminary trials in Latvia is shortly characterized below.

‘Kaari’ and **KK 281-14 (‘Kalar’)** are productive and have good quality sweet fruits with storage till December, which puts them in the peak season of Latvian apple market. For this reason, they have difficulty to compete with the main commercial cultivar in Latvia ‘Auksis’, which has similar fruit look and the same harvest time, but significantly longer consumption period, even with common storage.

‘Karamba’ is a small, but very tasty, sweet apple ripening in autumn. Tree is productive, late flowering, medium susceptible to mildew. Interesting for home gardens.

‘Kasper’ is very early ripening, has high productivity. With proper thinning fruits are tasty, but still small and unattractive in colour. Interesting for home gardens.

‘Krista’ has large, very uniform, firm fruits which can be stored several months but have poor flavour. Fruits drop easily. Flavour could be better in Northern Latvia, as shown by results in Estonia (Kask et al., 2010).

‘Reuno’ is so similar ‘Liivi Kuldrenett’ (‘Vidzemes Zelta Renete’) that often the fruits cannot be told apart; in Latvia their harvest time and storage length are similar. The tree is very productive and has more spreading habit than ‘Liivi Kuldrenett’; like it, is medium susceptible to scab and mildew.

Crabapple ‘Kuku’ is the best tasting in its group; it has small tree with drooping branches and regular good yields. Fruits can be consumed fresh and processed; only in conditions of extreme drought stress (year 2018) may develop some bitterness. The other crab apples ‘Ritika’, ‘Ruti’ show some off-taste, but are suitable for processing.

Hybrids from year 1997 crosses with scab resistance gene *Rvi6* selected by K. Kask were received at our institute in years 2014–2016 and have been evaluated for a short time. These were ‘Virve’, KK 8-5, KK 4-11, KK 4-11 and KK 5-16 (‘Kelin’). Of these only **'Kelin'** is already included in a second stage trial, planted in 2015 (trial results: below). Their fruit tasting data in preliminary testing are given in Table 2.

Table 2. Taste panel evaluation of Estonian apples in preliminary testing since 2014 (*Sx* – standard error)

Cultivar	Year	Look	*Sx*	Taste	*Sx*	Aroma	*Sx*	Firm-ness	*Sx*	Juici-ness	*Sx*
Auksis	2014	4.2	0.3	4.2	0.3	4.0	0.5	4.0	0.5	4.0	0.4
(control)	2015	4.5	0.4	4.4	0.4	3.9	1.0	3.9	0.6	3.9	0.8
	2016	4.3	0.4	4.3	0.5	3.7	0.6	4.0	0.4	3.8	0.6
	2017	4.5	0.4	3.9	0.4	3.2	1.0	3.6	0.5	3.3	0.8
	2018	4.6	0.3	4.3	0.4	4.2	0.6	4.1	0.6	3.9	0.7
	2019	4.4	0.5	4.2	0.7	3.8	1.0	3.8	0.4	3.6	0.5
	Range	4.2-4.6		3.9–4.4		3.2–4.2		3.6–4.1		3.3–4.0	
KK 8-5	2016	4.3	0.4	3.7	0.6	3.4	0.9	3.9	0.4	4.0	0.2
	2018	4.4	0.5	4.0	0.6	3.7	1.2	4.2	1.0	4.2	0.8
	2019	4.4	0.5	3.3	0.9	3.3	1.0	4.1	0.5	4.0	0.5
	Range	4.3–4.4		3.3–4.0		3.3–3.7		3.9–4.2		4.0–4.2	
KK 4-11	2019	4.5	0.3	4.1	0.6	4.0	0.8	4.1	0.3	4.2	0.7
Kelin	2017	4.2	0.6	3.8	0.6	3.4	1.0	4.0	0.7	3.7	0.7
	2018	4.6	0.3	3.9	0.6	3.5	1.0	3.4	0.8	3.3	0.7
	2019	4.4	0.4	4.2	0.4	3.6	1.0	3.8	0.5	3.6	0.6
	Range	4.2–4.6		3.8–4.2		3.4–3.6		3.4–4.0		3.3.–3.7	
Virve	2017	4.3	0.4	3.8	0.5	3.5	0.9	3.4	0.6	3.8	0.4
	2018	4.4	0.3	4.0	0.3	3.8	0.8	4.0	0.8	3.8	0.8
	2019	4.2	0.4	4.1	0.9	4.0	0.9	3.8	0.7	3.8	0.6
	Range	4.2–4.4		3.8–4.1		3.5–4.0		3.4–4.0		3.8	

'Virve' ('Lobo' x 'Remo') has large and tasty fruits, stored till December or January. Tree is productive, with dense crown. The cultivar was planted in a wider trial in 2020.

Hybrid KK 8-5 ('Imrus' o.p.) has productive tree and large fruits with medium to good flavour, ripening in autumn and similar in look to the old cultivar 'Streifling Herbst'.

Hybrid KK 4-11 ('Lobo' x 'Remo') has given only the first yield of bright red, medium size fruits with good eating quality and storage potential. The tree is early bearing, productive and easy in training. Planting in a wider trial is planned in 2022.

By results of preliminary testing, cultivars 'Aule', 'Kastar', 'Kersti', KK 201-2 ('Karlote'), KK 2812 and KK 5-16 ('Kelin') were selected for second stage trials, planted in 2011, 2014 and 2015. Their results are discussed below.

Results of the trial planted in 2011

Productivity and growth. All cultivars in this trial started bearing fruit in 2013. In average of all years, there were no significant differences between cultivars in fruit number, yield per tree and average fruit mass (Table 3). Yet there were significant differences in yield amount between individual years, determined by alternance of bearing.

Table 3. Production apple cultivars and hybrids in a trial planted in 2011 on rootstock B.9

Cultivar, hybrid	Harvest date	Fruit number per tree	Yield, kg per tree	Average fruit mass, g	Non-standard fruits, %	Type of non-standard
	2013					
Auksis (control)	12.09.	2.0ab	0.4a	311.7	0.0	
Aule	12.09.	1.7a	0.4a	266.7	7.8	seedless
Kastar	13.09.	15.7c	4.2b	274.6	4.0	cracking; small
KK 201-2 (Karlote)	12.09.	9.0bc	2.4b	271.7	19.2	scab
	2014					
Auksis (control)	08.09.	30.0	4.2	139.2a	10.1a	russet
Aule	18.09.	26.2	4.2	171.0ab	0.0a	
Kastar	03.10.	40.7	7.3	198.5b	35.1b	cracking
KK 201-2 (Karlote)	03.10.	37.5	5.8	157.9ab	8.9a	cracking
	2015					
Auksis (control)	-	0.0a	0.0a	-	-	
Aule	29.09.	16.5a	3.2a	186.9	0.8a	fruit rot
Kastar	29.09.	67.3b	11.0b	165.4	12.6b	cracking
KK 201-2 (Karlote)	-	0.0a	0.0a	-	-	
	2016					
Auksis (control)	07.09.	59.3*	8.0	133.4*	1.4a	scab
Aule	23.09.	72.8	13.3	196.0**	2.0a	scab; small
Kastar	29.09.	70.0	12.1	173.8	23.8b	cracking
KK 201-2 (Karlote)	29.09.	132.3**	19.4	147.7*	6.4a	small
	2017					
Auksis (control)	14.09.	31.7a	5.3b	113.8a	14.3	small
Aule	25.09.	28.7a	3.0ab	165.2b	39.5	aphids; scab
Kastar	17.10.	104.0b	14.6c	140.8ab	26.0	cracking
KK 201-2 (Karlote)	-	0.0a	0.0a	-	-	
	2018					
Auksis (control)	31.08.	94.3b	11.6b	118.9	6.7a	small
Aule	14.09.	78.8b	10.8b	137.7	1.7a	small
Kastar	01.10.	7.5a	1.1a	150.0	100.0b	cracking; rot
KK 201-2 (Karlote)	17.09.	102.0b	11.6b	113.2	15.9a	small
	2019					
Auksis (control)	-	0.0	0.0a	-	-	
Aule	18.09.	41.7	7.7b	191.6b	85.4	frost
Kastar	15.10.	96.5c	12.6b	130.7a	62.3	cracking
KK 201-2 (Karlote)	-	0.0a	0.0a	-	-	
	Average of all years (^{x} for yield - sum of 7 years)					
Auksis (control)	IX 1. [1]	28.5	^{x}27.3	163.8	7.1ab	
Aule	IX 2. [1]	38.1	^{x}42.7	178.1	22.0a	
Kastar	X 1. [1]	54.5	^{x}60.9	182.7	34.2b	
KK 201-2 (Karlote)	IX 3. [1]	46.4	^{x}46.2	176.7	11.3ab	

Notes. In the same year, the cultivars marked with different letters (a, b, c) differed significantly by *Tukey HSD*; with ** differ significantly from marked with * by LDS_{05}; – no data; [1] month, decade.

'Kastar' had the highest yield in 2013, 2015, 2017 and 2019. It had also significantly lower alternance index than other cultivars, 0.13 in the period of 2015–2016 and 0.09 in 2016–2017 'Aule' showed the lowest alternance index (0.21) in 2018–2019 and yield similar with 'Auksis' in most years. 'Auksis' had no yield in 2019, KK 201-2 ('Karlote') in 2017 and 2019. Yield amount in 2019 was affected by spring frost, but 'Karlote' in 2017 was also weakened by excess yield of 2016.

Measurements of trunk diameter in 2019 showed that trees of 'Aule' are much more vigorous than 'Auksis', with TCSA respectively 293.4 cm^2 and 167.6 cm^2.

Fruit quality

Average fruit mass of 'Aule' was significantly the highest in 2016, 2017 and 2019. Fruits were very uniform in size and shape. Significant differences in all years were found for amount of non-standard fruits. The highest amount of damaged fruits had 'Kastar', which has a high tendency to cracking at calyx, on average 34.2%. In 2018 cracking reached 100%. On the other side, control cultivar 'Auksis' had only 7.1% non-standard fruits on average.

In 2019, 'Aule' had significant spring frost damages, 85.4% of fruits. These damages were similar with 'Auksis' in the same orchard plot on trees bearing fruits.

Harvest time and storage

Harvest date of 'Aule' was in the 2^{nd} half of September, while control 'Auksis' was harvested in beginning of September. Their length of storage was similar, till mid-February or mid-March, but fruits of 'Aule' hold on tree much better than 'Auksis'. 'Kastar' was picked later, usually beginning of October, and could be stored till 2^{nd} half of March. 'Karlote' produced fruits only in 3 years, its picking dates varied from mid-September to October, and storage length from mid-December till mid-February.

Fruit tasting for these cultivars was carried out since preliminary trials, and so includes many year data, with some exceptions (Table 4). It showed the best results for 'Auksis' and only slightly lower for 'Aule', although in the hot summer of 2019 the quality of 'Aule' decreased, as fruits soon became overripe. Tasting results of 'Kastar' and 'Karlote' were poor in several years.

Results of the trial planted in 2014

Productivity and growth

Both cultivar 'Kersti' and control 'Zarya Alatau' in this trial started bearing fruit in 2016. All years 'Kersti' had lower number of fruits per tree, but higher fruit mass, 218 g on average. As fruits of 'Zarya Alatau' were smaller, yield in kg per tree did not differ significantly either in separate years or on average of all years (Table 5).

Both cultivars in the first years had vigorous, upright trees of similar size, with TCSA in 6^{th} year 19.8 cm^2 for 'Kersti' and 21.2 cm^2 for 'Zarya Alatau'. Strong growth was determined by low yields till 2017, when 'Kersti' gave 2.3 kg and 'Zarya Alatau' 4.2 kg per tree. By year 2019 the average yields per tree had reached 16.2 kg for 'Zarya Alatau' and 9.5 kg for 'Kersti'. Several older trials have shown that 'Zarya Alatau' is highly productive (Rubauskis & Skrivele, 2007), while trees of 'Kersti' planted in 2011, in preliminary trial on rootstock MM106, have not yet produced good yield.

Fruit quality

In 2019, 83.6% fruits of 'Kersti' were damaged by spring frost, significantly more than 'Zarya Alatau', but in 2018 'Zarya Alatau' had the highest amount of undersize fruits, 37.7%.

Taste panel evaluation of 'Kersti' could be carried out only in 2 years, including preliminary testing; the rating was good (Table 4).

Table 4. Taste panel evaluation of Estonian apples in second stage trials (*Sx* – standard error)

Cultivar, hybrid	Year	Look	*Sx*	Taste	*Sx*	Aroma	*Sx*	Firm-ness	*Sx*	Juici-ness	*Sx*
Auksis (control)	2009	4.5	0.4	4.6	0.3	4.4	0.5	4.4	0.5	4.8	0.2
	2010	4.3	0.4	4.3	0.4	3.9	0.3	3.7	0.5	4.6	0.5
	2011	4.5	0.3	4.3	0.3	4.3	0.4	4.1	0.4	4.4	0.5
	2012	4.2	0.4	4.1	0.5	3.8	0.7	4.0	0.8	4.6	0.5
	2013	4.5	0.4	4.4	0.4	3.7	1.0	4.0	0.7	4.2	0.8
	2014	4.2	0.3	4.2	0.3	4.0	0.5	4.0	0.5	4.0	0.4
	2015	4.5	0.4	4.4	0.4	3.9	1.0	3.9	0.6	3.9	0.8
	2016	4.3	0.4	4.3	0.5	3.7	0.6	4.0	0.4	3.8	0.6
	2017	4.5	0.4	3.9	0.4	3.2	1.0	3.6	0.5	3.3	0.8
	2018	4.6	0.3	4.3	0.4	4.2	0.6	4.1	0.6	3.9	0.7
	2019	4.4	0.5	4.2	0.7	3.8	1.0	3.8	0.4	3.6	0.5
	Range	4.2–4.6		3.9–4.6		3.2–4.4		3.6–4.4		3.3–4.8	
Aule	2009	4.4	0.4	4.3	0.4	3.9	0.6	4.1	0.5	4.9	0.2
	2010	4.6	0.4	4.5	0.4	4.0	0.6	4.3	0.5	4.9	0.2
	2011	4.3	0.4	3.9	0.8	3.9	0.6	3.6	0.9	4.5	0.5
	2012	4.5	0.2	4.5	0.3	4.2	0.4	4.3	0.5	4.7	0.4
	2014	3.9	0.4	4.2	0.4	3.8	1.0	4.1	0.6	4.2	0.4
	2015	4.4	0.4	3.9	0.6	3.8	0.9	4.1	0.5	4.0	0.7
	2017	4.4	0.4	4.1	0.7	3.8	0.8	3.9	0.5	3.9	0.6
	2018	4.3	0.2	4.0	0.9	3.7	1.0	3.7	0.5	4.0	0.6
	2019	3.9	0.4	3.9	0.7	3.3	0.9	3.4	0.5	3.2	0.7
	Range	3.9–4.6		3.9–4.5		3.3–4.2		3.4–4.3		3.2–4.7	
Karlote	2009	4.4	0.4	4.2	0.4	4.3	0.4	4.2	0.3	5.0	0.1
	2010	4.4	0.4	4.0	0.7	4.1	0.5	3.8	0.4	4.8	0.3
	2011	4.1	0.5	3.9	0.6	3.7	0.5	3.9	0.9	4.3	0.4
	2013	4.1	0.6	4.0	0.7	3.5	0.9	3.5	0.7	3.3	0.8
	2016	4.4	0.4	3.9	0.5	3.6	0.9	3.7	0.5	4.1	0.5
	2018	4.1	0.5	3.2	0.8	2.9	1.0	3.3	0.4	3.2	0.5
	Range	4.1–4.4		3.2–4.2		2.9–4.3		3.3–4.2		3.2–5.0	
Kastar	2009	4.3	0.2	3.8	0.5	3.9	0.6	3.7	0.6	4.9	0.2
	2010	4.0	0.7	3.3	0.6	3.7	0.7	2.8	0.8	4.9	0.3
	2011	4.5	0.3	4.1	0.4	4.3	0.5	3.3	0.9	4.5	0.5
	2012	3.7	0.6	3.7	0.7	4.2	0.7	3.3	1.1	4.7	0.4
	2013	3.9	0.6	3.7	0.8	3.5	1.1	4.2	0.4	3.3	1.3
	2015	4.2	0.4	3.3	0.6	3.1	0.8	3.8	0.9	3.0	0.8
	Range	3.7–4.5		3.3–4.1		3.1–4.3		2.8–4.2		3.0–4.9	
Kersti	2014	4.1	0.7	4.2	0.5	3.6	0.8	4.3	0.4	4.1	0.4
	2017	4.4	0.4	4.3	0.4	3.9	0.9	4.2	0.7	4.1	0.6
	Range	4.1–4.4		4.2–4.3		3.6–3.9		4.2–4.3		4.1	
KK 2812	2018	4.5	0.5	4.5	0.4	4.1	1.0	4.0	0.7	3.8	0.6
	2019	3.9	0.4	4.5	0.4	3.6	0.9	3.9	0.5	3.8	0.6
	Range	3.9–4.5		4.5		3.6–4.1		3.9–4.0		3.8	

Table 5. Production of apple cultivars in a trial planted in 2014 on rootstock B.9

Cultivar, hybrid	Harvest date	Fruit number per tree	Yield, kg per tree	Average fruit mass, g	Non-standard fruits, %	Type of non-standard
	2016					
Kersti	-	0.2a	0.3	-	-	
Zarya Alatau (control)	26.09.	6.1b	1.0	169.7	10.0	bitter pit
	2017					
Kersti	09.10.	10.7	2.3	219.0b	4.2	bitter pit, russet
Zarya Alatau (control)	09.10.	30.4	4.2	131.0a	16.0	small; fruit rot
	2018					
Kersti	13.09.	23.2a	4.8	211.4b	1.3a	bitter pit
Zarya Alatau (control)	02.10.	58.7b	8.9	149.3a	35.7b	small; cracking
	2019					
Kersti	20.09.	45.0a	9.5	224.0b	83.6b	frost
Zarya Alatau (control)	11.10.	128.9b	16.2	129.2a	41.5a	frost, hail
	Average of all years (^{x} for yield - sum of 4 years)					
Kersti	IX 2. [1]	19.8a	^{x}16.8	218.0b	32.9	
Zarya Alatau (control)	X 1. [1]	56.0b	^{x}30.4	142.9a	27.0	

Notes. In the same year, the cultivars marked with different letters (a, b) differed significantly by *Tukey HSD*; – no data [1] month, decade.

Harvest time and storage

Fruit harvest of 'Kersti' for most years was the 2nd half of September, they stored till February or March. Control - late winter control cultivar 'Zarya Alatau' was harvested in beginning of October and stored till April.

Results of the trial planted in 2015

Productivity and growth

Control cultivar 'Antei' and KK 5-16 ('Kelin') in this trial started bearing first few apples already in 2016, while KK 2812 in 2017, and has given only few fruits (Table 6). Significant cultivar differences in all-year average yield per tree were not statistically provable, but differences were found in 2018 and 2019). 'Antei' had the highest yield both these years, reaching 10.8 kg in 2019, when 'Kelin' gave 7.9 kg. Trunk measurements in 2019 showed weaker vigour for 'Kelin' than 'Antei', with TCSA respectively 9.5 cm^2 and 12.6 cm^2 in the 5th year of growth.

Fruit quality

The average fruit mass of 'Kelin' in all years was the lowest*)* as compared with both other cultivars producing very large fruits, but still acceptable, 152.4 g. The highest average amount of non-standard fruits had KK 2812*),* mostly due to frost and hail damages in 2019 reaching 64.2%.

Harvest time and storage

Fruits of 'Antei' in this trial were harvested in beginning of October (except the extremely hot year 2019) and stored till February or March. The best harvest time and storage length of 'Kelin' and especially KK2812 still need to be found, as the first pickings were tentative, lacking previous knowledge.

Table 6. Production of apple cultivars and hybrids in a trial planted in 2015 on rootstock B.9

Cultivar, hybrid	Harvest date	Fruit number per tree	Yield, kg per tree	Average fruit mass, g	Non-standard fruits, %	Type of non-standard
	2016					
Antei (control)	26.09.	1.6	0.3	154.7	0.0	
KK 5-16 (‘Kelin’)	26.09.	3.3	0.5	139.8	0.0	
KK 2812	-	0.0	0.0	-	-	
	2017					
Antei (control)	09.10.	0.7	1.5	251.7	0.0	
KK 5-16 (‘Kelin’)	26.09.	0.0	0.0	-	-	
KK 2812	-	0.4	0.0	-	-	
	2018					
Antei (control)	03.10.	14.0b	3.8b	273.9b	1.7a	small
KK 5-16 (‘Kelin’)	03.10.	12.0b	1.4ab	166.9a	0.6a	cracking
KK 2812	03.10.	3.4a	1.0a	297.1b	17.3b	watercore
	2019					
Antei (control)	11.10.	49.1b	10.8b	223.3b	23.6a	hail, frost, bitter pit
KK 5-16 (‘Kelin’)	19.09.	62.3b	7.9a	128.8a	24.4a	frost, hail
KK 2812	18.09.	29.6a	7.3a	257.0b	64.2b	frost, hail
	Average of all years (^{x} for yield - sum of 4 years)					
Antei (control)	X 1.[1]	16.4	^{x}15.2	243.0b	8.7a	
KK 5-16 (‘Kelin’)	IX 3. [1]	19.4	^{x}10.4	152.4a	8.7a	
KK 2812	IX 3. [1]	8.3	^{x}8.4	253.5b	45.5b	

Notes. In the same year, cultivars marked with different letters (a, b, c) differed significantly by *Tukey HSD*; – no data; [1] month, decade

‘Kelin’ seems to have a long harvest window, from 2nd half of September till early October, as they hold very well on tree, but with late picking they have low juiciness as shown by tasting results in 2018 (Table 2). In general, its taste rating was good and may improve with finding appropriate harvest time. Fruits can be stored till February, maybe longer, without any storage damages.

DISCUSSION

Most Estonian apple cultivars in Latvia ripen in midseason and for this reason have hard competition with the most widely grown commercial apple ‘Auksis’. Estonian cultivars can compete with it only if they combine high yields, excellent fruit quality, good fruit storage and disease resistance. From this aspect, new scab resistant *(Rvi6)* selections ‘Virve’, and possibly KK 4-11 look very promising, as they seem to combine all mentioned characteristics, yet they need more testing.

More results were obtained in the trials planted from 2011 to 2015 at Dobele, where six Estonian cultivars and selections showed various results. Their value for planting in Latvia is discussed below.

‘Aule’

Early winter cultivar with attractive, uniform fruits, looking similar with ‘Auksis’, but with different, refreshing flavour. The over-colour often is weaker, striped. Trees are vigorous, easy to train and have good productivity. Resistance to scab is medium. Fruits may be damaged by spring frost to the same extent as ‘Auksis’. Its value lays both in fruit visual similarity to ‘Auksis’ and later harvest season, as well a good fruit holding on tree. Fruits may have poorer storage and shelf life after hot summers. It is a promising cultivar, recommended for farm trials.

‘Kastar’

Winter cultivar with high productivity and sweet fruits, resembling Latvian cultivar ‘Stars’; like it, may somewhat lack juiciness. Has a strong tendency to fruit cracking at eye, which makes its commercial growing in Latvia non-profitable.

KK 201-2 (‘Karlote’)

This winter apple has very unreliable performance, with good yields and fruit quality only in some years. Not promising.

‘Kersti’

This winter cultivar has large, tasty fruits, but poor productivity and late bearing, too large trees in Latvian conditions. Possibly longer and warmer summers are responsible for the too vigorous growth. The fruit surface often is bumpy, looking like aphid damage. Not promising.

KK 5-16 (‘Kelin’)

Early winter apple with attractive sweet and firm fruits which hold strongly on tree and store very well. Medium juicy, with late picking may be lacking juiciness. First results show relatively weak tree vigour and medium productivity. Resistant to scab *(Rvi6)* and not injured by mildew, unlike ‘Tiina’. It is worth wider testing.

KK 2812

Was selected as a large ‘Antonovka’ type apple with compact tree, but so far has yielded very poorly. More observations are needed, but unlikely to be promising.

Cultivar **‘Liivika’**, which was included in earlier trials, certainly also deserves mentioning here. It has bright yellow, tasty fruits; average 10-year taste panel rating is 4.2 for fruit look, 4.1 for flavour. Harvested in mid-September and can be stored till January. Optimal harvest maturity (Streif index) is 0.07–0.1, must be picked when colour is well-developed. Trees are below medium vigour, production medium (12 kg per tree on B.9) and biennial. Has fruit quality as good, but better storage and smaller trees than the similar cultivar ‘Liivi Kuldrenett’ (‘Vidzemes Zelta Renete’) popular in Latvia. Although medium productivity and lack of uniformity in fruit shape may limit its use for commercial planting, it is recommended for home gardens and organic growing (Ikase, 2015).

The trial place in Dobele, southern Latvia has warmer climate than other regions in Latvia, and this certainly affected trial results. High mildew infection on Estonian cultivars like 'Tiina' and 'Tiit' is regularly observed in Dobele, while farmers from Northern and Eastern Latvia report no such problem. Summer temperatures exceeding 30 °C, which occur more frequently in latest years, lead to over-ripening and poor storage of apples, and were the main reason for discarding of many selections in preliminary testing. Early softening of fruits often causes poorer coloration and taste (Warrington et al., 1999; Lin-Wang et al., 2011). In the discussed trials, such negative effect was observed for 'Aule' in 2019. Also, number of cultivars and selections in preliminary testing showed lower acidity than observed in Estonia, rated in tasters notes as sweet or insipid while in Estonia they are sub-acid (Kask, 2010; Kivistik, 2014). This may be explained by faster fruit maturing. On the other side, climate in Latvia varies significantly between regions, the same as in Estonia (Kask et al., 2010). As northern regions of Latvia lie close to Estonia, it is only logical to suggest that Estonian cultivars there will perform better, and their high winter-hardiness may be the decisive factor planting 'Kaari' or 'Krista' instead of 'Auksis', as these cultivars have shown good performance in Estonia.

CONCLUSIONS

1. Estonian apple cultivars 'Liivika' and 'Aule' are recommended for trial planting at farms in different regions of Latvia.
2. By first results, scab resistant *(Rvi6)* apples 'Virve', KK 5-16 ('Kelin') and KK 4-11 may have promise for growing in Latvia but need longer trials.
3. Cultivars 'Kastar', 'Kersti' and hybrid KK 201-2 ('Karlote') cannot be recommended for planting in Latvia.
4. Midseason ripening Estonian apple cultivars have hard competition in Latvia with the most widely grown commercial apple 'Auksis', and can compete with it only combining high yields, very good fruit quality, good storage and disease resistance.
5. Performance of most Estonian cultivars in southern Latvia is affected by higher summer temperatures, and the results may be better in Northern Latvia.

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Comparison of Polyphenols and Anthocyanin Content of Different Blackcurrant (*Ribes nigrum* L.) Cultivars at the Polli Horticultural Research Centre in Estonia

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Abstract. The evaluation of blackcurrant cultivars and their fruit properties at the Polli Horticultural Research Centre has been active since 1945. In addition to the assessment of biological and economic properties of cultivars, it is essential to pay attention to fruit quality. In 2014, the laboratory building of Polli Horticultural Research Centre was reconstructed within the PlantValor competence centre project, enabling to introduce HPLC methods for the determination of polyphenolic compounds in fruit quality analysis. In 2017 and 2018, the fruit quality of 37 blackcurrant cultivars of different geographical origin (Belarus, Estonia, Finland, Latvia, Lithuania, Norway, Poland, Russia, Scotland, Sweden and Ukraine) was analysed. All cultivars were grown in the genetic resources collection (2008–2019) located at the Polli Horticultural Research Centre. The main aim of the study was to analyse the content of polyphenols and anthocyanins for selecting suitable blackcurrant genotypes for breeding programmes, fruit production and possible product development. In two consecutive years of the study, the total polyphenols content in the fruits of different cultivars varied 290–634 mg 100 g^{-1} fresh weight (fw) and the anthocyanins 183–471 mg 100 g^{-1} fw.

Key words: anthocyanins, biochemical composition, blackcurrant, fruit quality, polyphenols.

INTRODUCTION

Blackcurrant (*Ribes nigrum* L.) is an important soft fruit crop in Estonia. The yield of blackcurrant and its resistance to winter damage in Estonian climate is highly cultivar dependent (Kikas & Libek, 2020). In the breeding programme of blackcurrant, in addition to biological and economic properties, research on fruit yield and its quality is also very important (Sasnauskas et al., 2009; Masny et al., 2018; Sasnauskas et al., 2019; Jarret et al., 2020). Blackcurrants of high quality contain significant amounts of ascorbic acid as well as other antioxidants such as polyphenols and anthocyanins. Delphinidin-3-*O*-rutinoside, cyanidin-3-*O*-rutinoside, delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside are the most abundant monomeric anthocyanins in blackcurrants (Nour et al., 2013; Tian et al., 2019). Besides anthocyanins, the fruits also contain derivatives of phenolic acids (p-coumaric acid, coffeic acid), flavonols and proanthocyanidins (Mattila et al., 2011; Jimenez-Garcia et al., 2013; Vagiri et al., 2013; Mattila et al., 2016; Tian et al., 2019).

The chemical profiles of blackcurrant fruits vary significantly among cultivars (Bakowska-Barczak & Kolodziejczyk, 2011; Jarret et al., 2018), the growing year (Tian et al., 2019), fruit development stage and maturity (Jarret et al., 2018). Besides the genotype, the quality of fruits, including content of nutrients depends on weather conditions and cultivation site (Yang et al., 2010; Krüger et al., 2011; Kaldmäe et al., 2013; Vagiri et al., 2013; Woznicki et al., 2015; Kikas et al., 2017; Paunović et al., 2017; Allwood et al., 2019; Tian et al., 2019). Still, the genotype has larger effect on the content of bioactive compounds than the location of the experimental plots and the year of investigations (Vagiri et al., 2013).

In addition to the sensory properties of blackcurrant, the colour and content of bio-functional compounds of fruits are highly appreciated (Hilz et al., 2005). Therefore, blackcurrants rich in bioactive components are a valuable source for product development in the food industry (Kozák et al., 2009). The increased demand for preservation of the high quality of food products and maintaining their nutritional value, for example canned goods, is the driver for research on the quality of raw material used in food production (Strizhevskaya et al., 2019). Moreover, the selection of various fruit processing technologies helps to increase the amount of polyphenolic compounds in different products (Rätsep et al., 2020). The effect of blackcurrant extract on both antioxidant and anti-inflammatory properties was reported by Lyall et al. (2009). Some scientific proof was found for anti-cancer properties of blackcurrant (Bishayee et al., 2011; Khoo et al., 2012). Furthermore, blackcurrant extract containing cyanidin-3-*O*-glucoside was considered effective for the therapy to prevent and/or to treat smoking-related periodontal diseases (Desjardins et al., 2012). Blackcurrant anthocyanins were also in the focus of several sport nutrition studies, and their effect on fat oxidation and exercise performance was recently presented (Cook et al., 2015; Cook et al., 2017; Cook & Willems, 2019; Hurst et al., 2019).

The main aim of the present research was to compare the biochemical composition of blackcurrant fruit focusing on the content of polyphenols and anthocyanins in order to provide a more complex information for further selection of suitable blackcurrant genotypes for plant breeding, fruit production and possible product development.

MATERIALS AND METHODS

Site and experiment description

All 37 blackcurrant cultivars were grown in the field of the genetic resource collection (2008–2019) located at the Polli Horticultural Research Centre of Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences (58°7'26"N, 25°32'43"E), Estonia. The field collection was established in autumn 2008 with two-year-old plants, three bushes per cultivar with a planting density of 3.0×1.0 m. Each spring a conversion rate (dose) of 300 kg ha^{-1} of the complex fertilizer Cropcare 6-14-23 (Kemira OY) was applied. The plant rows were mulched with milled peat and the space in between the rows was covered with grass and mown regularly during the vegetation period. The experimental plot was established on medium heavy loamy soil which had appropriate water and air conditions for the root system, but no irrigation system was used for the experimental plants.

Evaluated cultivars

In 2017 and 2018, the fruit quality of 37 blackcurrant cultivars of different geographical origin in Europe was analysed. Among the tested cultivars there were three Belarussian (‘Belorusskaya Sladkaya’, ‘Katyusha’, ‘Pamyat Vavilova’), four Scottish (‘Ben Alder’, ‘Ben Avon’, ‘Ben Finlay’, ‘Ben Lomond’), three Finnish (‘Aström’, ‘Kajaanin Musta’, ‘Mortti’), one Latvian (‘Mara’), four Lithuanian (‘Dainiai’, ‘Gagatai’, ‘Pilenai’, ‘Smaliai’), three Norwegian (‘Hedda’, ‘Narve Viking’, ‘Varde Viking’), six Polish (‘Bona’, ‘Gofert’, ‘Ores’, ‘Ruben’, ‘Tiben’, ‘Tisel’), one Russian (‘Zagadka’), three Swedish (‘Intercontinental’, ‘Titania’, ‘Triton’), one Ukrainian (‘Syuita Kievska’) and eight Estonian cultivars (‘Almo’, ‘Asker’, ‘Ats’, ‘Elmar’, ‘Elo’, ‘Karri’, ‘Mairi’, ‘Varmas’). Cultivar ‘Titania’ was selected as a control.

Weather conditions

The metrological data for the experimental site was obtained from the Estonian Weather Service database (www.ilmateenistus.ee). Weather conditions varied during the experimental years as shown in Table 1. In 2017, the average temperatures were lower than long-term mean (1981–2010) by 0.3 °C in May, by 0.5 °C in June and 1.9 °C in July. In May 2017, the recorded rainfall was 28.2 mm less than the long-term average. In 2018, the average temperatures from May to August were higher compared to long-term mean. The temperatures were higher than the average of 1981–2010 by 4.7 °C in May, by 1.1 °C in June, and 2.7 °C in July. In the second experimental year the precipitation rate in May was 16.6 mm less and in July 32.2 mm less than the long-term mean. Moreover, the second decade of July in 2018 was recorded as extremely warm and dry.

Table 1. Average monthly temperatures (°C) and precipitation (mm) during vegetation period in experimental years (2017–2018) in Viljandi compared to long-term mean (1981–2010)

Year	Month			
	May	June	July	August
	Average T °C			
2017	10.1	13.9	15.5	16.3
2018	15.1	15.5	20.1*	18.1
1981–2010	10.4	14.4	17.4	16.3
	Sum of precipitation, mm			
2017	13.8*	60.9	79.4	81.7
2018	25.4	60.6	39.8*	94.3
1981–2010	42	69	72	83

*Month characterised with extreme meteorological conditions.

Experimental methodology

200 g of pooled samples of fully ripened berries from three bushes per cultivar were collected in the middle of the harvesting season in each experimental year (in July 2017 and 2018) and stored frozen at -20 °C until biochemical analysis. Fifty fruits from each sample were selected randomly, weighed (g) and the average fruit mass was calculated.

For the determination of biochemical parameters, 200 g of berries were homogenised using a kitchen blender (Philips Speed Touch 800 W, Netherlands). The soluble solids content (SSC; %) in the samples was measured at 20 °Cusing an ABBE refractometer (Abbe WYA-1S, Optic Ivymen System, Comecta S.A, Spain). Titratable acids content (TA; %, citric acid equivalent - eqv.) was determined by titration with

0.1 M NaOH using the Metrohm 905 Titrando automatic titrator driven by Tiamo 2.4 software (Metrohm, Switzerland). Polyphenolic compounds (including anthocyanins) were determined using UHPLC-DAD-MS/MS Shimadzu Nexera X2 system (Shimadzu, Japan). Polyphenols (including anthocyanins) were extracted from the homogenised samples using 50% EtOH solution acidified with 1% HCl (sample solvent ratio 1:20 w/v). Chromatographic separation of polyphenols was implemented on ACE Excel 3 C18-PFP (100 mm × 2.1 mm × 3 µm) column maintained at 40 °C. The total flow of the mobile phase was 0.25 mL min^{-1} and the injection sample size was 1 µL. The mobile phase gradient was mixed of two solvents: 1% formic acid in Milli-Q water (A) and 1% formic acid in methanol (B). The following multistep gradient program was used to separate the compounds: linear 15%–80% of mobile phase B from 0.01 to 27 min, linear 80%–100% B from 27 to 29 min, isocratic 100% from 29 to 33 min, the column was brought back to initial conditions with linear 100%–15% B from 33–39 min. The total polyphenols (TPC; mg 100 g^{-1}, chlorogenic acid eqv.) and total anthocyanins (TACY; mg 100 g^{-1}, cyanidin-3-*O*-glucoside eqv.) were quantified at the wavelengths of 280 nm and 520 nm respectively. Individual anthocyanins were identified by comparing retention times, UV spectra and parent and daughter ion masses with those of the standard compounds purchased from Sigma-Aldrich, Extrasynthese, Carbosynth and Chromadex. All the determinations were performed in triplicates on fresh weight (fw) basis.

Statistical analysis

Collected data of an average fruit weight and biochemical composition for the tested blackcurrant cultivars were analysed statistically using one-way ANOVA. The least significant differences (*LSD* 0.05) were also calculated. Different letters in tables and figures mark significant differences at $P \leq 0.05$. The linear correlation coefficients were calculated between the fruit weight and biochemical compounds with the significance of coefficients being $P \leq 0.01$. The strength of the relationships was estimated as $r \leq 0.30$ (weak), $0.31 \leq r \leq 0.70$ (moderate) and $r \leq 0.71$ (strong).

RESULTS AND DISCUSSION

The results of the average fruit weight and biochemical composition of the tested blackcurrant cultivars are presented in Tables 2, 3 and 4. The fruit weight (size), appearance, taste and its quality are the essential parameters when concerning the fresh consumption of blackcurrants. The average fruit weight of blackcurrant cultivars ranged between 0.6 and 1.4 g in both experimental years (Table 2).

Significantly highest fruit weight (1.3–1.4 g) was determined for 'Karri', 'Mara', 'Intercontinental', 'Syuita Kievska' and 'Mairi', when compared to control cultivar 'Titania' (0.9 g). These cultivars had large fruits also in earlier experiments and studies (Sasnauskas et al., 2009; Kikas & Libek, 2020). The fruit weight of the remaining cultivars ranged between 0.6 and 1.2 g and were not significantly different from the control. Some of the Estonian cultivars produced larger fruits when compared to others. Fruit weight has been one of the main aims of the blackcurrant breeding programme realized until present day.

Table 2. Fruit weight, biochemical composition and soluble solids: titratable acid ratio of the tested blackcurrant cultivars, average results of two experimental years (2017–2018)

Origin	Cultivar	FW, g	SSC, %	TA, %	SS to TA ratio	TPC, mg 100 g^{-1}	TACY, mg 100 g^{-1}
Belarus	Belorusskaya Sladkaya	0.8 ^{def}	22.9 ^{a}	2.9 ^{f}	7.9 ^{d}	401 ^{nop}	276 ^{jk}
	Katyusha	0.7 ^{ef}	16.3 ^{n}	2.6 ^{h}	6.4 ^{kl}	408 $^{m-p}$	278 ^{jk}
	Pamyat Vavilova	0.7 ^{ef}	16.6 ^{m}	2.9 ^{f}	5.7 ^{p}	354 st	237 ^{mn}
Estonia	Almo	1.2 ^{abc}	17.1 ^{l}	2.3 ^{k}	7.4 ^{e}	493 ^{def}	330 ^{ef}
	Asker	1.1 $^{a-d}$	17.1 ^{l}	2.6 ^{h}	6.6 ^{ij}	372 ^{qrs}	247 ^{lm}
	Ats	0.9 $^{c-f}$	18.3 ^{h}	2.5 ^{i}	7.3 ^{ef}	506 ^{cde}	358 ^{cd}
	Elmar	0.9 $^{c-f}$	17.1 ^{l}	3.2 ^{d}	5.4 ^{q}	414 ^{mno}	280 ^{ijk}
	Elo	1.2 ^{abc}	20.2 ^{d}	2.3 ^{k}	8.7 ^{b}	324 ^{u}	200 ^{pq}
	Karri	1.4 ^{a}	18.0 ^{ij}	2.3 ^{k}	7.8 ^{d}	390 ^{opq}	233 ^{mn}
	Mairi	1.3 ^{ab}	19.1 ^{f}	2.3 ^{k}	8.3 ^{c}	327 ^{u}	196 ^{pq}
	Varmas	1.1 $^{a-d}$	18.1 ^{ij}	2.7 ^{g}	6.8 ^{h}	454 ^{hij}	304 ^{gh}
Finland	Aström	0.7 ^{ef}	19.3 ^{ef}	2.7 ^{g}	7.2 ^{fg}	534 ^{b}	368 ^{cd}
	Kajaanin musta	0.9 $^{c-f}$	16.7 ^{m}	2.3 ^{k}	7.1 ^{g}	428 ^{lm}	278 ^{jk}
	Mortti	0.7 ^{ef}	17.9 ^{j}	2.3 ^{k}	7.9 ^{d}	466 ^{ghi}	313 ^{fgh}
Latvia	Mara	1.4 ^{a}	15.5 ^{p}	2.7 ^{g}	5.7 ^{p}	358 st	243 ^{lmn}
Lithuania	Dainiai	1.2 ^{abc}	17.1 ^{l}	2.4 ^{j}	7.1 ^{g}	328 ^{u}	207 ^{op}
	Gagatai	0.9 $^{c-f}$	16.0 ^{o}	2.0 ^{m}	8.2 ^{c}	470 ^{fgh}	322 ^{fg}
	Pilenai	1.0 $^{b-e}$	16.2 ^{n}	2.5 ^{i}	6.5 ^{jk}	420 ^{lmn}	277 ^{jk}
	Smaliai	0.9 $^{c-f}$	16.0 ^{o}	2.4 ^{j}	6.7 ^{hi}	453 $^{h-k}$	304 ^{gh}
Norway	Hedda	0.9 $^{c-f}$	16.3 ^{n}	3.8 ^{a}	4.3 ^{s}	290 ^{v}	183 ^{q}
	Narve Viking	0.8 ^{def}	16.3 ^{n}	2.3 ^{k}	7.1 ^{g}	477 ^{fgh}	322 ^{fg}
	Varde Viking	0.9 $^{c-f}$	19.2 ^{f}	2.5 ^{i}	7.8 ^{d}	634 ^{a}	471 ^{a}
Poland	Bona	1.2 ^{abc}	14.7 ^{q}	2.2 ^{l}	6.7 ^{hi}	362 ^{rst}	242 ^{lmn}
	Gofert	0.7 ^{ef}	22.3 ^{b}	2.5 ^{i}	9.1 ^{a}	391 ^{opq}	275 ^{jk}
	Ores	0.8 ^{def}	16.6 ^{m}	3.5 ^{c}	4.7 ^{r}	493 ^{def}	352 ^{d}
	Ruben	0.9 $^{c-f}$	17.2 ^{l}	3.0 ^{e}	5.8 ^{op}	440 ^{jkl}	305 ^{gh}
	Tiben	0.8 ^{def}	19.4 ^{e}	3.6 ^{b}	5.4 ^{q}	515 ^{bcd}	355 ^{cd}
	Tisel	1.1 $^{a-d}$	20.5 ^{c}	3.2 ^{d}	6.5 ^{jk}	385 ^{pqr}	261 ^{kl}
Russia	Zagadka	1.0 ^{cde}	16.2 ^{n}	2.7 ^{g}	6.1 ^{m}	460 ^{hij}	314 ^{fgh}
Scotland	Ben Alder	0.6 ^{f}	18.1 ^{i}	2.9 ^{f}	6.3 ^{l}	489 ^{efg}	330 ^{ef}
	Ben Avon	0.9 $^{c-f}$	17.7 ^{jk}	2.9 ^{f}	6.1 ^{m}	613 ^{a}	446 ^{b}
	Ben Finlay	0.7 ^{ef}	14.2 ^{r}	2.4 ^{j}	6.0 ^{mn}	469 ^{fgh}	300 ^{hi}
	Ben Lomond	0.9 $^{c-f}$	17.6 ^{k}	2.9 ^{f}	6.1 ^{m}	513 $^{b-e}$	348 ^{de}
Sweden	Intercontinental	1.3 ^{ab}	17.6 ^{k}	3.0 ^{e}	5.9 ^{no}	343 ^{tu}	222 ^{no}
	Titania*	0.9 $^{c-f}$	19.3 ^{ef}	2.9 ^{f}	6.8 ^{h}	441 $^{i-l}$	300 ^{hi}
	Triton	0.7 ^{ef}	18.6 ^{g}	2.9 ^{f}	6.4 ^{kl}	529 ^{bc}	375 ^{c}
Ukraine	Syuita Kievska	1.3 ^{ab}	13.7 ^{s}	2.5 ^{i}	5.5 ^{q}	424 ^{lmn}	296 ^{hij}

Different letters in columns mark significant differences at $P \leq 0.05$ (one-way ANOVA). FW – fruit weight, g; SSC – soluble solids content (%); TA – titratable acidity (%); SS to TA ratio – soluble solids and titratable acids ratio; TPC – total polyphenols content, chlorogenic acid equivalents; TACY – total anthocyanins content, cyanidin-3-glucoside equivalents; *Titania is the control cultivar.

Parameters, such as the soluble solids content (SSC), titratable acids (TA) and soluble solids to titratable acids (SS to TA) ratio, are important concerning fruit taste and the suitability for fresh consumption. The average SSC in fruit samples of analysed cultivars varied between 13.7 and 22.9% (Table 2). Fruits of cultivars 'Belorusskaya Sladkaya', 'Gofert', 'Tisel' and 'Elo' had significantly higher (20.2–22.9%), but 'Tiben', ‘Aström’, ‘Mairi’ and ‘Varde Viking’ had similar SSC when compared to control cultivar ‘Titania’ (19.3%). Pluta & Żurawicz (2014) reported that the fruit of ‘Gofert’ was richer in SSC than standard cultivars. The rest of the cultivars had significantly lower soluble solids in their fruits, but the lowest amount was determined in ‘Syuita Kievska’ (13.7%). As it was reported by Tian et al. (2019), the content of soluble solids mostly depends on weather conditions during fruit ripening and their final ripeness during harvesting.

The TA in the fruits of the tested blackcurrant cultivars was 2.0–3.8% (Table 2). Cultivars ‘Elmar’, ‘Hedda’, ‘Intercontinental’, ‘Ores’, ‘Ruben’, ‘Tiben’ and ‘Tisel’ had a significantly higher content of organic acids in fruits (3.0–3.8%), when compared to ‘Titania’ (2.9%). No significant difference in acid content was found in fruits of the control cultivar and ‘Beloruskaya Sladkaya’, ‘Ben Alder’, ‘Ben Avon’, ‘Ben Lomond’, ‘Pamyat Vavilova’ and ‘Triton’. The lowest TA was determined in the fruits of ‘Gagatai’ (2.0%). Similar results for the cultivar ‘Gagatai’ were reported previously by Sasnauskas et al. (2009) and Tian et al. (2019). It should be noted that the fruit of blackcurrant cultivars of Polish origin, except ‘Gofert’, tended to have a high organic acid content but Scottish cultivars had a somewhat lower acidity. These differences were likely due to the variable genetic background of the tested cultivars in this study and the diverse locations for cultivation including environmental factors, as reported by Tian et al. (2019).

The soluble solids to titratable acid (SS to TA) ratio varied 4.3–9.1 (Table 2). The ratio depended on the distribution of SSC and TA in the fruits. Cultivars ‘Almo’, ‘Aström’, ‘Ats’, ‘Belorusskaya Sladkaya’, ‘Dainiai’, ‘Elo’, ‘Gagatai’, ‘Gofert’, ‘Kajaanin musta’, ‘Karri’, ‘Mairi’, ‘Mortti’, ‘Narve Viking’ and ‘Varde Viking’ had a significantly higher SS to TA ratio (7.1–9.1) when compared to the control (6.8). There were no significant differences among the fruits of ‘Bona’, ‘Smaliai’, ‘Varmas’ and ‘Titania’. Similarly to the sugar to acid ratio, the SS to TA ratio can be used as a parameter to describe the sensory perception of the sweetness of the fruit.

Over the two experimental years, the TPC in the fruits of the tested blackcurrant cultivars was on average 290–634 mg 100 g^{-1} fw, and total anthocyanins ranged from 183 to 471 mg 100 g^{-1} fw (Table 2). The TPC in 2017 was somewhat lower than in 2018, 424 mg 100 g^{-1} fw and 449 mg 100 g^{-1} fw respectively. This was probably because in 2018 the temperatures were up to 5 °Chigher during the fruit ripening period when compared to 2017. The fruits of cultivars ‘Varde Viking’, ‘Ben Avon’, ‘Aström’, ‘Triton’, ‘Tiben’, ‘Ben Lomond’, ‘Ats’, ‘Ores’, ‘Almo’, ‘Ben Alder’, ‘Narve Viking’, ‘Gagatai’ and ‘Ben Finlay’ contained a significantly higher amount of polyphenols (466–634 mg 100 g^{-1} fw), while there were no differences between the control cultivar ‘Titania’ and ‘Zagadka’, ‘Varmas’, ‘Smaliai’, ‘Ruben’, ‘Kajaanin Musta’, ‘Mortti’, ‘Syuita Kievska’ and ‘Pilenai’ (420–460 mg 100 g^{-1} fw). The fruits of cultivar ‘Hedda’ had the lowest TPC (290 mg 100 g^{-1} fw). Correlation analysis revealed the negative relationship between the fruit weight and TPC in fruits (r = -0.473; Table 3). The compounds are more abundant in smaller fruits, because the fruit skin to pulp ratio is higher and the fruit skin contains more polyphenols than the pulp. Tian et al. (2019) reported a somewhat higher TPC in different blackcurrant cultivars grown in Finland, 598–2,798 mg 100 g^{-1} in dry weight (dw). In addition, these authors indicated a higher TPC in fruits of Scottish cultivars when compared to others. That phenomenon could be observed in our study too, as the four Scottish cultivars were leading in the fruit TPC, while Finnish cultivars were more variable in the content of these compounds.

Table 3. Correlation coefficients for the fruit weight and biochemical composition of the tested blackcurrant cultivars, average results of two experimental years (2017–2018)

Parameters	FW, g	SSC, %	TA, %	SS to TA ratio	TPC, mg 100 g^{-1}
SSC, %	-0.180				
TA, %	-0.219	0.175			
SS to TA ratio	0.082	0.520	-0.734		
TPC, mg 100 g^{-1} fw	-0.473	0.056	0.010	-0.002	
TACY, mg 100 g^{-1} fw	-0.485	0.080	0.058	-0.030	0.989

FW – fruit weight, g; SSC – soluble solids content (%); TA – titratable acidity (%); SS to TA ratio – soluble solids and titratable acids ratio; TPC – total polyphenols content, chlorogenic acid equivalents; TACY – total anthocyanins content, cyanidin-3-glucoside equivalents.

The total content of anthocyanins in fruits of tested blackcurrant cultivars differed on average of two years of studies and within each year. The average of total anthocyanin content for all cultivars was 287 mg 100 g^{-1} fw in 2017, but 305 mg 100 g^{-1} fw in 2018 (data not shown). Again, Tian et al. (2019) obtained much higher total content of anthocyanins (1,501 ± 587 mg 100 g^{-1} dw) in Finnish conditions. Similarly to polyphenols, the increased temperatures during fruit ripening in 2018 affected the content of total anthocyanins as well. In the present studies, the exception was the late ripening cv. ‘Varde Viking’, its fruits had higher content of anthocyanins in 2017 than in 2018. Overall the cv. ’Varde Viking’ had a remarkable content of anthocyanins. Similar results were also obtained in Lithuania (Stanys et al., 2019). According to Woznicki et al. (2015), it was revealed that there was a negative relationship between temperature and anthocyanin accumulation in the fruits of ‘Varde Viking’ in experiments conducted in Norway. Therefore, the cultivars’ response to temperature fluctuations differed probably depending on genotype. Cultivars ‘Ben Avon’, ‘Triton’, ‘Aström’, ‘Ats’, ‘Tiben’, ‘Ores’, ‘Ben Lomond’, ‘Ben Alder’, ‘Almo’, ‘Narve Viking’, ‘Varde Viking’ and ‘Gagatai’ had a significantly higher content of total anthocyanins in fruits, but ‘Zagadka’, ‘Mortti’, ‘Ruben’, ‘Varmas’, ‘Smaliai’, ‘Ben Finlay’, ‘Syuita Kievska’ and ‘Elmar’ contained a similar level (280–314 mg 100 g^{-1} fw) of these compounds as the control cultivar ‘Titania’ (300 mg 100 g^{-1} fw). As it was reported by Bakowska-Barczak & Kolodziejczyk (2011), fruits of ‘Ben Alder’ had also a high content of anthocyanins. The fruits of the rest of the tested cultivars had a significantly lower content of total anthocyanins compared to ‘Titania’, with the lowest amounts in ‘Elo’, ‘Mairi’ and ‘Hedda’ (183–200 mg 100 g^{-1} fw). In previous research conducted by Viškelis et al. (2012), the negative relationship between the anthocyanin content and fruit weight of blackcurrants was found, that is in agreement with the present study (r = -0.485; Table 3).

On the other hand, Finnish researchers found no significance between fruit weight and anthocyanins in the evaluated blackcurrant cultivars (Mattila et al., 2016). Most likely the content of these natural pigments depended rather on the genotype than fruit weight (Tian et al., 2019). However, anthocyanins could be affected by the fruit maturity stage of the crop (Raudsepp et al., 2010; Jarret et al., 2018). The highest concentrations of anthocyanins were accumulated in overripe small fruit berries (Viškelis et al., 2012). In our studies, a strong positive correlation between the contents of polyphenols and anthocyanins in the tested blackcurrant fruits (r = 0.989; Table 3) was found, as expected.

Table 4. Most abundant individual anthocyanins in the fruits of the tested blackcurrant cultivars, average results of two experimental years (2017–2018)

Origin	Cultivar	Dp-3-*O*-rut, mg 100 g^{-1}	Cy-3-*O*-rut, mg 100 g^{-1}	Dp-3-*O*-glu, mg 100 g^{-1}	Cy-3-*O*-glu, mg 100 g^{-1}
Belarus	Belorusskaya Sladkaya	114^{cd}	97^{bc}	35^{cd}	19^{bc}
	Katyusha	120^{cd}	86^{c}	43^{cd}	22^{b}
	Pamyat Vavilova	100^{d}	86^{c}	29^{de}	19^{bc}
Estonia	Almo	138^{cd}	111^{b}	46^{c}	24^{b}
	Asker	123^{cd}	70^{cd}	36^{cd}	15^{c}
	Ats	170^{bc}	120^{b}	44^{c}	19^{bc}
	Elmar	122^{cd}	85^{c}	44^{c}	21^{b}
	Elo	80^{de}	76^{cd}	28^{de}	14^{c}
	Karri	108^{d}	83^{c}	24^{de}	14^{c}
	Mairi	76^{de}	76^{cd}	29^{de}	15^{c}
	Varmas	143^{c}	110^{b}	31^{d}	15^{c}
Finland	Aström	162^{bc}	128^{ab}	47^{c}	24^{b}
	Kajaanin Musta	122^{cd}	97^{bc}	34^{cd}	16^{c}
	Mortti	131^{cd}	93^{c}	61^{b}	22^{b}
Latvia	Mara	117^{cd}	73^{cd}	34^{cd}	12^{c}
Lithuania	Dainiai	80^{de}	93^{c}	16^{e}	11^{c}
	Gagatai	140^{cd}	114^{b}	37^{cd}	21^{b}
	Pilenai	112^{cd}	101^{bc}	38^{cd}	22^{b}
	Smaliai	150^{bc}	86^{c}	44^{c}	18^{c}
Norway	Hedda	57^{e}	55^{d}	42^{cd}	20^{b}
	Narve Viking	161^{bc}	90^{c}	49^{bc}	16^{c}
	Varde Viking	218^{a}	134^{ab}	77^{a}	27^{b}
Poland	Bona	82^{de}	100^{bc}	33^{d}	26^{b}
	Gofert	108^{d}	85^{c}	50^{bc}	20^{b}
	Ores	141^{cd}	130^{ab}	44^{c}	25^{b}
	Ruben	122^{cd}	105^{bc}	48^{bc}	22^{b}
	Tiben	181^{b}	93^{c}	56^{bc}	18^{c}
	Tisel	108^{d}	76^{cd}	54^{bc}	20^{b}
Russia	Zagadka	131^{cd}	92^{c}	64^{ab}	24^{b}
Scotland	Ben Finlay	109^{d}	113^{b}	62^{ab}	40^{a}
	Ben Alder	179^{b}	153^{a}	71^{ab}	35^{ab}
	Ben Avon	124^{cd}	93^{c}	53^{bc}	22^{b}
	Ben Lomond	161^{bc}	119^{b}	34^{cd}	16^{c}
Sweden	Intercontinental	103^{d}	72^{cd}	33^{d}	13^{c}
	Titania*	129^{cd}	86^{c}	57^{bc}	19^{bc}
	Triton	214^{a}	113^{b}	26^{de}	11^{c}
Ukraine	Syuita Kievska	162^{bc}	91^{c}	33^{d}	14^{c}

Dp-3-*O*-rut – Delphinidin-3-*O*-rutinoside; Cy3-*O*-rut – Cyanidin-3-*O*-rutinoside; Dp3-*O*-glu – Delphinidin 3-*O*-glucoside; Cy-3-*O*-glu – Cyanidin 3-*O*-glucoside. Different letters in columns mark significant differences at $P \leq 0.05$ (one-way ANOVA); * Titania is the control cultivar.

The content of individual anthocyanins in fruits was typical for blackcurrants (Table 4). It was previously reported that glycosides of cyanidin and delphinidin (3-*O*-glucoside and 3-*O*-rutinoside) accounted for up to 97% of total anthocyanins in blackcurrants (Nour et al., 2013; Tian et al., 2019). In the present study, delphinidin-3-*O*-rutinoside was the most abundant anthocyanin in blackcurrants followed by cyanidin-

3-*O*-rutinoside, delphinidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside. The content of delphinidin-3-*O*-rutinoside was significantly higher in fruits of cultivars 'Varde Viking', 'Triton', 'Tiben' and 'Ben Alder' (179–218 mg 100 g^{-1} fw), but lower only in 'Hedda' (57 mg 100 g^{-1} fw) when compared to 'Titania' (129 mg 100 g^{-1} fw). The rest of the tested cultivars had a similar level of the compound with the control. According to Rubinskiene et al. (2005), the cyanidin and delphinidin rutinosides were the most stable during storage of the blackcurrant extract for 12 months at 8 °C. The blackcurrant fruits with a high content of these individual anthocyanins could have a good potential to be used in food products that are stored in cold for long periods.

The content of cyanidin-3-*O*-rutinoside was significantly higher in the fruits of the following cultivars: 'Almo', 'Ats', 'Varmas', 'Gagatai', 'Varde Viking', 'Ores', 'Triton', 'Aström', 'Ben Finlay', 'Ben Alder' and 'Ben Lomond' (110–153 mg 100 g^{-1} fw), but the lowest in 'Hedda' (55 mg 100 g^{-1} fw), (Table 4). The fruits of other tested cultivars contained a medium level of the compound and did not differ significantly when compared to control cv. 'Titania' (86 mg 100 g^{-1} fw). In previous studies, it was found that cyanidin-3-*O*-rutinoside was the most thermally stable anthocyanin (Rubinskiene et al., 2005). Therefore, these cultivars containing a high level of this particular anthocyanin could be of great interest in terms of food preservation and thermal processing.

Cultivar 'Varde Viking' had significantly the highest amount of delphinidin-3-*O*-glucoside in its fruits (77 mg 100 g^{-1} fw). Fruits of the other tested cultivars 'Elo', 'Karri', 'Mairi', 'Varmas', 'Intercontinental', 'Triton', 'Syuita Kievska' and 'Pamyat Vavilova' had a remarkably lower content (16–33 mg 100 g^{-1} fw) of this individual anthocyanin in comparison to 'Titania' (57 mg 100 g^{-1} fw). Most of the analysed blackcurrant cultivars had a similar content of the compound in fruit samples as the control and no significant differences were identified (Table 4).

A significantly higher amount of cyanidin-3-*O*-glucoside was determined in the fruits of Scottish origin 'Ben Finlay' (40 mg 100 g^{-1} fw), while the rest of the tested cultivars (11–35 mg 100 g^{-1} fw) did not differ significantly in the content of this individual anthocyanin in their fruit when compared to control cultivar 'Titania' (19 mg 100 g^{-1} fw). In general, from the tested cultivars all Scottish cultivars, Estonian 'Almo' and 'Ats', Finnish 'Aström' and Swedish 'Triton' revealed their high potential for individual anthocyanins.

CONCLUSIONS

The results of the present study give a broad overview of the biochemical properties of 37 blackcurrant cultivars with different origins, cultivated during 2017–2018 at Polli Horticultural Research Centre, Estonia. This essential information on fruit weight and the contents of polyphenols and anthocyanins provide guidelines for selecting suitable blackcurrant genotypes for the breeding programme, fruit production and possible product development. Generally, the total polyphenol and anthocyanin contents of blackcurrant fruits vary depending on cultivar, its origin and the year of investigation.

By country of origin, blackcurrant cultivars are summarized on the basis of the results obtained in comparison with the control cultivar 'Titania'. The fruit weight of Belarussian cvs. was similar to control, but 'Belorusskaya Sladkaya' had the highest

SSC and higher SS to TA ratio. Most of the Scottish cvs. evaluated were characterised by high TPC and TACY (except ‘Ben Finlay’), and fruits of ‘Ben Alder’ had the highest level of cyanidin-3-*O*-rutinoside. The Finnish cvs. were characterised by high SS to TA ratio. The Latvian cv. ‘Mara’ was distinguished with the largest fruits (1.4 g). From the Lithuanian cvs., ‘Dainiai’ had the largest fruits, whereas ‘Gagatai’, ‘Smailiai’ presente higher TPC and TACY. The Norwegian cvs., except ‘Hedda’, were characterised by high SS to TA ratio, TPC and TACY, moreover, ‘Varde Viking’ had the highest level of delphinidin-3-*O*-rutinoside and delphinidin-3-*O*-glucoside in its fruits. The Polish cvs. had similar fruit weight compared to control, while ‘Gofert’ and ‘Tisel’ presented high SSC and ‘Gofert’ had the highest SS to TA ratio. Russian cv. ‘Zagadka’ presented lower SSC, TA and therefore decreased SS to TA ratio as well. Swedish cvs. ‘Intercontinental’ had large fruits and higher TA; while ‘Triton’ showed higher TPC and TACY. Ukrainian cv. ‘Syuita Kievska’ had large fruits. Estonian cvs. ‘Karri’ and ‘Mairi’ were characterised by large fruits, ‘Almo’, ‘Ats’, ‘Elo’, ‘Karri’ and ‘Mairi’ had higher SS to TA ratio, but ‘Almo’ and ‘Ats’ had higher TPC and TACY.

The content of individual anthocyanins in blackcurrant fruits could be considered for the purpose of innovative product development. The results are preliminary collected from two years only, therefore further research is needed for final conclusions as the yearly differences in biochemical composition were affected by variable weather conditions. The future prospect of the studies is the evaluation and determination of technological properties of fruits of potential blackcurrant cultivars.

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Evaluation of the main biological and production traits of Latvian apple cultivars in the conditions of Central Russia

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Abstract. Apple selections of Latvian breeding were evaluated in the Central zone of Russia since 1980, in total 32 cultivars and hybrids. After long-term evaluation, the following can be recommended for use in breeding of scab resistant cultivars with high quality fruits - 'Dace' (gene *Rvi6*), 'Arona', and good storage - 'Edite' (*Rvi6*), 'Forele', 'Olga', 'Madona', for breeding of early cultivars - 'Roberts' and DI-93-4-8, both resistant to scab (gene *Rvi6*) and fruit rots. Cultivars and hybrids with the best cold resistance of vital tissues were selected by artificially modelling winter-hardiness components − early colds (1st component) and mid-winter colds up to -38 °C (2nd component), showing reversible damages not exceeding 2.0 points: 'Daina', 'Ella', 'Atmoda', 'Gita', 'Saiva', of which the last 3 maintained high hardiness of bark, cambium and xylem with slight increase of bud damages also at -40 °C. Cultivars 'Daina' and 'Ella' showed resistance of buds and vital tissues on the level of 'Antonovka' after modelling a thaw with following freezing to -25 °C (3rd component), which suggests tolerance to fluctuating winter temperatures. These cultivars demonstrated good adaptation to different environment conditions and may be considered in breeding of new adaptive apple cultivars with high fruit quality.

Key words: winter-hardiness, disease resistance, productivity, fruit quality, storage, breeding.

INTRODUCTION

Cultivars with good adaptation potential to various environment conditions, maintaining both productivity and fruit quality, have the highest chances of success in growing. Success of breeding work significantly depends on right evaluation and choice of parent material possessing high levels of valuable traits and ability to transfer these traits to their descendants. Already N. I. Vavilov had stated the problem of initial material for research in genetics and breeding. He wrote: 'Success of breeding work, as it is known, to a high degree depends on the initial material. This refers equally to self-pollinating and cross-pollinating plants'. Presenting a scientific base for the necessity to preserve and use the enormous diversity of the world's genetic resources, he stressed: 'The need for fundamental change of cultivars, in accordance with our harsh continental climate conditions, gives a first rate importance to wide involvement of new breeding material' (Vavilov, 1951). At present, problems of preservation and utilisation of plant genetic resource collections are of national and strategic importance for every country (Evans et al., 2012; Dzyubenko, 2015; Bramel & Volk, 2019).

Long-term experience of breeders worldwide shows the high effectivity of the right choice of hybridization parents and donors of valuable traits in the creation of new adaptive, competitive cultivars. In this aspect, comprehensive research of apple-tree winter-hardiness, tolerance to diseases and pests is necessary, along with other economically valuable traits, followed by genetic research and selection of sources and donors of these traits.

Most preferable for apple breeding is the involvement of promising cultivars and genotypes with maximal expression of valuable traits in their phenotype, having high genetic stability, low variability of characteristics in changeable environment conditions. To expand the genetic diversity of breeding and pre-breeding material, the modern apple breeding programs involve genetic material obtained by inter-species hybridization and complex pyramiding crosses (F2, F3 and F4), combining a range of valuable traits (winter-hardiness, scab resistance etc.) transferred through breeding to new adaptive, high quality apple cultivars (Evans, 2013; Kazlauskaya et al., 2013; Sansavini & Tartarini, 2013; Kozlovskaya, 2015; Kellerhals et al., 2020).

The oldest pomological institution in Russia - All-Russian Research Institute of Fruit Crop Breeding (further - VNIISPK) in 2020 will celebrate its 175-year anniversary. For all this time, VNIISPK maintains the established traditions. The genetic resources collection at the Institute is regularly extended with new genotypes from Russia and abroad. The main goal has always been not mechanical collecting, but obtaining of breeding material for hybridization, along with selection of sources and donors for most valuable traits.

The pomological collection of VNIISPK at present includes 860 accessions of apple cultivars and genotypes of different genetic origin from various geographic zones. Primary and collection evaluation is done for the main agronomical and biological characteristics: winter-hardiness, precocity, productivity, fruit market and consumption quality, length of storage, resistance to diseases, and suitability for growing in modern intensive plantations.

In the Central Russian zone of horticulture, the main limiting factor for successful cultivation of fruit trees are severe winters with low temperatures in autumn and mid-winter, thaws at the end of winter and deficit of warmth in summer. Other stress situations (drought, leaf water potential loss, soil water logging etc.) also have negative influence on fruit trees (Bhusal et al., 2019 and 2020). In these unfavourable conditions cultivars able to combine production characteristics with tolerance to abiotic factors have a special value.

Western European apple cultivars in this zone have shown sensitivity to low winter temperatures and perish after severe winters. Many-year investigations have shown that these cultivars are unsuitable for cultivation in the Central Russia zone also because of insufficient positive temperatures for fruit development. For this reason, a priority of research is investigation and choice of pre-breeding material and donors for creation of competitive new Russian apple cultivars, to establish intensive, profitable and adaptive orchards in this zone of fruit growing. The base for breeding is selection of initial material which is winter-hardy, drought resistant and tolerant to main diseases (Sedov, 2014; Sedov et al., 2014).

Since 1980 the collection includes hybrids and cultivars from Latvia, Institute of Horticulture. They originate from the Latvian apple breeding program aimed at disease tolerance, fruit quality, productivity, winter-hardiness and adaptation to the local climate (Ikase & Dumbravs, 2004; Ikase & Lacis, 2013). These cultivars and hybrids were obtained in the cool climate of Latvia with active growth period (temperatures over 10 °C) of 135−150 days and active temperature sum of 1,700−2,100 °C (Kļaviņš, 2016). Winter temperatures below −30 °C repeat each 5−10 years. The average annual air temperature is 6.4 °C, in February -3.7 °C, in July 17.4 °C, annual precipitation 692 mm. As Latvia is situated in the Northern European apple growing region with specific demands, apple cultivars are selected for their winter-hardiness and necessary length of vegetation period. Winter and spring temperature fluctuations as well as relatively abundant precipitation make these problems deeper. Results of their investigation at VNIISPK present significant interest in determining their value as initial material for further breeding in Russia, as well as their adaptability to climatic conditions more severe than in their homeland.

The aim of the study was to evaluate winter-hardiness of Latvian cultivars and hybrids by use of modelling of the components of winter-hardiness, as well as their productivity, disease resistance, fruit quality and storage, and to select cultivars suitable for use in further breeding.

MATERIALS AND METHODS

Samples of apples of Latvian breeding were studied in collection plots of All-Russian Research Institute of Fruit Crop Breeding (VNIISPK) in Orel region from 1980 till 2017. The Latvian cultivars and hybrids evaluated at VNIISPK included three groups. Cultivars selected before 1990 are 'Aivariņš', 'Alro', 'Ausma', 'Dainis', 'Forele', 'Iedzenu', 'Ilga', 'Sando', 'Stars'. The next group was obtained in repeated crosses of Latvian cultivars 'Iedzenu', 'Forele' etc. with 'Lawfam', 'Lobo', 'Melba', 'Starkrimson', 'Stark', 'Slava Peremozhtsam' and others. It includes cultivars 'Agra', 'Atmoda', 'Baiba' (Co), 'Daina', 'Eksotika', 'Ella', 'Madona', 'Olga', 'Saiva', 'Sapnis', hybrids AMD-12-15-15 (Andris), AMD-19-15-21 (Arona), AMD-12-9-13, AMD-12-9-16, DI-93-13-16. Further, these were involved in crosses with scab resistance donors (*Rvi6*) 'Liberty' and BM41497. This newest group includes scab resistant cultivars and hybrids 'Dace', 'Edite', 'Gita', 'Paulis', 'Roberts', DI-2-90-117, DI-93-4-8, and relatively resistant hybrids - DI-93-4-14 ('Dobeles Sidrābols'), D-18-94-15. The total number of evaluated cultivars and hybrids was 32.

VNIISPK is situated in the middle of Central Russian highlands at 203 m above sea level, in a zone of temperate continental climate. The average annual air temperature is 4.6 °C, absolute minimum -39.9 °C, absolute maximum 40 °C (2012). Sum of temperatures above 10 °C is 2,250 °C. The average annual precipitation is 550 mm, in April and May droughts and dry winds occur. The vegetation season is 175−185 days, active growth season (above 10 °C) is 130−149 days. Periodical extreme weather conditions in winter negatively affect the productivity of fruit crops, showing the need for adaptive cultivars. Comparing with Latvia, summer temperatures are slightly higher, but the climate is drier, with colder winters and more constant snow cover.

The harshness of winter colds can be characterized by a sum on average negative temperatures from November to March. During the research period the most unfavourable winters had sum of negative temperatures over 1,000 °C, continuing colds below -30 °C and average air temperature in winter below 0 °C (Table 1).

Table 1. Characteristics of winter conditions in Orel (Meteorological Station of VNIISPK)

Year	Sum of average negative temperatures, °C	Minimal temperature, °C		Average temperature in winter, °C	Days with thaws (December to February)
		air	at snow level		
1990/1991	605.4	-26.8	-25.3	-5.7	30
1991/1992	425.1	-22.1	-25.0	-4.7	40
1992/1993	592.9	-25.3	-25.2	-5.0	33
1993/1994	932.1	-29.7	-30.0	-5.8	48
1994/1995	537.6	-31.5	-36.7	-5.4	39
1995/1996	1,225.7	-32.0	-31.6	-10.3	8
1996/1997	708.5	-37.5	-28.0	-6.8	38
1997/1998	635.4	-31.6	-24.7	-6.0	35
1998/1999	832.6	-33.0	-34.2	-5.3	41
1999/2000	640.6	-28.4	-23.5	-4.6	54
2000/2001	642.7	-26.8	-28.3	-5.8	31
2001/2002	681.7	-30.0	-32.0	-6.2	42
2002/2003	1,099.6	-30.5	-33.0	-10.2	8
2003/2004	502.5	-24.5	-25.5	-5.2	17
2004/2005	746.8	-26.5	-30.5	-4.9	21
2005/2006	1198.1	-36.5	-39.3	-9.3	6
2006/2007	421.0	-27.2	-24.5	-3.1	43
2007/2008	424.4	-21.2	-22.5	-4.2	15
2008/2009	536.4	-19.5	-19.7	-4.9	16
2009/2010	1,033.0	-32.0	-31.0	-9.8	23
2010/2011	946.0	-34.2	-34.5	-8.6	5
2011/2012	694.1	-39.9	-34.0	-6.8	26
2012/2013	951.5	-31.7	-28.9	-6.6	35
2013/2014	576.1	-31.0	-29.3	-5.9	20
2014/2015	486.1	-24.5	-26.0	-5.1	31
2015/2016	499.3	-29.3	-26.3	-4.3	62
2016/2017	601.5	-24.0	-17.0	-6.6	28

Evaluation of the main agronomical and biological characteristics was done in trials planted in 1980 and 2005 on vigorous rootstocks (‘Antonovka’ seedlings) at distances 6×3 m, as well as top-grafted on a hardy, low-vigour frame-builder 3-17-38 in 2004–2006, planted at distances 5×2 m. For each cultivar 5 trees were planted. Cultivar ‘Antonovka’ was used as control. Evaluation was performed according with the accepted methods of cultivar testing (Sedov et al., 1999; Tyurina, 2002).

It must be noted that fruit look and taste were rated by a 5-point scale. The taste panel included 7–8 persons. Tasting was done at optimal eating maturity. Fruit look was rated as follows: 5 – fruits large, with regular shape, bright ground and over colour; 4 – fruits of acceptable size, attractive; 3 – fruits below medium size, mediocre attractivity; 2 – fruits small, poor colour and shape; 1 – fruits very small and unattractive. Fruit taste was rated as: 5 – excellent taste, dessert apple; 4 – good taste, table apple; 3 – mediocre taste; 2 – not suitable for fresh eating; 1 – inedible. The flesh structure was evaluated as coarse or fine, firm, soft or mealy. Flesh juiciness was evaluated as juicy, medium juicy or dry.

In field trials, winter damages of plant tissues (xylem, bark) and organs (buds, annual shoots, framework branches, trunk) were evaluated by a 5-point scale, where: 0 – no visual winter damage; 1 – very weak damages: xylem yellowish, small superficial bark damages on trunk and branches; 2 – weak damages: xylem light brown, weak superficial scald of bark, drying-out of annual shoots; 3 – significant damages: xylem brown, significant damages of bark with necrosis to xylem, die-back of some branches; 4 – very strong damages: xylem dark brown, heavy scald of bark, loss of a large part of tree crown; 5 – tree perished. All 5 trees of a cultivar were evaluated, with 'Antonovka' as control.

The apple cultivar testing program included evaluation of cold damages both in field and in controlled conditions, to determine the resistance potential by the following main components of the winter-hardiness complex (Krasova et al., 2013): 1st – resistance to early colds at end of November and beginning of December (with gradual temperature decrease to -5 °C, -10 °C, -25 °C and -5 °C, -10 °C, -30 °C); 2nd – maximum level of cold resistance after adaptation in January and February (-5 °C, -10 °C, -38 °C and -5 °C, -10 °C, -40 °C; -5 °C, -10 °C, -42 °C); 3rd – resistance in periods of thaw (-5 °C, -10 °C, +2 °C, -25 °C and -5 °C, -10 °C, +2 °C, -30 °C); 4th – ability to restore resistance by renewed adaptation after thaws (-5 °C, -10 °C, +2 °C, -5 °C, -10 °C, -30 °C).

Artificial freezing by modelling the damaging factors was done for Latvian cultivars and hybrids together with other cultivars in the genetic resources collection during 2009–2011 in freezing chamber ESPEC PSL-2KPN. Standard size annual shoots were collected in two replications. The length of temperature regimes was as following: adaptation at -5 °C (5 periods of 24 hours) and -10 °C (5 periods of 24 hours); after that decreasing temperature by 5 °C an hour till the critical temperature; critical temperatures for 8 hours; thaw +2 °C (2 periods of 24 hours).

Evaluation of damages of bud and shoot tissues was done by binocular microscope MBS-2 by the degree of browning, using a 5-pont scale: 0 – no damage, 5 – tissues and buds perished after the recovery period.

Evaluation of apple scab (*Venturia inaequalis* (Cooke) G. Winter) damages was done in field on the background of natural infection, in years favourable for disease development. The scab damage degree was evaluated by a 5-point scale, where: 0 – no visible infection, 1 – very weak infection with single lesions on leaves, small point-like lesions on fruits; 2 – up to 10% leaves with few, small lesions, on fruits small lesions, sporulation weak tor medium; 3 – up to 25% of leaf surface with infection, on fruits small and large lesions, sporulation medium; 4 – strong infection to 50% of leaves, lesions large, with dark abundant sporulation, on fruits many large lesions with cracking; 5 – very strong infection with large lesions on > 50% leaf surface, on fruits numerous merging lesions with cracks and dark sporulation. The average rating of leaf infection was determined by evaluation of 5 trees (25 leaves from 4 sides of tree). For evaluation of scab infection of fruits, a sample of 100 fruits from different trees was taken during harvest.

By the results of leaf and fruit scab infection levels evaluation, the cultivars were classified into the following groups: highly resistant (including gene *Rvi6* carriers), resistant, medium resistant and susceptible. The presence of resistance gene Rvi6 was determined at Institute of Horticulture, Latvia by molecular markers (Ikase & Lacis, 2013).

Processing of experimental data was done by dispersion analysis using *Microsoft Excel*, with significance level $p = 0.05$.

RESULTS

Winter-hardiness

Apple cultivars and hybrids of Latvian breeding demonstrated different resistance to unfavourable winter conditions in Orel region. During the trial period, the most unfavourable was winter of 2009–2010 with a sum of average negative temperatures 1,033 °C and average temperature in winter -9.8 °C (lower than the long-term average by 5.2 °C). January was the coldest, with average -16.4 °C which is 7.2 °C lower than the long-term average. Air temperature below -20 °C held for a long period and at the end of January fell to -32 °C. The soil freezing was insignificant. Also, in the winter 2011/2012 the minimum temperature was -39.9 °C. The autumn months before these winters were favourable for fruit tree adaptation. Significant winter damages of fruit tree vegetative parts were observed after extended warm autumn periods or in the end of winter when colds occurred after long-term thaws (years 2010, 2014 and 2015).

Artificial freezing by modelling factors of damage by components of winter-hardiness was done in 2009–2011. In common autumn conditions with gradual decrease of air temperature till the end of November or mid-December, most local cultivars developed resistance to early colds (1^{st} winter-hardiness component). This was true also for most Latvian cultivars subjected to artificial freezing, which showed high ability to increase resistance at modelling a temperature fall in mid-December to -25 °C after adaptation, without injury to buds, bark, cambium and xylem. When the shoot freezing temperature was lowered to -30 °C in the beginning of winter, the observed damages of buds were 0.7–1.5 points (control 'Antonovka' - no damage), damages of cambium, bark and xylem were insignificant (Table 2).

Table 2. Damage degree of Latvian apple cultivars and hybrids after modelling of damage factors in controlled conditions in mid-winter (average in 2009−2011), points

Cultivar, hybrid	Damage degree of buds: bark: cambium: xylem in points											
	-5°, -10°, -38 °C				-5°, -10°, -40 °C				5°, -10°, -42 °C			
	buds	bark	cambium	xylem	buds	bark	cambium	xylem	buds	bark	cambium	xylem
'Antonovka' (control)	0.6^{e}	0.4^{d}	0.4^{c}	0.7^{d}	1.5^{e}	1.4^{d}	0.8^{c}	1.0^{e}	1.9^{d}	1.9^{c}	1.4^{d}	2.2^{c}
'Aivariņš'	2.2^{ab}	1.6^{b}	1.6^{a}	1.5^{c}	2.7^{a}	2.0^{b}	1.5^{b}	1.9^{c}	2.7^{b}	2.0^{c}	1.9^{c}	2.6^{bc}
'Andris'	2.0^{b}	1.5^{b}	$1,0^{b}$	2.8^{a}	2.9^{a}	2.7^{a}	1.9^{a}	2.5^{b}	2.9^{b}	2.8	2.8	3.4^{a}
'Atmoda'	1.6^{c}	1.5^{b}	0.9^{bc}	1.1^{d}	2.0^{c}	1.8^{c}	1.3^{b}	1.5^{d}	2.3^{c}	2.0^{c}	1.8^{c}	4.0^{a}
'Ausma'	1.5^{c}	1.4^{c}	0.9^{bc}	2.6^{a}	2.4^{b}	2.1^{b}	1.6^{ab}	3.0^{a}	2.9^{b}	2.2^{c}	2.0^{c}	3.4^{a}
D-18-94-15	2.6^{a}	2.5^{a}	1.9^{a}	1.1^{d}	–				–			
'Daina'	1.8^{bc}	1.5^{b}	1.1^{b}	1.1^{d}	–				–			
'Dainis'	2.1^{b}	1.8^{b}	1.7^{a}	2.2^{b}	–				–			
DI-93-4-8	2.1^{b}	1.8^{b}	1.5^{a}	1.6^{c}	2.2^{b}	2.0^{b}	1.8^{a}	1.6^{d}	2.4^{c}	2.1^{bc}	2.1^{b}c	3.5^{a}
'Ella'	1.7^{c}	1.3^{c}	1.3^{b}	1.9^{b}	2.5^{ab}	2.4^{a}	2.0^{a}	2.0^{c}	3.4^{a}	3.1^{a}	3.1^{a}	3.3^{ab}
'Gita'	1.9^{b}	2.0^{b}	1.2^{b}	1.5^{c}	2.2^{b}	1.9^{bc}	1.5^{b}	1.7^{d}	3.6^{a}	3.1^{a}	2.1^{bc}	2.4^{c}
'Saiva'	1.7^{c}	1.4^{c}	1.2^{b}	1.2^{d}	2.1^{bc}	1.9^{bc}	1.7^{a}	1.9^{c}	3.2^{a}	2.7^{a}	2.6^{ab}	3.1^{b}

Note: different letters in the same column show significant differences between cultivars at $p = 0.05$ significance level.

Freezing to -38 °C showed that majority of tested cultivars had stronger damages than ‘Antonovka’. Statistical analysis showed that the tissue damage levels differed significantly (Ff > Ft) at $p = 0.05$ level. Yet damages of vital tissues and buds did not exceed 2.0–2.5 points and were reversible. Low damages at the level of ‘Antonovka’ had ‘Atmoda’, ‘Daina’, ‘Saiva’, D-18-94-15 for xylem, ‘Atmoda’ and ‘Ausma’ - for cambium (Table 2).

Artificial freezing of shoots to -40 °C increased bud and tissue damages. Most cultivars were injured stronger than ‘Antonovka’, and significant differences of damage degree of vital tissues were observed - for bark from 1.8 points (‘Atmoda’) to 2.7 points (‘Andris’), for xylem from 1.5 (‘Atmoda’) to 3.0 points (‘Ausma’). Yet for all tested cultivars the damages of cambium after artificial freezing at -40 °C were reversible - from 1.3 (‘Atmoda’) to 2.0 points (‘Ella’). Reversible (not exceeding 2.0 points) also were the bark damages of cultivars ‘Aivariņš’, ‘Gita’, ‘Saiva’, ‘Atmoda’, hybrid D-93-4-8 (Table 2).

Some cultivars had tissue damages on the level of ‘Antonovka’ also after artificial freezing to -42 °C. Damages of bark and cambium for ‘Atmoda’ and ‘Aivariņš’ and cultivar ‘Ausma’ for cambium did not exceed 2.0 points, and for ‘Aivariņš’ damages of xylem also were relatively low. Their buds showed slightly higher damages. Other cultivars and hybrids were damaged stronger than ‘Antonovka’. Significantly higher damages of buds and tissues were observed for ‘Andris’, ‘Ella’, ‘Saiva’, ‘Gita’, much exceeding control ‘Antonovka’.

In the end of winter and early spring as well as after thaws plant resistance to cold decreases, and if lower temperatures occur later, tree vegetative and generative parts can be damaged. Resistance of fruit trees to drying out due to water loss and tissue damage in this period is critical. Modelling of temperature below -25 °C after 2-day thaw at +2 °C (3rd component) allowed to ascertain the hardiness of buds and tissues on the level of ‘Antonovka’ for cultivars ‘Dainis’ and ‘Ella’, hardiness of bark, cambium and xylem for ‘Atmoda’, ‘Daina’, ‘Gita’, bark and cambium for ‘Saiva’. Higher damages than ‘Antonovka’ and other tested cultivars had ‘Andris’ and D-18-94-15 (Table 3). If modelling thaw was done with lower following temperature of -30 °C, all evaluated cultivars had damages of buds stronger than for ‘Antonovka’. By hardiness of cambium, bark and xylem cultivars ‘Atmoda’ and ‘Saiva’ were equal to ‘Antonovka’.

Table 3. Damage degree of Latvian apple cultivars and hybrids after modelling of returned cold after thaw in controlled conditions (temperature regime -5 °C, -10 °C, +2 °C, -25 °C), average in 2009−2011, points

Cultivar, hybrid	Damage degree of buds: bark, cambium: xylem in points			
	buds	bark	camb	xylem
‘Antonovka’ (control)	0.6^{c}	0.9^{c}	0.7^{d}	0.1^{b}
‘Aivariņš’	1.6^{a}	1.8^{a}	1.5^{ab}	0.8^{a}
‘Andris’	1.6^{a}	1.8^{a}	1.5^{ab}	0.8^{a}
‘Atmoda’	1.5^{a}	0.9^{c}	0.4^{d}	0.5^{b}
‘Ausma’	0.8^{c}	1.3^{b}	0.9^{c}	0.6^{a}
D-18-94-15	1.8^{a}	$1,7^{a}$	1.9^{a}	0.9^{a}
‘Daina’	1.5^{a}	1.0^{c}	0.7^{d}	0.4^{b}
‘Dainis’	0.9^{c}	0.2^{d}	0.0^{d}	0.2^{b}
DI-93-4-8	1.6^{a}	1.2^{b}	0.8^{c}	0.6^{a}
‘Ella’	1.0^{c}	0.9^{c}	0.7^{d}	$0,4^{b}$
‘Gita’	1.2^{b}	1.0^{c}	0.6^{d}	0.5^{b}
‘Saiva’	1.3^{a}	0.7^{c}	0.5^{d}	0.7^{a}

Note: different letters in the same column show significant differences between cultivars at $p = 0.05$ significance level.

Some cultivars were tested also for the ability to restore cold resistance of tissues after artificial thaw and following decrease of temperature in a regime -5 °C, -10 °C, +2 °C, -5 °C, -10 °C, -30 °C (4th winter-hardiness component). High restoration ability had 'Aivariņš', 'Ausma', 'Andris', 'Atmoda', 'Ella'. When temperature was decreased to -35 °C, 'Agra', 'Daina', 'Magone' and 'Iedzenu' had severe damages of buds.

Resistance to diseases

Most Latvian apple cultivars and hybrids by their resistance to apple scab (*Venturia inaequalis* (Cooke) G. Winter) significantly surpassed 'Common Antonovka', fruits and leaves of which could be damaged to 2.0–2.5 points (5-point scale). High resistance to scab and fruit rots was found for 'Dace', 'Edite', 'Gita', 'Paulis', 'Roberts', DI-93-4-8, DI-2-90-117, with scab resistance determined by the oligogene *Rvi6*. Highly resistant to scab and fruit rots were also 'Agra', 'Arona' and 'Forele' (Tables 4−6).

Productivity

Evaluation of apple cultivars in the trial planted in 1980 allowed to select the earliest yielding cultivars - 'Forele' which gave 5.8 kg per tree on seedling rootstock in 3rd year, and 'Stars' which yielded in 4th year. Other cultivars in this trial bore fruits much later. During long-term evaluation 'Forele' showed average productivity on the level of 'Antonovka' (26.9 kg per tree), from 20 to 50 kg annually. Similar productivity had also 'Iedzenu' (27 kg). Significantly lower and biennial production had 'Stars', AMD-12-9-13 and 'Alro' (Table 4).

Table 4. Productivity and fruit quality of Latvian apple cultivars and hybrids grown on vigorous seedling rootstocks (planted 1980, evaluated 1999−2002)

Cultivar, hybrid	Average yield, kg per tree*	Average fruit mass, g	Type of flavour	Fruit look/ taste, points 1−5	Flesh	Scab resistance	Length of storage, days
'Alro'	10.6^{a}	130	subacid	4.2/4.2	firm	good	65
AMD-12-9-13	17.8^{c}	120	subacid to acid	4.4/4.3	medium firm, fine	good	65
'Forele'	26.9^{a}	150	subacid to sweet	4.5/4.5	firm, crisp, aromatic	high	180
'Iedzenu'	27.1^{a}	150	subacid	4.5/4.3	firm, crisp, fine	medium	85
'Stars'	25.6^{b}	130	subacid	4.3/4.2	medium firm, fine	medium	65
'Antonovka' (control)	30.7^{a}	130	subacid to acid	4.3/4.2	medium firm, very aromatic	medium	65
LSD_{05}	4.7						

* differences of average yield are significant at $p = 0.05$ level.

In the trial planted in 2005 the earliest yielding cultivars were 'Dobeles Sidrābols', 'Edite', 'Gita', 'Paulis', 'Roberts', DI-93-13-16, having first flowers and fruits in 3rd year. In the age of 5−10 years 'Dobeles Sidrābols', 'Edite', 'Paulis', DI-93-13-16 as well as DI-2-90-117, D-18-94-15 had average yields significantly higher than 'Antonovka'. Other cultivars and hybrids gave yields like the control (Table 5).

Table 5. Productivity and fruit quality of Latvian apple cultivars grown on vigorous seedling rootstocks (planted 2005, evaluated 2009−2012)

Cultivar, hybrid	Average yield, kg per tree*	Average fruit mass, g	Type of flavour	Fruit look/ taste, points 1−5	Flesh	Scab resistance **	Length of storage, days
'Baiba' (Co)	7.0^{d}	160	sub-acid	4.3/4.5	juicy, fine	good	80
'Dace'	8.2^{d}	160	sub-acid	4.3/4.5	fine, mild, juicy, aromatic	high (*Rvi6*)	110
DI-18-94-15	12.0^{c}	180	sweet to sub-acid	4.7/4.3	fine, mild, juicy	good	150
DI-2-90-117	12.0^{c}	160	sub-acid	4.3/4.2	firm, juicy	high (*Rvi6*)	90
DI-93-13-16	13.9^{b}	130	sweet	4.4/4.2	medium firm, juicy	good	10 (very early)
DI-93-4-8	9.4^{d}	150	sub-acid to acid	4.3/4.2	melting, juicy	high (*Rvi6*)	120
Dobeles Sidrābols	16.1^{a}	130	sub-acid	4.3/4.3	firm, crisp	good	150
'Edite'	15.1^{b}	135	sub-acid	4.3/4.3	firm, juicy, aromatic	high (*Rvi6*)	90
'Gita'	10.1^{c}	140	sub-acid	4.3/4.2	firm, juicy	good (*Rvi6*)	90
'Paulis'	19.1^{a}	140	sub-acid	4.4/4.4	crisp, juicy	high (*Rvi6*)	90
'Roberts'	10.2^{c}	150	sub-acid	4.8/4.3	crisp, juicy, aromatic	high (*Rvi6*)	40 (early)
'Antonovka' (control)	8.0^{d}	130	sub-acid to acid	4.3/4.2	medium firm, very aromatic	medium	65
LSD_{05}	3.2						

* differences of average yield are significant at p = 0.05 level; ** − *Rvi6* − scab resistance oligogene.

In the top-grafted trial established in 2004−2006, first flowers and fruits in the 2nd year had 'Arona', 'Ella', 'Gita' and 'Olga'. In the age of 5−10 years 'Gita' significantly surpassed the control. Its average yield was 36 kg per tree (36 tons per ha). Other evaluated cultivars yielded on the level of the highly productive control 'Antonovka'. Among them 'Agra', 'Atmoda', 'Daina', 'Edite', 'Ella' exceeded 20 tons per hectare (Table 6).

Fruit quality and consumption period. Of the evaluated cultivars the earliest ripening was 'Agra', harvested in the end of July or early August, like 'White Transparent'. Its fruits were of medium size (130 g), with bright red over-colour, sub-acid, firm flesh, below medium juiciness. Fruits of hybrid DI-93-13-16 matured a few days later, but before 'Melba', its fruits were attractive and sweet. Late summer cultivar 'Baiba' with columnar tree had fruits of high market quality, medium to large, with attractive colour and fine, juicy and very tasty flesh; fruits could be stored till beginning of December, like 'Melba'. Fruits of the other late summer apple 'Roberts' were distinguished by attractive elongated shape and attractive, uniform dark red over-colour, juicy flesh and balanced taste (Tables 4−6).

Table 6. Productivity and fruit quality of Latvian apple cultivars on low-vigour frame builder 3-17-38 (top-grafted 2004–2006, evaluated 2009−2012)

Cultivar	Average yield, kg per tree*	Average fruit mass, g	Type of flavour	Fruit look/taste, points	Flesh	Scab resistance **	Length of storage, days
'Agra'	23.8^{b}	130	sub-acid	4.4/4.2	low juiciness	high	12 (very early)
'Aivariņš'	12.5^{c}	160	sub-acid	4.4/4.3	soft, fine	good	120
'Sapnis'	15.7^{c}	120	sub-acid	4.3/4.2	firm, fine	high	120
'Andris'	9.5^{d}	130	sub-acid	4.4/4.3	Firm	good	130
'Arona'	18.8^{c}	120	sweet	4.5/4.5	firm, juicy	high	110
'Atmoda'	21.3^{b}	125	sub-acid to acid	4.2/4.1	firm, juicy, aromatic	good	180
'Ausma'	19.5^{c}	140	sub-acid	4.4/4.3	medium firm	good	80
'Daina'	21.3^{b}	140	sub-acid	4.6/4.5	juicy, fine	good	95
'Edite'	24.7^{b}	160	sub-acid	4.4/4.5	firm, juicy, fine, aromatic	high (*Rvi6*)	160
'Ella'	21.7^{b}	150	sub-acid to sweet	4.3/4.5	soft, juicy, fine	good	130
'Gita'	36.0^{a}	160	sub-acid	4.5/4.4	firm, juicy	high (R*vi6*)	110
'Ilga'	18.0^{c}	160	sub-acid	4.5/4.5	firm, juicy, fine	good	105
'Madona'	19.7^{c}	150	sub-acid	4.5/4.3	juicy, aromatic	good	160
'Olga'	18.8^{c}	150	sub-acid	4.3/4.5	firm, juicy	good	160
'Saiva'	19.8^{c}	130	sub-acid	4.5/4.5	medium firm, juicy	good	95
'Sando'	18.8^{c}	140	sub-acid	4.4/4.3	firm, juicy	good	110
'Antonovka' (control)	18.0^{c}	140	sub-acid to acid	4.3/4.2	medium firm, very aromatic	medium	65
LSD_{05}	8.0						

* differences of average yield are significant at p = 0.05 level; ** − *Rvi6* − scab resistance oligogene.

The midseason cultivar 'Gita' had high market quality, large or over medium size, attractive, with dark red over-colour and greyish bloom, juicy, fine, pleasant flesh, and could be stored till beginning of January. 'Ausma' also ripened in autumn and in some years could be stored till end of January. Its fruits were of medium size, with attractive blush, pleasantly subacid, sometimes with excess acidity which decreased during storage. Short storage period (mostly till end of November) had midseason cultivars 'Stars', 'Alro' and hybrid AMD-12-9-13.

Cultivars ‘Daina’, ‘Ilga’, ‘Saiva’ had balanced sub-acid taste, juicy, fine flesh and high evaluation both of taste and appearance. Their fruits could be consumed till end of December or mid-January. Very good sweet taste with slight pleasant acidity, as well as bright attractive colour had late ripening ‘Ella’ and hybrid DI-18-94-15. Late cultivar ‘Iedzenu’ was strongly affected by storage diseases.

For full year fruit supply the cultivar ability to maintain good quality over an extended period is essential. Among the tested cultivars, good storage till end of February or beginning of March had ‘Edite’, ‘Madona’ and ‘Olga’. The longest storage had ‘Forele’ with attractive fruits keeping till April.

DISCUSSION

In the climate of Central Russia (Orel region) Latvian apple cultivars showed different levels of field resistance to unfavourable winter conditions. Previous investigation of Latvian apple genotypes in field (Krasova et al., 2014) allowed to find cultivars with good winter-hardiness on the level of old local cultivars from Central Russia and Baltic region - ‘Antonovka’ (‘Common Antonovka’), ‘Borovinka’ (‘Charlamowsky’), ‘Papirovka’ (‘White Transparent’) and ‘Osenneye Polosatoye’ (‘Streifling Herbst’). These were ‘Agra’, ‘Iedzenu’, ‘Dainis’, ‘Magone’. During unfavourable winters in the trial period they had low injury of xylem to 1.5 points. In Latvia, ‘Agra’ and ’Iedzenu’ also have shown high winter-hardiness in field, while data on ‘Dainis’ are insufficient, as its plantings are very few and only in the best orchard areas.

In the field study, a number of cultivars and hybrids showed medium winter-hardiness with regeneration of damages when evaluated in field, in the range of 2.1–2.5 points (stronger than for ‘Antonovka’): ‘Aivariņš’, ‘Arona’, ‘Baiba’, ‘Dace’, ‘Dobeles Sidrābols’, ‘Eksotika’, ‘Ella’, ‘Gita’, ‘Madona’, ‘Olga’, ‘Paulis’, ‘Roberts’, ‘Saiva’, ‘Sapnis’, AMD-12-9-16, DI-2-90-117. When temperature in field conditions fell below -30 °C and especially after thaws, stronger damages (3.0 points) had ‘Andris’, ‘Ausma’, ‘Daina’, ‘Edite’, ‘Sando’, DI-93-13-16, D-18-94-15, DI-93-4-8 (Krasova et al., 2014). In the milder climate of Latvia, all these have shown good or very good winter-hardiness in field (Drudze, 2000 and 2004; Ikase & Dumbravs, 2001).

To obtain more precise evaluation, the cold resistance potential of individual cultivars was determined by artificially modelling the components of winter-hardiness. The investigated Latvian cultivars and hybrids demonstrated high ability to reach adapted condition when subjected to modelling of gradual temperature decrease till -25 °C and -30 °C in mid-December, without damages of buds, bark, cambium and xylem (1st component).

Modelling of freezing temperatures -38 °C, -40 °C and -42 °C in mid-winter allowed establish the cultivar and hybrid resistance potential to very low temperatures and select the best genotypes by this component of winter-hardiness (2nd component).

In mid-winter (2nd component) after modelling of gradual temperature decrease to -38 °C, for majority of cultivars damages of vital tissues did not exceed 2.0 points and were reversible. Cultivars and hybrids with damages on the level of ‘Antonovka’ were ‘Daina’, ‘Saiva’, D-18-94-15 for xylem, ‘Atmoda’ and ‘Ausma’ for cambium. When temperature was lowered to -40 °C, several cultivars also showed good resistance of cambium, bark and xylem with damages of 2.0 points - ‘Aivariņš’, ‘Atmoda’, ‘Gita’, ‘Saiva’, hybrid DI-93-4-8. For these cultivars -40 °C is the threshold of hardiness after temperature adaptation. These data in general comply with previous field investigations (Krasova et al., 2014), but part of these cultivars in field had demonstrated only medium hardiness, which may be explained by the more complex character of field conditions.

Cultivars combining good resistance of all vital tissues to early colds and negative temperatures to -38 °C in mid-winter, with reversible damages not exceeding 2.0 points, were 'Ella', 'Daina', 'Atmoda', 'Gita', 'Saiva'. The last 3 maintained good resistance of bark, cambium and xylem with slight increase of bud damages also at -40 °C. It must be noted that in the Baltic climate these cultivars are considered to be only medium hardy (Drudze, 2000 and 2004; Univer & Univer, 2015), which may be explained by a shorter vegetation period and more frequent temperature fluctuations in winter.

Cultivars 'Atmoda' and 'Aivariņš' had bark and cambium damages on the level of 'Antonovka' even at -42 °C, and for 'Ausma' cambium damages also did not exceed 2.0 points. Other cultivars at this temperature had stronger injuries, and tissue damages of 'Andris', 'Ella', 'Saiva' were significantly higher than for 'Antonovka'. At the same time, while damages of bark and cambium for 'Atmoda' and DI-93-4-8 were low, their xylem showed poor hardiness at modelling temperature fall to -42 °C (damages 3.6−4.0 points). This complies with their low field hardiness observed during strong (below -40 °C) and prolonged colds in mid-winter (Krasova et al., 2014).

Modelling of thaws with following lowering of temperature to -25 °C (3rd component) showed good bud and tissue hardiness on the level of 'Antonovka' for cultivars 'Dainis' and 'Ella'.

In conditions of relatively high humidity favourable for the development of fungal diseases, selection of highly disease resistant cultivars gains increased importance. High resistance to scab and fruit rots was found for Latvian cultivars and hybrids with gene *Rvi6* inherited from BM 41497 and 'Liberty', which complies with authors' data (Ikase & Dumbravs, 2001, 2004). Highly resistant in the conditions of Orel were also some cultivars not possessing known resistance oligogenes - 'Agra', 'Arona' and 'Forele'. The first two have shown high disease resistance also in the moister climate of Latvia, while 'Forele' there is susceptible both to scab and fruit rots (Drudze, 2000). Reported cases of breakdown of gene *Rvi6* scab resistance with appearance of more virulent pathogen races (Kazlauskaya et al., 2009; Bus et al., 2011; Masny, 2017; Fisher et al., 2018) raises the need for search and utilisation in breeding of new sources of monogenic and high polygenic resistance sources.

High precocity and productivity surpassing control 'Antonovka' were found in 'Gita', 'Edite', 'Dobeles Sidrābols', DI-93-13-16. Equal to 'Antonovka' in this aspect were 'Arona', 'Forele', 'Olga'. Medium precocity, but fast production increase showed 'Paulis', D-2-90-117, D-18-94-15, while 'Agra', 'Ausma', 'Ella', 'Ilga', 'Madona' gave average, but annual yields. These last cultivars in Latvia have shown higher, but less regular production (Drudze, 2000 and 2004).

Fruit quality of the evaluated cultivars was good and similar to observations in Latvia. So, lack of juiciness for 'Agra' has been observed also in Latvia during hot, dry summers. On the other side, influence of climate differences was observed for some cultivar maturing and storage. Cultivar 'Ausma' in Orel ripens in autumn, while in Latvia it is a late cultivar with long storage. Also, fruits of 'Ilga' in Latvia after cooler summers develop poor colour but can be stored till March or longer. In Orel the longest storage had 'Atmoda', 'Edite', 'Forele', 'Madona' and 'Olga'. They show good storage potential also in Latvia (Drudze, 2000 and 2004), but 'Edite' there is sensitive to physiological disorders (Juhnevica-Radenkova et al., 2016). Cultivar 'Edite' in Orel had larger and brighter fruits on low vigour frame builder.

Cultivar 'Gita' showed very good results when top-grafted on a low-vigour frame builder - very early start of bearing, yields significantly higher than 'Antonovka', uniform ripening, bright colour, while on vigorous seedling rootstocks the yields did not surpass control, fruits were smaller and had weaker colour. In Latvia it shows some promise for commercial plantations and has been highly productive on dwarfing rootstocks (Rubauskis et al., 2015; Rubauskis & Skrivele, 2017).

CONCLUSIONS

- A number of Latvian cultivars showed good performance in the colder and more continental climate of central Russia, thus demonstrating good adaptation potential to different environment conditions, and may be considered in breeding of new adaptive apple cultivars with high fruit quality.
- By the results of many-year evaluation in Central Russia, the following can be recommended for use in breeding of scab resistant cultivars with high quality fruits - 'Dace' (gene *Rvi6*), 'Arona', and good storage - 'Edite' (*Rvi6*), 'Forele', 'Olga', 'Madona'. These cultivars after gradual cold adaptation can tolerate early colds to -30 °C and mid-winter colds to -38 °C with reversible damages.
- For breeding of early cultivars can be recommended 'Roberts' and hybrid DI-93-4-8 ('Liberty' x 'Latkrimson'), both resistant to scab and fruit rots.
- Cultivars and hybrids with the best cold resistance of buds, bark, cambium and xylem by artificially modelling winter-hardiness components - early colds (1^{st} component) and mid-winter colds to -38 °C (2^{nd} component) - were: 'Daina', 'Ella', 'Atmoda', 'Gita', 'Saiva'; the last 3 maintained high hardiness of bark, cambium and xylem with slight increase of bud damages also at -40 °C.
- Cultivars 'Daina' and 'Ella' showed resistance of buds and vital tissues on the level of 'Antonovka' after modelling a thaw with following freezing to -25 °C (3^{rd} component), which suggests good adaptation to fluctuating winter temperatures.

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Influence of vermicompost on strawberry plant growth and dehydrogenase activity in soil

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Abstract. Vermicompost is increasingly becoming popular as an organic fertiliser used for different crops. Effects of vermicompost on strawberry plant growth and soil properties were studied in this investigation. The research was performed in LatHort from 2015 to 2017. Strawberry was grown on open field in rows. Two trials were established. In Trial 1, the application of vermicompost with a dose of 50 mL per plant was compared to growing without any fertilization. In Trial 2, several treatments were used: 1) only inorganic mineral fertilization applied; 2) vermicompost applied with a dose of 100 mL per plant in planting holes, later mineral fertilization applied; 3) vermicompost applied with a dose of 100 mL per plant in planting holes, no additional fertilization applied; 4) vermicompost applied two times per season on the ground around plants with dose of 50 mL per plant, no mineral fertilization applied. The plant growth was assessed two times per season by evaluating the amount of leaves and plant height. Soil dehydrogenase activity was evaluated during all growing seasons as indicator of soil microbial activity. The application of vermicompost positively influenced plant growth in comparison to growing without fertilization. In Trial 2, plant growth varied among years. During first two growing seasons better plant growth was observed for plants fertilized by inorganic mineral fertilizers, while later the growth levelled off for all treatments applied. The application of vermicompost had positive influence on the soil dehydrogenase activity in contrary to fertilization by mineral fertilizers.

Key words: *Fragaria x ananassa* Duch., fertilization, vegetative growth, dehydrogenase activity.

INTRODUCTION

Strawberry is one of the most widely grown small fruits in Latvia with a tendecy to slowly increase area. In 2019, the plantations of strawberry reached 553 ha (Central Statistical Bureau of Latvia, 2019). Nowadays from the side of customers there is increased interest on fruits that are grown organically or with reduced use of chemical products. Chemical fertilizers are widely used to increase the yield and fruit quality, while at the same time they can reduce soil fertility over the years (Sinha et al., 2009). To replace chemical fertilizers, different organic materials can be used, and vermicompost is one of the possibilities. Vermicompost is the excreta of earthworm, where biodegradable wastes such as farm wastes, kitchen wastes, market wastes, bio-wastes of agro based industries, live- stock wastes etc. are converted passing through the worm-gut to nutrient rich compost (Adhikary, 2012). It is described as improver of soil health and nutrient status (Adhikary, 2012), the miracle plant growth promoter

(Sinha et al., 2009). It increases germination, growth, flowering, fruit production and accelerates the development of a wide range of plant species (Lazcano & Domínguez, 2011). The addition of compost improves soil physical properties by decreasing bulk density and increasing the soil water holding capacity (Weber et al., 2007). Further, soil application of vermicompost as supplementary dose has ability to improve availability of soil nitrogen, phosphorus and potassium (Singh & Sharma, 2016). Vermicomposts are rich in microbial populations and diversity and have large particulate surface areas that provide many microsites for microbial activity as well (Arancon et al., 2004). However, the effects of vermicompost on plant-soil systems are not yet fully understood when mechanism of enzymatic activities is concerned. Besides, the most of research is done in the USA and southern countries, while it is a less known organic fertilizer for northern European climate. The current study is confined to the response of plant growth in strawberry and dehydrogenase activity in soil after application of vermicompost alone or in combination with mineral fertilizers.

MATERIALS AND METHODS

Field experiments and treatments

The experiments were carried out at the Institute of Horticulture (LatHort) in Pūre, Tukums region, Latvia (57°02` N and 22°52` E). The experimental site was situated on a sandy loam soil with dolomite mother rock, pH_{KCl} - 5.9, organic matter - 2.5%, K_2O - 151 mg kg^{-1}; P_2O_5 - 194 mg kg^{-1}; Mg - 195 mg kg^{-1}; Ca - 1,050 mg kg^{-1}. Due to low potassium content, potassium sulphate was applied as a basic fertilizer for all field in a dose 26 g m^{-2} in the spring of 2015. Later soil analysis were performed within treatments in the autumn of 2015 and 2016. Results are shown in the Table 1.

Table 1. Results of soil analysis in Trials 1 and 2 in years 2015 and 2016

Indicator, sampling time / treatment		Trial 1: Unfertilized	Trial 1: Vermi-compost, 50 mL $plant^{-1}$	Trial 2: Only mineral fertilizers	Trial 2: Mineral fertilizers + vermi-compost, 100 mL $plant^{-1}$	Trial 2: Vermi-compost, 100 mL $plant^{-1}$	Trial 2: Vermi-compost, 50 mL $plant^{-1}$, 2x
Soil pH (KCl 1 n)	2015	6.0	6.0	6.0	5.9	6.0	5.9
	2016	6.3	6.2	5.9	6.0	6.3	6.4
Organic matter, %	2015	2.9	2.8	3.2	3.1	2.8	3.7
	2016	1.9	2.1	2.0	2.0	2.1	2.1
P_2O_5, mg kg^{-1}	2015	161	153	168	138	166	167
	2016	102	114	125	101	130	144
K_20, mg kg^{-1}	2015	90	106	124	91	121	107
	2016	170	196	248	383	156	230
Ca, mg kg^{-1}	2015	1,150	1,120	1,140	1,200	1,080	1,160
	2016	890	904	910	813	918	920
Mg, mg kg^{-1}	2015	231	243	274	231	243	249
	2016	177	168	170	151	185	203

Strawberries were planted on May 20, 2015 in rows with a planting distance 1.0 m between rows and 0.3 m between plants in rows. Cultivar 'Induka' was used in the investigation. Two trials were established. In Trial 1, two treatments were evaluated: 1) no fertilization was used during strawberry growth; 2) the vermicompost was applied with a dose of 50 mL per plant in planting holes before planting. In Trial 2, following treatments were used: 1) only inorganic mineral fertilization applied; 2) vermicompost was applied with a dose of 100 mL per plant in planting holes, later inorganic mineral fertilization applied; 3) vermicompost was applied with a dose of 100 mL per plant in planting holes during planting, no additional fertilization applied; 4) the vermicompost applied two times per season (in spring and at the end of summer) on the ground around plants with a dose of 50 mL per plant, no mineral fertilization applied. In both trials, treatments were replicated four times with the 15 plants per plot.

In Trial 2, in treatments with application of mineral fertilizers, 1.2 g plant^{-1} of N was applied in 2015 during season, 3.0 g plant^{-1} of N and 3.7 g plant^{-1} of K_2O were applied in 2016 and 2.6 g plant^{-1} of N and 1.9 g plant^{-1} of K_2O were applied in 2017 as inorganic fertilizers. In 2017, the doses were reduced because plants were not grown full season. The vermicompost used in both trials was produced by BIOEC (Bioorganic Earthworm Compost) Ltd from cattle manure and green grass using Californian earthworms. According to producer, it contains at minimum 0.5% N, 0.3% P_2O_5, 0.5% K_2O, microelements Fe, Ca, Mn, Mg, Zn, Cu, B, pH 7–8; organic matter - at minimum 30%.

Drip irrigation was used in the plantations. Plants were irrigated six times (from the middle of June to the end of August) in 2015, four times in 2016 (during May, June) and one time in 2017 (July). One liter of water per row meter was applied every irrigation time. Weeds were controlled mechanically and by hand weeding. During fruit ripening time the straw mulch was applied between rows. No chemical plant protection products were used in the trials. To prevent spreading of pests and diseases, all leaves and runners were cut and removed from the field after finishing of fruit harvesting in 2016.

Measurements and dehydrogenase activity analysis

The evaluations were performed for three growing seasons: 2015 to 2017. The plant growth was assessed in August of 2015, May and July of 2016 and May and August of 2017 by evaluating the amount of opened leaves and measuring plant height. Randomly selected 20 plants per treatment were evaluated.

Soil dehydrogenase activity (DHA) was evaluated during seasons 2016 and 2017 as indicator of soil microbial activity. The soil samples from every treatment were collected 6 times per season from the beginning of June till the end of August.

DHA activity was detected according to Kumar et al. (2013) method as modified by Dane & Šterne (2016). One gram of soil sample was exposed to 0.2 mL of 0.4% INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride) and 0.05 mL of 1% glucose in 1 mL distilled water for at least 6 hours. The formed INTF (p-iodonitrotetrazolium formazan) is extracted by adding 10 mL methanol and actively shaking for 1 min. INTF is measured spectrophotometrically at wave length 485 nm. DHA activity (amount of INTF) was calculated by formula:

$$\text{Amount of INTF } (\mu\text{L} \times L^{-1} \times \text{h}) = \frac{(-3a^2 + 4a) \times 86{,}400}{(60 \times h) + min}$$

where a – reading from spectrophotometer; h – incubation time in full hours; min – minutes over full hour.

Meteorological data

Meteorological data were obtained from an automatic field meteorological station (Luft) located at Pūre. Data from each year were different (Table 2). The season of 2015 characterized by very low amount of precipitation in June and hot and dry weather in August. In 2016, the average air temperature during the vegetation period was somewhat higher than in 2015 and there were more precipitation in this year than in previous. July was the hottest and wettest month in the season, while the lowest amount of precipitation was in September.

In season of 2017, the average temperature was lower than in previous years. Spring frosts were observed even at the beginning of June and there were large temperature fluctuations in May. While August was the warmest month of the season according to average air temperature. Almost all season was rather dray, while in September there were heavy rainfalls, though experiments were already finished on that time.

Table 2. Average air temperature and amount of precipitation during vegetation periods of 2015–2017

Month	Year	Precipitation, mm	Air temperature, °C		
			min	max	average
April	2015	34	-2.5	18.8	6.2
	2016	38	-5.5	16.7	6.2
	2017	35	-8.2	19.5	4.6
May	2015	39	-1.5	21.3	10.4
	2016	52	0.1	27.3	13.8
	2017	12	-5.1	32.3	10.7
June	2015	5	0.1	26.2	14.3
	2016	38	1.6	33.0	16.5
	2017	45	-0.4	28.6	15.0
July	2015	81	6.0	30.4	16.7
	2016	75	8.3	30.7	18.4
	2017	20	3.6	27.7	16.7
August	2015	15	3.2	32.3	18.0
	2016	70	4.5	28.3	16.6
	2017	12	4.1	31.7	17.0
September	2015	33	-1.0	26.6	13.2
	2016	18	1.9	27.6	13.4
	2017	174	0.5	22.4	12.7

Data analysis

Descriptive statistics, analysis of variance, followed by Fisher`s *LSD* (least significant difference) test ($P \leq 0.05$) and Pearson`s correlation were used for data analysis. The statistical analyses were performed using the MS Excel 2013.

RESULTS AND DISCUSSION

Plant growth

During investigation period strawberry plant growth changed within years mainly due to plants aging. It was also influenced by fertilizers applied. Changes were observed for both investigated parameters - amount of leaves and plant height.

Leaves are a very important part of a plant as they are the primary sites of photosynthesis and manufacture food for plants. The amount of leaves increased with the age of plants in all treatments and both trials. Some slight decrease was observed in the summer of 2017, which was influenced by cutting off all leaves in the previous summer to improve phytosanitary situation in the plantation. In Trial 1, application of vermicompost with the dose 50 mL per plant at planting increased the amount of leaves compared to growing without any fertilization (Fig. 1). While significant difference was observed only in the first growing seasons.

According to previous research, the vermicompost can contain significant quantities of plant growth hormones and humic acids, which act as plant regulators and can increase plant growth, and contains enzymes, which can break down the organic matter in the soil to release the nutrients and make it available to the plant roots that can succeed plant growth too (Nielson, 1965; Arancon et al., 2006b; Adhikary, 2012). Besides, vermicompost provides all nutrients in readily available form and enhances uptake of nutrients by plants (Adhikary, 2012). Research has shown that vermicompost has an effective role in improving growth of different field crops, including vegetables, ornamentals, cereals, nuts and fruit plants (Buckerfield & Webster, 1998; Reddy & Ohkura, 2004; Basheer & Agrawal, 2013; Kaur et al., 2015; Raha, 2015; Joughi et al. 2018; Dechassa et al., 2019). Several studies have also been done on it for strawberries (Arancon, et al., 2003; Arancon et al., 2004; Arancon et al., 2006a; Arancon et al., 2006b; Ameri et al., 2012; Singh et al., 2015; Zuo et al., 2018).

In the Trial 2, where different applications of vermicompost were compared to mineral fertilization, the amount of leaves significantly did not differ among treatments, except in May of 2016, when, in the treatment with application of vermicompost with dose 100 mL per plant, the amount of leaves was significantly lower than in the treatment with only mineral fertilization (Fig. 2). Later the amount of leaves increased in this treatment.

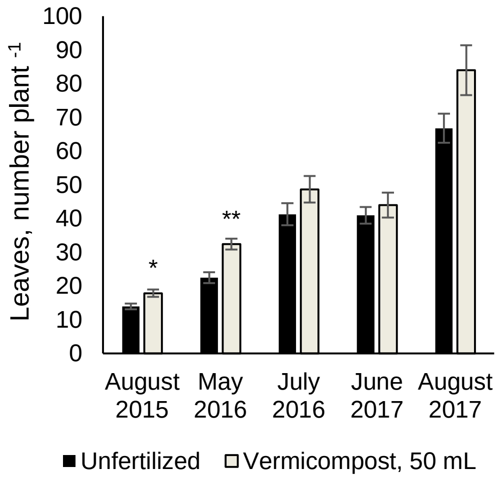

Figure 1. Number of leaves per plant in unfertilized plots and fertilized with vermicompost, dose 50 mL plant^{-1}, during three growing seasons and standard error bars. * – the difference among treatments significant at $P \leq 0.05$; ** – the difference among treatments significant at $P \leq 0.01$.

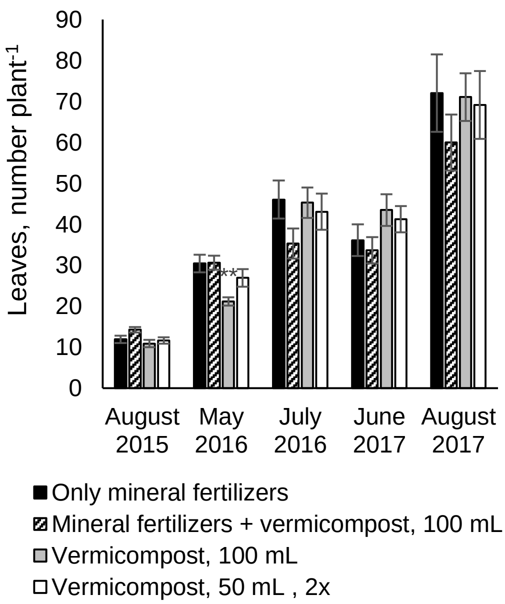

Figure 2. Number of leaves per plant in plots fertilized by mineral fertilizers and/or vermicompost during three growing seasons and standard error bars. ** – the difference among treatments significant at $P \leq 0.01$ compare to treatment with application of only mineral fertilizers.

The amount of leaves significantly correlated with the plant height ($P < 0.01$) in both trials. During growing years, the highest plant height was observed in the second growing season (Figs. 3, 4).

In the Trial 1, the application of vermicompost had positive effect on the increase of plant height compared to the unfertilized plots which might be associated with ability of vermicompost to improve photosynthesis rate, free radical scavenging and soil enzymatic activity as complemented by Zuo et al. (2018). While significant difference was observed only during the first growing seasons that was similar to the situation with leaf amount. The lower growth in the third season can be explained by utilising nutrients and plant growth stimulators provided, as vermicompost was given only at the planting, and increased plant age.

In Trial 2 in total, the biggest plant height was observed in the treatment with only mineral fertilization applied. In August 2015 and May 2016, in all other treatments the plant height was significantly lower ($P < 0.01$) than in the treatment with only mineral fertilization (Fig. 4). In July 2016, it was significantly lower in treatments with application of both - mineral fertilizers and vermicompost, and treatment with only vermicompost application two times per season with a dose of 50 mL per plant. Later plant growth levelled off in all treatments.

Vermicompost and inorganic fertiliser combination increased strawberry growth significantly in trials in the USA, Ohio, where vermicompost-treated plots were supplemented with appropriate

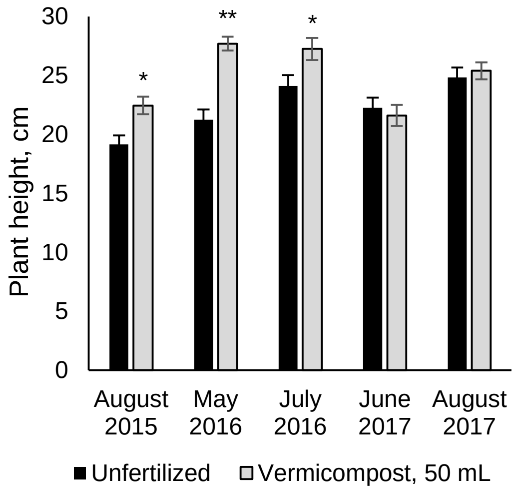

Figure 3. Plant height in unfertilized plots and fertilized with vermicompost, dose 50 mL plant^{-1}, during three growing seasons and standard error bars. * – the difference among treatments significant at $P \leq 0.05$; ** – the difference among treatments significant at $P \leq 0.01$.

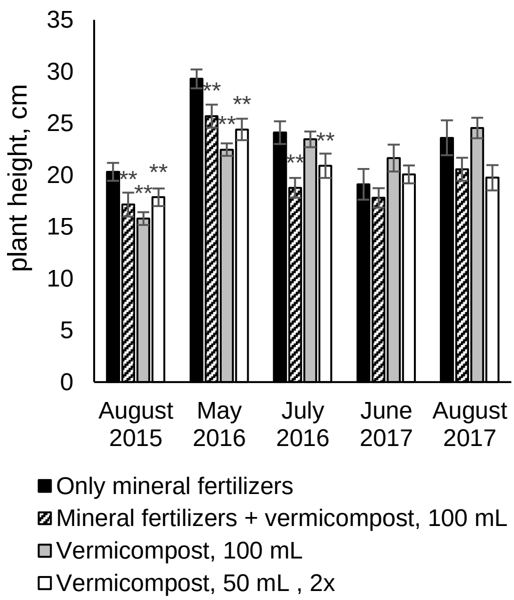

Figure 4. Plant height in plots fertilized by mineral fertilizers and/or vermicompost during three growing seasons and standard error bars. ** – the difference among treatments significant at $P \leq 0.01$ compare to treatment with application of only mineral fertilizers.

amounts of inorganic fertiliser to equalize the total recommended full rate of main macronutrients (Arancon et al., 2003; Arancon et al., 2004). In our trial, the vermicompost was given additionally to recommended doses applied by inorganic mineral fertilisers and the positive effect of combination of inorganic fertilization and vermicompost on strawberry plant growth was not observed, probably because of too high concentration of mineral nutrients. There was observed high increase of plant available potassium content in the soil in this treatment (Table 1).

Dehydrogenase activity in soil

The dehydrogenase enzyme activity was measured, as these enzymes are considered to be good indices of overall microbial activity (Nannipieri et al., 1990). According to Arancon et al. (2006a), levels of dehydrogenase activity are correlated positively and significantly with amounts of soil microbial biomass.

In our Trial 1, where application of vermicompost with dose 50 mL per plant at planting was compared to without fertilization, no significant difference was observed between treatments in dehydrogenase activity (data not shown). Probably the dose applied was too small to influence soil microbiota. Albiach et al. (2000) have reported about non-effectiveness of low doses of vermicompost on the increase of microbial biomass content and enzymatic activities after the application to a horticultural soil.

In Trial 2, where different applications of vermicompost were compared to mineral fertilization, significant differences were observed between treatments in the second (Fig. 5.) and third (Fig. 6) growing year, as well as significant differences were observed between sampling dates. In 2016, the lowest dehydrogenase activity was at the beginning of June and the highest it was at the end of July. While in 2017, it increased in June and decreased in July. It can be explained by different weather conditions during growing years. In 2016, the end of May and beginning of June were very dry, while in 2017, July and August were dry and it was not possible to irrigate sufficiently.

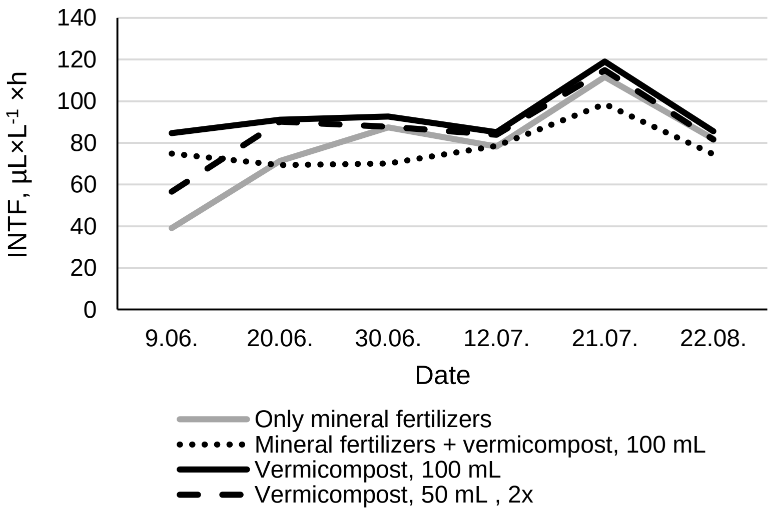

Figure 5. Soil dehydrogenase activity in plots fertilized by mineral fertilizers and/or vermicompost in the second growing year (2016). INTF, µL× $L{-1}$ × h

In the second growing year in general, both treatments with application of only vermicompost showed significantly higher ($p = 0.001$) soil microbial activity compared to treatments where mineral fertilizers were applied. In the treatment with split application of vermicompost (50 mL plant^{-1} two times per season), at the beginning of June the dehydrogenase activity was low, while later it increased. Whereas in the treatment with vermicompost application 100 mL per plant at planting, already at the beginning of June the activity was high and was the highest during all season. It shows that 100 mL of vermicompost is an amount big enough to be adequate for successful microbiota life cycle.

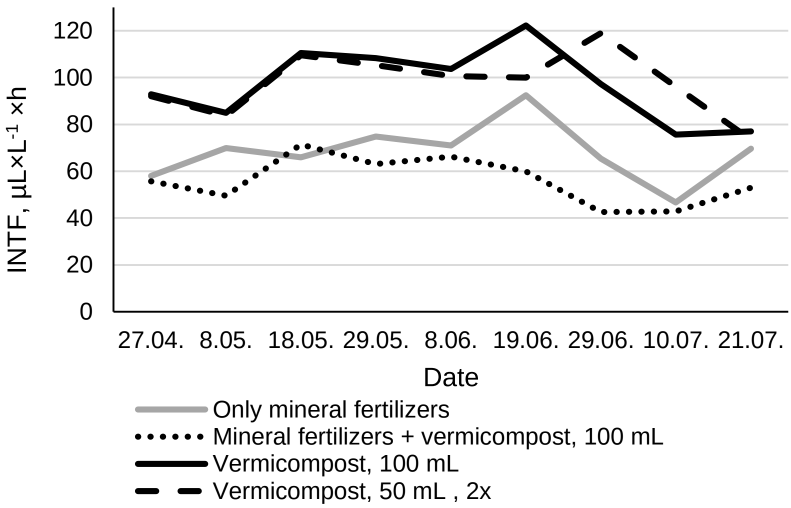

Figure 6. Soil dehydrogenase activity in plots fertilized by mineral fertilizers and/or vermicompost in third growing year (2017).

The same trend can be seen in the third growing year, where both treatments with application of only vermicompost showed significantly higher ($p < 0.001$) soil microbial activity compared to treatments where mineral fertilizers were applied, and the gap between mineral fertilized and only vermicompost fertilized treatments had become bigger. There have been several other reports that vermicompost fertilizers can increase soil microbiological activity (Atiyeh et al., 2001; Arancon et al., 2006a; Sinha et al., 2009), while mineral fertilizers adversely affect beneficial soil microorganisms and soil chemistry (Sinha et al., 2009).

CONCLUSIONS

Obtained results confirm that vermicompost has a positive effect on strawberry plant growth compare to non fertilization and can be used as organic fertiliser ir strawberry plantations. The dose 50 mL per plant at planting maintained positive effect for the first two growing seasons, while this dose did not influence overall microbial activity in soil. The application of only mineral fertilizers had a higher effect on strawberry plant growth than vermicompost, and combined fertilization (vermicompost and mineral fertilizers) during the first two growing seasons, while later this effect decreased. The application of mineral fertilizers reduced soil microbial activity compared to only vermicompost application that could negatively influence soil fertility in the future. More investigations on doses and application times for vermicompost are necessary.

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Effect of planting scheme on photosynthetic activity and dry matter accumulation in apple leaves

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Abstract. This study aims to identify changes photosynthetic rate and dry matter accumulation in apple leaves with decreasing plant to plant distance of the trees. Apple tree 'Auksis' was grafted on dwarfing P60 rootstock and planted at different in distances: 0.5 m, 0.75 m, 1 m and 1.25 m between plant to plant distance in rows. Photosynthetic indices were measured at 1.00–1.20 m above ground inside the canopy. 20 randomly selected leaves from the whole apple tree canopy were used to determine leaf area, fresh and dry weight. Measurements were made in three different stages in May, June and September. By decreasing the distance between apple trees from 1.5 m to 0.5 m, photosynthetic rate decreases correspondingly, decreasing by 23% in spring, and decreasing by 31% in autumn. Distance between trees has no significant impact on leave mass per area (LMA), however in spring is higher by 33–51% compared to summer and 42–78% compared to autumn. Dry and fresh weight ratio (DW/FW) significantly increased in summer - by 27% and in autumn - by 37% compared to spring, also DW/FW significantly decreased by the decreasing distance from 1.5 m to 0.5 m by 4–6%. In summary, the decreasing distance reduces the photosynthetic rate, the accumulation of dry matter. Also, photosynthetic rate decreases from spring to harvest time, and on the contrary, the accumulation of dry matter increases as autumn approaches. After evaluating the obtained results, the aim is to further delve into the use and transpiration of water and the impact of the planting scheme on fruit quality.

Key words: photosinthetic rate, competitive stress, *Malus domestica,* dry matter, Lithuania.

INTRODUCTION

The efficiency of photosynthesis depends on a complex of environmental factors: temperature, water content, CO_2 concentration, light intensity, nutrient content, etc. (Long, 2006; Yamori, 2016; Suvorova et al., 2017). In order to increase the productivity yield per unit area, the distance between seedlings per unit area is reduced, which causes competitive stress of the fruit trees on the main elements of photosynthesis (Fernández-de-Uña et al., 2016; Al-Namazi, 2017). Increasing density of trees reduces leaf illumination, increases shading, and reduces photosynthetic processes (Ishii, 2012; Manoli, 2017). Light becomes one of the most limiting factors and a key tool for regulating photosynthesis productivity in garden plants (Posada, 2009; Pengelly, 2010). The densely planted, tall, and vigorous trees have reduced light penetration in the lower and interior parts of the canopy, thereby reducing fruit yield, quality, photosynthetic rate (Kami, 2010; Bhusal et al., 2017).

Photosynthetic rate decreases with the penetration of light to the canopy and the illumination of the leaves (Cai, 2011; Dong et al., 2012). The efficiency of photosynthesis is strongly correlated with biomass content, decreasing photosynthesis also inhibits the formation of biomass of the most of plants (Boussadiaa, 2009; Hassiotou, 2009; Ghasemzadeh, 2010; Hassiotou, 2010; Gibsona, 2011; Gaju et al., 2016; Hüner et al., 2016). Also, there is correlation between seasonality and photosynthetic rate, studies show that photosynthetic activity of plants with higher photosynthetic intensity changes more seasonally (Zhang et al., 2017). The illumination intensity of the leaves strongly influences not only mass of biomass but also the size and structure of the leaves as reflected by leaf mass per area (LMA) (Riva, 2016). According to recent studies, leaves with higher LMA in the warm season showed lower photosynthetic rate for woody species and herbaceous plants (He et al., 2019). Although photosynthesis, dry matter accumulation in light deficiency studies have been conducted worldwide, major studies have been performed on tropical and subtropical plants (Posada, 2009; He, 2012), spruce (Ishii, 2012) and others.

Data on changes in medium-broadleaved deciduous trees, garden plants, apple trees, their photosynthesis and dry matter accumulation through tree density changes are lacking. According to global studies, as the distance between trees decreases, it is likely that the rate of photosynthesis and dry matter accumulation will decrease significantly from a certain distance. Therefore, this study aims to identify changes photosynthetic rate and dry matter accumulation in apple leaves with decreasing distance between fruit trees.

MATERIALS AND METHODS

Plant material and growing conditions

A field experiment was carried out in an intensive orchard at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Lithuania. The apple tree (*Malus domestica* Borkh.) cv. Auksis was grafted on super-dwarfing rootstocks P60. All trees were planted in 2001. Trees were planted in distances: 0.5 m, 0.75 m, 1 m and 1.25 m between trees in rows, while space between rows was 3 m. Pest and disease management was carried out according to the integrated plant protection practices, the orchard was not irrigated. Soil conditions of the experimental orchard were as follows: clay loam, pH 7.3, humus 2.8%, P_2O_5 255 mg kg^{-1}, K_2O 230 mg kg^{-1}. Measurements were performed in 3 replicates, 3 trees in each replicate, 3 times per season: in the end of May (leaves fully expanded BBCH 20-25), in the middle of July (beginning of apple maturity BBCH 73-75) and at the end of August (harvest time BBCH 87-88). A completely sunny day is chosen for the measurements.

Measurements

Photosynthetic rate was determined at 9:00–12:00 am by using a LI-6400XT portable open flow gas exchange system (Li-COR Biosciences, Lincoln, USA). Reference air [CO_2] (400 μmol mol^{-1}), light intensity (1,000 μmol m^{-2} s^{-1}) and the flow rate of gas pump (500 mmol s^{-1}) were set. Twenty measurements were made from three leaves (Fully mature leaves are selected) on 1.00–1.20 m above ground.

To determine the leaf area (cm^2), twenty leaves were randomly sampled from all tree canopy and measured with a leaf area meter (AT Delta - T Device, UK). The dry mass of twenty leaves was determined by drying apple tree leaves in 70 °C for 48 h. (Venti cell 222, Medcenter Einrichtungen, Gräfeling, Germany) to constant weight.

Leaf dry mass per area (LMA) was calculated from twenty leaves dry mass divided from twenty leaves area.

Meteorological conditions

The meteorological data were collected from 'iMetos' meteorological station at the Institute of Horticulture, LAMMC. Both years temperature was close or higher then multiannual (Fig. 1). 2018 was dry, natural drought was announced in Lithuania in 2018 (Lithuania, lat. 55°N, 2018). However, the trees had sufficient water content. While 2019 had 14% more rainfall than perennial, with particularly heavy rainfall on harvest time in August.

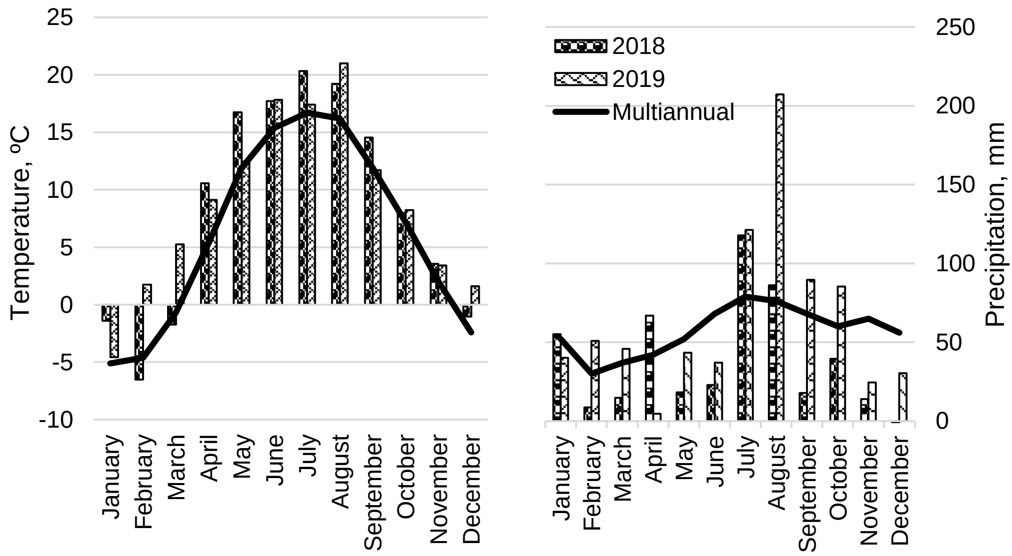

Figure 1. Meteorological conditions in two years and the total precipitation in 2018 and 2019 compared to multiannual (average of 100 years) conditions.

Statistical analysis

The data was processed using XLStat software (Addinsoft, 2019), and analyzed using Tukey (*HSD*) test at the confidence level $p = 0.05$. Data was processed using MS Excel software (version 7.0), standard deviation represents the mean of three replications.

RESULTS

The distance between trees had a significant effect on the photosynthetic rate. Although the season has influenced Photosynthetic rate (Pn) in individual years, no general trend has been observed, nevertheless, in spring the fall in photosynthetic rate with distance reduction is significantly less than in autumn. By decreasing the distance between apple trees from 1.5 m to 0.5 m, the rate of photosynthesis decreases correspondingly, decreasing by 23% in spring, and decreasing by 31% in autumn (Fig. 2).

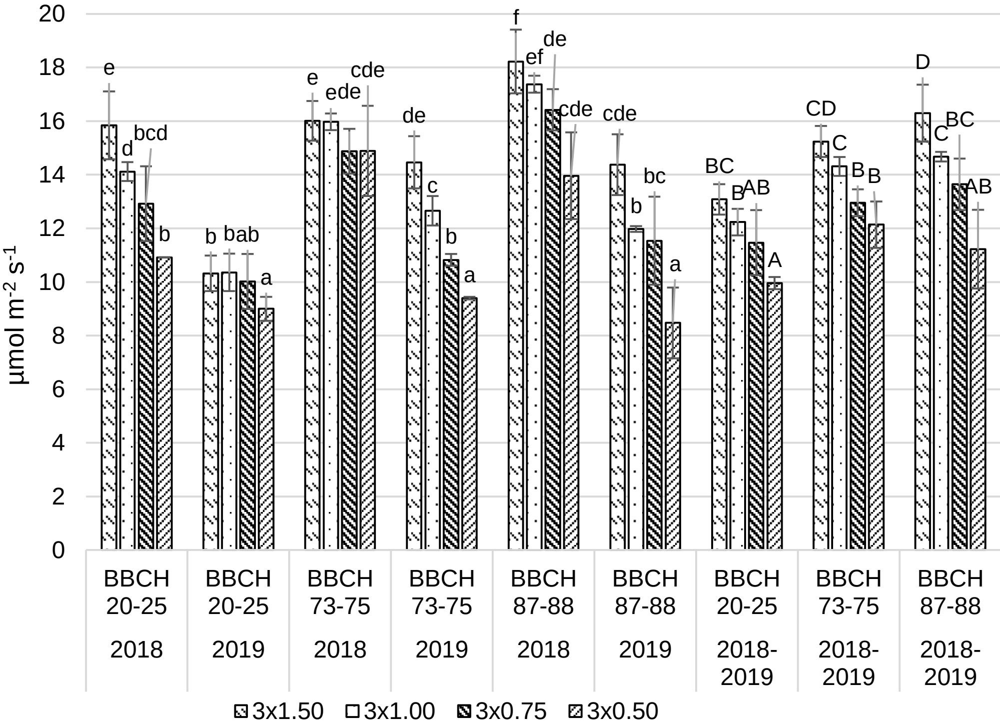

Figure 2. Changes in photosynthetic rate during the growing season on 'Auksis' apple trees leaves. Means followed by the same letter do not differ significantly at $P = 0.05$ according to Tukey multiple range; lower-case letters indicate significant differences in 2018 and 2019 and capital letters the significant differences in mean of years. Error bars shows standard deviation.

The increasing distance between apple trees had no significant effect on leaf mass per area (LMA), while the effect of seasonality was significant. The lowest LMA was found during the spring (BBCH 20-25), significantly higher in summer (BBCH 73-75) and autumn (BBCH 87-88), respectively 33–51% and 42–78% compared to spring (Fig. 3).

Dry and fresh weight ratio significantly different not only seasonally, but also by the increasing density of apple trees. DW / FW ratio increased by 27% (BBCH 73-75) and 37% (BBCH 87-88) compared to BBCH 20-25. As the distance between apple trees decreased, the DW / FW ratio decreased, with a significant decrease in the distance from 1.5 m to 0.5 m, which decreased by 4–6% in different seasons.

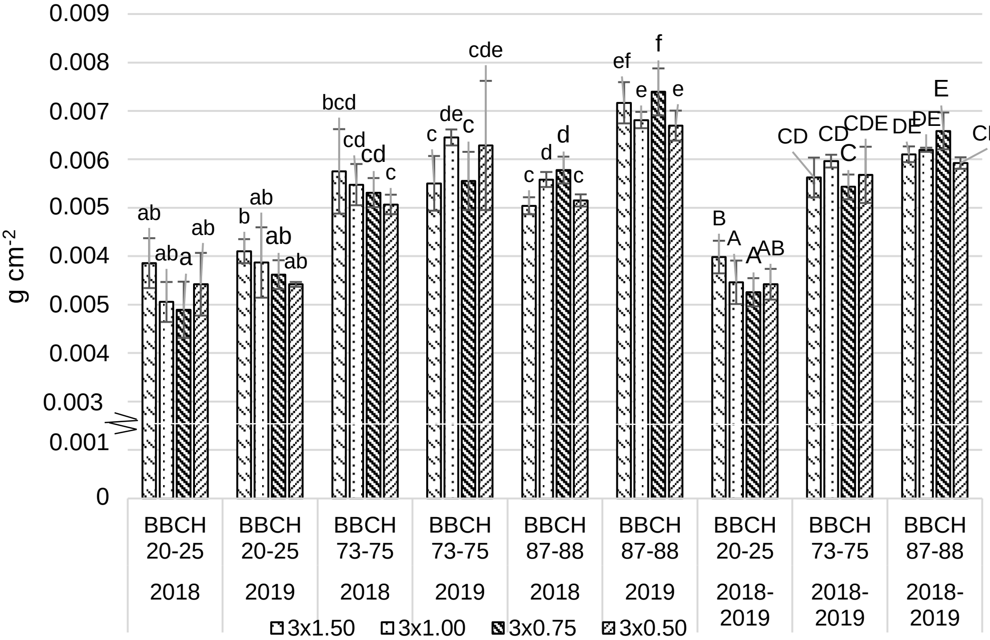

Figure 3. Leaf dry mass per area (LMA) in apple trees 'Auksis' during 2018 and 2019 (and average of two years) in three seasons of vegetation. Means followed by the same letter do not differ significantly at $P = 0.05$ according to Tukey multiple range; lower-case letters indicate significant differences in 2018 and 2019 and capital letters the significant differences in mean of years. Error bars shows standard deviation.

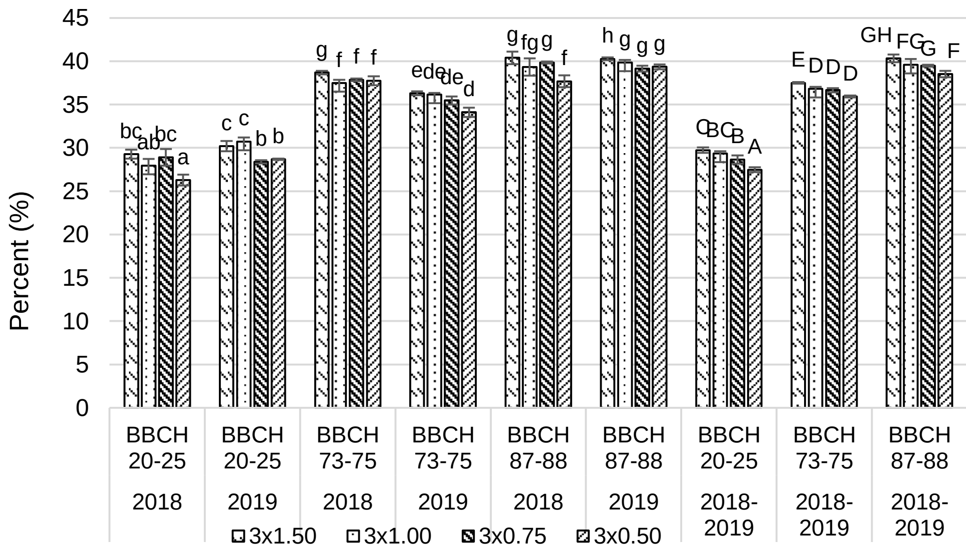

Figure 4. Dry and fresh weight ratio of 'Auksis' leaves during 2018 and 2019 (and average of two years) in three seasons of vegetation. Means followed by the same letter do not differ significantly at $P = 0.05$ according to Tukey multiple range; lower-case letters indicate significant differences in 2018 and 2019 and capital letters the significant differences in mean of years. Error bars shows standard deviation.

Positive strong and moderate correlations were found between the studied indicators (Fig. 5)

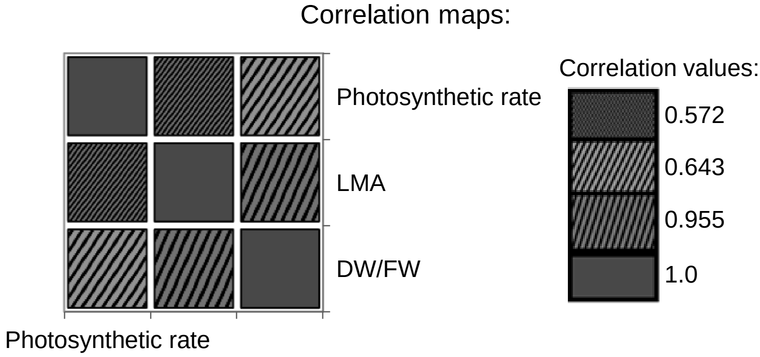

Figure 5. Correlation maps (by Pearson correlation matrix) between photosynthetic rate (Pn), leave mass per area (LMA) and Dry and fresh weight ratio (DW/FW).

DISCUSSION

The efficiency of photosynthesis is strongly correlated with biomass content, decreasing photosynthesis also inhibits the formation of biomass of the plant (Boussadiaa, 2009; Hassiotou, 2009; Ghasemzadeh, 2010; Hassiotou, 2010; Gibsona, 2011; Gaju et al., 2016; Hüner et al., 2016). However, results from our study show that dry matter accumulation per unit area varied slightly between apple trees planted at different distances (Fig. 2). High LMA (thick) leaves show low water loss due to thick cuticle layers and leaf thickness to be an important feature determining the efficiency of resource acquisition, water retention, and CO_2 assimilation and leaves with higher LMA in the warm season (Riva, 2016; Zhang et al., 2017; He et al., 2019) as well as when plant exposed to the scarcity of water (Bhusal et al., 2020). Although the leaf mass per area did not show a clear trend, the dry to green mass ratio was strongly correlated with the rate of photosynthesis (Fig. 4). Increasing tree density resulted in lower photosynthesis rate, more water retention and less dry matter accumulation in leaves (Fig. 2 and Fig. 4).

Decreasing light: under 30% sunlight, midday leaf net photosynthesis was reduced by 32%–44%, (Guenni et al., 2018). Excessive light can inhibit photosynthesis and biomass growth, shading could improve plant growth in *Torreya grandis* seedling, the optimum shade for these seedlings is 75% (Tang et al., 2015). Recent studies have shown that 20% shading increases the efficiency of apple photosynthesis, research also showed that shading substantially increased the size and weight of apples (Aoun & Manja, 2020). Our studies showed that at 3×1.5 m, apple trees, although shaded by one another, were active in photosynthesis, and with increasing competition and shading, the rate of photosynthesis tended to decrease (Fig. 2).

World-wide studies have found different photosynthetic responses to temperature. Some scientists have reported results that warmed plants have higher photosynthesis rates and higher optimum temperatures that maximize photosynthesis rates (Zhou et al., 2007; Niu et al., 2008; Gunderson et al., 2010; Crous, 2013). Others, meanwhile, say that warming of the temperature does not significantly affect the rate of photosynthesis (Bronson & Gower, 2010; Chi et al., 2013). However, changes in the length of the day go hand in hand with changes in temperature. Way & Montgomery (2015) in their research showed the importance of day length on tree growth. Some studies showed that photosynthetic capacity decreases with shortening day length. In addition, day length plays an important role in controlling the seasonal variation in gross ecosystem productivity (Busch et al., 2007; Bauerle et al., 2012; Stoy et al., 2014). Still, in Lithuanian conditions, when the day begins to shorten before the harvest, the photosynthesis rates increased, so the length of the day for apple trees of this variety had no negative effect on the intensity of photosynthesis (Fig. 2). Mean temperatures, however, remained similar, although the length of the day fell. From the obtained data we can state that photosynthesis rate of 'Auksis' apple trees was seasonally more influenced by temperature than day length (Fig. 1).

CONCLUSION

- By decreasing the distance between apple trees from 1.5 m to 0.5 m, photosynthetic rate decreases correspondingly.
- Distance between trees has no significant impact on LMA, however in spring is higher up to 50% compared to summer and up to 78% compared to autumn.
- DW/FW significantly increased in summer and autumn compared to spring, also DW/FW significantly decreased by the decreasing distance from 1.5 m to 0.5 m by 4–6%.
- In summary, the decreasing distance reduces the photosynthetic rate, and the accumulation of dry matter. Also, photosynthetic rate decreases from spring to harvest time, and the accumulation of dry matter inversely increases as autumn approaches.

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The effect of *Lamiaceae* plants essential oils on fungal plant pathogens *in vitro*

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Abstract. Fungal pathogens *Alternaria* spp., *Botrytis* spp. and *Colletotrichum* spp. cause a significant loss of horticultural crops and their yield annually. The most convenient way for controlling diseases caused by these pathogens is the use of chemical fungicides. However, current practices still result in soil, water and air pollution, contribute to the loss of biodiversity and climate change, also are harmful to human health. Therefore, there is a growing demand for environmentally friendly plant protection methods. Herbs, especially, volatile oils, are a natural source of active ingredients. The findings of antimicrobial and antifungal activities, low toxicity, and biodegradability of essential oils make them potential for use in plant protection against pathogens instead of chemicals. This research aimed to evaluate the ability of *Lamiaceae* plants essential oils to suppress the growth of *Alternaria* spp., *Botrytis* spp., and *Colletotrichum* spp. *in vitro*. The study was carried out at the LAMMC Institute of Horticulture, Lithuania. Essential oils from lavender (*Lavandula angustifolia*) and thyme (*Thymus vulgaris*) were obtained by hydrodistillation and poured to potato dextrose agar medium at 200–1,000 µL L^{-1} concentrations. The radial colony growth of each pathogen measured after placing mycelial plugs of each fungus on Petri dishes. Results demonstrated that thyme essential oil significantly suppressed the growth of all three investigated fungal pathogens at concentrations starting from 400 µL L^{-1} 7 days after inoculation as no growth of the pathogens observed. Meanwhile, lavender essential oil had lower antifungal activity than thyme. The most significant concentration of lavender essential oil was 1,000 µL L^{-1}. To conclude, thyme essential oil showed high antifungal activity, and lavender essential oil showed moderate antifungal activity for our tested horticultural crop fungal pathogens. Both oils can be applied as one of the eco-friendly ways to control plant pathogens.

Key words: antifungal, inhibition, *Lavandula angustifolia,* lavender, *Thymus vulgaris*, thyme.

INTRODUCTION

Nowadays about 80% of foods are of plant origin due to new consumer habits. The safety of agricultural products means much more than the quantitative and qualitative safety of the foods produced (Carvalho, 2017). There are many factors affecting plant health, and fungal pathogens are ones of them. Diseases caused by pathogens have a significant economic impact on plant production for different crops (Survilienė et al., 2010; Singh et al., 2015; Boddy, 2016; He et al., 2019). It may infect plants though miscellaneous parts at diverse growth stages, and resulting in high losses of the plants or whole yield (Patriarca et al., 2014; Neri et al., 2016; He et al., 2019; Rosero-Hernandez et al., 2019). Anthracnose, caused by *Colletotrichum* species, and grey mould, caused by *Botrytis cinerea,* are primary diseases of strawberry, nevertheless, could be found among other hosts as well (Boddy, 2016; Rasiukevičiūtė et al., 2018; He et al., 2019; Jakobija et al., 2020). A common fungal genus *Alternaria* spp. causes Alternaria leaf spot or Alternaria leaf blight that leads to pre- and postharvest damage to agricultural products, including vegetables, fruits, and cereal (Patriarca et al., 2014). In addition to spoiling a wide variety of foods, some *Alternaria* species can produce secondary metabolites considered as mycotoxins, which can be harmful to humans and animals, and phytotoxins, which play an essential role in the pathogenesis of plants (Lõiveke et al., 2004; Kütt et al., 2010; Patriarca et al., 2014).

Plant protection products, such as chemical fungicides, are used to protect crops from fungal diseases (Survilienė & Dambrauskienė, 2006; Abbey et al., 2019). Based on research in recent years, it can be confirmed that pesticide residues are present in about half of food products, and several of them can be detected quite often in various products (Carvalho, 2017). The combined effects may be complementary, but there are also cases where active substances in different chemical plant protection products enforce each other`s adverse effects. It is known that several plant protection products have a more substantial overall impact on each other`s presence. The current legislation does not address the overall effects of active substances (Sharma et al., 2019).

Moreover, surface waters and soil are contaminated with fungicides remains too, and the global warming increases toxicity of pesticides at higher temperatures (Op de Beeck et al., 2017; Sharma et al., 2019). Additionally, a significant impact on pathogen resistance is observed, which causes difficulties in controlling diseases and imbalance the microbial community in soil. According to the requirements of EU Regulation 2009/128/EC, reducing chemical products residues is one of the primary purposes of engaging in crop production. It is essential to use those active substances in disease control that address specific plant protection problems and pose the least risk to the environment and human health (OJEC, 2009).

Therefore, to solve pesticides contamination problems, several research groups have explored a natural source of effective ingredients to control soil-borne pathogens. The findings of low toxicity, biodegradability, antifungal and antimicrobial activities of plants essential oils allow us to use eco-friendly plant protection instead of chemical (Abdolahi et al., 2010; Hussein & Joo, 2017; Reang et al., 2020; Šernaitė et al., 2020). Essential oils have been widely studied and applied in the food, medicine and cosmetic industries (Zabka et al., 2014; Carvalho, Estevinho & Santos, 2016; Karpiński, 2020).

Lamiaceae family plants are found all over the world, and many are familiar garden herbs such as rosemary, oregano, mint, basil, lavender and thyme. As a result, it becomes a cheap raw material and the application of these herbs essential oils for horticultural crop fungal pathogens control as a biofungicides represents an alternative disease management strategy due to its ability to provide environmentally friendly disease control (Feng et al., 2011; Mamgain et al., 2013). Various essential oils for the management of *Alternaria* spp., *Botrytis* spp., *Colletotrichum* spp. and other fungal pathogens in field and greenhouse conditions have been investigated (Abdolahi et al., 2010; Sarkhosh et al., 2018a; Awais et al., 2020). However, in reviewing the available literature, it was observed that there is a lack of studies on the antifungal activity of thyme and lavender essential oils against horticultural crop fungal pathogens.

This research aimed to evaluate the ability of *Lamiaceae* plants essential oils to suppress the growth of *Alternaria* spp., *Botrytis* spp., and *Colletotrichum* spp. *in vitro*.

MATERIALS AND METHODS

The research was carried out at the LAMMC Institute of Horticulture (LAMMC IH) Laboratory of Plant Protection in 2018–2019. Essential oils (EOs) of lavender (*Lavandula angustifolia* Mill.) and thyme (*Thymus vulgaris* L.) herbs were selected for this study according to their antifungal and antimicrobial activities, which have the potential to be effective against plant pathogens such as *Alternaria* spp., *Botrytis* spp., and *Colletotrichum* spp.

Essential Oils Extraction

Thymus and lavender herbs were collected from the experimental fields of LAMMC IH and naturally dried. According to the Association of Official Analytical Chemists (AOAC, 1990) methods, one kilogram of each plant material was extracted using a Clevengerdistillation system (Glassco, India). The time of each materials extraction was 2 hours under normal atmospheric pressure.

Efficacy of Essential oils

The lavender and thyme essential oils separately poured into the potato dextrose agar (PDA) medium at concentrations of 200, 400, 600, 800, and 1,000 μL L^{-1}, homogenised and distributed in sterilised Petri dishes at a temperature of 45 °C. Mycelium plugs (6-mm-diameter) of 7-day old single spore isolates of *Alternaria* spp., *Botrytis cinerea*, *Colletotrichum* spp. (from LAMMC IH Laboratory of Plant Protection isolate collection)were cut and placed fungal side down in the centre on each Petri dish with the PDA and the tested EOs option. Pathogens obtained from infected carrot (*Alternaria* spp.) and strawberry fruits (*B. cinerea*, *Colletotrichum* spp.) were identified by using of 10x and 40x magnifications on the microscope evaluating their sporangiophores, sporangia, hyphae, conidiophores, conidia, colony texture and growth pattern (Simmons, 2007; Kumar & Kudachikar, 2018; Rasiukevičiūtė et al., 2018). There were four replications of the same treatment. Plates incubated at 22 ± 2 °C temperature in the dark for 7 days. After 2, 4, and 7 days inoculation (DAI), radial growth (cm) of mycelium (including the diameter of the disc) was measured and compared with the results of the control.

Statistical Analysis

SAS Enterprise Guide 7.1 program (SAS Institute Inc., Cary, NC, USA) was applied for the analysis of experimental data. Analysis of variance (ANOVA) procedure was processed, and Duncan`s multiple range test ($p < 0.05$) was used for the comparison of obtained means.

RESULTS AND DISCUSSION

The ability of EOs from *Lamiaceae* plants to suppress the growth of *Alternaria* spp., *B. cinerea*, *Colletotrichum* spp. was investigated on PDA under different concentrations.

The antifungal effect of thyme and lavender EOs on *B. cinerea* colony growth 2, 4 and 7 DAI is presented in Fig. 1. Different EOs exhibited various antifungal activities. The thyme EO concentrations of 400, 600, 800, and 1,000 µL L^{-1} significantly inhibited mycelium growth ($p < 0.05$) through the whole experimental period. However, the effect of 200 µL L^{-1} concentration was weaker at 7 DAI. The measured radial colony growth of the pathogen was 1.23 cm, while no radial colony growth was observed at other concentrations. Similarly to our results, Abdolahi et al. (2010) found that thyme EO strongly suppressed *B. cinerea* and *Mucor piriformis* growth when added at concentrations from 800 µL L^{-1}. However, thyme oil at 200 µL L^{-1} only presented a fungistatic effect against *B. cinerea* (Abdel-Rahim & Abo-Elyousr, 2017). In other research, Reang et al. (2020) evaluated five essential oils (*Syzygium aromaticum* L., *T. vulgaris* L., *L. angustifolia* L., *Cymbopogon citratus* and *Mentha piperita* L.) against grey mould at 0.5%, 1% and 1.5% concentration under *in vitro* conditions. Obtained results agree with our study: among the five essential oils, thyme oil showed maximum growth inhibition of *B. cinerea* at all concentrations. Meanwhile, *L. angustifolia* EO had a moderate effect on the radial growth of this fungus.

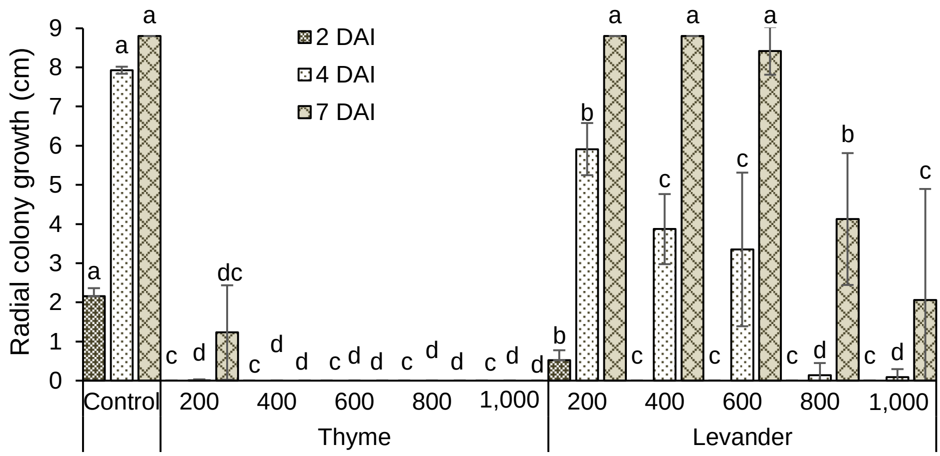

Figure 1. The antifungal effect of thyme and lavender essential oils on *B. cinerea* colony growth at different concentrations 2, 4, and 7 days after inoculation (DAI). The same letter indicates no significant differences between treatments ($p < 0.05$).

In our study, concentrations from 400 µL L^{-1} to 1,000 µL L^{-1} of lavender EO showed pathogen suppression ($p < 0.05$) 2 DAI ultimately. However, inhibition of the mycelium growth decreased 4 and 7 DAI. This EO demonstrated minimal inhibition at 200 µL L^{-1} at 2 DAI (0.52 cm), 4 DAI (5.91 cm), and 7 DAI (8.80 cm) compared to control (2.16, 7.93 and 8.80 cm). The radial growth of the *B. cinerea* was reducing with the increasing concentration of lavender oil. Soylu et al. (2010) described a 25.6 µg mL^{-1} concentration as having the best fungicidal properties while investigating *in vitro* and *in vivo* antifungal activities of volatile oils. Inhibition of spore germination was also observed, but at a sufficiently higher concentration (51.2 µg mL^{-1}).

The antifungal effect of thyme and lavender EOs on *Colletotrichum* spp. colony growth 2, 4, and 7 DAI is presented in Fig. 2. The graph shows that fungal growth was significantly inhibited ($p < 0.05$) by thyme EO, starting from minimal concentration (200 µL L^{-1}) during the research. A similar result was obtained by Sarkhosh et al. (2018b). Authors found that the minimum complete inhibitory concentration was determined to be 125 µL L^{-1}. In Vilaplana et al. (2018) study thyme oil demonstrated the best fungicidal effect against *Colletotrichum musae* at tested concentrations (100, 250, 500 and 1,000 µL L^{-1}). It exhibited significant mycelial growth inhibition ($p < 0.05$) compared to other essential oils tested 6 and 12 DAI. Another case revealed *in vitro* efficacy of the EO extracted from eight plant species (Sarkhosh et al., 2018a). The application rates were 100, 250, 500, 1,000, and 2000 µL L^{-1}. The results showed a 100% reduction of mycelium growth of *Colletotrichum, Botryosphaeria, Phytophthora*, and *Fusarium*, after applying thyme oil at all concentrations tested (Sarkhosh et al., 2018a).

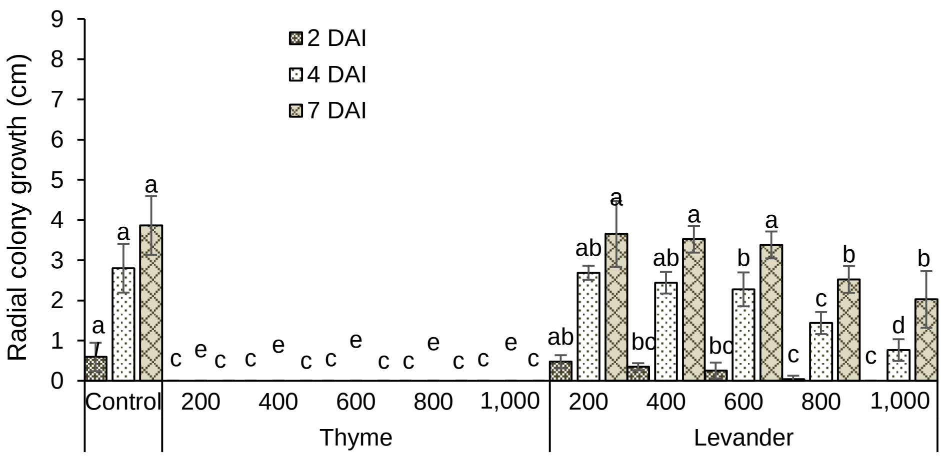

Figure 2. The antifungal effect of thyme and lavender essential oils on *Colletotrichum* spp. colony growth at different concentrations 2, 4, and 7 days after inoculation (DAI). The same letter indicates no significant differences between treatments ($p < 0.05$).

Unlike the thymus EO, the effect of lavender EO was not so high, and the growth of fungal colonies was very similar to that of the control. Only 1,000 µL L^{-1} of lavender EO showed significant growth repression 2 DAI. The same tendency was observed that with increasing concentration in the medium, radial colony growth decreased. Our results are similar to those of Sarkhosh et al. (2018b), who reported that mycelial growth of *C. gloeosporioides* at a higher application rate (1,000 µL L^{-1}) was completely inhibited. There was also a significant positive linear relationship between application rate and mycelial growth. Hoseini et al. (2019) study showed that *L. angustifolia* EO reached 84.5% mycelium growth inhibition at 1,000 µL L^{-1} 10 DAI.

The antifungal effect of thyme and lavender EOs on *Alternaria* spp. colony growth 2, 4, and 7 DAI is presented in Fig. 3. Thyme EO had a strong antifungal effect from 400 µL L^{-1} concentrations, as no *Alternaria* spp. colony growth was observed at 400–1,000 µL L^{-1} ($p < 0.05$). The concentration of 200 µL L^{-1} performed highly antifungal; however, the measured mycelium was 0.04 cm 7 DAI. Feng et al. (2011) study revealed that thyme EO at 500 μL L^{-1} showed a significant contact inhibition effect on *A. alternata* for 3 days both *in vitro* and *in vivo*. Either *A. citri* growth was completely prevented at 400 μL L^{-1} *in vitro* concentration of *T. vulgaris* EO. Moreover, the fungal growth steadily decreased with an increasing EO amount (Ramezanian et al., 2016).

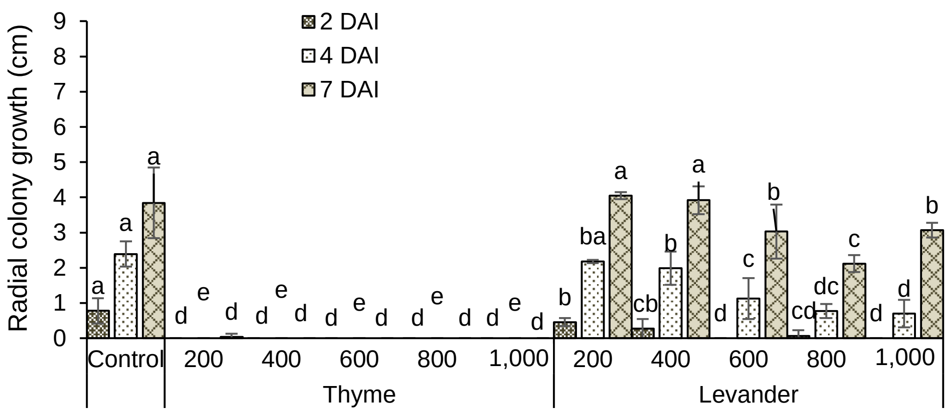

Figure 3. The antifungal effect of thyme and lavender essential oils on *Alternaria* spp. colony growth at different concentrations 2, 4, and 7 days after inoculation (DAI). The same letter indicates no significant differences between treatments ($p < 0.05$).

The lavender EO did not maintain the reduced *Alternaria* spp. growth at 200 μL L^{-1} and 400 μL L^{-1} 7 DAI and even promoted the spread of mycelium compared to control. The diameter of the colonies was 4.05 and 3.92 cm, respectively. The mycelium grew to 0.78 cm in the control treatment 2 DAI, 2.39 cm after 4 and 3.85 cm after 7. However, with increasing EO concentration, the antifungal effect intensified. The mycelium did not grow at a concentration range of 600–1,000 μL L^{-1} 2 DAI. Subsequently, mycelial growth of pathogen was not so effectively inhibited like after 2 days, although a smaller diameter was measured with increasing concentration at 4 and 7 DAI. According to Hussein & Joo (2017), distinctive antifungal activity against all fungi (*Alternaria*, *Fusarium*, *Sclerotinia*, C*ylindrocarpon* and *Botrytis* spp.) was showed under the influence of lavender EO 5 and 10% concentrations in Petri plates after 10 days of incubation. In Lu et al. (2019) work, lavender EO at 1,000 μL L^{-1} possessed high impact on radial colony growth among the 34 EOs at 3 DAI.

Analysing the antifungal effects of these EOs to radial growth of *Alternaria* spp., *B. cinerea*, and *Colletotrichum* spp., lavender EO was not so efficacy as thyme oil. In some cases discussed, radial colony growth was little different from the control treatment, which would mean investigating higher concentrations of the lavender EO in the future. Thyme EO can be stated as potential raw material for the development of eco-friendly plant protection products. The lowest concentration of thyme EO is 400 μL L^{-1} as no growth of the pathogens was observed 7 DAI. The effectiveness of thyme EO is perhaps due to its natural terpenoid thymol, which plays a vital role in inhibiting pathogens (Zabka et al., 2014).

CONCLUSIONS

To conclude, results demonstrated that thyme essential oil is an absolute inhibitor of all pathogenic fungi (*Alternaria* spp., *B. cinerea*, *Colletotrichum* spp.) growth during the whole experiment period. Meanwhile, the effect of lavender essential oil was short-lived and less powerful. The use of natural antifungal agents is increasingly encouraged nowadays. With more research on lavender essential oil in the future, both oils could be applied as environmentally friendly ways to control plant pathogens.

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Variability in yield of the lowbush blueberry clones growing in modified soil

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Abstract. In Latvia blueberry plantations are represented by approximately 486 ha in 2018, and about 44% of blueberry plantations are established in cutover peat bogs and approximately 40 ha of them are grown lowbush blueberry (*Vaccinium angustifolium* Ait.). In Latvia, lowbush blueberries are not grown on modified mineral soil (peat on the top of mineral soil). Ten lowbush blueberry clone were sampled from a commercial field to estimate potential productivity. The experiment was done at the Faculty of Agriculture, Latvia University of Life Sciences and Technologies. The plantation was established in peat (pH 3.8), which was covered in a layer of about 40 cm on mineral soil, rooted cuttings were planted in 2012, at a distance of 0.5×0.9 m, if necessary, the plantation was watered. The yield was estimated for a five year period, from 2015–2019. Significant differences in yield were found both by years and between clones. Over a five-year period, yields between clones ranged from 0.18 kg (2017) to 4.79 kg (2019) per bush. The high coefficient of variation (from 24.6 to 84.9%) indicate differences in yield between clones, with only 4 clones being below 30%. The average yield of clones by years was higher in 2019 (2.24 kg per bush), the lowest in 2017 (1.12 kg per bush). The results indicate variability on yield between the clones included in the experiment and year.

Key words: yield, one berry weigth, *Vaccinium angustifolium* Ait.

INTRODUCTION

Lowbush blueberry *Vaccinium angustifolium* Ait. (in some literature noted as a wild blueberry or Canadian blueberry) has a clonal growth habit; it is a prostrate shrub that spreads trough an underground network of rhizomes (Bell et al., 2010). In Europe this plant is relative new to cultivating (Hjalmarsson, 2006) due to its low climatic requirements.

Individual genotypes of lowbush blueberry exhibit significant differences in berry yield. In a several year study, Hepler & Yaraborough (1991) determined that the mean yield of 100 blueberry clones was 7.7 t ha^{-1} (yield ranged from 0.4 to 17 t ha^{-1}). In a three year study, Estonian researcher (Starast et al., 2007) determined that the yield of lowbush blueberry was from 1.3 to 5.5 t ha^{-1} depended significantly on soil pH.

Common cultivation practice in the U.S. and Canada has demonstrated that lowbush blueberry yields are maximized when the crops are grown in a two or three-year cropping cycle, with alternating vegetative and fruiting (yield) years (DeGomez, 1988).

According to data from the Rural Support Service, in Latvia blueberry plantations reaches approximately 486 ha in 2018 (Latvian Agriculture, 2019). According to the data of the Latvian Fruit Growers' Association (personal communication), about 44% of blueberry plantations are established in cutover peat bogs and approximately 40 ha of them are grown lowbush blueberry (*Vaccinium angustifolium* Ait.).

In Latvia there is a lack of information about lowbush blueberry yield. Currently berries are harvested by hand (at least the first berries intended for fresh consumption), then a hand-rake is used. The aim of this study was to determine the productivity of ten lowbush blueberries clones over a five-year period.

MATERIALS AND METHODS

Growing Conditions and Plant Material

The experiment was done at the Faculty of Agriculture, Latvia University of Life Sciences and Technologies (LLU) in Jelgava (56° 39' 47.1" N, 23° 45' 13.6"). The plantation was established in peat (pH 3.8) on top of mineral soil (about 40 cm). Rooted cuttings were planted in spring, 2012, at a distance of 0.5×0.9 m. The plant material for the root cuttings were selected from a commercial plantation grown from seedlings. Ten lowbush blueberry clones were evaluated (without replications).

The yield was estimated for a 5 year period, from 2015–2019. The crop was picked by hand two to three times (picking depended on berry mature, but the first pick was done when the approximately 75% of the berries were mature). Yield (kg per bush) and yield quality was determined during the experiment. A sample of 50 mature berries was weighed on each harvest date and used to calculate the average berry weight over the season. Cumulative yield over a five-year period for each clone was also calculated.

Plants were fertilized with 25 g m^{-2} granular fertilizer 12N–8P–16K (NH_4 7%, NO_3 5%, P_2O_5 8%, K_2O 16% + Mg 1.4%, S 10%, B 0.02%, Fe 0.06%, Zn 0.01%) one time per season (at the beginning of bloom). Five to six honey bee colonies were located near the experimental field, which provided the pollination of the clones. Netting was placed over the plants to exclude birds. If necessary, the plantation was watered. Lowbush blueberry clones were grown without pruning six year after planting, but in the spring of 2017 a half of the dormant bushes were pruned to assess new shoot formation (yield differences pruned/unpruned were not recorded).

Environmental conditions

The data of air temperature in the trial sites were recorded by data logger (MicroLite USB and EasyLog EL-USB-2-LCD+). The data were recorded in digital format every hour.

Air temperature differed between years (Fig. 1). In 2015 and 2017, the air temperature was similar, only November 2015 was characterized by a higher temperature (+10 °C in 2015 vs. +3.8 °C in 2017). In January, 2016 the air temperature was stable below 0 degrees, but in May sharp fluctuations in air temperature were observed. Meteorological conditions was unfavourable in 2017, where the vegetation period was characterized by lower temperatures during the study period and increased precipitation (data not shown) in August and September. The vegetation periods of 2018 and 2019 the air temperature was higher and drier than average.

Statistical analysis

Descriptive statistics were used for mathematical data processing, coefficient of variation was calculated, ANOVA and Tukey test were done to determined significance (P = 0.05) of differences. Different letters in figures and tables indicate significant differencies.

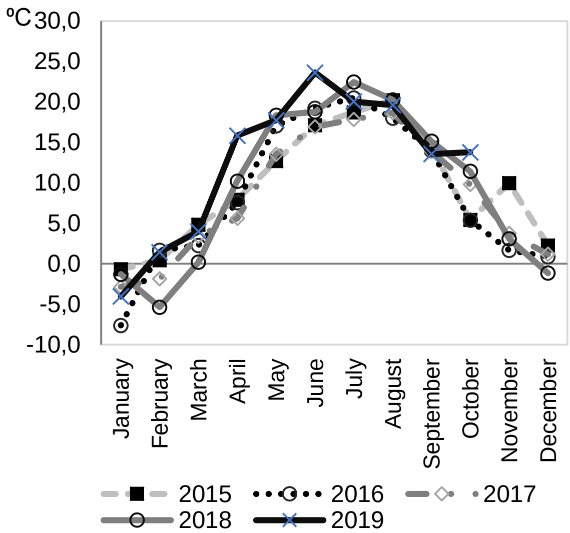

Figure 1. Monthly average air temperature during the experimental period.

RESULTS AND DISCUSSION

The year 2015 was characterized by fluctuating air temperature, which affected the ripening process of the berries. In this year the berries were picked only twice with an interval of 3 weeks of difference (1st time - on July 22, 2nd time - on August 6). May 2016 was characterized by high air temperature and large temperature fluctuations. The first berries were harvested on July 18, harvested 3 times with an interval of 8 to 10 days. In 2017, meteorological conditions affected the phenological development of plants - flower bud break occur later, flowering was also later in the season. The berries were harvested only once - on August 18, 2017. In 2018 the flowering period was characterized by an unusually high air temperature (average air temperature in May was 18.4 °C, in the period from 18 to 23 May the air temperature exceeded 30 degrees) and low rainfall (drought). The berries were harvested twice with a 14 day intervals (1st time on 13 July, 2nd time on 27 July). In 2019 the phenological development occur early, and also characterized by fluctuating air temperature. In 2019, the berry was harvested twice with an interval of 12 days (July 12 and July 24), but the last harvest for clone 1.1. was also done third time - on September 7, 2019.

Over a five-year period, yields between ten lowbush blueberry clones ranged from 0.18 kg (clone no. 2.4., 2017) to 4.79 kg (clone no. 1.1., 2019) per bush. Clone no. 2.5. showed the most stable yield in all years of the study, which is also indicated by the coefficient of variation (24.7%). Also clones no. 1.4., 1.5. and 2.1. showed relatively less yield fluctuations during the study period (coefficient of variation are from 27.1 to 29.3%). Only one (clone no. 1.1.) of the ten clones included in the experiment was characterized by large yield fluctuations, which is also shown by the very high (84.9%) coefficient of variation (Table 1).

Table 1. Average yield (kg per bush) of lowbush blueberry clones and coefficient of variation

Clone number	Average yield 2015	2016	2017	2018	2019	Average in five year period	Coefficient of variation (%)
1.1.	1.03	2.38	0.59	1.19	4.79	1.99 ab	84.9
1.2.	1.27	2.77	0.97	1.87	0.52	1.48 a	59.1
1.3.	1.45	2.32	1.10	1.23	1.84	1.58 a	31.4
1.4.	1.26	1.88	1.15	1.25	2.04	1.51 a	27.1
1.5.	2.25	1.37	1.85	2.48	3.05	2.20 ab	28.8
2.1.	0.72	1.48	1.17	0.86	1.39	1.12 a	29.3
2.2.	1.05	1.76	0.50	1.17	1.39	1.17 a	39.5
2.3.	0.72	1.60	0.54	1.60	1.39	1.17 a	43.3
2.4.	0.70	1.49	0.18	1.52	1.50	1.07 a	56.8
2.5.	2.59	2.74	2.87	3.71	4.50	3.28 b	24.7

Significant differences in yield were found between years (P = 0.002) and clones (P < 0.05). The clone factor (η^2% = 46.40) had the highest effect on lowbush blueberry yield, also significant was the whole year's weather factor (η^2% = 19.68).

The average yield of the ten lowbush blueberry clones was 1.66 kg per bush with a high coefficient of variation (57.87%). Yield ranged from 1.09 kg per bush in 2017 to 2.24 kg per bush in 2019 (Fig. 2). The results indicate a relatively small variability between the clones included in the experiment.

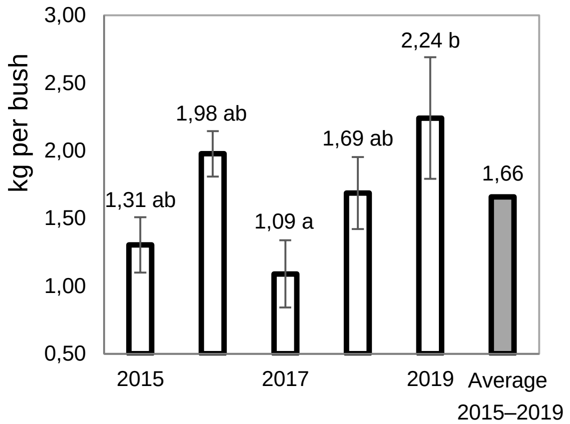

Figure 2. Mean yield of ten lowbush clones in trial by year. Bars represent standard error of the mean.

The mean berry weight of lowbush blueberry cones ranged from 0.5 to 0.6 g (clones no. 1.1, 1.3, 1.5., 2.4. and 2.5.) and from 0.8 to 0.9 g (clones no. 1.2., 1.4., 2.1., 2.2. and 2.3) with a coefficient of variation of 26.82% that indicate sufficient factor stability.

The most productive clone in the five-year period was clone no. 2.5, where its cumulative yield during the experiment was 16.41 kg from the bush. The lower cumulative yield were obtained from clones no. 2.4., 2.1., 2.3., and 2.2. (Fig. 3). The average cumulative yield was 8.30 kg per bush.

Recalculating the yield from one bush to one hectare (assuming that 22,000 plants are planted per 1 ha) potentially yield could be 28.7 t ha^{-1} in the third year after planting, and 49.3 t ha^{-1} in the seventh growing year (or 36.52 t ha^{-1} in average). The sdisparity between the average yield reported in literature (Hepler & Yaraborough, 1991; Albert et al., 2011) and the high yields obtained in this study may be due to the differences in pollination and irrigation. In this study the blueberry clones were subjected to a high density of bees for pollination. As mentioned in literature 25–60% of lowbush blueberry yields are affected by pollinators (Drummond, 2019), Wood (1969) determinate that lowbush blueberries can set up to 40% of their blossoms in field condition with native pollinators and 70% when supplemented with honeybees. Moisture is necessary for flower bud development and for increasing the weight of the berries, but proved that the lowbush lowbush blueberry has adapt to growth under limited moisture conditions (Glass et al., 2005).

In total, all clones formed a 0.5–0.6 m high shrub, with a large number of shoots (on average 8 to 40 annual shoots per bush - data not show in this article). Only three clones (Table 2) formed rhizomes, confirming the recent information indicates in literature that seedlings and micropropagated lowbush blueberries become established and spread faster than rooted cuttings (Morrison et al., 2000). Half of the evaluated lowbush blueberry clones were characterized by berries that were blue with surface wax, half - with black berries without visible surface wax (Table 2).

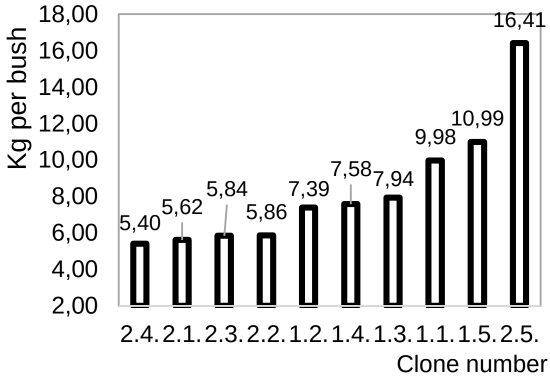

Figure 3. The cumulative yield of lowbush blueberry clones over a five-year period.

Table 2. Fruit characteristics of the ten lowbush blueberry clones

Parameters	Spread by rhizomes	Berry colour		Yield potential (kg per bush)		
		dark blue with surface wax	black without visible surface wax	low (< 1.5)	moderate (1.5–2)	high (> 2)
Clone no.	1.4	1.1	1.4	1.2	1.4	1.1
	2.2	1.2	2.1	2.1	1.3	1.5
	2.5	1.3	2.2	2.2		2.5
		1.5	2.3	2.3		
		2.5	2.4	2.4		

As mentioned in the literature (Bernard & Joubes, 2013; Samuels et al., 2008), the surface wax of the berry outer layer is the first protective barrier against abiotic and biotic stresses (protects the berry from drying out, extreme temperatures, UV radiation, pathogenic attack, etc.). Study in Latvia have shown (Klavins et al., 2019) that the surface wax composition varies between blueberry species and even cultivars, which may also affect the firmness and shelf life of the berries, etc. According to the observations in this experiment we hypothesize that for clone no. 2.3, the layer of surface wax could be significantly thinner than compared to the other clones. As mentioned Chu et al. (2018), the existence of natural wax help maintained the firmness of fruit and in delaying its softening.

CONCLUSIONS

The yield of lowbush blueberries fluctuate from year to year and are mainly influenced by the clone factor. The potential yield from evaluated clones can be very high and now there is a basic information about the lowbush blueberry yield potential in

Latvia conditions. Research should be continued, including investigating the effects of weather, mulch, fertilization and irrigation on the lowbush blueberry productivity.

ACKNOWLEDGEMENTS. The research leading to this report was supported by the Institute of Horticulture (LatHort) under project 'Examination of promising berry varieties for integrated plant cultivation in different regions of Latvia and development and improvement of their cultivation technologies'. Colleagues and students are gratefully acknowledged (for berry picking and calibration).

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Influence of Raspberry bushy dwarf virus on pollination of red raspberry (*Rubus idaeus* L.) cultivars

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Abstract Raspberry bushy dwarf virus (RBDV) is one of the major pollen-borne pathogens of the genus *Rubus* that causes drastic reduction of yield and degradation of berry quality. The aim of the study was to evaluate the quality of raspberry pollen and the effect of RBDV on pollination process. The research was carried out at the Institute of Horticulture. Within two years, 2017 and 2018, the pollen viability and pollen germination capacity of nine raspberry cultivars were analysed. The cross-pollination was done and the pollination quality of 31 crossing combinations was evaluated. The study found that although the pollen viability of cultivars infected with RBDV was higher than that of uninfected cultivars, there were no statistically significant differences. The viral contamination of the mother plant played a more important role in the pollination process. Pollination was better on uninfected mother plants and pollen germinated was faster than on infected plants. However, when the virus-infected cultivars were pollinated with infected pollen, the virus had an effect on the growth rate of pollen tubes, that decreased and the pollen tubes did not reach the ovary.

Key words: pollen germination, pollen viability, hybridization, fluorescence microscopy.

INTRODUCTION

Most of commercially grown raspberries are self-fertile (Daubeny, 1971). Insect activity helps to distribute pollen, increase the number of drupelets, and thus affect the size of the fruit (Cane, 2005). The number of drupelets may also be increased if different cultivars are crossed (Colbert & de Oliveira, 1990; Žurawicz et al., 2018), though the increase is largely dependent on the cultivars, which are used in crossing (Žurawicz, 2016).

Pollination is an essential step in the reproduction of flowering plants, while plant viruses can be spread via pollen during pollination process. There are known 46 plant viruses that may spread via pollen, where 18 of these can cause both: mother plant (horizontal transmission by pollen) and seed (vertical transmission by pollen) infection during pollination and fertilization (Card et al., 2007; Isogai et al., 2014). Raspberry bushy dwarf virus (RBDV) is one of them (Murant et al., 1974; Isogai et al., 2014).

The RBDV is widespread and infects wild and cultivated plants of the genus *Rubus* worldwide (Murant, 1976; Murant, 1987; Martin et al., 2013). The economic importance of RBDV is difficult to assess (Murant, 1987), but it significantly affects the yield and quality of raspberries (Murant et al., 1974; Daubeny et al., 1982). In addition, the virus spreads very rapidly and susceptible cultivars may be contaminated during the first two to three flowering seasons (Murant et al., 1974) and within five to six years the plants may be 100% infected with the virus (Bulger & Martin, 1990; Martin, 2002).

The virus spreads by transferring RBDV infected pollen with bees and other insects to uninfected plants during the pollination process (Chard et al., 2001). Infected pollen first land on the stigma and infect it, and then lead to systemic infection of the plant (Jones et al., 1982; Isogai et al., 2014; Isogai et al., 2015). Drupelet abortion is one of the most visible signs of virus infection in berries, called 'crumbly fruit', which consists of a few large, irregularly shaped drupelets, and, when picked, it crumbles into individual drupelets due to poor drupelet adhesion (Murant et al., 1974; Daubeny et al., 1978; Moore & Robbins, 1990; Martin et al., 2013).

Virus spreads very rapidly. Most of the reported susceptible cultivars can became infected during the first two to three flowering seasons, if the source of infection is close (Murant et al., 1974; Jones et al., 1982). Therefore the planting of resistant cultivars is the only method to control the virus (Murant, 1987). Studies on the mechanism of infection with RBDV in raspberry plants are available in the literature, while there is a lack of studies on the effect of the virus on viability of pollen and the pollination process. The aim of our study was to evaluate the viability of pollen infected with RBDV and the pollination process itself.

MATERIALS AND METHODS

The research was carried out at the Institute of Horticulture (LatHort) in 2017 and 2018. During previous studies done by the Unit of Plant Pathology and Entomology of LatHort, the infection by RBDV virus was determined for nine raspberry cultivars: 'Lubetovskaja', 'Ina', 'Glen Ample', 'Glen Rosa', 'Glen Moy', 'Glen Rosa', 'Glen Magna', 'Glen Doll', which were used in this study.

Raspberry pollen grains were collected shortly before pollination when the white petals appeared and the buds were still closed (BBCH 59). Pollen viability was determined by using the method of staining with acetic carmine solution, where the fertile pollen coloured in carmine red colour, whereas the sterile pollen remained light brown or pale pink and had an atypical shape.

Pollen germination capacity test was carried out *in vitro* on solid nutrient medium in various germination conditions. Two types of medium were used in 2017and 2018:

- 10% sucrose medium = 0.5 g agar + 5 g sucrose + 50 mL distilled water;
- 15% sucrose medium = 0.5 g agar + 7.5 g sucrose + 50 mL distilled water + 0.015 g boric acid.

The medium with pollen was placed in a box with moistened filter paper, which retains air humidity, and then placed in an incubation chamber (Memmert, Germany) at 22 °C and incubated for four hours. Leica DMLS microscope (objective 40×) was used for counting live but ungerminated and germinated pollen (a small tube longer than pollen length was visible). In order to evaluate whether pollen germination increases over time, pollen grains were germinated for more than 12 hours on 10% sucrose medium in Petri dishes. The temperature increase by 2 °C and 20% sucrose medium (0.5 g agar + 10 g sucrose + 50 mL water + 0.02 g boric acid) was used additional in 2018 to assess the difference between cultivars in diverse growing conditions.

In the study, two RBDV-infected cultivars: ‘Ina' and 'Glen Doll’, and two non-RBDV-infected: 'Lubetovskaja' and ‘Glen Ample’ were selected for cross-pollination. The flowers were counted on the canes of open pollination. On the canes evaluated for self-pollination, isolators were put on flowers and the flowers were not emasculated. In the treatment with cross-pollination, the flowers under isolators were emasculated. Emasculated flowers were pollinated according to the crossing plan 2 to 3 days after emasculation. In study, 31 crossing combinations were performed, including open pollination and self- pollination.

Fluorescence microscopy was used to determine the biocompatibility. Pollinated flowers were picked on days 1, 2, 3, 4 and 5 after pollination and then used for laboratory analysis. The flowers were fixed in FAA solution (80% ethyl alcohol, 37% formalin, 100% glacial acetic acid; ratio 8:1:1) for 24 hours, then rinsed for 3.5 h in water, 0.5 h in distilled water, and 70% ethyl alcohol was added after the final rinse. Preserved flowers began to be prepared 33 hours prior to microscopy. Firstly they were macerated in 8N NaOH for 12 h, then rinsed for 8 h in water and 1 h in distilled water. The flowers were stained in 0.1% aniline blue and 0.1N K_3PO_4 aqueous solution for at least 12 hours. Fluorescence was monitored by adjusting the settings of the Leica DMLS microscope, adding an additional device for fluorescent light, and working in the dark. Samples were viewed at 10× and 40× magnification. How far the pollen tubes have grown was evaluated visually (evaluated as: no pollination; fluorescent pollen, where the pollen tubes has grown to ¼; ½; ¾ or full length of the style). The percentage of non-pollinated stigmas was determined relative to the total number of counted pistils for every crossing and standard deviations calculated. The percentage of pollen germination rates on each sampling day were determined as well. Average values of 5 days for each crossing were used for further analysis. The averages were grouped into four groups based on a viral infection of the female and male plants:

1) uninfected mother plant pollinated with infected pollen;
2) uninfected mother plant pollinated with uninfected pollen;
3) infected mother plant pollinated with infected pollen;
4) infected mother plant pollinated with uninfected pollen.

To evaluate the potential impact of environmental factors on the pollen development process, meteorological data (air temperature, air relative humidity, precipitation and mean wind speed) in May and June of 2017 and 2018 were included in the data analysis. Meteorological data were collected by station ‘Lufft’ located in the orchard.

Data analysis was performed using Microsoft Office Excel 2007. The mean viability of the pollen and standard deviation between readings were calculated, the one-factor analysis of variance was performed. Mean germination rate and standard deviations were calculated for each growing condition and for each studied cultivar. One-factor analysis of variance was performed.

RESULTS AND DISCUSSION

Meteorological data

The data on meteorological conditions in May-June of 2017 and 2018 is presented in Fig. 1. The significantly lower average air temperature (11.78 °C) was recorded in May 2017 compare to May 2018. During this period the air temperature was between 1.71 and 19.85 °C, whereas in May 2018, the air temperature was between 10.38 and 21.74 °C. In both years, the minimum and maximum air temperatures during June were similar: 10.00 to 19.58 °C in 2017, and 10.30 to 21.23 °C in 2018. in June 2017, air humidity was 74.23% and it was significantly higher than in June 2018. Wind speed, which can affect pollen transport from plant to plant, was significantly higher in June 2017 (2.5 m s^{-1}) compare to June 2018, but significantly lower in May 2018 (1.54 m s^{-1}). The amount of precipitation was significantly higher (1.97 mm m^{-2}) in June 2017.

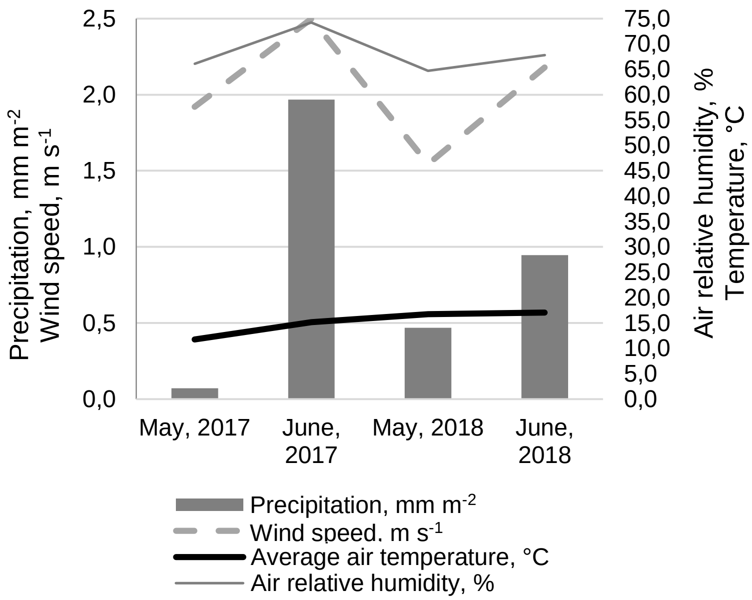

Figure 1. Precipitation (mm m^{-2}), wind speed (m s^{-1}), average air temperature (°C) and air relative humidity (%) in May and June of 2017 and 2018.

Viability

The average pollen viability of five cultivars used in the study was 92%, (*SD* 6%) in 2017 (Fig. 2). 'Glen Ample' (93.8%), 'Alvi' (94%), and 'Ina' (94.5%) infected with RBDV showed highest pollen viability, but no significant difference was found between cultivars in 2017 ($p = 0.09$).

Pollen viability of seven cultivars studied in 2018 ranged from 93% to 99%, the average viability was 97% (*SD* 3.1%) (Fig. 3). Significant difference was found between cultivars ($p = 3.65\times10^{-5}$). 'Lubetovskaja' (viability of pollen 93%) had significantly lower amount of viable pollen than other cultivars.

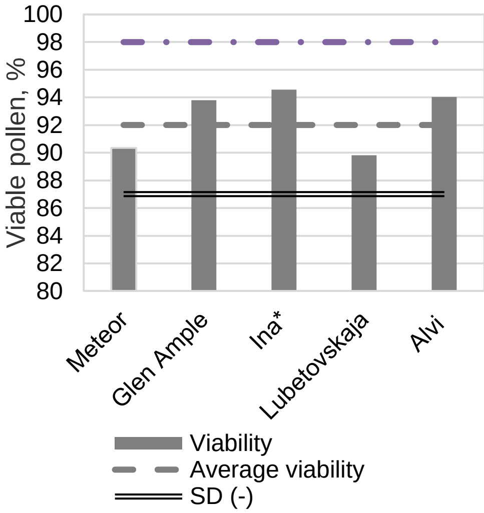

Figure 2. Pollen viability of raspberry cultivars 'Ina', 'Alvi', 'Glen Ample', 'Meteor' and 'Lubetovskaja' in 2017, expressed as a percentage of evaluated pollen grains.

*Infected cultivars by RBDV, *SD*(-) and *SD*(+) – min and max borders of mean standard deviation of pollen viability.

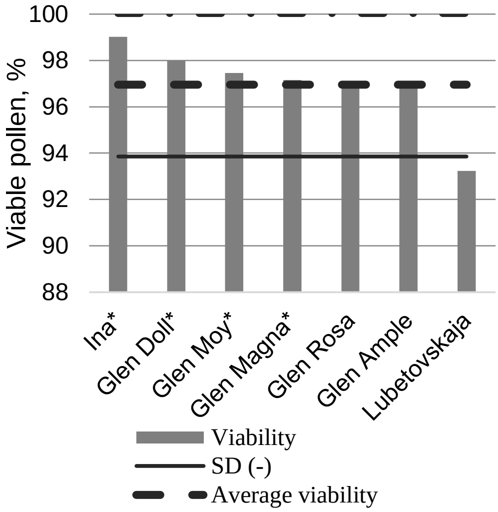

Figure 3. Pollen viability of raspberry cultivars 'Ina', 'Glen Doll', 'Glen Moy', 'Glen Magna', 'Glen Rosa', 'Glen Ample' and 'Lubetovkaja' in 2018, expressed as a percentage of evaluated pollen grains.

*Infected cultivars by RBDV, *SD*(-) and *SD*(+) – min and max borders of mean standard deviation of pollen viability.

The difference in results between years could be affected by the different meteorological conditions during the raspberry flowering and pollination time in May and June. For example, in May 2017, the average air temperature was significantly lower compare to May 2018. Minimum and maximum temperatures in May were also relatively different between the both years, 1.71 and 19.85 °C in 2017, respectively, while in May 2018, it was 10.38 and 21.74 °C, so in 2017, the pollen viability may had been affected by relatively much lower temperatures during pollen development and raspberry flowering, which were close to zero at some periods. Though, Otterbacher et al. (1983) is concluded that the reduction in pollen viability is caused directly by high-temperature stress. In our experiment, the pollen viability was above 93% for all cultivars in 2018, which is considered as high. In similar studies, fresh pollen viability for species *Rubus ellipticus* ranged from 32.5 to 97.7% (Pawar et al., 2017) and *Rubus paniculatus* S. 73.3% (Hiregoudar et al., 2019). In the study of Gercekcioglu et al. (2000), pollen viability in *Rosaceae* trees ranged from 71.5 to 81.8%.

Germination

Studied cultivars showed different pollen germination rate on 10 and 15% sucrose medium at 22 °C for 4 hours in 2017 and 2018 (Table 1).

Cultivated for more than 12 hours on 10% sucrose medium, 'Lubetovskaja' showed the best germination capacity (29%). The average germination rate of cultivars was 25.3%, with standard deviation 2.4%. There was no significant difference in the percentage of germinated pollen compared to cultivation for 4 hours on 10% medium.

However, it was visually observed that the length of the pollen tubes of all cultivars were significantly longer after maintenance of 12 hours.

Table 1. Average pollen germination rate of raspberry cultivars, expressed as a percentage of the listed pollen on 10 and 15% sucrose medium at 22 °C for 4 hours in 2017 and 2018

Cultivar	10% sucrose		15% sucrose		Average for cultivar	$SD_{cultivar}$
	2017	2018	2017	2018		
Meteor	15.1	-	24.9	-	20.0	6.9
Alvi	15.9	-	10.6	-	13.3	3.7
Glen Ample	13.9	23.9	18.0	40.0	23.4	11.5
*Ina**	22.6	24.4	27.2	62.5	34.1	18.0
Lubetovskaja	16.6	22.7	14.1	55.3	27.2	19.1
Glen Doll	-	30.0	-	33.6	31.8	2.5
Glen Moy	-	25.4	-	18.8	22.1	4.7
Glen Rosa	-	30.5	-	44.2	37.4	9.7
Glen Magna	-	22.5	-	37.7	30.1	10.7
average	16.8	25.6	19.0	43.3	26.2	
SD_{medium}	3.4	3.3	6.9	16.4	7.2	

*RBDV infected cultivars are presented in italic.

In general, comparing the data of both study years and pollen germination on different media in different growing conditions, it was observed that the best pollen germination was on 15% sucrose media, at 22 °C for 4 hours - 43.3% (*SD* = 16.4%). Cultivars showed the lowest germination capacity on 20% sucrose medium at 24 °C for 12 hours - 10.9% (SD_{medium} = 2.1%).

It was observed that in each germination condition one of the cultivars studied showed better pollen germination than the other, so it can be concluded that each cultivar has its own optimal pollen germination conditions. However, the hypothesis at the beginning of the study, that pollen from the RBDV infected cultivar has a lower pollen germination capacity, was not confirmed. The composition of the medium and the germination time had a greater influence on the pollen germination capacity. Pollen germination was performed on 10, 15 and 20% sucrose medium. Although there are studies that have shown that increasing the concentration of sucrose in the medium promotes pollen germination (Hiregoudar et al., 2019), the best medium for raspberry pollen in this study was the 15% sucrose medium. This medium is also optimal option for germinating of pollen from other *Rosaceae* plants, such as apple tree *(Malus)* (Abdel, 1999). For cherry laurel (*Prunus laurocerasus* L.) the best results were observed after 24h of germination on 15% sucrose medium (Sulusoglu & Cavosuglu, 2014). This germination time was also optimal to *Rubus paniculatus* S. (Hiregoudar et al., 2019), but in this study the optimal medium was 25% Sucrose + 5ppm Gibberellic acid.

In N. Pawar study (Pawar et al., 2017), 25% sucrose medium was also determined as optimal for *Rubus ellipticus* pollen germination, while there was added 0.4% boric acid to medium and the germination was observed after 48 h.

Interestingly, the cultivars 'Ina' and 'Lubetovskaja', which showed the highest and lowest levels of pollen viability, respectively, showed the highest susceptibility to germination media in the germination test. For cv. 'Ina' the standard deviation between the media was 17% and for cv. 'Ļubetovskaja' the standard deviation was 16.2%.

Cross-pollination

More non-pollinated stigmas were found in crosses, where an infected cultivar was used as a female plant (Fig. 4). By using self-pollination the uninfected cultivars had slightly more non-pollinated stigmas (20.6 ± 1.7%) than infected cultivars (16.4%), while the standard deviation was higher - 12.2%. The least amount of non-pollinated stigmas was in open pollination, 8.2 ± 0.3% for uninfected cultivars and 2.9 ± 3.3%for infected cultivars.

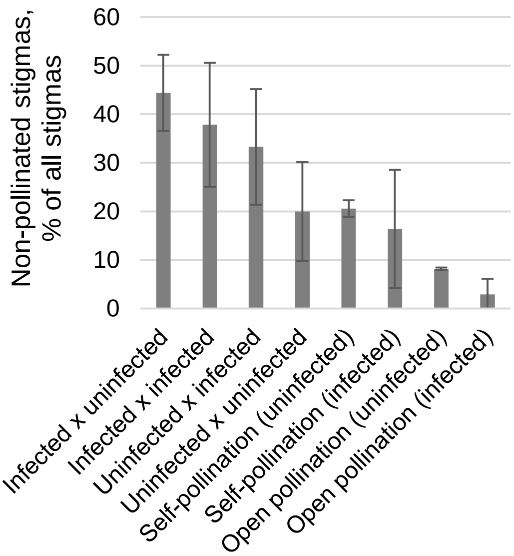

Figure 4. Relative amount of non-pollinated stigmas for different crossing combinations, compared to self-pollination and open pollination of infected and uninfected cultivars.

The highest relative amount of fluorescent pollen was found for infected cultivars using the open pollination - 57.6% (Fig. 5). The average relative amount of fluorescent pollen for all groups was 35.6 ± 12.2%. The highest relative amount of pollen germinated up to ¼ was in crosses, where the infected cultivar was pollinated with both uninfected pollen (32.3%) and infected pollen (31.6%). The least relative amount of pollen at this stage was in the open pollination, 4.3% for infected cultivars and 6.8% for uninfected cultivars. On average for all groups, 25.1 ± 6.7% of the pollen tubes were germinated to ½ of the style length. There was significantly more pollen at this stage if uninfected mother plant was pollinated with uninfected pollen (33.7%), but significantly less in open pollination of infected cultivars (14.3%). On average, 15.6 ± 4.6% of the all pollen was germinated up to ¾ of the style length. The least relative amount of germinated pollen at this stage was detected if an infected cultivar was crossed with an infected cultivar (7.1%). On average, 4.2 ± 2.3% of the pollen tubes were germinated to the ovule. The highest relative amount of pollen at this stage were in the open pollination of the infected cultivar (7.6%), but the least relative amount of if the infected cultivar was used as the mother plant.

Initially looking at the pollen germination process at each crossing on each sampling day, it was expected that at each subsequent day, the most of pollen tubes would have germinate further on the stylus and in the day 5, the most pollen would have reached the ovule. One of the reasons why this correlation did not materialize is due to the limited number of flowers in isolators. During first days collected flowers were of visually better quality, whereas the quality of the samples collected in subsequent days was not so high. Considering that air temperatures at the time of pollination in late May, early June, reached 30 °C, and isolators may have raised the temperature even further, the quality of the samples may have been affected by high temperature stress (Otterbacher et al., 1983), which may have caused a reduction in pollen viability and germination capacity. A negative effect of increased air average temperature on pollination was observed also in another Latvian study, about sour cherry (Feldmane et al., 2017). The results may also have been influenced by the characteristics of the cultivars used in the study, as there may be cultivars that are genetically or physiologically incompatible with each other.

It was hypothesized that infectivity by pollen plays an important role in pollination. This hypothesis was partially confirmed in our study, because the infectivity of mother plant played a more important role in the pollination process. The pollination was better and pollen tubes grew faster for uninfected mother plants than for infected. However, if pollination was done with infected pollen on the infected mother plant, the rate of pollen tubegrowth decreased, the pollen tubes did not reach the ovule and fertilization did not occur. This could be explained by the delay in the effective pollination period (EPP) the ovule is aged when the pollen tubes reach it (Williams, 1965). EPP and ovule aging are also mediated by high temperatures (Sanzol & Herrero, 2001).

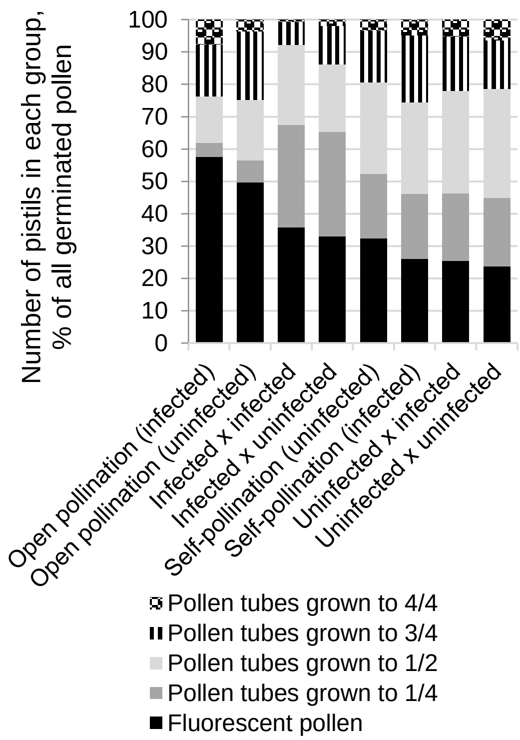

Figure 5. Development of pollen tubes in style and amount of florescent pollen in different crossing combinations, expressed as percentage of all pollinated pistils.

CONCLUSION

The results of the study showed that pollen infected with RBDV germinates equally well to uninfected ones in the studied cultivars. They have the same or slightly higher viability, but during the pollination process, the virus has an effect on pollen tubes growth rate.

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Suitability of blue honeysuckle (*Lonicera caerulea* L.) cultivars of different origin for cultivation in the Nordic-Baltic climate

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Abstract. The rising trend of blue honeysuckle has led to the increase in new plantations and berry production in recent years in Nordic-Baltic region, including Estonia. This crop is naturally distributed in the temperate climate zone of Northern Hemisphere. Estonia is also located in the same climate zone, but differs only from warm maritime air. The main aim of this research was to find out cultivars' adaptation to the changing weather conditions regarding winter hardiness, fruit weight, yield and occurrence of secondary flowering. The data was recorded from two closely situated plantations in Polli village, Viljandi County, Estonia. Eighteen cultivars of blue honeysuckle with different origin (Russia, Canada, Poland and Czech Republic) were tested. In 2016, greater winter damage was recorded when compared to the period of 2017–2020 with just marginal damage. In conclusion, the Canadian cultivars ('Borealis', 'Indigo Gem', 'Indigo Treat' and 'Tundra') and Polish 'Duet', presented their best properties and suitability to Estonian climatic conditions.

Key words: edible honeysuckle, fruit weight, *Lonicera caerulea* L., production, secondary flowering, winter hardiness.

INTRODUCTION

Edible honeysuckle (*Lonicera caerulea* L.) also known as blue honeysuckle or Haskap is naturally distributed in Russia and Asia (Chaovanalikit et al., 2004). The berries have been collected for food and used for medical purposes for a long time. However, in Estonia, this crop has gained popularity in recent years. Formerly it was grown solely in home gardens. Recently, an increasing number of producers have established small plantations of blue honeysuckle (the exact statistics is absent). It has gained the appreciation of growers because of their sufficient cold hardiness in our climate and early ripeness of the berries. As there are many cultivars available in nurseries of different origin, appearance and fruit ripening time, therefore it is difficult to select the right cultivar that is productive, but also suitable for variable weather conditions and resistant to other biotic and abiotic stressors.

Despite many advantages of blue honeysuckle, still the suitability of cultivars for cultivation in a changing weather conditions in Estonia needs to be confirmed. For example in the autumn, occasionally occurring warm periods may cause the delay in the process of preparation for winter dormancy of the plants. Therefore, secondary flowering of the plants can be observed (Arus & Kask, 2007). Secondary flowering of edible honeysuckle may occur due to plants' low requirement of chilling hours and on account of fluctuating temperatures in autumn (Kolasin & Pozdnyakov, 1991; Plekhanova et al., 1993; Gerbrandt et al., 2018a). Leonovna (2019) stated that during ten years of research, plants usually started flowering prematurely in October or November, and in some extreme years even ovaries were formed.

In addition, fluctuations in air temperature during the wintertime coming with warmer periods that frequently end with frosts may reduce the bioweight or destroy the whole plant (Lazdiņa et al., 2016). Early growth cessation and leaf drop has been associated with susceptibility to changes of temperature in the winter (Gerbrandt et al., 2018a). More than 30 years ago, the suitability of edible honeysuckle for cultivation in temperate climate was questionable due to early bud break during increased winter temperatures (Plekhanova, 1986). However, at present, the wide selection of different cultivars has changed the viewpoint. The local Japanese species of edible honeysuckle (*L. caerulea* var. *emphyllocalyx*) is considered to be more adapted to moderate climate (Thompson & Barney, 2007) than those used for breeding of the Russian cultivars (Plekhanova et al., 1993). It was reported that other species in the genus of *Lonicera* such as *L. tatarica* L. and *L. maackii* (Rupr.) Maxim. had both phases of exogenous and deep dormancy (Brailko & Gubanova, 2014). The exogenous dormancy lasts for 68–72 days and is followed by 20–23 days of deep dormancy. The trials performed in Ukraine revealed that the exogenous dormancy started in the genus of *Lonicera* from II–III decade of August (Rura & Opanasenko, 2000).

As well as phenological adaption, the appearance and economical parameters act as key factors in terms of fresh consumption. The fruits of blue honeysuckle are quite small, an average weight is ranging from 0.56 to 2.18 g (Gawroński et al., 2014; Gerbrandt et al., 2018b). As it was reported by Arus & Kask (2007) the yield of fully-grown plants of blue honeysuckle in Estonia was rather low (700–2,220 g per bush) compared to mainstream fruit crops like blackcurrant (2,000–6,300 g) depending on the growing sites (Kahu et al., 2009). Still, it was found that phenological adaption had an important role in reaching extremes in fruit weight and yield (Gerbrandt et al., 2018b).

The aim of this research was to compare 18 edible honeysuckle cultivars in order to find out their suitability for Nordic-Baltic climate and cultivation properties in terms of winter hardiness, fruit weight, productivity, yield and occurrence of secondary flowering.

MATERIAL AND METHODS

Experimental site and cultivars

Eighteen edible honeysuckle cultivars originated from Russia ('Amfora', 'Bakcharskij Velikan', 'Lebedushka', 'Leningradski Velikan', 'Morena', 'Moskovskaja 23', 'Nimfa', 'Roksana', 'Tomichka', 'Chulymskaya', Viola', 'Volhova'), Canada ('Borealis, 'Indigo Gem', 'Indigo Treat', 'Tundra'), Poland ('Duet') and Czech Republic

('Modry Triumph') were evaluated from 2016 to 2019. Two experimental plots were established in 2014 with the same cultivars at both locations: (1) cultivar collection of Polli Horticultural Research Centre (58°7' N, 25°32'E) with three plants of each cultivar per replication for manual harvesting; and (2) Seedri nursery (58°6' N, 25°33'E) with 30 plants of each cultivar per replication for machine harvesting. Bushes were planted with a spacing of 1.5 m between plants and 3.0 m between rows. Plants were grown in rows mulched with tree bark in Polli, and textile cover in Seedri nursery. In both places no additional irrigation of experimental plants was applied.

Meteorological conditions

Table 1. Weather conditions of Viljandi according to Estonian Weather Service during the experimental years (2016–2020) compared to long-term average (1961–1990)

Month	Mean monthly temperature, °C						Total monthly precipitation, mm					
	Year 2016	2017	2018	2019	2020	Long-term average	2016	2017	2018	2019	2020	Long-term average
January		-3.2	-2.2	-5.3	2.5	-4.4		37	40	54	50	62
February		-3.1	-8.3	-0.2	1.0	-5.1		39	23	42	106	43
March		1.1	-3.8	1.2	2.2	-1.0		48	26	55	38	43
April		3.2	6.7	7.3	4.9	5.3		50	49	3	47	36
May		10.3	15.2	10.9		11.3		14	25	52		48
June		13.9	15.6	18.3		14.9		61	61	73		87
July		15.6	20.2	16.0		17.5		79	40	57		83
August		16.3	18.1	16.4		16.1		82	94	65		91
September	12.8	11.9	13.7	11.5		11.0	26	134	108	92		67
October	4.0	5.0	7.2	6.8		6.0	36	113	78	112		81
November	-0.9	2.4	2.4	2.6		0.6	85	60	35	61		64
December	-0.1	0.3	-2.4	1.9		-3.1	45	84	34	68		60

Months of autumn of 2016 were less humid than those of long-term average (1961–1990). However, rainfall in November was 21 mm more than the average. October was two degrees colder than the mean. Overall, the weather from October (2.4 °C) to December 2017 (0.3 °C) was a few degrees colder than in 2016 (-0.9 °C, -0.1 °C). Temperatures in April were lower (3.2 °C) compared to the average (5.3 °C), as of May (10.3 °C), June (13.9 °C) and July (15.6 °C). May was the driest month (14 mm), whereas, in September, the greatest rainfall was recorded (134 mm). Also, October 2017 was very humid (113 mm). However, December of 2017 was considerably warm (0.3 °C) compared to the previous years (-3.1 °C). There was a fluctuation in February 2018. The temperatures in the period from February to March in 2018 were 3.2 °C and 2.8 °C less than the long-term average. On the other hand, the months of April and May and meteorological autumn months were up to 3.9 °C warmer compared to the long-time average. Again, in May 2018 there was a drought with only 25 mm of rainfall, but the highest precipitation (108 mm) was recorded in September. The winter of 2019 occurred earlier, in February the average temperature was warmer (-0.2 °C) than the long-term average (-5.1 °C). Also, meteorological spring months (March and April) were up to 2 °C warmer than the average. The precipitation rate of April in 2019 was extremely low (only 3 mm), but it increased in May (52 mm). There was a 31 mm higher rainfall in October of 2019 when compared to long-term mean. The beginning of 2020 was drastic for plant overwintering. The temperatures in the months of January, February and March did not drop below 0 °C and were fluctuating up to 7 degrees. In February of 2020, the precipitation level was also different from average with the rainfall of more than double (106 mm).

Phenological data

The vegetation period in 2017 started in May. The start of the vegetation period is defined by a period when the temperature stays constantly below 5 °C. The beginning of blue honeysuckle plant growth is defined by the bud burst, which started in March 27th–31th. The beginning of flowering is defined when 5% of the petals of flowers on a single bush is open, and in 2017 cultivars ‘Volhova’ and ‘Nimfa’ were the first ones flowering on May 10th. The beginning of flowering was recorded until the 17th of May. On June 13th–25th first fruits started the colouration to blue. First fruits ripened in the middle of June, 15th–July 4th. The earliest ripening cultivar was ‘Viola’ and the latest ‘Duet’.

The vegetation period in 2018 started in April, and the bud break of blue honeysuckle was observed during April 11th–April 21th. Cultivar ‘Viola’ had the first flowers on April 28th, and the beginning of bloom was recorded until 7th May. The colouration of blue honeysuckle fruits started on May 25th to June 6th. The fruits of blue honeysuckle started to ripen from May 25th to June 30th. Cultivars with the earliest ripening were ‘Tomichka’ and ‘Volhova’, their fruits ripened from June 2nd. The ripening of fruits continued until June 16th.

The vegetation period in 2019 started in April, but the bud break in 2019 was observed already from March 22nd to April 13th. The start of flowering was recorded on April 20th–28th. It was found that cultivar ‘Nimfa’ had the first flowers. The colouration of fruits started from May 30th to June 4th. Fruits started ripening in the II decade of June, 17th–July 1st. Cultivar ‘Volhova’ and ‘Leningradski Velikan’ were the earliest and ‘Tomichka’, ‘Tundra’, ‘Viola’, ‘Duet’ and ‘Amfora’ the latest ones.

Evaluations and determinations

The evaluation of different parameters was done by using a 9-point ranking scale (1–9) at both experimental plantations as follows: (1) winter hardiness was evaluated from the end of April to the beginning of May depending on the year (1 = dead to the ground; 5 = moderate injury, 40–50% of branches with visible damage; 9 = no injury); (2) secondary flowering was observed visually on the bushes in November (1 = secondary flowering is absent, 5 = secondary flowering is moderate, 9 = very high secondary flowering); (3) productivity per plant was evaluated a week before harvest (1 = very low yield up to 100 g per bush, 5 = moderate productivity with 400–700 g per bush, 9 = very high yield more than 1,000 g).

For determination of average single fruit weight, 50 fruits were weighed and the weight was divided by 50. Yield per bush was calculated as follows: yield for manually harvested bushes - each bush was harvested separately and weighed per bush; yield for machine harvested bushes - all the bushes of each cultivar were harvested and the yield was divided per bush (15 June – 4 July 2017; 2–25 June 2018; 14 June – 1 July 2019). The ‘Joanna-4’ berry harvester (capacity 0.1–0.15 ha per hour, Weremczuk Ltd, Poland) was used for mechanical harvesting of blue honeysuckle at Seedri nursery. All determinations and evaluations were performed in triplicate.

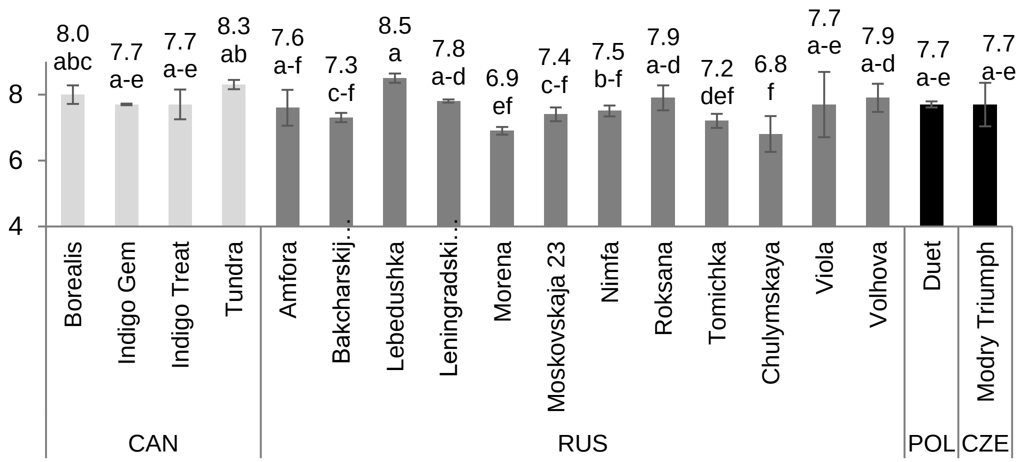
Statistical analysis

Data was expressed as means (± standard deviation - *SD*) in Table and Figures. Results of the fruit weight, productivity and fruit yield of each genotype were analysed using one-way ANOVA in an individual years of investigation and as average for all years of studies. The least significant differences (*LSD*_0.05) were also calculated.

RESULTS AND DISCUSSION

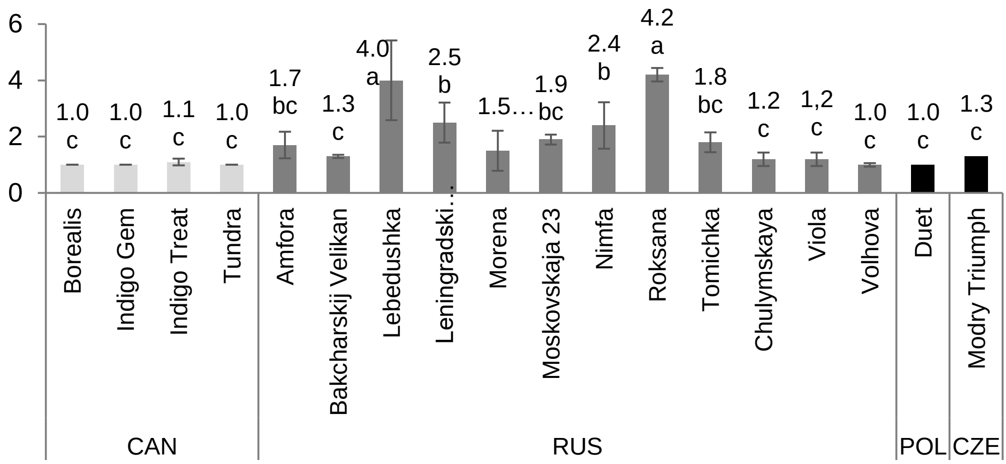
Winter hardiness and secondary flowering

The local climate limits the area of blue honeysuckle cultivation, mainly due to the occurrence of winter damage of plants. The results of winter hardiness showed that over the experimental years (2016–2020), each cultivar had only slight winter damage (8.5–7.6; Fig. 1), this means that some vegetative parts did not survive the winter.



Fugure 1. Average results of winter hardiness of plants of tested edible honeysuckle cultivars in 2016–2020, using 1–9 point ranking scale, 1 = dead to ground, 5 = moderate injury, 9 = no injury. CAN = Canada, RUS = Russia, POL = Poland, CZE = Czech Republic. Different letters (a, b…) indicate significant ($p < 0.05$) differences among cultivars. Data expressed as means (± standard deviation bars on columns).

The least damage was recorded for cultivars of Canadian, Polish and Czech origin. In the group of Russian cultivars, there were more of those that had higher winter injury in comparison with the average injury (7.6). However, in our study, the plants of cultivar ‘Chulymskaya’ had the highest rate of winter injury (6.8). In the research conducted in Russia, it was observed that plants of ‘Leningradski Velikan’ was tolerant to low temperatures (Shpitalnaya & Titok, 2016). The study also showed that plants of cultivars ‘Nimfa’ and ‘Morena’ were winter hardy, but in our study, some injuries occurred in plants of both genotypes. Although plants of blue honeysuckle can withstand -45 °C (Hummer, 2006), it was concluded that fluctuating temperatures might still damage the plants (Plekhanova et al., 1993). In addition, the precipitation in November of 2016, October 2017 and 2019 was above average. Therefore, somewhat higher rainfall in the autumn of these experimental years could have interrupted the plants’ entrance to winter dormancy by enhancing their growth instead. The latter may harm the plants due to suitable temperatures for their growth causing winter injuries after a sudden temperature drop (Gerbrandt et al., 2018a). During December of 2016, 2017 and 2019, the temperatures were higher than usual. The very exceptional time interval was 2019/2020 when there were small fluctuations and the temperature stayed above zero. In conclusion, it seemed that plants of cultivars from Russian origin were more prone to winter damage when compared to the group of Canadian ones and others. It was also determined that the Canadian cultivars with Japanese blue honeysuckle species in their pedigree were most adapted to moderate temperate climate (Thompson, 2006).

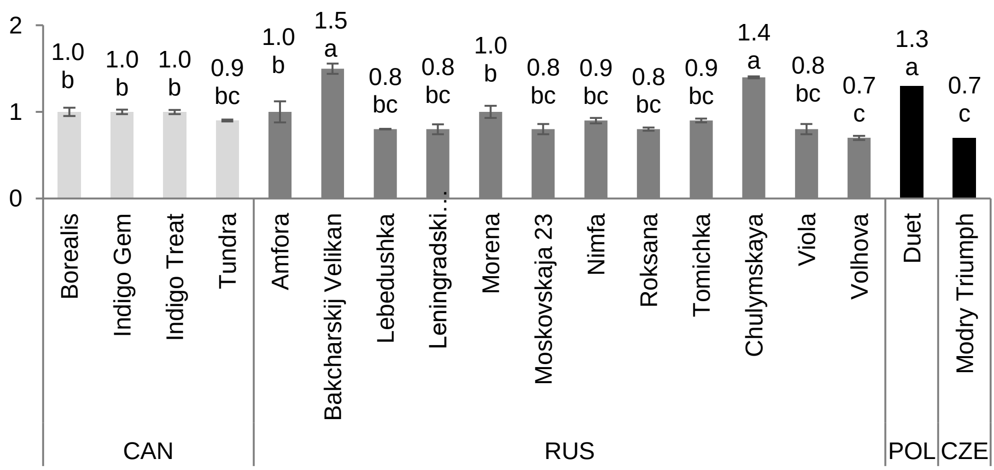


Fugure 2. Average results of the secondary flowering of plants of tested edible honeysuckle cultivars in 2016–2019, using 1–9 point ranking scale; 1 = secondary flowing is absent, 5 = secondary flowering is moderate, 9 = very high secondary flowering. CAN = Canada, RUS = Russia, POL = Poland, CZE = Czech Republic. Different letters (a, b…) indicate significant ($p < 0.05$) differences among cultivars. Data expressed as means (± standard deviation bars on columns).

The secondary flowering of blue honeysuckle plants in the autumn is the next important concern, as it may decrease the next years' yield (Gerbrandt, 2017). During our evaluations in the years of 2016–2019, average results of secondary flowering of tested cultivars varied between 1.0 and 4.2 points (Fig. 2). Generally, it could be seen that cultivars of Russian origin were more subjected to the secondary flowering (up to 4.2 points) than cultivars from Canada and another origin, which nearly did not have any flowering in the autumn (up to 1.3 points only). Plants of cultivars: 'Roksana' (4.2) and 'Lebedushka' (4.0) had the highest rate of the secondary flowering. The moderate rate of autumn flowering was observed on plants of cultivars: 'Nimfa' and 'Leningradski Velikan' (2.4 and 2.5, respectively). The similar results for these previously mentioned cultivars were also recorded earlier in Minsk, Belarus (Firsova et al., 2019) and in the oblast of Kirov, Russia (Shpitalnaya & Titok, 2016). The secondary flowering was absent in the plants of Canadian cultivars 'Borealis', 'Indigo Gem', 'Tundra', Russian origin 'Volhova' and Polish cultivar 'Duet' did not show any signs of late flowering over the experimental years. In Russia (oblast of Tambov), the occurrence of secondary flowering was observed on the plants of different blue honeysuckle cultivars in the warm and dry conditions in autumn (Kirina, 2010). In our study, the favourable weather conditions for the secondary flowering were observed in the years of 2016 and 2018. In 2016, the phenological autumn months (September and October) had up to 45 mm less rain, and the air temperature in September was 1.8 °C warmer than the long-term average. In 2018, quite warm temperatures were recorded in September and October with 2.7 and 1.2 °C more, respectively, and with up to 29 mm less precipitation in November when compared to the long-term mean.

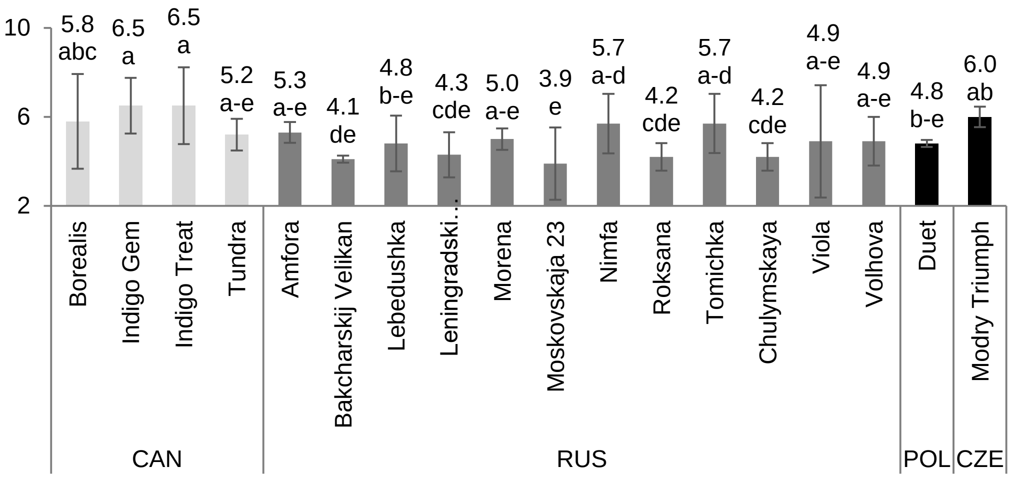
Fruit weight, productivity and yield

The fruit weight is an important characteristic in terms of fresh fruit consumption. Fruit size of blue honeysuckle can significantly differ by cultivar. The average fruit weight of the tested cultivars varied from 0.7 to 1.5 g (Fig. 3). It was calculated a 2.14-fold difference between the average highest and lowest fruit weight among evaluated cultivars. Results obtained in our study were in an agreement with those of Gawroński et al. (2014). 'Bakcharskij Velikan', 'Chulymskaya' and 'Duet' had the highest fruit weight (1.5 g, 1.4 g and 1.3 g, respectively). In contrast, other studies conducted in Poland recorded a high range of fruit weight for 'Duet' (1.4–1.9 g), (Małodobry et al., 2013; Gawroński et al., 2014). In our study, smaller fruit weight of tested cultivars may be because no additional irrigation system was used for plants in the experimental field. According to Nowakowski et al. (2019) irrigation of plants increased fruit size and weight of Canadian originated cultivars, which had fruit weight of 1.0 g or close to it. These results were similar to those obtained in Canada with cultivars 'Borealis', 'Tundra' and 'Indigo Gem' (1.0 g, 1.0 g, 1.0 g) (Gerbrandt, et al., 2018b). Weather conditions during fruit development can have an impact on fruit weight (Bożek, 2012). Lower temperature than the long-term mean in 2017 and quite high rainfall in 2019 could have had an important impact on fruit weight. Also, the fruit set depends highly on pollinators and the lack of pollinating insects can lead to poor fruit set (Bożek, 2012).



Fugure 3. Results of average fruit weight (g) of tested edible honeysuckle cultivars in 2017–2019. CAN = Canada, RUS = Russia, POL = Poland, CZE = Czech Republic. Different letters (a, b…) indicate significant ($p < 0.05$) differences among cultivars. Data expressed as means (± standard deviation bars on columns).

The lowest average fruit weight below 1.0 g was found in Czech cultivar 'Modry Triumph' (0.7 g) and Russian cultivar 'Volhova' (0.7 g). Somewhat higher average fruit weight (0.9 g) of cultivar 'Volhova' was found in Ukrainian research (Leonovna, 2019). The average fruit weight of 'Amfora' (1.1 g) in our study was consistent with that obtained by Zaripova et al. (2019). Nevertheless, there were no significant differences in the fruit weight among other cultivars of Russian origin 'Viola', 'Leningradski Velikan', 'Lebedushka', 'Roksana', 'Moskovskaja 23', 'Tomichka' and 'Nimfa'. Our results of fruit weight for 'Leningradski Velikan' ranged from 0.8 to 1.2 g in investigated years and were in an agreement with those reported by Shpitalnaya & Titok (2016). In a study conducted in Estonia by Arus & Kask (2007) recorded a similar fruit weight for cultivars 'Roksana' and 'Tomichka' (0.9 g and 0.8 g, respectively). Slightly higher, but comparable results of fruit weight of 'Morena' and 'Nimfa' (1.0–1.5 g and 0.8–1.0 g, respectively) were obtained in two independent studies conducted in Minsk (Belarus) and Republic of Bashkortostan (Russia) (Shpitalnaya & Titok, 2016; Zaripova et al., 2019). Somewhat higher fruit weight was presented in the studies conducted in Belarus and Russia showing dependency on the location of the plantation. In our study, lower fruit weight compared to the results of other authors might be related to insufficient moisture conditions occurring in most of the experimental years. In addition, the temperatures during fruit ripening increased in May 2018 and June 2019, respectively 3.9 and 3.4 °C higher than the long-term mean.



Fugure 4. Results of average fruit productivity per bush of edible honeysuckle tested cultivars in 2017–2019, using 1–9 point ranking scale; 1 = very low yield, 5 = moderate productivity, 9 = very high yield. CAN = Canada, RUS = Russia, POL = Poland, CZE = Czech Republic. Different letters (a, b …) indicate significant ($p < 0.05$) differences among cultivars. Data expressed as means (± standard deviation bars on columns).

The fruit productivity of blue honeysuckle is considerably low. In the need for profitable production, it is essential to grow high yielding cultivars. Evaluated cultivars of blue honeysuckle had low to moderate productivity per bush, the scores ranged from 3.9 to 6.5 which corresponds to the 400–700 g per bush (Fig. 4). The highest score of fruit productivity was recorded for Canadian cultivars 'Indigo Gem' and 'Indigo Treat', but there were no significant differences when compared to 'Modry Triumph', 'Borealis', 'Tomichka', 'Nimfa', 'Amfora', 'Tundra' and 'Morena'. The lowest productivity was found for 'Bakcharskij Velikan' (4.1) and 'Moskovskaja 23' (3.9). The weather could have had an impact on the productivity of the tested cultivars, as extremely warm temperatures and low precipitation were recorded in May 2018. Again, if there had been an additional irrigation system, it could have had a positive effect on yield as reported by Nowakowski et al. (2019).

One of the most important reasons which could increase the production acreages of blue honeysuckle in Estonia is making the fruit harvesting more efficient by using the mechanical harvester. Still, in our study, the fruit yield harvested manually was much higher when compared to mechanical harvesting, except for cultivar 'Viola' (Table 2). Lower yield of mechanical harvesting may be due to the losses of berries which can be related to the design of the machine (Casamali et al., 2016). Moreover, the efficiency of mechanical harvest depends on the shape of the bush, berries should be easily detached and the ripening period should be concentrated (Dale et al., 1994). The yield of hand-harvested fruits of the tested cultivars ranged widely from 166 to 1,883 g per plant (Table 2). The lowest hand-harvested yield was recorded for cultivar 'Viola' (166 g), but there were no significant differences among the other cultivars: 'Leningradski Velikan', 'Chulymskaya', 'Bakcharskij Velikan', 'Modry Triumph', 'Duet', 'Moskovskaya 23', 'Nimfa' and 'Roksana'. Hand-harvested fruit yields of Canadian cultivars were significantly higher, mostly due to their larger fruit weight. In our experiment 'Indigo Gem' appeared to be the highest yielding cultivar (hand-harvested), but there were no significant differences among the following cultivars: 'Amfora', 'Indigo Treat', 'Tundra' and 'Morena'. In our study, the average fruit yield of cultivar 'Duet' obtained by manual harvesting was 629 g per plant. It was reported that 3–4-year-old plants of 'Duet' produced yield from 718 to 1,560 g in Polish conditions (Małodobry et al., 2013; Gawroński et al., 2014). Other authors have reported the average fruit yield of blue honeysuckle in the range from 1,100 g to 5,000 g per bush (Hummer, 2006; Zavalishina et al., 2017), although in Estonia the average yield of this crop was significantly lower, 700–2,220 g per bush (Arus & Kask, 2007). Presumably, these differences can be related to the climatic conditions and geographical areas selected for cultivation of blue

Table 2. Fruit yield of manually and mechanically harvested blue honeysuckle cultivars in average of two experimental years (2018–2019), g per plant

Country of origin	Cultivar	Manual harvesting	Mechanical harvesting
Canada	Borealis	957^{cdef}	625^{a}
	Indigo Gem	$1,883^{a}$	584^{ab}
	Indigo Treat	$1,569^{abc}$	293^{a-e}
	Tundra	$1,523^{abc}$	366^{a-e}
Russia	Amfora	$1,639^{ab}$	530^{abc}
	Bakcharskij Velikan	580^{efg}	254^{b-e}
	Lebedushka	$1,184^{b-e}$	258^{b-e}
	Leningradski Velikan	459^{fg}	120^{de}
	Morena	$1,307^{a-d}$	406^{a-e}
	Moskovskaja 23	655^{efg}	130^{de}
	Nimfa	$660d^{efg}$	209^{cde}
	Roksana	$737d^{efg}$	372^{a-e}
	Tomitchka	$1,159^{b-f}$	305^{a-e}
	Chulymskaya	536^{efg}	275^{a-e}
	Viola	166^{g}	209^{cde}
	Volhova	966^{cdef}	274^{a-e}
Poland	Duet	629^{efg}	473^{a-d}
Czech Rep.	Modry Triumph	560^{egf}	105^{e}

honeysuckle. In general, the fruit weight, yield and productivity tend to be higher in warmer climatic conditions.

The average fruit yield of mechanically harvested blue honeysuckle cultivars was low and ranged from only 105 to 625 g per plant (Table 2). In our study, the highest yield was recorded for Canadian cultivar 'Borealis' (625 g per plant) and there were no significant differences in comparison to 'Indigo Gem', 'Amfora', 'Duet', 'Morena', 'Roksana', 'Tomichka', 'Chulymskaya' and 'Volkova'. The lowest fruit yield was harvested from a cultivar of Czech origin 'Modry Triumph' (105 g). The average fruit yield of all mechanically harvested cultivars were generally significantly lower when compared to manual harvesting. In order to apply the mechanical harvesting technology, cultivars should be selected according to their suitability for that purpose.

CONCLUSIONS

The current research represent the results of the winter hardiness, secondary flowering, fruit weight, productivity and fruit yield of 18 blue honeysuckle cultivars evaluated in the Nordic-Baltic climate region. Russian cultivars 'Chulymskaya', 'Roksana', 'Moskovskaja 23' and Czech cultivar 'Modry Triumf' did not perform well in these conditions. The Canadian cultivars revealed their better suitability for the Nordic-Baltic climate during the experimental years of 2016–2020 at Polli Horticultural Research Centre and Seedri nursery, Estonia. The prospective blue honeysuckle cultivars of interest are Canadian origin 'Borealis' and 'Tundra', Russian origin 'Volhova' and Polish origin 'Duet'. Plants of these cultivars presented good winter hardiness, low occurrence of secondary flowering, large fruits and sufficient productivity with high yield. Based on the provided results, these cultivars can be recommended for growing in the changing climate of Estonia. However, further investigations need to be done on flower pollination, plant resistance to pests and diseases, ripening time and fruit biochemical composition of cultivars in order to gain additional information for the purpose of cultivation and fruit quality of this crop.

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Precision fertilisation technologies for berry plantation

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Abstract. Increased cost-effectiveness in crop production can be achieved by automating technological operations. This is also the case for berry cultivation in plantations. Starting any berry cultivation automation process should, quite naturally, begin with fertilisation, since this is the first technological operation to be carried out during the vegetation period and is a relatively simple one. The main task here is to apply the correct amount of fertiliser under the canopy of plants. Blueberry plantations that have been established on milled peat fields have plants that have been planted in parallel rows at a pre-designated interval. The fertilisation of plants must take place individually in the first years of their growth, so that each plant is fertilised separately. This form of fertilisation can be referred to as precision fertilisation. The aim of this paper was to provide an overview of the levels of technology now available when it comes to precision fertiliser equipment and to introduce the concept of a new precision-automated fertiliser unit, while also justifying the efficiency of using automated equipment. The automated fertiliser unit that is to be designed will be autonomous, will move unmanned through the plantation, and will include the necessary sub-systems for the precision fertilisation of individual plants, such as a plant detection system, a fertilising nozzle, a motion system and, additionally, a service station. On the basis of the results obtained, it can be argued that the use of an automated precision fertilisation unit increases productivity levels by approximately 2.25 times and decreases the specific fertiliser costs by approximately 8.4 times when compared with the use of a portable spot fertiliser.

Key words: berry plantation, agricultural robotics, precision fertilising, product design and development.

INTRODUCTION

The blueberry cultivation system consists of the following technological operations: soil preparation, planting, plantation maintenance, plant fertilisation, plant protection, harvesting, post-harvesting processing, and cutting back the plants or carrying out rejuvenation pruning (Starast et al., 2002; Olt et al., 2013; Zydlik et al., 2016¸ Retamales & Hancock, 2018).

These technological operations can be carried out either manually or using a machine (Scherm et al., 2010; Olt et al., 2013; Arak & Olt, 2014), with the latter method of cultivating blueberries being more productive and efficient than the former (Käis &

Olt, 2010; Takeda et al., 2017). Blueberry plantations have been established on mineral soils, but also on depleted peat milling fields (Peatland Ecology Research Group, 2009). Machinery has been developed that can carry out all of the technological operations that are involved in blueberry plantations that have been established on mineral soils.

Peat milling fields have a pH level and moisture regime that is suitable for blueberry cultivation (Noormets et al., 2003; Smagula & Litten, 2003; Arak et al., 2018); however, the ground used here has a low load-bearing capacity and, therefore, machinery with very low levels of specific pressure can be used and, unfortunately, such machinery has not been the centre of attention for larger machinery-building companies. A few smaller companies have produced the appropriate machinery though, and other units that potentially can be used in plantations that have been established on peat milling fields (Olt et al., 2013; Takeda, et al., 2017).

The main possibility offered in terms of reducing the unit cost of blueberry cultivation is by implementing machinery-based solutions. The use of machinery in blueberry cultivation sets out specific requirements for the plantation: the use of machinery is possible in continuously-maintained and pruned plantations; in order to ensure normal operations in terms of servicing and harvesting machinery, the plantation ground must be level and should remain level during use; service or technical roads must be established; machine harvesting requires the periodic pruning of old branches; first rejuvenation pruning is carried out between the eighth and tenth years of operations, and thereafter every three or four years.

The efficiency of machine cultivation around berry plants, including blueberries, can further be increased by using methods that involve precision cultivation (Chang et al., 2012) and by automating the technological operations that are involved in the process. In the implementation of precision cultivation, unmanned platforms (Dubbini et al., 2017; Grimstad et al., 2018) and field robots (Hayashi et al., 2010; 2014; Yamamoto et al., 2010; 2014) are increasingly being introduced into the process of carrying out the various technological operations.

Beginning the automation of blueberry cultivation from the point of fertilising the plantation is entirely reasonable when it comes to carrying out that process by using an automated fertiliser unit. With any berries, blueberries included, the fact that the availability of nutrients in the soil significantly affects the productivity of the plants is a factor that must be taken into account (Farooque et al., 2012; Chen et al., 2014). Average blueberry yield can go from 1.49 t ha^{-1} to 5.02 t ha^{-1} with the right fertilisation technics (Karlsons & Osvalde, 2019). Greater fertilisation norms (with nitrogen levels reaching up to 150 kg ha^{-1}) serve to significantly improve the growth of the plants and improve yields (Ehret et al., 2014), especially with soils that are low in nutrients (Starast et al., 2007; Paal et al., 2011). A strong positive relation has been found between the availability of nutrients and the vegetative parameters of the blueberry plant - in terms of its height and the total area of the leaves (Liet, 2017; Vainura, 2018).

However, fertilisation depends both upon the properties of a specific soil and the plant's age, which results in a specific norm for each fertiliser. From the point of view of the plant's age, the fact should be kept in mind that the root grows each year and this will result in a larger area to be fertilised. In the first year, the fertiliser should be spread across a smaller area of about 20×20 cm around the plant; at an age of between six to eight years, the area around the plant's roots have achieved their maximum dimensions (about 100×100 cm), and this also depends upon the density of the plantation: if the distance between plants in a row is 150 cm, then the area to be fertilised is 150×150 cm.

The aim of this research was to provide an overview of the current situation regarding fertilisers and their analogues, to introduce the concept of advanced fertiliser technology that will need to be developed, and to justify the effectiveness of using automated fertilisation equipment when compared to other well-known technological solutions.

MATERIALS AND METHODS

This work comprised the first phase of product development, the substance of which was to determine the current situation regarding technological equipment that can be used in fertiliser production, to define the available functionality, to create a concept, to search for and select constructive solutions, and to justify the effectiveness of those solutions (Ulrich & Eppinger, 2011).

The patent databases, Espacenet and World Intellectual Property Organization (WIPO), were used to determine the current situation. According to current regulations, the scope of the patent investigation was global and its depth covered a span of at least twenty years. In order to design the precision fertiliser technology for a berry plantation - in particular a blueberry plantation - it is necessary to define its core and individual functions, which are as follows:

1) in the blueberry plantation that has been created, the plants are arranged in rows with a set gap between them (between 1.0 m and 1.5 m, and possibly less or more), and therefore the automated fertiliser unit must move through the field, mainly in a straight line along the plant row;

2) the fertilisation of blueberry plants must take place individually in the first years of their growth, with each plant being fertilised separately according to the needs of the plant. It is expedient to apply spot fertilisation, with the fertiliser having to be spread around the plant and under its canopy (Hart et al., 2006);

3) the blueberry plants should be fertilised two or three times during the season by dosing the fertiliser at a rate of between 30–80 g for each plant (less in the first few years, more later) (Hart et al., 2006); therefore the automated fertilisation unit's dosing equipment should be set up to dispense the required amounts of fertiliser. Carpet fertilisation cannot be used in a blueberry plantation because it would allow weeds to flourish and that would unpredictably increase maintenance costs;

4) the automated fertilisation unit's functions must also include not only precise dosage of the fertiliser but also the identification of the plant's location (Milella et al., 2018; Brahmanage & Leung, 2019).

If the blueberry plants were located in one continuous row with a specific gap between them then it would be relatively easy to design an automated fertilisation unit - robot. But in real-life plantations, plant rows are not straight and the distance between plants varies. According to sources (Arak & Olt, 2020), deviations from the central axis of a plant row can reach up to ± 365 mm and the distance between plants - their spacing - can vary by 137.2 ± 166 cm on average in a plant row, being somewhere within the range of between 91.5 cm to 180.0 cm. The values for the transverse and longitudinal dimensions of the projection of the foliage vary widely between 5.0 cm and 48.0 cm, and between 4.0 cm to 44.0 cm respectively. For example, the mean height of two year-old plants is measured at 22.0 cm, but this also differs within a large range which can be anything between 6.0 cm and 39.0 cm. A type of fertiliser that adapts to these conditions can be described as a precision fertiliser, and a suitable stand-alone unit as a precision automated fertilisation unit.

The cost effectiveness levels in terms of berry cultivation can be determined through the specific cost of any technological operation as e_A (EUR ha^{-1}), which includes the fixed and variable costs involved in the operation as follows:

$$e_A = \frac{1}{W}(C_F + C_V), \quad (1)$$

where W – the fertilising unit's productivity levels; C_F – the fixed cost, € h^{-1}; C_V – the variable cost, € h^{-1}.
with the fixed cost C_F being expressed as follows:

$$C_F = \frac{1}{T}\left(C_f + C_a + C_h\right), \quad (2)$$

where T – the fertilising unit's seasonal load i.e. its total operating time during the season, h; C_f – the fertilising unit's depreciation, €; C_a – its battery's depreciation, €; C_h – its service station's depreciation, €.

Taking into account that

$$C_f = \frac{C_{fb} \cdot a_{fc}}{100}, C_a = \frac{C_{ab} \cdot a_{ac}}{100}, C_h = \frac{C_{sb} \cdot a_{sc}}{100}, \quad (3)$$

where C_{fb} – the carrying volume for the automated fertilising unit, €; C_{ab} – the cost of its battery (or batteries), €; C_{sb} – the carrying volume for its service station, €; a_{fc} – the depreciation rate of the automated fertilisation units, %; a_{ac} – the depreciation rate of its battery (or batteries), %; a_{sc} – the depreciation rate of its service station, %, we can express the fixed cost as follows:

$$C_F = \frac{1}{100T}\left(C_{fb} \cdot a_{fc} + C_{ab} \cdot a_{ac} + C_{sb} \cdot a_{sc}\right). \quad (4)$$

The variable cost C_V is expressed as:

$$C_V = \frac{C_m}{T} + C_e + C_w, \quad (5)$$

where C_m – the annual cost of servicing (involving maintenance and repairs) for the automated fertilising unit, €; C_e – the cost of electricity to recharge the battery, € (kWh)$^{-1}$; C_w – related labour costs (wages and taxes), € h^{-1}, whereas the specific cost C_e (€ (kWh)$^{-1}$) of electricity to charge the battery is calculated as follows:

$$C_e = Q_e \cdot (r_e + r_c + r_r + r_a) \quad (6)$$

where Q_e – the hourly electricity consumption rate (kWh) h^{-1}; r_e – the purchase price of electricity, € (kWh)$^{-1}$; r_c – the network fee, € (kWh)$^{-1}$; r_r – the renewable energy fee, € (kWh)$^{-1}$; r_e – the electricity excise duty, € (kWh)$^{-1}$; and labour costs C_w are calculated as follows:

$$C_w = M_c \cdot q_p \cdot k_p, \quad (7)$$

where M_c – the area of the plantation that is being fertilised by the automated fertilising unit or, in other words, the automated fertilising unit's productivity levels, ha h^{-1}; q_p – remuneration for the operator of a manually-operated fertilising unit in terms of fees to be paid for fertilising one hectare of the plantation, € ha^{-1} (or in the case of an automated fertilising unit being used, $q_p = 0$); k_p – the factor accounting for labour taxes; $k_p = 1.34$.

Given the relationships (5) – (7), we get the formula for calculating the variable cost as follows:

$$C_V = \frac{C_m}{T} + Q_e \cdot (r_e + r_c + r_r + r_a) + M_c \cdot q_p \cdot k_p. \tag{8}$$

The productivity area W_A (ha h^{-1}) for the precision automated fertilisation unit can be calculated as follows:

$$W_A = 0.36 \cdot v_k \cdot B_h, \tag{9}$$

where v_m – the average operating speed of the automated fertilisation unit, m s^{-1}; B_h – the operating width, m.

Considering that $v_m = s_f/(t_m + t_s)$ and the working width is equal to the row width $B_h = s_l$, the productivity area can be expressed as follows:

$$W_A = 0.36 \cdot \frac{s_f \cdot s_l}{t_m + t_s}, \tag{10}$$

where s_f – the distance between plants, ie. the 'step' between plants in a row, m; s_l – the row width, m; t_m – the time taken in moving from plant to plant; t_s – the time taken for standstills and/or dosing the fertiliser.

The operations of a working fertiliser are better characterised by shift productivity levels - its working day productivity levels $W_{A,d}$, which are expressed as follows:

$$W_{A,d} = 0.36 \cdot \frac{s_f \cdot s_l}{t_m + t_s} \cdot T_d \cdot \tau, \tag{11}$$

where T_d – the total length of a working day or of a single shift in the plantation, h; $_d = 8$ h; τ – the working time usage factor, whereas

$$\tau = \frac{T_e}{T_d} = \frac{T_e}{T_e + T_p + T_t + T_l + T_o}, \tag{12}$$

where T_e – the effective working time h of the fertilising unit, ie. the time involved in the fertilising unit moving along the plant row and fertilising the plants; T_p – the time taken to turn on the turning strip and to move from one work path to another; T_t – the time taken to move to the service point and to return to the work in progress; T_l – the time taken to fill up the fertiliser bunker and to set up the fertilising unit; T_o – the organisational time involved, including the time taken for short-term rests.

In this work, the cost effectiveness levels involved in the fertilisation of berry plants has been compared with that of manual fertilisation, with the use of a portable spot fertiliser and a precision automated fertilisation unit being taken as an example.

DESIGN AND DEVELOPMENT OF THE FERTILISING ROBOT

A determination of the current situation regarding fertiliser technology

A patent investigation revealed that, according to patent document CN209192086U, there exists an automated field unit which contains a frame, driving and rear wheels, a drive, and a work tool. A shortcoming of this already-available automated field unit is the fact that its drive is equipped with an internal combustion engine which generates noise, has a high purchase cost, emits carbon dioxide and other harmful chemical compounds into the atmosphere as a result of fuel combustion, and involves high levels of cost in terms of regular technical maintenance during its use. According to patent document CN108551783A, there currently exists an automated field unit that consists of a frame, driving and support wheels, a drive, and sowing and fertiliser tools. This unit is a mobile machine for row-based sowing of seeds and the application of fertilisers, and its main drawback is the fact that it sows seeds and fertilises along a set row patterns and is unable to react to deviations from that row. According to patent document CN109196995A, there is an extant automated fertiliser unit which contains a frame, a fertiliser tool, a transmission section, a control unit, and accessories. A shortcoming of this particular automated fertiliser unit is its lack of functionality. The main problem with those automated fertiliser units that can be found in a search through the available patent records is that they lack a plant recognition function.

According to document EE 01058U1, there exists a fertilising unit that has a precision dosing function, while also including a fertiliser bunker, a volume-based dosing module with a regulator and a drive, and a fertiliser line and dosing nozzle. This unit's dosing module is a volume-based dispenser which is connected to a drive that contains a control unit. The dispenser's drive unit is an electric drive which consists of a step motor which allows the fertiliser quantity to be set up and used as a precision fertiliser in a berry plantation, by means of dispensing the prescribed quantity of fertiliser individually to each plant. The main shortcoming of the fertilising unit described above is that it is a portable unit which is carried and operated manually, which means that its operating time per day is limited (to eight hours) and it is directly dependent upon the operator's work capacity.

Automating the fertilising process

The authors of this work aimed to be able to automate the fertilisation process in connection with blueberry plants, which consists of automating the fertilisation unit - making it autonomous and self-regulating. This fertilising unit will contain a fertiliser bunker that is rigidly attached to its frame by means of a structural grid, as well as possessing self-adjusting guide wheels, rear wheels, a dosing unit that is attached to the lower part of the fertiliser bunker and a fertiliser line that is connected to the dosing unit's upper section, a traction drive, a control unit for the fertilising unit as a whole, a sensor block, a precision fertilising tool, an onboard computer, and a battery. The automated fertilising unit's control unit (Fig. 1) contains a steering wheel drive which allows the unit to turn its steering wheels, a traction drive to ensure that the automated fertilising unit moves forwards, and a brake drive to stop it during the fertiliser dosing process. The automated unit has a wheeled chassis with two steering wheels and two rear wheels. Electric drives have been used for a steering wheel drive, a traction drive, a brake drive, and the dispensing nozzle's drive. The fertiliser line is connected to the dispenser by its upper end. The dispenser is a volume-based unit which is equipped with a regulator and is connected to a drive that contains a control unit in the form of a step motor.

The fertiliser line is a flexible, tubular element with a dosing nozzle at its lower end, and which is attached to a linear guide. The function of the linear guide is to move the dispensing nozzle in an oblique direction in relation to the fertilising unit's motion target.

The sensor block contains plant recognition cameras, an energy storage indicator, a navigation sensor, and a level-recognition sensor in the fertiliser bunker. The precision fertilising machine includes a plant detection camera, a dosing nozzle drive, a linear guide, and an automatic switch on the dosing unit. One plant detection camera which is designed to detect the location of a plant row is attached to the front end of the automated fertilising unit and another, designed to identify the plant to be fertilised, is placed near its precision fertilising unit.

The automated fertiliser unit's service system includes a remote database for data transmission, a digital twin and a service station containing a fertiliser tank which is equipped with a filling unit for the fertiliser unit's fertiliser bunker, a level sensor for the fertiliser tank, and a charging unit for charging the fertiliser unit's energy storage unit. Other units may also be operating within the service system of a berry plantation. Information on the functioning of the service system will be provided to the farmer or to the operator who is responsible for making decisions when it comes to controlling the automated fertilisation unit.

The service station contains a fertiliser tank that is equipped with a filling unit for the fertiliser unit's bunker, a level sensor for monitoring the fertiliser tank's level, and a charging unit for charging the fertiliser unit's energy storage battery. The filling unit for the fertiliser's bunker consists of a screw conveyor.

The automated fertiliser unit works as follows: the fertiliser bunker in the automated unit is filled with fertiliser at the service station and its energy storage unit is charged. The automated fertiliser unit, now fully prepared for fertilisation, will be directed from the service station to move along the rows of berry plants within the plantation and will move in a shuttling manner, progressing from plant to plant.

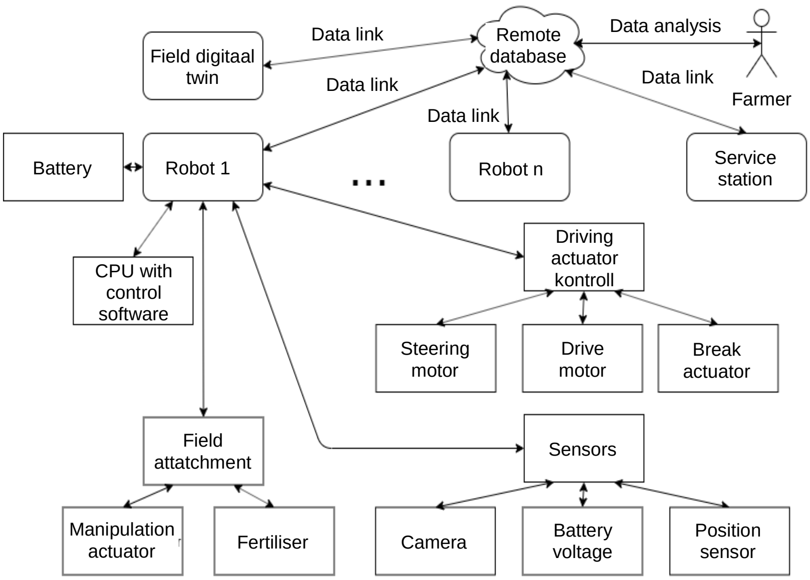

Figure 1. A block diagram showing the automated fertilising unit and its service system.

The automated fertilisation unit's service system is connected through a data communication relay to a remote database (Fig. 1), where the unit's main logs are kept and access to current data and calculation results is provided to other parties. The automated fertilisation unit and its maintenance station provide data uploads to the remote database and download instructions from the remote database. In addition, other robots can work within the berry plantation's system, with these also being coordinated through the remote database. Information on the operations of the service system is provided through the remote database to the farmer or to the responsible operator who is making the control decisions. The digital twin receives the input required for simulation from the remote database, in order to predict the need for maintenance and to allow for the digital reproduction of a deviation situation in a simulation environment. The automated fertilisation unit's sensor block provides feedback from the sensors to the onboard computer where data processing is carried out by the required software. On the basis of the processed data, the performance of assigned tasks is assessed and the necessary control effects are transmitted to the precision fertilising unit and to its control unit. The navigational sensor and the plant detection cameras are used to identify the position of the automated fertilisation unit within the plantation in relation both to the plant row and to the specific plant. Based on information received on the location of the automated fertilising unit, a sequence of activities is planned with the aim of fertilising the centre of the plant. From the image received from the plant camera, the angle is calculated between the plant row and the direction of the fertilising unit's movement, which is then attempted to be reduced by a correction impulse to the steering wheel drive. When the detected plant comes within the precision fertiliser unit's work area, the traction drive is stopped, the brake drive is applied, and the automated fertilisation unit is stopped while the plant detection camera is used to check that the dosing nozzle has reached the optimum location for fertiliser delivery, sending an impulse to the control drive for the dosing nozzle. The automatic dose switch is activated and fertiliser moves from the dosing unit through the fertiliser line to the dosing nozzle, where it drops to the designated spot when it reaches the ground. A transverse displacement device will move the dosing nozzle in a transverse direction compared to the automated fertilisation unit's direction of movement so that it can dispense the fertiliser to the intended location. After the fertilisation operation has been carried out, the traction drive is started and the brake drive releases the brake so that the automated fertilisation unit can move over to the location of the next plant. The plant fertilisation cycle will continue to be repeated until either the reading on the level sensor in the fertiliser bunker or the charge indicator on the energy storage unit falls below the set point; thereafter the automated fertilisation unit proceeds to the maintenance station, uploading to the maintenance station's remote database the location at which it stopped providing its intended service. At the maintenance station, the automated fertilisation unit stands still until its fertiliser bunker is filled with fertiliser and its battery has reached its intended level of charge or has been replaced. Technical maintenance can also be carried out on the automated fertilisation unit at the maintenance station if necessary. After leaving the service station, the automated fertilisation unit will return to its unfinished task or is directed by the remote database to start a new task.

In stand-alone driving, the automated fertilisation unit uses software algorithms to avoid obstacles, taking into account not only its own presence but that of other automated units within the plantation, based on information in the remote database. To be able to avoid random objects, the locations of the bodies are used as they are calculated from the image stream on the plant detection camera.

In addition, other robots can work in the berry plantation system, being coordinated through a remote database. Information on the operation of the system will be provided through the remote database to the farmer or to the responsible operator so that control decisions can be taken. The automated fertilisation unit's onboard computer checks for task execution based on feedback from the sensor block. The navigation sensor and the plant detection camera are used to detect the position of the automated fertilisation unit in the plantation in relation both to the plant row and to the plant. Based on information received on the location of the automated fertilisation unit and from the plant detection camera, a sequence of activities is planned with the aim of bringing the dosing nozzle to the plant so that the intended amount of fertiliser can be supplied. If the reading on the fertiliser bunker's level sensor or the energy storage unit's charge indicator falls below the set point, the automated fertilisation unit will head to the maintenance station. Once the required level for the fertiliser bunker and/or the charge level on the energy storage unit have been reached again, the automated fertilisation unit will return to its last location and continue any task that it has not yet completed.

The first prototype of the automated fertilisation unit has been built on the platform of an electric ATV Hecht 56150 (Fig. 2). The specifications for the automated fertilisation unit's prototype are given in Table 1.

Table 1. Technical specifications for the original prototype of the automated fertilisation unit

Parameter	Unit	Value
The unit's dimensions (l × w × h)	mm	1,450×1,020×960
The unit's weight	kg	120
Engine power	W	1,200
Batteries	Ah	20
Movement speed	km h^{-1}	40
Load-bearing capacity	kg	120
Working speed	km h^{-1}	1.8
Fertiliser bunker volume	L	20

Figure 2. The original prototype of the automated fertilisation unit.

Specific cost of spot fertilising operations

We compare the specific costs involved in spot fertilising with the use of three different technical means, namely fertilising:

1) with a bucket and a measuring shovel;
2) with a portable spot fertiliser;
3) with an automated precision fertilisation unit.

Based on the data provided in sources (Arak & Olt, 2020), the distance between plants in the spreadsheet calculations amounts to 1.4 m between rows, with the length of plant rows being 280 m. Each plant row contains 200 plants. In all cases the fertilisation rate was equal to 30 g per plant. The number of plants to be fertilised for different variants has been selected on the basis of an indicative daily norm (Table 2).

Table 2. Initial data for spreadsheet calculations

Indicator	Manual fertilising with a bucket and a measuring shovel	Portable contact spot fertiliser	Automated precision fertilisation unit
Number of plants to be fertilised, N	2,400	3,000	6,000
Length of the plant row l, m	280	280	280
Distance between plants in a row, s_f m	1.4	1.4	1.4
Spacing between plant rows, s_l m	1.4	1.4	1.4
Number of plants in a row, n	200	200	200
Number of plant rows	12	15	30
Volume of fertiliser bunker, L (kg)	10 (9)	20 (18)	100 (90)
Fertilisation norm, g plant^{-1}	30	30	30
Number of times the fertiliser bunker needs to be filled per day	8	5	2
Total weight of fertiliser given to plants, kg	72	90	180

Measurements and timings were taken for all of the usual operations, including the time taken to move from one plant to another, the standstill time, the time taken to administer the fertiliser, the time taken to turn to the next plant row at the end of the current plant row, the service time including time taken to fill the fertiliser bunker, and the time taken to replace the battery, as well as time for technologically-related matters, including the time taken to travel to the maintenance plant, such as the location at which the fertiliser bunker is to be filled, and the time taken to return to the work site. The organisational time, in terms of the operator's rest period which is ninety minutes a day. The data obtained was used to find an indicative time-use factor τ (Table 3).

Table 3. The technological characteristics of spot fertilising when using different technical means

Indicator	Manual fertilising with a bucket and a measuring shovel	Portable contact spot fertiliser	Automated precision fertilisation unit
Time taken to move from one plant to another along the row, t_m s	4.6	4.2	2.8
Time taken for standstills and to administer the fertiliser, t_s s	5.3	4.0	1.9
Time taken to turn to the next plant row, s	5.0	4.5	3.0
Effective operating time, T_e s	23,760	24,600	28,200
Total time taken to move from one plant row to another, T_p s	55	63	87
Technological time - total time taken to move to the service point or station and back, T_t s	2,255	1,590	160
Distance to the service point and back, m	2,480	1,740	330
Service time - total time to fill the fertiliser bunker and to replace the battery, T_l s	680	390	60
Organisational time (operator's rest time, coffee breaks, etc), T_s s	5,400	5,400	0
Time taken to fertilise plants, T_d s	32,150	32,043	28,507
Working time usage factor, τ	0.739	0.767	0.990
Potential productivity area, ha h^{-1}	0.071	0.086	0.150
Shift productivity, ha shift^{-1}	0.420	0.527	1.188
Productivity area, ha h^{-1}	0.052	0.066	0.149

RESULTS

Detailed results from the analysis are presented in Tables 3 and 4. Table 3 shows that if the fertiliser bunker is filled at one end of the field, this initial task takes a lot of time for a manual fertiliser to move to the service point and back to the working spot and to fill the fertiliser bunker. Working time is best used when working with an automated precision fertilisation unit. It is also important that any automated precision fertilisation unit does not require any rest periods or coffee breaks. It is noteworthy that in the case of using a manual fertiliser, a total of 2,400 plants take eight hours, 49 minutes, and 28 seconds to fertilise, or 13.27 seconds per plant. When using a portable spot fertiliser, a total of 3,000 plants can be fertilised in eight hours, 51 minutes, and three seconds, or 10.62 seconds per plant. An automated precision fertilisation unit fertilises a total of 6,000 plants in seven hours, 55 minutes, and seven seconds, or 4.75 seconds per plant.

Table 4. Indicators of economic efficiency for spot fertilising

Indicator	Manual fertilising with a bucket and a measuring shovel	Portable contact spot fertiliser	Automated precision fertilisation unit
Cost of equipment, EUR	12	729	8,900
Fertiliser unit's depreciation, %	–	10	10
Fertiliser unit's usage time, h	–	1,700	4,760
Fertiliser unit's standard depreciation, EUR h^{-1}	–	0.429	1.869
Cost of the service station, EUR	–	–	5100
Maintenance plant's depreciation, %	–	–	10
Maintenance plant's usage time, h	–	–	9200
Maintenance plant's standard depreciation, EUR h^{-1}	–	–	0.554
Electrical energy consumption, kWh $shift^{-1}$	–	–	4
Purchase price of electrical energy, EUR $(kWh)^{-1}$	–	–	0.0428
Transmission of electricity, EUR $(kWh)^{-1}$	–	–	0.0423
Renewable energy fee, EUR $(kWh)^{-1}$	–	–	0.0113
Electricity excise duty, EUR $(kWh)^{-1}$	–	–	0.001
Cost of electricity to charge the batteries, EUR h^{-1}			0.022
Labour costs, EUR h^{-1}	6.5	6.5	–
Labour costs, EUR h^{-1} (including taxes at 34.4%)	8.74	8.74	–
Fixed costs, EUR h^{-1}	≈ 0	0.429	2.423
Variable costs, EUR h^{-1}	8.74	8.74	0.049
Productivity area, ha h^{-1}	0.052	0.066	0.149
Specific operating cost, EUR ha^{-1}	168.00	138.92	16.59

As a result, during one shift of eight hours a total of 2,170 plants can be fertilised with a hand bucket and a measuring shovel, while 2,711 plants can be fertilised with a portable spot fertiliser unit, and 6,063 with an automated fertilisation unit. Therefore the automated fertilisation unit is 2.25 times more productive than the portable spot fertiliser unit. At this point it should be taken into account that the duration of a human working shift is eight hours a day, but only weather conditions can limit the working time of the automated fertilisation unit. The economic efficiency indicators for spot fertilisers are summarised in Table 4. The efficiency calculations have taken into account the fact that the maintenance station is also used for servicing a cleaning robot, which is why its usage time per year is longer than that of the automated fertilisation unit.

Table 4 shows that any mechanisation of the process produces greater levels of efficiency than does manual operation, and automation is even more efficient. It is characteristic that the application of precision fertiliser technology requires a significantly higher one-off investment than in other technological options, notably in terms of the acquisition of an automated fertilisation unit and a maintenance station, but the specific costs involved in the automated fertilisation unit are much lower than those involved with other technological options. Therefore, automated fertilisation unit will be more cost effective on larger plantations.

The data in Table 4 show that one automated precision fertilisation unit is suitable for serving plantations of at least 30 hectares, and taking 201 hours for a single fertilising operation, or approximately seventeen days or 2.5 weeks if working days are up to twelve hours long. The use of a portable local fertiliser is associated with a human factor, which allows for a maximum of eight hours for a shift and a maximum of 30 hectares for the same time (2.5 weeks) to be fertilised, using four units or working in shifts with two units. This calculation does not take into account the cost of the fertiliser, so the costs that are presented are not specific fertiliser costs, but reflect only the cost involved in carrying out the technological side of the operation.

CONCLUSIONS

The work has defined the current situation regarding the use of technological equipment for fertilising a berry plantation (the patent study itself), from which it was found that autonomous precision fertilisation in berry plantations is still something that has not been fully resolved in technological terms. This paper offers the concept of an automated precision fertilisation unit and its service system, providing definitions of the functions for such an automated unit, while selecting the robot platform, completing the original prototype, and providing an initial justification for the efficiency of using an automated precision fertilisation unit. On the basis of the results obtained, it can be argued that the use of an automated precision fertilisation unit increases productivity levels by approximately 2.25 times and decreases the specific fertiliser costs by approximately 8.4 times when compared with the use of a portable spot fertiliser. This will make it possible to reduce the realisation price for harvested crops and increase the competitiveness of berry farming. It should be added that the figures that have been obtained are provisional and still need to be checked under real production conditions.

In the subsequent R&D work, the automated precision fertilisation unit and its service system must be designed together with a modular maintenance station according to the concept. The parameters of the work tools must be optimised if necessary, work documentation must be prepared, the production technology must be prepared and made ready, the finished prototype must be manufactured, and a trial run must be carried out if necessary. The methodology to be resolved to be able to determine the individual fertiliser quantity for each plant. This would allow a saving to be made in terms of the cost of acquiring fertilisers, while preserving the balance between nutrients in the soil, reducing environmental pollution, and increasing the yield. Naturally, legal protection for the intellectual property resulting from the work must also be provided.

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Structure: Title, Authors (initials & surname; an asterisk indicates the corresponding author), Authors' affiliation with postal address (each on a separate line) and e-mail of the corresponding author, Abstract (up to 250 words), Key words (not repeating words in the title), Introduction, Materials and methods, Results and discussion, Conclusions, Acknowledgements (optional), References.

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- Use single line spacing and **justify the text**. Do not use page numbering. Use **indent 0.8 cm** (do not use tab or spaces instead).
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- Use *italics* for Latin biological names, mathematical variables and statistical terms.
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References

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- **For whole books**

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Shiyatov, S.G. 1986. *Dendrochronology of the upper timberline in the Urals*. Nauka, Moscow, 350 pp. (in Russian).

- **For articles in a journal**

Name(s) and initials of the author(s). Year of publication. Title of the article. *Abbreviated journal title* (*in italic*) volume (in bold), page numbers.

Titles of papers published in languages other than English, should be replaced by an English translation, with an explanatory note at the end, e.g., (in Russian, English abstr.).

Karube, I. & Tamiyra, M.Y. 1987. Biosensors for environmental control. *Pure Appl. Chem.* **59**, 545–554.

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- **For articles in collections:**

Name(s) and initials of the author(s). Year of publication. Title of the article. Name(s) and initials of the editor(s) (preceded by In:) *Title of the collection (in italics)*, publisher, place of publication, page numbers.

Yurtsev, B.A., Tolmachev, A.I. & Rebristaya, O.V. 1978. The floristic delimitation and subdivisions of the Arctic. In: Yurtsev, B. A. (ed.) *The Arctic Floristic Region*. Nauka, Leningrad, pp. 9–104 (in Russian).

- **For conference proceedings:**

Name(s) and initials of the author(s). Year of publication. Name(s) and initials of the editor(s) (preceded by In:) *Proceedings name (in italics)*, publisher, place of publishing, page numbers.

Ritchie, M.E. & Olff, H. 1999. Herbivore diversity and plant dynamics: compensatory and additive effects. In: Olff, H., Brown, V.K. & Drent R.H. (eds) *Herbivores between plants and predators. Proc. Int. Conf. The 38th Symposium of the British Ecological Society*, Blackwell Science, Oxford, UK, pp. 175–204.

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