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ADOMAS HREBNICKIS – THE FAMOUS HORTICULTURIST AND POMOLOGIST

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The article represents a brief biography of the famous horticulturist and pomologist professor Adomas Hrebnickis, reviews his pedagogic and editorial activities, traverses his scientific heritage and discusses the role of his works in the development of horticulture. A. Hrebnickis was a very productive scientist, broadminded horticulturist, good organizer and remarkable pedagogue. He carried out innovative biological investigations on parthenocarpy and flowering of fruit trees. A. Hrebnickis discovered and described cultivars still present in Lithuanian cultivars standard assortment (still popular among Lithuanian gardeners) and his works highlighted many cultivars originated from Lithuania worldwide. A. Hrebnickis plays a significant role in horticulture development not only in Lithuania but also in the whole Eastern Europe.

Key words: Adomas Hrebnickis, horticulture, pomology.

Brief Biography of Adomas Hrebnickis. According to the passport of Adomas Hrebnickis he was born on the 24th December, 1857 (the old Julius calendar) (Ališauskaitė, 1987 m.). According to new calendar of Grigalius, the birth date is 6th January, 1858. Professor was born in former Vitebsk gubernya, Lepel district, Cioche manor. His parents Stanislav Hrebnickis and Konstancia Samiščia were poor landlords. They lost the manor, the birthplace of the future professor A. Hrebnickis, when he was only school-aged. Thereafter Adomas lived with his grandmother in Turosą manor with a huge garden and plenty of garden
plant cultivars. This garden undoubtedly had a great impact on A. Hrebnickis interests.

**Education.** During 1870–1878 A. Hrebnickis studied in Daugavpils (Dvinsk) gymnasiu. There his aptitude for the Nature Science and drawing abilities revealed. After finishing gymnasiu he entered the St. Petersburg Institute of Forestry, where the discipline of horticulture was taught. In 1883 A. Hrebnickis successfully presented his thesis under the title “Starch as a storage material in our trees” (Гребницкий, 1883). This work was published as a single edition and A. Hrebnickis was offered a place of assistant at the Institute. There he studied under support of famous botany professors J. P. Borodin and N. A. Monteverde. After studying works of horticulture of many foreign and Russian scientists, he became interested in horticulture science, which was subject matter of discussion in amateur horticulturists clubs. As a result an idea of own research base arose, however A. Hrebnickis did not possess any land or money for that.

**The beginning of ‘Rojus’ establishement.** While studying at the St. Petersburg Institute of Forestry A. Hrebnickis made friends with Vladas Stankevičius from Lithuania. He visited V. Stankevičius home a few times. A. Hrebnickis married V. Stankevičius sister Stanislava and settled in Beržininkai near small town Dukštas. After creating his own family A. Hrebnickis got an opportunity to realize his ideas about horticultural investigations in his own garden. In 1886 A. Hrebnickis started to grow planting material in his father’s in law garden that turned into pomological garden called “Rojus” (“Paradise”). This garden was unsurpassable in Lithuanian history. The garden was being developed till 1936. Plants were not only propagated by A. Hrebnickis himself but also obtained from Ukraine, Poland and Germany. A few varieties were brought from the journey to USA where A. Hrebnickis took part in international exhibition of horticulture as an expert in 1895. There were 1197 different varieties of apple, pear, plum, sour and sweet cherry already growing in the garden in 1922.

**Pedagogic activity.** At the age of 35 A. Hrebnickis began to give his lectures of horticulture at the Institute of Forests in Petersburg. There he worked as an assistant from 1884 to 1891, later from 1902 to 1918 as a lecturer and head of department and after election as a professor in 1918 (Седов, 1997) continued working until 1922 when he was granted a title of professor honour.


In 1922 A. Hrebnickis returned to “Rojus”, which was a part of Poland then, and actively joined the development of Poland horticulture, wrote articles, collected, observed, described, propagated and disseminated landraces. A. Hrebnickis was elected as a full member of pomology committee of Poland horticulture association, which was responsible for regionalism and standardization of cultivars. In 1923 professor A. Hrebnickis was admitted to citizenship of Poland.
After the return of Vilnius land to Lithuania in 1939, professor A. Hrebnickis was invited to Lithuanian Chamber of Agriculture as handler for horticulture.

Professor A. Hrebnickis died on 13th October, 1941 and was buried in the grave of Stankevičius family in Dukštas town.

Scientific heritage. The scientific heritage of professor A. Hrebnickis contains fundamental works of pomology, publications in scientific periodical journals, identified and described landrace and apple cultivars selected by professor himself.

The first publication of A. Hrebnickis was his original thesis prepared at the Institute of Forests in Petersburg in 1883. Intensive scientific work of Professor A. Hrebnickis lasted for 53 years until 1936 when the last publications showed. Scientific – literary heritage of A. Hrebnickis contains translations, original scientific works including specific kind of information – answers in various journals, especially “Пёдоводство”, to questions concerning horticulture. Overall there are almost 500 publications of A. Hrebnickis. Figure 1 represents the accumulation curve of A. Hrebnickis publications.

![Figure 1](image.png)

**Fig. 1.** Trends of A. Hrebnickis publications accumulation

The decade from 1898 to 1908 gave the biggest number of A. Hrebnickis scientific publications. During that period the highest increase of original publications was reached. During that time professor wrote many science popularizing publications, however the most productive period was 1908–1913.

A. Hrebnickis published his publications in various periodicals popular at that time in Russia (Fig. 2). The highest number of publications was published in most known among horticulturists periodical “Пёдоводство“.
F i g. 2. Distribution of publications in periodicals
2 p a v. Publikacijų pasiskirstymas pagal leidinius
7 – Научное плодоводство

All publications of professor A. Hrebnickis in periodicals can be grouped according to the subject matter (Fig. 3). The highest number of publications is devoted to description of varieties and investigation of assortment in various regions. A. Hrebnickis was the first in Lithuania to show that varieties of south origin only in exceptional cases give satisfactory results. A. Hrebnickis started regionalizing varieties in Lithuania and in 1904–1906 suggested list of fruit trees to different regions. In 1911 professor created the first nonofficial Lithuanian standard fruit tree assortment, he also gathered and described Lithuanian landraces: ‘Beržininkų ananasas’, ‘Ilgai išsilaišantis’, ‘Panemunės baltasis’, ‘Jono pepinas’, ‘Gerkonių avietinis’.

The second group of publications consists of 17 articles devoted to physiological – biochemical researches of horticultural plants, flowering biology and parthenocarpy problems. These publications contain original very innovative and very progressive results at that time, which were taken into account by many European scientists. Prof. A. Hrebnickis tried to describe and to investigate every variety in biological aspect. In 1908 a unique research was made, which showed that some varieties have tendency to parthenocarpy, many fruit tree crossing and pollination experiments.

There are 28 articles in the scientific heritage of prof. A. Hrebnickis dedicated to different nursery problems. In these articles different grafting methods, rootstock selection questions are analysed.

The fourth group contains articles devoted to problems of orchard establishment: choice of place for orchard, soil preparation, tree planting.

The fifth group contains publications devoted to orchard maintenance. Especially big attention in this group (even 47 publications) is devoted to plant protection from diseases and pests. Interesting thoughts A. Hrebnickis presendetabout orchard plant fertilization. His notices about fertilizer placing are described in 11 publications.

In literary heritage of prof. A. Hrebnickis there are 37 publications for specific
horticulture questions, there are 3 publications concerning fruit storage, 9 publications concerning decorative plants, which can be used for orchard lee zones, 4 publications for vegetable growing in order to use garden soil rationally. All this characterise wide area of thought of prof. A. Hreblickis.

**Fig. 3.** Distribution of publications according to subject.
1 – Variety descriptions; 2 – Assortment choice; 3 – Physiological–biochemical researches; 4 – Flowering researches; 5 – Parthenocarpy; 6 – Nurseries; 7 – Garden establishment; 8 – Soil preparation for orchard; 9 – Tree planting; 10 – Orchard supervision/care; 11 – Fertilization; 12 – Plant protection; 13 – Watering; 14 – Pruning; 15 – Special questions of horticulture; 16 – Orchard plants; 17 – Fruit storage; 18 – Decorative plants; 19 – Olericulture.

The biggest fundamental pomological work is “Арёас пёодов”. Under supervision of prof. A. Hreblickis above mentioned work was prepared by about 30 authors. All pomological works published in Russia till that time were written and by one author (Pereéчь, 1868, Усиков, 1901, Симиренко, 1901, Пашкевич, 1899), they included regional aspects of pomology.

At the end of the XIXth century in Russia there was a lack of pomological literature. Horticulture in the country started to become more industrial. That increased the interest in varieties of orchard plants. Imperial horticulturists association in Petersburg in 1894 organized international horticulture exhibition (Одинцов, 1999), during which it was decided to prepare well illustrated pomological atlas. In 1895 a
request for financing that publication was handed to the government. Government satisfied the request. Association requested that publication was practical; varieties described in the publication were potentially valuable in the industry, and publication was available for wide public (Гребницкий, 1906). There was made an announcement to the public with request (i) to give a list of varieties that need to be described; (ii) to inform who can describe selected varieties; (iii) inform who can make illustrations (Рауш фон Траубенберг, 1896).

From 60 invited horticulturists only 32 responded. There were suggested 250 apples, 130 pear and less other plant varieties. From these lists pomological commission of association leaded by Duke A. E. Gagarin selected 100 varieties and published their list in journal “Плодоводство“ in 1897. This list was corrected according to suggested remarks. Confirmed lists of varieties were sent to authors to get variety descriptions, however not all authors gave variety descriptions. Such kind of variety descriptions was given by 30 authors. Prof. A. Hrebnickis described 44 varieties. Every description was sent to people who recommended including that variety into atlas for remarks. Editor A. Hrebnickis summarized every description. A. E. Gagarin made final edition of the text. Publication was illustrated using chromolithography method from original drawings and colour photographs.

Some illustrations were taken from foreign publications. Prof. A. Hrebnickis drew for the atlas 46 illustrations. Atlas was published in Sankt Petersburg in a run of four thousand copies. From rise of an idea till its realization 12 years passed.

Big number of varieties described in the atlas make the publication unique. The atlas includes assortment of varieties from southern to northern regions. Work was done by leading professionals of this field.

In the publication “Atlas plodov” varieties of Lithuanian origin were made known all over the world: ‘Lietuvas pepinas’, ‘Lietuvas cukrinis’ and others.

A. Hrebnickis was making plant breeding in some extent. After death of the professor in his garden there were found seedlings of various origin. Apple variety ‘Šlechta’ and variety ‘Nugalėtojas Žvirka’ are created in the orchard of A. Hrebnickis. There were selected 2 summer, 5 autumn, and 2 winter apple seedlings, late ripening plum and very early sweet cherry.

Summarizing heritage of prof. A. Hrebnickis, it is possible to state that he was very productive scientist, wide area of thought horticulturist, excellent organizer and fine educator, he made known to the public varieties created in Lithuania, he found and described varieties, which still can be found in Lithuanian standard assortment, he also made innovative biological fruit tree blossom and parthenocarpy researches, he occupies the place of honour both in Lithuanian and in Eastern European horticulture evolution.

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ADOMAS HREBNICKIS – ŽYMUSIS SODININKAS, POMOLOGAS

V. Stanys, T. Univer

Santrauka

Straipsnyje pateikiama trumpa žymaus sodininko, pomologo profesoriaus Adomo Hrebnickio biografija, aprašyta jo pedagoginė, redakcinė veikla, išanalizuotas mokslinis palikimas, aptartas vaidmuo sodininkystės plėtrai. Parodyta, kad profesorius A. Hrebnickis buvo labai produktyvus mokslininkas, platas akračio sodininkas, puikus organizatorius ir geras pedagogas, pagarsinę savo darbuose Lietuvoje kilusias veisles, atrašęs ir aprašęs veisles, kurios ir dabar yra Lietuvos standartiniai sortamenti, atlikęs novatoriškus biologinius vaismedžių žydėjimo bei partenokarpijos tyrimus, užimantis garbingą vietą ir Lietuvos, ir visos Rytų Europos sodininkystės raide.
THE YIELD AND FRUIT QUALITY OF TWO PLUM CULTIVARS ON DIFFERENT ROOTSTOCKS

Edite KAUFMANE, Mara SKRIVELE, Edgars RUBAUSKIS, Laila IKASE

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Myrobalan (Prunus cerasifera) seedlings are the most popular rootstocks for plums in Latvia. Only recently wider investigations were begun about the vegetatively propagated rootstock suitability to conditions of Latvia, their influence on plum cultivar productivity and fruit quality.

In 1996–1999 several trials were planted, where different plum cultivars were grafted on several vegetatively propagated rootstocks – vigorous AP-1 and Myruni, dwarf St. Julien A, GF 655/2 and semi-dwarf Druzhba and SVG 11-19, as well as two forms of Prunus cerasifera seedlings. The trials included several cultivars. Two of them – diploid ‘Kometa’ and hexaploid ‘Victoria’ are the most grown cultivars in Latvia and the subject of this study.

In the period of full yield the data of four years were analyzed to evaluate the influence of rootstocks on yield and fruit weight of both mentioned cultivars. On the selected seedling form PU-20651 of Prunus cerasifera and on Myruni the diploid cultivar ‘Kometa’ was more productive than on the other rootstocks, which was statistically significant also. The smallest yield per tree of this cultivar was obtained on the rootstocks GF 655/2 and St. Julien A. The average fruit weight depended on the yield value.

The hexaploid cultivar ‘Victoria’ yielded significantly better on rootstock AP–1 than on Druzhba and PU-20651 seedlings. Influence of yield or rootstock forms on the average fruit weight was not found.

Key words: fruiting, growth, plums, Prunus cerasifera, Prunus domestica, plum rootstocks.

Introduction. In Latvia plum trees are grown mainly on Prunus cerasifera ssp. divaricata seedlings. These rootstocks have some negative points – insufficient compatibility with some cultivars, too vigorous growth, only average winter-hardiness. It shows the necessity of alternative rootstocks for intensive orchards.

The first yielding data obtained in several trials at Dobele during previous years, with four cultivars on six different rootstocks, showed that the interaction between rootstocks and cultivars was not significant. Average fruit weight depended more of cultivar traits and the amount of yield, while the influence of rootstock was not significant (Kaufmane et al., 2007). It was important to establish the influence of rootstocks on yield parameters also at full cropping. Especially necessary was to find the most suitable rootstocks for cultivars ‘Victoria’ and ‘Kometa’ – two most widely grown plums in Latvia and also several other countries.

Materials and methods. During 1996 to 1999 several trials were planted,
where six different plum cultivars were grafted on several vegetatively propagated rootstocks – vigorous AP-1 and Myruni, dwarf St. Julien A, St. Julien GF 655/2 and semi-dwarf Druzhba and SVG 11-19, as well as two forms of *Prunus cerasifera* seedlings – PU-20651 and common *P. cerasifera* ssp. *divaricata*. The experiments were established at Dobele, in the southern part of Latvia on sod-podsolic and carbonate soil with sandy clay loam, with or without fertigation (Kaufmane et al., 2007; Rubauskis et al., 2002). Planting distances were 3 × 5 m, tree training was traditional. Soil management consisted of frequently mowed grass in the alleyways and 1-m-wide strips, treated with herbicides or mechanically.

As the trials were established in a small area and in uniform growth conditions, but had different planting years, for the mathematical analysis of yield data and to prove the statistical credibility of the differences, we used only four-year data at full cropping period (fifth to eighth growth year), instead of calendary yield data. For the current study two plum cultivars were chosen – the diploid ‘Kometa’ and hexaploid ‘Victoria’, as having the most variance of rootstocks in the trials. For ‘Kometa’ the yield and average fruit weight data were obtained for combinations with seven generatively or vegetatively propagated rootstocks, but the trials with ‘Victoria’ were done only on three rootstocks, which had good compatibility with it.

The significance of the results was computed according to the analysis of variance. The significant (p-value < 0.05) groups in tables and figure are marked with letters (*a* and *b*) according to Tukey_{0.05}.

The index of alternance (bienniality) of two plum cultivars in dependence of rootstocks was calculated using a method developed by Ivars Dimza and described in previous experiments with apple (Skrivele et al., 2000).

Rootstocks St. Julien A, St. Julien GF 655/2 and Myruni are well known in Europe, so descriptions only of rootstocks selected in Russia are given here: Druzhba, AP-1, SVG 11-19, and *P. cerasifera* form PU-20651 selected at the Latvia State Institute of Fruit-Growing.

**AP-1 (Kuban 86)** is a hybrid between *Prunus cerasifera* and *Prunus persica*. It is a semi-vigorous clonal rootstock, universal for plum, apricot and peach. It is easy to propagate with softwood cuttings, compatible with scion cultivars (Kaufmane and Andersone, 1997). The productivity of 10–12 years-old trees on AP-1 is reported to be about 30% bigger if compared with trees on seedlings of *P. cerasifera*. The winterhardiness of roots is about the same as for myrobalan plum. The rootstock shows promise in good orchard sites, especially on sod carbonate soils. In other places the soil needs improvement and lime. Grows well on heavy, moist soils. Resistant to diseases (Eremin, 2003).

**Druzhba** is a hybrid between *Prunus armeniaca* and *Prunus besseyi*. It is a semi-dwarfing clonal rootstock for plum and apricot, which is promising for the small tree size, easy propagation with softwood cuttings and very precocious cropping. According to the data of Krymsk Experimental Station, for ‘Reine-Claude d’Althan’ this rootstock proved to be the best in the group of low vigour rootstocks (Eremin, 2003).

**SVG 11-19** is a hybrid between *Prunus besseyi* and *Prunus salicina* ssp. *ussurienisis*. It is a semi-vigorous clonal rootstock for plum, well compatible with diploid plum cultivars. Good winter hardness, the roots tolerate -18°C. Easily
propagated with softwood cuttings. This rootstock is rapidly spreading in the hard-climate areas of Russia (Eremin, 2003).

PU-20651 is a seedling rootstock for plums selected at the Latvia State Institute of Fruit-Growing. It is an open pollinated seedling of Prunus cerasifera ssp. divaricata, possibly a cross with P. salicina spp. ussuriensis. The trees are more winter-hardy and smaller in size than common myrobalan rootstock P. cerasifera ssp. divaricata. The flower buds are medium hardy, flowering is early. Tolerance to temperature fluctuations in winter is satisfactory. Productivity is 10–30 kg/tree, yielding is not always regular. Fruits are very small (6–9 g), freestone, 100 seed weight is 54 g (7.2% of fruit weight). Seed germination is good, needs 4-month stratification. Compatibility in nursery with P. salicina ‘Skoroplodnaya’, ‘Kometa’ and P. domestica ‘Victoria’ is good, with ‘Reine-Claude d’Oullins’ satisfactory. The rootstock somewhat reduces tree size of cultivar ‘Victoria’, as shown by preliminary trials (1989–1995). It can be also propagated with softwood cuttings.

**Results and discussion.** The rootstock influence on cultivar ‘Kometa’ yield per tree during the full cropping period was proved statistically. The highest yields were obtained on Myruni and on PU-20651 (Fig. 1). Both are vigorous myrobalan (P. cerasifera) rootstocks, yet in Latvia size-reducing rootstocks can be used only at the best sites, because for crops with reduced winter-hardiness, like plums, low temperatures and temperature fluctuations during winter often cause substantial damage of fruit spurs and flower buds on the lower branches – like it was observed also in 2007 in the whole territory of Latvia.

**Fig. 1.** The average, minimum and maximum yield of plums during four-year period of full cropping, depending on rootstock

![Graph showing yield comparison](image)

The yield of ‘Kometa’ on Myruni was significantly higher than on common myrobalan P. cerasifera ssp. divaricata seedlings (Fig. 1). Although it could not be proven statistically, the yield of ‘Kometa’ on PU-20651 seedlings was also somewhat
higher than on common myrobalan seedlings.

On the vigorous rootstock AP-1 the cultivar ‘Kometa’ had smaller yields per tree, contrary to the results of trials in Russia, where AP-1 increased the yield of this cultivar for 30% as compared with *P. cerasifera* seedlings (Eremin, 1985).

The differences of average fruit weight for ‘Kometa’ were small; still the mathematical credibility of rootstock influence was quite high (p-value – 0.006). The largest fruits were obtained on rootstock St. Julien A with the lowest yield, yet on the productive rootstocks Myruni and PU-20651 the fruit weight decreased only by a few grams and was not statistically different (Table 1). The data obtained by German researchers in State Training and Research Center at Weinsberg also show that plum cultivars on Myruni have both good yield and average fruit weight.

Of the three rootstocks used for the cultivar ‘Victoria’, the highest yield per tree was obtained on AP-1 (Fig. 1). To find out the influence of rootstock vigour on the yield amount, further trials may be needed. The influence of yield and rootstock on the average fruit weight was not significant (Table 1).

**Table 1. The average fruit weight of two plum cultivars during four-year period of full cropping, depending on rootstock**

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>‘Kometa’</th>
<th>‘Victoria’</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>minimum</td>
<td>maximum</td>
</tr>
<tr>
<td>SVG 11-19</td>
<td>25</td>
<td>37</td>
</tr>
<tr>
<td>AP-1</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td><em>P. cerasifera</em></td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td>PU-20651</td>
<td>28</td>
<td>39</td>
</tr>
<tr>
<td>GF 655/2</td>
<td>28</td>
<td>39</td>
</tr>
<tr>
<td>St. Julien A</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>Myruni</td>
<td>27</td>
<td>38</td>
</tr>
<tr>
<td>Druzhba</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td>Mean</td>
<td>29</td>
<td>36</td>
</tr>
</tbody>
</table>

The differences of yield among years can be explained not only by unfavourable weather conditions, but also by a tendency to bienniality (Skrivele et al., 2000; Rubauskis et al., 2003), caused by too abundant cropping typical for both cultivars.

**Table 2. The index of alternation of two plum cultivars during four-year**
period of full cropping, depending on rootstock

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>'Kometa'</th>
<th>'Victoria'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>years 5 to 6</td>
<td>years 7 to 8</td>
</tr>
<tr>
<td></td>
<td>5-6 season</td>
<td>7-8 season</td>
</tr>
<tr>
<td>SVG 11-19</td>
<td>0.23</td>
<td>0.62</td>
</tr>
<tr>
<td>AP-1</td>
<td>0.32</td>
<td>0.27</td>
</tr>
<tr>
<td>P. cerasifera</td>
<td>0.65</td>
<td>0.78</td>
</tr>
<tr>
<td>PU-20651</td>
<td>0.57</td>
<td>0.64</td>
</tr>
<tr>
<td>GF 655/2</td>
<td>0.62</td>
<td>0.72</td>
</tr>
<tr>
<td>St. Julien A</td>
<td>0.58</td>
<td>0.77</td>
</tr>
<tr>
<td>Myruni</td>
<td>0.66</td>
<td>0.58</td>
</tr>
<tr>
<td>Druzhba</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean</td>
<td>0.54</td>
<td>0.64</td>
</tr>
</tbody>
</table>

and on Myruni the diploid cultivar ‘Kometa’ was more productive than on the other rootstocks. The average fruit weight depended on the yield value.

The hexaploid cultivar ‘Victoria’ yielded better on rootstock AP–1. Influence of yield or rootstock forms on the average fruit weight was not found.

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Kaukazinės slyvos (*Prunus cerasifera*) sėjinukai yra populiariausi slyvų poskiepiai Latvijoje. Tik neseniai buvo pradėti išsamesni tyrimai apie vegetatyviškai dauginamų poskiepių tinkamumą Latvijos sąlygomis, jų įtaką slyvų veislės produktyvumui ir vaisių kokybei.


Pilno derėjimo laikotarpiu buvo išanalizuoti ketverių metų duomenys, siekiant įvertinti poskiepių įtaką abiejų minėtų veislų derliui ir vaisių masei. Su atrinkta *Prunus cerasifera* sodinuko forma PU-20651 ir su Myruni diploidinė veislė ‘Kometa’ buvo derlingesnė nei su kitu poskiepium, o tai patikima ir statistiškai. Mažiausias šios veislės derlius nuo vaismedžio gautas naudojant poskiepius GF 655/2 ir St. Julien A. Vidutinė vaisiaus masė priklausė nuo derliaus vertės.

Heksaploidinė veislė ‘Victoria’ su poskiepiu AP–1 derėjo patikimai geriau negu su poskiepiais Družba ir PU-20651. Derliaus ar poskiepių formų įtaka vidutinei vaisiaus masėi nepastebėta.

Santrauka

Kaukazinės slyvos (*Prunus cerasifera*) sėjinukai yra populiariausi slyvų poskiepiai Latvijoje. Tik neseniai buvo pradėti išsamesni tyrimai apie vegetatyviškai dauginamų poskiepių tinkamumą Latvijos sąlygomis, jų įtaką slyvų veislės produktyvumui ir vaisių kokybei. 


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Heksaploidinė veislė ‘Victoria’ su poskiepiu AP–1 derėjo patikimai geriau negu su poskiepiais Družba ir PU-20651. Derliaus ar poskiepių formų įtaka vidutinei vaisiaus masėi nepastebėta.

CONTENT OF POLYPHENOLIC COMPOUNDS AND THEIR ANTIOXIDATIVE PROPERTIES IN HARVESTED BLACK MULBERRY (*Morus nigra* L.) FRUIT AT DIFFERENT RIPENESS PHASES

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Black mulberry fruit is known for its high nutritious value and widely used in natural medicine because of its therapeutic properties. In recent years, black mulberry fruit has become an object of research as a source of antioxidants. The aim of this paper has been to determine the content of polyphenolic compounds and their antioxidative properties in extracts of black mulberry fruit harvested in 2006 at different maturity phases. The content of polyphenolic compounds (total phenols, anthocyanins) in black mulberry fruit extracts correlated with the date of harvest. The highest concentration of polyphenolic compounds was determined in extracts from fruit collected at the full maturity phase. Extracts obtained from black mulberry fruit revealed quite high antioxidative activity.

**Key words**: black mulberry, polyphenols, anthocyanins, antioxidative activity.

**Introduction.** Black mulberry (*Morus nigra* L.) belongs to the family Moraceae. Lańska (1992) reports that black mulberry tends to be grown in warmer parts of Europe, including France, Italy and Spain. However, certain investigations carried out at the Chair of Horticulture, the University of Warmia and Mazury in Olsztyn, Poland (Waźbinska, 2001), proved that the plant could be cultivated successfully under the climatic conditions prevailing near Olsztyn. Besides, black mulberry does not demand any special soil conditions. Moreover, its therapeutic properties were recognized back in the Middle Ages. Black mulberry fruit is 2.5 cm long, reddish in colour before it is ripe and black when fully mature. In shape, it resembles raspberries. Mulberries are harvested in July and August. In addition to being a popular source of nutrients, they are also used broadly in natural medicine owing to their therapeutic properties (Darias-Martín et al., 2003). Furthermore, black mulberries are being studied as raw product for natural food dyes. On the other hand, they are a perishable foodstuff since they soften very quickly, which makes their handling, transport and marketing extremely difficult (Gerasopoulos and Stavroulakis, 1997; Suh et al., 2003).

Recently, fruit of black mulberry has raised an interest as a source of antioxidants such as anthocyanins, e.g. cyanidin 3-glucoside and cyanidin 3-rutinoside. These cyanidins are used in therapy of rheumatoid, carcinogenic and cardiologic illnesses...
It seems advisable to look for new sources and methods of obtaining anthocyanins from black mulberry because of their antioxidative activity.

The purpose of the study has been to determine the content of polyphenolic compounds (polyphenols) and their antioxidative properties in extracts of black mulberries harvested at different phases of fruit ripeness. In addition, correlation between the content of total phenols and anthocyanins was established.

**Material and methods.** The test material consisted of black mulberry (*Morus nigra* L.) fruit collected in 2006 at different fruit ripeness phases: I – 14th July (light red fruit), II – 19th July (red fruit), III – 25th July (dark red fruit), IV – 26th July (black fruit) and V – 31st July (black fruit gathered from earth).

Citric acid (pH 2) was used as a solvent for solid-liquid phase extraction. Extracts were initially cleansed by filtering through Whatman No 1 paper. The content of total phenols in extracts was determined according to Folin-Ciocalteau method, where it was converted to amounts of gallic acid (Singleton et al., 1999). The content of anthocyanins in fresh extracts was determined using the method elaborated by Niketić-Aleksić and Hrazdina (H method) (1972), whereas the total content of anthocyanins was established according to Wrlöstad’s method (W method) (1976). Antioxidative activity was determined by Yen and Hung’s method (2000), which involved analysis of scavenging effectiveness of 1,1 diphenyl 2 picrylhydrazyl (DPPH) radicals in extracts from black mulberry fruit. The results were presented as inhibition percentage.

For measurements of concentrations of polyphenolic compounds, a UV-Vis 6405 spectrophotometer (Jenway, England) was used.

All reagents used for analyses were of analytic purity.

The results of chemical determinations obtained in three replications underwent statistical analysis for one-factor experiments using Duncan’s test at $\alpha = 0.05$. In addition, correlation between contents of total phenols and anthocyanins was derived.

**Results.** Statistically significant differences were found when comparing concentrations of total phenolic compounds in extracts of black mulberry fruit collected at different fruit ripeness phases (Fig. 1). The content of total phenols was the highest in extracts of black mulberry fruit harvested on harvest date IV (452 mg 100 g$^{-1}$ fruit) and the lowest – in extracts obtained from fruit gathered on harvest date I (95 mg 100 g$^{-1}$ fruit). As regards the other harvest dates, the content of total phenols was on a similar level (164–222 mg 100 g$^{-1}$ fruit).

The content of anthocyanins in black mulberry fruit extracts determined by two methods – Wrlöstad (W) and Niketić-Aleksić, Hrazdina (H) – was the highest in the case of fruit picked on harvest date IV, reaching 170 and 223 mg 100 g$^{-1}$ fruit, respectively (Fig. 2). On the other dates of harvest, levels of anthocyanins were comparable and did not show any statistically significant differences.
Fig. 1. Content of total phenolic compounds in extracts of black mulberry fruit

1 p a v. Bendrųjų fenolio junginių kiekis juodojo šilkmedžio uogų ekstraktuose

Fig. 2. Content of anthocyanin compounds in black mulberry fruit extracts

2 p a v. Antocianinų junginių kiekis juodojo šilkmedžio uogų ekstraktuose

Fig. 3. Antioxidative activity of black mulberry fruit extracts in relation to concentration of anthocyanins in extracts

3 p a v. Antioksidacinio juodojo šilkmedžio uogų ekstrktų poveikio priklausomybė
Analysis of polyphenolic compounds showed that extracts produced from black mulberry fruit were characterised by high antioxidative activity (from 74 to 76%), which was not correlated with the harvest dates.

In order to study antioxidative properties, authors chose a range of concentrations of anthocyanins in extracts (0.05–0.15 mg cm$^{-1}$), within which the antioxidative activity was high. The antioxidative activity of the compounds tended to increase as the concentration of an antioxidant rose, although the increase continued only up to a certain level, which the model curve in Fig. 3 shows.

**Discussion.** The content of total phenols in black mulberry fruit extracts was the highest on harvest date IV, when the berries were fully ripe. In contrast, it was the lowest in extracts from unripe fruit collected on the first harvest date (Fig. 1). Regarding the remaining harvest dates, concentrations of phenolic compounds in black mulberry fruit were comparable. Fruit collected on harvest dates II and III had not reached full maturity, thus the level of total phenolic compounds determined in extracts from that fruit was lower.

Similar relationships were revealed in the case of anthocyanins. The content of anthocyanin compounds in extracts from black mulberry fruit, determined by two methods, was the highest on harvest date IV, when fruit was black and fully ripe (Fig. 2). On the other harvest dates, the levels of anthocyanins determined in the fruit extracts were similar and showed no significant differences. However, compared to the amount of anthocyanins in fully ripe fruit (date IV), the results obtained on the other harvest dates were lower. Also the extract produced from fully ripe fruit gathered from the ground (harvest date V) contained low quantities of polyphenolic compounds (total phenols and anthocyanins), which may have been due to loss occurring when fruit fell from trees and quickly began to spoil (Gerasopoulos and Stavroulakis, 1997; Suh et al., 2003).

There is a close relationship between the content of total phenols and anthocyanins in fruit extracts. Our study confirmed a strong correlation between the content of total phenols and that of anthocyanins in black mulberry fruit extracts, determined by two different methods. Moreover, the correlation obtained by method W ($R^2 = 0.934$) is similar to that produced by method H ($R^2 = 0.928$).

Levels of polyphenolic compounds depend on a number of factors such as: plant species, environmental conditions, agronomic treatments and ripeness of edible parts of fruit (Asami et al., 2003; Kalt et al., 2001; Ważbińska et al., 2006). While collecting black mulberry fruit on different harvest dates, we took into consideration different fruit maturity phases. On date IV the fruit ripeness was optimum, which was reflected in the highest content of polyphenolic compounds in extracts produced from that fruit.

The polyphenolic compounds present in black mulberry fruit possessed strong antioxidative properties (from 74 to 76%). Tsai et al. (2004) as well as Yildirim (2006) affirmed that wine from black mulberry fruit was characterised by high antioxidative activity (70.71% and 58%, respectively).

Antioxidative properties of polyphenolic compounds are conditioned by several factors, some of which (fruit species and composition, chemical structure of compounds, extraction methods) were discussed previously (Pliszka et al., 2003, 2005). It was then
concluded that extracts from European elder fruit were high in antioxidative activity (82 to 89%), which was predominantly determined by the presence of cyanidin glycosides (cyanidin 3-glucoside and cyanidin 3-rutinoside), which are good antioxidants (Chen et al., 2006; Kim and Park, 2006).

The content of polyphenolic compounds and their antioxidative properties in extracts from black mulberry fruit are weaker than those determined for extracts from European elderberry fruit extracts (Pliszka et al., 2005; Ważbińska et al., 2006). This might have been caused by a loss of polyphenolic compounds in perishable black mulberry fruit while making extracts and performing chemical analyses.

**Conclusions.** 1. Content of polyphenolic compounds (total phenols, anthocyanins) in extracts of black mulberry fruit depended on harvest dates.

2. The highest concentration of polyphenolic compounds was determined in extracts of black mulberry fruit harvested at the full maturity phase.

3. Extracts obtained from black mulberry fruit were high in antioxidative properties.

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POLIFENOLIO JUNGINIŲ KIEKIS IR JŲ ANTIOKSIDACINĖS SAVYBĖS NUSKITOSE JUODOJO ŠILKMEDŽIO (MORUS NIGRA L.) UOGOSE SKIRTINGAI ETAPAIS

B. Pliszka, J. Waźbińska, G. Huscza-Ciolkowska, B. Płoszaj
Santrauka


Reikšminiai žodžiai: juodasis šilkmedis, polifenoliai, antocianinai, antioksidacinis poveikis.
SCREENING OF STRAWBERRY RESISTANCE TO *PHYTOPHTHORA CACTORUM*

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Crown rot of strawberry, *Phytophthora cactorum*, has been a problem in Finnish strawberry production since 1990. Susceptible cultivars can suffer from severe plant losses without fungicide treatment. Resistance of cultivars to crown rot has been tested in Finland since 1993. The method used during the first years was the crown test where mycelium of *P. cactorum* is placed in a small wound in the crowns of strawberry plants. Zoospores are now used in the NFT system to inoculate small plants. The runner plants used in the tests were young. The crown test and NFT both give the same result in most cases, but certain cultivars may be more resistant when zoospores are used and the plants are not wounded. Unwounded plants can show the level of resistance or susceptibility to crown rot they would have in field conditions. During recent years, the resistance of clones obtained from the Finnish strawberry breeding program have been screened both with the crown test and the NFT test. The clones and cultivars have to be screened in spring or autumn. In summer they may show higher susceptibility to crown rot, in winter their susceptibility is lower. The screening methods show results in four weeks. The inoculation method has further been improved to make the test more reliable.

**Key words**: crown rot, *Fragaria × ananassa*, reliability, screening methods.

**Introduction.** J. Schröt was the first who detected that *Phytophthora cactorum* (Lebert & Cohn) cause crown rot and plant death of strawberry in Finland in 1990 (Parikka, 1991). The disease caused severe plant losses in fruit production and nurseries. At the beginning of 1990s there was little knowledge of crown rot resistance of strawberry cultivars grown in Finland and those potentially suitable for Finnish conditions. Resistance of strawberry cultivars has been evaluated at MTT Agrifood Research Finland, Plant Protection, since 1993 using the crown test (Parikka, 2003). Approximately 60 cultivars had been tested in greenhouse by different methods till 2003, (Parikka, 2006).

Crown test (Pitrat and Risser, 1977; Seemüller, 1977) was first used for screening cultivar resistance to crown rot. In other studies, zoospores were also used both in crown tests and in tests on unwounded plants. Eikemo et al. (2000) found that unwounded, not cold-stored plug plants showed no symptoms when zoospore sprays were used as the inoculum, while wounded plants developed crown rot symptoms. According to Bell et al. (1997), there are, however, no differences between the crown test and the test with zoospore spray. Zoospore tests are used in screening resistance of breeding material. They are easier to perform than crown tests and more effective but, according to Simpson et al. (1994), symptom development is slow and a large
number of replications are required for reliable results. In the first test in hydroponics with zoospores the test period used was several months (Rijbroek et al., 1997).

The nutrient film technique (NFT) has been used at MTT for screening of resistance to crown rot since 1998 (Parikka, 2006). Earlier trials with zoospore sprays had not been successful, probably because of lack of humidity during the infection. Adding the P. cactorum inoculum as sporangia to circulating irrigation water makes it possible to spread the inoculum rapidly and evenly throughout the plant material and without any need for extra humidity in the greenhouse to produce infection (Parikka, 2006). The two screening methods have been compared with the same strawberry cultivars in similar greenhouse conditions. Resistance or susceptibility of strawberry cultivars to leather rot caused by P. cactorum has not been tested.

The aim of these studies was to get information of plant resistance to P. cactorum inoculating them by different methods. Information of resistance is needed when fungicide treatments are planned. Screening methods are being improved for the needs of strawberry breeding to test resistance of selected clones.

**Material and methods.**

**Plant material. Crown test:** Young runner plants with 2 to 4 leaves were used for screening. The basic material of the cultivars comprised micropropagated mother plants kept and propagated in a greenhouse. The runners were rooted in Sphagnum peat substrate for 3 to 4 weeks and then planted into 0.5 litre plastic pots and fertilizer as described by Parikka (2003).

**NFT test:** Runners with one small leaf were rooted in 5 cm rockwool cubes (AO 36/40, Grodania A/S, Denmark) in a greenhouse for 2 to 3 weeks in 20°C (day) and 18°C (night) with a 16-hour day.

**Dipping test:** Runners were rooted in rockwool cubes as in the NFT test. Alternatively, small runner plants with one leaf were rooted in Vefi pots (VP 96) in peat substrate (Kekkilä Oy).

**Production of inoculum**

**Phytophthora cactorum** mycelium used for crown inoculations was collected from 3-week-old PDA plates. For inoculations in NFT, P. cactorum mycelium was grown on a potato dextrose medium for at least 4 weeks. The plates were then stored in a refrigerator and taken into room temperature (22–24°C) a day before use. To produce sporangia, 20 ml of distilled water was poured on the plates kept on the mycelium for 24 hours (Parikka, 2006). The number of sporangia was adjusted to about 20,000 per ml of inoculum (0.5 L).

**Inoculation**

The plants were inoculated 2 to 3 days after planting into pots. A modified crown test (Seemüller, 1977) was used as the test method (Parikka, 2003).

Unwounded, rooted runner plants were used in the NFT tests. Some comparative trials were also made with plants wounded in the crowns before placing into hydroponics.

The rockwool cubes with rooted plants were then put into the NFT system. Before inoculation the plants were irrigated in the system for one day with pure water. The inoculum was added to the 60-L water tank of the NFT system for a final concentration of about 16500 sporangia/L. A mixture of three P. cactorum isolates was used for the inoculum. Different concentrations of sporangia (5000–80,000 per ml) were tested.
with cultivar Jonsok and some other cultivars.

For the dipping test, inoculum was produced as for the NFT test. The rooted strawberry runners in rockwool cubes were placed in a 10-L plastic container and the inoculum was added in the container (1 L/container). The plants were removed after 24 hours and placed into the NFT system for the test period.

For the runners rooted in small pots, the inoculation was carried out as for the plants in rockwool cubes. The trays were inoculated in the plastic container and removed after 24 hours and placed into a greenhouse for the test period.

**NFT system**

The growing system was made of white PVC pipes 65 mm wide, with 17 cm between plants (Vefi AS, Norway). A submersible pump was placed in a 100-L tank filled with 60 L of tapwater. It circulated about 4 L water/min in the system and the overflow was returned to the tank. The plants in rockwool cubes were put into planting sites and the cubes were covered with plastic strips to avoid algal growth. Fertilizer was added into the system one day after inoculation (Parikka, 2006).

**Test conditions and evaluation**

The test time for the two techniques was 4 weeks and the temperature in the greenhouse was kept at 24°C (day) and 18°C (night) with a 16-hour day. The tests were carried out mainly during spring and autumn to have as similar conditions for the tests as possible. The susceptible cultivar ‘Jonsok’ and a resistant cultivar ‘Sara’ were used as controls in the crown and NFT tests. Disease assessments of the crown tests were made weekly on a scale of 0–5 (Parikka, 2003). In NFT tests the plants were evaluated after the test period using the same scale, where 0 = dead and 5 = very good. The crowns were cut longitudinally and the amount of browning in the tissues was observed and evaluated (0 = no discoloration, 1 = brown).

**Results. Efficiency of screening methods.** In all test types, the first wilt symptoms became visible in 3 to 5 days in susceptible cultivars. The control cultivar ‘Jonsok’ shows the same level of susceptibility with both methods, the crown test and the NFT system. Inoculation by dipping the plants gives the same result as adding the sporangia in circulating irrigation water. Dipping the small pots seems effective according to the preliminary trials on the susceptible cultivar ‘Jonsok’.

The symptoms of crown rot develop within 4 weeks and a longer test time does not change the level of infection. The rate of symptom expression in the crown test does, however, depend on the cultivar (Fig.). The time of the year when the plants are inoculated also affects symptom expression on some cultivars.

In NFT system, the symptom expression rate has not been calculated. Wounding the crowns of rooted runner plants before inoculation in NFT did not affect the susceptibility of cultivar ‘Arking’.

**Concentration of sporangia in inoculum**

Different concentrations of sporangia in the inoculum in NFT was studied on some cultivars. Concentrations of 5000 to 80,000 sporangia/ml in the basic suspension gave the same result on ‘Jonsok’. On some other cultivars the results varied in different tests. The results were, however, comparable to those obtained by the crown test (Table 1.). The inoculum concentration chosen for screening was, however, higher (20 000 sporangia/ml) to guarantee an adequate amount of active zoospores in the
irrigation water.

**Fig.** Disease development on some cultivars during the 4-week test period.

The crowns were inoculated in autumn or spring.

**Pav.** Kai kurių veislių ligos vystymasis per 4 testo savaites.

Plant vigour: 5 – very good, 0 – dead.

**Table 1.** Different concentrations of sporangia in the NFT test: the efficiency of inoculation on some cultivars, susceptibility of cultivars with the inoculum concentration used and resistance level compared with the crown test

<table>
<thead>
<tr>
<th>Sporangia in inoculum</th>
<th>&quot;Jonsok&quot;</th>
<th>&quot;Canarosa&quot;</th>
<th>&quot;Lima&quot;</th>
<th>&quot;Gerida&quot;</th>
<th>&quot;Senga Sengana&quot;</th>
<th>&quot;Sara&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 × 10⁶</td>
<td>0.06</td>
<td>4.7</td>
<td>0.5</td>
<td>0.8</td>
<td>3.7</td>
<td>4.9</td>
</tr>
<tr>
<td>1.5 × 10⁶</td>
<td>0–0.5</td>
<td>11.2</td>
<td>0.8</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0 × 10⁶</td>
<td>0.1–0.5</td>
<td>3.0–3.2</td>
<td>3.1</td>
<td>2.5–2.7</td>
<td>4.4–4.5</td>
<td>3.9</td>
</tr>
<tr>
<td>2.5 × 10⁶</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0 × 10⁶</td>
<td>0.6–0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0 × 10⁶</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.0 × 10⁶</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Resistant to crown test: 0–0.8 S 3.1 I 3.0–3.2 I 1.4–3.6 S–R 2.5–4.9 I–R 4.8–5.0 R.

#S = susceptible, I = intermediate, R = resistant.

#S = jautrios, I = vidutinės jautrio, R = atsparios.

Rageliai buvo inokuluoti rudenį arba pavasarį.

Augalo gyvybingumas: 5 – labai geras, 0 – augalas negyvas.

The cultivars tested were ranked into three classes according to the test results: resistant, intermediate and susceptible. Earlier crown test results were reported using 5 classes (Parikka, 2003). Fluctuation of resistance level was obvious between tests. Some cultivars were more resistant in the NFT than in the crown test, but also the converse
was seen. The most common strawberry cultivars in fruit production in 2006 were mainly susceptible, but variation in resistance between test methods is prominent on ‘Florence’ and ‘Korona’ (Table 2). Other tested cultivars were also mainly susceptible (Table 3). Most of the tested cultivars showed the same level of resistance in both tests. The variation in the test results during different seasons is comparable to that found in the crown tests, and the level of resistance of most cultivars was similar with both methods when the tests were performed in the same season.

Discussion. Crown rot caused by *Phytophthora cactorum* has become common
in planting material during the last ten years. According to the questionnaire of the Finnish Fruit and Berry Growers' Association, the main cultivar currently planted by Finnish strawberry growers is ‘Polka’, which covers more than half of the production area. This cultivar is very susceptible to crown rot and it has to be treated with fungicide at planting to avoid plant losses. The more resistant cultivars like ‘Senga Sengana’ and ‘Bounty’ are not common in fruit production and most of the new cultivars introduced to production are susceptible to *Phytophthora* infection. The main goal in screening

<table>
<thead>
<tr>
<th>Cultivar Name</th>
<th>Crown test</th>
<th>Variation</th>
<th>Resistance</th>
<th>Highest value</th>
<th>Lowest value</th>
<th>NFT</th>
<th>Variation</th>
<th>Resistance</th>
<th>Highest value</th>
<th>Lowest value</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Goosecarg'</td>
<td>0</td>
<td>S</td>
<td>XI</td>
<td>0.5</td>
<td>S</td>
<td>VI</td>
<td>S</td>
<td>XI</td>
<td>VI</td>
<td>S</td>
</tr>
<tr>
<td>'L'Acadie'</td>
<td>0.15</td>
<td>S</td>
<td>IX</td>
<td>2.0</td>
<td>S</td>
<td>VII</td>
<td>S</td>
<td>XI</td>
<td>VII</td>
<td>II</td>
</tr>
<tr>
<td>'Rosie'</td>
<td>0.3</td>
<td>S</td>
<td>VI</td>
<td>1.6</td>
<td>S</td>
<td>VIII</td>
<td>X</td>
<td>I</td>
<td>I</td>
<td>IX</td>
</tr>
<tr>
<td>'Lara'</td>
<td>1.15</td>
<td>S</td>
<td>IX</td>
<td>2.4</td>
<td>S</td>
<td>VIII</td>
<td>X</td>
<td>I</td>
<td>I</td>
<td>IX</td>
</tr>
<tr>
<td>'Oda'</td>
<td>0.15</td>
<td>S</td>
<td>IX</td>
<td>3.0</td>
<td>S</td>
<td>VIII</td>
<td>X</td>
<td>I</td>
<td>I</td>
<td>IX</td>
</tr>
<tr>
<td>'Oda'</td>
<td>1.5</td>
<td>S</td>
<td>XI</td>
<td>1.6</td>
<td>S</td>
<td>VIII</td>
<td>X</td>
<td>I</td>
<td>I</td>
<td>IX</td>
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<tr>
<td>'Rita'</td>
<td>1.5</td>
<td>S</td>
<td>XI</td>
<td>0.7</td>
<td>S</td>
<td>II</td>
<td>S</td>
<td>XI</td>
<td>II</td>
<td>S</td>
</tr>
<tr>
<td>'Pavana'</td>
<td>2.1</td>
<td>S</td>
<td>VI</td>
<td>3.45</td>
<td>S</td>
<td>I</td>
<td>X</td>
<td>VI</td>
<td>VI</td>
<td>I</td>
</tr>
<tr>
<td>'Cilady'</td>
<td>3.0</td>
<td>I</td>
<td>I</td>
<td>4.1</td>
<td>R</td>
<td>II</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>'Lana'</td>
<td>3.1</td>
<td>I</td>
<td>VI</td>
<td>1.8</td>
<td>S</td>
<td>IX</td>
<td>III</td>
<td>R</td>
<td>R</td>
<td>IX</td>
</tr>
<tr>
<td>'Mara de Bois'</td>
<td>3.1</td>
<td>I</td>
<td>IX</td>
<td>4.5</td>
<td>R</td>
<td>IX</td>
<td>R</td>
<td>IX</td>
<td>R</td>
<td>IX</td>
</tr>
</tbody>
</table>


resistance of strawberry cultivars to crown rot was in the 1990s to bring knowledge of the cultivars available to the growers and to inform them about disease resistance (Parikka, 2003). The increased trade of strawberry plants has not reduced the need for this information.

The need for a more natural inoculation method started the work with NFT. The crown test is reliable because it gives the same results if the plants are inoculated during the same season of the year. The results of the crown test have been well comparable with Norwegian results of the zoospore test (Eikemo et al., 2000). The hydroponic test of Rijboek et al. (1997) was a long-term test and we aimed at a short-term test with small runner plants. Our test with running irrigation water gives the results as quickly as the crown test. The restrictions are, however, the same as in the crown test: the test time has to be restricted to spring or autumn to get reliable results. The variation in results is also a problem. Many cultivars show different levels of resistance in the crown and NFT tests (Parikka, 2006). The tests in NFT can fail for many reasons. The viability of zoospores can be reduced or completely lost. To maintain viability, the inoculum has to be handled carefully and the sporangia must be collected in 24 hours after exposure to water. Too old sporangia release the zoospores before they get into the irrigation water and may not infect the plants. The hydroponic environment should not contain substances that are harmful to zoospores. Dipping is as effective an inoculation method as running water and it can save costs in the greenhouse environment.

*P. cactorum* can survive in soil as oospores and infect both crowns, roots and fruit of strawberry. Resistance to fruit infections has not been studied here. The recent
study of Golebniak et al. (2006) reports that in trials some cultivars show resistance to leather rot, but mainly the tested cultivars are susceptible to the disease. Some of these results agree with our findings of cultivar resistance to crown rot.

Screening resistance of cultivars gives a good basis for testing breeding material. It helps to identify the problems of reliability of screening methods and to choose the best practices for testing. Improvements to the methods make trials easier to perform and reduce demand of labour and other costs.

**Conclusions.** The screening reported here shows the relative susceptibility to *P. cactorum* of the strawberry varieties grown in Finland and some of the cultivars introduced to field trials. The results achieved in resistance testing should serve growers when estimating the risk of disease and deciding of measures for control of crown rot.

The NFT system makes it possible to use small, unwounded strawberry runner plants in screening for resistance. Unlike normal zoospore tests, the NFT system gives the results within the same time as the crown test when small fresh runner plants are used. There is still work to be done to improve the reliability of the system. The system is expensive and there may be problems with the viability of *Phytophthora* zoospores. Inoculation by dipping the tray plants in pots could solve these problems. The variation between the crown test and the NFT results probably reflect the susceptibility to natural infection.

During recent years, resistance of the clones obtained from the Finnish strawberry breeding programme has been screened both with the crown test and the NFT test. The screening was carried out in a greenhouse in similar conditions. The clones have to be screened in spring or autumn. In summer they may show higher susceptibility to crown rot, in winter their susceptibility is lower.

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SODININKYSTĖ IR DARŽININKYSTĖ. MOKSLO DARBAI. 2007. 26(3).

**BRAŠKIŲ ATSPARUMO PUVINUI *PHYTOPHTHORA CACTORUM* TYRIMAS**

P. Parikka

*Santrauka*


**Reikšminiai žodžiai:** *Fragaria × ananassa*, patikimumas, šaknies kaklelio puvinys, tyrimo metodai.
Fruiting potential and biochemical composition of eleven cultivars and clones of *Actinidia kolomikta* were investigated at the Kaunas Botanical Garden of Vytautas Magnus University in 2001–2005. The most perspective cultivars and clones were selected. Morphometric assessment revealed significant differences in berry size. The accessions of kolomikta kiwi were grouped according to berry size agreeably to the *Actinidia* genus descriptor. The cultivars and female clones of *A. kolomikta* were compared in respect of the total number of fruiting shoots and the average mass of berries per meter of length of two-year-old branch. Reliable differences between accessions were ascertained in the amounts of ascorbic acid, chlorophylls, titratable acidity and sugars. Skin firmness was evaluated as exceptionally important index of berry quality as well.

**Key words**: biochemical composition, clone, cultivar, fruiting shoot.

**Introduction.** Investigations of new horticultural plants with high biological and economical potential and enlarged viability are the most important problem of modern horticulture. The species *Actinidia kolomikta* (Maxim.) Maxim. is one of the most perspective plant, the berries of which accumulate a complex of biologically active substances (Skriptchenko, 2001; Ferguson and MacRae, 1992). The popularity of *A. kolomikta* is rising regarding the new productive cultivars, which were created in recent years (Huang et al., 2003; Pranckietis and Pranckietienė, 2000; Wang et al., 2003).

The first scientific evaluations of acclimatization and introduction of the genus *Actinidia* were conducted at Kaunas Botanical Garden of Vytautas Magnus University in the forth decade of the twentieth century (Minkevičius, 1936). The main precondition for the comprehensive studies was establishing of the modern collection of *A. kolomikta* genetic resources in 1996. Resistance to biotic and abiotic factors of environment as well as growth and development of different cultivars and clones was evaluated in 1996–2005 (Daubaras et al., 2002; Česonienė, 2004).

The main attention in breeding of new cultivars of *A. kolomikta* should be fixed on the productivity and biochemical composition of berries, particularly ascorbic acid. It
is important to ascertain the accumulation of biologically active compounds in berries and select the accessions with the largest productivity.

The aim of this study was to compare the fruiting characteristics and berry quality of cultivars and clones of *A. kolomikta*.

**Materials and methods.** Completely yielding female cultivars and clones of *A. kolomikta* have been chosen for the investigations. From three to six plants of each accession were planted at a distance of 2.3 × 3.0 meters. The triple wire system was used for the supporting of plants. The morphological investigations were carried out in 2001–2005. The average length, width and berry mass were determined analyzing samples of 30 berries.

The fruiting potential was referred as the number of fruiting shoots and berries as well as the total mass of berries per meter of length of fruiting branch in 2001–2003 (Zhang and Thorp, 1986).

Biochemical investigations have been conducted on berries of 11 cultivars and clones in 2001–2003. The samples of berries were collected at the technical maturity stage. Dry soluble solids have been determined by the manual Carl Zeiss Abbe refractometer, dry substances gravimetrically – drying them at 105°C up to the constant mass. The total amount of sugars has been ascertained by the Bertrand method and ascorbic acid by the Tilmans reaction: it was titrated by the solution of 2.6-dichlorophenolindophenol sodium salt. The titratable acidity has been ascertained titrating with the solution of 0.1 N sodium alkali and having calculated into the amount of citric acid (Ермаков, 1987). The amount of chlorophylls has been determined by spectrophotometer (Гавриёenko et al., 1975). Skin firmness has been ascertained by penetrometer IDP-500, by needle – 1 mm².

The statistical analysis of the data was carried out using program packet *SELEKCIJA*, which operate as applied to the basic program in the *EXCEL* packet (Tarakanovas and Raudonius, 2003).

**Results.** The fruiting potential of female cultivars and clones was assessed in 2001–2003. The fruiting shoots of 2001 were formed only on branches grown in 2000 from resting buds after late frost in spring. The Lithuanian cultivars ‘Landė’ and ‘Paukštės Šakarva’ as well as cultivars of Russian origin ‘VIR–2’ and ‘Krupnoplodnaja’ produced fruits only on short generative shoots in 2001. The number of generative shoots per meter of length of two-year-old branch attained from 9.1 (‘Krupnoplodnaja’) to 17.6 (‘Landė’). Other cultivars, as well as the clones F1M1 and F1, produced fruits on mixed shoots too. The average number of mixed shoots ranged from 0.5 shoots /m (F1) to 3.5 shoots /m (‘VIR-1’). All investigated female cultivars and clones produced fruits both on generative and mixed shoots in 2002 and 2003.

The results obtained in 2001–2003 revealed the statistically reliable differences of female cultivars and clones in the total number of fruiting shoots per meter of length of two-year-old branch (Fig. 1).

**Fi g. 1.** The total number of fruiting shoots per meter of length of two-year-old branch: in 2001 LSD₀.⁰⁵ = 3.9; in 2002 LSD₀.⁰⁵ = 3.2; in 2003 LSD₀.⁰⁵ = 2.9.
1 pav. Bendras derančių ūglių kiekis: 2001 m. $R_{05} = 3,9$; 2002 m. $R_{05} = 3,2$; 2003 m. $R_{05} = 2,9$.

Cultivars ‘Landé’ and ‘VIR-1’ were typical of a constant large number of fruiting shoots (over 15 shoots/m). Other cultivars (‘Lankė’, ‘Laiba’ and ‘Pavlovskaja’) were typical of very significant variation of the number of shoots in different years. It reveals that other factors also considerably influenced the number of fruiting shoots.

The cultivars also differed in the total mass of berries harvested per meter of length of two-year-old branch (Fig. 2).

**Fig. 2.** The total mass of berries per meter of length of two-year-old branch: in 2001 $LSD_{05} = 14.5$; in 2002 $LSD_{05} = 15.9$; in 2003 $LSD_{05} = 16.6$.

2 pav. Derančios šakos vidutinė uogų masė, g/m: 2001 m. $R_{05} = 14.5$; 2002 m. $R_{05} = 15.9$; 2003 m. $R_{05} = 16.6$.

The correlation coefficient $r$ between average number and total mass of berries per meter of length of two-year-old branch ranged from 0.42 to 0.55, whereas the same between average mass of a berry and total mass of berries per meter of length ranged from 0.21 to 0.34.

The berries of investigated female samples have been assessed according to the criteria of the general description of *Actinidia* Lindl. genus. It has been found that the
berries of investigated cultivars fall into the group of very small (up to 2 g), small (2–3 g) and moderate (3–5 g) berries. Comparing the cultivars of *A. kolomikta*, three groups of cultivars and clones have been distinguished according to the average mass of a berry: large berries (‘Paukštės Šakarva’, ‘Krupnoplodnaja’, F1) – the mass of a berry exceeded 3 g; moderate berries (‘Landė’, ‘Sentiabrskaja’, ‘Pavlovskaja’, F1M1, ‘VIR–2’) – 2–3 g; small berries (‘Laiba’, ‘Lankė’, ‘VIR-1’) – the mass of a berry did not exceed 2 g (Fig. 3).

**Fig. 3.** The average mass of a berry of *A. kolomikta* cultivars and clones in 2001–2005

Different cultivars and clones were compared according to the amounts of different biochemical compounds. The cultivars ‘Sentiabrskaja’, ‘Landė’ and ‘Paukštės Šakarva’ had the largest amount of sugars that was found to be 6.90; 6.89 and 6.80%, respectively. The cultivars accumulated from 3.34 (‘Sentiabrskaja’) to 4.67% (‘Landė’) of inverted sugar. In accordance with titratable acidity cultivar ‘VIR-1’ (2.0%) was distinguished. The determined ratio of sugars to acidity (index of sweetness) attained from 2.68 (‘VIR-1’) to 4.40 (‘Sentiabrskaja’).

The average amount of ascorbic acid in the berries of the cultivars studied was 876 mg/100g. The largest amount of ascorbic acid was typical of the cultivars ‘VIR-1’, ‘Landė’ and ‘Krupnoplodnaja’, the berries of which accumulated during technical maturity stage on average 1145.7, 1044.2 and 1022.3 mg/100g of ascorbic acid, respectively. They should be used in breeding with the aim to create new cultivars that typically have large amount of ascorbic acid (Table 1).

**Table 1.** The amounts of ascorbic acid and chlorophylls in the *A. kolomikta* berries, 2001–2003
Reliable differences in the amount of chlorophylls were ascertained: the total amount of chlorophylls in the berries attained from 3.18 mg/100 g (‘Sentiabrska’) to 5.30 mg/100 g (F1M1). Chlorophyll \( a \) amounted on the average to 2.4 mg/100 g and chlorophyll \( b \) to 1.8 mg/100 g.

A comparison has been made between the cultivars and clones according to the amount of dry solids and dry soluble solids. No essential differences between them has been found since Fisher’s criterion was: \( F_{0.05\text{act}} = 1.92 < F_{0.05\text{theor}} = 2.35 \) and \( F_{0.05\text{act}} = 1.17 < F_{0.05\text{theor}} = 2.35 \), respectively. In the technical maturity stage female cultivars and clones on average accumulated from 14.4 to 17.0% of dry solids and from 10.6 to 12.4% of dry soluble solids.

The berries of clone F1 were distinguished by the firmest skin (on average 300 N/cm\(^2\)). Firm skin was characteristic of only one Lithuanian cultivar ‘Landė’ (277 N/cm\(^2\)), while the skin firmness of berries of cultivar ‘Lankė’ was 188 N/cm\(^2\).

A comparison of the biochemical composition of berries of \( A. \) kolomikta was made in 2001–2003. Reliable differences were determined between the total amounts of sugars, saccharose, inverted sugar, dry matter and dry soluble solids in case the probability level was 99% (Table 2).

<table>
<thead>
<tr>
<th>Cultivar or clone</th>
<th>Ascorbic acid (mg/100 g)</th>
<th>Chlorophylls (mg/100 g)</th>
<th>Total dry matter (%)</th>
<th>Chlorophyll ( a )</th>
<th>Chlorophyll ( b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Landė’</td>
<td>1044.2 def</td>
<td>4.18 abcdef</td>
<td>2.60</td>
<td>1.59 ab</td>
<td>1.73 ab</td>
</tr>
<tr>
<td>‘Paukštės Šakarva’</td>
<td>925.9 cd</td>
<td>4.75 bcdef</td>
<td>2.80</td>
<td>2.00 ab</td>
<td>2.10 ab</td>
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<tr>
<td>‘Lankė’</td>
<td>743.9 a</td>
<td>6.63 ab</td>
<td>2.50</td>
<td>2.10 ab</td>
<td>2.20 ab</td>
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<tr>
<td>‘Lairė’</td>
<td>760.9 a</td>
<td>3.73 ab</td>
<td>1.93</td>
<td>1.73 ab</td>
<td>1.99 ab</td>
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<tr>
<td>‘Sentiabrska’</td>
<td>839.6 ab</td>
<td>3.18 a</td>
<td>1.90</td>
<td>1.26 a</td>
<td>2.00 ab</td>
</tr>
<tr>
<td>‘Krapnoplodėja’</td>
<td>1022.3 cdef</td>
<td>3.95 ab</td>
<td>2.10</td>
<td>1.99 ab</td>
<td>2.20 ab</td>
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<tr>
<td>‘Pavlė’</td>
<td>790.8 ab</td>
<td>4.36 ab</td>
<td>2.33</td>
<td>2.00 ab</td>
<td>2.20 ab</td>
</tr>
<tr>
<td>‘VIR’-1</td>
<td>1145.7 f</td>
<td>3.75 ab</td>
<td>2.00</td>
<td>1.80 ab</td>
<td>2.20 ab</td>
</tr>
<tr>
<td>F1M1</td>
<td>789.1 ab</td>
<td>5.30 d</td>
<td>3.13</td>
<td>2.20 e</td>
<td>2.50 ab</td>
</tr>
<tr>
<td>F1</td>
<td>854.8 abc</td>
<td>4.70 bc</td>
<td>2.50</td>
<td>2.20 e</td>
<td>2.50 ab</td>
</tr>
<tr>
<td>‘VIR’-2</td>
<td>720.6 abc</td>
<td>3.83 ab</td>
<td>2.00</td>
<td>1.80 ab</td>
<td>2.20 ab</td>
</tr>
<tr>
<td>Average</td>
<td>876.2</td>
<td>4.2</td>
<td>2.4</td>
<td>1.8</td>
<td></td>
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<tr>
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</table>

Table 2. The comparison of the amounts of biochemical components in the berries of \( A. \) kolomikta and skin firmness, 2001–2003
most significant amount of ascorbic acid in the berries of technical maturity was also determined in 2002, approximately 930 mg/100 g. Reliable differences in the amount of titratable acidity and ascorbic acid were ascertained at the 95% probability level. The results of chemical investigations showed no statistically reliable differences

<table>
<thead>
<tr>
<th>Biochemical component</th>
<th>The year of investigations</th>
<th>LSD R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2001</td>
<td>2002</td>
</tr>
<tr>
<td>Total amount of sugars</td>
<td>5.7</td>
<td>7.2</td>
</tr>
<tr>
<td>Benzas olivus (%)</td>
<td>3.9</td>
<td>5.0</td>
</tr>
<tr>
<td>Inverted sugar</td>
<td>1.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Saccharose</td>
<td>1.7</td>
<td>2.2</td>
</tr>
<tr>
<td>Titratable acidity</td>
<td>3.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Total amount of chlorophylls</td>
<td>803</td>
<td>930</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>4.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Total amount of chlorophylls</td>
<td>9.8</td>
<td>13.3</td>
</tr>
<tr>
<td>Skin firmness</td>
<td>265</td>
<td>234</td>
</tr>
</tbody>
</table>

* - the least significant difference, in case α = 0.05;  | - the least significant difference in case α = 0.01; * - significant difference is absent.

The results of investigations of skin firmness indicated that in the technical maturity stage this index was 230–320 N/cm\(^2\). Therefore, the berries of cultivars ‘Lankė’, ‘Sentiabrska’ and clone F1M1, the skin of which was less firm (respectively, 188 N/cm\(^2\), 220 N/cm\(^2\) and 197 N/cm\(^2\)), should be gathered at the beginning of the technical maturity stage.

**Discussion.** It is important to detect the characteristics of yield formation on female plants of *A. kolomikta*. As different authors have reported, the generative buds of genus *Actinidia* develop on short generative or mixed shoots (Ferguson, 1984; Snowbal, 1995; Tiyayon, 2003).

According to different authors, the number of fruiting shoots, the number and average mass of berries per meter of length of a branch were characterized as the fruiting potential of other species (*A. deliciosa, A. arguta*) and the object of breeding (Zhang and Thorp, 1986; Samanci, 1997; Kulczewski, 2003).

A comparison of fruiting peculiarities revealed the significant differences between the cultivars and clones of *A. kolomikta*. On the basis of the obtained results
it is possible to state that the total number of fruiting shoots and the average mass of berries per meter of length of two-year-old branch are important indexes assessing and selecting cultivars of *A. kolomikta*. The high fruiting potential could be predict for the accessions with large number of berries per meter of length of two-year-old branch.

The area of *Actinidia* plantations enlarges because of valuable biochemical composition of berries (Huang et al., 2004). The differences in amounts of ascorbic acid between species are significant. The cultivars originated from *A. deliciosa* accumulate from 40 to 260 mg/100 g of ascorbic acid, whereas the cultivars bred from *A. arguta* accumulate on average 70–130 mg/100 g (Ferguson and Mac Rae, 1992; Richardson et al., 2004). The results of this study confirmed that the cultivars of *A. kolomikta* accumulate from 720 to 1145 mg/100 g of ascorbic acid at the technical maturity stage. The best cultivars in this respect are the Lithuanian cultivars ‘Landė’, ‘Paukštės Šakarva’ and the Russian cultivar ‘VIR-1’. They could be used in breeding as the potential donors of valuable properties.

The amount of ascorbic acid, the average mass of a berry, as well as the fruiting potential, are the most important indexes assessing *A. kolomikta* genetic resources.

**Conclusions.** 1. The main part of *A. kolomikta* berry yield ripens on short generative shoots. Cultivars ‘Landė’ and ‘VIR-1’ typically have constant large number of fruiting shoots.

2. The total number of fruiting shoots and the mass of berries per meter of length of a branch are the most important indexes for the estimation of fruiting potential of female accessions of *A. kolomikta*.

3. The berries of cultivars and clones accumulated from 720 mg/100 g to 1145 mg/100 g of ascorbic acid at the technical maturity stage. The most valuable cultivars ‘VIR-1’, ‘Landė’ and ‘Krupnoplodnaja’ were selected in respect of amount of ascorbic acid.

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MARGALAPĖS AKTINIDIJOS (*ACTINIDIA KOLOMIKTA*) DERĖJIMO POTENCIALO IR UOGŲ KOKYBĖS TYRIMAI

L. Česonienė, P. Viškelis

Santrauka


Reikšminiai žodžiai: biocheminė sudėtis, derantis ūgis, klonas, veislė.
EVALUATION OF COLUMNAR APPLE HYBRIDS ON DWARFING ROOTSTOCKS

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Columnar apple cultivars of foreign origin so far have not shown good results in Latvia, as they lack winter hardiness and resistance to canker *Nectria galligena*; few have good fruit quality and storage. The aim of this trial was to evaluate the yielding, fruit quality, disease resistance and winter hardiness of new columnar apple hybrids. Five hybrids from the Latvian breeding programme and two from Russia were included in the trial. They were planted in 2002 on rootstock Pure-1 in three or six replications at spacing of 1 × 4 m and grown using standard techniques and sprayings. After five years hybrids D-7-94-15 and D-3-94-2 were the most productive. The majority showed a tendency to biennial bearing. Most hybrids ripened early, in August or beginning of September; the earliest was D-7-94-10. Late ripening were KV-109 and D-3-94-2, which also had the longest storage. D-7-94-15 had the best eating quality and productivity. Several hybrids showed good winter hardiness and tolerance to *Nectria* canker. Their level of scab resistance also was good. *Monilia* rot significantly injured fruits of KV-103, and also D-7-94-10 and D-7-94-12. The highest scab injuries had KV-109 on fruits and D-7-94-2 on leaves, while D-7-94-10 had no visible scab injuries. No significant mildew injury was observed. Some trees of D-7-94-2 and D-7-94-10 were killed by canker. The highest winter injuries had KV-109, while D-7-94-12 and D-7-94-10 had practically no injuries. The hybrid D-7-94-15 has been selected as the best and handed in for DUS testing in 2006 with the name ‘Baiba’.

**Key words:** columnar apple, disease tolerance, fruit quality, *Malus domestica*, winter hardiness, yield.

**Introduction.** Columnar apple-trees are the target of breeding programs in many countries (Kelsey and Brown, 1992; Meulenbroek et al., 1999; De Wit et al., 2000; Gelvonauskienė et al., 2006; Кичина, 2002), including Latvia (Drudze, 2000; Ikase and Dumbravs, 2003). Since the discovery of the columnar Co gene (Lapins, 1976), the knowledge of the inheritance of columnar tree habit has improved, which also helps to understand the growth and development of columnar apple-trees (Meulenbroek et al., 1999; De Wit et al., 2000; Kenis and Keulemans, 2004). Research has shown the influence of rootstock on the growth and yielding of columnar apple trees, with dwarfing rootstocks not only reducing tree size and promoting start of production, but as well decreasing the amount of branching (Inomata et al., 2005; Gelvonauskienė et al., 2006; Качаёнкин, 2003). So the growth and productivity of columnar apple-trees depends on several factors – genotype, rootstock, training system and adaptation to the climate at the place of growth (Кичина, 2002, Качаёнкин, 2003). This explains variable success of columnar apple breeding programs and the difficulties of the introduction of
columnar apple cultivars into areas with different growth conditions. The latest is one of the main factors so far hindering successful growing of columnar apples in Latvia, where winter-hardiness and tolerance to such diseases as canker *Nectria galligena* and apple scab *Venturia inaequalis* become crucial (Ikase and Dumbravs, 2003).

The aim of this study was to evaluate the yielding, fruit quality, disease resistance and winter hardiness of new columnar apple hybrids, grown on dwarfing rootstocks.

**Materials and methods.** The trial was established in 2002 on the dwarfing rootstock Pure-1 in three or six replications at spacings of 1 × 4 m (2 500 trees/ha) and grown using standard techniques and pesticide sprayings. Five hybrids from the Latvian breeding programme (D-7-94-2, D-7-94-10, D-7-94-15 and D-3-94-2) and two hybrids from Russia (KV-103, KV-109) were included in the trial. The trees were planted as 1-year old whips and were not headed back at planting. They were trained by removing, or heading back all lateral shoots longer than 7 cm; this was done several times during summer as necessary. Although the trees were not staked at planting, they had to be staked after the beginning of cropping. The fruitlets were hand-thinned after June drop, which did not completely prevent bienniality.

The following parameters were evaluated for each tree:
- annual growth of the leader and total tree height in spring (cm);
- overall tree health in spring (points 1–10);
- flowering intensity (points 1–10);
- fruit count and yield in kg;
- disease damages (points 1–10) – scab on leaves, mildew, canker;
- winter injuries of lateral shoots, tree top and trunk base (points 0–5) – in the tables the highest damage rating of all these is given.

For fruits, the dates of fruit picking were registered for each hybrid. A collected sample of fruits was taken from all trees of each hybrid – no less than 30 fruits, except if the yield was lower. The sample was weighed and the average fruit weight (g) was calculated. Fruits with scab and fruit rot injury were counted and their amount then expressed in %. The number of standard fruit (without injuries, standard size) was also expressed in %. Fruit attractiveness and flavour were evaluated by a taste panel of 10 persons – for early hybrids soon after picking, for late ripening ones after storage. The fruits were stored at 3°C.

After the winter of 2006–2007, when the temperatures in December and January were over 5°C, followed by -28°C in February, the injuries of fruit spurs were evaluated in two zones – 0–50 cm and 50-100 cm from soil level, counting 100 spurs on all trees and calculating injury in %.

The bienniality (alternance) index BI was calculated using the formula:

\[ BI = \frac{(yield_1 - yield_2)}{(yield_1 + yield_2)} \times 100\%
\]

The index was calculated for years 2005–2006 for each individual tree. Then the average of all trees was calculated for the hybrid. The grouping was: 21–40% low bienniality, 41–60% medium bienniality, 61–100% high bienniality.

The data were mathematically processed using analysis of variance and analysed by Tukey’s test (PS 0.05), where means followed by the same letter (a, b, c, d) do not differ significantly. Pearson correlation coefficients were calculated for the interaction of factors.
**Results and discussion.** The trees of some columnar apple hybrids on dwarfing rootstock Pure-1 started flowering in the planting year, but all flowers were removed to help tree growth. The majority flowered in 2003, yet fruit set was poor due to winter injury and extremely dry summer (non-columnar apple cultivars also had very poor yield in 2003). Spring frost in 2004 (−4°C during full bloom) did not significantly damage flower buds of the new columnar apple hybrids.

After the trees started to yield, it was necessary to tie them to short stakes, to prevent bending under the fruit weight. The need for tree staking runs contrary to some previous publications which suggest that no staking is necessary for columnar apple-trees on any rootstock (Tobutt, 1984; Кичина, 2003). The bending over of trees was especially expressed after heavy rainfall and can be explained both by the shallow root system of dwarfing rootstocks and by smaller tree diameter on these rootstocks as compared with semi-dwarf rootstocks (Gelvonauskienė et al., 2006). Significantly, D-7-94-2 and KV-103 with the most vigorous trees did not bend.

After five years hybrids D-3-94-2 and D-7-94-15 were the most productive (Table 1). The average yield per tree of columnar apples on Pure-1 was low and did not exceed 2.5 kg, which would be 6.25 tons/ha. This might be compensated by increased planting density, up to 6 600 trees/ha. There was low positive correlation between yield and tree height (r = 0.248, significant at 0.05 level), yet the most productive hybrids did not have the tallest trees (Table 2).

<table>
<thead>
<tr>
<th>Hybrid Hybrids</th>
<th>Yield (kg/tree)</th>
<th>mean bidurkis</th>
<th>Bienniality index Prancūžų rodykis, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2004</td>
<td>2005</td>
<td>2006</td>
</tr>
<tr>
<td>D-3-92-2</td>
<td>1.38</td>
<td>0.88</td>
<td>2.41</td>
</tr>
<tr>
<td>D-7-94-15</td>
<td>1.68</td>
<td>0.50</td>
<td>2.45</td>
</tr>
<tr>
<td>KV-109</td>
<td>0.10</td>
<td>1.80</td>
<td>0.80</td>
</tr>
<tr>
<td>KV-103</td>
<td>0.37</td>
<td>0.70</td>
<td>2.03</td>
</tr>
<tr>
<td>D-7-94-10</td>
<td>0.63</td>
<td>0.60</td>
<td>1.13</td>
</tr>
<tr>
<td>D-7-94-2</td>
<td>0.70</td>
<td>0.93</td>
<td>0.67</td>
</tr>
<tr>
<td>D-7-94-15</td>
<td>0.25</td>
<td>0.25</td>
<td>0.40</td>
</tr>
<tr>
<td>Total / Bibis</td>
<td>0.97</td>
<td>0.68</td>
<td>1.72</td>
</tr>
</tbody>
</table>

Table 1. **Average yield of columnar apple hybrids on rootstock Pure-1**

The tree height on Pure-1 was between 0.9 m and 1.9 m in the fifth year of growth. Tree height varied significantly among hybrids, with D-7-94-12 and D-7-94-15 having the smallest trees, D-7-94-2 and KV-103 the largest ones (Table 2). The annual growth of trees also varied significantly; D-7-94-10 and D-7-94-12 grew bearing – all hybrids with the lowest productivity. This again proves the high necessity of early fruit thinning for columnar apples.

The tree height on Pure-1 was between 0.9 m and 1.9 m in the fifth year of growth. Tree height varied significantly among hybrids, with D-7-94-12 and D-7-94-15 having the smallest trees, D-7-94-2 and KV-103 the largest ones (Table 2). The annual growth of trees also varied significantly; D-7-94-10 and D-7-94-12 grew...
most slowly. Yet it did not vary significantly between years as could be expected, and no correlation was found between yield and annual growth. Still, new growth of abundantly cropping trees was close to zero in some cases, which shows the necessity to improve tree vigour, as they may not have enough leaf surface.

**Table 2.** Average tree height and annual growth of columnar apple hybrids on rootstock Pure-1

| Hybrid | Average tree height | Average annual growth | | | |
|--------|---------------------|-----------------------|---|---|
|        |                      |                       | 2004 | 2005 | 2006 |
| D-392-2 | 86.7               | 109.5                | 135.8<sup>a</sup> | 27.0 | 26.3 | 34.3 |
| D-7-94-15 | 83.7              | 102.5                | 119.0<sup>b</sup> | 18.8 | 16.5 | 21.3 |
| KV-109 | 91.0               | 100.5                | 130.0<sup>b</sup> | 28.0 | 29.5 | 24.0 |
| KV-103 | 106.3              | 127.3                | 161.3<sup>b</sup> | 21.0 | 37.3 | 30.0 |
| D-7-94-10 | 96.3             | 112.7                | 124.7<sup>b</sup> | 18.3 | 7.3  | 3.3  |
| D-7-94-2 | 130.3             | 160.0                | 191.0<sup>b</sup> | 29.7 | 31.0 | 29.0 |
| D-7-94-12 | 70.5              | 84.0                 | 93.5<sup>b</sup> | 18.5 | 11.0 | 3.5  |
| Total/ Average | 93.8               | 113.6                | 136.3 | 23.0 | 22.6 | 23.0 |

(mean values, cm)

Table 3. Average tree health, flowering, disease and winter injuries of

... (Table 3). After the winter of 2006–2007 serious damages of fruit spurs were observed on some hybrids, especially at the level closer to ground. Such injury may permanently decrease productivity of columnar apple-trees, because the renewal of spurs often is poor, and the fruiting zone remains only at the top of the tree (Кичина, 2003). Yet such injuries were not observed on KV-109 and D-7-94-10, while KV-103 and D-3-94-2 had very low injuries (Table 3).

The highest scab injuries had KV-109 on fruits (statistically not proven) and D-7-94-2 on leaves, while D-7-94-10 had no visible scab injuries (Tables 3, 4). No significant mildew injury was observed in the trial.

Part of trees of D-7-94-2 and D-7-94-10 were killed by canker (Table 3). Canker injury had negative correlation with tree flowering (r = -0.318, significant at 0.01 level), yield (r = -0.262, significant at 0.05 level) and new growth (r = -2.293, significant at 0.05 level).

Most hybrids ripened early, in August or at the beginning of September; the earliest ripening was D-7-94-10 (Table 4). Late ripening were KV-109 and D-3-94-2, which also had the longest storage. *Monilia* rot significantly injured fruits of KV-103, and also D-7-94-10 and D-7-94-12.

Table 3. Average tree height, flowering, disease and winter injuries of
columnar apple hybrids on rootstock Pure-1 in 2004–2006

Table 4. Picking dates, average fruit weight and quality assessment of columnar apple hybrids on rootstock Pure-1 in 2004–2006

The best eating quality had D-7-94-15, D-7-94-12 and D-7-94-2, but the last two
had very low productivity (Tables 1, 4).

The hybrid D-7-94-15 has been selected as the best and was handed in for DUS testing in 2006 with the name ‘Baiba’. It is an early ripening columnar apple with fruits similar to ‘Melba’, but less acid, of good quality. ‘Baiba’ has shown no susceptibility to scab or other diseases. The trees are of medium vigour and productive, but tend to bear biennially. The winter hardiness of trees is good, including Northern Latvia.

**Conclusions.** 1. The average yield per tree of columnar apples on the dwarfing rootstock Pure-1 was low. There was low positive correlation between yield and tree height ($r = 0.248$, significant at 0.05 level), yet the most productive hybrids did not have the tallest trees. More vigorous rootstocks may be important for columnar apples in locations where winter injury of fruit spurs closer to the soil level is possible. So the productivity may be increased either by increasing planting density or by using semi-dwarf rootstocks like B118.

2. Several of the new columnar apple hybrids used in the trial showed good suitability to Latvian climate, including winter hardiness and tolerance to *Nectria* canker. Their level of scab resistance also was good.

3. The new columnar apple cultivar ‘Baiba’ was selected as the best in the trial and may be promising for home gardens.

References


Santrauka


**Reikšminiai žodžiai:** atsparumas žiemą, derlius, koloninės obelys, ligų toleravimas, *Malus domestica*, vaisių kokybė.
INVESTIGATION OF BLUE-BERRIED HONEYSUCKLE LINES AND CULTIVARS IN VILNIUS UNIVERSITY BOTANICAL GARDEN COLLECTION

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2Department of Botany and Genetics of Vilnius University, M.K. Ėiurlionio 21, Vilnius, Lithuania. E-mail: Donatas.Naugzemys@gf.vu.lt
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Key words: blue-berried honeysuckle (Lonicera L.), berries characteristic, distribution of diseases, genetic variation.

Introduction: The blue-berried honeysuckle is called non-traditional horticultural plant in Lithuania. In Lithuania blue-berried honeysuckle grows only in individual’s plots and botanical gardens (Lietuvos pomologija, 1996). The extra-early ripening of berries (before first strawberries) is the most economically important feature of this plant.

Blue-berried honeysuckle (Lonicera L.) belongs to the family Caprifoliaceae Juss. section Isika Reh., subsection Caeruleae Rehd. (Krüssmann, 1977). The volume of the subsection is already many years under investigation and discussions. Different authors single out one to 10–11 species within the subsection (Žilinskaitė, 2002). Blue-berried honeysuckle is a perennial deciduous shrub growing to 2 m and producing extra-early
ripening berries, which are dark navy to purple in color. Berries are an excellent source of many valuable vitamins, mineral and biological active substances and can be used as natural antioxidants and natural colorants (Plekhanova, 2000; Chaovanalikit, 2004). One more positive character of the shrub is frost hardiness, short growing season, and rather simple growing conditions. Blue-berried honeysuckle is commercially promising plant. Fruits of blue-berried honeysuckle are widely harvested in Russia, Canada and Japan (Hummer, 2006).

Although the blue-berried honeysuckle was harvested from the wild for centuries in Japan and Russia, breeding programs began since 1950 in Russia and since 1980 in Japan. Only in 1990 two breeding programs in North America began at Oregon State University and the University of Saskatchewan (Hummer, 2006). Since 1814 (Skridaila, 2001) the blue-berried honeysuckle was known as dendrologic object in the collection of Vilnius University Botanical Garden. The blue-berried honeysuckle was investigated as berry plant when the Pomology Department was established in 1974. Since 2004, the study was supported by the Lithuanian Government Science Program “Scientific Research of the Plant Genetic Resource in Lithuania” (2004–2008).

The collection of blue-berried honeysuckle of Vilnius University Botanical Garden contains four species, four subspecies, 26 cultivars and 36 lines. Blue-berried honeysuckle grows in three plantations: basic, selected lines, species and cultivars. The plants of basic plantation are grown of seeds obtained from Russia in 1979. A large variation in taste and berry size was documented in this plantation. Selected plants, which did not have a bitter taste of berries, were propagated by green cuttings and planted in the plantation of lines in 1996. The youngest plantation was established in 1999–2000 and contains species and cultivars granted mainly by Saint Petersburg Institute of Plant Industry (VIR), Kaliningrad University Botanical Garden and Garden Plant Variety Investigation Station (Vilnius district, Nemėžis). In 2003–2006 the attention was focused on collection of lines to investigate and select the best of them.

**Materials and methods.** We have been studying 34 lines of blue-berried honeysuckle. The lines were compared with 9 cultivar-standards. Cultivars ‘Baktcharskaja’, ‘Golubuje vereteno’, ‘Morena’, ‘Narymskaja’, ‘Siniaja ptica’, ‘Tomitchka’, ‘Vasjuganskaja’, ‘Viola’, ‘Zoluschka’ are included in the list of the best cultivars of Russia (Нетрадиционнūе садовūе куётворū, 2003; Ягоднūе куётворū, 2001). In 2003–2006 the key criteria for selection were size, quality of berries and distribution of plant diseases. Plants were estimated according to descriptors (Catalogue, 1977), and subdivided into classes according to the mark intensity. The chemical composition of fruit was determined by standard methods.

Phytosanitary condition of plants was evaluated: spreading of plant diseases was determined basing on the amount of the infected plants, and disease intensity was defined estimating the area of a plant infected by the pathogen. Record of the plant diseases was performed according to the manual „Žemės ūkio augalų kenkėjai, ligos ir jų apskaita“ (2002). The infected plant samples are deposited in the Herbarium of the Institute of Botany (BILAS). The plant disease agents were identified employing the following methods: visual observation, moist chamber, isolation of pure cultures for identification of plant disease agents, microscopy. Plant disease agents were identified following M. B. Ellis, J. P. Ellis (1997), B. Grigaliūnaitė (1997), M. Ignatavičiūtė, A. Treigienė (1998). The running number and number of plants are
The study of genetic variation and relationships among some blue-berried honeysuckle lines and cultivars was based on RAPD analysis. DNA was isolated from fresh young plant leaves using Genomic DNA purification kit (MBI Fermentas). DNA concentrations were measured using an Eppendorf BioPhotometer.

RAPD amplification. Decamer deoxyoligonucleotide primers (170-5, 380-5) for RAPD analysis were purchased from Carl Roth. One µl of template DNA (50 ng) was amplified in 25 µl PCR mixture consisting 2.5 µl 10 × Taq reaction buffer, 3.0 mM MgCl₂, 0.2 mM dNTP, 1 µM primer, 1 U Taq DNA polymerase (MBI Fermentas). RAPD-PCR was carried on in the thermocycler (Eppendorf Mastercycler personal) according to the following program: 1 cycle of 4 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C, and finally by 1 cycle of 5 min at 72°C. The electrophoresis and documentation of RAPD-PCR products were performed as described earlier (Naugžemys et al., 2006).

Data analysis. The RAPD data obtained were used to construct UPGMA (Unweighted Pair – Group Method of arithmetic Averages) dendrogram using TREECON software package v. 1.3 b (Van de Peer & De Wachter, 1994). The dendrogram was constructed on the basis of Nei and Li genetic distance (Nei and Li, 1979). The significance level of dendrogram branches was determined using 1000 bootstrap replicates.

**Results.** Characterization of investigated lines and cultivars. The studied plants were morphologically different. All studied plants were accessed according descriptors (Catalogue, 1977) and were arranged in two groups according the most important morphological and phenological characters (Table 1):

1) Bushes about 2 m, very early bloom and ripening of large blue berries, short rest period of generative buds – the most (41) of studied examples;

2) Short bushes (about 0.8 m), late bloom and late ripening of round blue berries, frost and drought resistance, long rest period of generative buds – *L. caerulea* subsp. *kaantschatika*, ‘Kairēnai’, ‘L69-3’, ‘639-8’.

**Table 1. Characteristic of honeysuckle lines and cultivars**

<table>
<thead>
<tr>
<th>Characteristic and cultivars</th>
<th>Lines and cultivars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bushes about 2 m in height, very early bloom and ripening (beginning of June) of large blueberries; short rest period of generative buds</td>
<td>Var. L. <em>caeruleae</em></td>
</tr>
</tbody>
</table>
Berry characteristic. Weight of berries is one of the most important criteria. Distribution of honeysuckle lines and cultivar-standards according to berry weight is presented in Table 2. In 2003–2006 it was a large variation in berry size (0.44–1.29 g) documented. Both cultivar-standards and lines have different weight of berries. Two groups (plants with small and medium berries) have nearly the same number of examples (17 and 18). Economically perspective lines must have berries of more than 0.70 g weight. 17 lines met this requirement: ‘2E’, ‘2S’, ‘32’, ‘22T12’, ‘1B43’, ‘1E’, ‘96-3’, ‘2-1’, ‘96-4’, ‘639-8’, ‘1L’, ‘2R’, ‘2C’, ‘3-5’, ‘Kairėnai’, ‘L69-3’, ‘3U’.

Table 2. Distribution of honeysuckle lines and cultivar-standards according to average berry weight in 2003–2006

<table>
<thead>
<tr>
<th>Berry weight</th>
<th>Line, cultivar-standard (average berry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.41–0.60</td>
<td>‘Golubce vereteno’ (0.44 ± 0.07), ‘10-32’ (0.48 ± 0.06), ‘L. caerulea’ (0.51 ± 0.05), ‘Sinajia gryca’ (0.53 ± 0.06), ‘11’ (0.54 ± 0.06), ‘1M’ (0.55 ± 0.08), ‘1U’ (0.55 ± 0.07), ‘Narytnyskaia’ (0.56 ± 0.05), ‘96-1’ (0.56 ± 0.07), ‘1P’ (0.58 ± 0.04), ‘3-96’ (0.58 ± 0.08), ‘10’ (0.58 ± 0.06), ‘3-79’ (0.59 ± 0.03), ‘96-2’ (0.59 ± 0.02), ‘2D’ (0.59 ± 0.08), ‘IV’ (0.59 ± 0.04), ‘2C63’ (0.60 ± 0.02)</td>
</tr>
<tr>
<td>0.61–0.90</td>
<td>‘3R’ (0.62 ± 0.07), ‘19’ (0.63 ± 0.05), ‘2K’ (0.65 ± 0.08), ‘1G16’ (0.68 ± 0.05), ‘Vsiola’ (0.76 ± 0.08), ‘Tomitchka’ (0.73 ± 0.07), ‘Parabeskaia’ (0.73 ± 0.08), ‘2E’ (0.73 ± 0.04), ‘2S’ (0.74 ± 0.05), ‘Vsiola’ (0.76 ± 0.08), ‘Vasjuganskaja’ (0.77 ± 0.02), ‘Zoluschka’ (0.78 ± 0.05), ‘32’ (0.81 ± 0.05), ‘Balecharskaja’ (0.81 ± 0.05), ‘22T12’ (0.83 ± 0.05), ‘1B43’ (0.84 ± 0.05), ‘1E’ (0.82 ± 0.07), ‘Morena’ (0.86 ± 0.05)</td>
</tr>
<tr>
<td>0.91–1.2</td>
<td>‘96-3’ (0.90 ± 0.04), ‘2-1’ (0.95 ± 0.07), ‘96-4’ (0.95 ± 0.07), ‘639-8’ (0.98 ± 0.07), ‘1L’ (1.02 ± 0.03), ‘2R’ (1.03 ± 0.04), ‘2C’ (1.04 ± 0.04), ‘L. subsp. novogratziana’ (1.05 ± 0.05), ‘3-5’ (1.09 ± 0.09), ‘Kairėnai’ (1.09 ± 0.05), ‘L-69-3’ (1.11 ± 0.08), ‘1U’ (1.29 ± 0.05)</td>
</tr>
</tbody>
</table>

Total / Bviso: 17

Total / Bviso: 18

Total / Bviso: 12
(21.03%), the lowest – berries of ‘2C’ lines (12.21%). Concentration of sugar showed a large variation and ranges 1.75 times: 9.22% (L. caerulea) and 5.02% (‘1L’). The acidity had more variations and ranged 2.4 times: 2.45% (‘2C’) and 1.03% (‘Baktcharskaja’). It was an individual proportion of investigated parameters of different lines and cultivars.

Table 3. **Berry chemical composition of some honeysuckle lines and cultivar-standards in 2003–2006**

<table>
<thead>
<tr>
<th>Line, cultivar</th>
<th>Berry weight (Ugros mass) (g)</th>
<th>Chemical composition</th>
<th>Acidity (Rt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. caerulea</td>
<td>0.51 ± 0.05</td>
<td>18.72 ± 0.08</td>
<td>2.17 ± 0.05</td>
</tr>
<tr>
<td>L. c. subsp. caerulea</td>
<td>1.05 ± 0.05</td>
<td>13.87 ± 1.05</td>
<td>1.05 ± 0.05</td>
</tr>
<tr>
<td>‘1G16’</td>
<td>0.68 ± 0.05</td>
<td>17.33 ± 0.08</td>
<td>2.17 ± 0.05</td>
</tr>
<tr>
<td>‘1E’</td>
<td>0.82 ± 0.07</td>
<td>20.25 ± 2.21</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘1L’</td>
<td>1.02 ± 0.03</td>
<td>14.31 ± 1.03</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘1N73’</td>
<td>0.77 ± 0.06</td>
<td>20.04 ± 0.21</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘1P’</td>
<td>0.84 ± 0.04</td>
<td>25.03 ± 1.01</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘2C’</td>
<td>0.10 ± 0.03</td>
<td>12.21 ± 0.99</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘2R’</td>
<td>1.03 ± 0.04</td>
<td>15.81 ± 1.12</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘3-1’</td>
<td>0.95 ± 0.07</td>
<td>14.87 ± 1.21</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘3-5’</td>
<td>0.90 ± 0.04</td>
<td>15.54 ± 0.99</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘96-3’</td>
<td>0.95 ± 0.07</td>
<td>14.87 ± 1.21</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘96-4’</td>
<td>1.05 ± 0.07</td>
<td>15.44 ± 0.99</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘Kairenai’</td>
<td>1.09 ± 0.05</td>
<td>15.15 ± 0.37</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘Baktcharskaja’</td>
<td>0.81 ± 0.03</td>
<td>15.73 ± 0.65</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘Morena’</td>
<td>0.86 ± 0.08</td>
<td>15.90 ± 0.68</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘Parabolska’</td>
<td>0.73 ± 0.08</td>
<td>13.11 ± 0.91</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘Vasuganska’</td>
<td>0.77 ± 0.02</td>
<td>15.83 ± 0.67</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘Viosa’</td>
<td>0.86 ± 0.04</td>
<td>16.88 ± 0.32</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘L69-3’</td>
<td>1.11 ± 0.08</td>
<td>12.95 ± 0.89</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>‘639-8’</td>
<td>1.11 ± 0.08</td>
<td>15.92 ± 0.74</td>
<td>1.03 ± 0.05</td>
</tr>
</tbody>
</table>

Distribution of diseases. In 2003–2006 diseases of the aboveground part of bushes were investigated. There were 171 samples from infected plants collected. The following fungal disease agents were identified: Alternaria alternata (Fr.) Keissl., Botrytis cinerea Pers., Fusarium oxysporum var. orthoceras (Appel & Wollenew) Bilai (Fusarium oxysporum f. sp. conglutinans W. C Snyder & H. N. Hansen), Marssonina sp., Microsphaera lonicerae (DC.) G. Winter (Erysiphe lonicerae DC.), Leptosphaeria vagabunda Sacc., Seimatosporium lonicerae (Cooke) Shoemaker, Cladosporium cladosporioides (Fresen) G. A. de Vries, Fumago vagans Pers. (Calderiomyces fumago Woron.). In general, the impact of the fungal disease agents was not crucial for the extinction of the investigated plants. The maximum distribution of diseases (60–90%) was registered on cultivar ‘Baktcharskaja’, also on the line ‘1J’ in 2003 and on cultivars ‘Baktcharskaja’ and ‘Sinaja ptica’ in 2004, 2005 and 2006. Disease intensity made up 20–40%. In 2003–2006 less sensitive

Genetic variation and relationships among 3 cultivars and 6 lines (Table 4) were characterized by random amplified polymorphic DNA.

**Table 4. Characteristic of honeysuckle lines and cultivars investigated by RAPD method**

4 lentėlė. RAPD metodu tirtų sausmedžio linijų ir veislių charakteristikos

**Table 4 continued**

4 lentelės tęsinys

<table>
<thead>
<tr>
<th>Cultivar, line</th>
<th>Shrub characteristic</th>
<th>Origin</th>
<th>Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Bakcharskaja’</td>
<td>Shrub about 1.5 m, drop-shaped berries 20 = 12 mm, 0.81 g medium early ripening</td>
<td>Russia, VIR, seedling of elite form 16/63</td>
<td>L. caerulea, L. caerulea subsp. kamschatka</td>
</tr>
<tr>
<td>‘Viola’</td>
<td>Shrub about 1.5 m, cylindrical berries 23×10 mm, 0.76 g medium early ripening</td>
<td>Russia, VIR, results of far hybridization</td>
<td>L. caerulea, L. caerulea subsp. kamschatka</td>
</tr>
<tr>
<td>‘Morena’</td>
<td>Shrub about 1.0 m, drop-shaped berries 27 = 11 mm, 0.90 g early ripening</td>
<td>Russia, VIR, results of hybridization L. ( \times ) L. ( \times ) subsp. aromatica and jambica</td>
<td>L. caerulea, L. caerulea subsp. aromatica</td>
</tr>
<tr>
<td>‘96-1’</td>
<td>Shrub about 1.0 m, drop-shaped berries 22 = 12 mm, 0.95 g early ripening</td>
<td>Lithuania, VU BG, seedling</td>
<td>L. caerulea, L. caerulea subsp. kamschatka</td>
</tr>
<tr>
<td>‘96-4’</td>
<td>Shrub about 1.0 m, drop-shaped berries 17 = 11 mm, 1.03 g early ripening</td>
<td>Lithuania, VU BG, seedling</td>
<td>L. caerulea, L. caerulea subsp. kamschatka</td>
</tr>
<tr>
<td>‘28’</td>
<td>Shrub about 1.5 m, drop-shaped berries 30 = 11 mm, 1.29 g early ripening</td>
<td>Lithuania, VU BG, seedling</td>
<td>L. caerulea, L. caerulea subsp. kamschatka</td>
</tr>
</tbody>
</table>
RAPD analysis was based on the 88 molecular markers (polymorphic DNA fragments) established in previous study (Naugžemys et al., 2007 in press). The results presented in Fig. 1 confirmed the genetic identity of studied plants. Genetically related (according pedigree data) individuals were grouped together and formed separate clusters. Cultivar ‘Morena’ and line ‘L69-3’ bred by means of far hybridization are genetically distinct from the other lines or cultivars. Two individuals of line ‘639-8’ according RAPD analysis can be considered as clones of the same genotype.

**Discussion.** In general it is little known about blue-berried honeysuckle genetics.
For the study of such anonymous genomes RAPD analysis is widely used. The results obtained in our study demonstrate that RAPD method can be effectively used to genotype Lonicera examples. These investigations are valuable and will be continued.

The berries are the most investigated objects of blue-berried honeysuckle. It is enough information about chemical composition of berries (Plekanova, 2000; Chaovanalikit, 2004). There are 10–17% of dry substances in berries, from it 5–10% of saccharides. It is mostly glucose (75%) and less of fruit sugar, galactose, sucrose and ramnose in the berries. Content of organic acids is 1.5–4.5%. Citric acid prevails (90%) but also apple and amber acids are represented. The content of pectin is 0.4–0.8% of fresh weight. There are also amino acids: asparagine, glutamine, leucine and alanine. The investigation of basal components of berries demonstrates an individual proportion of investigated parameters of different lines and cultivars in our collection.

Honeysuckle bushes yield a good harvest up to 20–25 years. However, they still happen to be damaged by various disease agents. Therefore, it is very important to evaluate the plants for resistance to fungal diseases. There were 171 samples from infected plants collected and 9 fungal disease agents were identified in collection of blue-berried honeysuckle. In general, the impact of the fungal disease agents was not crucial for the extinction of the investigated plants. It is interesting that in Russia blue-berried honeysuckle are resistant to fungal diseases, in Japan plants are nonresistant to disease agent Botrytis sp., in Canada – to Microsphaera sp. (Bors, 2006). The fungal disease agent Botrytis cinerea Pers. and Microsphaera lonicerae (DC.) G. Winter was established on plants of our collection. We want once more to foreground, that blue-berried honeysuckles are introduced plants in Lithuania and the climate conditions are atypical and not optimal to these plants. It determines other resistance to diseases. Plants of L. caerulea, L. caerulea subsp. kamtschatika, 14 lines and 7 cultivars were less sensitive to the impact of fungal disease agents. There are 17 lines with berries of more than 0.70 g weight too. In summary, there are 6 lines (‘1L’, ‘2C’, ‘3U’, ‘3-5’, ‘32’, ‘96-4’) that include both features (large berries and resistance to disease). These 6 lines are perspective to obtain the status of Lithuanian genetic resources.

Conclusions. In 2003–2006 the attention was focused on genetic lines collection. According to the weight of berries (one of the most important criteria), 17 prominent lines were selected. Plants of L. caerulea, L. caerulea subsp. kamtschatika, 14 lines and 7 cultivars were less sensitive to the impact of fungal diseases agents. There are 6 lines (‘1L’, ‘2C’, ‘3U’, ‘3-5’, ‘32’, ‘96-4’), which include both features (large berries and resistance to disease).

Acknowledgments. The study was supported by the Lithuanian Government Science Program “Scientific Research of the Plant Genetic Resource in Lithuania” (2004–2008).

SODININKYSTĖ IR DARŽININKYSTĖ. MOKSLO DARBAI. 2007. 26(3).
MELSVAUOGIŲ SAUSMEDŽIŲ LINIJŲ IR VEISLIŲ ĮVERTINIMAS
VILNIAUS UNIVERSITETO BOTANIKOS SODO KOLEKCIJOJE

S. Žilinskaitė, D. Naugžemys, D. Radaitienė, D. Žvingila

Santrauka


Reikšmingiai žodžiai: atsparumas ligoms, genetinė įvairovė, melsvauogiai sausmedžiai (Lonicera L.), uogų charakteristika.

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FRUIT YIELD AND CONTENT OF MACROELEMENTS IN FRUITS OF SELECTED ECOTYPES OF WILD EUROPEAN ELDERBERRY (*Sambucus nigra* L.) IN THE LAKE DISTRICT OF WARMIA AND MAZURY, POLAND

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**Abstract.** In 2003–2006 a study on selected ecotypes of European elderberry (*Sambucus nigra* L.) forms growing in wild was completed at the Department of Horticulture, the University of Warmia and Mazury in Olsztyn. The study covered sites far from roads and other sources of pollution in 8 towns and villages of Warmia and Mazury, a region in north-eastern Poland (Giżycko, Gietrzwałd, Korsze, Mrągowo, Mikołajki, Pasym, Olsztynek and Węgorzewo). The aim was to evaluate fruit yield and content of macroelements in fruit of wild European elder.

The highest fruit yield was collected from elder shrubs growing in Mikołajki (16.02 kg), followed by those in Mrągowo (13.60 kg), Giżycko (11.90 kg) and Korsze (11.19 kg). The fruit yield at the other test sites varied from 6.90 to 9.07 kg per plant.

The highest 100 fruit weight was obtained from the elder plants, which produced the highest yields (0.45 g in Mikołajki, 0.40 g in Giżycko, 0.33 g in Mrągowo and 0.30 g in Korsze).

**Key words:** European elder, yield, macroelements.

**Introduction.** Interest in European elderberry has grown in the recent years. It can be expected that in the near future the plant will join other plants traditionally grown in horticulture (Waźbińska, 1998, 2000).

In many European countries elder is grown in plantations, where special farms produce healthy fruit from commercial cultivars. In Poland, efforts are undertaken to popularise the plant among gardeners. Many authors (Borowska et al., 2000; Pliszka et al., 2005; Waźbińska, 1997, 1998; Waźbińska and Puczel, 2004) have proven that commercial cultivars of European elder produce fruit rich in organic acids, sugars, vitamin C and polyphenolic compounds. Raw material for food processing industry obtained from European elder is a recognised source of antioxidants and substances, which may prevent cancer (Abuja et al., 1998; Moszczyński, 1996; Obidowska, 1998; Oszmiański and Lamer-Zawadzka, 1995). European elder fruit can be used for production of jellies and juices.

Until present, there has not been much research done on ecotypes of wild growing European elder forms in an unpolluted area such as the Province of Warmia and Mazury.

The purpose of the study has been to evaluate the fruit yield and content of macroelements in fruit of selected ecotypes of wild European elder growing near Giżycko, Gietrzwałd, Korsze, Mrągowo, Mikołajki, Pasym, Olsztynek and Węgorzewo.
Material and methods. The study on selected ecotypes of European elderberry (*Sambucus nigra* L.) wild forms was conducted by the Department of Horticulture, the University of Warmia and Mazury in 2003–2006. The study comprised sites in 8 towns and villages in the Province of Warmia and Mazury (Giżycko, Gietrzwałd, Korsze, Mrągowo, Mikołajki, Pasy, Olsztynek and Węgorzewo) far from roads and sources of pollution. At each of the above localities, 6 shrubs were taken for examination. The shrubs, growing near one another, were of approximately the same height and breadth and yielded abundant corymbs. During the harvest, the volume of yield was tested on randomly chosen shrubs (6 at each site) and the fruit was used for determination of macroelements with the following methods:

- nitrogen content (N) – PB 07,
- phosphorus (P<sub>2</sub>O<sub>5</sub>) – PB 04,
- potassium (K<sub>2</sub>O) – PB 03-75101,
- magnesium (MgO) – PB 06,
- calcium (CaO) – PB 05,
- sodium (Na<sub>2</sub>O) – PB 03.

The results obtained in three replicates underwent analysis of variance using the MS Excel 97 software.

Before these examinations, soil at each site was analysed to determine its pH, humus content and concentration of available forms of macroelements in the top soil layer (Table 1).

<table>
<thead>
<tr>
<th>Location</th>
<th>Content of granulometric fraction: 0.02 (%)</th>
<th>pH (1 M KCl)</th>
<th>Available compound content (mg·100 g&lt;sup&gt;−1&lt;/sup&gt; of soil)</th>
<th>Humus content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giżycko</td>
<td>23</td>
<td>Sandy loam</td>
<td>Prionitis</td>
<td>6.6</td>
</tr>
<tr>
<td>Gietrzwałd</td>
<td>16</td>
<td>Loose sandy soil</td>
<td>Prionitis</td>
<td>6.9</td>
</tr>
<tr>
<td>Korsze</td>
<td>18</td>
<td>Heavy sandy soil</td>
<td>Stanisławow</td>
<td>6.7</td>
</tr>
<tr>
<td>Mrągowo</td>
<td>19</td>
<td>Heavy sandy soil</td>
<td>Stanisławow</td>
<td>6.7</td>
</tr>
<tr>
<td>Mikołajki</td>
<td>8</td>
<td>Sandy loam</td>
<td>Prionitis</td>
<td>6.9</td>
</tr>
<tr>
<td>Pasy</td>
<td>13</td>
<td>Sandy soil</td>
<td>Stanisławow</td>
<td>6.8</td>
</tr>
<tr>
<td>Olsztynek</td>
<td>22</td>
<td>Sandy loam</td>
<td>Prionitis</td>
<td>7.4</td>
</tr>
<tr>
<td>Węgorzewo</td>
<td>16</td>
<td>Sandy loam</td>
<td>Prionitis</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Before these examinations, soil at each site was analysed to determine its pH, humus content and concentration of available forms of macroelements in the top soil layer (Table 1).
ranged from 0.97 to 2.13%. At all the sites the soil reaction was neutral, ranging from 6.6 to 7.4 pH in 1 M KCl.

The fruit yield per shrub and content of macroelements in fruit were similar in all the years covered by the study, which is why the results were averaged – as means for four years, subjected to analysis of variance and run by Duncan’s test at p = 0.05 for a field trial.

Results. The highest elder fruit yield was obtained from a plant growing in Mikołajki (16.02 kg), followed by shrubs in Mrągowo (13.60 kg), Giżycko (11.90 kg) and Korsze (11.19 kg) (Table 2). Shrubs growing at the remaining four sites yielded between 6.90 kg to 9.07 kg per plant.

Table 2. Yield per plant (kg) and 100 fruit weight (g) of wild growing ecotypes of European elder. Means of four years (2003–2006)

The highest 100 fruit weight was produced by plants growing at the sites characterised by the highest yield (0.45 g in Mikołajki, 0.40 g in Giżycko, 0.33 g in Mrągowo and 0.30 g in Korsze).

Concentrations of macroelements assayed are presented in Table 3. Fruit of European elder contained 0.69–1.49% of nitrogen, 0.32–0.87% of phosphorus and 2.38–3.80% of potassium per 1 kg of dry matter. The highest concentration of those 3 elements was found in the fruit of wild European elder growing at the sites, which were richer in mineral components and humus (Mikołajki, Mrągowo, Korsze and Giżycko). Similar relationships were observed in regard to content of magnesium, calcium and sodium in elder fruit.


Discussion. The results of the study completed in 2003–2006 at different sites in Giżycko, Gietrzwałd, Korsze, Mrągowo, Mikołaji, Pssym, Olszynek and Węgorzewo
showed significant differences among yields harvested from those sites (Table 2).

The highest yield of European elder fruit was collected from a shrub growing in Mikołajki (16.02 kg). High yields were also harvested in Mrągowo (13.60 kg), Giżycko (11.90 kg) and Korsze (11.19 kg). At the other four sites, fruit yields varied between 6.90 and 9.07 kg per plant. Kadarova (1986) reports a study completed in Slovakia. Kaack (1989) carried out a study in Denmark (Copenhagen and Arstler), in which local ecotypes were compared with commercial cultivars (cv. ‘Sambo’ and ‘Haschbery’). It was found out that the commercial cultivars produced higher yields. Botu (1984) informs about a study on new cultivars of European elder in Romania, in the Danube River delta. Three cultivars, ‘Ina’, ‘Norna’ and ‘Vilcea’, which had been bred there, were characterised by fast growth of plants and produced about 10 kg of fruit per plant. Waźbińska (1998) reported that ecotypes of wild growing European elder forms propagated from woody grafts and growing near Olsztyn produced 6 kg of fruit per plant, whereas those selected from the Lake District of Warmia and Mazury yielded from 16 to 15 kg of fruit per plant (Waźbińska et al., 2007).

The weight of fruit is a very important factor as heavier fruit make it easier and faster to harvest the yield, which in turn reduced costs and labour (Laack, 1989). In our study, the highest 100 fruit weight was determined for the European elder shrubs growing at the sites characterised by the highest yields (Mikołajki, Mrągowo, Korsze and Giżycko).

Our results are consistent with the data reported in relevant references. Kaack (1989) wrote that 100 fruit weight for particular cultivars and ecotypes ranged between 15 and 31 g, whereas Popraczy and Laszlo (1984) reported a wider variation in fruit weight. According to an ecotype they studied, the weight of 100 fruit varied from 9 to 45 g.

European elder shrubs growing in soil less abundant in mineral components or humus produced lower yields and smaller fruit.

The concentrations of particular elements in European elder fruit can be found in Table 3. The examined fruit sample contained 0.69–1.49% of nitrogen per kg of dry matter, 0.32–0.87% of phosphorus per kg of d.m. and 2.38–3.80% of potassium per kg of d.m. The highest concentrations of these elements were determined in fruit of European elder plants growing at the sites where soil was richer in minerals and humus (Mikołajki, Mrągowo, Korsze and Giżycko). Similar relationships were observed in the case of magnesium, calcium and sodium in fruit. The present results on the content of macroelements in European elder fruit are similar to those cited by
Conclusions. 1. Yield from selected ecotypes of wild growing plants of European elderberry in the Lake District of Warmia and Mazury was varied, and largely depended on the soil abundance in minerals and humus.

2. The highest 100 fruit weight was determined in the case of elder plants growing at the sites located in Mikołajki, Mrągowo, Korsze and Giżycko.

3. A clear difference was observed in the yield and 100 fruit weight between the 8 sites. Shrubs growing in soil more abundant in nutrients and humus produced higher yields and larger fruit.

4. The concentration of macroelements in fruit produced by wild growing ecotypes of European elder was also correlated with a site. Sites richer in mineral components and humus were associated with European elder plants producing fruit richer in macroelements.

References


11. Waźbińska J. Zawartość niektórych składników chemicznych w owocach bzu


atrinktų ekotipų tyrimai. Tyrimai apėmė toli nuo kelių ir kitų taršos šaltinių esančias vietoves aštuoniuose Varmijos ir Mazūrijos miestuose bei kaimuose – šiaurės rytiniame Lenkijos regione (Giżycko, Gietrzwałd, Korsze, Mrągowo, Mikołajki, Pasym, Olsztynek ir Węgorzewo). Tyrimų tikslas buvo įvertinti laukinio europinio šeivamedžio uogų derlių ir makroelementų kiekį uogose. Didžiausią derlių davė šeivamedžio krūmai, augantys Mikołajki (16,02 kg), po to ėjo krūmai, augantys Mrągowo (13,60 kg), Giżycko (11,90 kg) ir Korsze (11,19 kg). Kitose tirtose vietovėse derlius svyravo nuo 6,90 iki 9,07 kg nuo augalo. Didžiausia 100 uogų masė gauta nuo tų šeivamedžių, kurie davė didžiausią derlių: Mikołajki – 0,45 g, Giżycko – 0,40 g, Mrągowe – 0,33 g ir Korsze – 0,30 g.

Reikšminiai žodžiai: derlius, europinis šeivamedis, makroelementai.
THE YIELD AND CONTENT OF SOME CHEMICAL COMPONENTS IN FRUITS OF COMMON MEDLAR (*MESPILUS GERMANIKA* L.)

PART I

YIELD

**Jadwiga WAŹBIŃSKA**

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The study was carried out at the Experimental Garden of the University of Warmia and Mazury in Olsztyn in 2002–2004. Trees of common medlar (*Mespilus germanika* L.) of two cultivated varieties (‘Bredase Reus’ and ‘Westerveld’) were used as the experimental material; the trees had been brought from an experimental nursery in Boskoop (the Netherlands). The plants were planted at the spacing of 3 × 3.5 m in four replications. Each year fruit was collected and some of its morphological characteristics were measured, including the weight and diameter of one fruit and the yield per tree. The results were subjected to the analysis of variance, and the mean values were tested according to Duncan’s t test at the level of significance *p* = 0.05.

The measurements reveal that the variety ‘Bredase Reus’ was characterised by higher weight (22.45–26.30 g), larger diameter (3.45–4.78 cm) and bigger yield per tree (4–5 kg). The examined varieties bore fruits of higher weight and larger diameter and gave a bigger yield per tree.

**Key words:** *Mespilus germanica*, morphological features, yield.

**Introduction.** Medlar is found in natural sites in Central Asia and in Asia Minor as well as in the Caucasus (Seneta, Dolatowski, 2000; Sękowski, 1993). Currently, it is found as separate ornamental tree in parks and gardens in the South and West Europe (Kremer, 1989; Łańska, 1992, Noordhuis, 1997; Vermeulen, 1977).

Medlar bears spherical or pear-like fruits, about 3 cm in diameter, with five preserved sepals, which do not dry out after blossoming but unfold together with fruits (Hrynkiewicz – Sudnik, Sękowski and Wilkiewicz, 2001; Kremer, 2000; Noordhuis, 1997; Seneta, Dolatowski, 2000; Vermeulen, 1977; Waźbińska, 1998; Waźbińska, 1999; Wit, 1997).

The trees require good, fertile soil, tolerate pollution of the urban environment and are largely resistance to frost (Łańska, 1992; Mika, 1994; Noordhuis, 1997).

The species can be multiplied generatively, while large-fruited varieties are usually multiplied vegetatively by grafting or budding, on the rootstock of whitethorn, pear and quince. (Hrynkiewicz – Sudnik, Sękowski, Wilczkiewicz, 2001; Mika, 1994; Waźbińska, 2001).

Considering the interesting form of its fruits, the tree should be more frequently
planted in parks and gardens (Reichholf and Steinbach, 1995). It bears fruit regularly as it blossoms late and spring frosts do not damage its flowers (Waźbńska, 1989).

The aim of this study was to conduct a morphological assessment of fruits and the yielding of two varieties of common medlar, grown in Olsztyn.

**Materials and methods.** The medlar experiment was laid down at the Experimental Garden of the University of Warmia and Mazury in Olsztyn in 1999. Trees of common medlar (*Mespilus germanika* L.) of two cultivated varieties (‘Bredase Reus’ and ‘Westerveld’) were used as the experimental material; the trees had been brought from an experimental nursery in Boskoop (the Netherlands). The plants were planted at the spacing of 3 × 3.5 m in four replications. Each year fruits were collected and some of their morphological characteristics were measured, including the weight and diameter of one fruit and the yield per tree. The results were subjected to the analysis of variance, and the mean values were tested with Duncan’s t test at the level of significance p = 0.05.

**Results.** Of the two examined varieties (‘Bredase Reus’ and ‘Westerveld’), ‘Bredase Reus’ bore fruit of higher weight (22.45–26.30 g), while the weight of the other variety, ‘Westerveld’, was smaller (11.22–19.15 g). The highest weight was achieved by the fruits of both varieties in 2004. As in the case of the fruit weight, the ‘Bredase Reus’ fruit had a larger diameter (3.45–4.78 g) than those of the ‘Westerveld’ variety (2.57–2.90 g). The highest yield was also achieved by the ‘Bredase Reus’ variety. In different years of the experiment it ranged from 4.10 to 6.80 kg per tree, whereas for the ‘Westerveld’ variety it ranged from 2.76 to 3.99 kg per tree. Both varieties gave better yield in 2002 and 2004, when the weather in Olsztyn favoured plant growth (Table 1).

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<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>I</td>
<td>-1.3</td>
<td>-1.1</td>
<td>-1.0</td>
<td>-3.6</td>
</tr>
<tr>
<td>II</td>
<td>0.4</td>
<td>-1.6</td>
<td>2.8</td>
<td>-4.7</td>
</tr>
<tr>
<td>III</td>
<td>2.0</td>
<td>0.7</td>
<td>3.6</td>
<td>1.2</td>
</tr>
<tr>
<td>IV</td>
<td>10.7</td>
<td>7.2</td>
<td>7.8</td>
<td>6.0</td>
</tr>
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<td>V</td>
<td>14.0</td>
<td>12.8</td>
<td>16.2</td>
<td>14.1</td>
</tr>
</tbody>
</table>

Table 1. Average air temperatures and total precipitation in 2000–2004 according to the Institute of Meteorology and Water Management in Olsztyn

Table 1 continued

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<tbody>
<tr>
<td>I</td>
<td>-1.3</td>
<td>-1.1</td>
<td>-1.0</td>
<td>-3.6</td>
<td>-6.4</td>
<td>-3.0</td>
<td>35.3</td>
<td>16.7</td>
<td>41.6</td>
<td>32.9</td>
<td>29.8</td>
<td>28.3</td>
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<td>II</td>
<td>0.4</td>
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<td>2.8</td>
<td>-4.7</td>
<td>-0.9</td>
<td>-2.8</td>
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<td>13.4</td>
<td>53.4</td>
<td>4.9</td>
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<tr>
<td>III</td>
<td>2.0</td>
<td>0.7</td>
<td>3.6</td>
<td>1.2</td>
<td>2.0</td>
<td>1.0</td>
<td>48.5</td>
<td>41.2</td>
<td>43.2</td>
<td>14.9</td>
<td>33.7</td>
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<td>7.8</td>
<td>6.0</td>
<td>7.3</td>
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<td>54.9</td>
<td>14.2</td>
<td>35.5</td>
<td>46.5</td>
<td>32.8</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>14.0</td>
<td>12.8</td>
<td>16.2</td>
<td>14.1</td>
<td>11.0</td>
<td>12.6</td>
<td>53.5</td>
<td>33.2</td>
<td>81.5</td>
<td>30.2</td>
<td>79.3</td>
<td>49.4</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Comparison of some morphological characteristics of fruits and the yield of common medlar in 2002–2004

<table>
<thead>
<tr>
<th>Years</th>
<th>Fruit weight</th>
<th>Fruit diameter</th>
<th>Yield per 1 bush</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Varieties (g)</td>
<td>Varieties (cm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>'Bredase Reus'</td>
<td>'Westerveld'</td>
<td>'Bredase Reus'</td>
</tr>
<tr>
<td>2002</td>
<td>24.86</td>
<td>17.21</td>
<td>4.11</td>
</tr>
<tr>
<td>2003</td>
<td>22.45</td>
<td>11.22</td>
<td>3.45</td>
</tr>
</tbody>
</table>

Discussion. Of the two examined varieties, ‘Bredase Reus’ bore fruit of higher weight (22.45–26.30 g), while the weight of the fruits of the other variety, ‘Westerveld’, was smaller (11.22–19.15 g). The highest weight was achieved by the fruit of both varieties in the last (26.30 g; 19.15 g) and first (24.86 g; 17.21 g) year of the experiment. It can be explained by favourable values of temperature and rainfall in those years (Table 1). As in the case of the fruit weight, variety ‘Bredase Reus’ gave larger fruits (3.45–4.78 cm in diameter), while the fruits of variety ‘Westerveld’ were smaller, 2.66–2.90 cm. Mika (1994) reports that fruits of some common medlar varieties ‘Dutch Giant’ can be as large as 5–6 cm. In their study, Waźbińska et al. (1999) obtained fruit of the same variety with diameter of 4.0 cm, while the diameter of variety ‘Royal’ was only 2.40 cm. As with other morphological features of the fruits of common medlar (weight and diameter), the highest yield was given by the plants in 2002 and 2004.

It was 4.10 and 6.80 kg per tree of variety ‘Bredase Reus’ and 2.76 to 3.99 kg per tree of variety ‘Westerveld’. The weather conditions dominating in those years in Olsztyn favoured the growth of the plants (Table 1). Waźbińska 1988, Waźbińska et al. (1999) obtained a similar yield of the ‘Dutch Giant’ and ‘Royal’ in the first year after planting.

Conclusion. The fruits borne by variety ‘Bredase Reus’ have heavier and larger fruit and bigger yield.
References


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**BALTAŽIEDĖS ŠLIANDROS (MESPILOUS GERMANIKA L.) DERLIUS IR CHEMINIŲ JUNGINIŲ KIEKIS VAISIUOSE I DALIS**
DERLIUS
J. Ważbińska

Santrauka


Matavimai parodė, kad ‘Bredase Reus’ veislės vaisiai išsiskyrė didesne mase: 22,45–26,30 g, didesniu skersmeniu: 3,45–4,78 cm ir didesniu derliumi: 4–5 kg.

Reikšminiai žodžiai: derlius, Mespilus germaniška L., morfologinės savybės.
THE YIELD AND CONTENT OF SOME CHEMICAL COMPONENTS IN FRUITS OF COMMON MEDLAR (MESPILUS GERMANICA L.)

PART II

CONTENT OF SOME CHEMICAL COMPONENTS IN FRUITS OF COMMON MEDLAR (MESPILUS GERMANICA L.)

Jadwiga WAŹBIŃSKA

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The study was conducted in the experimental Garden of the University of Warmia and Mazury in Olsztyn and at the Chair of Horticulture in 2002–2004. Fruits of two varieties of common medlar (‘Bredase Reus’ and ‘Westerveld’) were used as the experimental material. The fruits were picked between 20th and 30th of October (before the ground frost period) and examined for the contents of dry substance, sugars, vitamin C and acidity. The examined varieties of common medlar were similar in terms of dry substance content: on the average ‘Bredase Reus’ contained 25.82%, while ‘Westerveld’ – 24.33%. The total sugar content in the fruits of common medlar was 4.46 g in variety ‘Westerveld’ and 4.88 g in ‘Bredase Reus’. The examined varieties contained much more simple sugar than oligo- and polysaccharides. Their content in the examined fruits ranged from 1.67 to 1.73 g / 100 g.

The total acidity was about 1.03 g / 100 g in ‘Westerveld’ and 1.22 g / 100 g in ‘Bredase Reus’. The statistical analysis proved the existence of statistically significant differences in vitamin C content between varieties. The fruits of the ‘Bredase Reus’ contained more vitamin C (19.55 mg in 100 g of fresh substance) than these of ‘Westerveld’ (18.66 mg in 100 g of fresh substance).

Key words: acids, dry substance, Mespilus germanica L., sugars, vitamin C.

Introduction. Common medlar (Mespilus germanica L.) belongs to the family Rosaceae. It is attractive looking tree or shrub because of the plant dimensions, with nice leaves and flowers and fruits of unusual shape. Medlar can be as high as 5 m. It develops a wide, low set crown. It has elliptic leaves, up to 14 cm long, serrated, with a white moss-like coating on the bottom side, in autumn changing their colour to yellow; 5-petal white flowers, up to 5 cm in diameter (Senata and Dolatowski, 2000; Waźbińska, 1998).

Medlar bears spherical or pear-like fruits, which are not directly consumable as the pulp is very hard and stony, distinctly sour and slightly tart (Hoduj, 1998; Kremer, 1996; Mika, 2001; Pieniżek, 1981). The fruit can be picked before the period of frost (Hodun, 1998; Mika, 2001) and stored in a cool place in a single layer. In the course
of the process, the pulp becomes soft and turns brown; it becomes juicy, sweet and tasty. The inside of the fruit resembles baked apple. Ripe fruit can be stored for many weeks (Hodun, 1998; Lańska, 1992; Mika, 2001). It has good dietetic and therapeutic properties as it contains considerable amounts of tannins, protein, sugars, organic acids, pectins, minerals, vitamin C, provitamin A and others (Lańska, 1992).

Medlar grows best on damp, sunny sites (Noordhuis, 1997). Medlar fruit can be used to make jellies and preserves (Waźbińska, 1998). In the regions where they are grown, medlar fruit is used as a wine additive because of the tannins and organic acids contained in it. Unripe fruit can be marinated (Haciseferošullari et al., 2005; Hoduj, 1998; Kremer, 1996).

**Materials and methods.** The experiments were conducted at the Experimental Garden of the University of Warmia and Mazury in Olsztyn and at the Chair of Horticulture in 2002–2004. Fruit of two varieties of common medlar (‘Bredase Reus’ and ‘Westerveld’) picked between 20th and 30th of October (before the period of ground frosts) were used as the experimental material.

The following were determined in the fruits:
- dry matter – the drier method at 105°C – PN – 71 (A-75101);
- sugar contents – reduction titration with weakly alkaline copper reagent according to Luff Schorll – PN –71 (A-75101);
- total acidity - expressed as malic acid, by titration with 0.1 m Na OH – PN – 71 (A-75101);

The experiment results varied from year to year, so the results from three years were juxtaposed as mean values and subjected to analysis of variance. The mean values were tested according to Duncan’s t-test at the level of significance p = 0.05.

**Results.** Slight differences were found to exist in the dry matter content between the common medlar varieties (Table). ‘Bredase Reus’ contained 25.82% of dry substance, whereas ‘Westerveld’ – 24.33%.

The total sugar content in common medlar fruit was 4.46 g in ‘Westerveld’ and 4.88 g in ‘Bredase Reus’ (Table). The examined varieties contained much more monosaccharides than oligo- and polysaccharides. Their content in the examined fruits ranged from 1.67 to 1.73 g / 100 g.

The medlar fruit did not contain much acid (Table). Total acidity was 1.03 g / 100 g in ‘Westerveld’ and 1.22 g / 100 g in ‘Bredase Reus’.

The statistic analysis proved the existence of statistically significant differences in vitamin C content between the varieties. The fruits of the ‘Bredase Reus’ variety contained more vitamin C (19.55 mg in 100 g of fresh substance) than ‘Westerveld’ (18.66 mg in 100 g of fresh substance).

**Table.** The contents of some chemical compounds found in fruit of common medlar (mean values of 2002–2004)

<table>
<thead>
<tr>
<th>Compound Type</th>
<th>‘Bredase Reus’</th>
<th>‘Westerveld’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td>25.82</td>
<td>24.33</td>
</tr>
<tr>
<td>Total sugar (g/100g)</td>
<td>4.46</td>
<td>4.88</td>
</tr>
<tr>
<td>Total acidity (g/100g)</td>
<td>1.03</td>
<td>1.22</td>
</tr>
<tr>
<td>Vitamin C (mg/100g)</td>
<td>19.55</td>
<td>18.66</td>
</tr>
</tbody>
</table>

Lentelė. Cheminių junginių kiekis baltažiedės šliandros vaisiuose (pateiktos
2002–2004 m. vidutinės vertės)

Discussion. Slight differences were found to exist between the dry substance content in the fruits of both varieties (Table). ‘Bredase Reus’ contained 25.82% of dry substance on average, whereas ‘Westerveld’ contained 24.33%. Similar results were obtained by Haciseferošullari et al. (2005) and Romero-Rodriguez et al. (2000).

<table>
<thead>
<tr>
<th>Content</th>
<th>‘Bredase Reus’</th>
<th>‘Westerveld’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>25.82 a</td>
<td>24.33 b</td>
</tr>
<tr>
<td>Sausa masė (%)</td>
<td>3.21 a</td>
<td>2.73 b</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>1.67 a</td>
<td>1.73 a</td>
</tr>
<tr>
<td>Monosacharidai (%)</td>
<td>4.88 a</td>
<td>4.46 b</td>
</tr>
<tr>
<td>Complex sugars</td>
<td>1.22 a</td>
<td>1.03 b</td>
</tr>
<tr>
<td>Polisacharidai (%)</td>
<td>19.55 a</td>
<td>18.66 b</td>
</tr>
</tbody>
</table>

Total sugar content in the fruits of common medlar was 4.46 g in ‘Westerveld’ and 4.88 g in ‘Bredase Reus’. The examined varieties contained much more monosaccharides than oligo- and polysaccharides. Monosaccharides – fructose and glucose – are the main sugars in medlar fruit (Hacżseferošullarż et al., 2005). Their content in the examined fruits was 1.67–3.11 g / 100 g, with the total sugars content amounting to 4–4.97 g / 100 g. Lanska (1999) reports that the total sugar content in medlar fruit amount to 11–13 g / 100 g, which is much more than in the fruit examined in this study. Romero-Rodriguez et al. (2000) reported an even higher value – 21.23 g / 100 g. Glew et al. (2003) found the fruit to contain similar amounts of sugar (0.04 to 3.12 g / 100 g) depending on how ripe the fruit was. Medlar fruit contained little acids (Table).

The total acidity was 1.03 g / 100 g in ‘Westerveld’ and 1.22 g / 100 g in ‘Bredase Reus’. According to Hacżseferošullarż et al. (2005) and Romero Rodriguez et al. (1992), organic acid content in medlar fruit can range from 0.28 to 5.63 g / 100 g. Other authors (Glew et al., 2003a; Lanska, 1992) report values of slightly over 1 g.

Statistical analysis has proved the existence of significant differences in vitamin C content between the varieties. ‘Bredase Reus’ contained more (19.55 mg in 100 g of fresh substance), than ‘Westerveld’ (18.66 mg in 100 g of fresh substance). Romero Rodriguez et al. (1992) report very low concentrations of vitamin C in medlar fruit (0.3 mg / 100 g), in contrast to this study, where much higher values (18.66–19.55 mg / 100 g) were found.

Conclusion. The fruits of two examined medlar varieties (‘Bredase Reus’ and Variety ‘Bredase Reus’ contained more dry substance, total sugars, acids and vitamin C.

Gauta 2007 06
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**BALTATŽIEDĖS ŠLIANDROS (MESPILUS GERMANIKA L.) DERLIUS JŪŲ CHEMINIŲ JUNGINIŲ KIEKIS VAISIUOSE**
II DALIS
CHEMINIŲ MEDŽIAGŲ KIEKIS VAISIUOSE

J. Waźbińska

Santrauka


Bendras rūgštingumas ‘Westerveld’ veislėje buvo apie 1,03 g / 100 g, o ‘Bredase Reus’ – 1,22 g / 100 g. Vitamino C kiekio statistinė analizė parodė patikimus skirtumus tarp veislių. ‘Bredase Reus’ veislės vaisiuose nustatyta daugiau vitaminų C (19,55 mg / 100 g šviežios masės) nei ‘Westerveld’ veislės vaisiuose (18,66 mg / 100 g šviežios masės).

Reikšminiai žodžiai: Mespilus germanika L., sausa masė, rūgštys, vitaminas C, cukrūs.
One of the most important problems of sea buckthorn breeding program in Belarus is producing wilt-resistant cultivars that are suitable for machine harvesting. To introduce new parental forms into the breeding process, the examination of wild sea buckthorn plantations at the territory of South Warmia of Poland (Olsztyn, Poland) was carried out in 2006. First of all, the absence of visual wilt symptoms was taken into account. Also, the following criteria of suitability for machine harvesting were considered: character of pull-out force, spadix density, fruit stalk length. As a result of the observation 7 female forms, 1 male form and the clone of cultivar ‘Podarok sadu’ were selected. Wilt symptoms were absent in all the selected forms. The fruit stalk length varied from 2.3 to 6.4 mm, pull-out force varied from 1.8 to 3.0 H, number of fruits in one fruiting bud varied from 3.0 to 5.8. General defect of the forms, except for the forms of ‘Kortovo-1’ and the clone of cultivar ‘Podarok sadu’, is their small-fruits (up 0.4 g).

**Key words:** germplasm, sea buckthorn, suitability for machine harvesting, wilt resistance.

**Introduction.** Sea buckthorn is one of the most promising crops for fruit growing in Belarus. Cultivation of sea buckthorn at the industrial scale is restricted by insufficient cultivar assortment, which is mainly presented by the introduced cultivars selected at the Botanical gardens of the MSU (Moscow, Russia), e.g. ‘Avgustinka’, ‘Aromatnaja’, ‘Botanicheskaja’, ‘Krasnoplodnaja’, ‘Niwelena’, ‘Podarok sadu’, ‘Trofimovskaja’, and ‘Plamennaja’, developed by a team of breeders from Nizhniy Novgorod State Agricultural Academy, Allrussian research institute for plant protection and the RUE “Institute for Fruit Growing”, Belarus. In addition to the abovementioned fact there is no effective method for machine harvesting (Государственный реестр, 2007).

Sea buckthorn breeding program that is being run at the RUE “Institute for Fruit Growing” is aimed at the development of highly productive varieties with features-indicators of suitability to machine harvesting, e.g. dry and easy tearing, long fruit stalk and moderate vigor. Recent research has shown that certain cultivars are susceptible to wilt, which in some cases causes 100% destruction of plants. This fact proves that it is necessary to carry out breeding for wilt resistant varieties (Шаёкевич, 2005).

One of the major stages in the breeding process is studying the germplasm and selecting the initial material. Natural thickets and plantations, established by layers and seedlings, are valuable sources for initial forms due to the polymorphism.

Natural sea buckthorn thickets cover about 810 thousand ha (in Russia, China
and Mongolia) (Sun, 1995).

Research carried out in several institutions proved sea buckthorn accessions of the Baltic region to be promising for breeding process (Фефеёв et al., 2002; Albrecht, 2003; Aksenova and Dolgacheva, 2003; Rumpunen, 2004; Шаёкевич, 2005; Смертин, 2006).

Wild sea buckthorn plantations of the Baltic region are located in Kaliningrad and Leningrad regions of Russia, in Lithuania, Poland and Germany. The most studied are plantations covering about 1000 ha in Kaliningrad region (Кондрашов, 1980; Мишуёина et al., 1982; Гаранович, 1983; Фещенко, 2002).

Research on evaluation of wild sea buckthorn accessions on the territory of South Warmia (Poland) has not been carried out before.

The research is aimed at the selection of initial material in the territory of north-eastern Poland. The initial material will be used for breeding high productive, wilt resistant cultivars, with the complex of traits indicating suitability for machine harvesting.

**Materials and methods.** Objects of the research were wild sea buckthorn plantations in the territory of north-eastern Poland (Olsztyn and surrounding area, Krynica Morska) and the sea buckthorn collection at the University of Warmia and Mazury. It is a common fact that the forms introduced from similar climatic regions are the most resistant to the complex climatic factors, thus, it explains the choice of the objects. Furthermore, we chose these objects due to the absence of publications on sea buckthorn in Warmia. The research was carried out in August–September, 2006. In the selection process the absence of visual wilt symptoms, such as fading fruit and shoots, orange-red spots or swellings on bark, was primarily taken into account.

Plants without visual symptoms of wilt were further evaluated. Visual yield assessment, mean fruit weight, number of fruits in a fruit bud and indicators of suitability for machine harvesting, e.g. character of tearing and pull-out force, spadix density, fruit stalk length, were taken into account.

Visually yield was assessed by a 5-grade scale: 1 – solitary fruits, 2 – weak, 3 – average, 4 – good, 5 – abundant fruit bearing (the Program and strategy..., 1980).

Mean fruit weight was defined by weighing 100 typical fruits in four replications. Cultivars were classified according to “Кёассификатор рода Hippophae rhamnoides L.” (1993): very small (< 0.2 g); small (0.2–0.4 g); medium (0.5–0.7 g); large (0.8–1.0 g); very large (> 1.0 g).

Number of fruits from a flower bud was determined as mean value from 20 samples taken from the middle of shoots in 3 replications.

Type of tearing fruit from the stalk was considered as wet (> 75% with rapture of fruits), semi-dry (30–75%), and dry (< 30%) (“Кёассификатор рода Hippophae rhamnoides L.”, 1993).

Pull-out force was measured by the spring dynamometer. 20 fruits from the middle of two-year shoots at the stage of full maturation in three replications were assessed. Distribution of cultivars by groups was performed according to “Программа и методика сортоизучения пёодовých, ягоднých и орехопёоднých культур” (1999), as
follows: 1.0–1.5 N – weak, 1.6–2.0 N – medium, more than 2.0 N – strong pull-out force.

Length of fruit stalk was determined by taking 20 fruits in four replications. Cultivars were classified according to “Классификатор рода Hippophae rhamnoides L.” (1993) as short (< 4 mm); medium (4–7 mm); long (> 7 mm).

Moreover, shoot prickliness was taken into account for male forms.

The results were statistically processed by one-way analysis of variance by software STATISTICA 6.0.

**Results and discussion.** In the examined plantations the forms without visual symptoms of wilt dominated. There occurred forms with wilt marks on separate shoots and all over the plants. Meanwhile the examination of wild sea buckthorn plantations on the territory of Kaliningrad region, bordering on Warmia and Mazury district, carried out by the Russian scientists in 1980’s did not detect plants with visual wilt symptoms (Мишунёна et al., 1982). We used forms with total absence of visual wilt symptoms for further research.

Describing Kaliningrad population of sea buckthorn some scientists (Мишунёна et al., 1982) noted high fruit bearing of the forms, on the contrary, other researchers (Трофимов, 1988) depict weak fruit bearing. Our research showed that the majority of forms were characterized by abundant fruit bearing (Table, Fig.). We used forms with yield graded 4-5 points for further study.

**Table. Characteristics of sea buckthorn forms selected from wild plantations in Olsztyn (2006)**

<table>
<thead>
<tr>
<th>Form Forma</th>
<th>Yield assessment (points)</th>
<th>Number of fruits in flower bud (pcs)</th>
<th>Fruit weight</th>
<th>Length of fruit stalk (cm)</th>
<th>Pull-out force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kortowo-1</td>
<td>5</td>
<td>5.4a</td>
<td>0.51b</td>
<td>5.7b</td>
<td>2.0d</td>
</tr>
<tr>
<td>Podarok sadu (clone)</td>
<td>5</td>
<td>4.2b</td>
<td>0.89a</td>
<td>5.6bg</td>
<td>2.1cd</td>
</tr>
<tr>
<td>Parkowa-1</td>
<td>4</td>
<td>3.4c</td>
<td>0.40c</td>
<td>3.8d</td>
<td>1.8e</td>
</tr>
<tr>
<td>Sportownaja-1</td>
<td>5</td>
<td>3.4c</td>
<td>0.36d</td>
<td>3.6d</td>
<td>2.5b</td>
</tr>
<tr>
<td>Shossejnaja-2</td>
<td>5</td>
<td>5.8a</td>
<td>0.35d</td>
<td>3.3a</td>
<td>2.6a</td>
</tr>
<tr>
<td>Shossejnaja-3</td>
<td>5</td>
<td>3.1c</td>
<td>0.36d</td>
<td>3.6d</td>
<td>2.5b</td>
</tr>
<tr>
<td>Shossejnaja-4</td>
<td>5</td>
<td>3.1c</td>
<td>0.35d</td>
<td>5.5bc</td>
<td>2.2c</td>
</tr>
<tr>
<td>LSD0.05</td>
<td></td>
<td>0.57</td>
<td>0.020</td>
<td>0.35</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Differences between forms on the number of fruit in a flower bud, mean fruit weight, productivity indicators were significant. Most of the selected forms had a number of fruit in a flower bud amounting to 3.0–3.4 with the exceptions of the form
‘Kortowo-1’, which had this index 1.6–1.8 times higher, and ‘Podarok sadu’. It is known that forms from Kaliningrad population of the Baltic climate have less fruits in a fruit bud compared to forms from the Siberian climate (Trofimov, 1988). According to V. T. Kondrashov (1980), number of fruit in a flower bud is not higher than 4. Our results confirm this conclusion.

Fruit weight is one of the most important economic indexes, which is the most reliable indicator for selection of promising seedlings, along with the number of flower buds. Literature references prove that sea buckthorn of the Baltic climate has small size of fruit. According to the Russian scientists, mean fruit weight accounts for 0.1–0.3 g and does not exceed 0.6 g for the most promising selections (Кондрашов, 1980; Шпитаейная, 2006). Our results are in agreements with this opinion. All forms but ‘Kortowo-1’ (middle fruit) and clone of cultivar ‘Podarok sadu’ (large fruit) were characterized as small-fruited. The indicator was probably influenced by unfavorable weather conditions (water deficiency and high temperature) in July when the fruits were formed.

Special characteristics of sea buckthorn is absence of ablative layer between fruit stalk and shoot. It is supposed that this feature was formed as a result of natural selection of the forms that kept fruit on shoots till spring (Пантеевеева, Гунин, 2003). Many forms have fruit stalk that is torn with pieces of skin and it leads to juice loss. This is a significant disadvantage of sea buckthorn, hence, solving this problem is one of the directions in breeding programs. Study of the type of pull-out force allowed to select 7 forms with dry tearing from wild plantations. Dry tearing of fruit was characteristic for the clone of ‘Podarok sadu’.

Literature references indicate wide diversity of wild sea-buckthorn forms by
the length of fruit stalk. In particular, this indicator varies from 0.9 to 4.2 mm for Kaliningrad populations, and it is stable in various paedoclimatic regions (Kondrashov, 1980). We have also determined significant differences on fruit stalk length between the forms. Thus, the best form ‘Shossejnaja-3’ had fruit stalk 2.78 times longer than the fruit stalk of the worst form ‘Shossejnaja-1’.

The important criterion for selection of initial forms is a value of pull-out force. Samples with pull-out force less than 1.5 N are suitable for machine harvesting by vibration samples (Habarov, Bartenev, 2002). Pull-out force values for selected forms were significantly different. No forms were discovered with low values. The forms ‘Kortowo-1’ and ‘Parkowaja’ had middle value of the indicator. The other forms were strong pull-out forms. The form ‘Shossejnaja-2’ was significantly worse by this index.

Since sea buckthorn is a diclinous plant, the success in breeding depends on male forms as well. The male plants were studied and selected along with the evaluation and selection of female forms. On the territory of Krynica Morska a winterhardy form with the absence of visual fading symptoms and low degree of prickliness was selected. All selected forms will be used in further breeding research.

**Conclusions.** Sea buckthorn forms from wild plantations in the north-east of Poland differ significantly by the complex of economic and biological features. 1 male and 7 female forms were selected as initial material for breeding. All selected female forms were characterized by the absence of visual wilt symptoms, high productivity and dry tearing-off of fruits. The form ‘Shossejnaja-3’ was characterized by the maximum fruit stalk length (6.4 mm), whereas the form ‘Parkovaja’ had the minimum value of the pull-out force (1.8 N). A winterhardy male form with the absence of visual fading symptoms low degree of prickliness was selected.

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Viena iš svarbiausių dygliuotojo šaltalankio veisimo programos problemų Baltarusijoje yra vytuliui atsparių veislių, tinkamų automatiniam derliaus nuėmimui, išvedimas. Siekiant įvesti naujas tėvines formas į veislų kūrimo procesą, 2006 metais buvo ištirtos laukinių dygliuotųjų šaltalankių plantacijos Pietų Varmijos (Olštinas, Lenkija) teritorijoje. Pirmiausia buvo pastebėtas akivaizdus vytulio simptomų nebuvas. Be to, atsižvelgta į šiuos tinkamumo automatiniam derliaus nuėmimui kriterijus: traukimo jėgos pobūdį, burbuolės tankumą, žiedkočio ilgį. Stebėjimai leido atrinkti 7 moteriškas formas, 1 vyrišką ir veislės ‘Podarok sadu’ kloną. Vytulio simptomų nebuvo nė vienoje atrinktoje formoje. Žiedkočio ilgis buvo nuo 2,3 iki 6,4 mm, traukimo jėga – nuo 1,8 iki 3,0 H, uogų skaičius iš vieno žiedpumpurio – nuo 3,0 iki 5,8. Bendras visų formų, išskyrus ‘Kortovo-1’ ir veislės ‘Podarok sadu’ kloną, trūkumas buvo jų mažos uogos (iki 0,4 g).

Reikšminiai žodžiai: atsparumas vytuliui, dygliuotas šaltalankis, genofondas, tinkamumas automatiniam derliaus nuėmimui.
Several strawberry cultivar trials have been conducted in Pūre HRS (Latvia) since 1997. 46 newly introduced cultivars and hybrids were tested in total. The phenological and pomological properties were studied. Studies were carried out on open field using sustainable cultivation systems. In the trial of 1997–1998 the best results were obtained with cultivars ‘Induka’ and ‘Suurpriz Olimpiade’. These cultivars were productive, of good fruit quality, good winterhardiness and satisfactory resistance to diseases. Cultivars ‘Korona’ and ‘Holiday’ showed also good fruit quality. In the trial of 1999–2001 the best results were obtained with cultivars ‘Jonok’, ‘Eldorado’, ‘Bounty’, ‘Pegasus’ and ‘Pandora’. Cultivar ‘Pandora’ because of very late production time and good size of fruits roused special interest. In the trial of 2001–2003 the best results showed cultivar ‘Polka’, which had better productivity and fruit quality than standard cultivars. Cultivar ‘Marmolada’ had also good fruit quality, but its winterhardiness under our conditions was rather low and productivity poor. In the trial established in 2004, after two years of investigations the highest yield with good quality was obtained from hybrid 40-1 of Pūre HRS breeding.

**Key words:** *Fragaria × ananassa*, resistance to diseases, ripening time, yield, quality, winterhardiness.

**Introduction.** Strawberry today is the most popular commercial berry crop in Latvia. In 1996 there were only about 500 ha of strawberries in Latvia, and today it is more than 1000 ha. The most of production is used for fresh local markets. The old cultivars are not more in line with present market demands. Therefore, growers are looking for new, better cultivars. Productivity, high quality of berries and resistance to diseases and pests are the main properties for acceptance. Winterhardiness is also important under Latvian conditions. Due to increasing of competition among growers, there is increased interest in extending of production season while using cultivars with very early and late production time.

Research work with strawberries, including evaluation and introduction of new foreign cultivars in Latvia, is concentrated at the Pūre Horticultural Research Station (Pūre HRS) in collaboration with Pūre Horticultural Research Centre. These studies summarize research results evaluating strawberry cultivars during the last 10 years.

**Materials and methods.** The studies described here were carried out at the Pūre Horticultural Research Station since 1997 to 2006. Four strawberry cultivar trials were established during this time. The first trial was established in the autumn of 1995.
Evaluation was done in 1997–1998. The trial, according to cultivar ripening time, was arranged in two blocks with 8 cultivars in each and including two standard cultivars. Second trial was established in the spring of 1998. Evaluation was done in 1999–2001. 12 cultivars were tested including two standards. Third trial was established in the autumn of 1999. Evaluation was done in 2001–2003. 12 cultivars and one hybrid (from Poland) including two standard cultivars were tested. Fourth trial was established in the spring of 2004. Evaluation was done in 2005–2006. 11 cultivars and two hybrids of Pure HRS breeding including two standard cultivars were tested. In all trials widely grown cultivars ‘Zefyr’ (early) and ‘Senga Sengana’ (medium late) were used as standards.

In all trials plants were planted in single rows at spacing of 30 × 100 cm. The matted rows of 20 cm wide were formed. Sustainable cultural practices were applied, without mulching and drip irrigation and with low input of chemicals. A completely randomised block design with four replicates and thirty plants per plot was used.

Characteristics evaluated were as follows: course of phenophases, winterhardiness, total yield and amount of 1st class, 2nd class and unmarketable berries, berry size (by weighting of 50 fruits at each picking time), fruit sensory evaluation (flavour, firmness and appearance), susceptibility to grey mould (Botryotinia fuckeliana) (by weighting of damaged fruits at each picking time) and leaf diseases – white leaf spot (Mycosphaerella fragariae) and leaf scorch (Diplocarpon earlianum). To note the cultivar earliness the index of earliness was calculated (Bite et al., 1997). Winterhardiness was evaluated visually in spring, using scale 0–5 (where 0 – no visual winter injury, and 5 – totally injured/dead plants). Fruit sensory evaluation was done using scale from 1–5, where 1 – the lowest evaluation, and 5 – the highest positive evaluation. The susceptibility to leaf spot diseases weas evaluated visually after fruit harvesting using scale 0–5 (where 0 – no visual damages, and 5 – totally injured leaf surface).

The data were subjected to statistical analysis (probability 95%). Duncan’s multiple range test was used to separate the means.

Results. Trial 1 (1997–1998). In this trial the earliest production time was observed for cultivar ‘Darunok Vchiteliu’ (Table 1). Cultivars ‘Roxana’, ‘Desnanka’ and ‘Festivalnaya Romascha’ had also early production time, similar to standard cultivar ‘Zefyr’. The later production time than standard cultivar ‘Senga Sengana’ had only cultivars ‘Kokinskaya Pozdnaya’ and ‘Bogota’. Other cultivars were of medium ripening time.

Cultivar ‘Induka’ was the most productive among the tested cultivars, and the lowest marketable yield was obtained from cultivar ‘Darunok Vchiteliu’, which had also the lowest fruit weight (Table 1). Cultivars ‘Desnanka’, ‘Korona’, ‘Holiday’, ‘Taganka’, ‘Syurpriz Olimpiadi’ and ‘Kokinskaya Pozdnaya’ showed similar productivity to standards. Cultivar ‘Kokinskaya Pozdnaya’ had the highest percentage of the 1st class fruits and the highest average fruit weight, while the fruits were soft and with average flavour. Cultivar ‘Elsanta’ also had high percentage of the 1st class fruits and good fruit quality, while its yield was low. In the fruit sensory evaluation cultivars ‘Korona’ and ‘Bogota’ had the highest score for fruit flavour, and ‘Roxana’ – the lowest.

Table 1. Index of earliness, marketable yield, amount of 1st class berries,
berry size and flavour of strawberry cultivars. The average of two production years (1997–1998)

On the average during two testing years cultivars ‘Induka’, ‘Syurpriz Olimpiade’ and ‘Taganka’ had the lowest winter damages, which were similar to standard cultivars (Table 2). Cultivars ‘Bogota’, ‘Elsanta’, ‘Darunok Vchiteliu’ and ‘Gaja’ had the highest winter damages.

The spreading of leaf spots during testing years was quite low. White leaf spot was more spread than leaf scorch. Cultivar ‘Darunok Vchiteliu’ had the lowest damage by white leaf spots; ‘Korona’ and ‘Bogota’ were the most susceptible (Table 2). Cultivars ‘Induka’ and ‘Senga Sengana’ had the lowest damage by leaf scorch; ‘Festivalnaya Romascha’ and ‘Gaja’ were the most susceptible. Cultivars ‘Bogota’ and ‘Elsanta’ showed the highest resistance to grey mold; cultivar ‘Kokinskaya Pozdnaya’ was the most susceptible.

Table 2. Winterhardiness and resistance to diseases of strawberry cultivars.
The average of two testing years (1997–1998)

Trial 2 (1999–2001). In this trial none of tested cultivars were of earlier production time than early standard cultivar ‘Zefyr’ (Table 3). Cultivar ‘Jonsok’ had similar time of production beginning as ‘Zefyr’, but, according to index of earliness, it was later. Cultivars ‘Bounty’, ‘Pegasus’, ‘Laura’ and ‘Pandora’ had later production time than standard cultivar ‘Senga Sengana’. Cultivars ‘Laura’ and ‘Pandora’ had especially late production time.

None of tested cultivars had significantly higher marketable yield than standard cultivars, according to average of three production years (Table 3). ‘Jonsok’ and ‘Bounty’ were the most productive among tested. Rather good yield with high amount of the 1st class fruits and big average fruit weight was obtained also from cultivars ‘Pandora’ and ‘Pegasus’. The highest percentage of the 1st class fruits was obtained from standard cultivar ‘Zefyr’ and ‘Eldorado’. ‘Eldorado’ also had the highest average fruit weight among the tested cultivars. Cultivar ‘Laura’ had big berries with very good firmness, but many of them were unmarketable because of misshapes. According to the fruit sensory evaluation, the highest score for fruit flavour had cultivar ‘Pegasus’, and ‘Laura’ – the lowest.
Table 3. Index of earliness, marketable yield, amount of 1st class berries, berry size and flavour of strawberry cultivars. The average of three production years (1999–2001)

According to the average of three testing years, only cultivar ‘Jonsok’ had similar winterhardiness to standard cultivars (Table 4). Other cultivars had significantly higher score for winter damages than standards.

The spreading of leaf spot during testing years was quite low, but leaf spot disease was more spread than leaf scorch. Cultivars ‘Lambada’ and ‘Pegasas’ had the lowest damage by white leaf spot, and ‘Senga Sengana’ was the most susceptible (Table 4). Cultivar ‘Laura’ had the lowest damage by leaf scorch, and ‘Jonsok’ was the most susceptible. There was high incidence of grey mold in 2001, when the amount of damaged fruits varied from 11 to 31% from total yield depending on cultivar. According to the average of three production years, cultivars ‘Zefyr’, ‘Jonsok’, ‘Honeoye’, ‘Emily’ and ‘Lambada’ had the lowest damage, ‘Pandora’ and ‘Laura’ – the highest.

Table 4. Winterhardiness and resistance to diseases of strawberry cultivars.
The average of three testing years (1999–2001)

This trial only cultivar ‘Lvovskaya Rannaya’ showed earlier production time than standard cultivar ‘Zefyr’ (Table 5). Cultivar ‘Lihamma’ had similar production time to ‘Zefyr’. Cultivars ‘Marmolada’ and ‘Rhapsody’ were the latest. Other cultivars according to index of earliness were of medium ripening time.

The yield in this trial was quite low. According to the average of three production years, only cultivar ‘Polka’ had significantly higher marketable yield than standard cultivars (Table 5). Extremely low yield was obtained from cultivars ‘Lovovskaya Rannaya’, ‘Marmolada’, ‘Bargerglow’, ‘Rhapsody’ and ‘Lihamma’. Cultivars ‘Gerida’, ‘Polka’ and hybrid LPR-805-4 had the highest percent of the 1st class fruits, which was similar to standard cultivar ‘Zefyr’. Cultivar ‘Lvovskaya Rannaya’ had the lowest amount of the 1st class fruits with the lowest average fruit weight. ‘Marmolada’ had the highest average fruit weight with the best firmness among the tested cultivars. Good fruit size was observed also for hybrid LPR-805-4 and cultivar ‘Gerida’. Cultivar ‘Polka’ had the highest score for fruit flavour, while ‘Rhapsody’, ‘Bargerglow’ and LPR-805-4 – the lowest.

According to the average of three testing years, none of tested cultivars had higher winterhardiness than standard cultivar ‘Zefyr’ (Table 6). Only cultivar ‘Bargerglow’ had similar winterhardiness to ‘Zefyr’. Cultivars ‘Rhapsody’, ‘Gerida’ and ‘Marmolada’ were the most damaged during winter.
Table 5. Index of earliness, marketable yield, amount of 1st class berries, berry size and flavour of strawberry cultivars. The average of three production years (2001–2003)

The spreading of leaf spot during testing years was very low. Cultivars ‘Bargerglow’ and ‘Lvovskaya Rannaya’ had the lowest damage by white leaf spot, while ‘Senga Sengana’ and LPR-805-4 were the most susceptible (Table 6). Cultivars ‘Polka’, ‘Lhamma’, ‘Lvovskaya Rannaya’ and ‘Gerida’ had the lowest damage by leaf scorch, while ‘Syriusz’ was the most susceptible. There was high incidence of grey mold in the trial, especially in 2001. According to the average of three production years, cultivars ‘Zefyr’ and ‘Lvovskaya Rannaya’ had the lowest damage, while ‘Marmolada’ and ‘Rhapsody’ – the highest.

Table 6. Winterhardiness and resistance to diseases of strawberry cultivars.
After two years of investigations, according to index of earliness, none of tested cultivars showed earlier production time as ‘Zefyr’. Cultivar ‘Oka’ had similar production time to ‘Zefyr’ (Table 7). Cultivars ‘Alice’, ‘Florence’, ‘Sophie’ and hybrid 40-1 (Pure HRS breeding) had later production time than standard cultivar ‘Senga Sengana’ and cultivar ‘Sophie’ was characterized by very late production time among them.

Very good winterhardiness was observed for cultivar ‘Oka’. Cultivar ‘Dange’ and hybrids 38-3 and 40-1 showed similar winterhardiness to standards (Table 7). Cultivars ‘Florence’ and ‘Alice’ were the most damaged during winter.

According to the average of two production years, only hybrid 40-1 had significantly higher marketable yield than standard cultivars (Table 7). Especially low yield was obtained from cultivar ‘Aura’, which had also low amount of E and 1st class fruits (data not shown). ‘Oka’ had the highest average fruit weight among the tested cultivars, but its fruits were of low firmness. Good fruit size was observed also for cultivars ‘Rosie’, ‘Alice’, ‘Florence’ and hybrid 38-3. According to the fruit sensory evaluation, cultivar ‘Sophie’ had the highest score, while ‘Aura’ – the lowest (data not shown).

The incidence of grey mold was low in years 2005 and 2006. According to the average of two production seasons, cultivars ‘Zefyr’, ‘Aura’, ‘Rosie’, ‘Florence’ and hybrid 40-1 had the lowest damage, while ‘Oka’ and ‘Feiervé’ – the highest one (Table 7).
Table 7. Index of earliness, winterhardiness, marketable yield, berry size and grey mold damage of strawberry cultivars. The average of two production years (2005–2006)


<table>
<thead>
<tr>
<th>Cultivar/ Vydis</th>
<th>Index of earliness</th>
<th>Winter damage*</th>
<th>Marketable yield</th>
<th>Average fruit weight</th>
<th>Grey mold kekerinio puvinio pažeidimai (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Oka'</td>
<td>183 a**</td>
<td>0.7 a</td>
<td>3.1 abcd</td>
<td>14.3 e</td>
<td>2.9 b</td>
</tr>
<tr>
<td>'Zefyr'</td>
<td>183 a</td>
<td>1.3 b</td>
<td>5.3 ef</td>
<td>8.9 b</td>
<td>0.6 a</td>
</tr>
<tr>
<td>'Aura'</td>
<td>184 b</td>
<td>1.9 c</td>
<td>1.2 a</td>
<td>7.4 a</td>
<td>0.6 a</td>
</tr>
<tr>
<td>'Rosié'</td>
<td>184 b</td>
<td>2.4 d</td>
<td>4.2 bdef</td>
<td>10.9 cd</td>
<td>0.6 a</td>
</tr>
<tr>
<td>'Real'</td>
<td>185 c</td>
<td>1.9 c</td>
<td>5.6 f</td>
<td>9.3 b</td>
<td>1.1 ab</td>
</tr>
<tr>
<td>'38-5'</td>
<td>187 d</td>
<td>1.7 bc</td>
<td>3.2 abcd</td>
<td>10.8 cd</td>
<td>1.1 ab</td>
</tr>
<tr>
<td>'Feierverk'</td>
<td>187 d</td>
<td>1.9 c</td>
<td>2.6 abc</td>
<td>9.3 b</td>
<td>2.7 b</td>
</tr>
<tr>
<td>'Dange'</td>
<td>188 de</td>
<td>1.2 b</td>
<td>4.6 cdef</td>
<td>8.9 b</td>
<td>1.0 ab</td>
</tr>
<tr>
<td>'Sengas Sengana'</td>
<td>189 e</td>
<td>1.3 b</td>
<td>3.4 bode</td>
<td>7.5 a</td>
<td>1.8 ab</td>
</tr>
<tr>
<td>'Alice'</td>
<td>190 f</td>
<td>2.9 ef</td>
<td>4.8 def</td>
<td>11.6 d</td>
<td>1.4 ab</td>
</tr>
<tr>
<td>'40-1'</td>
<td>191 f</td>
<td>1.3 b</td>
<td>8.9 g</td>
<td>9.1 b</td>
<td>0.7 a</td>
</tr>
<tr>
<td>'Florencs'</td>
<td>193 g</td>
<td>3.2 f</td>
<td>3.1 abcd</td>
<td>10.6 c</td>
<td>0.6 a</td>
</tr>
<tr>
<td>'Sophie'</td>
<td>197 h</td>
<td>2.6 de</td>
<td>2.4 ab</td>
<td>8.6 b</td>
<td>1.6 ab</td>
</tr>
</tbody>
</table>

* – evaluation given in scores, where 5 being the best.
** – values in a column followed by the same letter do not differ significantly according to Duncan’s multiple range test (P = 0.05).
*** – values in a column followed by the same letter do not differ significantly according to Duncan’s multiple range test (P = 0.05).

Discussion. The evaluated cultivars and hybrids significantly differed in production time, productivity, yield, fruit quality and resistance to diseases.

Latvian strawberry growers are interested in good, very early and late ripening cultivars for prolonging of production season and getting higher price of berries (Bite et al., 1997). Early standard cultivar ‘Zefyr’ is still dominant among the earliest in Latvia (Laugale, 2004). It has good winterhardiness and quality of berries, but the yield is not stable and fluctuates among years. In our trials only cultivars ‘Lvovskaya Rannaya’ and ‘Darunok Vchiteliu’ showed earlier production time than ‘Zefyr’. Unfortunately, they had lower winterhardiness, yield and smaller berries than ‘Zefyr’. It agrees with observations of Stanislavjević et al. (1997) that cultivars with somewhat earlier fruit ripening have smaller yields. Cultivars ‘Roxana’, ‘Desnanka’, ‘Festivalnaya Romascha’, ‘Lihamma’ and ‘Oka’ had similar production time to ‘Zefyr’, but most of them had lower yield and poorer fruit quality than ‘Zefyr’. Only cultivar ‘Desnanka’ showed similar productivity, but fruits were smaller and softer than these of ‘Zefyr’. Cultivar ‘Jonsok’ had similar beginning of production as ‘Zefyr’, but, according to the index of earliness, it was later, because of slower production rate. It had similar yield and fruit size, but lower evaluation of fruit flavour.

Some of the tested cultivars had late and very late ripening time. Ripening time of cultivars ‘Bogota’, ‘Laura’, ‘Pandora’, and ‘Sophie’ was especially late. Unfortunately, only cultivar ‘Pandora’ had productivity similar to standard cultivars and good fruit quality. Cultivar ‘Bogota’ was characterized by high percentage of the 1st class berries
and good fruit evaluation, while the yield was low, because of low winterhardiness. Cultivar ‘Sophie’ had productivity similar to ‘Senga Sengana’ and better fruit size and fruit sensory evaluation, but low winterhardiness.

The most of tested cultivars were of medium ripening time. Cultivars ‘Induka’, ‘Syurpriz Olimpiadi’, ‘Korona’, ‘Holiday’, ‘Eldorado’, ‘Polka’ and ‘Real’ showed the best results of them.

Latvian climate is characterised by humid and cold winters with snow covering, though quite often there are thaws and glazed frosts, which have negative influence on strawberry wintering (Laugale, 2000). In our trials all of tested cultivars were more or less damaged during winters. Only cultivar ‘Oka’ showed higher winterhardiness than the standard cultivar ‘Zefyr’, which has good winterhardiness under Latvian conditions. Cultivars ‘Bargeglow’ and ‘Induka’ had similar winterhardiness to ‘Zefyr’ and higher than standard cultivar ‘Senga Sengana’, which is mentioned as medium winterhardy (Nes, 1997). According to Haffner and Vesterheim (1997), winter injury affects the plant’s further productivity. It agrees also with our investigations that most cultivars with higher winter injuries had low productivity.

Botrytis rot is one of the most important diseases of strawberry, reducing yield and quality before and after harvest (Berrie et al., 2000). There was observed high infection by Botrytis rot in 2001, especially on late ripening cultivars, because of abundant precipitation during harvesting. The early cultivars suffered less. Cultivars ‘Korona’, ‘Jaune’, ‘Bogota’, ‘Elsanta’, ‘Jonsok’, ‘Emily’, ‘Honeoye’, ‘Lambada’, ‘Wega’, ‘Lvovskaya Rannaya’ and ‘Gerida’ had lower susceptibility to grey mould than standard cultivar ‘Senga Sengana’, which is known as susceptible to grey mould (Zurawicz and Daubeny, 1995). In contrary, in 2005 and 2006 there was draught during harvesting and the grey mould infection in Trial 4 was low.

Leaf spot diseases were widespread in all plantings, but they did not cause severe damage. The damage severity increased with the age of planting. The white leaf spot were usually more widespread than leaf scorch. White leaf spot is of more importance according to other investigations too (Delhomez et al., 1995; Schmid et al., 2004). Cultivars ‘Darunok Vchitelu’, ‘Taganka’, ‘Gaja’, ‘Elsanta’, ‘Lambada’, ‘Pegasus’, ‘Bargerglow’ and ‘Lvovskaya Rannaya’ were the most resistant to this disease in our trials.

Conclusions. From the tested cultivars the following were selected as the most appropriate for growing under Latvian conditions in commercial plantings: ‘Jonsok’ from early cultivars, ‘Induka’, ‘Syurpriz Olimpiadi’, ‘Korona’, ‘Holiday’, ‘Eldorado’, ‘Polka’ from cultivars with medium ripening time and ‘Bounty’, ‘Pegasus’, ‘Pandora’ from cultivars with late and very late ripening time.

Moreover, the following strawberry cultivars were selected for breeding as donors of certain characteristics: ‘Darunok Vchitelu’ and ‘Lvovskaya Rannaya’ – for earliness and resistance to leaf spot, ‘Bogota’ – for lateness, fruit size, flavour and resistance to grey mould, ‘Laura’ – for lateness and fruit firmness, ‘Kokinskaya Pozdnaya’ – for lateness and fruit size, ‘Marmolada’ – for fruit quality and resistance to leaf diseases, ‘Elsanta’ – for fruit quality and resistance to white leaf spot and grey mould.

In the newest trial after two years of investigations hybrid 40-1 of Pūre HRS
breeding had shown the best results. The evaluation of this trial will be continued next year.

Gauta 2007 06
Parengta spausdinti 2007 06

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SODININKYSTĖ IR DARŽININKYSTĖ. MOKSLO DARBAI. 2007. 26(3).

BRAŠKIŲ VEISLIŲ TYRIMAI PŪRĖS (LATVIJA) SODO AUGALŲ TYRIMO STOTYJE PER PASTARUOSIUS 10 METŲ
V. Laugale, L. Lepse

Santrauka


Reikšminiai žodžiai: atsparumas ligoms, atsparumas žiemai, derlius, Fragaria × ananassa, kokybė, sunokimo laikas.
Pūre Horticultural Research Station’s black currant collection includes more than 130 accessions. Every year the collection is supplemented with new cultivars and selections of different origin. The preliminary evaluation of accessions is carried out to clarify the suitability of accessions for using in breeding and commercial growing under Latvian conditions. Phenological traits, winterhardiness, yield, fruit quality, and resistance to diseases and gall mite of 30 accessions were evaluated in 2002–2005. Many cultivars were found to be of commercial and breeding importance. Cultivars ‘Laimiai’, ‘Kupoliniai’, ‘Orlovskii Vals’ were the most productive among those tested. ‘Gagatai’, ‘Laimiai’, ‘Azhurnaya’ and ‘Ekzotika’ had the largest berries. Very good taste was observed for cultivars ‘Kupolinaiai’, ‘Zelonaya Dimka’ and hybrid 79-204-2. Cultivars ‘Hedda’, ‘Joniniai’, ‘Gagatai’, ‘Shiryayevskaya’, ‘Lentyai’, ‘Ryasnaya’ were the most winterhardy. Cultivars ‘Bogatyr’, ‘Joniniai’, ‘Ryasnaya’, ‘Azhurnaya’ had the lowest infection by leaf spots. Cultivars ‘Storklas’, ‘Hedda’, ‘Bagira’, ‘Azhurnaya’, ‘Ekzotika’ and hybrid 79-204-2 showed the highest resistance to mildew. Cultivars ‘Bogatyr’, ‘Gagatai’, ‘Laimiai’ and ‘Strata’ had the highest resistance to gall mite.

Key words: bush habit, fruit quality, phenology, Ribes nigrum L., yield, resistance, winterhardiness.

Introduction. Black currant is one of the most widely grown berry crops in Latvia. The fruits are used for fresh market and processing. Growers are interested in high yielding cultivars with good fruit quality and resistance to pests and diseases. The winterhardiness is also important under Latvian conditions. Latvian winters are characterised by sharp temperature fluctuations. Under such conditions cultivars originated in Western Europe and in the continental climate of Russia often are seriously cold damaged during winter. This demonstrates the necessity for a perusal evaluating of cultivars and selections before recommending them to the growers or using in breeding. The collection of black currants in Pūre Horticultural Research Station (Pūre HRS) has more than 130 accessions from throughout the world, but mainly from countries of the former Soviet Union territory. Every year the collection is supplemented with new cultivars and selections of different origin. This study was conducted to obtain preliminary information about some newly obtained accessions in the collection and to determine their possible usefulness for fruit production under local conditions and breeding.

Materials and methods. 30 cultivars were evaluated in the Pūre HRS field
collection in 2002–2005. Plants were planted in the spring and autumn of 1999 in rows with spacing 2.5 m between rows and 1.0 m between plants in row. Three bushes of each accession were used for testing. ‘Glebovskaya’ (in spring planting) and ‘Seyanets Golubki’, ‘Shiryayevskaya’ (in autumn planting) were included as control cultivars.

Weeds were controlled mechanically between rows and by hand weeding and herbicides in rows. Plants were pruned by hand each spring. No chemical treatments for pest and disease control were applied.

In this study we estimated the following: phonological observations, winterhardiness (ratings range from 1 to 5, where 1 – very low winterhardiness, 5 – very high winterhardiness), total yield, average fruit weight, fruit appearance and flavour (ranged from 1 to 5, where 1 – very bad, 5 – very good), skin thickness (ranged from 1 to 3, where 1 – thin, 2 – medium thick, 3 – very thick), resistance to gall mite and diseases: leaf spots and American powdery mildew (visually on scale 0 to 5, where 5 – severe symptoms and 0 – no symptoms). The severity of diseases was assessed at the end of summer, when higher level of infection was observed, but gall mite damage – in early spring. Buds infected with gall mite were removed by hand in early spring. During period of dormancy the bush habit was evaluated as erect, medium erect or broad.


**Table 1.** Winterhardiness of evaluated black currant accessions

<table>
<thead>
<tr>
<th>Good winterhardiness (low damage)</th>
<th>Medium winterhardiness (medium damage)</th>
<th>Low winterhardiness (high damage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geras atsparumas (maziai pažeidimą)</td>
<td>Vidutinio atsparumo (pasakų pažeidimų)</td>
<td>Stipresnių atsparumo (daugiau pažeidimų)</td>
</tr>
</tbody>
</table>
period, which enables better organising of harvesting and higher income (Pluta and Hummer, 1996). The evaluated accessions differed at the beginning of vegetation, flowering and ripening time. They were either early, or medium or late. Phenological phases, especially the beginning of flowering, were strongly influenced by weather conditions and differed between years. ‘Seyanets Golubki’, and ‘Iyunskaya Kondrashovoi’ had the earliest bud burst, but ‘Silvergieters Schwarze’ and ‘Rosenthal Langtraubige’ – the latest (data not shown). ‘Seyanets Golubki’ and ‘Kriviai’ were characterized by the earliest beginning of flowering, while ‘Bogatyr’, ‘Ben More’ and ‘Strata’ – by the latest between the tested accessions (data not shown). Cultivars ‘Seyanets Golubki’ and ‘Iyunskaya Kondrashovoi’ were the earliest in ripening time and ‘Ben More’, ‘Stor Klas’ and ‘Lentyai’ were the latest (Table 2). The late production time of cultivars ‘Ben More’ and ‘Storklas’ is also accented in other investigations (Stanislavljevic et al., 2002; Trajkovski et al., 2000). Most cultivars were of medium flowering and ripening time.

Yield. The yield is very important trait, which determines the profit of a blackcurrant plantation. Productivity is determined by genetic factors, especially by degree of self-fertility, and also by environmental factors, e.g. weather during differentiation of flowers and flowering, growing conditions of plants etc. (Wenn, 1978). According to investigations of Pluta (1994), yield has a high heritability coefficient in black currants and it is important for selecting of genotypes for breeding. In our collection black currant yield was quite low and significantly varied between years and accessions. The lowest average yield was harvested in 2003, while the highest – in 2002 (data not shown). In 2003, during black currant flowering time spring frosts (up to -4°C) and drought were observed. That caused the premature drop of flowers. Strong spring frosts during flowering time were observed also in 2004 (up to -6°C). Some accessions strongly suffered in winter of 2004/2005, which was characterized by sharp fluctuation of temperature and very cold beginning of March. It reduced yield in 2005. It must be taken in consideration that any fungicide and insecticide were not used in the planting. It caused the spread of diseases and pests and reduced yield. According to average of all testing years, hybrid 79-204-2 and cultivars ‘Ben Sarek’ and ‘Storklas’ were the most productive in the planting of the spring of 1999 (Table 2). Lithuanian cultivars ‘Laimiai’, ‘Kupoliniai’, ‘Kriviai’ and Russian ‘Orlovskii Vals’ were the most productive in the planting of the autumn of 1999. In trial in Poland cultivar ‘Joniniai’ was the most productive and ‘Laimiai’ also had good yield (Kawecki et al., 2000). In our collection ‘Joniniai’ gave lower yield than ‘Laimiai’.

The demand for cultivars that produce a maximum yield in their first years of life has increased in recent years, because of the trend to reduce the period of plot exploitation (Kuminov, 1996). Cultivars ‘Kupoliniai’ and ‘Gagatai’ and hybrid 79-204-2 showed the highest yielding in the first production years.

Table 2. Black currant yield and fruit evaluation in Pūre HRS collection (average of 2002–2005)

<table>
<thead>
<tr>
<th>Accession</th>
<th>Yield 2002</th>
<th>Yield 2003</th>
<th>Yield 2004</th>
<th>Yield 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Seyanets Golubki'</td>
<td>5.6</td>
<td>4.2</td>
<td>6.0</td>
<td>3.8</td>
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<tr>
<td>'Iyunskaya Kondrashovoi'</td>
<td>5.2</td>
<td>3.8</td>
<td>5.4</td>
<td>4.0</td>
</tr>
<tr>
<td>'Silvergieters Schwarze'</td>
<td>4.8</td>
<td>3.6</td>
<td>5.0</td>
<td>3.2</td>
</tr>
<tr>
<td>'Rosenthal Langtraubige'</td>
<td>4.4</td>
<td>3.2</td>
<td>4.6</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Table 2. Pūre STS kolekcijos juodųjų serbentų derlius ir uogų įvertinimas

<table>
<thead>
<tr>
<th>Accession</th>
<th>Uogų derlingumo klasė</th>
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<tbody>
<tr>
<td>'Seyanets Golubki'</td>
<td>8.0</td>
</tr>
<tr>
<td>'Iyunskaya Kondrashovoi'</td>
<td>7.5</td>
</tr>
<tr>
<td>'Silvergieters Schwarze'</td>
<td>6.5</td>
</tr>
<tr>
<td>'Rosenthal Langtraubige'</td>
<td>6.0</td>
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95
(2002–2005 m. vidurkiai)

**Table 2 continued**

<table>
<thead>
<tr>
<th>Accession</th>
<th>Ripeing time</th>
<th>Average yield, kg per bush</th>
<th>Average weight</th>
<th>Flavour</th>
<th>Skin thickness</th>
</tr>
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<tbody>
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</table>

<table>
<thead>
<tr>
<th>Accession</th>
<th>Ripeing time</th>
<th>Average yield, kg per bush</th>
<th>Average weight</th>
<th>Flavour</th>
<th>Skin thickness</th>
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</tbody>
</table>

*Note: Data from 1999 season.*
Fruit quality. Berry size is one of more important productivity components. Black currants are mostly grown for processing. The size of berries for processing, if harvested mechanically, is not a restrictive trait for variety distribution (Šikšnianas, 2001). Big and average-sized fruits are preferred by the processing industry. Cultivars with sizable berries are more convenient for manual harvesting and preferable for fresh market. Cultivars ‘Laimiai’, ‘Azhurnaya’, ‘Ekzotika’, ‘Gagatai’ and ‘Lentyai’ had the highest average fruit weight among the tested accessions (Table 2). Cultivars ‘Kupoliniai’, ‘Ben Sarek’, ‘Iynskaya Kondrashovoi’, ‘Joniniai’, ‘Orlovskii Vals’ and hybrid 79-204-2 had also average fruit weight above one g per berry. According to other investigations, cultivar ‘Lentyai’ has shown also the high berry weight in trial in Estonia (Libek and Kikas, 2002) and in Dobele PBHRS, Latvia (Kampuss, 2005), while cultivar ‘Laimiai’ – in Poland (Kawecki et al., 2000).

Recently, some European countries are increasingly interested in black currant cultivars with dessert fruit for fresh market. Berries for fresh consumption are expected to be of large size, good-looking, with good or very good taste and aroma (Trajkovski et al., 2000). Of our tested accessions, only hybrid 79-204-2 and cultivars ‘Joniniai’, ‘Laimiai’, ‘Bagira’, ‘Kupoliniai’ and ‘Lentyai’ had large berries with quite good flavour (Table 2). Cultivars ‘Joniniai’ and ‘Lentyai’ are marked also as potential donors in breeding for fresh consumption by Kampuss (2005). In organoleptic evaluation panel hybrid 79-204-2 and cultivars ‘Zelonaya Dimka’ and ‘Kupoliniai’ had the highest evaluation of fruit flavour, while cultivars ‘Storklas’ and ‘Ben Sarek’ – the lowest. Cultivars ‘Gagatai’, ‘Lentyai’ and ‘Azhurnaya’ had the highest scores for fruit appearance, while ‘Tinker’ – the lowest.

The thickness of fruit skin is important for mechanical harvesting of fruits and transportation, while thin skin is more preferable for fresh consumption. In our collection the thickest fruit skin were observed for cultivar ‘Stor Klas’, and the thinnest

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<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>‘Gagatai’</td>
<td>medium</td>
<td>1.01 ± 0.66</td>
<td>1.32 ± 0.16</td>
<td>4.6</td>
<td>3.9</td>
<td>2.1</td>
<td></td>
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<tr>
<td>‘Delikates’</td>
<td>medium</td>
<td>0.10 ± 0.10</td>
<td>0.78 ± 0.21</td>
<td>3.6</td>
<td>3.8</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>‘Orlovskii Vals’</td>
<td>medium</td>
<td>1.22 ± 0.79</td>
<td>1.02 ± 0.25</td>
<td>4.3</td>
<td>3.8</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>‘Shiryaevskaya’</td>
<td>medium</td>
<td>0.38 ± 0.24</td>
<td>0.66 ± 0.14</td>
<td>3.9</td>
<td>3.7</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>‘Zelonaya Dimka’</td>
<td>medium</td>
<td>0.43 ± 0.32</td>
<td>0.88 ± 0.34</td>
<td>4.3</td>
<td>4.5</td>
<td>1.7</td>
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<tr>
<td>‘Moka’</td>
<td>medium</td>
<td>0.11 ± 0.07</td>
<td>0.65 ± 0.32</td>
<td>3.6</td>
<td>4.3</td>
<td>1.4</td>
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<tr>
<td>‘Strata’</td>
<td>medium</td>
<td>0.05 ± 0.05</td>
<td>0.83 ± 0.32</td>
<td>3.8</td>
<td>4.0</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>‘Chomii Zhenschag’</td>
<td>medium</td>
<td>0.16 ± 0.10</td>
<td>0.89 ± 0.14</td>
<td>4.3</td>
<td>3.7</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>‘Lentyai’</td>
<td>medium</td>
<td>0.96 ± 0.66</td>
<td>1.24 ± 0.17</td>
<td>4.7</td>
<td>4.4</td>
<td>1.7</td>
<td></td>
</tr>
</tbody>
</table>

* - ratings range from 1 to 5, where 1 is lowest positive evaluation and 5 – highest.
** - all ratings 1 to 5 (1 – very thin, 2 – thin, 3 – medium, 4 – thick).

Table 2. Biological characteristics of the tested black currant accessions.
– for cultivars ‘Moka’, ‘Seyanets Golubki’ and ‘Ekzotika’.

Resistance to diseases. Black currant cultivars were seriously infected by diseases. The following diseases were observed on currants: powdery mildew (*Sphaerotheca mors-uvae* (Shw.) Berk.) and leaf spots (*Pseudopeziza ribis* Kleb and *Septoria ribis* Desm.).

Powdery mildew is one of the most widespread diseases affecting black currants in Latvia (Bite and Laugale, 2002). It was the most common and damaging disease in our collection planting. It caused damage of leaves and shoots and reduced plant yield. The level of plant damage by powdery mildew varied between accessions and growing years. The highest infection for most of cultivars was observed in 2004, the lowest – in 2002. Powdery mildew symptoms in all testing years were not observed only on cultivar ‘Hedda’. ‘Hedda’ is described as free from mildew and one of predominant early black currant cultivars in Norway (Måge, 1993). The rating of accessions according to infection level you can see in the Table 3.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Low susceptibility (average score 0–1.0)</th>
<th>Medium susceptibility (average score 1.1–3.0)</th>
<th>High susceptibility (average score &gt; 3.0)</th>
</tr>
</thead>
</table>
Only cultivars ‘Bogatyr’, ‘Glebovskaya’, ‘Laimia’ and ‘Strata’ had no damaged buds, but ‘Ben More’, ‘Lentai’, ‘Orlovskii Vals’, ‘Storklas’, ‘Tinker’ and ‘Zelonaya Dimka’ had the highest damage rate. The most of accessions had only few damaged buds. According to other investigations, ‘Seyanets Golubki’ and ‘Bogatyr’ have shown low susceptibility to gall mite in Estonia (Libek and Kikas, 2002), but cultivar ‘Storklas’ had high damage in SLU- Balsgard, Sweden (Trajkovski et al., 2000).

**Bush habit.** Accessions had either erect, medium erect and broad bush habit. The rating of accessions according to bush habit are summarised in the Table 5.

<table>
<thead>
<tr>
<th>Bush habit of evaluated accessions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Erect</strong></td>
</tr>
<tr>
<td>Accessions</td>
</tr>
</tbody>
</table>

**Conclusions.** In total, cultivars ‘Ben Sarek’, ‘Bogatyr’, ‘Ekzotika’, ‘Kriviai’, ‘Azhurnaya’, ‘Joniniai’, ‘Laimia’ and ‘Bagira’ had the highest overall evaluation with the highest amount of desirable traits and were selected for further investigations.

The following black currant cultivars were selected as the most perspective for commercial growing: ‘Ben Sarek’, ‘Kriviai’ and ‘Joniniai’ and cultivars ‘Ekzotika’, ‘Joniniai’, ‘Kupoliniai’, ‘Bagira’, ‘Laimia’, ‘Lentai’ and hybrid 79-204-2- for fresh market:

The following blackcurrant genotypes were selected for breeding as donors of certain characteristics: winterhardiness – ‘Hedda’, ‘Ryasnaya’, ‘Joniniai’, ‘Bagira’,

**References**


Reikšminiai žodžiai: atsparumas žiemos šalčiams, derlius, fenologija, krūmo habitas, Ribes nigrum L., uogų kokybė.
The aim of the investigations carried out in the Central Russia was to study the inheritance of the most useful progeny features and find out strawberry genotypes promising as donors and sources of characteristics, which influence high yield and its quality, time of maturing, and high adaptability to climatic conditions. Cultivars are usually octoploids, and most features, including productivity, are inherited quantitatively. However, non-additive effects are important in the inheritance of yield components, especially of fruit size and inflorescence number. It means that some alleles or genes controlling one of the components reveal both dominance over each other and modifying effect towards the alleles or genes influencing opposed components. ‘Alpha’, ‘Estafeta’, and ‘Senga Sengana’ gave a significant part of high yielding seedlings; a few genotypes were sources of some yield components, but only ‘Alpha’ being used as a parent steadily passed feature of high productivity to progeny. Progenies of ‘Rubinovy Kulon’ from crosses with some cultivars had high percentage of large-fruited seedlings, but when the cultivar was intercrossed with two small-fruited testers, the genes controlling fruit size character revealed themselves only in the progeny of the tester with earlier time of ripening. Therefore, modifying genes may be present in the small-fruited clones. ‘Pandora’ is a donor of late flowering/ripening and male sterility of flowers linked to each other. The imperfection of flowers can be the cause of late flowering. If anthers are the place of synthesis of a flowering hormone and its quantity is dependent on pollen development, lower fertility of pollen and especially absence of stamens may lead to later flowering. It is found that ‘Sumas’ is possessed of a dominant gene controlling the highest level of frost tolerance among cultivars, and the cultivar is homozygous by the gene.

**Key words**: breeding, cross, donor, *Fragaria × ananassa* Duch., gene, parent, progeny.

**Introduction.** The garden strawberry (*Fragaria × ananassa* Duch.) has an advantage compared to many other horticultural crops associated with its octoploid status – the genomic constitution originally derived from different strawberry species (Bringhurst, 1990) and wider variability of characters allowing more chances to select genotypes having desirable trait sets. On the other hand, inheritance of them is usually complex. Many characters are inherited quantitatively when definite effects are summarized, while contribution of each additional gene may considerably differ. Seedlings often have traits intermediate between those of parent cultivars, although different ways can lead to the results. Inheritance of other important characters is much more complicated being dependent additionally on modifying genes, which reveal themselves as non-additive effects when combining abilities of parent cultivars are analyzed. This sometimes hampers the progress of obtaining selections with useful
characters in their highest degrees, which could meet current requirements of consumers and fruit producers. Occasions with simple inheritance of a character are extremely rare in the garden strawberry. It is frequently impossible to find out genetic control of characters completely, and any finding is important for successful breeding work. Cultivar and selection useful traits, significant part of which are inherited by their progenies, are most valuable because using them in crosses leads to quicker results. Most breeding programmes in the world are focused on extending the picking season in the early and late period, increasing fruit size and improving fruit quality (Faedi et al., 2002; Simpson, 2002). The same objectives are of high importance in our country. Also cold tolerance is one of the main aims as in other locations with severe climate conditions (Daubeny, 1990; Daugaard, 1998). Finding out genotypes, which could be used as donors and sources of useful characters influencing yield height and quality, time of maturing, high adaptability to climatic conditions, and studying peculiarities of their inheritance were the main goals of the studies.

Materials and methods. The progenies from a complete diallel scheme, including reciprocal crosses, where ‘Festivalnaya’, ‘Holiday’, ‘Rubinovy Kulon’, and ‘Troubadour’ were tested as parent cultivars (12 crosses in total), were planted in 1996 and 1997 and evaluated in 1997–1998 and 1998–1999, respectively to investigate inheritance of yield and yield components. The studies were designed as five randomized complete blocks. 40 plants per cross were presented in each block. The distance between rows was 0.8 m; plants within a row were spaced 0.35 cm apart. In addition, plants of each parent cultivar were planted for comparing. All runner plants were regularly removed. Truss numbers and total flower numbers were counted for each plant. Total flower number divided by truss number was flower number per truss. All marketable berries (no less than 1.8 mm wide in the maximum diameter) from each plant were counted and weighed during each harvest to compute average weight of a marketable fruit.

In order to trace the inheritance of fruit size character, progenies from crosses of ‘Feierverk’, ‘Konservnaya Plotnaya’, and ‘Rubinovy Kulon’ with a clone of Fragaria virginiana ssp. platipetala and those from ‘Feierverk’, ‘Festivalnaya’, ‘Holiday’, ‘Redgauntlet’, ‘Rubinovy Kulon’, and ‘Senga Sengana’ crossed with a clone of Fragaria ovalis as testers were evaluated in 1998 and 2000, respectively. Seedlings obtained from cross combinations of ‘Rubinovy Kulon’ × Or 159-43-64 (‘Kokinskaya Rannyaya’ × S₂ of ‘Festivalnaya’), ‘Senga Sengana’ × ‘Alpha’, ‘Rubinovy Kulon’ × ‘Alpha’, ‘Feierverk’ × ‘Alpha’, ‘Estafeta’ × ‘Alpha’, and ‘Estafeta’ × ‘Naidyona Dobraya’ were estimated in 2002 for their efficiency to obtain high-yielding descendants with useful traits using percentage indices of the latter. Progenies from crosses of ‘Alpha’ with ‘Honeoye’, ‘Korona’, and Or 975-12-72 (‘Rubinovy Kulon’ × ‘Festivalnaya’), between ‘Festivalnaya’ and ‘Alpha’, and from crosses of ‘Pandora’ with ‘Alpha’ and ‘Lord’ were evaluated in 2005 with the same purpose. The progenies obtained from the two latter crosses were studied in addition to find out peculiarities of inheritance of very late flowering/fruit ripening and male sterility. Plants of ‘Sumas’, which is known to be winterhardy, progenies raised from crosses of the cultivar with ‘Kama’, Or 975-12-72, and those from open pollinated seed were estimated in field conditions in the spring of 2003, after extremely low negative
temperatures in December 2002, for cold tolerance. The estimation of injury caused to the plants was carried out using the 6-point scale ranging from 0 (no injury) to 5 (the heaviest degree of injury, plant is dead).

Computation of effects and variances of general (GCA) and specific (SCA) combining abilities to state additive and non-additive components in the inheritance of yield and yield components was performed using method 3 by Griffing (1956). Calculation of standard errors and correlation analysis between yield and fruit size to reveal sorts of progenies, in which yield is more often linked to the character, were carried out in accordance with the appropriate t-test and CORR procedure of SAS Institute (User’s guide, version 5; SAS Institute, Cary, N.C., 1989).

Results. Yield and yield components. Out of cultivars involved in the diallel crosses which were studied in 1997–1998, and again in 1998–1999, ‘Festivalnaya’ and ‘Troubadour’ usually produced many inflorescences and high yields, significantly higher in the second cropping year, particularly the former, while ‘Rubinovy Kulon’ and to a lesser extent ‘Holiday’ had approximately equal yields in both first and second crops. Large berries distinguished ‘Festivalnaya’ and ‘Rubinovy Kulon’. ‘Holiday’ and ‘Troubadour’ had smaller fruit, but initiated more flowers per inflorescence, especially the latter. Significant percentages of high-yielding seedlings were obtained only in the progenies from crosses between ‘Troubadour’ and ‘Festivalnaya’ (18.3%), ‘Troubadour’ and ‘Holiday’ (16.7%), ‘Festivalnaya’ and ‘Troubadour’ (15.4%), and ‘Festivalnaya’ and ‘Rubinovy Kulon’ (11.4%). ‘Festivalnaya’ and ‘Troubadour’ passed on high yield in second cropping year being possessed of the highest GCA effects, while the highest values of GCA effect and variance for marketable yield in the first season was found for ‘Rubinovy Kulon’ (Table 1). At the same time, the seedlings derived from ‘Festivalnaya’ and ‘Troubadour’ inherited the lowest yields in first year. SCA variance being significant in both years played more often unimportant role in yield inheritance, especially compared to GCA components except those of ‘Rubinovy Kulon’ (in first year) and ‘Holiday’ (in second year), both in combination with ‘Festivalnaya’. However non-additive effects (SCA) played significantly more important role in inheritance of some yield component characters, for instance, of inflorescence number, first of all in the progenies of ‘Festivalnaya’ and ‘Troubadour’. The same might be tracked in the inheritance of flower number per inflorescence in first year where ‘Troubadour’ was possessed of an especially high value of SCA variance. The differences for both GCA and SCA variances in this character for second cropping year were insignificant.

Non-additive effects were still higher in the inheritance of fruit size character. Values of SCA variance were higher than those of GCA for all the cultivars studied (Table 1), and the difference between them was particularly evident for ‘Rubinovy Kulon’ in both years. Nevertheless, additive effect in the inheritance of the trait by the progenies of the cultivar was also the highest. The other cultivars passed on small fruit to most seedlings. Only ‘Holiday’ in first year and ‘Festivalnaya’ and ‘Troubadour’ in second year had positive values of GCA effects and variances.

Table 1 continued

However SCA variances of ‘Troubadour’ and ‘Festivalnaya’ computed for first year as
| Cultivar | Year | 'Festivałnaya' | 'Holiday' | 'Rabintscy Kulik' | 'Trumbucha' | Standard error of the trait mean | Standard error of the trait mean
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<tr>
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<td>1</td>
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<tr>
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well as that of ‘Holiday’ for second year exhibited that they could be valuable parents in some combinations (as a rule, with ‘Rubinovy Kulon’). For instance, the crosses between ‘Festivalnaya’ and ‘Rubinovy Kulon’ gave the biggest quantities of large-fruited seedlings (15.9% in the population of ‘Festivalnaya’ × ‘Rubinovy Kulon’ and 16.1% from the reciprocal cross). Rather high percentage of large-fruited seedlings occurred in most progenies of ‘Rubinovy Kulon’, but mean fruit weights of seedlings in them varied noticeably more than in the others. The seedlings with the largest berries were also derived from ‘Rubinovy Kulon’. ‘Festivalnaya’ being a large-fruited cultivar is a significantly poorer parent in breeding for the trait.

Marketable fruit number is dependent on many yield components. Nevertheless, the parents, which produced higher numbers of marketable berries per plant, passed on this trait to higher percentage of seedlings in their progenies, one way or another. Intercrossing between ‘Festivalnaya’ and ‘Rubinovy Kulon’ provided seedlings with bigger quantities of marketable fruit in first year, while in second year seedlings derived from ‘Festivalnaya’ and ‘Troubadour’ produced more marketable berries per plant (data not presented).

Additional information about inheritance of fruit size character could be got from the progenies obtained from crosses of several cultivars with two wild octoploid species as testers: a clone of *F. ovalis* which was early ripening and extremely small-fruited (0.4 to 0.6 g), and a clone of *F. virginiana* ssp. *platipetala* (average fruit weight was 1.3 g) with mid-late term of maturing. Out of the cultivars involved in the study and used as female parents in crosses with the same clone of *F. ovalis* (Table 2), all were large-fruited, only ‘Holiday’ and ‘Senga ‘Sengana’ were somewhat inferior in the character, while fruit of ‘Feierverk’ were slightly larger compared to the others. The progeny from intercrossing between ‘Rubinovy Kulon’ and the small-fruited tester developed noticeably larger fruit on average, and more than half of seedlings produced fruit with mean weight exceeding 4 g. Significant amounts of comparatively
large-fruited plants were discriminated in the progenies of ‘Feierverk’, ‘Redgauntlet’, and ‘Senga Sengana’. Although seedlings obtained from the cultivars with somewhat smaller berries had lesser fruit weight on average, the difference was not adequate. In addition, the seedlings derived from ‘Rubinovy Kulon’ had significantly larger berries compared to those of ‘Feierverk’. Most seedlings of ‘Feierverk’, ‘Redgauntlet’, and particularly ‘Rubinovy Kulon’ produced moderate fruit quantities, while those of ‘Festivalnaya’ and ‘Senga Sengana’ rather frequently had numerous, but small berries.

**Table 2.** Fruit size of cultivars and their progenies from crosses with the same clone of *F. ovalis* (2000)

<table>
<thead>
<tr>
<th>Cross</th>
<th>Quantity of seedlings</th>
<th>Mean fruit weight (g)</th>
<th>Amount of seedlings with fruit weight (%)</th>
<th>Average fruit weight in progeny (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sodalinės kiekis</td>
<td>Valdantų uogos masė</td>
<td>front 3 to 4 g</td>
<td>more than 4 g</td>
</tr>
<tr>
<td>‘Feierverk’ × <em>F. ovalis</em></td>
<td>325</td>
<td>11.5</td>
<td>58.6</td>
<td>18.6</td>
</tr>
<tr>
<td>‘Festivalnaya’ × <em>F. ovalis</em></td>
<td>186</td>
<td>10.5</td>
<td>67.9</td>
<td>26.3</td>
</tr>
<tr>
<td>‘Holiday’ × <em>F. ovalis</em></td>
<td>196</td>
<td>9.7</td>
<td>72.6</td>
<td>22.5</td>
</tr>
<tr>
<td>‘Redgauntlet’ × <em>F. ovalis</em></td>
<td>154</td>
<td>10.7</td>
<td>59.8</td>
<td>22.6</td>
</tr>
<tr>
<td>‘Rubinovy Kulon’ × <em>F. ovalis</em></td>
<td>1028</td>
<td>11.1</td>
<td>14.1</td>
<td>27.8</td>
</tr>
<tr>
<td>‘Senga Sengana’ × <em>F. ovalis</em></td>
<td>142</td>
<td>9.9</td>
<td>57.3</td>
<td>28.0</td>
</tr>
</tbody>
</table>

* – Standard error here and below is given at *P* = 0.05.

Two years earlier three crosses had been carried out where ‘Feierverk’, ‘Konservnaya Plotnaya’, and ‘Rubinovy Kulon’ were used as female parents, whereas a clone of *F. virginiana* ssp. *platipetala* was taken as the pollinator (Table 3). Fruit of ‘Konservnaya Plotnaya’ are usually somewhat smaller in comparison with the two other cultivars. Nevertheless, neither ‘Rubinovy Kulon’ that would exhibit itself as a promising parent in breeding for the fruit size character in the later study mentioned above nor the most large-fruited ‘Feierverk’ as parents were superior to ‘Konservnaya Plotnaya’, at least as concerns the amount of hybrids with the largest berries. The percentages were low and nearly equal to each other. An advantage of ‘Rubinovy Kulon’ revealed itself only in somewhat higher average fruit weight in its progeny which was however insignificant. At the same time both others were considerably superior in the amount of seedlings with comparatively high yield per plant because of higher truss and fruit numbers. All the cultivars being crossed with this small-fruited clone fruit of which are visibly larger than those of *F. ovalis* seemed less valuable in breeding for the character.

**Table 3.** Amount of hybrids with high yield per plant and comparatively large fruit in progenies of three cultivars crossed with the same clone of *F. virginiana* ssp. *Platipetala*
Table 4. Efficiency of selection of high-yielding and large-fruited seedlings in progenies of cultivars manifesting these features
Late ripening and male sterility. In the two progenies obtained from very-late ‘Pandora’ (Table 4), high percentage of hybrids matured late. Such seedlings amounted to 48.4% in the progeny obtained from the cross of ‘Pandora’ × ‘Alpha’ (the latter is mid-season) and 58.1% in that grown from crossing with ‘Lord’ (mid-late). Moreover, there was a significant part of hybrids ripening nearer to the term of ‘Pandora’, within four days before it or even later (23.9% and 26.4% respectively). Almost all the late-season seedlings with a few exceptions in the first progeny and except less than 10% in the second one were male sterile as well as the female parent. Seedlings with fruit ripening very late were all pistillate. This trait of ‘Pandora’ was inherited by about half of its seedlings. Quantity of such seedlings in the progeny of ‘Pandora’ × ‘Alpha’ amounted to 46.8% and in that of ‘Pandora’ × ‘Lord’ did to 49.3%.

Cold tolerance. Very low negative temperatures in December 2002 (below -20°C) without snow cover led to heavy injury of plants in most cultivars, but they favoured revealing the most winterhardy ones. ‘Sumas’ bred in Canada exhibited outstanding tolerance to these frosts in early winter. The plants were damaged rather insignificantly; even yield losses were not particularly high. It was the level much superior to the other cultivars. Furthermore, all the seedlings obtained from two cross combinations of ‘Sumas’ with ‘Kama’ and Or 975-12-72 as well as 40 seedlings raised from open-pollinated seed of the cultivar being subjected to the extremely cold conditions demonstrated practically the same level of frost hardiness. All the plants survived the winter with somewhat injured branch crowns and practically no root damage. The difference in score of crown injury between individuals did not exceed the value of
0.5. The damage score did not go over 1.8 out of 5.0, while even those of the other cultivars which most successfully withstood the extreme conditions showed the scores equal to or exceeding 2.5.

**Discussion.** Yield is proved by many researchers to be inherited quantitatively (Hancock et al., 1996). High values of GCA:SCA ratios in this study are also evidence of such inheritance. Rather high values of SCA variance in yield inheritance (for example that calculated for ‘Holiday’) might be conditioned by occasional causes, for instance weather (especially frost impact) and pollination conditions, seed germination, etc. Nonetheless, some non-additive effect still cannot be ruled out. Moreover, high values of SCA variance (non-additive effect) for some yield components and low GCA:SCA ratio values respectively illustrate complexity of relations between genes controlling yield components and, as a result, yield.

As it was found, yield components comparable in value are inherited by progenies of different parents differently. First of all, it concerns inflorescence number per plant and fruit size. In the end, it is not so important how many gene sets control these traits, but it is clear that there are at least several alleles (however all says for existing numerous ones) of the genes controlling both components. Some alleles in each set are dominant, at least partially. Besides, obviously some of them can be modifiers for some alleles of the gene(s) determining opposed components. Likely they often compensate effects of each other. High values of non-additive effects in the inheritance of yield components might mean three principal opportunities for gene × gene (or alleles of genes) interaction: 1) there exist a number of alleles of a gene, and some of them are dominant, at least partially; 2) apart from the main gene(s) there are modifying one(s) which influence upon some alleles of the former (epistatic or suppressors); and 3) modifying genes (possibly genes determining some other components may act as modifiers) influence some combinations of the alleles of the main gene(s). However, this interaction may be much more complex in fact.

Differing patterns of inheritance of fruit size character by the progenies of different parent cultivars, which are almost equally large-fruited and by the progenies of the same cultivar (‘Rubinovy Kulon’) from crosses with the small-fruited testers is evidence that different alleles of a gene or even different genes contribute to the character differently. In addition, some modifying genes interfere, and evidently not only those controlling yield components.

The two wild clones used in the crosses – *F. ovalis* and *F. virginiana* – differed in time of flowering and ripening which could influence the inheritance of yield component characters. First of all, *F. ovalis* is known to be the earliest ripening strawberry species. The clone of *F. virginiana* ssp. *platipetala* used here matured much later; it was rather mid-late. ‘Rubinovy Kulon’ was the earliest cultivar amongst those used in the crosses, while ‘Festivalnaya’, ‘Konservnaya Plotnaya’, and ‘Senga Sengana’ mature latest. It is known that earliness is partially dominant if early ripening genotypes are involved in crosses (Hancock et al., 1996), although when garden strawberry cultivars are intercrossed percentage of earlier ripening seedlings is not particularly high (Shaw and Larson, 2005). The progenies obtained from crosses of the strawberry cultivars with *F. ovalis* produced fruit, which ripened very early (almost as early as the wild species) and much earlier on average than those of *F. virginiana* in this study. The seedlings from
the crosses were evaluated in the first year of fruiting. Most descendents of *F. ovalis* produced rather few berries. Earlier studies showed that strawberry genotypes ripening early and in early to mid-season terms often produce larger fruit in first cropping year (Shokaeva, 2006), while high-yielding mid-season, mid-late, and late-season ones developed heavier on average fruit in second season. Maybe, if the progenies had been evaluated in the second seasons of fruiting, results could have been somewhat different. Nevertheless, the difference between the progenies from intercrossing with the two clones was evident. While being crossed with *F. ovalis* ‘Rubinovy Kulon’ contributed to the fruit size character of the seedlings noticeably, in the progenies from crossing with *F. virginiana* ssp. *platipetala* the same genes of the cultivar practically did not reveal themselves. Therefore, either gene(s) controlling time of ripening or that/those closely linked to them could behave as modifiers for the genes or alleles determining fruit size. It is also possible that the genes controlling the other component characters, for instance, inflorescence number could behave the same way. Evidently inheritance of the gene(s) which control fruit size in the cultivar can change depending on some other gene(s), for instance, the clone of *F. ovalis* may have a gene epistatic for the genes controlling large fruit trait, while that of *F. virginiana* may be possessed of a gene suppressing it, for example, that determining inflorescence and flower/fruit number per plant, etc.

Studying of the high-yielding cultivars as parents in breeding for the complex character exhibited high efficiency only in that case when very productive ‘Alpha’ was intercrossed with another rather high-yielding cultivar. The other crosses gave rather low percentages of high-yielding seedlings. Although yield is inherited quantitatively, any additional gene providing higher yield contributed to fruit productivity only slightly, whereas lack of it led to clearly lower average yield level of a progeny almost irrespective of time of maturing. Yield was correlated with fruit size character more strongly in the progenies where percentage of high-yielding seedlings was not particularly high. It coincided with earlier or later term of maturing of most hybrids. Higher yield per plant on average in most promising progenies was related in part to somewhat smaller fruit and bigger quantities of marketable berries. Selection for very high yield per plant is accompanied by selection for those alleles (genes) or their combinations, which control inflorescence and fruit numbers per plant and are partially dominant over fruit size character. Yield is correlated more strongly with fruit size character only in progenies where yield was not particularly high on average and there was “some space” for fruit expanding.

‘Pandora’ intercrossed with mid-season ‘Alpha’ and mid-late ‘Lord’ appeared to be a donor of both late flowering/fruit ripening and male sterility of flowers. Simpson (2002), who bred ‘Pandora’ and studied inheritance of those characters by its progenies, reported earlier of late flowering to be linked with pistillate flowers, and the inference has been confirmed by these results. It was revealed by many researchers that strawberry genotypes flowering late often had low fertility of pollen. Supposedly high air temperatures lead to failure of normal pollen development. However, it is highly possible that just imperfection of flowers can be the main reason of late flowering and ripening. These studies on late flowering character and finding its linkage to male sterility of flowers allow the converse supposition that exactly under-
developed anthers (or their absence) and low fertility of pollen can be the reason of late flowering. Let us assume that anthers are the place where synthesis of a certain hormone inciting flowering takes place, and quantity of the flowering hormone stuff is dependent on anther and pollen development and maturing. The lower fertility of pollen is the lesser quantities of hormone stuff are synthesized. Absence of stamens which could be producers of the flowering hormone may lead to especially delayed flowering that was observed in ‘Pandora’ and the pistillate seedlings derived from the cultivar. Or 171-15-5, which is a descendent of ‘Pandora’, planted in a study with some other cultivars and selections with different time of flowering and maturing flowered irrespective of sum of effective temperatures, while all the others flowered markedly earlier in accordance with mean temperatures and their sum when spring conditions were warmer (Shokaeva, 2005).

The progeny of ‘Pandora’ from crosses with hermaphrodite cultivars segregated in approximately 1 : 1 ratio. This means that either a gene controlling this trait is dominant or there is another modifying gene (highly possible a mutation of a gene) because of which development of stamens is blocked. In any case ‘Pandora’ is heterozygous by the gene. It is known that pistillate plants occur in wild populations of *F. virginiana* and *F. chiloensis*, a clone of which (the latter) was used as the original source to produce ‘Pandora’ (Simpson, 2002). When hermaphrodites are selfed, a low percentage of pistillate plants are obtained among seedlings (Hancock et al., 1996). Solitary male sterile hybrids appear sometimes in progenies from crossing of ‘Protem’ and ‘Sumas’ with other cultivars (personal observations). So emergence of male sterile plants may be conditioned by either a mutation/recombination of certain linked genes after which the main gene and a modifier appear to be placed in the same chromosome near to each other or a mutation of the gene which is responsible for stamen development. In addition, presence of two or more modifiers suppressing each other cannot be ruled out. Obviously male sterility is connected with failure of normal ferment synthesis when protein molecules are unable to form tertiary and quaternary structure leading to normal activity that blocks processes associated with development of stamens.

Outstanding cold tolerance of ‘Sumas’ and its progenies means that the cultivar has a dominant gene ensuring outstanding hardening of the plants allowing withstanding extremely severe frosts, at least in early winter, and the cultivar is homozygous by the gene. It is difficult to judge whether the gene is presented with a pair of homologous alleles in the chromosome set of the cultivar or this allele is repeated more than two times. Additional studies of the cultivar by its progeny in appropriate conditions are needed to find it out. However, existence of only one pair of homologous alleles seems to be more probable. It is possible that this is not an allele of the gene controlling cold tolerance in other cultivars, but a different gene as its effect is significantly higher compared to those in the others.

**Conclusions.** 1. Yield is usually proved to be inherited quantitatively. However non-additive effects are highly significant and sometimes play even more important role in the inheritance of yield component characters, especially of fruit size and inflorescence number. It means that some alleles of a gene or even different genes controlling one of the characters reveal both dominance over each other and modifying effects towards alleles (or combinations of them) of the gene or genes controlling
opposed component characters.

2. ‘Alpha’ being used as a parent passes on high productivity steadily. It means that the genome of the cultivar includes more alleles ensuring high yield compared to the other cultivars studied.

3. ‘Rubinovy Kulon’ being intercrossed with some cultivars gives high percentage of large-fruited seedlings. However, progenies from crosses of the cultivar with two small-fruited clones of wild species differed in the inheritance of the genes controlling fruit size character. They revealed themselves only in the seedlings obtained from one of the combinations, which had earlier time of ripening. This could be explained by presence of different modifying genes in chromosome sets of the small-fruited clones.

4. ‘Pandora’ is a steady donor of late flowering/fruit ripening and male sterility of flowers, which are linked to each other. It is possible that anthers may be the place where synthesis of a flowering hormone takes place, and quantity of the hormone stuff is dependent on anther and pollen development. The lower fertility of pollen is the lesser quantities of hormone stuff can be synthesized. Absence of stamens may lead to especially delayed flowering.

5. Emergence of seedlings with pistillate flowers in progenies of hermaphrodite cultivars may be associated with a mutation of the gene controlling stamen development or a mutation (recombination) of another gene, which becomes a modifier (suppressor) for the former. Presence of more than one modifier is also possible.

6. ‘Sumas’ is possessed of the dominant gene controlling the highest level of frost tolerance among the cultivars studied, and the cultivar is homozygous by the gene. The progeny derived from ‘Sumas’ was practically as frost resistant as the cultivar.

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SVARBŪS BRAŠKIŲ GENOTIPŲ POŽYMIAI IR PAVELDĖJIMO YPATUMAI

D. Šokaeva

Santrauka


Reikšminiai žodžiai: donoras, *Fragaria × ananassa* Duch., genas, kryžminimas, palikuonys, tėvinė forma, veislių išvedimas.
EVALUATION OF APPLE CULTIVARS ON M.9 ROOTSTOCK IN INTENSIVE PLANTING SYSTEM

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Abstract. Five introduced apple cultivars (‘Jonagold King’, ‘Jonagold Decosta’, ‘Sampion’, ‘Pinova’ and ‘Pilot’) on M.9 rootstock were tested at the Lithuanian Institute of Horticulture in Babtai in 2000–2006. Cv. ‘Pinova’ gave significantly higher average yield in the young orchard (15.3 t/ha) and Cv. ‘Sampion’ was the most productive in full bearing orchard (36 t/ha). Cvs. ‘Jonagold King’, ‘Jonagold Decosta’ and ‘Pinova’ had significantly lower yield. The highest percentage of Extra class fruits was from apple trees of Cvs. ‘Jonagold King’ and ‘Sampion’. Cvs. ‘Pinova’ and ‘Pilot’ had smaller fruits. Fruits of Cv. ‘Sampion’ got the highest evaluation scores for taste and fruit appearance.

Key words: Malus, flowering time, yield, fruit quality.

Introduction. The last decade intensive technologies are introduced in commercial fruit growing in Lithuania. The main task for local fruit growers is to grow high quality apples in order to compete with imported fruits. For this purpose it is important to choice correctly cultivars, which could be adapted to Lithuanian climate conditions.


A large scale apple variety trials are constantly fulfilled at the Lithuanian Institute of Horticulture (Bandaravičius et al., 2001; Sasnauskas et al., 2006). In recent years at the Orchard technology department apple variety trials are performed aiming to identify the optimal cultivar – rootstock combination and to develop the most suitable orchard constructions. The trial conducted in 1994–2001 with twenty commercial valuable apple cultivars on M.26 rootstock let to develop more intensive planting systems for certain cultivars (Uselis, 2001; 2002). Trials with more dwarfing rootstocks showed higher cultivar precocity and productivity (Uselis, 2003; Kviklys, 2002).

The aim of the trial was to evaluate performance of cvs. ‘Jonagold King’, ‘Jonagold Decosta’, ‘Sampion’, ‘Pinova’ and ‘Pilot’ on M.9 rootstock in the intensive planting systems.
system under Lithuanian climate conditions.

**Material and methods.** Five apple cultivars ‘Jonagold King’, ‘Jonagold Decosta’, ‘Sampion’ (included in the National List of Plant Varieties), ‘Pinova’ and ‘Pilot’ on M.9 rootstock budded at 15 cm height were tested at the Lithuanian Institute of Horticulture in Babtai (Central Lithuania 55° 60' N, 23° 48' E) in 2000–2006. The orchard was planted in the spring of 2000. Planting distances 3 × 1 m. The trial consisted of four replicates with 5 trees in each. Replicates were randomised.

The soil was epicalcari-endohypogleyic cambisol (RDg4-K1) with following agrochemical properties: \( \text{pH}_{\text{KCl}} = 7.3 \), humus – 2.8%, \( \text{P}_2\text{O}_5 \) – 255 mg/kg, \( \text{K}_2\text{O} \) – 230 mg/kg. Trees were trained as slender spindles. Fertilization mainly with nitrogen was applied according to soil analysis before flowering and during intensive fruitlet growth.

Dates of the beginning of flowering and full bloom were observed. Flowering abundance was evaluated by 5-point scale according Szepanski and Rejman (1987). Fruit average weight was counted of the sample of 50 fruits. 50 fruits sample was graded following EC quality standards for apples. Fruit harvesting time was established by starch – iodine test (Streif, 1996). Fruits were stored in cold storage at +1–2°C. Fruit appearance and taste was evaluated by Pomological commission of the Lithuanian Institute of Horticulture. Results were statistically elaborated by the analysis of variance.

**Results.** According to the average of 6 years, all cultivars flowered abundantly (Table 1), especially cvs. ‘Sampion’ (4.6 points) and ‘Pilot’ (4.5 points), though no significant differences in flowering abundance were found among tested cultivars. According to the beginning of flowering, cvs. ‘Sampion’ and ‘Pinova’ are moderate early flowering and ‘Jonagold King’, ‘Jonagold Decosta’ and ‘Pilot’ are moderate late flowering cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Flowering abundance, points</th>
<th>Beginning of flowering, date</th>
<th>Full bloom, date</th>
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<tbody>
<tr>
<td>‘Sampion’</td>
<td>4.6</td>
<td>05-12</td>
<td>05-18</td>
</tr>
<tr>
<td>‘Jonagold King’</td>
<td>4.3</td>
<td>05-14</td>
<td>05-18</td>
</tr>
<tr>
<td>‘Jonagold Decosta’</td>
<td>4.1</td>
<td>05-14</td>
<td>05-19</td>
</tr>
<tr>
<td>‘Pilot’</td>
<td>4.5</td>
<td>05-15</td>
<td>05-20</td>
</tr>
<tr>
<td>‘Pinova’</td>
<td>4.3</td>
<td>05-13</td>
<td>05-18</td>
</tr>
</tbody>
</table>

**Table 1. Flowering data of apple cultivars**

Obelų veislių žydėjimo duomenys

Significant differences among tested cultivars in yielding capacity were recorded starting from the first year of cropping. The highest yield in the young orchard was from cv. ‘Pinova’ (4.59 kg/tree or 15.3 t/ha) (Table 2). cv. ‘Jonagold Decosta’ was the least productive during first two years. At full bearing stage (4–7th year) cv. ‘Sampion’ gave the highest average yield (36 t/ha). Significantly lower yields were recorded for both cv. ‘Jonagold’ clones and cv. ‘Pinova’.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Flowering abundance, points</th>
<th>Beginning of flowering, date</th>
<th>Full bloom, date</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Sampion’</td>
<td>4.6</td>
<td>05-12</td>
<td>05-18</td>
</tr>
<tr>
<td>‘Jonagold King’</td>
<td>4.3</td>
<td>05-14</td>
<td>05-18</td>
</tr>
<tr>
<td>‘Jonagold Decosta’</td>
<td>4.1</td>
<td>05-14</td>
<td>05-19</td>
</tr>
<tr>
<td>‘Pilot’</td>
<td>4.5</td>
<td>05-15</td>
<td>05-20</td>
</tr>
<tr>
<td>‘Pinova’</td>
<td>4.3</td>
<td>05-13</td>
<td>05-18</td>
</tr>
</tbody>
</table>

**Table 2. Average yield of apple cultivars**
According to the average of six years, the biggest fruits were of cv. ‘Jonagold King’ (162.9 g), though no significant differences were recorded with cvs. ‘Jonagold Decosta’ and ‘Sampion’. Significantly smaller fruits were of cvs. ‘Pinova’ and ‘Pilot’. When fruits were graded by size, more than 90% of fruits of cvs. ‘Jonagold King’ and ‘Sampion’ were Extra class (Table 3). Significantly lower amount of Extra class fruits and higher amount of fruits less than 60 mm were of cvs. ‘Pilot’ and ‘Pinova’.

Table 3. Distribution of apples according to classes (%)

According fruit evaluation performed by Pomological commission, cv. ‘Sampion’ had the highest score for fruit appearance (4.70), taste (4.55) and overall estimation (4.62). Fruits of cv. ‘Pinova’ had lower but not significant evaluation. The lowest score was of cv. ‘Pilot’ fruits: fruit appearance (4.12), taste (4.04) and overall estimation (4.11).

Under Lithuanian climatic conditions the harvesting time of cvs. ‘Sampion’ and ‘Pinova’ is the first decade of October, ‘Jonagold King’, ‘Jonagold Decosta’ and ‘Pilot’ – second decade of October. Fruits of ‘Pinova’ and ‘Sampion’ can be stored in ordinary cold storage until March, ‘Jonagold King’ and ‘Jonagold Decosta’ until middle of April and ‘Pilot’ – until the end of May.

Discussion. Introduction of new apple cultivars could be limited by climatic conditions. Comparing with the main apple growing regions, Lithuania has shorter vegetation period and lower sum of active temperatures. It is very important carefully to investigate all properties of introduced cultivars before recommendation for commercial growing. For example, earlier performed trial with standard cv. ‘Jonagold’ showed that fruits lacked colour and other fruit quality parameters and taste were not sufficient.
every year (Kviklienė, Kviklys, 2001). In this trial average fruit weight of ‘Jonagold’ clones was around 160 g, what is usual weight under Lithuanian climate conditions (Kviklys, Kviklienė, 2002). In Poland average fruit weight of cv. ‘Jonagold’ varies from 170 g to more than 250 g (Czynczyk et al., 2006; Skrzynski, Gastol, 2006).

Commercial apple fruit growers in Lithuania have a shortage of high quality winter cultivars. Harvesting season of many new cultivars starts at the second half of October, when the risk of autumn frosts appears. Tested cultivars, except cvs. ‘Sampion’ and ‘Pinova’, should be harvested in late term too. Earlier picked fruits usually have not enough colour, taste and size. Such results raise a doubt of the suitability to grow late harvesting season apple cultivars in Lithuania. Even in Poland where vegetation season is longer, harvesting time of ‘Jonagold’ starts at the end of October (Czynczyk et al., 2006).

Cv. ‘Sampion’ is one of the most widely planted apple cultivar in Lithuanian commercial orchards. However, there is a need to search proper rootstock and adapt thinning programme for this cultivar. In recent trial cv. ‘Sampion’ gave the highest crop. High yield and excellent fruit quality of cv. ‘Sampion’ was recorded in many trials too (Bielicki et al., 2002; Wrona, Kot, 2002). Cv. ‘Jonagold’ and its clones are one of the main varieties in Netherlands, Belgium, Germany and Poland. Cv. ‘Jonagold’ usually also gives high yield. One of the reasons of significantly lower yields of cv. ‘Jonagold’ clones in this trial could be more pronounced variable bearing. Slightly higher average productivity (up to 30 t/ha) was recorded in our early performed trials with standard ‘Jonagold’ (Kviklys et al., 2000). In other countries usually at such planting density the yield of cv. ‘Jonagold’ on M.9 rootstock is more than 50 t/ha (Vercammen et al., 2006; 2007).

Cvs. ‘Pinova’ and ‘Pilot’ are interesting cultivars for organic orchards and commonly used in Netherlands, Germany and Poland. Low percentage of Extra class apples of cvs. ‘Pinova’ and ‘Pilot’ can be explained by abundant fruit set. Fruitlet thinning programs should be developed for these cultivars in order to increase apple size. Cv. ‘Pinova’ is more suitable for growing in Lithuania taking in account better fruit quality established by evaluation of the Pomological commission.

Conclusions. 1. Cv. ‘Pinova’ gave the highest average yield in the young (15.3 t/ha) and cv. ‘Sampion’ in full bearing orchard (36 t/ha). Cvs. ‘Jonagold King’, ‘Jonagold Decosta’ and ‘Pinova’ had significantly lower yield.

2. Cvs. ‘Jonagold King’ and ‘Sampion’ had the highest percentage of Extra class fruits (> 90%), when ‘Pinova’ and ‘Pilot’ – the lowest (respectively, 47 and 57%).

3. Fruits of cv. ‘Sampion’ had the highest evaluation scores for taste and fruit appearance.

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Gauta 2007 06
Parenšta spausdinti 2007 06
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Reikšminiai žodžiai: derlius, Malus, vaisių kokybė, žydėjimo laikas.
INVESTIGATION OF APPLE CV. ‘JONAGOLD’ CLONES IN THE YOUNG ORCHARD

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Abstract. Seven clones of cv. ‘Jonagold’: ‘Red Jonaprince Red Prince’, ‘Jonagold Boerekamp Early Queen’, ‘Jonagored Supra’, ‘Jonaveld First Red’, ‘Decosta Jonagold DeCoster’, ‘Jonagold Novajo’ and ‘Jonabel’ were tested at the Lithuanian Institute of Horticulture in Babtai in 2003–2006. In the young orchard significant differences among cv. ‘Jonagold’ clones were recorded in vegetative growth, yield and fruit weight. Cvs. ‘Red Prince’ and ‘Novajo’ had smaller stem diameter, comparing with other tested clones. The highest total yield in the young orchard was recorded for cvs. ‘Early Queen’, ‘Red Prince’ and ‘Supra’, the lowest – for cv. ‘Novajo’. Average fruit weight of cv. ‘Novajo’ was significantly lower too.

Key words: Malus sp., vegetative growth, yield, fruit quality.

Introduction. Lithuanian climatic conditions determine introduction possibilities of apple cultivars. Shorter vegetation period and growing hours, winter cold are the limiting factors for the cultivars originated from the west and south of Europe. In earlier performed rootstock trial with standard cv. ‘Jonagold’ fruits lacked colour, especially on more vigorous rootstocks (Kviklienė, Kviklys, 2001). At the same time, other fruit quality parameters and taste were not sufficient every year (Kviklys, Kviklienė, 2002). The same colouring problems were noticed in other countries (Vercammen et al., 2007a; Vercammen et al., 2007b). To overcome this shorting, during the last decades, in commercial growing several colour mutations of cv. ‘Jonagold’ have been introduced. These ‘Jonagold’ clones differ in colour and colour pattern, being bright red, dark red, striped or solid blushed (Hampson, Kemp, 2003; Kemp et al., 1995). There were also declared differences among clones for their suitability to grow in cooler climate conditions (Czynczyk, pers. comm., 2005). No significant differences in earlier internal ripening could be determined between clones with the same crop load and the same virus status. Earlier picking is possible only due to better colouring (Hampson, Kemp, 2003; Jager, Kemp, 2000).

Until recent years, ‘Jonagold’ is one of the main varieties in the Netherlands, Belgium, Germany and Poland. Main clones grown and currently planted in these countries are ‘Jonagored Supra’, ‘Decosta Jonagold DeCoster’, and ‘Red Jonaprince Red Prince’ (Anonymous, 1999; Jager, Kemp, 2000). After discovering the Russian market, a new wave of establishing commercial orchards with the well-coloured clones of cv. ‘Jonagold’ is taking place in Belgium and the Netherlands (Deckers,
The aim of the trial is to evaluate suitability of cv. ‘Jonagold’ clones for growing under the Lithuanian climatic conditions.

**Material and methods.** Seven clones of cv. ‘Jonagold’: ‘Red Jonaprince Red Prince’ (further ‘Red Prince’), ‘Jonagold Boerekamp Early Queen’ (further ‘Early Queen’), ‘Jonagored Supra’ (further ‘Supra’), ‘Jonaveld First Red’ (later ‘First Red’), ‘Decosta Jonagold DeCoster’ (further ‘Decosta’), ‘Jonagold Novajo’ (further ‘Novajo’) and ‘Jonabel’ were tested at the Lithuanian Institute of Horticulture in Babtai (Central Lithuania 55° 60’ N, 23° 48’ E) in 2003–2006. The orchard was planted in spring of 2003. Planting distances were 3 × 1 m. The orchard was established without irrigation. The soil was epicalcari-endohypogleyic cambisol (RDg4-K1) with following agrochemical properties: pH\textsubscript{KCl} – 7.3, humus – 2.8%, P\textsubscript{2}O\textsubscript{5} – 255 mg/kg, K\textsubscript{2}O – 230 mg/kg. Planting material was propagated in the Netherlands as knip – trees and delivered by Vermeerderingstuinen Nederland (Propagation Gardens Netherlands).

Fertilization mainly with nitrogen was applied according to soil analysis before flowering and during intensive fruitlet growth.

Tree growth was evaluated by total shoot length (cm), tree height (cm) and stem diameter (mm), 30 cm above soil surface. Fruit average weight was counted of a sample of 100 fruits.

The trial consisted of four replicates with 5 trees each. Replicates were randomised. Results were statistically elaborated by the analysis of variance using Duncan’s multiple range test.

**Results.** The strongest shoot growth during the year of planting was recorded of cvs. ‘Red Prince’ and ‘Early Queen’ (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Vegetative growth of ‘Jonagold’ clones</th>
<th>Table 1. ‘Jonagold’ klonų vegetatyvinis vystymasis</th>
</tr>
</thead>
</table>

Their total shoot length differed significantly from other tested clones with the exception of cv. ‘Jonabel’. Significantly weakest shoot growth was observed for cv. ‘Novajo’. Its total shoot length was by 100% less than one of strong growing cultivars. Vegetative growth of cv. ‘Novajo’ expressed as stem diameter was suppressed during all years of investigations too. Two other clones, ‘Red Prince’ and ‘First Red’, had also smaller stem diameter. Cv. ‘Decosta’ exhibited strongest growth in the young orchard among
the tested clones, although there were no significant differences comparing with cvs. ‘Early Queen’, ‘Supra’ and ‘Jonabel’.

Significant differences among clones in yielding capacity were recorded starting from the first year of cropping. The highest yield was from cvs. ‘Red Prince’, ‘Early Queen’ and ‘Jonabel’ (1.50–1.83 kg/tree) (Table 2). ‘Early Queen’ remained on the top positions every year and had highest total yield in the young age (18.3 kg/tree). Only the total yield of two other cvs. ‘Red Prince’ and ‘Supra’ was significantly not different. Cv. ‘Supra’ was the least productive during first two years, but the most in 2006.

Table 2. Yield of ‘Jonagold’ clones (kg/tree)

<table>
<thead>
<tr>
<th>Clone</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Red Prince’</td>
<td>1.50 ab</td>
<td>6.68 ab</td>
<td>8.47 bc</td>
<td>16.7 ab</td>
</tr>
<tr>
<td>‘Early Queen’</td>
<td>1.83 a</td>
<td>6.49 ab</td>
<td>9.99 ab</td>
<td>18.3 a</td>
</tr>
<tr>
<td>‘Supra’</td>
<td>0.27 c</td>
<td>4.23 c</td>
<td>11.8 a</td>
<td>16.3 ab</td>
</tr>
<tr>
<td>‘First Red’</td>
<td>1.13 lb</td>
<td>5.54 bc</td>
<td>8.72 ab</td>
<td>15.4 bc</td>
</tr>
<tr>
<td>‘Decosta’</td>
<td>1.05 lb</td>
<td>4.58 bc</td>
<td>9.63 ab</td>
<td>15.3 bc</td>
</tr>
<tr>
<td>‘Novajo’</td>
<td>1.06 lb</td>
<td>4.16 c</td>
<td>8.96 ab</td>
<td>14.2 ab</td>
</tr>
<tr>
<td>‘Jonabel’</td>
<td>1.50 ab</td>
<td>7.24 a</td>
<td>6.02 c</td>
<td>14.8 bc</td>
</tr>
</tbody>
</table>

Values followed by the same letters within the columns are not statistically different at P ≤ 0.05.

significant differences were recorded only with cv. ‘Novajo’ (Table 3).

Table 3. Average fruit weight of ‘Jonagold’ clones (g)

<table>
<thead>
<tr>
<th>Clone</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Red Prince’</td>
<td>149 a</td>
<td>126 bc</td>
<td>152 a</td>
<td>142 ab</td>
</tr>
<tr>
<td>‘Early Queen’</td>
<td>145 a</td>
<td>128 bc</td>
<td>151 a</td>
<td>141 ab</td>
</tr>
<tr>
<td>‘Supra’</td>
<td>140 a</td>
<td>137 ab</td>
<td>159 a</td>
<td>145 ab</td>
</tr>
<tr>
<td>‘First Red’</td>
<td>148 a</td>
<td>126 bc</td>
<td>153 a</td>
<td>142 ab</td>
</tr>
<tr>
<td>‘Decosta’</td>
<td>146 a</td>
<td>147 a</td>
<td>162 a</td>
<td>152 a</td>
</tr>
<tr>
<td>‘Novajo’</td>
<td>154 a</td>
<td>116 c</td>
<td>131 b</td>
<td>134 b</td>
</tr>
<tr>
<td>‘Jonabel’</td>
<td>145 a</td>
<td>118 c</td>
<td>165 a</td>
<td>143 ab</td>
</tr>
</tbody>
</table>

Values followed by the same letters within the columns are not statistically different at P ≤ 0.05.

The average fruit weight was different during the trial time. The dry summer of 2005 resulted in much smaller fruits compared to fruit size in 2004 and 2006. The biggest changes of average fruit weight during the trial were recorded for cvs. ‘Novajo’ (38 g) and ‘Jonabel’ (47 g). The most stable fruit weight was of cvs. ‘Decosta’ (16 g) and ‘Supra’ (22 g).

Discussion. During first two years in the orchard, vegetative growth of trees
depended on the development of planting material. Though all trees were formed in the nurseries as two-year-old trees with one-year-old branches, the number of branches, stem diameter and tree height were not the same. It explains the strongly expressed significant differences among clones in total shoot length in the orchard. Influence of quality of planting material was noticed in stem diameter too. Later differences in stem diameter among clones decreased; nevertheless even in the fourth year after planting they still were significant. Considering growth peculiarities in the young orchard, it could be stated that ‘Jonagold’ clones will remain differences in vegetative development. Such tendency was mentioned by H.Kemp and Van Dieren (1996), but it was connected with virus status of planting material.

Three cultivars that had the highest total shoot growth during planting year were the most precocious in the second year. ‘Red Prince’, ‘Early Queen’ and ‘Jonabel’ yielded more than 1.5 kg/tree or more than 5 t/ha. Such yield is normal for cv. ‘Jonagold’ in the second leaf (Czynczyk et al., 2006; Vercammen et al., 2006). Yield of all tested clones increased during the first 4 years in the orchard, except cv. ‘Jonabel’. Higher total yield in the young orchard was recorded for cvs. ‘Early Queen’, ‘Red Prince’ and ‘Supra’, the lowest – for ‘Novajo’. Differences among yield of ‘Jonagold’ clones were recorded in the trials performed in Belgium too (Vercammen et al., 2006).

Average fruit weight of cv. ‘Jonagold’ varies from 170 g to more than 250 g in Poland (Czynczyk et al., 2006; Skrzynski, Gastol, 2006), which has comparable but more favourable growing conditions than in Lithuania. Under Lithuanian climatic conditions, the biggest fruits were in 2006 reaching more than 150 g. Of course the combination of dwarfing rootstock and an orchard without irrigation should be taken into account, meaning that average fruit weight could be increased if drought stress factor will be eliminated.

Significant differences of fruit weight among different selections of cv. ‘Jonagold’ usually are not strongly expressed if virus free plant material is used (Kemp, Van Dieren, 1996). However, ‘Novajo’ is known for its smaller fruit and different (more flat) fruit shape (Anonymous, 1999; Kemp, Van Dieren, 2003). In our trial, the smaller fruits of cv. ‘Novajo’ could be attributed to lower growth, but at the same time the yield was also relatively low. In comparison, fruit weight of weak growing and high yielding cv. ‘Red Prince’ was higher by 10 g. In other countries average fruit weight of cv. ‘Novajo’ on M.9 rootstock reaches 240-255 g (Vercammen et al., 2006).

Conclusions. 1. In the young orchard significant differences among cv. ‘Jonagold’ clones were recorded in vegetative growth, yield and fruit weight.

2. Cvs. ‘Red Prince’ and ‘Novajo’ had smaller stem diameter, comparing with other tested clones.

3. The highest total yield in the young orchard was recorded for cvs. ‘Early Queen’, ‘Red Prince’ and ‘Supra’, the lowest – for cv. ‘Novajo’.

4. Average fruit weight of cv. ‘Novajo’ was significantly lower in the tested group.

Acknowledgement. Authors acknowledge Vermeerderingstuinen Nederland (Propagation Gardens Netherlands) for a kind delivery of planting material.
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SODININKYSTĖ IR DARŽININKYSTĖ. MOKSLO DARBAI. 2007. 26(3).

‘JONAGOLD’ OBELŲ VEISLĖS KŁONŲ TYRIMAI JAUNAME SODE

D. Kviklys, N. Kviklienė, H. Kemp

Santrauka

THE EFFECT OF SOIL MULCHING ON YIELD OF  
SCHISANDRA CHINENSIS  

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Restated effects refer to cultivation lower Schisandra chinensis applying various litters. Mulching with the dung and the pine-bark from ammonium nitrate caused the significant increase in yield dependently on the year of research, as well as on the applied litter. The dung caused considerable increase of fruits; the black foil also increased yield of Schisandra chinensis. Masses of each group and of single fruits depended on the type of the litter. Plants mulched with dung and black foil had significantly bigger groups as well as the masses of each fruit.

Key words: growth, yield, mulching, Schisandra chinensis.

Introduction. Schisandra chinensis Ball. is a perennial climbing plant, which belongs to the family of Schisandraceae. This plant is known in the world under many different names. The most popular one is “limonnik kitajski” and “Five-Flavor Seed”. In pharmacology, raw material, known under name Schisandrae fructus is applied to production of drugs. Schisandra chinensis is most often grown in back-house gardens. However, it has recently been grown on manufactured plantations. Kawecki et al., (2001) observed that except ornamental value, the processed fruits of Schisandra are edible and as raw material are suitable for further production. In cultivation of Schisandra chinensis, Mariańska-Cichoń (2006) recommended soil fertilization and mulching due to beneficial effects of such treatment on growth and yielding of plants.

Material and methods. 45 plants (vegetative reproducing) of Schisandra chinensis were planted at the spacing of 60 cm on 15 plots (5 plants in each) in 2002. The experiment was conducted in the Experimental Station of the University of Warmia and Mazury in Olsztyn. 5 different mulches for weed control: black plastic, bark and additionally ammonium nitrate, manure (20 t ha⁻¹) and control treatment (without fertilization) were applied. Yielding and growth of plant and biometric parameters of fruit were determined in 2005 and 2006.

Results and discussion. The result of our studies is the stated differences in growth of plant. It was found that manure and bark with supplement of ammonium nitrate caused the growth of shoots to about 3 m. The largest amount of the shoots was ascertained using black plastic, although they were not longest. However, no effect of manure on the number of shoots was observed.

The first small yield of Schisandra chinensis produced 30% of shoots in 2004 on the control and black plastic treatments.
Augalo augimo priklausomybė nuo naudotos dangos

**Table 2.** Yield and mass of bunch and fruit of *Schisandra chinensis*

<table>
<thead>
<tr>
<th>Treatment Variations</th>
<th>Height Aukščis (cm)</th>
<th>Mean diameter Valtimis skersmuo (cm)</th>
<th>Length of one-year-old Shoot Vienarūs metų ilgis (cm)</th>
<th>Number of shoots, units Ciklo skyrius, vnt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black plastic</td>
<td>266.6</td>
<td>279.9</td>
<td>86.6</td>
<td>98.4</td>
</tr>
<tr>
<td>Iodo pevèle</td>
<td>264.9</td>
<td>282.2</td>
<td>87.9</td>
<td>93.7</td>
</tr>
<tr>
<td>Bark</td>
<td>279.9</td>
<td>299.8</td>
<td>90.5</td>
<td>108.5</td>
</tr>
<tr>
<td>Żievė</td>
<td>285.5</td>
<td>307.6</td>
<td>98.4</td>
<td>105.9</td>
</tr>
<tr>
<td>Bark + Ammonium nitrate</td>
<td>271.8</td>
<td>244.4</td>
<td>85.5</td>
<td>101.2</td>
</tr>
</tbody>
</table>

| Factor I, year       | n. s | 9.86 | 1.20 | 2.49 |
| Factor II, cover     | 13.38 | 8.06 | n. s | 1.09 |
| Factor II, cover     | n. s | 6.06 | 0.15 | 0.89 |

The largest amount of the shoots was obtained using black plastic. The average mass of plant was similar and amounted up to 30 g. According to other authors (Bogatko and Mazuchowski, 1992; Kędzi, 2002; Upton, 1999), the flowering and fructifying of *Schisandra chinensis* were observed in the fourth year after planting. On the other hand, the first fruits in the present experiment were obtained as early as in the third year. Probably it was due to vegetative reproduction. According to Szewczyk (1992),
vegetative reproduced plants produced similar faster yield (in the third year after seating).

The largest yield at the period of two years was obtained using manure and black plastic. No differences between masses of particular fruits were found at the first year of our observations. However, mass of bunch was diversified. Bark with addition of ammonium nitrate and manure affected increase in the mass of one bunch. Large fruits were obtained in the second year of experiment using manure and black plastic. According to Lipecki (2001), mulching with various materials also made a note of the significantly better increase in plants using bark, dung and black foil.

**Conclusions.** Soil mulching with manure and bark with supplement of ammonium nitrate significantly affected better growth of plant. On the other hand, the largest amount of shoots was obtained using manure as well as black plastic.

The best yield of *Schisandra chinensis* was obtained mulching with manure and black plastic. Mass of particular bunch was the largest on those treatments, as compared with other treatments.

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Parenga spausdinti 2007 06*

**References**


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DIRVOS MULČIAVIMO ĮTAKA SCHISANDRA CHINENSIS DERLIUI

Z. Kawecki, Z. Tomaszewska

Santrauka


Reikšminiai žodžiai: augimas, derlius, mulčiavimas, Schisandra chinensis.
EVALUATION OF STRAWBERRY CULTIVARS IN ESTONIA

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14 strawberry cultivars (‘Anne-Lise’, ‘Darunok’, ‘Gerida’, ‘Jewel’, ‘Melody’, ‘Pandora’, ‘Pegasus’, ‘Rhapsody’, ‘Symphony’, ‘Syrius’, ‘Vantage’, and ‘Wega’), together with ‘Senga Sengana’ and ‘Venta’ as standards, were evaluated in the experimental field of Polli Horticultural Research Centre of the Institute of Agricultural and Environmental Sciences of Estonian University of Life Sciences in 2005–2006. Winterhardiness, phenology, damage of blossoms by spring frosts and blossom weevil (*Anthonomus rubi*), yield, quality of fruits and hardiness of fruits to grey mould (*Botrytis cinerea*) and other fruit rots were investigated. The cultivars ‘Jewel’, ‘Syrius’ and ‘Wega’ appeared to be prospective for cultivating in Estonia. These three cultivars had good yield and firm fruits. ‘Pandora’ is of interest for being a late ripening cultivar with large fruits with relatively high vitamin C content (40 mg/kg fresh fruits) but in case of rainy weather the quality of fruit is declining. This cultivar cannot be grown as monoculture as it has only female flowers. Being a late flowering plant ‘Pandora’ escapes the spring frost damage.

Key words: *Anthonomus rubi*, blossoms, strawberry yield, weight of fruit.

Introduction. According to the data of Estonian statistics, strawberry plantations comprised 865 hectares in Estonia in 2005. The yield per hectare is varying from year to year, but on the average it is around 3 tons per hectare. Strawberry yield greatly depends on the weather conditions during the dormancy and growth periods (Nes, 1997, Battery et al., 1998; Hieteranta, Matala, 2002; Shokaeva, 2006) resulting in large yield differences between years. Instability in the yield over the years can be seen in the current trial as well. Accordingly, there is a need for cultivars that would not respond so strongly to extreme weather conditions and would give a good and high quality yield meeting the needs of both the grower and the consumer. Severe damage to yield is caused by spring frosts, occurring during bloom, and strawberry weevil, the damage of these being dependent on the weather conditions and resistance of the cultivars. In Estonia strawberries are mostly produced for fresh market and therefore fruit size, its attractiveness and shelf life are particularly important. Increased focus on nutritional health aspects of berries stresses the importance of the nutrient content of fruits. In order to improve the range of cultivars in use, the cultivars that have been prospective in other regions are introduced and tested in local climatic conditions. ‘Senga Sengana’ is used as a standard, since it has been in the list of recommended cultivars for a long period. ‘Senga Senga’ is a cultivar with good winterhardiness and stable yields, and in spite of having some deficiencies the consumer interest in it has not faded. ‘Venta’ was included in the trial as a second standard. ‘Venta’ is being
valued for its tasty and attractive fruit and very good winterhardiness and it is a popular cultivar for home gardens.

**Material and methods.** The experiment was established at Polli in the spring of 2004 with fourteen cultivars: ‘Anne-Lise’, ‘Darunok’, ‘Gerida’, ‘Jewel’, ‘Melody’, ‘Pandora’, ‘Pegasus’, ‘Rhapsody’, ‘Symphony’, ‘Syrius’, ‘Vantage’, and ‘Wega’, together with ‘Senga Sengana’ and ‘Venta’ as standards. The plot was planted with four replicates of 20 plants per cultivar with plant spacing of $1.20 \times 0.30$ m. Black plastic mulch was used only in the second experiment. During two subsequent years, 2005 and 2006, common phenological examinations, determination of winter damage, floral injury due to spring frost, yield, berry mass, taste and attractiveness evaluations were made. Damage due to diseases and pests, grey mould (with other fruit rots), and blossom weevil was evaluated. Dispersion analysis was used for statistical treating of data.

**Results.** Development of the yield over the years. In both years overwintering of cultivars was good as there was enough snow that could protect the bushes from low temperature damage (Table).

**Table.** Some biological and economically important characteristics of the strawberry cultivars. Average of the two trial years 2005–2006 m.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Inflorescences per plant</th>
<th>Flowers per plant</th>
<th>Spring frost damage in 2006</th>
<th>Damaged fruit</th>
<th>Winter hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>avg</td>
<td>avg</td>
<td>avg (%)</td>
<td>avg (%)</td>
<td>avg (%)</td>
</tr>
<tr>
<td>'Senga Sengana'</td>
<td>9.8</td>
<td>79</td>
<td>0.6</td>
<td>6.2</td>
<td>9</td>
</tr>
<tr>
<td>'Anne-Lise'</td>
<td>11.2</td>
<td>98</td>
<td>15.8</td>
<td>5.5</td>
<td>9</td>
</tr>
<tr>
<td>'Darunok'</td>
<td>9.8</td>
<td>66</td>
<td>12.5</td>
<td>1.8</td>
<td>9</td>
</tr>
<tr>
<td>'Gerida'</td>
<td>4.2</td>
<td>31</td>
<td>27.4</td>
<td>7.4</td>
<td>9</td>
</tr>
<tr>
<td>'Jewel'</td>
<td>9.8</td>
<td>81</td>
<td>10.4</td>
<td>3.6</td>
<td>7</td>
</tr>
<tr>
<td>'Melody'</td>
<td>7.5</td>
<td>58</td>
<td>5.7</td>
<td>3.4</td>
<td>9</td>
</tr>
<tr>
<td>'Pandora'</td>
<td>8.9</td>
<td>61</td>
<td>0</td>
<td>8.8</td>
<td>9</td>
</tr>
<tr>
<td>'Pegasus'</td>
<td>6.2</td>
<td>50</td>
<td>3.5</td>
<td>2.0</td>
<td>9</td>
</tr>
<tr>
<td>'Rhapsody'</td>
<td>7.5</td>
<td>54</td>
<td>2.3</td>
<td>5.0</td>
<td>7</td>
</tr>
<tr>
<td>'Symphony'</td>
<td>9.0</td>
<td>70</td>
<td>3.5</td>
<td>5.6</td>
<td>7</td>
</tr>
<tr>
<td>'Syrius'</td>
<td>8.4</td>
<td>72</td>
<td>1.9</td>
<td>2.8</td>
<td>9</td>
</tr>
<tr>
<td>'Vantage'</td>
<td>6.9</td>
<td>56</td>
<td>10.3</td>
<td>2.0</td>
<td>7</td>
</tr>
<tr>
<td>'Venta'</td>
<td>8.5</td>
<td>64</td>
<td>7.8</td>
<td>3.8</td>
<td>9</td>
</tr>
<tr>
<td>'Wega'</td>
<td>12.6</td>
<td>102</td>
<td>22.1</td>
<td>4.0</td>
<td>9</td>
</tr>
</tbody>
</table>

In 2005 the strawberries started to flower earlier than in 2006, but the beginning of fruit ripening was rather simultaneous in both years. The basis for a good yield is laid already during flowering. Within the range of tested cultivars the number of inflorescences and number of flowers was higher in 2006 than in 2005, only ‘Anne-Lise’ and ‘Wega’ had more flowers per plant in 2005. ‘Wega’ had also the highest
average numbers of flowers and inflorescences per plant over two years (Table). The overall average yield was three times lower in 2006 than in 2005. Average of number of flowers per inflorescence (including data of two years) varied between 7.0–8.9 and there was no significant difference between cultivars. In 2005 majority of cultivars had more flowers per inflorescence than in 2006.

In 2005 there no spring frost damage was observed in blossoms and damage from blossom weevil was not remarkable either, being the highest with ‘Venta’ (4.8%). In 2006, however, there was spring frost damage in blossoms and the damage was most severe in cultivars ‘Gerida’ and ‘Wega’. The same pattern could be observed with blossom weevil damage; in 2006 it was remarkably higher than in 2005; overall average was more than four times higher – 2.0 and 9.5%, respectively (Fig. 1). On the average cultivars ‘Anne-Lise’, ‘Darunok’, ‘Gerida’, ‘Melody’, ‘Rhapsody’, ‘Syriusz’ and ‘Venta’ were more affected than standard cv. ‘Senga Sengana’. The most severe damage was observed in cv. ‘Venta’ (12.3%). ‘Senga Sengana’ and ‘Wega’ were relatively fairly affected in both years.

Effect of the year and cultivar.

* indicates significant (p < 0.05) differences from the standard ‘Senga Sengana’.

In 2005 all cultivars gave rather good yields. The yield of three cultivars ‘Wega’, ‘Jewel’ and ‘Venta’ exceeded this one of the standard cv. ‘Senga Sengana’ (Fig. 2). Very modest yields were collected from ‘Gerida’ and ‘Rhapsody’ due to severe damage from strawberry mite (*Phytonemus pallidus* ssp. *fragariae* Zimm.) (damage rates 9 and 7, respectively).

Although the number of flowers was high in 2006 the yield remained at extraordinary low level. To some extent the plants were less vigorous due to the strawberry mite damage the year before followed by the unfavourable growth period of drought and hot weather. The average yield of the cultivars was almost three times lower than in 2005. In this year the best yielding one was ‘Syriusz’. On the average over the years the best yielding cultivars were ‘Wega’, ‘Venta’ and ‘Syriusz’.
In 2005 average berry weight was twice as high as in 2006. In both years of the experiment the largest fruits produced cultivars ‘Pandora’, ‘Syriusz’ and ‘Venta’. Cultivars ‘Pandora’, ‘Pegasus’, ‘Rhapsody’, ‘Symphony’, ‘Syriusz’ and ‘Wega’ on the average developed larger fruits than the standard cv. ‘Senga Senga’. The percentage of damaged fruit was higher in 2005, but still remained on a rather low level. More damaged fruits produced cultivars ‘Pandora’ and ‘Gerida’ – 13.6 and 12.9%, respectively. The cause of high percentage of inferior quality fruit of ‘Pandora’ was fruit cracking after heavy rainfall; the fruits of ‘Gerida’ were spoilt as a result of strawberry mite damage.

**Discussion.** The number of flowers and inflorescences was higher in 2006 than 2005 – in the second year of growth the plant had more crowns than in the first year. On the other hand, in August 2004, that is the period of flower bud initiation, it was cooler and dryer in than in the similar period in 2005, thus conditions were more favourable for termination of growth and floral bud differentiation (Kinnanen, Säko, 1979). It is
known that there is a strong positive correlation between the number of flowers and
the yield (Kikas, Libek, 2005). The current trial did not reveal this correlation as there
were other factors that had significant impact on the yield levels.

The years during the trial were very different in respect of weather conditions,
revealing the negative impact of unfavourable climate on fruit weight and formation
of the yield. At the same time the cultivars responded diversely to the unfavourable
weather conditions. In spite of disadvantageous weather, cv. ‘Syriusz’ gave a
considerable yield in 2006. However, ‘Wega’, which had given very good yield in
2005, had a very low yield in 2006. Based on the data from the first year, cv. ‘Jewel’
was considered to be one of the best in the range of cultivars tested, as it had shown
a good yield with tasty fruits that were firm and easy to handle, but in 2006 its yield
remained moderate. In 2006 the yield level was substantially reduced also by the spring
frost damage. The endurance of blossoms at freezing temperatures differs with cultivars.
‘Gerida’ and ‘Wega’ had blossoms that were most sensitive to frost. In the same year
rather severe damage from blossom weevil also occurred. Blossom weevil is known
to be a cultivars specific pest (Simpson et. al., 1997; Kikas, Libek, 2005; Libek and
Kikas, 2002). It has been noted earlier that blossom weevil likes cv. ‘Venta’ (Kikas,
Libek, 2002). Similar effect was observed in the present study were ‘Venta’ was most
severely affected and ‘Senga Sengana’ was not remarkably affected by blossom weevil.
Rather severe damage from blossom weevil occurred also with cultivars ‘Darunok’
and ‘Gerida’; cv. ‘Jewel’ and ‘Vantage’ got relatively fair damage. On the average
over the two years ‘Wega’, ‘Venta’ and ‘Syriusz’ gave better yields than the standard
‘Senga Sengana’. Two of the above mentioned cultivars had given good yields also in
the similar trial in Lithuania (Uselis, Rašinskienė, 2000). ‘Jewel’ also had rather high
yield. Similar to our observations on blossom weevil damage on ‘Pegasus’ and ‘Wega’
that remained at the same level with ‘Senga Segana’ was reported in the Polish trial
(Labanowska, 2004). Fruit weight is a cultivars specific feature that can be affected by
the growth conditions. The average fruit weight is mostly influenced by the damage
caused either by spring frost or blossom weevil. When first emerging flowers are
affected, it influences significantly the average fruit weight and yield in general as
the subsequently emerging fruit never grows into the size the first fruits would have
reached. (Faby et al., 2004). When the last blossoms are affected the damaged is not
remarkable. In this trial ‘Pandora’, ‘Syriusz’ and ‘Venta’ produced the largest fruits.

On the base of these preliminary results from the range of cultivars that were
included in the trial, for the first time two would be suitable for growing in Estonian
climatic conditions. The two are ‘Syriusz’, a cultivar that had stable and high yields, and
‘Jewel’ – also high yieling cultivar with attractive firm fruit, although it responded more
easily to unfavourable weather conditions. ‘Jewel’ was also less affected by the blossom
weevil. ‘Wega’ was a good yielding cultivar but reacted strongly to unfavourable
environmental conditions. Its fruits were not firm and attractive. ‘Pandora’ is a very
late cultivar bearing large beautiful fruit. In order to give final estimates about the
suitability of these cultivars for growing in Estonia a longer testing period is needed.
References


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BRAŠKIŲ VEISLIŲ ĮVERTINIMAS ESTIJOJE
Santrauka


Reikšminiai žodžiai: Anthonomus rubi, braškių derlius, uogų masė, žiedai.
INVESTIGATION OF GROWTH VIGOUR, YIELDING AND BERRY QUALITY OF THE PROMISING RASPBERRY CULTIVARS IN LITHUANIA

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12 raspberry cultivars developed in Russia, Ukraine, Estonia, England, Canada and USA were investigated at the Lithuanian Institute of Horticulture in 2003–2006. The most winterhardy were standard cultivars ‘Novokitajevskaja’ (stem cold injury – 0.5 scores) and ‘Beglianka’ (stem cold injury – 0.4–0.9 scores). Stems of raspberries ‘Meeker’ (2.6–4.5 scores) and ‘Glen Moy’ (2.2–3.7 scores) were the most cold injured. The most productive raspberry cultivars were ‘Siveli’, ‘Novokitajevskaja’, ‘Zorinka’, ‘Beglianka’, ‘Sputnica’, ‘Zviozdocka’ and ‘Husar’ (5.08–4.11 t ha⁻¹), the least productive ones – ‘Meeker’ and ‘Glen Moy’ (1.81–2.87 t ha⁻¹). The biggest berry weight was of cultivars ‘Glen Moy’, ‘Aborigen’, ‘Miraz’ and ‘Meeker’ (2.04–2.68 g). Berries of cultivar ‘Otava’ distinguish themselves with the significantly biggest amount of dry soluble solids (13.7), bigger amount of sugars (7.02%), ascorbic acid (24.4 mg 100 g⁻¹) and anthocyanins (22.4 mg 100 g⁻¹). In the berries of cultivar ‘Glen Moy’ it was found the bigger amount of anthocyanins (36.96 mg 100 g⁻¹) and ascorbic acid (23.6 mg 100 g⁻¹). The berries of cultivar ‘Husar’ distinguish themselves with big amount of dry soluble solids (11.8%), ascorbic acid (20.4 mg 100 g⁻¹) and anthocyanins (32.03 mg 100 g⁻¹). The berries of cultivars ‘Miraz’ and ‘Meeker’ distinguish themselves with big amount of ascorbic acid – 24.80 mg 100 g⁻¹ and 24.4 mg 100 g⁻¹, respectively.

Key words: berry biochemical composition, berry weight, cultivars, raspberry, sensual evaluation, yield, winterhardiness.

Introduction. Raspberries are valuable berry plants, which distinguish themselves with unique healing and dietetic properties. Raspberries start yielding soon, already in the second year after planting. Their berries ripen early – after honeysuckle and strawberries they prolong the season of fresh berries. Raspberry berries are aromatic, there are many biologically active substances in them and the ratio of sugars and organic acids produces good taste and tone up human organism (Причко, 1999).

From the biochemical point of view, raspberries are valuable for ascorbic acid (50–70 mg 100 g⁻¹), catechins (up to 80 mg 100 g⁻¹), anthocyanins (100–250 mg 100 g⁻¹), which distinguish themselves with antioxidative properties and determine raspberry colour and berry attractiveness (Weber, Rui Hai Liu, 2002). The composition of these colouring substances depends on genetic properties of cultivars (Begona et al., 1999). These are more important vitamins in raspberry cultivars – B₉, B₁₂, E, etc. (Казаков, 1994). These berries have healing properties. Their oil-cakes, in which elatic acid
is being found, are especially valuable. It is used in medicine as extremely strong anticancer compound (Loarca-Pina et al., 1998).

Berry quality and biochemical composition of raspberries strongly depend on cultivar properties and growth conditions (Morell, Harrison, 1999). Wide assortment of raspberry cultivars is being investigated in Lithuania already for many years. In raspberry berries grown under our climatic conditions there are: 10.5–12.5% of dry soluble solids, 4.9–6.6% of sugars, and 20.0–27.8 mg 100 g⁻¹ of ascorbic acid. They are estimated as averagely (1.7–2.2%) acid (Viškelis et al., 2006). Colouring substances, anthocyanins, already are investigated in the berries of different cultivars (Viškelis, Bobinaitė, 2007).

Comercial raspberry plantations in Lithuania cover approximately 260 ha. Most raspberry cultivars from European countries and USA are productive, but not winterhardy and sensitive to temperature variation (Gwozdecki, 1993; Zornić et al., 1993; Исачкин et al., 2001). This problem especially urgent under climatic conditions of our country, where often it becomes warmer in winter and after that suddenly gets colder. Raspberry productivity depends on their winterhardiness (Казаков, 1994; Kikas et al., 2002; Danek, 2004). Light, humus rich soils are suitable for raspberries. Many raspberry plantations in Lithuania are planted in the soils of hard granulometric composition. Therefore it is very difficult to evaluate biological-economical plant properties, when growing raspberries under such agroclimatic conditions. Raspberry cultivars created in Russia, Ukraine, Estonia, England, Canada and USA, were chosen for investigations (Исачкин et al., 2001; Kikas et al., 2002; Danek, 2004; Подорожный, 2004).

The aim of the investigation – to investigate and evaluate growth vigour, yield and berry quality of new promising raspberry cultivars under Lithuanian agroclimatic conditions.


Raspberries were planted at the distances of 3.0 x 0.5 m in the spring of 2001. They were grown according to intensive berry growing technologies. The width of plant belt was about 60 cm, the number of stems unfixed. Length and width of record plot – 3 m, area – 9 m². Experiment was carried out in for replications.

Soil – epicari-endohypogleyic cambisoil (RDg4-k1), average hard and hard loam. Agrochemical soil characteristic: pH_kCl – 7.1, humus – 2.3%, P₂O₅ – 290 mg kg⁻¹, K₂O – 180 mg kg⁻¹. Traits evaluated in the experiment: the number of raspberry annual stems in 1 m of the plant belt, their diameter and height; stem cold injury after cold winters (evaluated in 5 scores scale: 0 – not injured, 5 – completely dead); yield (t ha⁻¹); the average berry weight (during the first, third and the last berry picking, applying randomized method for selection of 100 berries in plot).

The content of soluble solids was measured by digital refractometer (ATAGO PR-32), sugar content (inverted sugar and sucrose) – by Bertrand method, titratable acidity – titrating by 0.1 N NaOH solution and evaluating the amount of citric acid
Ascorbic acid (vitamin C) content was measured by titration with 2,6-dichlorophenolindophenol sodium salt solution, using chloroform for intensely colored extracts (AOAC, 1990). The anthocyanins were extracted from 5 g of berries with acidified (0.1N HCl, v/v) 95% (v/v) food grade ethanol. The berries were ground with quartz sand and the extraction was continued with 20 ml portions of solvent until the sample became colorless. The extract was diluted with acidified ethanol. The absorption was measured on a spectrophotometer (Genesys-5) at 535 nm. The concentration of anthocyanins was determined from the calibration curve, which was constructed by measuring the absorption of cyd-3-glu (Wrolstad, 1976).

The data of investigations were statistically evaluated by dispersion analysis method for the blocks of randomized replications.

Meteorological conditions during investigations were rather different: the most favourable conditions for raspberry growing and yielding were in 2006, the least favourable – 2003. In May and June of 2003 it was dry. July was rainy, August and September – rather warm and dry. In the spring of 2004 there were recorded frosts up to -4.9°C. It was extremely dry in April: precipitation reached only 3.8 mm (multiannual average – 71.8 mm). July was dryer and colder than usually, August – very rainy. In 2005 July was very dry and hot; August was cooler and rainy. June of 2006 was dry, July – warmer and in half dryer than usually, August and September – warm and more rainy in comparison with multiannual data. Cold injury of raspberry stems was evaluated after the winter of 2003–2004. December of 2003 was rather warm, and in January suddenly it got cold. Nevertheless the stems of most raspberries were stronger injured in the winter of 2004–2005, when at the end of January and especially in February sudden fluctuations of temperatures occurred (it was significantly colder at the first half of May also).

**Results. Raspberry growth vigour and stem cold injury.**

During the period of investigations in 2004–2006 (Table 1) out of 12 raspberry cultivars ‘Siveli’ plants height was lowest (127.8 cm), and the stems of 8 cultivars (‘Glen Moy’, ‘Sputnica’, ‘Otava’, ‘Zviozdocka’, ‘Miraz’, ‘Zorinka’, ‘Beglianka’, ‘Husar’) were essentially higher (150–161 cm) in comparison with the standard cultivar ‘Novokitajevskaja’ (140.2 cm).

Stem diameter of raspberry cultivars ‘Husar’, ‘Sputnica’, ‘Miraz’ and ‘Meeker’ was bigger (11.4–11.6 mm) in comparison with the standard cultivar (10.6 mm). Raspberry belt density is characterized by the number of stems per 1 m of plant belt. In 2004–2006 raspberry cultivars ‘Glen Moy’, ‘Zviozdocka’ and ‘Zorinka’ produced significantly more (44.4–54.4 unt. m⁻¹), and ‘Miraz’ and ‘Siveli’ – less (25.5 unt. m⁻¹) annual stems per 1 m of plant belt in comparison with the standard cultivar (33.3 unt. m⁻¹).

Very important trait of raspberry is winterhardiness. After the winter of 2003–2004 stems of raspberry cultivars ‘Novokitajevskaja’, ‘Husar’ and ‘Beglianka’ were the least injured (cold injury less than 1 score) (Table 1). Stems of raspberry cultivars ‘Meeker’ (2.6 scores), ‘Glen Moy’ and ‘Miraz’ (2.2 scores) were injured most of all. Raspberries of most cultivars were more injured in the winter of 2004–2005. Even though that winter wasn’t very cold, weather was changeable and after frequent thaws, frosts began in February. Raspberries, which had long rest period, suffered least of all. Raspberry cultivars ‘Siveli’, ‘Beglianka’ and ‘Novokitajevskaja’
were the most winterhardy (stem injury – 0.1–0.5 scores), and the stems of raspberry cultivars ‘Meeker’ (4.5 scores) and ‘Glen Moy’ (3.7 scores) were cold injured most of all. Raspberry stems of 5 cultivars (‘Otava’, ‘Husar’, ‘Aborigen’, ‘Zviozdocka’ and ‘Sputnica’) were injured at 2–2.4 scores. This shows that raspberries are rather sensitive to sudden temperature fluctuations in winter, especially at the end of winter. During four years of investigations raspberry cultivars ‘Novokitajevskaja’ and ‘Beglianka’ were the most winterhardy.

Table 1. Indices of raspberry growth vigour and cold injury of raspberry stems in winter

<table>
<thead>
<tr>
<th>Cultivar Yeisle</th>
<th>Stem height (cm)</th>
<th>Stem diameter (mm)</th>
<th>Number of stems, mt.</th>
<th>Cold injury of stems, scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Novokitajevskaja’ (standard)</td>
<td>149.2</td>
<td>10.6</td>
<td>33.3</td>
<td>0.5</td>
</tr>
<tr>
<td>‘Aborigen’</td>
<td>141.2</td>
<td>10.9</td>
<td>27.3</td>
<td>1.3</td>
</tr>
<tr>
<td>‘Beglianka’</td>
<td>152.2</td>
<td>11.3</td>
<td>34.4</td>
<td>0.9</td>
</tr>
<tr>
<td>‘Glen Moy’</td>
<td>161.0</td>
<td>11.3</td>
<td>44.4</td>
<td>2.2</td>
</tr>
<tr>
<td>‘Husar’</td>
<td>150.0</td>
<td>11.6</td>
<td>27.3</td>
<td>0.6</td>
</tr>
<tr>
<td>‘Meeker’</td>
<td>142.2</td>
<td>11.4</td>
<td>27.6</td>
<td>2.6</td>
</tr>
<tr>
<td>‘Miraz’</td>
<td>155.8</td>
<td>11.5</td>
<td>25.5</td>
<td>2.2</td>
</tr>
<tr>
<td>‘Otava’</td>
<td>157.0</td>
<td>11.1</td>
<td>38.2</td>
<td>1.4</td>
</tr>
<tr>
<td>‘Siveli’</td>
<td>127.8</td>
<td>9.8</td>
<td>25.5</td>
<td>1.5</td>
</tr>
<tr>
<td>‘Sputnica’</td>
<td>159.5</td>
<td>11.6</td>
<td>35.0</td>
<td>1.1</td>
</tr>
<tr>
<td>‘Zoriska’</td>
<td>155.2</td>
<td>10.1</td>
<td>54.4</td>
<td>1.2</td>
</tr>
<tr>
<td>‘Zviozdocka’</td>
<td>156.5</td>
<td>10.2</td>
<td>48.8</td>
<td>1.7</td>
</tr>
</tbody>
</table>

‘Beglianka’ was significantly higher (3.87 t ha\(^{-1}\)) in comparison with the standard cultivar ‘Novokitajevskaja’ (2.54 t ha\(^{-1}\)), and the yield of cultivars ‘Aborigen’, ‘Meeker’ and ‘Miraz’ (0.67–1.4 t ha\(^{-1}\)) – lower (Table 2). In 2004 raspberries of 8 cultivars yielded essentially worse than the standard cultivar. Stem cold injury in the winter of 2003–2004 was the reason of significantly decrease of cultivars ‘Miraz’ and ‘Meeker’ yield. After the changeable winter of 2004–2005 plants of cultivars ‘Meeker’ and ‘Glen Moy’ were cold injured most of all and produced the lowest yield (1.42–2.17 t ha\(^{-1}\)). The highest yield (7.08–6.74 t ha\(^{-1}\)) produced raspberries of the most winterhardy cultivars ‘Siveli’, ‘Beglianka’ and ‘Novokitajevskaja’. The most favourable weather for the yielding of raspberries was in 2006: the average yield of 12 raspberry cultivars reached 6.06 t ha\(^{-1}\) (in 2003–2.26 t ha\(^{-1}\)).
Table 2. Raspberry yield (t ha\textsuperscript{-1})

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>’Novokitajevska’</td>
<td>2.54</td>
<td>4.74</td>
<td>6.74</td>
<td>6.25</td>
<td>5.07</td>
</tr>
<tr>
<td>(standard / standartinė)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>’Aborigen’</td>
<td>0.67</td>
<td>1.95</td>
<td>4.08</td>
<td>5.53</td>
<td>3.06</td>
</tr>
<tr>
<td>’Beglianka’</td>
<td>3.87</td>
<td>3.70</td>
<td>6.58</td>
<td>5.42</td>
<td>4.89</td>
</tr>
<tr>
<td>’Glen Moy’</td>
<td>1.80</td>
<td>2.35</td>
<td>2.17</td>
<td>5.17</td>
<td>2.87</td>
</tr>
<tr>
<td>’Husar’</td>
<td>2.03</td>
<td>4.17</td>
<td>3.70</td>
<td>6.52</td>
<td>4.11</td>
</tr>
<tr>
<td>’Meeker’</td>
<td>1.24</td>
<td>1.81</td>
<td>1.42</td>
<td>2.76</td>
<td>1.81</td>
</tr>
<tr>
<td>’Miraz’</td>
<td>1.40</td>
<td>1.80</td>
<td>4.77</td>
<td>4.10</td>
<td>3.02</td>
</tr>
<tr>
<td>’Otava’</td>
<td>2.48</td>
<td>2.71</td>
<td>4.31</td>
<td>3.38</td>
<td>3.72</td>
</tr>
<tr>
<td>’Siveli’</td>
<td>2.70</td>
<td>2.58</td>
<td>7.08</td>
<td>5.88</td>
<td>5.08</td>
</tr>
<tr>
<td>’Sputnica’</td>
<td>2.67</td>
<td>3.58</td>
<td>4.00</td>
<td>6.98</td>
<td>4.31</td>
</tr>
<tr>
<td>’Zorinka’</td>
<td>2.55</td>
<td>4.30</td>
<td>4.73</td>
<td>8.05</td>
<td>4.91</td>
</tr>
<tr>
<td>’Zviozdocka’</td>
<td>3.17</td>
<td>2.37</td>
<td>2.74</td>
<td>8.54</td>
<td>4.21</td>
</tr>
<tr>
<td>LSD\textsubscript{05} / R\textsubscript{05}</td>
<td>1.101</td>
<td>1.13</td>
<td>2.124</td>
<td>2.077</td>
<td>1.026</td>
</tr>
</tbody>
</table>

‘Zviozdocka’ produced significantly higher yield (8.54 t ha\textsuperscript{-1}) in comparison with the standard cultivar (6.25 t ha\textsuperscript{-1}), ‘Meeker’ (2.76 t ha\textsuperscript{-1}) and ‘Miraz’ (4.10 t ha\textsuperscript{-1}) yielded essentially less. The yield of the raspberry cultivars ‘Zorinka’ and ‘Zviozdocka’ depend on the stem number per area unit. Stem diameter had no effect on raspberry yield. During four years of investigations the highest yield produced raspberry cultivars ‘Siveli’ (5.08 t ha\textsuperscript{-1}), ‘Novokitajevska’ (5.07 t ha\textsuperscript{-1}), ‘Zorinka’ (4.91 t ha\textsuperscript{-1}), ‘Beglianka’ (4.89 t ha\textsuperscript{-1}), ‘Sputnica’ (4.31 t ha\textsuperscript{-1}), ‘Zviozdocka’ (4.21 t ha\textsuperscript{-1}) and ‘Husar’ (4.11 t ha\textsuperscript{-1}). The lowest yield because of the stems cold injury was of cultivars ‘Meeker’ (1.81 t ha\textsuperscript{-1}) and ‘Glen Moy’ (2.87 t ha\textsuperscript{-1}).

During the years of investigations raspberry cultivars ‘Glen Moy’, ‘Aborigen’, ‘Miraz’ and ‘Meeker’ produced significantly biggest berries (2.04–2.68 g) in comparison with the standard cultivar ‘Novokitajevska’ (1.72 g) (Table 3). The average berry weight of the other investigated raspberry cultivars didn’t differ significantly.

Raspberry berry quality indices. Sensual indices. Sensual quality of raspberry berries (taste and appearance) was established by degustation. In comparison with the standard, the best appearance had berries of cultivars ‘Miraz’ (4.8 scores), ‘Husar’ (4.5 scores), ‘Otava’, ‘Siveli’ (4.4 scores), ‘Glen Moy’, ‘Sputnica’ (4.3 scores). Raspberry cultivars ‘Otava’ and ‘Siveli’ distinguished themselves with the best taste (4.6 scores). Berries of cultivars ‘Beglianka’, ‘Husar’ and ‘Miraz’ distinguished themselves with taste properties. It was established that in comparison with the standard, raspberry cultivars ‘Miraz’, ‘Husar’, ‘Otava’, ‘Siveli’ and ‘Meeker’ distinguished themselves with significantly the best sensual properties. Poor appearance of raspberry cultivars ‘Zorinka’ and ‘Zviozdocka’ negatively influenced total evaluation of their sensual properties. Both appearance and taste of the berries of cultivar ‘Aborigen’ were evaluated the worst (Table 3).
Table 3. Average berry weight (g) and sensual evaluation of raspberry berries, scores

<table>
<thead>
<tr>
<th>Cultivar Veisle</th>
<th>Average berry weight (g)</th>
<th>Appearance</th>
<th>Taste</th>
<th>Total evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Novokitajevskaja’ (standard)</td>
<td>1.72</td>
<td>4.0</td>
<td>4.3</td>
<td>4.2</td>
</tr>
<tr>
<td>‘Aborigen’</td>
<td>2.16</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td>‘Beglianka’</td>
<td>1.67</td>
<td>4.0</td>
<td>4.5</td>
<td>4.2</td>
</tr>
<tr>
<td>‘Glen Moy’</td>
<td>2.04</td>
<td>4.3</td>
<td>4.2</td>
<td>4.3</td>
</tr>
<tr>
<td>‘Husar’</td>
<td>1.91</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>‘Meeker’</td>
<td>2.69</td>
<td>4.0</td>
<td>4.4</td>
<td>4.4</td>
</tr>
<tr>
<td>‘Miraz’</td>
<td>2.51</td>
<td>4.8</td>
<td>4.5</td>
<td>4.7</td>
</tr>
<tr>
<td>‘Otava’</td>
<td>1.75</td>
<td>4.4</td>
<td>4.6</td>
<td>4.5</td>
</tr>
<tr>
<td>‘Sivel’</td>
<td>1.98</td>
<td>4.4</td>
<td>4.6</td>
<td>4.4</td>
</tr>
<tr>
<td>‘Sputnica’</td>
<td>1.84</td>
<td>4.3</td>
<td>4.2</td>
<td>4.3</td>
</tr>
<tr>
<td>‘Zorinka’</td>
<td>1.56</td>
<td>3.9</td>
<td>4.4</td>
<td>4.2</td>
</tr>
<tr>
<td>‘Zviozdocka’</td>
<td>1.60</td>
<td>3.8</td>
<td>4.3</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Biochemical composition. Raspberry berry biochemical composition shows their quality best of all. Berry biochemical investigations were carried out according to the methods used at the Laboratory of Biochemistry and Technology at the Lithuanian Institute of Horticulture. There was found in raspberry berries rather big amount of dry soluble solids, which is one of the most important berry quality evaluation indices. According to the data of our investigations, the berries of raspberry cultivars accumulate from 9.9% up to 13.7% of dry soluble solids. In comparison with the standard, the berries of cultivar ‘Novokitajevskaja’, it was found significantly bigger amount of dry soluble solids in cultivars ‘Sivel’ and ‘Otava’ – 13.7–12.7%. Berries of cultivars ‘Beglianka’, ‘Glen Moy’ and ‘Aborigen’ accumulate significantly less amount of dry soluble solids. In raspberries of other cultivars their amount vary insignificantly – from 11.2% up to 11.8% (Table 4). According to the accumulated amount of sugars, berries of cultivars significantly exceed standard. There weren’t established significant differences in the amount of sugars between other raspberry cultivars and berries of cultivar ‘Novokitajevskaja’. According to our data, raspberry berries accumulate small amount of ascorbic acid, on the average 21.4 mg 100 g⁻¹. With the exception of raspberry cultivars ‘Beglianka’, ‘Sputnica’ and ‘Zviozdocka’, in comparison with the standard, it was accumulated significantly bigger amount of ascorbic acid in the berries of other cultivars. Berries of cultivars ‘Miraz’ (24.8 mg 100 g⁻¹), ‘Meeker’ and ‘Otava’ (24.4 mg 100 g⁻¹) distinguished themselves with the biggest amount of vitamin C.

Titratable acidity describes the total amount of organic acids in berries. In comparison with the standard, in the cultivars ‘Beglianka’, ‘Sputnica’ and ‘Zviozdocka’ there were established significantly bigger amounts of titratable acidity. In the berries of other cultivars, in comparison with cultivar ‘Novokitajevskaja’, significant differences among the amounts of organic acids weren’t detected (Table 4).
Colouring substances of the berries of these cultivars, anthocyanins, were investigated for the first time in Lithuania. It was established in raspberry berries total amount of anthocyanins, which determine their colour. The biggest amount of red pigments was found in cultivar ‘Sputnica’ (42.11 mg 100 g\(^{-1}\)), which berries are dark red (Table 4). Also rather big amount of anthocyanins was established in raspberries ‘Glen Moy’ and ‘Husar’ – 36.96 and 32.03 mg 100 g\(^{-1}\). In comparison with berries of cultivar ‘Novokitajevskaja’, significantly smallest amounts of anthocyanins were established in red raspberries of cultivar ‘Meeker’. There weren’t found significant differences among amounts of pigments accumulated by ‘Zorinka’, ‘Zviozdocka’ and standard. Yellow raspberry cultivar ‘Beglianka’ also accumulates anthocyanins, but their amount is insignificant and reaches only 2.10 mg 100 g\(^{-1}\) (Table 4).

**Discussion.** It was established that productivity of 12 raspberry cultivars didn’t depend on stem height, diameter and their number per unit of area. For example, raspberry cultivar ‘Siveli’ grew the lowest stems and the least their number per unit of area, but its berry yield was the biggest one. Raspberry productivity was mostly influenced by stem winter injury. Raspberry cultivars, which have long deep rest period, are the most winterhardy (Казаков, 1994). Raspberry stems tolerate rather low temperatures, but they are sensitive to sudden temperature fluctuations in winter, especially at its end. Since during the recent years our winters become more changeable and after often thaws frosts start, raspberries winterhardiness becomes one of the most important biological-economic properties. During four years of investigations raspberry cultivars ‘Novokitajevskaja’ and ‘Beglianka’ distinguish themselves with winterhardiness. During this period of investigations cultivars ‘Siveli’, ‘Novokitajevskaja’, ‘Zorinka’, ‘Beglianka’, ‘Sputnica’, ‘Zviozdocka’ and ‘Husar’ yielded the most abundantly (5.08–4.11 t ha\(^{-1}\)). Because of the stem winter injury the least yield produced cultivars ‘Meeker’ and
‘Glen Moy’ (1.81–2.87 t ha⁻¹). Moreover, Estonian scientists emphasized that raspberry productivity strongly depends on winterhardiness of the cultivar (Kikas et al., 2002). According to our data, the biggest berries were produced by extremely winterhardy raspberry cultivars ‘Meeker’ (USA), ‘Glen Moy’ (England), and cultivars ‘Aborigen’ and ‘Miraz’ created by plant breeder V. Kicina in Russia. Scientists of South Russia out of 11 raspberry cultivars according to the complex of properties distinguished cultivar ‘Husar’ as one of the best (Подорожный, 2004).

Raspberries are averagely vitaminous berries (Viškelis, Česnauskas, 2000; Wieneniska, Danek, 1999; Kampuse et al., 2002; Wieneniska et al., 2005). In our investigations it was found on the average 20.4 mg 100 g⁻¹ of ascorbic acid. Its amount in the berries of perspective cultivars fluctuates from 15.2 to 24.8 mg 100 g⁻¹.

It was observed in the investigations and indicated in the literature that pigment concentration in berries strongly depends on genetic properties of cultivars (Kampuse et al., 2002; Wieneniska et al., 2005). It was established that this index varies in wide scope – 15.2–129.5 mg 100 g⁻¹ (Table 4).

Sugar accumulation in raspberry berries is influenced by cultivar properties and methods of growing. Polish scientists observed that sugar amount in raspberries varies from 7.6% up to 10.7% (Bieniaz et al., 2005). According to our data, during the years of investigations berries accumulated 5.2–7.24% of sugars. It is stated in literature that titratable raspberry acidity varies in wide scope – 1.54–3.16% (Tešovic et al., 2003). In the berries of the investigated cultivars the total amount of the organic acids fluctuates from 1.43% up to 2.14% (Table 4). Such variation of organic acids in the case of different sugar amount influences raspberry sweetness degree and taste. This index in the cultivars of the investigated raspberries varies from 2.4 (‘Sputnica’) up to 4.4 (‘Siveli’).

Conclusions. 1. Stems of raspberry cultivars ‘Glen Moy’, ‘Sputnica’, ‘Otava’, ‘Zviozdocka’, ‘Miraz’, ‘Zorinka’, ‘Beglianka’ and ‘Husar’ were essentially higher (150–161 cm) than of the standard cultivar ‘Novokitajevskaja’. Stems of cultivar ‘Siveli’ were the lowest (127.8 cm). Stem diameter of cultivars ‘Husar’, ‘Sputnica’, ‘Miraz’ and ‘Meeker’ was the biggest (11.4–11.6 mm). Cultivars ‘Glen Moy’, ‘Zviozdocka’ and ‘Zorinka’ produced significantly more (44.4–54.4 unt. m⁻¹) and cultivars ‘Miraz’ and ‘Siveli’ – less (25.5 unt. m⁻¹) of annual stems per 1 m plant belt than the standard cultivar.

2. The most winterhardy cultivars were ‘Novokitajevskaja’ and ‘Beglianka’ (stem injury – 0.4–0.9 scores). Stems of raspberry cultivars ‘Meeker’ and ‘Glen Moy’ were the most cold injured (2.2–4.5 scores).

3. Raspberry cultivars ‘Siveli’, ‘Zorinka’, ‘Beglianka’, ‘Sputnica’, ‘Zviozdocka’ and ‘Husar’ were of the same productivity as standard cv. ‘Novokitajevskaja’ (5.08–4.11 t ha⁻¹). The least productive ones were ‘Meeker’ and ‘Glen Moy’ (1.81–2.87 t ha⁻¹). Berries of cultivars ‘Glen Moy’, ‘Aborigen’, ‘Miraz’ and ‘Meeker’ were the biggest (2.04–2.68 g). Berries of cultivars ‘Miraz’, ‘Husar’ and ‘Otava’ had the best taste properties.

4. Berries of cultivar ‘Otava’ distinguish themselves with the significantly biggest amount of dry soluble solids (13.7%), bigger amount of sugars (7.02%), ascorbic acid (24.4 mg 100 g⁻¹) and anthocyanins (22.4 mg 100 g⁻¹). In the berries of cultivar ‘Glen
Moy’ it was found the bigger amount of anthocyanins (36.96 mg 100 g$^{-1}$) and ascorbic acid (23.6 mg 100 g$^{-1}$). The berries of cultivar ‘Husar’ distinguish themselves with big amount of dry soluble solids (11.8%), ascorbic acid (20.4 mg 100 g$^{-1}$) and anthocyanins (32.03 mg 100 g$^{-1}$). The berries of cultivars ‘Miraz’ and ‘Meeker’ distinguish themselves with big amount of ascorbic acid – 24.80 mg 100 g$^{-1}$ and 24.4 mg 100 g$^{-1}$, respectively.

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References


SODININKYSTĖ IR DARŽININKYSTĖ. MOKSLO DARBAI. 2007. 26(3).

PERSPEKTYVIŲ LIETUVOJE AVIEČIŲ VEISLIŲ AUGUMO, DERĖJIMO IR UOGŲ KOKYBĖS TYRIMAI

L. Buskienė, M. Rubinskienė, P. Viškelis

Santrauka

Lietuvos sodininkystės ir daržininkystės institute 2003–2006 m. buvo iširta 12 aviečių veislų, sukurtų Rusijoje, Ukrainoje, Estijoje, Anglioje, Kanadoje ir JAV. Išvermingiausios žiemą buvo standartinės ‘Novokitajevskaja’ (stiebų pašalimas – 0,5 balo) ir ‘Beglianka’ (stiebų pašalimas – 0,4–0,9 balo) veislų avietės. Labiausiai pašalo ‘Meeker’ (2,6–4,5 balo) bei ‘Glen Moy’ (2,2–3,7 balo) aviečių stiebai. Derlingiausios aviečių veislės buvo ‘Siveli’, ‘Novokitajevskaja’, ‘Zorinka’, ‘Beglianka’, ‘Sputnica’, ‘Zviozočka’ ir ‘Husar’ (5,08–4,11 t ha–1), mažiausiai derėjo ‘Meeker’ ir ‘Glen Moy’ (1,81–2,87 t ha–1) avietės. ‘Glen Moy’, ‘Aborigen’, ‘Miraž’ ir ‘Meeker’ aviečių uogos buvo stambiausios (2,04–2,68 g). ‘Otava’ veislės uogos gausiu tirpių sausųjų medžiagų (11.8%), askorbo rūgšties (20,4 mg 100 g–1) ir antocianinų (92,0 mg 100 g–1) kiekiu. Daugiausiai antocianinų (129,5 mg 100 g–1) ir daugiau askorbo rūgšties (23,6 mg 100 g–1) rasta ‘Glen Moy’ aviečių uogose. Gausiu tirpių sausųjų medžiagų (11.8%), askorbo rūgšties (20,4 mg 100 g–1) ir antocianinų (92,0 mg 100 g–1) kiekiu pasižymėjo ‘Husar’ uogos. Askorbo rūgšties kiekiu išsiskyrė ‘Miraž’
(24,80 mg 100 g\textsuperscript{−1}) ir ‘Meeker’ (24,4 mg 100 g\textsuperscript{−1}) avietės.

Reikšminiai žodžiai: avietės, derlius, ištvermingumas žiemą, juslinis įvertinimas, uogos masė, uogų cheminė sudėtis, veislės.
YIELD AND FRUIT QUALITY OF FIFTEEN APPLE CULTIVARS

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Yield, harvesting time, storage life, quality and biochemical composition of fruits of 15 new apple (Malus domestica Mill.) cultivars were studied at the Lithuanian Institute of Horticulture in 2002–2006. Trees were grafted on M.26 rootstock at a spacing of 4 × 2 m.


Key words: apple, cultivars, quality of fruits, productivity.

Introduction. Fruit yield and quality has recently become more and more important for both consumers and producers. Fruit quality includes not only visual and gustatory, but also health promoting properties, which reduce the risk of several serious chronic diseases, such as cancer, coronary heart disease, type II diabetes (World Cancer Research Fund, 1997) and food safety characteristics. Consumer studies are generally aimed at determining the opinions and preferences of consumers with a view to introduce new cultivars into the market (Stainer et al., 1996; Sansavini, 2006, Palara and Colombo, 2006) and evaluate the acceptation of particular aspects related with fruit quality (Durner et al., 1992; Zanela, 2006).

Intensive research on the productivity and fruit quality at the Lithuanian Institute of Horticulture was carried out more than twenty years ago (Bandaravičius et al., 2000). Some cultivars responded differently to climatic conditions than others (Blažek and Varga, 2001; Sasnauskas et al., 2005; Sasnauskas et al., 2006).

The objective of this study was to evaluate yield and fruit quality in fifteen introduced apple cultivars.

Materials and methods. Trial years and place. The trial, which involved 15 apple cultivars, was planted at the Lithuanian Institute of Horticulture in the spring of 2002. Trees were grafted on M.26 rootstock. Evaluation and characterization of the cultivars and hybrids was performed in 2004–2006. Before orchard bare fallow was maintained where weeds were controlled mechanically and with herbicides.
Meteorological conditions. Temperature is the most important factor determining apple yield, particularly minimal temperatures in winter and spring. In 2002–2003 the temperature in December (5°C) and February (1.7°C) was lower, while in 2003–2004 the temperature in December (1.9°C) and February (2.5°C) was higher than multiannual value. During years of investigations the late spring frost at the beginning of bloom injured blossoms. At this time the minimal air temperature above the ground dropped from -0.3°C to -4.9°C, what injured fruit settings.

Plant material. The following introduced apple cultivars were compared with the standard cv. ‘Auksis’ (Lithuania): ‘Ausma’ (Latvia), ‘Caravel’ (Canada), ‘Charles Ross’ (United Kingdom), ‘Ciganochka’ (Ukraine), ‘Elegia’ (Ukraine), ‘Greensleeves’ (United Kingdom), ‘Honeycrisp’ (USA), ‘Katre’ (Estonia), ‘Katerina’ (Ukraine), ‘Ornament’ (Ukraine), ‘Perlina Kijeva’ (Ukraine), ‘Radogost’ (Ukraine), ‘Teremok’ (Ukraine) and ‘Velte’ (Latvia).

Experimental design. The trees were planted at the distance of 4 × 2 m. The trial was established in five replications. Each plot contained 1 fruit-tree. They were formed as spindle. Growing, fertilizing, pest, disease and weed control, soil cultivation, pruning, shaping and care of apple cultivars and promising hybrids were maintained as recommended for commercial orchards.

Observations and statistical analysis. The observations on fruits and trees have been done according to the standards used by the EUFRIN working group on apple and pear cultivar evaluation. In the trial the following characters of apple hybrids and cultivars was established: yield, t ha⁻¹; classification of fruits according to diameter, %; dates of harvesting time and storage life; fruit weight, g; quality of fruits, scores; biochemical compositions, %; output of apple juice, %; firmness of apple skin and flesh, N/cm². All data were subjected to analysis of variance. The significance of differences between the cultivars and hybrids was estimated at 0.05 level (Fisher’s Protected LSD and Duncan’s Multiple Range Test).

Results. Yield. Apple trees of cv. ‘Teremok’ (7.72 t ha⁻¹) and ‘Honeycrisp’ (9.34 t ha⁻¹) produced a higher yield in the third year in orchard, while trees of cv. ‘Caravel’ (0.16 t ha⁻¹) and ‘Katerina’ (0.28 t ha⁻¹) bear fruits lower (Fig. 1). In the fourth year in orchard apple trees of cv. ‘Auksis’ (24.8 t ha⁻¹) produced a higher yield, while cv. ‘Ornament’ (3.5 t ha⁻¹) produced a lower. In the fifth year of apple tree growth in orchard apple trees produced a higher yield. The cumulative yield of 5 years of apple cultivars ranged from 14.4 to 58.6 t ha⁻¹. Cvs. ‘Teremok’ (58.6 t ha⁻¹), ‘Ciganochka’ (42.9 t ha⁻¹), ‘Auksis’ (41.8 t ha⁻¹), ‘Perlina Kijeva’ (41.5 t ha⁻¹) and ‘Honeycrisp’ (40.2 t ha⁻¹) produced the highest yield. Cvs. ‘Katre’ (14.4 t ha⁻¹), ‘Charles Ross’ (15.6 t ha⁻¹) and ‘Velte’ (18.2 t ha⁻¹) produced a lower, while ‘Teremok’ (19.5 t ha⁻¹), ‘Ciganochka’ (14.3 t ha⁻¹), ‘Auksis’ (13.9 t ha⁻¹), ‘Perlina Kijeva’ (13.8 t ha⁻¹) and ‘Honeycrisp’ (13.4 t ha⁻¹) produced the highest yield.

Fig. 1. Cumulative yield of apple cultivars (t ha⁻¹)
Classification of fruits according to diameter established that cvs. ‘Auksis’, ‘Caravel’ and ‘Elegia’ produced fruits of the highest class (Table 1). 1 and 2 classes of apples ranged between 2–32%. Cvs. ‘Katerina’ and ‘Radogost’ produced not specific fruits.
Storage life. The earliest picking of fruits had cv. ‘Auksis’ (09-14) and ‘Velte’ (09-17), latest – cv. ‘Charles Ross’ (09-30) (Table 2).

Table 2. Harvest date, end of storage and fruit quality parameters of apple cultivars
2 lentelė. Obelų vaisių skynimo laikas, laikymosi pabaiga ir kokybės rodikliai

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Harvest date</th>
<th>End of storage</th>
<th>Fruit weight</th>
<th>Appearance</th>
<th>Taste</th>
<th>Quality evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>month, day</td>
<td>Laikymosi pabaiga</td>
<td>Vaisių masė (g)</td>
<td>Patrauklinas</td>
<td>Skonis</td>
<td>Vaisių skynimo kokybė</td>
</tr>
<tr>
<td>‘Auksis’</td>
<td>09-14 a*</td>
<td>03-12 ef</td>
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<td>7.6 e</td>
<td>7.6 f</td>
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<tr>
<td>‘Austria’</td>
<td>09-20 cdef</td>
<td>03-10 ef</td>
<td>120.0 cdef</td>
<td>7.4 cd</td>
<td>7.3 cd</td>
<td>7.3 cd</td>
</tr>
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<td>‘Caravel’</td>
<td>09-22 bo</td>
<td>12-09 bo</td>
<td>111.6 a</td>
<td>7.0 a</td>
<td>5.6 c</td>
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<tr>
<td>‘Charles Ross’</td>
<td>09-10 f</td>
<td>02-20 de</td>
<td>201.3 f</td>
<td>7.6 efg</td>
<td>7.2 c</td>
<td>7.2 c</td>
</tr>
<tr>
<td>‘Ciganochka’</td>
<td>09-21 bc</td>
<td>05-20 g</td>
<td>143.6 abcd</td>
<td>7.6 efg</td>
<td>7.0 b</td>
<td>7.2 c</td>
</tr>
<tr>
<td>‘Elegia’</td>
<td>09-28 def</td>
<td>03-04 ef</td>
<td>142.0 abcd</td>
<td>7.2 b</td>
<td>7.2 c</td>
<td>7.2 c</td>
</tr>
<tr>
<td>‘Greensleeves’</td>
<td>09-28 ef</td>
<td>02-28 de</td>
<td>118.6 ab</td>
<td>7.4 cd</td>
<td>7.3 cd</td>
<td>7.3 cd</td>
</tr>
<tr>
<td>‘Honeycrisp’</td>
<td>09-26 cdef</td>
<td>12-29 ab</td>
<td>150.3 bcd</td>
<td>7.7 g</td>
<td>7.4 def</td>
<td>7.2 c</td>
</tr>
<tr>
<td>‘Karte’</td>
<td>09-25 def</td>
<td>01-19 cd</td>
<td>121.3 ab</td>
<td>7.2 b</td>
<td>7.2 c</td>
<td>7.2 c</td>
</tr>
<tr>
<td>‘Katerina’</td>
<td>09-29 def</td>
<td>03-07 ef</td>
<td>122.6 abc</td>
<td>7.3 bc</td>
<td>7.2 c</td>
<td>7.2 c</td>
</tr>
<tr>
<td>‘Ornament’</td>
<td>09-21 bc</td>
<td>02-23 de</td>
<td>132.0 abc</td>
<td>7.3 bc</td>
<td>7.4 cd</td>
<td>7.2 c</td>
</tr>
<tr>
<td>‘Perlinia Kijeva’</td>
<td>09-23 cdef</td>
<td>12-15 ab</td>
<td>163.5 def</td>
<td>7.6 efg</td>
<td>7.1 b</td>
<td>7.1 b</td>
</tr>
<tr>
<td>‘Radogost’</td>
<td>09-28 def</td>
<td>05-10 fg</td>
<td>122.3 a</td>
<td>5.9 b</td>
<td>5.9 b</td>
<td>5.9 b</td>
</tr>
<tr>
<td>‘Terentok’</td>
<td>09-21 bc</td>
<td>03-06 ef</td>
<td>163.0 def</td>
<td>7.5 de</td>
<td>7.5 de</td>
<td>7.5 ef</td>
</tr>
<tr>
<td>‘Velte’</td>
<td>09-27 ab</td>
<td>01-20 cd</td>
<td>130.0 abcd</td>
<td>7.9 a</td>
<td>7.6 b</td>
<td>7.6 b</td>
</tr>
<tr>
<td>Mean</td>
<td>09-24</td>
<td>02-15</td>
<td>143.5</td>
<td>7.34</td>
<td>7.21</td>
<td>7.22</td>
</tr>
</tbody>
</table>

* Means followed by the same letter are not significantly different at P = 0.05 (Duncan’s multiple range t-test).
* Tai reikšmingų, padarinių taisus patišiems radėtis, nėra esminų skirtojų, kai P = 0.05 (Dunkane kriterijus).
Data of cold storage durability show that fruits of cvs. ‘Ciganochka’ (05-20) and ‘Radogost’ (05-10) may be stored longer. Cvs. ‘Caravel’ (12-05), ‘Perlina Kijeva’ (12-15) and ‘Honeycrisp’ (12-29) were distinguished for short time of storage life.

**Quality parameters.** All cultivars demonstrated intermediate (5–6.9 scores) good (7–7.5 scores) and extremely good (more than 7.5 scores) fruit appearance. Cvs. ‘Honeycrisp’ (7.7 score), ‘Charles Ross’, ‘Ciganochka’ and ‘Perlina Kijeva’ (7.6 score) produced extremely good appearance (Table 2).

Fruits of cvs. ‘Auksis’ (7.6 score) and ‘Teremok’ (7.5 score) had very good taste. Cv. ‘Caravel’ (5.6 score) had intermediate fruit taste. Fruit taste of other cultivars ranged between 7–7.4 scores.

Results of organoleptic evaluation show that fruits of cvs. ‘Auksis’ (7.6 score) and ‘Teremok’ (7.5 score) had very good quality (general estimate – involves taste and appearance). Other cultivars and hybrids had good quality (7.0–7.4 scores), except cv. ‘Caravel’ (5.6 score).

The largest fruits produced cvs. ‘Charles Ross’ (201.3 g), ‘Teremok’ (163 g), ‘Perlina Kijeva’ (163.3 g) and ‘Ausma’ (170 g), while smallest – cv. ‘Caravel’ (111.6 g).

**Chemical composition.** The highest amount of soluble solids was established in apple cv. ‘Elegia’ (13.8%), while essentially lower – in cv. ‘Caravel’ (9.0%) and cv. ‘Teremok’ (9.2%) (Table 3). The amount of titratable acidity of all cultivars and hybrids varied from 0.20 to 1.05. Dry matter content in apples ranged from 12.9 to 15.6%. Reliable differences in this parameter were between cv. ‘Honeycrisp’ and cv. ‘Perlina Kijeva’.

### Table 3. Biochemical characteristics of apple cultivars (%)

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Dry soluble solids</th>
<th>Titratable acidity</th>
<th>Dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tetraiz sausinos</td>
<td>Tetraiz sausinos</td>
<td>Sausinos</td>
</tr>
<tr>
<td>‘Auksis’</td>
<td>13.0</td>
<td>0.62</td>
<td>14.3</td>
</tr>
<tr>
<td>‘Ausma’</td>
<td>10.8</td>
<td>0.76</td>
<td>15.2</td>
</tr>
<tr>
<td>‘Caravel’</td>
<td>9.0</td>
<td>1.05</td>
<td>13.9</td>
</tr>
<tr>
<td>‘Charles Ross’</td>
<td>12.6</td>
<td>0.81</td>
<td>15.0</td>
</tr>
<tr>
<td>‘Ciganochka’</td>
<td>11.5</td>
<td>0.20</td>
<td>14.1</td>
</tr>
<tr>
<td>‘Elegia’</td>
<td>13.8</td>
<td>0.62</td>
<td>15.5</td>
</tr>
<tr>
<td>‘Greensleeves’</td>
<td>12.1</td>
<td>0.54</td>
<td>14.2</td>
</tr>
<tr>
<td>‘Honeycrisp’</td>
<td>10.6</td>
<td>0.46</td>
<td>12.9</td>
</tr>
<tr>
<td>‘Katre’</td>
<td>11.2</td>
<td>0.49</td>
<td>13.0</td>
</tr>
<tr>
<td>‘Katerina’</td>
<td>12.2</td>
<td>0.53</td>
<td>14.7</td>
</tr>
<tr>
<td>‘Ornament’</td>
<td>9.9</td>
<td>0.54</td>
<td>14.0</td>
</tr>
<tr>
<td>‘Perlina Kijeva’</td>
<td>12.6</td>
<td>0.65</td>
<td>15.6</td>
</tr>
<tr>
<td>‘Radogost’</td>
<td>11.4</td>
<td>0.62</td>
<td>14.3</td>
</tr>
<tr>
<td>‘Teremok’</td>
<td>9.2</td>
<td>0.68</td>
<td>13.2</td>
</tr>
<tr>
<td>‘Veltė’</td>
<td>10.6</td>
<td>0.64</td>
<td>14.6</td>
</tr>
<tr>
<td>Mean</td>
<td>11.3</td>
<td>0.61</td>
<td>14.3</td>
</tr>
<tr>
<td>LSD₉₅ / R₉₅</td>
<td>0.40</td>
<td>0.01</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Babtai, 2005–2006
Output of apple juice varied from 66.9% (‘Auksis’) to 79.1% (‘Caravel’) (Fig. 3). Cvs. ‘Elegia’, ‘Honeymcrisp’, ‘Katre’, ‘Ornament’, ‘Perlina Kijeva’, ‘Radogost’ and ‘Teremok’ had 70.1–73.3% of apple juice.

**Fig. 3.** Output of apple juice (%)
Firmness of apple skin ranged between cultivars. Most thin skin was observed on fruits of cvs. ‘Teremok’ (241 N/cm²), ‘Auksis’ (271 N/cm²) and ‘Velte’ (273 N/cm²). On the other hand, most thick skin had fruits of cvs. ‘Caravel’ (397 N/cm²), ‘Radogost’ (398 N/cm²) and ‘Ornament’ (425 N/cm²) (Fig. 4).

F i g. 5. Firmness of apple flesh (N/cm²)

Fruits of cvs. ‘Ausma’ (101 N/cm²), ‘Ciganochka’ (107.5 N/cm²) and ‘Perlina Kijeva’ (108.7 N/cm²) had most firm flesh, while ‘Velte’ (72.6 N/cm²), ‘Teremok’ (73.8 N/cm²) and ‘Auksis’ (77.1 N/cm²) had most soft flesh (Fig. 5).

Discussion. Our data show that different genotypes might have different strategy for productivity. During five years in orchard apple trees of cvs. ‘Teremok’, ‘Ciganochka’, ‘Auksis’, ‘Perlina Kijeva’ and ‘Honeycrisp’ produced the highest yield. Most unproductive were apple trees of ‘Katre’, ‘Charles Ross’ and ‘Velte’.

Quality is usually defined in terms of all of the characteristics of the food that lead a consumer to be satisfied with the product (Harker et al., 2003). The concept of quality can be studied from different perspectives; that of the consumer, or of the producer and the fruit industry (Molina et al., 2006). Establishing the optimum harvest date is an important factor in obtaining quality fruits. The best way to provide customers with good quality apples is therefore to select the most appropriate harvest date to guarantee consumer acceptance (Streif, 1996). The earliest harvest of fruits had cv. ‘Auksis’ and ‘Velte’, the latest – cv. ‘Charles Ross’. A long storage life is the most important factor in deciding, which cultivars are to be grown in a commercial orchard. The investigation shows that fruit of cvs. ‘Ciganochka’ and ‘Radogost’ can be stored longer.

To apple growers, fruit size is strongly correlated with profits (Salvador et al., 2006). Size, together with shape and color, is one of the most important fruit
quality characteristics to consumers (Schotzko, 1985). In our trial cv. ‘Charles Ross’, ‘Teremok’, ‘Perlina Kijeva’ and ‘Ausma’ produced the largest fruits.

Appearance, taste and texture are quality attribute that is critical in determining the acceptability of apple fruits by consumers (Jaeger et al., 1998). During the investigation period all fruit quality parameters were specific for each tested cultivars and depended on the year. Results of organoleptic evaluation show that fruits of cv. ‘Auksis’ and ‘Teremok’ had better appearance and taste. Dry soluble solids, titratable acidity and dry matter are associated with taste. The best chemical composition was found in fruits of cv. ‘Elegia’. The best output of apple juice had ‘Caravel’. The firmest skin had fruits of cv. ‘Ornament’, the firmest flesh – cv. ‘Perlina Kijeva’.

Based on the results from the present study, the most valuable cultivar was ‘Teremok’.

**Conclusions.**
1. The best cultivar, according to the whole complex of traits, was ‘Teremok’.

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SODININKYSTĖ IR DARŽININKYSTĖ. MOKSLO DARbai. 2007. 26(3).

PENKIOLIKOS OBELŲ VEISLIŲ DERLIUS IR VAISIŲ KOKYBĖ

A. Sasnauskas, D. Gelvonauškienė, P. Viškelis

Santrauka

2002–2006 m. Lietuvos sodininkystės ir daržininkystės institute tirta penkiolikos introdukuotų obelų veislių derlius, skynimo laikas, laikymosi pabaiga, vaisių kokybė ir biocheminė sudėtis. Dvimečiai obelų sodinukai su M.26 poskiepiu pasodinti 2002 m. pavasarį. Sodinimo schema – 4 × 2 m, po vieną vaismedį laukelyje penkiai pakartojimais.

Reikšminiai žodžiai: derlius, obelys, vaisių kokybė, veislės.
SEASONAL PATTERNS OF CARBOHYDRATES IN APPLE TREE CV. ‘AUKSIS’ ON DIFFERENT ROOTSTOCKS

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This study examined the effect of rootstock genotype on seasonal variation of non-structural carbohydrate (fructose, glucose, maltose) concentrations in apple tree shoot bark. Experiment was carried out at the Lithuanian Institute of Horticulture in 2005–2007. Investigations included cv. ‘Auksis’ on B series (B.9, B.146, B.396), P series (P 2, P 22, P 60) and M series (M.9, M.26) rootstocks. All groups of rootstocks are under research, followed the tendency of more intensive accumulation of monosaccharides in the orchards with more vigorous rootstocks in summer. By the way, accumulation of maltose was found to be more active in the rootstocks of P group in the investigated fruit trees. Intensive maltose synthesis in the fruit trees on P group rootstocks lead to the greater storage before the dormancy period. The biggest general quantity of sugars was found in the bark tissues of dwarf rootstock P 22. When growth and development is intensified, the most active transport of sugars in all investigated groups followed apple trees with less vigorous rootstocks.

Key words: apple tree, carbohydrate, rootstock.

Introduction. The importance of rootstocks is more and more widely recognized, which, in terms of their influence on yield productivity, are not less important than the grafted scions. Various apple rootstocks, as inhibitors or stimulators, can influence growth, fruiting initiation, crop, fruit quality and other biological and economical properties. According to the research made in Lithuania and other countries, rootstocks influence the beginning of the grafted scion, yield, quality of fruits and physiological features of the fruit trees (Fallahi et al., 2002; Marini et al., 2002; Kviklys et al., 2006; Kviklienė, Kviklys, 2006; Šabajevienė et al., 2006) However, physiological and morphological mechanisms, which determine morphological differences, are not completely clarified.

Many researchers state that the amount and variety of carbohydrates found in plants differ in various plant organs and conditions all throughout the growing season. Although storage of polysaccharides have significance in plant physiology, mono and disaccharides play a central role in metabolism, soluble sugars help regulate many developmental and physiological processes in plants. (Smeekens, 1998; Gibson, 2000). Carbohydrates reserves allow completing phenological developments without the benefit of current photosynthesis. In apple trees, leaf and early fruit development
in the spring depend on carbohydrate reserves within the tree (Mcqueen et al., 2004). Thus the storage of adequate supplies of carbohydrate is critical for both fruit yield and quality. Knowledge of the status of carbohydrate reserves is important for experiments involving manipulation of the carbon balance of fruit trees (Tustin et al., 1992).

**The aim** of this research was to compare the seasonal changes of carbohydrates and the dependence of their differences on the rootstock in bark tissues of apple tree cv. ‘Auksis’.

**Materials and methods.** Investigations of rootstock effect on apple tree seasonal variation of non-structural carbohydrate (TNC) concentrations were carried out at the Lithuanian Institute of Horticulture. Investigations included cv. ‘Auksis’ on B series (B.9, B.146, B.396, B.491), P series (P 2, P 22, P 60) and M series (M.9, M.26) rootstocks. The orchard was planted in the spring of 2001. Planting distances were 4 × 1.5 m. Trees were trained as slender spindles. The trial consisted of four replications with 3 trees in each. Replications were randomised.

Carbohydrate samples were prepared by grinding ~1 g of fresh weight (FW) material and extracted with 4 mL hot bidistilled water. After 24 h extract was filtered through cellulose and membrane (pore diameter 0.2 µm) filters. Chromatographic analysis was carried-out using Shimadzu 10A HPLC system with refraction index detector (Shimadzu, Japan) and Adsorbosil NH₂-column (150 mm × 4.6 mm; Alltech, USA). Mobile phase: 75% acetonitrile. Flow rate: 1 mL/min.

Data analysis was performed using MS Excel software.

According growth vigour control, all tested rootstocks were grouped in the following way: less vigorous than M.9 – P 22, the same as M.9 – Pure 1, B.396, P 60, B.9 and P 2, between M.9 and M.26 – B.491, the same or more vigorous as M.26 – Bulboga and B.146.

**Results.** The variation of investigated non-structural carbohydrate (TNC) (fructose, glucose and maltose) concentrations were determined by different rootstock genotypes and plant development stages (Fig. 1, Fig. 2, Fig. 3).

Considering the rootstock genotype and different year, the lowest total carbohydrate, fructose, glucose and maltose contents of bark tissues were detected in August (Fig. 1). In 2005 most of glucose was gained by the rootstocks of P group, in 2006 – by rootstocks of B group. According to the results, orchards on rootstocks P 2, B.146, and M.26 more intensively accumulated monosaccharides within the period.

Synthesis of hexoses in the investigated fruit trees in October went significantly up (Fig. 2). In 2005 glucose – 13.77–26.82 mg g⁻¹; fructose – 1.52–4.8 mg g⁻¹, and in 2006 glucose – 14.28–24.83 mg g⁻¹; fructose – 1.317–2.85 mg g⁻¹. By the way, accumulation of maltose was found to be more active in the rootstocks of P group in the investigated fruit trees. The greatest general quantity of sugars was found in the bark tissues of P 22 fruit tree rootstocks.

**Fig. 1.** Carbohydrate distribution in bark tissue of apple tree cv. ‘Auksis’ on different rootstocks in August (1-2005/2006, 2-2006/2007).

The results of investigation followed in March showed more active accumulation of maltose and fructose in the bark tissues of the fruit trees (Fig. 3). Synthesis of glucose in the fruit trees on different rootstocks took place differently. The quantity of glucose grew in the rootstocks of M group. It declined or became stable in the left part of the rootstocks. At the same time synthesis of fructose seemed to grow in the rootstock of fruit trees, except the one on P 22 rootstock, which accumulated less fructose in their bark tissue after dormancy period. According to the results, the highest concentrations of total sugars were found in cultivars grafted on P 22, B.396, M.9 rootstocks.

Discussion. In other reports it has been noticed, that different rootstock genotype affect fruit tree physiology (Fallahi, 2002; Šabajevienė et al., 2006).
These investigations present that rootstock genotype determines accumulation of carbohydrates. Sugars regulate growth activities by modulation gene expression and enzymes activities in both carbohydrate exporting and importing tissues. This ensures optimal synthesis and use of carbon and energy resources. (Koch, 1996, Coruzzi and Bush, 2001; Gupta and Kaur, 2005). As a result, carbohydrates react differently in terms of growth and development (Bianco et al., 1999), yield (Caruso et al., 1999), quality (Wang and Camp, 2000), bud and root formation (Maust et al., 2000), foliage (Niinemets, 1999), periodicity (Nzima et al., 1999), dormancy (Salisbury and Ross, 1991), and cold resistance (Palonen, 1999) in fruit trees.

The smallest total carbohydrate, fructose, glucose and maltose contents of bark tissues in all cultivars investigated in this research were in late summer. During this period the highest concentration of sugars was accumulated in the trunk and roots of fruit trees. Other scientists maintained that photosynthesis is activated by smaller content of sugars (Gupta and Kaur, 2005). The lowest concentrations of sugars were detected in orchards on M group rootstocks. All groups of rootstocks being under research followed the tendency of more intensive accumulation of monosaccharides in the orchards on more vigorous rootstocks at this period.

Total carbohydrates investigated in the research increased from summer to winter. The fruit trees suppose to gain enough storage of carbohydrates and start to use them on the second part of autumn. Khan et al. (1998) proposed that high winter-storage carbohydrate levels in the shoot would lead to high yield as in the following season. Synthesis of hexoses ran remarkably faster before the wintertime of studied fruit trees. By the way, accumulation of maltose was found to be more active in the rootstocks of P group in the investigated fruit trees. Intensivity of maltose synthesis in the fruit trees on P group rootstocks lead to the greater storage before the dormancy period. The biggest general quantity of sugars was found in the bark tissues of dwarf rootstock P 22.

Content of the detected sugars in bark tissues of studied fruit trees were at lower or similar levels during spring. Accumulated storage of carbohydrates actively moves from trunk to buds at this time. When growth and development is intensified, the most active transport of sugars in all investigated groups followed apple trees with less vigorous rootstocks.
Conclusions. All groups of rootstocks being under research followed the tendency of more intensive accumulation of monosaccharides in the orchards on more vigorous rootstocks in summer.

Infectivity of maltose synthesis in the fruit trees on P group rootstocks lead to the greater storage before the dormancy period. The greatest general quantity of sugars was found in the bark tissues of dwarf rootstock P 22.

When growth and development is intensified, the most active accumulation of sugars in all investigated groups followed apple trees on less vigorous rootstocks.

Gauta 2007 06
Parenšta spausdinti 2007 06

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Reikšminiai žodžiai: angliavandeniai, obelis, poskiepis.
ADAPTATION OF FOREIGN APPLE AND PEAR VARIETIES IN SWEDEN

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Fruit growing has a long tradition in Sweden with indigenous Nordic as well as foreign varieties frequently being grown in private gardens and commercial orchards. In this article we analyse the geographical origins of these varieties. The assortment of apples and pears in the most influential Swedish pomologies was investigated along with that of the national fruit gene bank mandate variety list. Furthermore, we discuss how fruit genetic resources from other countries with different climates have influenced the Swedish pomological heritage. In total we found 358 apple varieties, whereof 45% were Swedish, 44% foreign and 11% of uncertain origin. Of the foreign cultivars 15% were from outside Europe (mainly North America). As for pears, a total of 138 varieties were identified. Among these 17% originated from Sweden, 70% from abroad and 13% were of uncertain origin. Ten percent of the foreign pear varieties were found to be non-European. The main donor countries for apples were England, followed by Germany, Denmark and France. The corresponding countries for pears were France, Belgium, Germany and England. Interestingly, all of these countries are characterized by a warmer climate than Sweden and thus it can be concluded that certain apple and pear genetic resources from more southern latitudes do adapt well to the colder Swedish climate.

Key words: climate adaptation, cultivar, Malus domestica Borkh., Pyrus communis L., pomology.

Introduction. The progenitors of the cultivated apple (Malus domestica Borkh.) and pear (Pyrus communis L.) were probably first gathered in Central Asia, where many fruit species have their centres of diversity. According to Fischer (2004), apples have been used as human foodstuff for more than 10 000 years. European cultivation of apples and pears is described in the work of Homer about 800-600 B.C. Both Greeks and Romans knew about vegetative propagation and distinguished between cultivars. An early interest for apples can also be noted on northern latitudes. At Alvastra (central Sweden) remnants of dried apples have been reported from settlements dating back to the Scandinavian middle Neolithic (approx. 3000–2000 B.C.) (Anon., 2007). Most probably these apples originated from wild growing trees of the indigenous species Malus sylvestris Mill., which is one of the progenitors of M. domestica. Furthermore, apple seeds were found in the excavations of the Norwegian Oseberg ship from the Viking Age (Holmbo, 1921).

Fruit cultivation began in Sweden during the Middles Ages at monasteries, where
foreign plant material was introduced by monks (Nilsson, 1986). During the 17th and 18th century fruit growing became popular among the Swedish nobility (Påhlman, 1950), which imported large quantities of apple and pear trees from nurseries in southern Europe. Both named cultivars and seedlings were thus brought to the country. Some of the imports survived and became well known varieties with either their original names or with given local names. Other varieties and tree individuals could not withstand the Scandinavian climate and were sorted out. The majority of the cultivated fruit trees died in the winter of 1709 and those, who survived like ‘Snilsäpplet’ in Dalarna (central Sweden) were considered extremely hardy.

The first attempt to systematically describe and classify the fruit varieties grown in Sweden was made by Eneroth, who published his pomology in 1866. This was followed by a revised edition in the 1890s (Eneroth and Smirnoff, 1896–1899). The next Swedish pomology to be written was by Dahl, who published his first edition in 1929 and an extended second in 1943. In addition to their writings, both Eneroth and Dahl were engaged in testing and evaluating new foreign varieties under Swedish climatic conditions, a work which was reflected in their books.

In the 1980s a new Swedish pomology was published in two volumes by Nilsson (1986, 1989). By this time the main focus was on genuine Swedish varieties, rather than on imported ones. Since 2003 the interest for old plant material is manifested through the national program for cultivated plants (Programmet för Odlad Mångfald – POM). Today’s conservation efforts include Swedish mandate varieties, i.e. varieties of Swedish origin, which either has been named and spread locally or bred and marketed by Swedish plant breeders (Hjalmarsson, 2003). Foreign varieties for which longstanding growing traditions in Sweden can be documented are also included.

The aim of this paper was to carry out analyses of the assortment of apples and pears in Swedish pomology literature and in the Swedish mandate variety list. It documents and highlights the substantial influence of foreign apple and pear genetic resources in Swedish horticulture.

**Materials and methods.** For our study the following editions of Swedish pomologies were selected: Eneroth 1866 (apples and pears), Dahl 1943 (apples and pears), Nilsson 1986 (apples) and Nilsson 1989 (pears). Eneroth’s first edition was chosen because it is the earliest Swedish pomology, while Dahl’s second edition was chosen because it is more complete than the first. Nilsson pomology is the most recent and has not been subjected to revision.

With the exceptions mentioned below, all varieties in the studied material were arranged in two tables, one for apples, and one for pears. Furthermore, each variety was checked for synonyms and its country of origin was noted. The complete material includes 358, to our knowledge, unique apple varieties and 138 pear varieties.

In Eneroth’s pomology some foreign pear varieties that were recently imported for assessment are mentioned. When it was apparent to us that a variety never came into cultivation it was omitted. Additionally, we decided to omit colour mutants unless they were well known and described separately from the original variety. ‘P. J. Bergius’, the dark red mutant of the Swedish 19th century apple variety ‘Sävstaholm’, which was described in detail by Florin and Florin in 1918, is an example of a mutant fulfilling the criteria for inclusion.
Results. Apples. In total 358 apple varieties were identified (Table 1). On average each variety appeared in the study twice. Of the 358 registered varieties, 45% were indigenous, 44% originated from other countries and 11% had uncertain origin. Fifteen percent of the foreign varieties were from outside Europe (mainly North America). Among European donor countries England, Germany and Denmark were the most important contributing with 43, 36 and 19 varieties respectively, i.e. 63% of the total foreign material.

Table 1. Apples. Origin of Swedish mandate varieties and varieties appearing in the pomology books of Eneroth (1866), Dahl (1943) and Nilsson (1986).

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>No of varieties in Pomology skaters</th>
<th>No of Mandate varieties (2003)</th>
<th>Total no. of Divisio</th>
<th>Number of Divisio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweden</td>
<td>28 (Eneroth 1866)</td>
<td>144 (Nilsson 1986)</td>
<td>162</td>
<td>323</td>
</tr>
<tr>
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<td>1 (Nilsson 1986)</td>
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</tr>
<tr>
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<td>2</td>
<td>4</td>
</tr>
<tr>
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<td>5 (Nilsson 1986)</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
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<td>5 (Nilsson 1986)</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>England</td>
<td>13 (Eneroth 1866)</td>
<td>5 (Nilsson 1986)</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td>France</td>
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<td>5 (Nilsson 1986)</td>
<td>15</td>
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<td>11 (Nilsson 1986)</td>
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<td>68</td>
</tr>
<tr>
<td>Austria</td>
<td>1 (Eneroth 1866)</td>
<td>1 (Nilsson 1986)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>North America</td>
<td>1 (Eneroth 1866)</td>
<td>1 (Nilsson 1986)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Latin America</td>
<td>4 (Eneroth 1866)</td>
<td>8 (Nilsson 1986)</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>Russia</td>
<td>1 (Eneroth 1866)</td>
<td>1 (Nilsson 1986)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Other foreign</td>
<td>5 (Eneroth 1866)</td>
<td>5 (Nilsson 1986)</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Variety unknown</td>
<td>30 (Eneroth 1866)</td>
<td>27 (Nilsson 1986)</td>
<td>57</td>
<td>114</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>269</td>
<td>358</td>
<td>713</td>
</tr>
</tbody>
</table>

Following the time axis represented by the pomologies it can be seen that the total number of listed varieties more than doubled between 1866 and 1986. The percentage of varieties with uncertain origins declined from 25% in 1866 to 10% thereafter. On the
other hand, the percentage of varieties of Swedish origin increased from 24% in 1866 to 51% in 1986. However, a decline was noted in 1943 when indigenous varieties only amounted to 20%. Varieties from North America were almost unknown in 1866, but during the 20th century their influence became comparable to that of Danish varieties. Compared to the pomologies the assortment in the mandate list is somewhat biased towards Swedish varieties.

**Pears.** The study comprised 138 pear varieties. As can be seen from Table 2, each variety, as for apples, appeared approximately twice in the studied material. Of the 138 varieties 13% had uncertain origin. Seventeen percent originated from Sweden and 70% where from other countries, whereof 10% had non-European background.

**Table 2. Pears.** Origin of Swedish mandate varieties and varieties appearing in the pomology books of Eneroth (1866), Dahl (1943) and Nilsson (1989).

<table>
<thead>
<tr>
<th>Country of origin</th>
<th>No of varieties in</th>
<th>No of Mandate varieties (2005)</th>
<th>Total no of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweden</td>
<td>6</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Belgium</td>
<td>16</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>Denmark</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Denmark</td>
<td>5</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>France</td>
<td>14</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>Germany</td>
<td>12</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>North America</td>
<td>6</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Russia</td>
<td>1</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Other foreign</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Russian</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Summary</td>
<td>64</td>
<td>80</td>
<td>83</td>
</tr>
<tr>
<td>% of total</td>
<td>46</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td>Swedish</td>
<td>19</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Danish</td>
<td>9</td>
<td>5</td>
<td>22</td>
</tr>
<tr>
<td>Other</td>
<td>72</td>
<td>92</td>
<td>77</td>
</tr>
</tbody>
</table>

Of the latter varieties five came from North America. In addition, three bergamotts were noted as Asian. France and Belgium were the most important donor countries.
contributing with 37 and 26 varieties respectively, i.e. 65% of the total foreign material.

Following the time axis represented by the pomology books the number of varieties increased from 64 in 1866 to 83 in 1989, that is by 30%. The number of varieties of uncertain origin was substantially higher in 1866 (19%) than in 1943 (3%) and 1986 (11%). Notably the number of varieties with Swedish background was below 10% in 1866 and 1943, a percentage that more than doubled to 22% in 1989. Similarly to apples, American pears were almost unknown in 1866, but in contrast there was no significant influx of varieties during the 20th century. Also for pears, the mandate list is biased towards Swedish varieties.

Discussion. In the pomological literature varieties tend to be described with their history. Our geographical classifications were based on this information. However, the study revealed some difficulties. The fact that the indigenous apple species, M. sylvestris Mill., only had a small impact on today’s apple gene pool and that all pear genetic material was brought to Sweden from abroad sometimes makes it difficult to define whether a variety is indigenous or not. Most difficulties were noted in 1866 when the highest percentage of varieties with uncertain origins was recorded. In modern times some of these uncertain varieties were in the absence of synonyms eventually classified as Swedish.

Apples. The total number of apple cultivars in the studied material increased between 1866 and 1986. New varieties were brought into cultivation due to continued import, selection of promising seedlings and organized national breeding programs. However, some 40 varieties are found in all of the studied pomologies, among them 50% are obvious imports such as ‘Alexander’, ‘Ananas reinette’, ‘Baumanns reinette’, ‘Blenheim Orange’, ‘Calville blanche d’hiver’, Charlamovsky’, ‘Danziger kantapfel’, ‘Gelber Richard’, ‘Keswick codlin’, ‘Mecklenburger köningsapfel’, ‘Reinette de Damason’ and ‘Ribston pippin’. Another 25% are classified as genuine Swedish varieties. ‘Arvidsäpple’, ‘Brunnsäpple’, ‘Kavlås’, ‘Ringstad’, ‘Rossvik’, ‘Stenkyrke’ and ‘Sävstholm’ are included in this category. ‘Åkerö’, ‘Ivö’, ‘Tornpipping’, ‘Madam Palm’ and ‘Salaholmsäpple’ are normally classified as local varieties, but are here considered to be foreign.

According to the literature, ‘Åkerö’ was imported in 1759 as a seedling from the Netherlands to the Åkerö estate (central Sweden), where the mother tree still bears fruits. Until this day ‘Åkerö’ remains one of the most popular varieties in the Nordic countries. ‘Ivö’ and ‘Tornpipping’, widely cultivated in the county of Scania (southern Sweden), are considered synonyms to the American ‘Monroe seedling’ and the Belgian ‘Reinette de Thorn’. Furthermore, ‘Madam Palm’ seems to be identical to the French ‘Pomme de notre Dame’ and ‘Salaholmsäpple’ with the Polish ‘Morega polska’.

The high percentage of foreign varieties in Dahl’s pomology (1943) can be explained by his extensive import and evaluation work at the Alnarp Institute of Horticulture (southern Sweden). In the early 20th century apples and pears were considered crops for the future and efforts were made to promote their development. Variety testing and national breeding programs were part of this strategy. Ten Swedish bred cultivars resulting from these efforts along with previously unknown Swedish local varieties identified in an inventory by the Nordic Gene Bank explain the increase of Swedish varieties in Nilsson’s pomology (1986).
Studying the origin of the imported material we found that countries with warmer climate than Sweden were main donors, i.e. England, Germany, Denmark and France. The introduction of varieties from Canada and northern U.S.A., where the climate is similar to that of Sweden, was important during the first half of the 20th century. In addition Russia, also situated to the north, made contributions to the Swedish apple assortment. When the national mandate variety list was established in 2003 conservation of indigenous genetic resources was in focus. Thus, the list contains more local varieties than any of the pomology books, but in all fewer varieties than Nilsson (1986). Out of 209 varieties 69% are of Swedish origin.

Pears. The total number of pear cultivars in the study was 138, substantially less than the 358 apple cultivars. This is due to pears being less hardy than apples. Consequently most pear cultivars can only be grown in the southern parts of Sweden and the local varieties are few. Between 1866 and 1989 the increase of varieties was from 60 to 83. In percentage terms this increase was similar to that of apples, and so were the reasons for it. Some 21 varieties were described in all of the pomologies, whereof two were of presumed Swedish origin (‘Grännapäron’ and ‘Hovsta’) and the remaining 90% foreign, among them ‘Bonne Louise of Jersey’, ‘Belle lucrative’, ‘Gansels bergamott’, ‘Epargne’, ‘Noiveau poiteau’, ‘Seckel’, ‘Windsor’, ‘Williams’ Bon Chrétien’ and ‘Yat’.

As with apples there are several examples of local names having replaced the original denominations. For instance ‘Gråpäron’, one of the most common varieties in Sweden, is synonym with ‘Föret d’été (eng. ‘Yat’), which was first described in France in 1628. Furthermore, the presumed local variety ‘Johantorp’, is probably identical to the cultivar ‘Jaminette’. Dahl (1943) differs from the two other pomologists by presenting very few varieties with uncertain origin (3%) and a high amount of foreign varieties (92%). These data reflect the pomological research at the time. They also emphasize the fact that the Swedish growing tradition and climate is more oriented towards apples than pears. Naturally, the influence of foreign cultivars in the past was much more pronounced for pears than apples.

Swedish pear breeding efforts have been very limited. A variety, ‘Carola’, was released in 1983 and two more, ‘Fritjof’ and ‘Ingeborg’, in the 1990s. The latter two were the result of a joint Swedish-Norwegian breeding program. Thus the increase of national varieties in the pomology of Nilsson (1986) is primarily due to the finding and describing of unknown and forgotten local cultivars rather than the introduction of new nationally bred varieties. Studying the origin of the imported cultivars it was found that the majority of cultivars, as for apples, came from countries with warmer climate than Sweden. The main donor was France, followed by Belgium. Additionally, Germany and England were important donors. As a percentage of the total number of varieties American (US) pears comprised 6%, half the number of that of apples.

Conclusions. Our review of the Swedish pomological literature and the Swedish mandate list revealed that 358 apple and 138 pear varieties were traditionally grown in Sweden. Even though problems may occasionally arise when trying to define the origin of individual varieties, our results clearly show that several of the historically most successful varieties originated from geographic areas outside Sweden. In spite of a strong focus on the national heritage of varieties in Nilsson (1986, 1989) the
percentage of imports is as high as 49% for apples and 77% for pears. In the Swedish mandate list, which reflects the national pomological heritage, the corresponding figures are 18% and 49%, respectively. Thus, it can be concluded that the foreign influence is strong, and more pronounced among pears than apples. The main donor countries for apples were England, Germany, Denmark and France, and for pears France, Belgium, Germany and England. All of these countries are characterized by warmer climate than Sweden, and thus we can also conclude that certain apple and pear genetic resources from southern latitudes may adapt well to the relatively harsh Swedish climate.

Gauta 2007 06
Parensta spausdinti 2007 06

References

I. Hjalmarsson, V. Trajkovski

Santrauka


Reikšminiai žodžiai: Malus domestica Borkh., prisitaikymas prie klimato, Pyrus communis L., pomologija, veislė.
Eight apple cultivars were maintained according to Griffing’s method 4 at the Lithuanian Institute of Horticulture. Seedlings of 28 crosses were planted in the orchard using a randomized complete-block design with 5 blocks. Ten plants per block represented every cross. European canker injuries of seedlings in orchard were assessed using a 0–5 scale: 0 – no canker injury symptoms on trunk or branches and 5 – trunk or branch is girdled. The least average number of apple tree trunk and branches was detected in crosses with ‘Kaunis’ and ‘Tellissaare’. General combining ability for resistance to European canker was highly significant (P < 0.01), but specific combining ability (SCA) – was not significant (P < 0.01). The results suggest that resistance to canker in this group of apple cultivars is controlled predominantly by additive gene action. According to our results, apple cultivars ‘Kaunis’ and ‘Tellissaare’ can be used in breeding programme as sources of resistance to Nectria galligena (Bres.).

Key words: combining ability, European canker, inheritance, Malus.

Introduction. European canker of apple (Malus domestica Borkh.) is caused by the fungus Nectria galligena Bres. The disease is common in many countries around the world and is recognized as a serious economic problem in horticulture. Biology and epidemiology of European canker and warning systems were developed depending on it and meteorological conditions of a region (Xu and Butt, 1994; Butt et al., 1994; Latorre et al., 2002). Several groups of researches are involved in fruit tree breeding programs and evaluation of plant genetic resources for resistance to N. galligena in Europe (Laurens, 1999; Skrivele et al., 1995; Kemp and Dieren, 1999; Sasnauskas et al., 2006; USA (Korban, 1986) and Canada (Johnson et al., 1982). Plant breeding is one of the most effective tools to receive new cultivars with desirable traits. Ten years ago six teams were involved in fruit breeding programmes for resistance to Nectria galligena canker in Europe (Laurens, 1999). Alston (1978) reported about the evaluation of canker resistance of 10 Malus species seedlings and the highest portion of resistant seedlings was ascertained in progenies of M. baccata. Advanced apple selections were received from crosses with cultivar ‘Klon 40’ in Germany (Laurens, 1999). Different resistance degree to canker was estimated of apple cultivars in Lithuania (Sasnauskas, 2006), Latvia (Skrivele et al., 1995), Belgium (Lateur, Populer, 1994), and Belarus (Kozlovskaya et al. 1999). Genetic variability was estimated by
analyzing RAPD and ribosomal DNA polymorphism in North American populations of *Nectria galligena* (Plante et al., 2002). Involving in crosses apple cultivars resistant to *Nectria galligena* should be effective way to receive canker resistant cultivars.

Objective of this work was to study heritability and to select sources for resistance to *Nectria galligena*.

**Material and methods.** Eight apple cultivars were maintained according to Griffing’s method 4 (Griffing, 1956): ‘Orlik’ (Russia), ‘Noris’, ‘Auksis’, ‘Kaunis’ (Lithuania), ‘Katja’ (Sweden), ‘Tellissaare’ (Estonia), ‘Prima’ and ‘Idared’ (USA). Seedlings of 28 crosses were planted in the orchard using a randomized complete-block design with 5 blocks. Ten plants per block represented every cross. The combining ability was calculated according to Griffing’s method 4. *Nectria galligena* canker injuries of seedlings in orchard were assessed using 0–5 scale: 0 – no canker injury symptoms on trunk or branches, 1 – small wound on trunk or branch, 2 – canker wound cover ¼ of trunk or branch girth, 3 – ½ of trunk or branch girth, 4 – ¾ of trunk or branch girth and 5 – trunk or branch is girdled.

**Results.** The age of apple seedlings evaluated for resistance to European canker was 13 years from seed germination. Data about the number of wounds caused by canker are presented in Table 1. The total number of wounds per cross varied from 6 (‘Noris’ × ‘Orlik’, ‘Kaunis’ × ‘Prima’) to 62 (‘Noris’ × ‘Idared’). Every cultivar was involved in 7 crosses and average number of wound per all crosses was different. The least average number (10.4) of injuries was detected in crosses with ‘Kaunis’ and the highest one (34.4) – in crosses with ‘Idared’. Seedlings of ‘Idared’ had wounds three times as much than it was estimated for ‘Kaunis’ seedlings.

<table>
<thead>
<tr>
<th>Table 1. Number of damages caused by European canker in crosses</th>
<th>Palykunynė. Europinio vėžio žaizdų skaičius įvairių kryžminimų kombinacijų</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSD&lt;sub&gt;0.05&lt;/sub&gt; = 14.9&lt;sup&gt;x&lt;/sup&gt;; LSD&lt;sub&gt;0.05&lt;/sub&gt; = 8.9&lt;sup&gt;y&lt;/sup&gt;; LSD&lt;sub&gt;0.05&lt;/sub&gt; = 9.7&lt;sup&gt;z&lt;/sup&gt;; LSD&lt;sub&gt;0.05&lt;/sub&gt; = 9.4&lt;sup&gt;w&lt;/sup&gt;</td>
<td><strong>R</strong>&lt;sub&gt;0.05&lt;/sub&gt; = 14.9&lt;sup&gt;x&lt;/sup&gt;; <strong>R</strong>&lt;sub&gt;0.05&lt;/sub&gt; = 8.9&lt;sup&gt;y&lt;/sup&gt;; <strong>R</strong>&lt;sub&gt;0.05&lt;/sub&gt; = 9.7&lt;sup&gt;z&lt;/sup&gt;; <strong>R</strong>&lt;sub&gt;0.05&lt;/sub&gt; = 9.4&lt;sup&gt;w&lt;/sup&gt;</td>
</tr>
<tr>
<td>Significant differences were found among crosses resistant to European canker (P &lt; 0.01) (Table 2). General combining ability (GCA) of this trait was highly significant (P &lt; 0.01), but specific combining ability (SCA) – was not significant (P &lt; 0.01). The results suggest that resistance to European canker in this group of apple cultivars is...</td>
<td></td>
</tr>
</tbody>
</table>
controlled predominantly by additive gene action. Non-additive genetic factors, as estimated by SCA, do not contribute to total variance of resistance to canker.

**Table 2. Analysis of variance of resistance to European canker**

<table>
<thead>
<tr>
<th>Source</th>
<th>European canker resistance (df)</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crosses</td>
<td>27</td>
<td>0.23</td>
<td>1.99**</td>
</tr>
<tr>
<td>Kombinacija</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCA</td>
<td>7</td>
<td>0.09</td>
<td>3.8**</td>
</tr>
<tr>
<td>BKG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCA</td>
<td>20</td>
<td>0.03</td>
<td>1.35</td>
</tr>
<tr>
<td>SKG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>0.023</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Values of general combining ability effects (\( g_i \)) of apple cultivar resistance to European canker**

<table>
<thead>
<tr>
<th>Parent cultivars</th>
<th>( g_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Orlik'</td>
<td>-0</td>
</tr>
<tr>
<td>'Noris'</td>
<td>0.12</td>
</tr>
<tr>
<td>'Auksis'</td>
<td>0.05</td>
</tr>
<tr>
<td>'Katja'</td>
<td>0.07</td>
</tr>
<tr>
<td>'Kaunis'</td>
<td>-0.25</td>
</tr>
<tr>
<td>'Tellissaare'</td>
<td>-0.10</td>
</tr>
<tr>
<td>'Prima'</td>
<td>0.08</td>
</tr>
<tr>
<td>'Idared'</td>
<td>0.04</td>
</tr>
</tbody>
</table>

LSD_{0.01} / R_{0.01} 0.11

('Kaunis' and other cultivars as well. GCA effects of other cultivars varied from -0.00 ('Orlik') to 0.12 ('Noris').

**Discussion.** Genes that determine resistance to European canker are not yet identified (Alston et al., 2000). Researches in several countries demonstrated that *Malus* species (Alston, 1978) and apple cultivars resistance to European cancer is different (Sasnauskas, 2006; Skrivele et al., 1995; Lateur, Populer, 1994; Kozlovskaya et al., 1999). The identified resistant cultivars should be used in apple breeding programmes.
and it was shown that it is possible to select cultivars resistant to apple canker (Alston, 1978; Laurens, 1999). Our work demonstrated that resistance to European canker is controlled predominantly by additive gene action, so the selection of parents on its phenotype should be effective for development of resistant apple cultivars.

Conclusions. After evaluation of seedlings resistance to European canker in progenies derived from eight apple cultivars crosses made according to half-diallel mating design it was ascertained that resistance to European canker in this group of apple cultivars is controlled predominantly by additive gene action and apple cultivars ‘Kaunis’ and ‘Tellissaare’ can be used in breeding programme as sources of resistance to *Nectria galligena*.

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**VĖŽIUI (*NECTRIA GALLIGENA* BRES.) ATSPARIŲ OBELŲ SELE-KCIJA**

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**Santrauka**


**Reikšminiai žodžiai:** kombinacinė galia, *Malus*, paprastasis vėžys, paveldėjimas.
THE CORE COLLECTION OF THE NORTHERN EUROPEAN GENE POOL OF *RIBES* CREATED BY RIBESCO PROJECT

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The RIBESCO project, partly funded by the European Community, has been designed to improve the level of characterisation and conservation of the Northern European *Ribes* germplasm, currants and gooseberry, utilising coordinated, international achievements. A core collection of the Northern European *Ribes* germplasm will be established and preserved with special intensity. In the first phase, the *Ribes* germplasm conserved in the national *ex situ* collections is subjected to phenotypic characterisation using internationally defined descriptors. Information obtained will be saved in a database and it will be used to select material to the *Ribes* core collection and to define the part of germplasm that will be identified using molecular markers. The data of molecular identification provide a suggestion for an optimal core collection, representing a maximum amount of genetic variation, with true-to-type varieties selected and duplicated accessions uncovered. The selection of the most valuable genotypes is also based on agronomic, historical or other important cultural values. The decentralised *Ribes* core collection is established to assure the preservation of selected germplasm, each plant accession to be conserved as safety duplicates. For conservation, both *ex situ* field collections, *in vitro* slow-growth collections and cryopreservation long-term collections are established. Easy-access documentation will be a part of the activity. The project promotes *Ribes* genetic material transfer and use on a whole European scale.

Key words: cryopreservation, currants, database, gene bank, germplasm, gooseberry, markers.

Introduction. Northern Europe is the leading production area of *Ribes* fruits
Ribes species are also known by their high level of active ingredients available for functional and healthy-food products (Tahvonen et al., 2005; Johnston et al., 2007; Pantelidis et al., 2007; Wu et al., 2007). The genus Ribes represents an important part of small fruit germplasm in the Northern European gene bank collections, but at present there is no trans-national coordination of these collections.

Due to the long history of domestication and cultivation of Ribes species (Hjalmarsson and Wallace, 2007), the national gene bank collections in the northern European region consist mostly of old and found varieties, with possibly several synonyms and even wrongly-named accessions. In addition, varieties have been renamed, possibly several times, and on the other hand varieties mutated but still named similarly may be included in the collections. The collections also contain several unnamed accessions.

During the last few years, dynamic developmental processes in the European agricultural research field have been experienced, including several institutional changes. These changes have also affected national plant archives, gene banks, adding pressure to limit and rationalise the germplasm preservation activities. This has especially concerned the collections of those horticultural plants that cannot be preserved as seeds but must be maintained in ex situ collections as plants. In addition, the on-farm conservation of clonally propagated material is risky, because the plants can be replaced by other, newer varieties.

In Northern Europe, both black and red currant species are grown wild, covering a valuable gene pool with high winter and spring frost tolerance, late blooming and a short fruit development phase (Lanham et al., 1995; Brennan et al., 2007). Collections of these wild or early-domesticated genotypes have been achieved in the past, but comprehensive documentation and conservation of the plant clones has not been organised with safety back-up collections. So far, there is no common coordination system of the Ribes germplasm collections. Under the European Cooperative Programme for Plant Genetic Resources (ECPGR), the European Central Ribes/Rubus Database has been created, but its development has been discontinued.

A four-year project, RIBESCO – Core collection of Northern European gene pool of Ribes, was launched in April 2007 to improve the level of characterisation and conservation of Northern European Ribes germplasm utilising coordinated, trans-national achievements. The project is funded half-and-half by the participating institutes using their national financing and by ‘the Community programme on the conservation, characterisation, collection and utilisation of genetic resources in agriculture’ established by the European Commission (EC).

The core collection does not exclude the possibility to maintain also larger, e.g. field collections, but plant breeders seldom access a diffuse germplasm collection. For breeding work a well-documented core collection offers a more efficient and targeted gene pool than diffuse large germplasm collections. The Ribes core collection thus promotes the utilisation of this germplasm.

**Approach and structure of the project.** The RIBESCO project will improve the
level of characterisation and conservation of the Northern European Ribes germplasm (currants and gooseberry) by coordinated implementation. The information and safety level of the collections of Ribes genotypes is raised, and the high quality of the collection is assured.

**Fig.** The structure of the project

The structure of the project is progressive, one stage leading to another, with some parallel actions (Fig. 1). A trans-national network is created, combining both scientists and breeders working on northern Ribes germplasm. Both phenotypic characterisation and national knowledge as well as molecular identification are utilised to define the core collection. The project leads to the increased conservation security and documentation of the most valuable part of Ribes germplasm in the northern area of the EC. The participating institutes are MTT Agrifood Research Finland (as coordinating partner), Estonian University of Life Sciences, The Research Institute of Pomology and Floriculture (Poland), Swedish University of Agricultural Sciences, Vilnius University (Lithuania), Lithuanian Institute of Horticulture, Bundessortenamt (Germany), Latvian State Institute of Fruit Growing, and The University of Copenhagen (Denmark).

**Phenotypic characterisation.** The first phase of the project is focused on the phenotypic characterisation of Ribes gene bank material conserved in *ex situ* collections. This characterisation is done by employing traditional practices, observation, measurement and documentation of plant traits. The primary characterisation serves also as a screening for microsatellite analyses, to keep the expenses of molecular studies at a moderate level.

Germplasm is evaluated for a number of characteristics. These characteristics include both agronomic traits – such as crop earliness and winterhardiness – but also more neutral, morphological characters. The characteristics are of heritable quality and only marginally affected by the environment. Because the present and most possible future use of Ribes species is based on fruit production, also several fruit quality factors, such as colour and even taste, contributes are evaluated. Keeping in mind the special needs of sustainable production methods, pest and disease resistance, for instance to mildew (*Shaerotheca mors-uvae*), is also evaluated.

Characterisation is designed to suit in the best possible way to the documentation of the genetic traits of the plant material in the gene bank collections. It is based on ‘the Guidelines for the Conduct of Tests for Distinctness, Homogeneity and Stability’ by the International Union for the Protection of New Varieties of Plants (UPOV), offered
separately for black and red currants and gooseberry. The additional evaluation criteria created by the Nordic Gene Bank and accepted by the Nordic and Baltic countries are also applied and modified when necessary.

Data collection and recording is harmonised in a way that it can be saved in a consistent database and then be transferred to the European Central Ribes Database of ECPGR. The phenotypic characterisation will improve the documentation level of collections and, as a consequence, the accessions will have a greater value for instance in breeding work.

**Molecular characterisation.** Molecular analyses are designed to give a reliable estimate of the genetic relationships among the candidate varieties for the core collection. The molecular data provide a suggestion for an optimal core collection, which would represent a maximal amount of genetic variation. It also assures the trueness-to-type of varieties to be selected to core collections and uncovers duplicated accessions.

In recent years a substantial amount of molecular markers have been developed and tested to accurately reveal polymorphism in Ribes (Lanham and Brennan, 1998; Lanham and Brennan, 1999; Lanham et al., 2000; Brennan et al., 2002; Brennan et al., 2007). The first objective is to standardise and calibrate microsatellite marker analysis methods (SSR, Simple Sequence Repeats and ISSR, Inter-Simple Sequence Repeats) for the cultivated Ribes species in the participating laboratories. The calibration of the methods is a prerequisite for the undependable comparison of the results from each partner’s analysis of their own candidate genotypes. The partners will first employ the previously selected primers in the analysis of the standard set of genotypes. The results will show what kind of adjusting is needed to make the results generally comparable. After the calibration phase, all the partners analyse their own group of candidate genotypes. The aim is to provide molecular marker data of all candidates of the core collection. Thereafter, estimates of genetic relationships among the candidate genotypes are provided, and an optimal group containing a maximum amount of genetic variation is defined.

**Core collection definition.** The most valuable Ribes germplasm is selected to a core collection that will include maximal genetic variation with a minimum of repetitiveness. The core collection candidates are defined partly on the basis of the data of phenotypic characterisation. In addition, the selection is partly based on agronomic, historical or other important cultural values. This evaluation of candidate genotypes is done both at national and international level. Each nation defines which part of the collection is important from the national point of view. In addition, the collected data are considered as a whole, and the selection of candidates is done at the level of all genetic material. This pre-core collection is estimated to contain 25% of the material of collections, varying according to the size of collections and the type of plant material (known varieties, land races or unidentified material).

The final definition of the core collection is made by combining the results of phenotypic characterisation and agronomic, historical or other important cultural values with the microsatellite analysis data. A part of the germplasm can be categorised to belong to the core collection on the basis of passport data only, if, for instance, the cultural value of an old and verified variety is unarguable. In the same way as
selecting the core collection candidates, the final selection is done both at national and international level; the genetic material is considered as a whole, but the national standpoints are taken into account. It is estimated that approximately 10% of all analysed genotypes will be included in the core collection.

Core collection establishment. Collection standards. Standards for the quality of *Ribes* core collection are established in accordance with the existing national guidelines and the international guidelines of the International Plant Genetic Resources Institute (IPGRI) (Reed et al., 2004). This includes specifying among other things the standards for location, planting conditions, minimum level of care, number of plants per accession, documentation follow-up, utilisation and renewing of the collections.

The safety of germplasm collections requires duplication at another site. Different storage methods will be used. The main conservation method for *Ribes* germplasm is maintaining plants in *ex situ* field collections. *In vitro* collections are needed both as duplicate storages and as temporary collections for the accessions to be cryopreserved. In long run, cryopreservation will be the objective in the long-term duplicate preservation of *Ribes* genetic resources. Duplication can be a combination of field plantings, *in vitro* culture and cryopreservation. The participating institutes will agree on standard procedures for handling the plant material.

Establishment of field collections. Either new plantings or collections already established can serve as core collection, if they fulfil the standards. Proper site is an important factor in maintaining the health of the collection and in minimising the potential environmental hazards. The plants of the core collection can be grown in greenhouses, screen houses and in the field. The plants are propagated vegetatively by cuttings. This method can most likely be applied to all *Ribes* genotypes. Also micropropagated material can be used. Plant health must be considered at establishing the collection. Propagules shall be taken only from a healthy looking stock and if virus free material is available, it should be used.

Establishment of *in vitro* collections. Also *in vitro* collections can provide the long-term conservation method of core collection genotypes. The advantage of establishing *in vitro* cultures through meristem culture is virus elimination at least in some degree. *In vitro* maintenance can be carried out using methodology established to *Ribes* species (Welander, 1985; Orlikowska et al., 1991). *In vitro* storage may be in warm or cool conditions. The selection of the method will depend on the genotype and the available techniques. Slow-grow storage in cool conditions decreases labour requirements, costs and chance for contamination of cultures and the risk of loosing germplasm through different handling errors. It also decreases the risk of genetic instability.

Establishment of cryopreservation collections. Also cryopreservation can be applied in some laboratories for long-term preservation of the selected germplasm (Wang et al., 2005; Johnston et al., 2007). This material shall first be established *in vitro*. One major advantage of cryopreservation is that it can also be used for virus eradication. Cryopreserved meristems are stored in liquid nitrogen at −196°C or in the gas phase of it below −150°C.

Benefits and future prospects. The main target of the RIBESCO project is to assure the high-level conservation of genetic variability of *Ribes* species. This leads to
several benefits. Both the starting point and the one major objective of the RIBESCO project are to establish the collaboration of the national agents responsible for the conservation of *Ribes* germplasm. Creating the core collection and improving the data availability can more effectively direct national resources to conservation, when overlapping resource allocation is removed.

Improved data availability also ensures easier access and utilisation of the collections. Breeding work and product development, for instance, will benefit from that (Šiksnianas et al., 2006). The achievements of the project will also improve the quality of collections. The level of characterisation is increased, and documentation will include the data of molecular identification. The project thus also increases the level of scientific information on the collections. This will add the value of the collections, and increase the exchange and utilisation of this genetic resource.

Although the RIBESCO project includes the Northern pool of European *Ribes* germplasm only, this genetic resource is also valuable to more southern European regions. The improved availability of data will also benefit these areas. The conservation of more southern genetic resources of *Ribes* can be developed accordingly, by adapting the operational mode applied in RIBESCO. The structure created in this project is also open to be expanded, finally to cover the whole European area.

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PROJEKTO “RIBESCO” SUKURTA BENDRA ŠIAURĖS EUROPOS RIBES GENTIES GENOFONDO KOLEKCIJA

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Reikšminiai žodžiai: agrastai, duomenų bazė, genų bankas, genofondas, serbentai, žymenys.
Possibility to receive hybrids between distantly related species is restricted by reproduction isolation barriers. To overcome such barriers different methods are explored. Our attempts to cross lilies from group AH (Asiatic hybrids) with distantly related species *L. candidum* for a long time was unsuccessful, until AH female was pollinated by pollen of *L. candidum* mixed with pollen of other incompatible species *L. regale* Wils. and *L. monadelphum* Bieb. To identify origin of such progeny and to screen offspring of *L. candidum* inheritance of male characteristic SOD (superoxid dismutase) isozymes were choosed. Progeny inherited SOD isoforms from *L. candidum* in juvenile stage possesed broad leaves – the trait characteristic for *L. candidum*. Among 30 progeny 16 were selected as distantly related interspecific hybrids derived from AH × *L. candidum*.

**Key words:** GDH – glutamate dehydrogenase, inter-specific hybrids, isozymes, *Lilium candidum*, SOD – superoxide dismutase.

**Introduction.** In the past 30 years the importance of lily as a horticultural crop has increased and the inter-specific hybridization played important role in breeding of new cultivars (Van Tuyl et al., 2000; Van Tuyl et al., 2003). Inter-specific hybridization in the genus *Lilium* has been conduced to produce novel hybrids, which can combine ornamental traits and resistance to virus and fungal diseases from distantly related species. Depending on origin hybrid lilies are presently categorized in eight divisions (McRae, 1998). Cultivars belonging to the same horticultural division are the result of intra-section crosses. For example, widely grown cultivars of lilies belonged to Asiatic (AH), Oriental (OH) and Trumpet (TH) hybrids derived from species of sections *Sinomartagon*, *Archeolyrion* or *Leucolyrion*, respectively. To perform inter-specific hybridization between species from the same section usually isn’t difficult and such hybrids possess fertility enough to receive next generations. Such inter-division isolation determinates that genetic potentiality of genus wasn’t explored enough.

The factors limiting inter-specific crosses in *Lilium* can be specified into pre- and
post-fertilization barriers (Van Tuyl et al., 1991). To overcome pre-fertilization barriers, various pollination techniques, such as pollination by mixed pollen of several species, intrastylary (cut-style), grafted style, in vitro placental and ovule pollination have been performed (Asano, Myodo, 1977; Van Tuyl et al., 1991; Chi, 2000). To overcome post-fertilization barriers and avoid abortion of hybrid embryos embryo rescue, ovary slicing and ovule culture have been used (Van Tuyl et al., 1991; Chi, 2002).

So, developing of methods to overcome pre-fertilization barriers allowed receive distant inter-specific hybrids that are temporary categorized as miscellaneous hybrids (Van Tuyl, Van Holsteijn, 1996; Chi, 2002). Recently as a result of inter-sectional hybridization new cultivars derived by crossing Trumpet hybrids × Oriental hybrids, L. longiflorum × Asiatic hybrids, L. longiflorum × Oriental hybrids, and Oriental hybrids × Asiatic hybrids innovated the lily assortment (Van Tuyl, 1997).

The cultivars of lilies belonging to the group of Asiatic hybrids (AH) are resistant to frost, many fungal and virus diseases, and easy adapt to cultivation conditions. That makes Asiatic hybrids popular and important in flower trade. However, AH cultivars lack fragrance and should be more ornamental.

L. candidum L. is cultivated as ornamental and attar plant from ancient time (McRae, 1998). This species possesses the pure white, sweetly fragrant flowers and is very ornamental. Other desired characteristics of L. candidum L. for flower forcing industry are low temperature and low light tolerance (Van Tuyl, Van Holsteijn, 1996).

However, susceptibility to virus and fungal diseases, weak growth vigour and demanding for soil condition made this species recalcitrant for cultivation. As species possessing high ornamental value L. candidum L. was involved in distant inter-specific hybridization programs, but crosses have always been difficult to perform. The first hybrid originated between L. candidum L. and phylogeneticaly related species L. chalcedonicum was L. × testaceum, which probably appeared as a chance hybrid seedling in a field of L. candidum L. in Germany or Holland about 1810 (McRae, 1998). The cross has been repeated in 1932 and hybrid L. × testaceum has been backcrossed to both its parents to produce some cultivars belonging to division Candidum hybrids (McRae, 1998). In 1971 Charles Robinson crossed L. candidum var. solanikae with L. monadelphum Bieb. to produce cultivar June Fragrance (McRae 1998). Some cultivars were created by Judith Freeman after crossing June Fragrance with AH (Asiatic hybrids) (McRae 1998). Such inter-division hybrids as L. longiflorum Thunb. × L. candidum L. and L. henryi Baker. × L. candidum L. were received in Japan and Holland by cut style pollination and cultivation of isolated hybrid embryos in vitro (Asano, 1980; Van Tuyl, Van Holsteijn, 1996). E. A. McRae comments that Wilbert Ronald has performed direct crosses of L. candidum with L. cernuum Kom. and various Asiatic hybrids (McRae, 1998). However, we anywhere found more information about these hybrids and any cultivars derived from such crossing were registered (McRae, 1998).

Several times we tried to receive hybrids from crossing AH and L. candidum L. However, neither neither native nor cut style pollination techniques allowed to perform direct (AH × L. candidum) or reciprocal (L. candidum × AH) crosses. Only
when pollination of AH was performed by mixed pollen of distantly related species *L. regale*, *L. monadelphum* and *L. candidum* progeny was received (Proscevičius, Strikulytė, 2004). In this research screening of hybrids derived from *L. candidum* L. among progeny with random paternity was performed. Progeny derived from AH and *L. candidum* L. was identified in juvenile stage by some phenotypes and by molecular markers characteristic for species *L. candidum* L.

**Materials and methods.** 30 progenies (family 02-3) derived after pollination of lily seedling 0-42-1 from division AH by mixed pollen of *L. candidum* L., *L. regale* Wils. and *L. monadelphum* Bieb. were received by embryo culture as described previously (Proscevičius, Strikulytė, 2004).

For isozyme analysis parental forms and putative hybrids were cultivated 4 weeks at 25°C *in vitro* on solid MS medium supplemented with 3% sucrose (Murasige and Skoog, 1962).

Proteins were extracted from leaves homogenized in potassium phosphate buffer pH 7.8 and electro-focused in 4–10% PAAG according to Laemmli (1970). Enzyme’s staining was performed according to the procedures described previously (Kleizaitė et al., 1996; Žukas et al., 2000).

**Results.** To start screening and selection of AH × *L. candidum* hybrids among progeny with random paternity characteristics of SOD (superoxide dismutase) and GDH (glutamate dehydrogenase) in maternal form 0-42-1 (AH) and paternal forms *L. candidum* L., *L. regale* Wils. and *L. monadelphum* Bieb. were investigated. GDH extracted from leaves of female 0-42-1 and male species *L. regale* Wils., *L. monadelphum* Bieb., and *L. candidum* L. display the same isozyme banding patterns. We detected that screening of progeny derived from AH after pollination with mixed pollen of *L. regale* Wils., *L. monadelphum* Bieb. and *L. candidum* L. by inheritance of GDH isoforms were unsuitable. Two similar isoforms of GDH were detected in all parental forms, which were used to receive hybrids. However, heterogeneities in electrophoretic banding were detected in case of SOD. Some electrophoretic bands of SOD that were poorly resolved were omitted by numbers. So, seven isozyme banding patterns were detected in maternal form 0-42-1 (Fig.). Species *L. monadelphum* Bieb. possessed 3 and *L. regale* Wils. – 2 readable isoforms of SOD. All isoforms detected in paternal species *L. monadelphum* Bieb. and *L. regale* Wils. were common with maternal form 0-42-1. Only in species *L. candidum* L. were detected 3 unique isoforms of SOD that were absent in species *L. monadelphum* Bieb. and *L. regale* Wils. as well, as in female 0-42-1. Inheritances of these isoforms in progeny were used to screen hybrids derived from AH and *L. candidum* L. (Fig.). Among 30 tested hybrids with random paternity 16 inherited paternal isoforms of SOD characteristic for *L. candidum* L. The isoforms of SOD characteristic for mother 0-42-1 in hybrids was presented too. These hybrids were selected as hybrid progeny derived from AH × *L. candidum* L.

The leaf shape of young plants was used as additional characteristic to recognize hybrid progeny derived from *L. candidum*. The broad leaves at juvenile stage are characteristic to *L. candidum* L. and *L. monadelphum* Bieb. However, young leaves of *L. candidum* L. possesses more ovate form than *L. monadelphum* Bieb. The leaves
of species *L. regale* Wils. are long and narrow. Lanceted leaves possessed maternal form 0-42-1. Though all progeny (16 hybrids), which inherited SOD isoforms of *L. candidum* L., possessed broad leaves, but they were not very same as in species. The other offspring (14 hybrids) with random paternity, which did not inherited SOD isoforms from *L. candidum* L., mainly possessed narrow leaves (Table).

**Table 1.** Characteristic of progeny derived after pollination of lilies from group AH by mixed pollen of *L. candidum* L., *L. regale* Wils. and *L. monadelphum* Bieb.

Table 1. Palikuonių, gautų apdulkinus AH grupės lelijas *L. candidum* L.,
L. regale Wils. ir L. monadelphum Bieb. žiedadulkių mišiniu charakteristika

Discussion. The pre-zygotic and post-zygotic isolation barriers prevent to receive inter-specific hybrids between Asiatic hybrids and L. candidum L. The pollination of cut style has been used to overcome incongruity in inter-specific crosses of lilies (Asano, Myodo, 1977; Van Tuyl et al., 1991). However, when long pollen tube forming species are used to pollinate cut style the efficiency of fertilization is low (Van Tuyl et al., 1991). We found that pollen of species that separately are incompatible with distantly related maternal species or cultivars in mixtures allowed perform inter-specific crossing (Proscvičius, Strikulytė, 2004). However, such method of pollination claims the verification of ancestry in hybrids. On the other hand, apomixis make it necessary to confirm whether seedlings obtained from distant crosses are indeed desired hybrids or not (Chi, 2002). Different methods of hybrid verification based on morphological, cytological and molecular markers are commonly applied (Asano, 1980; Obata et al., 2000).

The biochemical genetic markers known as isozymes were useful because they exhibit co-dominant expression and do not show environmental effects. Isozyme banding patterns in lilies was used for lily cultivar identification and to estimate the phylogenetic relationship among those cultivars (Arzate-Fernandez et al., 2005). Isoforms of SOD detected in leaves of L. candidum allowed to screen hybrids between AH and L. candidum L. among progeny with random paternity. Inheritance of SOD isoforms shows that the half of analyzed offspring possess in ancestry L. candidum L. ($\chi^2 = 0.37, P_{10} > 0.05$). It confirms morphology of leaves. All 16 hybrids screened as progeny of L. candidum L. possessed broad leaves (Table). Such phenotype wasn’t detected in other part of progeny, which ancestry wasn’t confirmed. Leaf shape characteristic to AH is inherited only by one offspring 02-3-2. It allows predict that this offspring derived by apomixes, but we can’t exclude that it is progeny of L. monadelphum Bieb. Other 13 unidentified hybrids with narrow leaves may be progeny of L. regale. However, for such conclusions it is necessary to find informative molecular markers specific to L. regale Wils. as well, as to L. monadelphum Bieb. We are in progress of testing DNA markers that should allow screen ancestry of this two species.

Conclusions. 1. In leaves of L. candidum L. were detected SOD isoforms that were absent in Asiatic hybrid 0-42-1 and species L. monadelphum Bieb., and L. regale Wils. 2. Almost half of progeny derived after pollination AH with mixed pollen of

<table>
<thead>
<tr>
<th>Inheritance of SOD isoforms from L. candidum</th>
<th>Number of screened progeny</th>
<th>Number of progeny by inheritance of leaf form</th>
<th>Broad leaves</th>
<th>Narrow leaves</th>
<th>Lancetted leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inherited</td>
<td>16</td>
<td>16</td>
<td>13</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Not inherited</td>
<td>14</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Totally tested</td>
<td>30</td>
<td>16</td>
<td>13</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Broad leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Narrow leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lancetted leaves</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>


3. Among 30 progenies with random paternity 16 were screened as inter-specific hybrids between AH and L. candidum L.

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\textbf{LILIAM \textit{CANDIDUM} L. HIBRIDINIŲ PALIKUONIŲ ATRANKA}

\textbf{J. Proscvičius, V. Kleizaitė, D. Dambrauskaitė}

\textit{Santrauka}


\textbf{Reikšminiai žodžiai:} tarprūšinė hibridizacija, izofermentai, GDH – glutamato dehidrogenazė, SOD – superoksidido dismutazė, \textit{Lilium candidum}. 

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THE EFFECT OF TWO MULTICOMPONENT FERTILIZERS ON YIELDING AND CONTENT OF ORGANIC COMPOUNDS IN PEPINO (SOLANUM MURICATUM) FRUIT
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Solanum muricatum is the little-known edible plant, however, grown willingly in the Mediterranean region. The applied fertilizing caused the big increase in the fresh masses of fruits, which depended on the quantity of the dose. On the basis of comparable doses of nitrogen it is possible to state that Polifoska 5 is operating more profitably than Polimag 5. Bigger dry mass of fruits was received applying Polifoska 5. Applying bigger doses of fertilizers significantly reduced contents of the extract and acidity of fruits and increased contents of vitamin C and sugar.

Key words: Solanum muricatum, fertilization, yielding, organic compounds.

Introduction. The pepino (Solanum muricatum) is a little-known, edible plant, which originated from South America, tropical regions of Andes. Pepino is grown in Mediterranean region for its tasty fruits. Nowadays, cultivation of the plant in Polish greenhouses and tunnels increases great interest in our country. Mineral fertilization is a very important factor affecting size and quality of fruit. Ruiz and Nuez (1997) observed that pepino is one of many plants, characterized by high susceptibility to changes of chemical composition as a result of fertilization. Taking into account different fertilization requirements of Solanaceae family plant, the effects of fertilizer doses on the improvement of quality in pepino fruit traits need to be determined. In recent years unary fertilizers have been replaced by multicomponent fertilizers and therefore, studies in this field may be given priority (Nurzyński, 2003). Due to this fact, there were conducted experiments in pots to compare acting of two multicomponent fertilizers on yielding and fruit quality of Solanum muricatum. Obtained results are presented in current article.

Material and methods. The experiment was carried out in 2005 and 2006 with vegetative shoots of Solanum muricatum. The shoots were produced from the seeds sown in 2004. After rooting they were planted to pots (type Kick-Brauchman) with 9 dm³ of capacity. Soil moisture was maintained at 60% of their maximum water capacity. Two fertilizers, Polimag S and Polifoska 5, at the N doses of 0.5, 1.0 and 2.0 g per pot were applied. Each year fertilizing was introduced one by one after planting. Dry matter of pepino fruit was determined gravimetrically, content of sugars – by Lane-Eynon method standard PN 90/A-75101/07, content of vitamin C according to PN 90/A75101/11. Extract standard PN 90/A-75101/02 and acidity standard PN 90/A-75101/04 of fruits were also determined. Data statistically was
Results and discussion. Obtained yielding results of pepino fruit (fresh mass and dry matter) are presented in Table 1. Applying fertilizers, dependently on their doses and type, resulted in the increase of fresh mass of fruit. In comparison to Polimag S, better effect of Polifoska 5, based on comparable N doses, was stated. Our data showed that fertilizing with Polifoska 5, as compared to Polimag S, increased yield of fruit fresh mass in 2005 and 2006 by 15.96% and 21.48%, respectively. During the period of two years the yield increased on average by 18%. Under comparable N doses it was stated that Polifoska 5 at amount of 0.5 g N increased yield by 33% and at amount of 2.0 g N only by 10% in 2006, as compared with Polimag S.

Similar yield of fruit dry matter was observed. Under equal N doses, it was stated that Polifoska 5, applied at amount of 0.5 g N, increased yield of fruit dry matter by 15.6% in 2005 and decreased by about 2% in 2006. Therefore, Polifoska 5 applied in doses of 1 g N and 2 g N increased yield by 35% and only by 7%, respectively. It was found that fertilizer type and size of doses did not affect fruit size.

Table 1. Effect of fertilization on pepino (*Solanum muricatum*) yield, 2005–2006

<table>
<thead>
<tr>
<th>Fertilization (N dose per pot, g)</th>
<th>Fresh mass (g per pot)</th>
<th>Dry matter (g per pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fertilizer (0)</td>
<td>372</td>
<td>346</td>
</tr>
<tr>
<td>Polimag S (0.5)</td>
<td>558</td>
<td>406</td>
</tr>
<tr>
<td>Polimag S (1.0)</td>
<td>584</td>
<td>627</td>
</tr>
<tr>
<td>Polimag S (2.0)</td>
<td>746</td>
<td>759</td>
</tr>
<tr>
<td>Polifoska 5 (0.5)</td>
<td>653</td>
<td>540</td>
</tr>
<tr>
<td>Polifoska 5 (1.0)</td>
<td>697</td>
<td>760</td>
</tr>
<tr>
<td>Polifoska 5 (2.0)</td>
<td>833</td>
<td>838</td>
</tr>
</tbody>
</table>

LSD0.01 years

<table>
<thead>
<tr>
<th></th>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilizer</td>
<td>12.55**</td>
<td>9.71**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose of fertilizer</td>
<td>4.70**</td>
<td>5.16**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Higher doses of fertilizers considerably decreased extract and fruit acidity and increased content of vitamin C and total sugar. Significant differences were found in the content of vitamin C as dependent on type and doses of used fertilizers. Fertilization significantly affected the increase in the content of vitamin C. Effect of Polifoska 5, as compared with Polimag S, was higher on content of these components. Fertilization also positively affected sugar content. Positive correlation was found applying Polimag S. It was further observed that higher doses in comparison to lower doses of both fertilizers affected the increase in value of analyzed features. According to Heyes et al. (1994)
and Redgwell and Turner (1986), pepino fruit contained on average 35 mg 100 g⁻¹ of vitamin C, from 5 to 9 g 100 g⁻¹ of total sugar, and about 0.14% of acidity.

Table 2. Content of organic compounds in fresh mass of Solanum muricatum fruit

<table>
<thead>
<tr>
<th></th>
<th>Vitamin C</th>
<th>Total sugars</th>
<th>Extract</th>
<th>Acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg-100g⁻¹)</td>
<td>(mg-100g⁻¹)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>No fertilizer</td>
<td>24.54</td>
<td>26.18</td>
<td>4.82</td>
<td>4.96</td>
</tr>
<tr>
<td>Polimag S (0.5)</td>
<td>28.5</td>
<td>29.7</td>
<td>4.81</td>
<td>5.01</td>
</tr>
<tr>
<td>Polimag S (1.0)</td>
<td>30.3</td>
<td>32.4</td>
<td>5.12</td>
<td>5.21</td>
</tr>
<tr>
<td>Polimag S (2.0)</td>
<td>34.4</td>
<td>34.9</td>
<td>5.33</td>
<td>5.52</td>
</tr>
<tr>
<td>Polifoska 5 (0.5)</td>
<td>28.7</td>
<td>29.6</td>
<td>4.66</td>
<td>4.98</td>
</tr>
<tr>
<td>Polifoska 5 (1.0)</td>
<td>32.3</td>
<td>33.5</td>
<td>5.03</td>
<td>5.44</td>
</tr>
<tr>
<td>Polifoska 5 (2.0)</td>
<td>33.9</td>
<td>34.8</td>
<td>5.12</td>
<td>5.41</td>
</tr>
</tbody>
</table>

Conclusions. Applying of Polifoska 5 fertilizer at N doses similar to those in Polimag S, increased yield of dry matter and fresh mass of Solanum muricatum fruit. Therefore, higher doses of fertilizers significantly decreased content of extract and acidity of fruits.

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References


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Reikšminiai žodžiai: derlius, ežinė kiauliauogė (Solanum muricatum), organiniai junginiai, tręšimas.
INVESTIGATION OF PRODUCTIVITY OF SEED STALKS OF EDIBLE CARROT AND RED BEET LITHUANIAN CULTIVARS

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Investigation of productivity of seed stalks of edible carrot (Daucus sativus Röhl.) and red beet (Beta vulgaris L. subsp. vulgaris convar. vulgaris var. vulgaris) cultivars was carried out at the Lithuanian Institute of Horticulture in 2005–2006. There were investigated four red beet cultivars (‘Kamuoliai 2’, ‘Joniai’, ‘Vytėnų Bordo’, ‘Ilgiai’) and two carrot cultivars (‘Garduolės 2’, ‘Vaiguva’). Plantings of the investigated cultivars of root-crop vegetables were stored in two ways: in refrigerator and stationary cellar. After storage biochemical investigations were conducted. Vegetable seed stalks were grown in turfic gleyic albic soil, which granulometric composition – light loam on loam, reaction – close to neutral. During plant growing there were fixed parameters, which characterize their reproduction properties, determine the amount and quality of seeds, and the influence of reproduction year conditions on the preservation of the stability of an example. Investigations showed that biochemical composition of red beet and carrot root-crops little depends on the ways of their storage. Red beet cultivar ‘Kamuoliai 2’ was distinguished for the bigger amount of dry soluble solids – 16.6%. Cultivars ‘Garduolės 2’ and ‘Vaiguva’ have similar biochemical properties. Investigations showed that cultivar genotype and meteorological conditions influence morphological characteristics of the grown vegetable seeds. In red beet seed stalks there were detected black bean aphids, and their natural enemies – lady-birds and plant bugs. Seed stalks of red beet cultivar ‘Vytėnų Bordo’ were significantly distinguished for the abundance of black bean aphids. The abundance of pests and their natural enemies found in the seed stalks of carrot cultivars didn’t differ significantly.

Key words: productivity, carrot, red beet, reproduction features, quality, seed, seed stalks.

Introduction. Vegetable seed quality depends on genetic nature of cultivar, agroclimatic conditions, the applied agrotechnique, etc. One of the main tasks in modern vegetable growing is to grow not only qualitative but also safe production, not effecting negatively the environment (Bobinas, 1999). When agrotechnical conditions and the methods of farming changes, the need for more perfect cultivars and hybrids appear. Production experience and the investigations of some scientific institutions show that sowing vegetable seeds grown under Lithuanian climatic conditions, productivity increases and vegetables became more resistant to diseases (Gaučienė, 2001; Armolaitienė, 1998; Petronienė, 2000; Petronienė, 2001; Karklelienė et al., 2005). Plant breeders constantly create new cultivars and hybrids, and seed growing creates favourable conditions to distribute them. It is indicated in the literature that
changing the assortment of cultivars productivity often increases 15–20%. Plants of
the created new cultivars better take over nutrients from the soil, more effective means
of agrotechnique; plants became more resistant to diseases and pests (Gaučienė, 2001;
Пивоваров, Ебебедева, 1995). For the growth and ripening of most vegetable seed the
longer vegetation period is necessary, therefore two-annual vegetable mother plants
are being planted early. It is established in Lithuania that the vegetation of carrot seed
stalks lasts for 150–170 days (Gaučienė, 2001). In order to produce good seed yield
it is necessary that plants took roots well. In cooler weather and sufficient humidity,
strong root system and flower stalks develop (Bobinas, 1999). Growing vegetable seeds
it is very important to establish the optimal conditions of the environmental factors
in various stages of plant organogenesis. It is ascertained that under our agroclimatic
conditions the most intensive light is in July-August. Therefore, when the favourable
conditions of soil humidity and mineral nutrition are created, it is possible to expect
good quality seeds (Duchovskis et al., 2001).

**Materials and methods.** Investigation of productivity of seed stalks of
edible carrot (*Daucus sativus* Röhl.) and red beet (*Beta vulgaris* L. subsp. *vulgaris*
convar. *vulgaris* var. *vulgaris*) cultivars was carried out at the Lithuanian Institute
of Horticulture in 2005–2006. There were investigated four red beet cultivars
(‘Garduolės 2’, ‘Vaiguva’). Mother plants of the investigated cultivars of root-crop
vegetables were stored in two ways: in refrigerator (at the stable temperature of +1°C
and relational humidity of 90–95%) and stationary cellar (at the temperature from +2 to
+7°C and relational humidity of 90–95%). After storage biochemical investigations were
conducted. Dry soluble solids were investigated by numerical refractometer ATAGO
(Методу, 1987); total amount of dry matter – gravimetrically at the temperature of
105°C up to the constant weight (Manuals of food quality control, 1986); the amount
of carotenoids – spectrophotometricaly (Davies, 1976); nitrates – with potentiometer
(AOAC, 1990). Vegetable seed stalks were grown according to the accepted for
each type agrotechnical requirements in turfic gleicyic albic soil, which granulometric
composition – light loam on loam, reaction – close to neutral. During plant growing
there were fixed parameters, which characterize their reproduction properties, determine
the amount and quality of seeds, and the influence of reproduction year conditions
on the preservation of the stability of an example (Гибридное семеноводство, 1998;
Сидак, Мирошниченко, 1993). In vegetable seed stalks the manifestation of pests
was investigated and their calculation was carried out (Pests of agricultural plants,
diseases and their calculation, 2002). The main parameters of carrot and red beet seeds
were evaluated and processed by dispersion analysis (Tarakanovas, Raudonis, 2003).

It was hot and dry weather in April of 2005. In July-August warm and humid
weather prevailed, and this factor delayed seed ripening. Weather temperature in 2006
little differed from the multiannual average. Dry, little rainy weather prevailed that
year (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Meteorological conditions during seed stalk vegetation. Kaunas meteorological station, 2005–2006</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meteorologinės sąlygos sėklojų vegetacijos metu. Kauno meteorologinė</strong></td>
</tr>
</tbody>
</table>

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Results. Investigations of 2005–2006 indicated that the different storage of carrot and red beet mother plants didn’t influence significantly biochemical composition. It was established that carrot cultivars ‘Garduolės 2’ and ‘Vaiguva’ have similar biochemical properties. Carrots, which have much carotene, preserved this valuable property after lasting storage. After storage the amount of carotene fluctuated in them from 20.0 to 21.3 mg%, respectively (Table 2). The amount of dry soluble solids and dry matter in carrot root-crops was equal to the average multiannual parameters. The differences of the amount of nitrates among cultivar and root-crop ways of storage were in the limits of error. It is possible to state that lasting storage of carrot cultivars ‘Garduolės 2’ and ‘Vaiguva’ in different places do not change biochemical parameters of crop-roots.

<table>
<thead>
<tr>
<th>Month</th>
<th>Weather temperature (°C)</th>
<th>Precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>13.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Balandis</td>
<td>11.2</td>
<td>11.0</td>
</tr>
<tr>
<td>Geoguiz</td>
<td>15.0</td>
<td>16.3</td>
</tr>
<tr>
<td>June</td>
<td>19.4</td>
<td>19.3</td>
</tr>
<tr>
<td>Biržėlis</td>
<td>18.2</td>
<td>17.5</td>
</tr>
<tr>
<td>Liepa</td>
<td>11.3</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Table 2. Carrot biochemical parameters after lasting storage
Babtai, 2005–2006

Biochemical differences of red beet cultivars after lasting storage are bigger in comparison to edible carrots. Red beet cultivar ‘Kamuoliai 2’ was distinguished for the bigger amount of dry soluble solids (16.6–16.7%). Cultivars ‘Joniai’, ‘Vytenų Bordo’,
‘Ilgiai’ accumulated 14.0% of dry soluble solids. The amount of nitrates in red beets does not exceed the permissible rates and relationally is small – usually this vegetable type tends to accumulate big amount of nitrates. According to the data of chemical analyses, cultivar ‘Kamuoliai 2’ accumulates 245.0–248.3 mg kg\(^{-1}\) of nitrates, ‘Joniai’, ‘Vytėnų Bordo’ and ‘Ilgiai’ accumulate 370.0–473.0 mg kg\(^{-1}\) (Table 3).

### Table 3. Red beet biochemical parameters after lasting storage

3 lentelė. Burokėlių biocheminiai rodikliai po ilgalaikio laikymo

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Dry soluble solids</th>
<th>Nitrates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tarpus amamos medžiagos (%)</td>
<td>Nitrato (mg kg(^{-1}))</td>
</tr>
<tr>
<td>‘Kamuoliai 2’ (stored in refrigerator)</td>
<td>16.7</td>
<td>245.0</td>
</tr>
<tr>
<td>‘Kamuoliai 2’ (stored in stationary cellar)</td>
<td>16.6</td>
<td>248.3</td>
</tr>
<tr>
<td>‘Joniai’</td>
<td>14.3</td>
<td>445.3</td>
</tr>
<tr>
<td>‘Vytėnų Bordo’</td>
<td>14.1</td>
<td>370.0</td>
</tr>
<tr>
<td>‘Ilgiai’</td>
<td>14.0</td>
<td>473.0</td>
</tr>
</tbody>
</table>

LSD\(_{0.05}\) / \(K_{0.05}\) | 0.55 | 28.97 |

Investigations of seed stalk growth periods of the main vegetable types showed that seed ripening very depends on meteorological conditions and genetic nature of the cultivar. During the years of investigation red beet reacted less to environment and ripened seeds more quickly. Averagely 120 days pass from the planting of mother plants till seed ripening (Table 4). The period of carrot seed ripening is the longest one (152 days). When evaluating the period from planting of mother plants till flower stalk rising and button formation, it was observed that in cooler spring of 2006 this period of seed stalk growth was longer than in 2005. The methods of storage of mother plants hadn’t big influence on the formation of morphogenetic structures of carrot seed stalks. After evaluation of flowering period of cultivar ‘Kamuoliai 2’ it was established that mother plants stored in refrigerator delay formation of flowers.

Comparison of carrot seed stalks showed that seed stalks of cultivar ‘Vaiguva’ form less amount of morphogenetic structures than these of cultivar ‘Garduolės 2’. Seed stalks of red beet cultivar ‘Joniai’ were distinguished for the bigger amount of morphogenetic structures (7.9 units). Seed stalk productivity was different not only among cultivars, but also among separate seed stalks of the same cultivar. Red beet cultivar ‘Vytėnų Bordo’ produced somewhat bigger seed yield (52.3 g), but it was very uneven – the yield of separate plants differed for 1–2 times (Table 5).

### Table 4. Development of carrot and red beet seed stalks, per days in 2005–2006.


### Table 5. Morphological parameters of vegetable seed stalks
In red beet seed stalks there were found black bean aphids and their natural enemies – lady-birds and plant bugs (Tables 6, 7). The abundance of black bean aphids found in seed stalks of cultivar ‘Vytėnų Bordo’ (VII.18) was significantly bigger that that one in seed stalks of cultivar ‘Kamuolai 2’. There weren’t found significant differences among other pests and entomophagous.
Table 6. Abundance of black bean aphids and lady-birds in red beet seed stalks
6 lentelė. Pupinių amarų ir boružių gaumas burokėlių sėklojuose
Babtai, 2005–2006

Table 7. Abundance of plant bugs in red beet seed stalks
7 lentelė. Žolinių blakių gaumas burokėlių sėklojuose
Babtai, 2005–2006

Fig. 1. Abundance of onion thrips in flowers of carrot seed stalks (thrips, units per flower)
1 pav. Tabakinių tripsų gaumas morkų sėklojų žiedynuose, tripsai, vnt. žiedyne
Babtai, 2005–2006

In seed stalks of cultivars ‘Garduolės 2’ and ‘Vaiguva’ these pests were found: onion thrips, plant bugs, carrot aphids and lady-birds (Fig. 1., Tables 8, 9). There weren’t found significant differences among pest abundance in plants of two carrot cultivars.

Table 8. Abundance of plant bugs in carrot seed stalks

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Plant bugs, mean units / plant</th>
<th>Lady-birds, mean units / plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VII 12</td>
<td>VIII 22</td>
</tr>
<tr>
<td>‘Kamuolai’</td>
<td>2.85</td>
<td>1.75</td>
</tr>
<tr>
<td>‘Vytėnų Bordo’</td>
<td>2.65</td>
<td>0.82</td>
</tr>
<tr>
<td>LSD 0.05 / R 0.05</td>
<td>2.427</td>
<td>1.302</td>
</tr>
</tbody>
</table>
Table 9. Abundance of lady-birds and carrot aphids in carrot seed stalks

Results of investigations showed that carrot cultivar ‘Vaiguva’ formed bigger seeds. Viability of carrot seeds is influenced by meteorological conditions, especially humid and cold weather. Seeds of red beet cultivar ‘Ilgiai’ were characteristic to cultivar – small (1000 seeds weighted only 18.1 g) (Table 10).

Discussion. Data of investigations revealed the influence of meteorological conditions on carrot seed weight. Some authors state that at the beginning of carrot seed stalk button formation and flowering at low temperature smaller seeds grow up (Atherton, 1990). The data of our investigations confirms this also. Warm and dry weather (16–18°C) is the most favourable for seed ripening. Our investigations showed
that in 2006, when the weather was dryer at the time of seed ripening (in August), the obtained seeds were of better viability. Data of other investigators confirms this also (Ygarova, 2003). When it rains often seeds ripen longer, and when it is very hot seeds don’t have time to grow, germ, endosperm don’t form, seeds dry up, become defective (Gaučienë, 2001; Hodkin, 1998).

According to the data of biochemical analyses, prolonged storage of carrot cultivars ‘Garduolės 2’ and ‘Vaiguva’ in different places of storage don’t change biochemical parameters of root-crops. Red beet cultivar ‘Kamuoliai 2’ was distinguished for the better biochemical properties because of the bigger amount of dry soluble solids and smaller amount of nitrates in root-crops comparing with cultivars ‘Joniai’, ‘Vytėnų Bordo’ and ‘Ilgiai’.

High temperature influences the abundance and development of pests (Degenhardt, 1992). This is also confirmed by the data of our investigations. The biggest amount of black bean aphids in red beet seeds was in July-August.

Conclusions. 1. Root-crops of Lithuanian selection carrot cultivars ‘Garduolės 2’ and ‘Vaiguva’ accumulate 20.0–21.3 mg% of carotene, 11.1 and 12.4% of dry soluble solids and 183.0–195.1 mg/kg of nitrates. Ways of mother plant storage don’t influence significantly carrot biochemical composition.

2. Red beet cultivar ‘Kamuoliai 2’ was distinguished for the bigger amount of dry soluble solids (16.6–16.7%) and smaller amount of nitrates (245.0–248.3 mg/kg). Cultivars ‘Joniai’, ‘Vytėnų Bordo’, ‘Ilgiai’ accumulated approximately 14.0% of dry soluble solids and 370.0–473.0 mg/kg of nitrates. Way of mother plant storage doesn’t change root-crop chemical composition.

3. Edible carrot and red beet seed stalk development and seed reproduction properties are determined by genetic nature of cultivar and growth conditions. In warmer and dryer years carrot seeds are bigger and more viable.

4. Seed stalks of red beet cultivars ‘Joniai’ and ‘Vytėnų Bordo’ form more morphogenetic structures. Seed stalks of red beet cultivar ‘Vytėnų Bordo’ are more productive – one plant produces averagely 52.3 g of seeds.

5. The abundance of pests and their natural enemies found in the seed stalks of the investigated vegetable cultivars didn’t differ significantly.

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VALGOMOSIOS MORKOS IR RAUDOJONO BUROKĖLIO LIETUVIŠKŲ VEISLIŲ SĖKLOJŲ PRODUKTYVUMO TYRIMAS
Productivity and quality of carrot (Daucus sativus Röhl.) and onion (Allium cepa L.) cultivars and hybrids

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Productivity and quality of carrot and onion grown in the field collections were investigated at the Lithuanian Institute of Horticulture in 2004–2005. Cultivar is one of the most important factors affecting yield. Two carrot hybrids (‘Svalia’ F₁, ‘Skalsa’) from Lithuania and eight hybrids from Netherlands (‘Champion’ F₁, ‘Puma’ F₁, ‘Adige’ F₁, ‘Tyne’ F₁, ‘Natalja’ F₁, ‘Nebula’ F₁, ‘Torro’ F₁, ‘Jaguar’ F₁) have been investigated. Ten long day onion cultivars (‘Babtų didieji’ (from Lithuania), ‘Kristine’, ‘Fiesta’, ‘Wolska’, ‘Kutnowska’ (from Poland), ‘Stamford’ F₁, ‘Spirit’ F₁, ‘Friso’ F₁, ‘Summit’ F₁, ‘Barito’ F₁, bred in Netherlands) were estimated. Onions and carrots were grown in sandy light loamy calcareous epihypogleic luvisol. N – 60 kg ha⁻¹, P – 60 kg ha⁻¹, K – 120 kg ha⁻¹ for fertilization was used. Morphological indices, yield and biochemical carrot composition of carrot root-crop and onion was established.

Obtained results showed, that the productivity and quality of plant depends on the genotype. The highest differences of productivity were obtained in the carrot hybrids ‘Champion’ F₁ and ‘Adige’ F₁. Onion hybrid ‘Barito’ F₁ was the most productive in the group of hybrids, and the biggest bulbs were obtained from the hybrid ‘Stamford’ F₁. ‘Kristine’ distinguished itself with the biggest diameter of bulb and productivity in the group of cultivars.

Key words: carrot, cultivar, hybrid, morphological indices, onion, quality.

Introduction. Lithuanian agroclimatic conditions are favourable to the growing of field vegetable, including edible onions and edible carrots. Carrots of Lithuanian selection distinguish themselves with big amount of carotene and productivity (Gaučienė, 1996; Gaučienė, 1997; Gaučienė, Viškelis, 2001). Carrot root-crop quality depends on genetic features of cultivar or hybrid, soil and growth conditions. Different carrot cultivars differently react on the changed growth conditions (Gaučienė, Viškelis, 2001; Rosenfeld et al., 1997; Wiebe, 1987). Carrot seeds germinate best of all when soil temperature is 16–18°C, weather temperature – 16–20°C ant it is sufficiently humid. During vegetation the most favourable temperature for carrot growing is 15–20°C. During intensive root-crop growth carrots need constant humidity, because its lack injures root-crop quality (Gaučienė, 2001). Onions most often (approximately 90%) are being sown in trade farms (Kamštaitytė, Bobinas, 2004). One of the main factors, which influence onion productivity, is suitable selection of the cultivar (Brewster, 1994). Onion yield and quality depends on meteorological conditions,
especially on the sum of active temperatures (Daymond et al., 1997). It is optimal to choose these cultivars and hybrids, which are included into national list of plant cultivars. Ten edible carrot hybrids and long day onion cultivars and hybrids, created in Lithuania, Poland and Netherlands were investigated at the Lithuanian Institute of Horticulture in 2004–2005. It was evaluated their productivity and similarity, the most suitable samples for growing under our climatic conditions were established.

The aim was to investigate and to compare Lithuanian and foreign cultivars and hybrids of edible carrot and edible onion, evaluating morphological features of both types of vegetables and biochemical carrot composition.

Materials and methods. Lithuanian and foreign cultivars and hybrids of carrots and onions were investigated at the Lithuanian Institute of Horticulture in 2004–2005.

Investigations were carried out in crop rotation of the experimental field. Soil – sandy light loamy calcareous ephipogleyic luvisol (IDg 8-k, Calc(ar)i – Epihypogleyc Luvisols – LVg-p-w-cc) (Buivydaite et al., 2001). Carrot sowing was carried out by manual sowing machine on the profiled surface, in two rows, inter-rows of 70 cm, on 12-05-2004 and 24-05-2005 (seed rate – 80 units/m²). In the year of investigation carrot yield was gathered on October 21. Area of record plot – 5.6 m². Experiment was carried out in three replications. Morphological features (length and diameter of root-crop) and yield data of carrot root-crop were processed by dispersion analysis method (Tarakanovas, Raudonius, 2003). Carotene was established at the laboratory of biochemistry and technology by Murri method (Åšģąźīā, 1987), dry soluble solids – by numerical refractometer ATAGO (Ермаков, 1987); nitrates – by potentiometer (AOAC, 1990).

The main agrotechnological elements of onions: sowing on 30-04-2004 and 19-04-2005 (seed rate – 100 units/m²); lifting of bulbs (when 75% of leaves lie down) on 20-09-2004 and 15-09-2005; harvesting on 27-09-2004 and 27-09-2005. After harvest bulbs were classified to marketable and not marketable, weighted. 10 marketable bulbs were weighted, and it was measured their diameter at the widest part of bulb and their height from bottom up to the top of internal peels. The data were statistically processed by computer programs STAT 1.55 from program packet „SELEKCIJA“ (Tarakanovas, 1999) and ANOVA for EXEL, vers. 2.1. The significance of results was estimated by dispersion analysis method. Applying the method of principle co-ordinate analysis and computer program SPSS it was established the layout of the investigated edible onion cultivars and hybrids in space of two dimensions according to productivity in 2004–2005.

In the spring of 2004–2005 dryer and cooler weather prevailed (Table 1). Carrot germinated unevenly, but in the middle of vegetation and up till the harvest they grew rather good. August and September distinguished themselves with bigger amount of precipitation. In sunnier years (July-August of 2005) carrots accumulated more carotene. Meteorological conditions almost didn’t influence onion growth; only September of 2004 was more favourable for harvesting.

| Table 1. Meteorological conditions during carrot and onion vegetation |
Results. Estimation of carrot yield and its structural elements showed that carrot hybrids less react to the environmental conditions. Their total yield reached from 55.2 t/ha to 69.2 t/ha, marketability – 69.5–87.1% (Table 2). Carrot cultivars ‘Champion’ F₁ and ‘Adige’ F₁ during the years of investigation were distinguished for productivity (69.2 and 68.3 t/ha) and marketable appearance (marketability – 87.1 and 85.4%). Carrot cultivar ‘Skalsa’ F₁ produced the smallest total yield (55.2 t/ha).

Table 2. Estimation of carrot yield

<table>
<thead>
<tr>
<th>Month</th>
<th>Carrot Yield (t/ha)</th>
<th>Marketability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>9.6</td>
<td>32.3</td>
</tr>
<tr>
<td>May</td>
<td>10.7</td>
<td>46.2</td>
</tr>
<tr>
<td>June</td>
<td>13.7</td>
<td>77.4</td>
</tr>
<tr>
<td>July</td>
<td>16.1</td>
<td>59.4</td>
</tr>
<tr>
<td>August</td>
<td>16.7</td>
<td>123.4</td>
</tr>
<tr>
<td>September</td>
<td>11.6</td>
<td>36.2</td>
</tr>
</tbody>
</table>

The productivity of the investigated edible onion cultivars and hybrids in 2004–2005 reached from 18.5 t ha⁻¹ to 28.4 t ha⁻¹ (Table 3). Cultivar ‘Kristine’ distinguished itself with significantly biggest productivity. Yield of bulbs was 24.0 t ha⁻¹.
‘Kutnowska’ and ‘Fiesta’ produced the smallest yield, correspondingly 18.5 t ha\(^{-1}\) and 19.0 t ha\(^{-1}\). Productivity of Lithuanian onion cultivar ‘Babtų didieji’ reached 20.1 t ha\(^{-1}\), but this cultivar was distinguished for the biggest bulb marketability – 87%. Marketability of cultivars ‘Fiesta’ and ‘Wolska’ was 81%, and the smallest amount of marketable bulbs was obtained growing onion cultivar ‘Kutnowska’.

**Table 3. The yield of various edible onion cultivars and hybrids**

<table>
<thead>
<tr>
<th>Cultivars, hybrids</th>
<th>Total yield (t ha(^{-1}))</th>
<th>Output of marketable yield (%)</th>
<th>Average weight of marketable bulb (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babtų didieji’</td>
<td>20.1</td>
<td>87</td>
<td>98.4</td>
</tr>
<tr>
<td>‘Kristine’</td>
<td>24.0</td>
<td>84</td>
<td>102.1</td>
</tr>
<tr>
<td>‘Fiesta’</td>
<td>19.0</td>
<td>81</td>
<td>88.5</td>
</tr>
<tr>
<td>‘Wolska’</td>
<td>21.5</td>
<td>81</td>
<td>90.3</td>
</tr>
<tr>
<td>‘Kutnowska’</td>
<td>18.5</td>
<td>73</td>
<td>81.4</td>
</tr>
<tr>
<td>‘Stamford’ F(_1)</td>
<td>23.3</td>
<td>85</td>
<td>114.4</td>
</tr>
<tr>
<td>‘Spirit’ F(_1)</td>
<td>20.0</td>
<td>79</td>
<td>110.5</td>
</tr>
<tr>
<td>‘Summit’ F(_1)</td>
<td>19.8</td>
<td>85</td>
<td>97.1</td>
</tr>
<tr>
<td>‘Friso’ F(_1)</td>
<td>23.8</td>
<td>89</td>
<td>89.2</td>
</tr>
<tr>
<td>‘Barito’ F(_1)</td>
<td>28.4</td>
<td>92</td>
<td>103.4</td>
</tr>
</tbody>
</table>

After the establishment of the average weight of marketable bulb, it became clear that hybrids produced heavier bulbs. Hybrids ‘Spirit’ F\(_1\) and ‘Stamford’ F\(_1\) produced the biggest bulbs according to the weight – the average weight of one marketable bulb reached correspondingly 110.5 g and 114.4 g. Onions ‘Friso’ F\(_1\) produced the smallest bulbs according to the weight. Among cultivars, ‘Babtų didieji’ and ‘Kristine’ produced the biggest bulbs, correspondingly 98.4 g and 102.1 g. The smallest average weight of marketable bulb (81.4 g) was this of onion cultivar ‘Kutnowska’.

Evaluation of the average weight of marketable root-crop showed that carrots ‘Skalsa’ F\(_1\) produce big (148.0 g) root-crops, carrots ‘Puma’ F\(_1\) and ‘Adige’ F\(_1\) – small ones (114.3 g and 115.7 g). Carrots ‘Champion’ F\(_1\) and ‘Jaguar’ F\(_1\) were distinguished for the longest root-crops. Carrots ‘Natalja’ F\(_1\), ‘Tyne’ F\(_1\), ‘Torro’ F\(_1\) produced the root-crops of the average length and diameter (Table 4).

**Table 4. Evaluation of carrot root-crop morphological indices**

<table>
<thead>
<tr>
<th>Cultivars, hybrids</th>
<th>Total yield (t ha(^{-1}))</th>
<th>Output of marketable yield (%)</th>
<th>Average height of root-crop (cm)</th>
<th>Average diameter of root-crop (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Skalsa’ F(_1)</td>
<td>23.3</td>
<td>85</td>
<td>120</td>
<td>14.2</td>
</tr>
<tr>
<td>‘Puma’ F(_1)</td>
<td>20.0</td>
<td>79</td>
<td>115</td>
<td>13.1</td>
</tr>
<tr>
<td>‘Adige’ F(_1)</td>
<td>19.8</td>
<td>85</td>
<td>114</td>
<td>12.9</td>
</tr>
<tr>
<td>‘Champion’ F(_1)</td>
<td>23.8</td>
<td>89</td>
<td>114</td>
<td>13.2</td>
</tr>
<tr>
<td>‘Jaguar’ F(_1)</td>
<td>28.4</td>
<td>92</td>
<td>120</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Babtai, 2004–2005
The form of edible onion bulbs is one of the main indices, which describe their economical value. The measurements of the investigated edible onion cultivar and hybrid bulbs showed that bulb index fluctuated from 0.8 to 1.1 (Table 5). Cultivar ‘Kutnowska’ produced the flattest bulbs, cultivar ‘Kristine’ – the most oval bulbs. ‘Babtų didieji’ produced flat round and round bulbs, the index of which was 0.9.

<table>
<thead>
<tr>
<th>Hybrids</th>
<th>Average weight of marketable root-crop</th>
<th>Root-crop morphological indices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vidutinė pekinio sakyvėsias masė (g)</td>
<td>length aigis (cm)</td>
</tr>
<tr>
<td>‘Svalia’ F₁</td>
<td>118.3</td>
<td>18.5</td>
</tr>
<tr>
<td>‘Skals’ F₁</td>
<td>148.0</td>
<td>17.2</td>
</tr>
<tr>
<td>‘Champion’ F₁</td>
<td>139.0</td>
<td>23.1</td>
</tr>
<tr>
<td>‘Puma’ F₁</td>
<td>114.3</td>
<td>20.0</td>
</tr>
<tr>
<td>‘Adige’ F₁</td>
<td>115.7</td>
<td>18.3</td>
</tr>
<tr>
<td>‘Tyne’ F₁</td>
<td>129.7</td>
<td>18.2</td>
</tr>
<tr>
<td>‘Natalja’ F₁</td>
<td>125.7</td>
<td>20.0</td>
</tr>
<tr>
<td>‘Nebula’ F₁</td>
<td>130.7</td>
<td>21.2</td>
</tr>
<tr>
<td>‘Torro’ F₁</td>
<td>122.7</td>
<td>20.8</td>
</tr>
<tr>
<td>‘Jaguar’ F₁</td>
<td>137.7</td>
<td>22.5</td>
</tr>
<tr>
<td>LSD₀.₀５ / R₀.₀５</td>
<td>9.39</td>
<td>1.85</td>
</tr>
</tbody>
</table>

Carrot quality is determined by their biochemical composition. One of the main indices is the amount of carotene in them. According to the data of our investigation, carrots of Lithuanian selection ‘Svalia’ F₁ and ‘Skals’ F₁ accumulated the biggest amount of carotene, correspondingly 23.9 mg 100 g⁻¹ and 22.6 mg 100 g⁻¹. Out of the investigated foreign hybrids, carrots ‘Tyne’ F₁ and ‘Torro’ F₁ were distinguished for
the biggest amount of carotene, correspondingly 16.3 mg 100 g⁻¹ and 16.2 mg 100 g⁻¹ (Table 6). During the years of investigation carrots ‘Jaguar’ F₁ and ‘Adige’ F₁ accumulated the least amount of carotene, correspondingly 13.5 mg 100 g⁻¹ and 13.6 mg 100 g⁻¹. Carrots ‘Svalia’ F₁ (12.3%) and ‘Skalsa’ F₁ (11.9%) were distinguished for the biggest amount of dry soluble solids. The biggest amount of nitrates was found in carrots ‘Natalja’ F₁ (403.3 mg/kg⁻¹) and the least amount – in ‘Adige’ F₁ (240.0 mg/kg⁻¹).

**Table 6. Carrot root-crop biochemical indices**

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Carotenes (mg 100 g⁻¹)</th>
<th>Dry soluble solids (%ED)</th>
<th>Nitrates (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Svalia’ F₁</td>
<td>23.9</td>
<td>12.3</td>
<td>304.0</td>
</tr>
<tr>
<td>‘Skalsa’ F₁</td>
<td>22.6</td>
<td>11.9</td>
<td>291.0</td>
</tr>
<tr>
<td>‘Schampion’ F₁</td>
<td>16.0</td>
<td>10.1</td>
<td>275.7</td>
</tr>
<tr>
<td>‘Puma’ F₁</td>
<td>15.2</td>
<td>9.6</td>
<td>288.7</td>
</tr>
<tr>
<td>‘Adige’ F₁</td>
<td>13.6</td>
<td>10.6</td>
<td>240.0</td>
</tr>
<tr>
<td>‘Tyne’ F₁</td>
<td>16.3</td>
<td>11.3</td>
<td>361.0</td>
</tr>
<tr>
<td>‘Natalja’ F₁</td>
<td>15.6</td>
<td>11.2</td>
<td>403.3</td>
</tr>
<tr>
<td>‘Nebula’ F₁</td>
<td>15.4</td>
<td>10.8</td>
<td>387.7</td>
</tr>
<tr>
<td>‘Torro’ F₁</td>
<td>16.2</td>
<td>10.7</td>
<td>262.3</td>
</tr>
<tr>
<td>‘Jaguar’ F₁</td>
<td>13.5</td>
<td>9.3</td>
<td>311.3</td>
</tr>
<tr>
<td>LSD05 / LSD10</td>
<td>3.6</td>
<td>1.85</td>
<td>97.60</td>
</tr>
</tbody>
</table>

Discussion. Investigations showed that vegetable internal and external quality depends not only on genotype of vegetable type, but also on soil and growth conditions (Brewster, 1994; Booc et al., 1990; Urapova, 2003). There were carried out investigations in Lithuania of the yield and quality of the most suitable for growing carrot; they showed that carrots of different genotype react on environment conditions differently (Gaučienė, Viškelis, 2001). Evaluation of the total carrot yield revealed that Lithuanian heterosis hybrids almost do not fall behind from the foreign hybrids and in some years even exceed them. According to the data of our two-year investigations, carrots ‘Schampion’ F₁ were the most productive (69.2 t ha⁻¹). ‘Svalia’ F₁ less reacted to the changes of growth conditions, because during the years of investigations almost all carrots produced root-crops of regular form (marketability reached 80.6–82.8%). After evaluation of carrot biochemical composition it was established that carrots of Lithuanian selection accumulate 7.4–8.7 mg 100 g⁻¹ more carotene than the average of the investigated carrots. Gaučienė and Viškelis got the similar data (2001).

According to the results of principle co-ordinate analysis (PK) of edible onion different cultivars and hybrids in the space of two dimensions, the investigated samples were classified into several small groups (Fig.). In one group there were cultivars and hybrids, which productivity reached from 8.5 t ha⁻¹ to 20.1 t ha⁻¹. Cultivar ‘Kristine’ and hybrid ‘Friso’ F₁, which distinguished themselves with big productivity, located in one PK1 space of two dimensions. Onion hybrid ‘Barito’ F₁, which distinguished
itself with biggest productivity, located separately from the main group in PK1 space of two dimensions with high positive value.

**Fig.** The layout of various edible onion cultivars and hybrids in space of two dimensions according to the results of principled co-ordinate analysis (PK) of productivity data in 2004–2005

![Graph showing the layout of various edible onion cultivars and hybrids in space of two dimensions according to the results of principled co-ordinate analysis (PK) of productivity data in 2004–2005.]

After evaluation of peculiarities of various onion cultivars and hybrids, it is clear that it is possible to classify the investigated samples into several groups, which differ in potential possibilities of yield formation. Cultivars and hybrids, which located in PK1 space with high positive value, create preconditions for practical aims to choose the most valuable selection samples under Lithuanian climatic conditions.

The comparison of onion cultivar and hybrid investigations with the earlier investigations showed that onions ‘Spirit’ $F_1$ produce round (onion index 1.0) and heavier (114.4 g) bulbs. Data by A. Čižauskas confirms this too (Čižauskas, 2003).

**Conclusions.**

1. Carrots ‘Schampion’ $F_1$ and ‘Adige’ $F_1$ distinguishes themselves with the biggest total yield (69.2 and 68.3 t ha$^{-1}$) and the best marketable appearance (marketability – 87.1 and 85.4%).

2. Carrots ‘Natalja’ $F_1$, ‘Tyne’ $F_1$, ‘Torro’ $F_1$ produce root-crops of average length (18.2–20.8 cm) and diameter (3.5–3.7 cm).

3. Investigations show that carrots ‘Svalia’ $F_1$ and ‘Skalsa’ $F_1$ accumulate the biggest amount of carotene out of all the investigated carrots – correspondingly 23.9 mg 100 g$^{-1}$ and 22.6 mg 100 g$^{-1}$. The biggest amount of nitrates was found in carrots ‘Natalja’ $F_1$ (403.3 mg/kg$^{-1}$).

4. Onion hybrid ‘Barito’ $F_1$ produced the biggest yield (28.4 t ha$^{-1}$). These onions were distinguished for the biggest output of marketable yield (92.0%).

5. Onion hybrid ‘Stamford’ produced the biggest bulbs according to the weight. Cultivar ‘Kutnowska’ produced the flattest bulbs, cultivar ‘Kristine’ – the most oval bulbs.
References

Santrauka

Lietuvos sodininkystės ir daržininkystės institute 2004–2005 metais tirtas lauko sąlygomis augintų morkų bei svogūnų derlingumas ir kokybė.


Reikšminiai žodžiai: hibridai, kokybė, morkos, morfologiniai rodikliai, svogūnai, veislės.
The results of investigation on the morphological and decorative properties of Lithuanian peony cultivars and hybrids (author O. Skeivienė), grown at the Kaunas Botanical Garden of Vytautas Magnus University, are presented in this work. It was established, that the morphological and decorative properties of Lithuanian peonies distinguish themselves in large diversity: blossom duration – 10–20 days, blossom productivity – 12–35 units per bush, blossom size – 14–20 cm in diameter, bush height – 70–114 cm, blossoms are single and double. The blossom productivity increases with plant age. 41.6% changeability of bush height, blossom diameter, blossom productivity was influenced by genotype. The detected pathogens Botrytis paeonia Oud., Fusarium oxysporum Schltdl., Septoria paeonia Vest. did not cause significant damage to plants.

Key words: Lithuanian cultivars and hybrids, morphological and decorative traits, Paeonia lactiflora Pall.

Introduction. Peony belongs to Paeoniaceae family, Paeonia genus. There are known 52 species in genus, mainly spread in Eastern and Central Asia, less spread in Southern Europe and North America (Antanaitienė, Stanienė, 2001a). The taxonomy of the genus Paeonia is controversial (Sang, 1995). The International register includes about 5000 peony cultivars (Иппоёйтова, 2005). 69% of all peony cultivars are originated from Chinese peony (Paeonia lactiflora Pall.). 30% comprise group of peony hybrids (Paeonia hybrida hort.) created by cross–pollination Chinese, common and other peony species. Only 1% of peony cultivars are originated from common peony (Paeonia officinalis L.). The majority of tetraploid peonies are allopolyploids derived from crosses between phylogeneticaly distinct diploid lineages (Sang, 1995; Sang et al., 2004). Cultivars of Chinese peony differ in blossom colour, form, blossoming time and duration, bush height and leaf colour (De-Yuan Hong, 2003). Shrubs are 60–130 cm high. Blossoms are white and red of various shades, with diameter of 15–20 cm. They can be of different forms: single, Japan-type, anemone, half-double,
double. According to form and distribution of inner petals, double blossoms are as follows: crown-like, half-spherical, rose, half-rose (Македонская, 1988). The breeder professional O. Skeivienė created a great number of cultivars and hybrids of peony. These peonies distinguish themselves by various morphological, decorative and biological properties (Varkulevičienė, Stankevičienė, 2005). Collection, preservation, investigation and evaluation of Lithuanian flower genefund are a new trend of scientific researches in our country. Lithuanian cultivars are original, adapted to the local climate condition and it is urgent task to conserve, investigate and foster them as a part of our culture (Dapkusienė et al., 2002). Three cultivars (‘Virgilijus’, ‘Garbė Motinai’, ‘Prof. K. Grybauskas’) and sixteen hybrids (‘Maironis’, ‘Freda’, ‘Darius-Girėnas’, ‘Skeivienės vėlyvasis’, ‘Žilvinas’, ‘Elena’, ‘Rytas’, ‘Kastytis’, ‘Ona’, ‘Jadvyga’, ‘Ramunis’, ‘Vakaris’, ‘Danutė’, ‘Jonas’, ‘Tadas’ and ‘Regina’) are confirmed by Orders of Minister of Environmental as National Plant Genetic Resources. These cultivars and hybrids will be preserved in the future.

The aim of this work was to investigate morphological and decorative traits of Kaunas Botanical Garden peony cultivars and hybrids originated in and to evaluate their ornamental quality.


Chinese peony seedlings are grown according to the generally accepted cultivation technology (Македонская, 1988). Peonies were planted in an open area in rows to the southwest direction. The distance between rows was 1 m, between individual plants – 0.8 m.

The samples of peonies were selected to National Plant Genetic Resources according to criteria for selection of ornamental plants to national plant genetic resources approved by Commission of Plant National Genetic Resources. Plants were selected following by the investigation methods of individual and population selection, introductive researches. Eleven criteria were evaluated in a 1–5 point scale system; point value increased for a better indication. Total point sum was divided by a number of criteria. The average point indicated value of selected plant. Objects with point value three and more were offered to attach to national genetic resources of ornamental plants.

For evaluation of the decorative and morphological properties of P. lactiflora cultivars and hybrids the phenology of plants was observed, the beginning and the end of blossoming were established, morphological measurements were accomplished according to J. Vaidelys’ methodology (Vaidelys, 2005).

The samples for fungi identification were collected from plants bearing symptoms of fungi diseases. Fungi disease agents were isolated in pure culture using moist–
chamber method and then identified with microscopic analysis according to M. B. Ellis and J. P. Ellis (1997).

Both effects of plant age and peony cultivars on the growth and productivity parameters were assessed by redundancy analysis (RDA) in the computer program Canoco® for Windows 4.0. Statistical tests of significance were carried out after an independent of distribution laws Monte Carlo permutation test (1 000 permutations). Statistical analysis was done using MEAN (MS EXCEL), GLM (STATISTICA 5.5).

Results. The decorative features of peony are definite by flowering duration, blossom size, form, colour, number and bush height. The decorative value was established – 4.73 for ‘Darius-Girėnas’, ‘Skeivienės vėlyvasis’ and 4.82 – for other peonies. According to the flowering time (Antanaitytė, Stanienė, 2001 b) based on average data obtained in 2000–2006, the investigated *P. lactiflora* cultivars and hybrids were grouped as medium, medium late and late. The blossoming of Lithuanian peonies begins in 1st–3rd week of June. The hybrids with single blossoms are earlier; double blossom hybrids begin to blossom 2–3 weeks later. The cultivars ‘Virgilijus’, ‘Prof. K. Grybauskas’, and hybrids ‘Freda’, ‘Elena’, ‘Rytas’, ‘Kastytis’, ‘Ona’, ‘Jadvyga’ and ‘Tadas’ have the longest flowering duration (15–18 days). Other cultivars and hybrids blossom 11–14 days. Blossoms are hollow and double, white, rose and red of various shades. According to the form and distribution of inner petals, double blossom hybrids ‘Freda’ and ‘Vakaris’ are crown-like. The blossoms of ‘Darius–Girėnas’, ‘Skeivienės vėlyvasis’, ‘Garbė Motinai’ are half-spherical double and ‘Prof. K. Grybauskas’ is spherical double (Table 1).

<table>
<thead>
<tr>
<th>Cultivar, hybrid</th>
<th>Year of origin</th>
<th>Blooming duration, month</th>
<th>Form of blossom</th>
<th>Colour of blossom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lietuviškų <em>Paeonia lactiflora</em> Pall. veislių ir hibridų dekoratyvinės savybės</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Garbė Motinai’</td>
<td>1958</td>
<td>06–22 – 07–05</td>
<td>Half-spherical, double</td>
<td>Light rose with violet shade</td>
</tr>
<tr>
<td>‘Virgilijus’</td>
<td>1958</td>
<td>06–14 – 07–02</td>
<td>Single</td>
<td>Rose red</td>
</tr>
<tr>
<td>‘Prof. K. Grybauskas’</td>
<td>1958</td>
<td>06–30 – 07–15</td>
<td>Spherical double</td>
<td>Dark red with white edging</td>
</tr>
<tr>
<td>‘Maironis’</td>
<td>1964</td>
<td>06–09 – 06–22</td>
<td>Single</td>
<td>White</td>
</tr>
</tbody>
</table>
Our data obtained in 2000–2006 revealed that bushes of ‘Ramunis’, ‘Kastytis’, ‘Jonas’ and ‘Vakaris’ were very high (more 100 cm), ‘Tadas’, ‘Jadvyga’ and ‘Žilvinas’ – middle high.

### Table 2. The morphological parameters of Lithuanian *Paonia lactiflora* Pall. hybrids

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Height of plants</th>
<th>Diameter of blossom</th>
<th>Number of blossoming shrub</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(cm)</td>
<td>(cm)</td>
<td></td>
</tr>
<tr>
<td>'Maironis'</td>
<td>90.10 ± 3.10</td>
<td>15.21 ± 2.50</td>
<td>24.35 ± 2.56</td>
</tr>
<tr>
<td>'Freda'</td>
<td>97.22 ± 2.94</td>
<td>14.72 ± 2.68</td>
<td>23.46 ± 2.45</td>
</tr>
<tr>
<td>'Darius–Gintas'</td>
<td>95.12 ± 2.05</td>
<td>14.63 ± 2.75</td>
<td>19.87 ± 3.25</td>
</tr>
<tr>
<td>'Skeivienės vėlyvis'</td>
<td>100 ± 1.10</td>
<td>15.81 ± 1.25</td>
<td>15.68 ± 3.02</td>
</tr>
<tr>
<td>'Žilvinas'</td>
<td>74.51 ± 1.21</td>
<td>14.35 ± 2.31</td>
<td>18.67 ± 2.56</td>
</tr>
<tr>
<td>'Kastytis'</td>
<td>102.10 ± 2.45</td>
<td>20.01 ± 0.98</td>
<td>21.52 ± 2.63</td>
</tr>
<tr>
<td>'Vakaris'</td>
<td>114.2 ± 2.98</td>
<td>18.35 ± 1.32</td>
<td>26.86 ± 2.87</td>
</tr>
<tr>
<td>'Elena'</td>
<td>97.41 ± 4.52</td>
<td>19.12 ± 1.05</td>
<td>35.01 ± 0.98</td>
</tr>
<tr>
<td>'Rytas'</td>
<td>88.35 ± 5.12</td>
<td>18.54 ± 1.03</td>
<td>20.58 ± 2.87</td>
</tr>
<tr>
<td>'Ona'</td>
<td>84.71 ± 4.35</td>
<td>18.77 ± 1.02</td>
<td>17.52 ± 3.45</td>
</tr>
<tr>
<td>'Jadvyga'</td>
<td>70.55 ± 3.50</td>
<td>16.18 ± 1.88</td>
<td>11.79 ± 1.85</td>
</tr>
<tr>
<td>'Ramunis'</td>
<td>105.00 ± 3.76</td>
<td>15.22 ± 1.35</td>
<td>18.42 ± 1.49</td>
</tr>
<tr>
<td>'Danute'</td>
<td>82.59 ± 5.42</td>
<td>18.46 ± 1.21</td>
<td>26.23 ± 1.67</td>
</tr>
<tr>
<td>'Jonas'</td>
<td>103.00 ± 2.50</td>
<td>18.60 ± 1.40</td>
<td>30.02 ± 1.98</td>
</tr>
<tr>
<td>'Tadas'</td>
<td>76.68 ± 4.95</td>
<td>15.20 ± 2.50</td>
<td>15.36 ± 1.76</td>
</tr>
<tr>
<td>'Regina'</td>
<td>94.03 ± 3.02</td>
<td>18.01 ± 1.20</td>
<td>21.45 ± 1.99</td>
</tr>
</tbody>
</table>
Other Lithuanian peonies were high (Fig. 1 a, Table 2). The hybrid ‘Kastytis’ has huge, ‘Elena’, ‘Rytas’, ‘Vakaris’, ‘Danutė’, ‘Ona’, ‘Jonas’, and ‘Regina’ – large blossoms (Table 2).

Cultivar ‘Virgilijus’ has the largest blossoms (in diameter about 18 cm) and high plants – about 87 cm. The smallest flowers had plants of complete cultivar ‘Prof. K. Grybauskas’ (in diameter about 14 cm), but they had long blossoming (about 15 days) and were high (up to 100 cm). (Fig. 1 a, b).

Flowering productivity mean increases from 26 (in 2000) to 52 units (in 2006). It was increasing with plant aging. Environment conditions could have had an influence to the uneven increasing. From 2000 till 2002 increasing was slight (from 25 to 29) and during 2003–2004 flowering productivity had hardly increased, it was about 33 units (Fig. 2 a).
According to the literature, the generative buds of the next year are being formed in August-September (Васиёўа, 1972). During the research period in 2002 in these months there was not much rainfall (14 mm in August and 42 mm in September; in 2003 – the rainfall in August was heavier – 53 mm, but less in September – 28 mm). Flowering productivity had highly increased in 2005 and 2006. This was influenced by the bigger amount of rainfall during generative bud formation: 2004 – 97 mm in August and 35 mm in September; in 2005 – 135 mm and 48 mm. The optimal conditions in spring could also influence (temperature in April-June was higher than the average temperature of the year – 7.5°C, 12.1°C, 15°C).
Effect of peony cultivar on the growth and productivity parameters was significant ($F = 35.8; p = 0.001$) and explained 41.6% of the observed variance, as shown by redundancy analysis (RDA) (Fig. 2 b).

The most harmful and widespread disease of peony is root rot (the agent *Botrytis paeonia* Oud.), damaging plants during all vegetation period, (Petrauskaitė, Vengeliauskaitė, 1978). According to our data, obtained in 2004–2006, favourable conditions for disease spreading were in 2004–2005. Affected stem bases and buttons were found in hybrids ‘Skeivienės vėlyvasis’ (2.5%), ‘Freda’ and ‘Darius-Girėnas’ (3.5%). *Fusarium oxysporum* Schltdl. was isolated from under the roots zone of ‘Virgilijus’ in 2005, and ‘Darius-Girėnas’ in 2006. In rare cases *Septoria paeonia* Vest. was detected on ‘Skeivienės vėlyvasis’, ‘Freda’, ‘Darius–Girėnas’; their damage was not significant.

**Discussion.** According to our data, flowering duration fluctuates for 5–7 days. Sometimes analogical data (Antanaitienė, Stanienė, 2001 b) were observed by R. Antanaitienė and G. Stanienė for ‘Virgilijus’ and ‘Maironis’, which blossom beginning depends on meteorological conditions.

The height of *P. lactiflora* Pall. shrubs is 80–100 cm (Vaidelys, 2005; Zhou et al., 2005). The heights of Lithuanian peonies were from ‘Jadvyga’ 70.55 to 114.2 (‘Vakaris’) cm. They were middle (60–80 cm) – 3 hybrids, high (81-100 cm) – 9 hybrids and 3 cultivars and very high (more than 100 cm) – 4 hybrids (Table 2; Fig. 1 a).

Peony blossoms according to size are classified as huge (diameter more than 20 cm) – 1 hybrid, large (17–20 cm) – 7 hybrids and cultivar ‘Virgilijus’, middle (13–16 cm) – 8 and ‘Garbė Motinai’ and small (less than 13 cm) – cultivar ‘Prof. K. Grybauskas’ (Table 2; Fig 1 b).

According to Y. Zhou et al. (2005), during evaluation of peony cultivars it is very important to estimate root system and its anatomy. The authors established, that *P. lactiflora* roots are cylindrical, pale white to reddish brown colour in surface (Zhou et al., 2005). In our case roots of Lithuanian peonies were reddish brown colour.

Pathogens *Ramularia paeoniae* Preuss., *Leptothyrium paeonae*, *Phyllotata paeoniae*, *Cronartium aslepiadeum* (Wild.) Fr. described on peony in literature (Petrauskaitė, Vengeliauskaitė, 1978) were not detected on Lithuanian peonies. M. Samuitienė and M. Navalinskienė tested Lithuanian peony cultivars (‘Virgilijus’, ‘Garbė Motinai’, ‘Prof. K. Grybauskas’) and 8 hybrids for visual viral symptoms (Navalinskienė, Samuitienė, 2004). Plants were found to be healthy, except one plant in the cultivar ‘Garbė Motinai’ and solitary instances in some hybrids, which showed symptoms of ringpot disease.

**Conclusions.** 1. The morphological and decorative properties of Lithuanian peony cultivars and hybrids created in Kaunas Botanical garden by O. Skeiviene distinguish themselves in large diversity: blossom duration – 10–20 days, blossom productivity – 12–35 in bush, blossom size – 14–20 cm in diameter, bush height – 70–114 cm, blossom forms are single and double.

2. 41.6% changeability of bush height, blossom diameter, and blossom productivity was influenced by genotype.

3. The detected pathogens *Botrytis paeonia* Oud., *Fusarium oxysporum* Schltdl., *Septoria paeonia* Vest. did not cause significant damage to plants.
References

KAUNO BOTANIKOS SODE SUKURTŲ BIJŪNŲ MORFOLOGINIŲ IR DEKORATYVINIŲ SAVYBIŲ TYRIMAS

S. Dapkūnienė, J. Varkulevičienė, A. Stankevičienė, O. Motiejūnaitė

Santrauka

Straipsnyje apibendrinama Vytauto Didžiojo Universiteto Kauno botanikos sode auginamo puikiojo bijūno (Paeonia lactiflora Pall.) Onos Skeivienės sukurtų 3 veislių ir 16 hibridų morfologinės ir dekoratyvinės savybės. Tirtų bijūnų veislių ir hibridų morfologinėms ir dekoratyvinėms savybėms būdinga didelė įvairovė: kero žydėjimo trukmė tęsiasi 10–20 dienų; žydėjimo produktyvumas – 12–35 žiedai kere; žiedo dydis 14–20 cm, kerų aukštis – 70–114 cm; žiedo forma tuščiavidurė ir pilnavidurė. Žydėjimo produktyvumas didėja su veislių amžiumi. 41,6% kero aukščio, žiedo skersmens ir žydėjimo produktyvumo kitimams darė įtaką genotipui. Vegetacijos metu aptikti ligų sukėlėjai (Botrytis paeonia, Fusarium oxysporum, Septoria paeonia) augalams didelės žalos nepadarė.

Reikšminiai žodžiai: lietuviškos veislių ir hibridai, morfologinės ir dekoratyvinės savybės, Paeonia lactiflora Pall.
**STORAGE OF STRAWBERRY PLANTS IN VITRO, THEIR STABILITY AND ECONOMICAL ASPECTS**

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Economical efficiency of different in vitro techniques depends on botanical species and the scale of production. We have shown that the profitability of micropropagation for commercial laboratories can achieve 160%. In system of healthy plant production sufficient costs are charged on regular virology testing. It is possible to avoid partly of these costs by means of keeping virus-free plants in vitro. We have investigated the influence of strawberry long-term storage on propagation and rooting capacity, appearance of non-true type regenerants, pollen fertility, yielding capacity and vegetative productivity. It was shown the relatively high stability of the main pomological characters of strawberry plants after 15 years of storage. For economical evaluations we have compared methods of deposition in vitro and the maintenance of usual field collections. For this purpose the expenses of both treatments were counted. Calculations were made for 50 varieties (20 copies per each variety, total quantity 1000 copies). According to our calculations, the storage of strawberry in vitro in refrigerating chambers is 3.2 times more economical than maintenance of field collection. Besides, there is significant economy of the soil areas. Also it is necessary to note, that stored in vitro material does not reduce the phytosanitary status. According to our researches, plants after in vitro storage produced more runner plants in comparison with traditionally propagated ones. Thus the potential economic profit from strawberry collection kept in vitro is significantly bigger, than it is shown by direct calculations.

**Key words:** biotechnology, economical effectiveness, long term storage, micropropagation, stability, strawberry.

**Introduction.** Mullin and Schlegel showed the possibility of in vitro storage of strawberry cultures in 1976 (Mullin, Shlegel, 1976). Since that investigation many different improvements have been made and reliable techniques for long-term storage of many species have been developed (Самсонова, Трушечкин, 1991). However, there were very few facts about stability of the main pomological characters after in vitro storage. The main methods of in vitro storage are well known (Вусоцкий, 2006, 2002). For horticultural species the most suitable is the technique of keeping material at minimal growth conditions (Вусоцкий, 2002). This method can be useful as a part of cryopreservation. It is clear that the problem of genetic stability during long-term storage is almost the same as the one during micropropagation (Swartz et al., 1981). At our Institute we check the stability of strawberry and raspberry plants after cryopreservation (Карпова et al., 2002). Nevertheless, the complete investigation on long-term storage material has been absent. In our work we studied the influence...
of *in vitro* storage on growth, propagation and rooting capacity during recovery stage and the growth of stored material in field.

**Material and methods.** Different types of strawberry varieties: common June-fruiting varieties ‘Dukat’, ‘Holiday’, ‘Kokinskaya pozdniya’, ‘Naydena Dobraya’, ‘Senga-Sengana’, ‘Red Gauntlet’, ‘Zenit’, ever bearing variety ‘Geneva’ and day-neutral variety ‘Tribute’ were taken for our experiments. All these varieties were maintained in collection of Institute of Plant Physiology named after K. A. Timiryazev of Russian Academy of Science for 12–15 years. As control treatment there were used explants of the same varieties grown *in vitro* during 3 subcultivations after introduction in sterile culture. The explants were cultivated on standard propagation media, rooting media, transferred into sterile soil and then in field conditions. Propagation rate, rate of rooting, percentage of survived plants, vegetative and generative productivity, pollen fertility, appearance of non true to type plants were taken into consideration during observations. The analyses of variance and estimation of error of mean were done.

**Results.** It was shown that during the first steps of *in vitro* cultivation, the difference between experimental and a control material was sufficient only for some varieties (Table 1). For propagation rate the greatest difference was obtained in explants of ‘Senga-Sengana’ and ‘Tribute’. The same situation was noticed and for the shoots size. The other parameters both of stored material and of the control one were very similar.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Variants</th>
<th>Number of additional shoots</th>
<th>Average number of leaves per shoot</th>
<th>Average size of explant (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Papilädes (g)</td>
<td>Valdantins lapšs (g)</td>
<td>eksploanto dydis (mm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-st</td>
<td>2-nd</td>
<td>3-rd</td>
</tr>
<tr>
<td>‘Naydena Dobraya’</td>
<td>Storage</td>
<td>Laikimas</td>
<td>4.7</td>
<td>4.6</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Kontrolė</td>
<td>5.2</td>
<td>3.9</td>
<td>4.1</td>
</tr>
<tr>
<td>‘Red Gauntlet’</td>
<td>Storage</td>
<td>Laikimas</td>
<td>3.8</td>
<td>4.9</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Kontrolė</td>
<td>4.9</td>
<td>4.5</td>
<td>5.3</td>
</tr>
<tr>
<td>‘Geneva’</td>
<td>Storage</td>
<td>Laikimas</td>
<td>4.6</td>
<td>5.6</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Kontrolė</td>
<td>5.4</td>
<td>5.2</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Table 1 continued
On the rooting stage the significant difference, as far as percentage of rhizogenesis concerns, was noticed only for ‘Dukat’ where explants of control treatment showed the higher rooting capacity ($F = 20.3^{***}$). The quantity of roots and their length were higher for control explants (Table 2) and depended on variety peculiarities.

This fact had direct influence on survival rate at the adaptation stage. The control plants could be more successfully transferred into non-sterile conditions. The data concerning probability of survival are represented in Fig. 1.

After acclimatization there was the possibility to analyze the appearance of non-true type regenerants. Such off true type plants occurred as among stored material so among the control one. The most often we observed the white striped leaves. For example, 19.5% of ‘Tribute’ plants carried this character after storage and 15.0% of control. 2.5–11% of abnormal leaves were noticed on plants ‘Geneva’, ‘Holiday’, and ‘Dukat’ depending on variety and treatment. Another types of deviations were variegated leaves, multiapices and chlorosis. There were 1.7–4.8% of them.

Vegetative productivity of stored plants with control material at the end of the first growing season was compared in the field. The results are shown in Table 3.

Table 2. Root development of strawberry shoots after long-term storage
It is clear that mean data concerning stolon number, number of runner plants, and number of leaves was the same. Significant difference was noticed only for some varieties. In general, reaction of field plants depended on genotype. So ‘Kokinskaya pozdniya’ and ‘Holiday’ control plants exceed the plants after long term stored in vitro in stolon number of runner plants and number of leaves. In contrast, plants of variety ‘Naydena Dobraya’ from stored material gave significantly more stolons and runner plants.

**Fig. 1.** Survival of strawberry plants in soil substrate after different treatment
Table 3. Vegetative productivity of stored and control plants after the first growing season in field conditions

Table 3 continued

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment Variantas</th>
<th>Number of stolons</th>
<th>Number of runner plants</th>
<th>Number of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Storage</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Geneva’</td>
<td>1 00.0</td>
<td>97.5</td>
<td>81.3</td>
<td>10.7</td>
</tr>
<tr>
<td>‘Holiday’</td>
<td>98.6</td>
<td>88.5</td>
<td>89.0</td>
<td></td>
</tr>
<tr>
<td>‘Red Gauntlet’</td>
<td>93.5</td>
<td>96.0</td>
<td>91.0</td>
<td></td>
</tr>
<tr>
<td>‘Zenit’</td>
<td>100.0</td>
<td>96.8</td>
<td>89.0</td>
<td></td>
</tr>
<tr>
<td>‘Dukat’</td>
<td>88.0</td>
<td>97.5</td>
<td>83.5</td>
<td></td>
</tr>
<tr>
<td>‘Tribute’</td>
<td>89.0</td>
<td>95.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Naydena Dobraya’</td>
<td>14.2**</td>
<td>11.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Zenit’</td>
<td>5.8</td>
<td>11.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Dukat’</td>
<td>3.8</td>
<td>6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Tribute’</td>
<td>2.5</td>
<td>3.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Geneva’</td>
<td>3.0</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Kokinskaya poodziana’</td>
<td>10.2***</td>
<td>11.7***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Only several plants gave flowers and fruits in first vegetative season. Therefore, the estimation of biological yielding capacity was done in second year. The data are given in Fig. 2. The results of field experiments demonstrated that the generative productivity of several varieties (‘Kokinskaya pozdniya’, ‘Naydena Dobraya’, ‘Tribute’, ‘Zenit’) was slightly higher than this of control plants and sufficiently higher than this of ‘Dukat’ plants. In contrast, biological yield of ‘Holiday’ and ‘Geneva’ was 3 and 2 times greater than this of stored plants. It confirms the prevalent role of variety’s peculiarities in reaction to long-term storage of strawberry plants in vitro.

**Fig. 2.** Biological yielding capacity of different strawberry varieties after long-term storage and control treatment in second year of vegetation

It is known that every technique will be accepted to the wide use if it is economically sound. That is why we appreciated in vitro storage also from economic point of view. There were estimated 1000 plants, which were stored in vitro in minimal
growth conditions and in the field. The results of estimation are presented in Table 4.

Table 4. Estimation of cost-effectiveness of in vitro storage of strawberry varieties (50 varieties, 20 plants per each) during 10 years

<table>
<thead>
<tr>
<th>Expense items</th>
<th>Storage under field conditions</th>
<th>Storage in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Laimynas laikymo laukanėse</td>
<td>Laimymo in vitro</td>
</tr>
<tr>
<td>1. Wages</td>
<td>2 735.0</td>
<td>3 000.0</td>
</tr>
<tr>
<td>Darbo užtikrinimas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Plant material</td>
<td>35 000.0</td>
<td>1 750.0</td>
</tr>
<tr>
<td>Augalinė žalva</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Fuel</td>
<td>7611.0</td>
<td></td>
</tr>
<tr>
<td>Degalū</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Electric power</td>
<td></td>
<td>4 196.0</td>
</tr>
<tr>
<td>Elektros enerija</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Fertilizers, pesticides and others</td>
<td>64 110.0</td>
<td></td>
</tr>
<tr>
<td>Trašos, pesticidų ir kit.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Costs for adaptation</td>
<td></td>
<td>402.0</td>
</tr>
<tr>
<td>Adaptacijos išlaidos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Chemical substances</td>
<td></td>
<td>945.0</td>
</tr>
<tr>
<td>Cheminės medžiagos</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Amortization</td>
<td>5 512.0</td>
<td>20 000.0</td>
</tr>
<tr>
<td>Amortizacija</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Designing</td>
<td>200.0</td>
<td></td>
</tr>
<tr>
<td>Planteimis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Other direct costs</td>
<td>5 758.0</td>
<td>1 515.0</td>
</tr>
<tr>
<td>Kitos tiekinių išlaidų(5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total direct costs</td>
<td>120 926.0</td>
<td>31 805.0</td>
</tr>
<tr>
<td>In vitro išlaidų</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Overhead expenses</td>
<td>2 474.0</td>
<td>6 900.0</td>
</tr>
<tr>
<td>Pradžių išlaidai(30%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Insurance funds</td>
<td>9 872.0</td>
<td>3 066.0</td>
</tr>
<tr>
<td>Dariusmas(8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total costs</td>
<td>133 272.0</td>
<td>41 805.0</td>
</tr>
<tr>
<td>In vitro</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion. The behavior of micropropagated in vitro strawberry plants under field conditions was investigated early (Алексеенко et al., 2005; Висоцкий, Алексеенко, 2000). It was noticed the increase of vegetative productivity during the first and second years. That’s why the micropropagated plants are recommended mainly for mother-stock plantation. In present investigation it has been shown that recovered plants after long-term storage on proliferation stage in vitro in general non-significantly differ from not long ago introduced ones in sterile culture. The lesser number of roots and their length could be explained by habituation of explants during long keeping in vitro and reducing of their sensitivity to exogenous auxins. Such poor development of root system of test-tube plants in final result caused reduction of output of the adapted plants in non-sterile conditions.

As far as appearance concerns, plants with different type of abnormalities are rather typical phenomenon for tissue culture technique. The same types of changes
were observed and for other plants (Lis, 1997). Relatively low frequency of appearance of such alterations could be the result of auto-selection during long-term storage when the abnormalities were eliminated due to lower growth or slower cells divisions.

The vegetative and generative productivity mainly depend on variety’s peculiarities. According to our results, this fact should be taken into consideration during long storage in vitro of different strawberry varieties.

As it was shown, large scale of in vitro propagation of different botanical species can be highly profitable (Куёиков et al., 2005). The economic profit from in vitro storage of strawberry collections can be also sufficient. According to our calculations, the maintenance, for example, of 50 varieties in simple refrigerating chamber is 3 and more time cheaper than the field maintenance. Taking into consideration the increasing propagation rate in field, the direct profit will be significantly higher.

Conclusions. The results of present investigation allowed drawing the conclusion about the possibility of long term maintenance (more than 12 years) of strawberry varieties and other valuable forms in vitro. It was demonstrated relatively high stability of the main variety’s characteristics after such storage on the stage recovering in vitro (proliferation, rooting, weaning) and following growth under field conditions. We have dealt mainly with morphological features. Certainly it would be better to use additionally specific genetic markers or RAPD-analysis for deeper investigation. It is planed in future work.

The cost of in vitro collections keeping is at least 3 times smaller in comparison to the maintenance of field collections, but such technique requires special laboratories. That’s why this technique can be recommended at present only for scientific institutions.

Acknowledgements. The authors would like to thank senior research worker of Institute of Plant Physiology named after K. A. Timiryazev of Russian Academy of Science Olga Vysotskaya for the material from long term stored strawberry collection.

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10. Сабонова О. Н., Трушечкин В. Г. Способ сохранения in vitro жизнеспособности растений // А. с. СССР № 1630708, МПК A0144/00 / Заявка № 4636303/13 от 24.11.88; опубл. 28.02.91. Б. И. № 8.

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**BRAŠKIŲ SAUGOJIMAS IN VITRO SISTEMOJE, JŲ STABILUMAS IR EKONOMINIAI ASPEKTAI**

I. Kulikov, V. Vysotskiy
Santrauka


**Reikšminiai žodžiai:** biotechnologija, braškė, ekonominis efektyvumas, ilgalaikis saugojimas, mikrodauginimas, stabilumas.
IN VITRO CULTIVATION OF GRAPE CULTURE UNDER SOLID-STATE LIGHTING

Anželika KURILČIK¹,², Renata MIKLUŠYTĖ-ČANOVA², Silva ŽILINSKAITĖ², Stasė DAPKŪNIENĖ², Pavelas DUCHOVSΚIS¹, Genadij KURILČIK³, Gintautas TAMULAITIS³, Artūras ŽUKAUSKAS³

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We report on the influence of illumination spectrum on grape morphogenesis. The plantlets were cultivated in vitro using an illumination system based on light-emitting diodes (LEDs). Four groups of LEDs emitting in red (at the wavelengths of 660 nm and 640 nm), blue (450 nm), and far-red (735 nm) were exploited and the ratios of the photon flux densities (PFDs) of these spectral components were maintained at selected values. The plantlets were simultaneously cultivated in five growth modules, which were identical except of a preset illumination spectrum. Culture of grape Vitis vinifera L. ‘Gailiūnė’ has been cultivated.

Morphological and biometric parameters and concentration of photosynthetic pigments in the plants were measured after 28 or 30 days of the treatment. The total PFD of 40–55 µmol m⁻² s⁻¹ was found to be optimal for growth of the grape plantlets. Our study was focused on elucidation of the morphogenetic influence of illumination in the blue and far-red regions on the development of grape explants.

We demonstrate that spectrally selective solid-state lighting is an effective tool for cultivation of grape culture in vitro and discuss the prospective for using this technique on a commercial scale.

Key words: in vitro plant cultivation, light-emitting diodes, morphogenesis, Vitis vinifera.

Introduction. Light is the energy source for photosynthesis and the main factor of plant development. Lighting conditions are very important for growth and development of plantlets in culture vessels of many in vitro grown species, including grapevines. The control and optimization of illumination is essential for a successful acclimatization, increase of the survival rate, and decrease of the acclimatization period (Amâncio et al., 1999). The common sources of light currently used for in vitro plant cultivation are fluorescent lamps. Metal halide, high-pressure sodium, and incandescent lamps are being also applied. However, these sources emit a large portion of light in spectral regions that are unnecessary for efficient growth of plants. Recently, solid-state lighting sources based on light-emitting diodes (LEDs) with narrow-band emission have been introduced for more efficient in vitro plant growth (Heo et al., 2006; Jao et al., 2005;
In comparison with conventional fluorescent lamps, LED illuminators have several advantages, such as longer lifetime, smaller mass and volume, lower thermal emission, and selective monochromic spectrum (Bula et al., 1991; Brown et al., 1995; Tanaka et al., 1998). LED-based illuminators provide an alternative to fluorescent lamps, as a light source with tailored spectrum that can meet specific needs of plants (Žukauskas et al., 2002). Recently, computer-controlled LED illuminators were successfully introduced into greenhouse plant cultivation (Bliznikas et al., 2004; Tamulaitis et al., 2005).

Grapevine (Vitis vinifera L.) is one of the most important fruit crops cultivated worldwide (Das et al., 2002). Harvest of the grapevine is used in wine industry and for the production of grapes and raisins. Grapevine is propagated by hardwood cuttings or via somatic embryogenesis in vitro (Martins et al., 2003; Croce et al., 2005). Grapevine tissue and organ culture have important practical applications in viticulture. The basic purpose of in vitro propagation is the elimination of grapevine diseases via meristem culture. In breeding programs, in vitro propagation can be successfully used as an alternative for grapevine rapid multiplication (Slavtcheva and Dimitrova, 1999).

The influence of illumination spectrum on in vitro growth of various plants has been studied by using solid-state illumination systems (Lian et al., 2002; Nhut et al., 2003; Jao et al., 2005). However, few reports are published on the micropropagation of grapevine by applying LED-based illumination. Heo et al. (2006) described the effect of light quality of a LED-based lamp on the growth of grapevine cultivated in vitro. However, the effects of illumination on morphogenesis of the in vitro cultured grapevine explants have not been carried out up to now.

The objective of the present study was to analyze the growth and morphogenesis of the grapevine plantlets cultured in vitro under various illumination spectra and total PFDs.

**Materials and methods.** Plant materials and culture condition. Grapevine plantlets (Vitis vinifera L. ‘Gailiūnė’) were grown in vitro in Murashige & Skoog (1962) modified nutrient medium (MS + IAA 0.2 mg/l + BAP 0.05 mg/l, ½ NH₄NO₃, ½ KNO₃, without vitamins, mio-inositol, and glycine) at 26/22°C (day/night) temperatures maintained within 1°C. Five millilitres of medium were dispensed in 16 × 150 mm tubes covered with PVC caps with air exchange. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20 min. One explant per tube was planted and 36 tubes per treatment were prepared. Explants in our experiments were taken from grapevine regenerants, which were grown up in vitro under white luminescent lamps OSRAM L 36 W/20. In sterile conditions, the top part of a shoot with one axillary bud was cut off and used as an explant. The length and fresh weight of the grapevine explants were 1±0.1 cm and 0.0105 ± 0.0012 g, respectively.

Light treatments. The cultures of in vitro plantlets were illuminated using red (at the wavelengths of 660 nm and 640 nm), blue (450 nm), and far-red (735 nm) LEDs powered by a self-designed driver. The plantlets were exposed to light for a 16 h photoperiod.
The first experiment was aimed at the optimization of the total PFD. The fractional PFDs of the spectral components are specified in Table 1. Before the experiment, the PFDs were measured at the level of plantlets using a radiometer-photometer (model RF-100 G.PAR-100, Sonopan, Poland). The fractional PFDs of the light components were maintained constant in all treatments. The fractions were fixed at 14% for 450-nm, 50% for 640-nm, 27% for 660-nm, and 9% for 735-nm components, respectively. The total PFD in different treatments was changed from 25 ± 5 in treatment A1 to 85 ± 5 µmol m$^{-2}$ s$^{-1}$ in treatment A5 (Table 1).

The second experiment was aimed at the study of explant morphogenesis. The fractional PFDs of the spectral components for five treatments are specified in Table 2. The total PFD was maintained constant in all treatments. The value of the total PFD was fixed at 43 ± 5 µmol m$^{-2}$ s$^{-1}$. The experiment was focused on the revealing of the influence of the 450-nm and 735-nm light components on the morphogenesis of the grapevine. Therefore, the PFDs of these two components were varied, while the red 660-nm component, which is the main component contributing to photosynthesis, was fixed at a constant level (22 µmol m$^{-2}$ s$^{-1}$).

Treatments B1 and B2 were arranged to reveal the influence of the far-red component in growth treatments without the blue component. The far-red 735-nm component was switched off in treatment B1 and maintained at 4 µmol m$^{-2}$ s$^{-1}$ in treatment B2. The blue component was fixed at 12 µmol m$^{-2}$ s$^{-1}$ in treatments B3, B4, and B5. These three treatments were arranged to reveal the influence of the increasing PFD of the far-red component on plantlet growth. The PFD of the far-red 735 nm component was maintained at 0, 4, and 9 µmol m$^{-2}$ s$^{-1}$ in treatments B3, B4, and B5, respectively.

The influence of the blue component at a fixed fractional PFD in the far-red region can be traced by comparison of the results of treatment pairs B1 versus B3 (no far-red illumination is applied) and B2 versus B4 (the PFD of the far-red component is maintained at 4 µmol m$^{-2}$ s$^{-1}$).

### Table 1. Photon flux densities (in µmol m$^{-2}$ s$^{-1}$) of illumination spectral components in growth treatments of the first experiment

<table>
<thead>
<tr>
<th>Treatment Variants</th>
<th>Total Band (100%)</th>
<th>450 nm (14%)</th>
<th>640 nm (50%)</th>
<th>660 nm (27%)</th>
<th>735 nm (9%)</th>
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<tr>
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<td>11.9</td>
<td>42.5</td>
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</table>

### Table 2. Photon flux densities (in µmol m$^{-2}$ s$^{-1}$) of illumination spectral components in growth treatments of the second experiment

<table>
<thead>
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<th>Treatment Variants</th>
<th>Total Band (100%)</th>
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<th>640 nm (50%)</th>
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<th>735 nm (9%)</th>
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skirtinguose antrojo eksperimento variantuose

Data collection and statistical analysis. The fresh and dry weight (FW and DW, respectively), stem and root length, number of leaves and roots, and amount of photosynthetic pigments of the grapevine plantlets were studied after 30
and 28 days of cultivation in the first and second experiment, respectively. 20 replicates were randomly selected for biometric analysis. To determine the DW, plantlets were oven-dried at 105°C until a constant mass was reached. The rest 15 replicates were used to measure the concentrations of the photosynthetic pigments. After extraction with 100% acetone according to the Wettstein (Gavrilenko et al., 2003) method, the total chlorophyll \( a \) and \( b \) and carotenoid content in leaf tissues per one gram of green foliage mass was analysed by a double-array spectrophotometer (model Genesys 6, Thermospectronic, USA).

Results. Optimization of the total PFD. The biometric parameters of the grapevine plantlets grown in vitro for 30 days under different photon flux densities are shown in Fig. 1. The parameters do not show a significant dependence on total PDF, which was varied from 25 to 85 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). The length of the shoots show a slight tendency to increase with increasing total PFD from 25 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in treatment A1 to 85 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in treatment A5 (Fig. 1A). The length and number of roots followed the same trend (Fig. 1A-B). Meanwhile, no significant differences in leaf number were observed in grapevine plantlets grown under different total PFDs (Fig. 1B).

Although a slight decrease of the fresh weight and leave weight (FW and LW, respectively) of the grapevine plantlets by increasing of the total PFD from 25 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in treatment A1 to 85 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in treatment A5 was observed, the differences between treatments were not significant (data not shown). The length of the largest leaf of the plantlets somewhat increased with the increase of the total PFD from 25 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in treatment A1 to 55 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in treatment A3 (Fig. 1C). The further increase of the PFD to 85 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in treatment A5 showed a tendency for a decrease of the length of the largest leaf. Meanwhile, no significant differences in leaf width were observed in grapevine plantlets grown under the total PFD in the range 25-70 \( \mu \text{mol m}^{-2} \text{s}^{-1} \). Only in treatment A5 (under 85 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)), this parameter was significantly lower (Fig. 1C).

The variations of the amount of photosynthetic pigments between treatments with different PFDs were not significant (Fig. 1D), except of a small decrease of all pigments by 15% in treatment A3 (55 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)). The ratio of chlorophyll \( a \) and \( b \) concentrations slightly increased with the increase of the total PFDs from 25 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in treatment A1 to 85 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) in treatment A5 (Fig. 1E). Meanwhile, the ratio
of the total chlorophyll concentration \((a + b)\) to the concentration of carotenoids was similar in all treatments (data not shown).

**Fig. 1.** Biometric parameters of grape explants grown *in vitro* under illumination at different total flux densities as specified in Table 1: shoot and root length (A), number of leaves and roots (B), parameters of the largest leaf (C), content of photosynthetic pigments in leaves (D), ratio of the concentrations of chlorophylls \(a\) and \(b\) (E).

Taking into account all small differences observed while changing the total PFD, the optimal total PFD for growth of grapevine plantlets *in vitro* was estimated to be between 40 and 55 \(\mu\)mol m\(^{-2}\)s\(^{-1}\) (treatments A2-A3). The value of the PFD was selected from this range for the second experiment.

**Fig. 2.** Parameters of grape explants grown under different illumination
regimes as specified in Table 2: shoot and roots length (A), number of leaves and roots (B), DW/FW ratio (C), contents of photosynthetic pigments in leaves (D), ratio of the concentration of chlorophylls \(a\) and \(b\), ratio of the total content of chlorophylls to the content of carotenoids (E).

Study of the influence of blue and far-red spectral components on plantlet morphogenesis. The impact of the blue component was revealed by comparison of the results obtained in two pairs of treatments: B1 versus B3 and B2 versus B4. The plantlets grown without the blue component had larger height and number of roots but lower length of roots, number of leaves and concentrations of all photosynthetic pigments (see Fig. 2A, B, D).

Under the PFD of the blue component fixed at 12 \(\mu\)mol m\(^{-2}\)s\(^{-1}\), the increase of the partial PFD of the far-red component from 0 to 9 \(\mu\)mol m\(^{-2}\)s\(^{-1}\) (treatments
B3-B5) resulted in a tendency for an increase of the shoot and root lengths and number of roots, while the number of leaves and concentrations of all photosynthetic pigments showed a tendency for a decrease (Fig. 2A, B, D).

The FW and DW were similar within experimental error in all treatments (data not shown), whereas the DW/FW ratio showed a decrease by the increasing of the far-red component PFD from 0 µmol m$^{-2}$s$^{-1}$ to 4 µmol m$^{-2}$s$^{-1}$, both with and without the blue component (see treatments B1 versus B2 and treatments B3 versus B4 in Fig. 2C).

The plantlets grown in treatment B1 without both the blue component and the far-red component had a maximum value of the concentration ratio of chlorophyll $a$ and $b$, but a minimum value of the ratio of the total chlorophyll concentration ($a + b$) to the concentration of carotenoids (Fig. 2E).

**Discussion.** The first experiment was aimed at the determination of the optimal total PFD for the growth and development of the grapevine plantlets. Selection of the range of the PFDs used in our experiments was based on the published data (Kim et al., 2004; Jao et al., 2005). In spite of the weak dependence of the plantlet parameters on the total PFD, the range 40-55 µmol m$^{-2}$s$^{-1}$ can be pointed out as the optimal total PFD for the grapevine plantlets. In comparison with regenerants of grapevines grown under fluorescent lamps (Dapkūnienė et al., 2004), plants grown under solid-state irradiation exhibited smaller height, reduced numbers of leaves and roots, as well as lower amounts of chlorophylls $a$ and $b$. Meanwhile the length of roots and fresh weight were found larger for plants grown under LED treatments.

It is worth noting that no abnormalities imposed to the grapevine plantlets by intense illumination at the maximal PFD used in our experiments were observed. This is in contrast to our results of similar study of chrysanthemum plantlets (to be published). This difference is possibly an indication that the grapevine plantlets need higher total PFDs than the chrysanthemum plantlets do.

Our second experiment shows that the presence of the 450-nm component in the illumination spectrum inhibits the plantlet elongation and simultaneously enhances concentrations of all photosynthetic pigments. Such effects were observed also by Nhut et al. (2003) in strawberry, Kim et al. (2004) in Chrysanthemum, and Jao et al. (2005) in Zantedeschia grown in vitro. Our study revealed a different influence of the blue component on the growth and development of roots and leaves. The plantlets grown under the treatments with the blue component had a lower height and number of roots but a larger length of roots and larger number of leaves per plantlet (see treatments pairs B1 versus B3, and B2 versus B4 on Fig. 2A, B).

This study did not show any significant differences in accumulation of fresh and dry weight, which have been demonstrated by Nhut et al. (2003) and observed in our study of chrysanthemum. Meanwhile, the influence of the far-red light PFD on the DW to FW ratio was established. The increase in PFD of the far-red component resulted in a decrease of the DW/FW ratio, both with and without the blue component (see treatment B1 versus B2 and treatments B3-B5 in Fig. 2C). A stronger impact was observed in treatments without the blue light. Thus both blue and far-red light components participate in this process via phytochrome and cryptochrome systems.

In addition, the results of treatments B1 versus B3 and B2 versus B4 demonstrate that the blue component also enhances the synthesis of the photosynthetic pigments.
Meanwhile, the far-red component inhibits this process, especially in the presence of the blue light (see treatments B1 versus B2 and B3-B5 on Fig. 2D). Our study showed that the blue and far-red components interfere in the control of the photosynthetic pigments development. This conclusion is supported by the decrease in the ratio of the total concentration of chlorophylls and the concentration of carotenoids in absence of blue light due to far-red component (treatments B1 and B2 in Fig. 2E) and by a low sensitivity of these parameters to far-red component in the presence of blue light (treatments B3-B5).

Our results imply that the blue (450 nm) and far-red (735 nm) spectral components are involved in the plantlets growth and control of photosynthetic pigment development by synergetic interactions between blue/red light photoreceptors, cryptochromes and phytochromes. We suggest that the response of plants to variation of illumination spectrum depends on plant species. Some features of this response are common for all plants (e.g., inhibition of shoot elongation by blue light), whereas other features are individual. Thus, a fine tailoring of the illumination spectrum for commercial growth of the grapevine plantlets in vitro using the LED-based lighting technology is feasible, but requires further optimization.

**Conclusions.** The optimal value of the total PFD for in vitro growth of grapevine plantlets using a four-component LED lamp was found to be in the range 40–55 µmol m\(^{-2}\) s\(^{-1}\). The presence of the blue component in the illumination spectrum has an inhibiting influence on the plantlet elongation. Simultaneously, blue light enhances leave formation and increases the concentrations of all photosynthetic pigments. An increase in PFD of the far-red light has a suppressive influence on the processes of DW/FW accumulation and synthesis of photosynthetic pigments. An additional study including analysis of endogenous hormones is necessary for a deeper understanding of the effect of the illumination spectrum on grapevine growth and development.

**Acknowledgements.** This study was partially supported by the Lithuanian State Science and Studies Foundation under project HORTILED.

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VYNMEDŽIŲ KULTIVAVIMAS IN VITRO KIETAKŪNIO APŠVIETIMO SĄLYGOMIS


Santrauka


Nustatyta, kad vynmedžių eksplantų, auginamų in vitro, optimaliausios augimo sąlygos yra apie 40–55 µmol m⁻²s⁻¹ suminio fotonų srauto ribose. Nustatytas mėlynosios šviesos inhibuojantis poveikis eksplantų stiebo tisimui, kartu didėjant lapų skaičiui ir fotosintezės pigmentų koncentracijai. Išvertintas tolimosios raudonosios šviesos dalyvavimas sausos ir žalios masių akumuliacijos procesuose bei fotosintezės pigmentų sintezėje.

Pademonstruota, kad derinamo spektro šviestuvai, pagaminti panaudojant puslaidininkinius šviestukus, yra universalūs ir lankstūs priemonė augalų kultivavimui in vitro ir leidžia optimizuoti vynmedžių eksplantų fotosiliozologinius procesus.

Reikšminiai žodžiai: augalų kultivavimas in vitro, morfogenezė, šviestukai, Vitis vinifera.
RAPD – MOLECULAR MARKER IN DETECTION OF HYBRIDS \textit{LA HYBRIDS}

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In Poland Oriental lilies, \textit{Lilium longiflorum} and its Asiatic hybrids, have been very popular. As a result of interspecific and intergroup hybridization, progeny plants are obtained which not always are hybrids. Apomixis occurring in \textit{Lilium regale, L. longiflorum, L. superbum, L. pumilum} requires confirming that the obtained seedlings are hybrids. Studies identifying hybrids, based on DNA analysis began in the 80-ies of XX century. Molecular techniques, with the most popular one – RAPD are used for identification. As a result of crossing \textit{Lilium longiflorum} with ‘Connecticut King’ belonging to Asiatic hybrids, progeny plants were obtained using embryo isolation method. The confirmation of their hybrid status was performed by RAPD, analyzing DNA genotypes of two parental forms and six progeny plants. DNA was isolated from the leaves of young lilies growing in the greenhouse 11 primers of 10–nucleotide sequences chosen at random, from LA pr 1 to LA pr 11 were used. Amplified markers by RAPD were separated on agar gel and visualized in UV light at the presence of ethidium bromide. Among evaluated genotypes five turned out to be hybrids and one had the same genotype as maternal plant.

\textbf{Key words}: apomixis, \textit{Lilium}, RAPD.

\textbf{Introduction}. According to Orlikowska et al. (2001) there is a possibility of apomixis in the genus \textit{Lilium}. Molecular markers enable analysis at an early stage of plant development and shorten the process necessary for breeding a new cultivar (Karp, 2000; Orlikowska et al., 2001). Different marker techniques (Yamagishi, 1995; Marasek and Orlikowska, 2001, Wiejacha et al., 2001) are used to confirm the hybrid status of \textit{Lilium} obtained as a result of distant crossing. The majority of more and more widely used molecular markers are based on Polymerase Chain Reaction (PCR). Among them the most common technique is the analysis of random amplified polymorphic DNA (Williams et al., 1990; Choi et al., 1999).

The aim of the present study was verification of their hybrid status, which seedlings obtain by distant crossing \textit{L. longiflorum} and ‘Connecticut King’.

\textbf{Materials and methods}. Parental forms of the following plants were tested: ‘Connecticut King’ – (‘Keystone’ × ‘Connecticut Lass’) with yellow – slightly orange flowers without dots; \textit{Lilium longiflorum} – with long white flowers and six seedlings \textit{Lilium longiflorum} × ‘Connecticut King’ obtained by ovule rescue.

Methods RAPD–PCR. DNA was isolated from the leaves of juvenile plants growing in the greenhouse using Genomic Mini from A&A Biotechnology. In PCR-
RAPD reactions 11 primers, whose sequences are given in Tables 1–2, were used.

Polymerase chain reactions (PCR) were performed in 25 µl of mixture containing 100 µM of each dNTP, 3 mM MgCl$_2$, 1 U Taq polymerase (Recombinant MBI Fermentas), 2.5 µl buffer 10 × PCR with (NH$_4$)$_2$SO$_4$, 2.5 µg of BSA, 35 ng of primer, 15 ng of template DNA. RAPD analyses were performed in two replications using PTC 200 DNA Engine Thermal Cycler produced by MJ Research. Polymerase chain reaction profile was the following: initial incubation – 94°C, 4 min., 45 cycles: denaturation – 94°C, 15 sec.; primer annealing – 36°C, 30 sec.; primer extension – 72°C, 90 sec.; final extension – 72°C, 5 min.

The PCR products were electrophoresised in TBE buffer (0.45 M TRIS-borate, 0.01 M EDTA) in 1.5% agarose gel containing 0.5 mg/dm$^3$ of ethidium bromide and documented by FluorS- MultImager produced by BioRad.

**Results.** All 10-nucleotide primers used in the study generated amplification products. The number of reproducible amplification products depended on the primer and ranged from 1–13 (Tables 1–2).

<table>
<thead>
<tr>
<th>Table 1. Analysis of maternal <em>L. longiflorum</em> (Ll) and paternal ‘Connecticut King’ (CK) markers in putative hybrids of <em>L. longiflorum</em> × ‘Connecticut King’ (L 1 – LA 6). Shadowed cells indicate bands, which are markers of hybrid identification.</th>
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<table>
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<tr>
<th>Marker</th>
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Totally 58 amplification products were obtained, over half of which (35) indicated polymorphism. Only one of the primers did not generate RAPD polymorphic products (L pr 1). A considerable variation in parental form RAPD marker profile enabled to verify the hybrid status of the examined objects obtained as a result of crossing *L. longiflorum* ‘Snow Queen’ and ‘Connecticut King’ (LA 1- LA 6). Among the detected RAPD polymorphic markers 16 bands were specific for paternal genome and appeared in five out of six forms being verified (LA 1, LA 3, LA 4, LA 5, LA 6). The profiles of marker bands for the second object under study (LA 2) contained neither of the products.
and were 100% similar to the profiles obtained for *Lilium longiflorum* (maternal form). The obtained results indicate that the plants LA 1, LA 3, LA 4, LA 5, LA 6 may be recognized as intergroup hybrids, whereas LA 2 was probably formed by apomixis (Fig. 1). On the basis of the obtained results it should be stated that distant crossing of *L. longiflorum* × ‘Connecticut King’ may result in obtaining well-developed seeds if *L. longiflorum* is a maternal form, but only after isolating embryos.

**Fig. 1.** RAPD-PCR products amplified (in two replications) using L pr 7, L pr 8 (1- DNA molecular marker) characteristic of parental genotypes *L. longiflorum* ‘Snow Queen’ (Ll) and ‘Connecticut King’

![Image]

Table 2. Analysis of maternal *L. longiflorum* (Ll) and paternal ‘Connecticut King’ (CK) markers in putative hybrids of *L. longiflorum* × ‘Connecticut King’ (L7 – L11). Shadowed cells indicate bands, which are markers of hybrid identification.
Motininės *L. longiflorum* (Ll) ir tėvinės ‘Connecticut King’ (CK) medžiagos žymenų tyrimas tikėtinuose *L. longiflorum × ‘Connecticut King’* (L 7 – L 11) hibriduose. Užtamsinti langeliai rodo brūkšnius, kurie yra hibridų identifikavimo žymenys.

**Discussion.** Due to the possibility of apomictic embryo development during interspecific crossing in *Lilium* genus, using molecular markers to identify hybrid forms at early stages allows to improve the effectiveness of selection (Orlikowska et al., 2001).

RAPD technique is not the only one, which may be used for this kind of analysis. However, it is a relatively economical, simple in use and enables the analysis without the information concerning specific sequences within the examined genomes. On the other hand its sensitiveness to the conditions, under which the analysis is conducted, may create problems with reproducing experiments under conditions of another laboratory. Due to the mentioned problem of reproducing RAPD–PCR reactions the analyses presented in this paper were performed in two replications. Taking into consideration the fact that the conclusions concerning the hybrid status of five out of six analysed objects are based on the set of sixteen reproducible RAPD markers generated by 10 different nucleotide primers they should not raise any doubts.

**Conclusion.** RAPD–PCR molecular technique used for verifying the obtained seedlings showed that one out of six seedlings was probably formed via apomixis.

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**RYTIEJIŠKŲ LELIJŲ HIBRIDŲ NUSTATYMAS NAUDOJANT AAPD MOLEKULINIUS ŽYMENIS**

**B. Ploszaj**

*Santrauka*


**Reikšminiai žodžiai:** apomiksė, *Lilium*, RAPD.
Regeneration experiment was started with aim to develop an efficient plant regeneration system for Cydonia oblonga Mill. cultivars. Variation of organogenic capacity of leaves depending on their position on the microshoot, photoperiod influence on differences in regeneration and interaction of these two factors was investigated in this experiment. Shoots were regenerated from leaf obtained from microshoots culture in vitro. Leaf explants of three genotypes were collected from microshoots and placed on Murashige and Skoog medium (MS) supplemented with 0.056 mg l\(^{-1}\) naphthylacetic acid (NAA) and 7.05 mg l\(^{-1}\) of thidiazuron (TDZ). Explants were exposed to continuous 16 h photoperiod for 8 weeks, or in the dark for 4 weeks and then transferred to 16 h photoperiod for 4 weeks. Leaf position and photoperiod had a significant effect. Significant interaction effects between these two factors on explant regeneration rate was obtained: 54.2% in K 11, 100% in K 16 and 93.8% in K 19 Cydonia oblonga cultivars, compared to that reported previously (about 43%). This improvement in vitro in plant regeneration system allows developing transformation protocol for Cydonia oblonga.

Key words: Cydonia oblonga, regeneration, tissue culture.

Introduction. Quince (Cydonia oblonga) is used as a dwarfing rootstock for pear, but it is sensitive to low temperatures, resulting in reduced yield due to bud injury, flower damage, poor fruit set, or even whole plant death in susceptible cultivars. Tolerance to cold stress through the introduction of cold responsive genes is being investigated using an Agrobacterium-mediated gene transfer system (Seelye et al., 1994). The critical step for successful genetic transformation is the development of an efficient in vitro regeneration system (Ahuja, 2000; Perez-Tornero et al., 2000).

Regeneration of shoot in vitro from leaf has been reported for Cydonia oblonga (Dolcet-Sanjuan et al., 1991). Genotype influence on morfogenetic reaction of Cydonia oblonga leaf was investigated by Stanienė and Stanys (2004), but the effect of maturity of the explant and dark period influence before 16 h photoperiod to the regeneration percentage was not established. The aim of our work was to investigate variation of organogenic capacity of leaves depending on their position on the shoot and photoperiod of Cydonia oblonga Lithuanian cultivars.

Materials and methods. The work was performed at the Department of Genetic...
and Biotechnology of Horticulture Plants at the Lithuanian Institute of Horticulture in 2007. Three Lithuanian origin cultivars K 11, K 16, K 19 of *Cydonia oblonga* Mill. were used for the research.

**Tissue culture maintenance.** Microshoot culture *in vitro* was grown at the temperature and humidity controlled chamber at 22°C and 50 gmol m⁻² s⁻¹ PPF. 16 h photoperiod was provided by cool white fluorescent light. Microshoots were grown *in vitro* on Murashige and Skoog (1962) nutrient medium, supplemented with 100 mg l⁻¹ inosite; 0.5 mg l⁻¹ thiamine; 0.5 mg l⁻¹ pyridoxine; 0.5 mg l⁻¹ nicotinic acid; 1 mg l⁻¹ ascorbic acid, 0.75 mg l⁻¹ BAP, and solidified with 7 g l⁻¹ Phyto agar. The pH of the medium was adjusted to 5.8 before the addition of agar and autoclaved at 121°C for 30 min. Shoot cultures were maintained on this medium, transferred to fresh medium every 5–6 weeks.

Adventitious shoot induction from leaf explants. Shoots were regenerated from leaf obtained from microshoots culture *in vitro*. Explants were excised from *in vitro* shoot, wounded transverse slices on four-cuts and placed abaxial side up in 100 x 20 mm Petri dishes containing 25 to 30 ml Murashige and Skoog (1962) nutrient medium, supplemented with 100 mg l⁻¹ inosite; 0.5 mg l⁻¹ thiamine; 0.5 mg l⁻¹ pyridoxine; 0.5 mg l⁻¹ nicotinic acid; 1 mg l⁻¹ ascorbic acid, 0.056 mg l⁻¹ naphthylacetic acid (NAA) and 7.05 mg l⁻¹ of thidiazuron (TDZ) and solidified with 7 g l⁻¹ Phyto agar. Explants were exposed to continuous 16 h photoperiod for 8 weeks, or in the dark for 4 weeks and then transferred to 16 h photoperiod for 4 weeks. The explant regenerated and number of adventitious shoots centers per regenerated explant was recorded after 8 weeks on MS medium.

Organogenic capacity of leaves depending on their position on the shoot. For each cultivar (K 11, K 16, K 19), about 120 explants were collected from three different positions on the shoot (from terminal, middle, and basal part of shoot). Each treatment consisted of four replicated Petri dishes containing 10 explants. Explants were exposed to continuous 16 h photoperiod.

Photoperiod influence on differences in regeneration. For each cultivar (K 11, K 16, K 19), about 80 leaf explants per treatment combination were cultured in two different conditions. Half culture dishes were maintained in continuous 16 h photoperiod for 8 weeks, and half in the dark for 4 weeks and then transferred to 16 h photoperiod for 4 weeks.

Effect of interaction of leaf position on the shoot and photoperiod on differences in regeneration. About 96 leaf explants for each cultivar (K 11, K 16, K 19) were collected from terminal part of shoot. Each treatment consisted of four replicated Petri dishes with 12 explants. Half culture dishes were maintained in continuous 16 h photoperiod for 8 weeks, and half in the dark for 4 weeks and then transferred to 16 h photoperiod for 4 weeks.

Data collection and statistical analysis. Percent regeneration was defined as the percentage of explants that produced at least one shoot. Mean number of shoot centers per regenerated explant was obtained by counting the total number of shoot centers from regenerating explants in a plate. We recorded regeneration percent and number of shoot centers per regenerated explant after 8 weeks. Statistical analysis was conducted using the computer program ANOVA, from program packet “SELEKCIJA”
(Tarakanovas, 1999). Significant differences of treatment means were determined by the Duncan’s multiple range test.

**Results.** Organogenic capacity of leaves depending on their position on the shoot. Effect of maturity of the explant had significant effect on shoot regeneration. Leaf explants from terminal part of shoot formed significantly more shoots than leaves from middle and basal parts of shoot in all three cultivar of *Cydonia oblonga* (Table 1). The regeneration frequency depended on the plant genotype (Table 1). The highest results of regenerated explants were obtained from explants of terminal part of shoot in K 16 cultivar – 92.3% with 1.94 shoot centers per regenerating leaf, 77.5% with 2.03 shoot centers per regenerating leaf were obtained in K 11 cultivar and 72.5% with 2.28 shoot center per regenerating leaf in K 19 cultivar. Differences in frequency of explant regenerated from middle and basal parts of microshoots were not significant and varied from 25.0 to 37.9% of cultured leaves with 1.29 to 2.09 shoot centers per regenerating leaf (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Effect of leaf positions on the microshoot on regeneration of shoot from leaves of <em>Cydonia oblonga</em> cultivars in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variant Variatūr</strong></td>
</tr>
<tr>
<td><strong>Terminal part</strong></td>
</tr>
<tr>
<td>Explant tested, units</td>
</tr>
<tr>
<td>Time of shoot decay, vit</td>
</tr>
<tr>
<td>Number of regenerating explants</td>
</tr>
<tr>
<td>Frequency of explant regenerated</td>
</tr>
<tr>
<td>Explant tested, units</td>
</tr>
<tr>
<td>Time of shoot decay, vit</td>
</tr>
<tr>
<td>Number of regenerating explants</td>
</tr>
<tr>
<td>Frequency of explant regenerated</td>
</tr>
<tr>
<td>Explant tested, units</td>
</tr>
<tr>
<td>Time of shoot decay, vit</td>
</tr>
<tr>
<td>Number of regenerating explants</td>
</tr>
<tr>
<td>Frequency of explant regenerated</td>
</tr>
<tr>
<td>Explant tested, units</td>
</tr>
<tr>
<td>Time of shoot decay, vit</td>
</tr>
</tbody>
</table>

**Photoperiod influence on regeneration.** The dark period before
16 h photoperiod had significant effect on organogenesis. Significantly more shoot developed leaf explants cultured in the dark for 4 weeks and then transferred to 16 h photoperiod for 4 weeks compared to continuous 16 h photoperiod for 8 weeks in all three cultivar of *Cydonia oblonga* (Table 2). The regeneration frequency depended on the plant genotype (Table 2). The highest results of regenerated explants were obtained in K 19 and cultivar – 87.9% of cultured leaves with 2.52 shoot center per regenerating leaf and in K 11 cultivar 87.8% of cultured leaves with 2.42 shoot center per regenerating leaf. In K 16 genotype it was obtained 65.0% explant regeneration of cultured leaves with 2.12 shoot center per regenerating leaf. Regeneration in continuous 16 h photoperiod for 8 weeks in all three cultivar of *Cydonia oblonga* varied from 20.0 to 30.0% of cultured leaves with 1.22 to 2.13 shoot center per regenerating leaf (Table 2).

**Table 2. Effect of photoperiod on regeneration of shoot from leaves of *Cydonia oblonga* cultivars in vitro**

<table>
<thead>
<tr>
<th>Cultivar of <em>Cydonia oblonga</em></th>
<th>K 11</th>
<th>K 16</th>
<th>K 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variants</td>
<td>Explant tested units</td>
<td>Primary explant regeneration</td>
<td>Regenerated explants units</td>
</tr>
<tr>
<td>K 11</td>
<td>40</td>
<td>30.0 a</td>
<td>1.83</td>
</tr>
<tr>
<td>8 weeks in 16 h photoperiod</td>
<td>36 weeks</td>
<td>4 weeks in dark and transferred to 16 h photoperiod for 4 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Explant tested units</td>
<td>41</td>
<td>87.8 b</td>
<td>2.42</td>
</tr>
</tbody>
</table>

Influence of interaction of leaf position on the shoot and...
photo period on differences in regeneration. Basing on the results of previous experiments, we used only leaves from terminal part of the microshoot and placed it in the most favorable cultivation conditions (dark for 4 weeks and then transferred to 16 h photoperiod for 4 weeks). The frequency of regenerated explants reached 100% with 3.31 shoot center per regenerating leaf in K 16 cultivar. Lowest regeneration was obtained in K 11 cultivar – 54.2% of cultured leaves with 2.08 shoot center per regenerating leaf (Table 3).

Table 3. Effect of photoperiod on regeneration of shoot from explants from terminal part of shoot of Cydonia oblonga cultivars in vitro

Discussion. The high variability of shoot regeneration was observed depending on their position on the shoot. It may be the result of the variation of organogenic capacity of different maturity leaves. The youngest leaves had higher organogenic competence and explant regeneration was obtained from 72.5% to 92.3%, depending on Cydonia oblonga genotype, comparing to explant regeneration obtained from middle or basal parts of microshoot, that varied from 25.0 to 37.9% depending on genotype. Our results
agree with results reported in *Prunus dulcis* – a higher percentage of shoot regeneration was obtained from juvenile leaf explants compared to adult leaves (Miguel et al., 1996). Perez-Tornero and colleagues (2000) reported, that regeneration capacity of young leaves in *Prunus armeniaca* comparing to older leaves increased twice.

A difference in adventitious shoot regeneration was observed between explants cultured in dark period before 16 h photoperiod and in continuous 16 h photoperiod for 8 weeks.

Regeneration from 65.0 to 87.9% of cultured leaves with 2.12–2.52 shoot center per regenerating leaf were observed when cultures were maintained in the dark for 4 weeks and then transferred to 16 h photoperiod for 4 weeks. These results are in agreement with previous reports on adventitious shoot regeneration of *Prunus* species (Gentile et al., 2002–2003; Miguel et al., 1996; Pooler and Scorza, 1995) and apple (Welander, 1988). The dark period may be important because of its possible influence on endogenous hormones of the levels and interaction with exogenously applied growth regulators that may promote adventitious shoot regeneration.

We investigated a combination of two factors stimulating shoot regeneration. Youngest leaves from microshoots were grown for 4 weeks in darkness followed by 4 weeks under 16 h photoperiod. The frequency of regenerating leaves reached 100% with 3.31 shoot centers per regenerating leaf in K 16 cultivar. Lower regeneration was obtained in K 11 cultivar 54.2% of cultured leaves with 2.08 shoot center per regenerating leaf. This indicates that adventitious shoot regeneration from isolated leaves is the result of plant genotype response to combination of various environmental factors. Attention should be taken to genotypic differences during development protocols for shoot regeneration in vitro. Genotypic effects were reported for *Prunus* species (Espinosa et al., 2006; Ainsley et al., 2000, 2001b; Bassi and Cossio, 1991; Gentile et al., 2002; Grant and Hammatt, 2000). For this reason, it is necessary to investigate various genotypes under the same conditions in order to select the genotype that gives the best results.

Our investigation is a part of protocol for adventitious shoot regeneration from *Cydonia oblonga* leaves in vitro. Further research should be done to investigate other factors involved in adventitious shoot regeneration of *Cydonia oblonga* in order to optimize protocols for use in genetic transformation.

**Conclusion.** It was found that position of explant on the shoot and dark period before 16 h photoperiod enhance adventitious shoot regeneration of quince (*Cydonia oblonga* Mill.) cultivars K 11, K 16, K 19. Interaction between these two factors enhanced shoot regeneration from leaf explant of *Cydonia oblonga* K 16 cultivar to 100%.

**Acknowledgements.** The work was supported by Lithuanian State Science and Studies Foundation. Project ATSPARUMAS ŠALČIUI, reg. No. N – 07014.

*Gauta 2007 06
Parengta spausdinti 2007 06*

**References**
Santrauka

Regeneracijos eksperimento tikslas buvo sukurti efektyvią augalų regeneracijos sistemą *Cydonia oblonga* Mill. veislėms. Šiame eksperimente buvo tiriama lapų organogeninių galimybių įvairovės priklausomybė nuo lapų padėties ant mikroūglio, fotoperiodo įtaka ūglių regeneracijai ir šių dviejų veiksnių sąveika. Ūglių regeneracijai buvo naudoti lapai, izoliuoti nuo mikroūglio įkultūros *in vitro*. Trijų *Cydonia oblonga* Mill. genotipų lapų eksplantai buvo pasodinti į Murashige ir Skoog terpę (MS), praturtintą 0,056 mg l$^{-1}$ nafylacetinės rūgšties (NAR) ir 7,05 mg l$^{-1}$ tidiazurono (TDZ). Eksplantai buvo kultivuojami 8 savaites 16 valandų fotoperiode arba 4 savaites tamsoje, po to perkelti į 16 valandų fotoperiodą 4 savaitėms. Lapų padėtis ant ūgliai ir fotoperiodas turėjo esminę reikšmę ūglių regeneracijai. Buvo nustatyta, kad dviejų veiksnių tarpusavio sąveikos esmė yra efektas ūglių regeneracijos dažniui: 54,2% K 1, 100% K 6 ir 93,8% K 19 *Cydonia oblonga* veislėse. Šis augalų regeneracijos sistemos *in vitro* paverčia sudaryti prielaidas tranformacijos protokolo tobulinimui *Cydonia oblonga* veislėse.

_Reikšminiai žodžiai:_ *Cydonia oblonga*, ląstelių kultūra, regeneracija.
INVESTIGATION OF PHYA AND PHYB GENE EXPRESSION OF THALE CRESS (ARABIDOPSIS THALIANA (L.) HEINGH.)

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E-mail: j.vinskiene@lsdi.lt

Aiming to investigate expression of thale cress (Arabidopsis thaliana (L.) Heyngh.) genes PHYA and PHYB, which are involved in signal reception and transmission, total RNA was isolated from plants, grown in different illumination. Light emission diodes (LED), constructed by VU Material and Applied Science Institute, were used for analysis. RNA of Arabidopsis exposed to luminescence light was used for comparison (flow of light photons 50 µmolm⁻²s⁻¹).

Constitutive GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene was used as a control of gene expression. Copy DNA (cDNA) of PHYA gene fragment (region of 176-1215 bp), PHYB gene fragment (region of 125-1004 bp) and GAPDH gene was synthesized using reverse transcriptase and polymerase chain reaction (PCR).

Different in length PHYA (1 000 bp, 750 bp and 400 bp) and PHYB (875 bp and 560 bp) transcripts were got after electrophoresis during expression studies in various lighting conditions. It was estimated, that light quality and time of exposition influences expression of PHYA gene. Supposedly, this might be related to different regulation of PHYA gene promoter dependent on light conditions. Expression studies of PHYB gene revealed that light quality didn’t have impact on this gene expression and amount of PHYB transcripts under light effect relatively is the same.

Key words: Arabidopsis thaliana, cDNA, gene expression, phytochrome.

Introduction. One of the most important environmental factors affecting plant growth and survival is light. Light perception is mediated through the action of several plant photoreceptors, of these the most intensively studied are the phytochromes (Neuhaus et al., 1997; Fry et al., 2002; Schäfer and Bowle, 2002). Each phytochrome can exist in two photoconvertible conformers: the red light absorbing form (Pᵣ), generally considered to be physiologically inactive, and the far-red absorbing form (Pᵣᵢ). Pᵣ has an absorption maximum at ca. 665 nm and is converted to Pᵣᵢ, which absorbs maximally at ca. 730 nm, being thereby converted back to Pᵣ (Parks, 2003; Reid et al., 2004). Phytochromes play a critical role regulating the photomorphogenic development of the plant being in Pᵣᵢ form (Neuhaus et al., 1997; Hall et al., 2001). The Pᵣᵢ form of phyA acts through heterotrimeric G proteins to stimulate gene expression that results in chloroplast development and anthocyanin biosynthesis (Neuhaus et al., 1993).

It is estimated that phytochromes of thale cress (Arabidopsis thaliana (L.)
Heingh.) are encoded by a small family of five genes (PHYA, PHYB, PHYC, PHYD and PHYE) (Hoecker et al., 1998; Sharrock and Clack, 2002; Schäfer and Bowle, 2002). Most biophysical and functional characteristics for the phytochromes are known for phytochromes A (phyA) and B (phyB). The relative amounts of these chromoproteins vary according to the ambient light conditions. In this regard, phyA was found to comprise the great majority of photoreceptor type in dark-grown (etiolated) tissue, whereas light-grown green tissue showed this balance shift toward phyB. This dramatic change in photoreceptor proportion results from differences in the expression and stability of the different phytochrome types (Parks, 2003). Exposure to light causes rapid loss of phyA, not only through degradation of Pfr, but also because phyA mRNA is highly unstable and because transcription of the PHYA gene is under feedback down-regulation by Pfr. Thus, in light-grown plants phyA is present at barely detectable levels. In contrast, phyB is constitutively expressed and relatively stable as Pfr form in both dark-grown and light-grown plants. PhyB is synthesized slowly and appear not to be under such strict transcriptional regulation as phyA (Hall et al., 2001; Parks, 2003; Reid et al., 2004).

It is less known how the light of different wavelength affects expression of PHYA and PHYB genes. The main goal of our research is to examine expression of phytochromes A and B given in different light regime conditions.

**Material and methods.** Total RNA was extracted from thale cress (Arabidopsis thaliana (L.) Heynh.) Columbia ecotype using Perfect RNA, Eukaryotic, Mini Kit (MBI Fermentas, Lithuania) according to producer recommendations. RNA was isolated from plants exposed to different light conditions for 4, 8 and 12 days. Five illuminators (light emission diodes – LED), constructed by VU Material and Applied Science Institute, were used for analysis. They spread the light of four different wavelengths (447 nm, 638 nm, 669 nm, 731 nm). The first light source was supplemented with the light of 380 nm wavelength, the second – 622 nm, the third – 595 nm, the fifth – 520 nm. RNA of Arabidopsis exposed to luminescence light was used for comparison (flow of light photons 50 µmolm⁻²s⁻¹). Constitutive GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene was used as a control of gene expression.

Copy DNA (cDNA) of PHYA gene fragment (region of 176-1215 bp), PHYB gene fragment (region of 125-1004 bp) and GAPDH gene was synthesized using RNA of Arabidopsis and RevertAid™ H Minus First Strand cDNA Synthesis Kit (MBI Fermentas, Lithuania) according to producer supplied methodology. Primers used for cDNA synthesis were: PHYAF1, PHYBF1 and GAPDHF1 (Table).

The amplification reaction (PCR) was conducted in a 20 µl reaction mixture that contained 1 × Taq Buffer with (NH₄)₂SO₄, 3.1 mM MgCl₂, 0.2 mM dNTP, 1 µM of oligonucleotide primers PHYAR1, PHYBR1, GAPDHR1 (Table), 1,25 units of Taq DNA polymerase (MBI Fermentas, Lithuania) and 0,4 mg cDNA.

**Table. Primers, used for PCR, and their sequences**

<table>
<thead>
<tr>
<th>Lentele</th>
<th>PGR naudoti pradmenys ir į jų sekos</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA denaturation at 94°C for amplification of PHYA and PHYB gene fragments</td>
<td></td>
</tr>
</tbody>
</table>
was performed for 4 min, 35 cycles at 94°C – 1 min 15 s, 62.3°C (for PHYA) or 60.2°C (for PHYB) – 1 min 15 s, 72°C – 2 min, in the end – 6 min at 72°C. DNA denaturation at 94°C for amplification of GAPDH gene was performed for 2 min, 35 cycles at 94°C – 30 s, 51°C – 30 s, 72°C – 30 s (Mastercycler®, Eppendorf).

Amplified PCR products were subjected to electrophoresis on a horizontal 1% agarose gel. Obtained results were estimated by UV light using Easy Win32 (Herolab) computer program. GeneRuler™ 1 kb DNR Ladder (MBI Fermentas, Lithuania) has been used as DNA size marker.

Primers of PHYA and PHYB gene fragments were chosen according to sequences NM_100828 and NM_127435, respectively, published in NCBI GeneBank (http://www.ncbi.nlm.nih.gov/entrez/viewer). Primers of GAPDH gene were chosen according to primer sequences, published in http://zygem.com/products.php.

Results. Analyzing expression of PHYA gene three different transcripts 1 000 bp, 750 bp and 400 bp were got in the electrophoregram after isolation of Arabidopsis RNA after 4, 8 and 12 days (Fig. 1). We can see that all wavelengths used in our research determine formation of 400 bp transcript when RNA was extracted after 4 days. cDNA transcript of the same length was detected after 12 days when Arabidopsis plants were exposed to 2nd, 4th and 5th light sources. Transcript of 1 000 bp wasn’t detected in those plants, which were exposed to 1st and 2nd light sources for 4 days. PHYA gene fragment of 750 bp was got after RNA extraction after 4 and 8 days. After 12 days the same in length transcript was obtained from plants exposed to 3rd light source. According to this, we can propose that light quality and time of exposition influences expression of PHYA gene. This can be related to plants physiological age, also.

After investigation of PHYB gene expression under different light regime conditions it was estimated that light quality and time of exposition didn’t have impact for this gene expression. Transcripts of 875 bp and 560 bp were got under all light regime conditions (Fig. 2).

Transcripts 1000 bp, 750 bp and 400 bp of PHYA gene were got in electrophoregram after isolation of RNA from plants exposed to luminescence light. Whereas amount of PHYB gene transcripts compared to amount of transcripts under different light regime conditions didn’t change. 875 bp and 560 bp DNA fragments were obtained (Fig. 3).

Fig. 1. Influence of light regime for PHYA gene expression of Arabidopsis thaliana

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHYA</td>
<td>PHYA-F1</td>
<td>5′- ATCTCTAGAAGAACCAACACCTACACAGGCACTCTCACT-3′</td>
</tr>
<tr>
<td>PHYA</td>
<td>PHYA-R1</td>
<td>5′- ATCTCTAGAAGAACCAACACCTACACAGGCACTCTCACT-3′</td>
</tr>
<tr>
<td>PHYB</td>
<td>PHYB-F1</td>
<td>5′- ATCTCTAGAAGAACCAACACCTACACAGGCACTCTCACT-3′</td>
</tr>
<tr>
<td>PHYB</td>
<td>PHYB-R1</td>
<td>5′- ATCTCTAGAAGAACCAACACCTACACAGGCACTCTCACT-3′</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GAPDH-F1</td>
<td>5′- GCAGGCGCTTCTTTGTC-3′</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GAPDH-R1</td>
<td>5′- GCAGGCGCTTCTTTGTC-3′</td>
</tr>
</tbody>
</table>

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Analyzing expression of *Arabidopsis GAPDH* gene it was determined that its expression doesn’t change under different light conditions. In all cases transcripts
GAPDH of 300 bp were got (Fig. 4).

**Fig. 4.** Electrophoregram of GAPDH gene of *Arabidopsis thaliana*

4 pav. Baštėžių vairenio GAPDH geno elektroforegrama

1 – ė = (447 nm, 638 nm, 669 nm, 731 nm) + 380 nm;
2 – ė = (447 nm, 638 nm, 669 nm, 731 nm) + 622 nm;
3 – ė = (447 nm, 638 nm, 669 nm, 731 nm) + 595 nm;
4 – ė = 447 nm, 638 nm, 669 nm, 731 nm;
5 – ė = (447 nm, 638 nm, 669 nm, 731 nm) + 520

**Discussion.** According to Canton and Quail (1999), dicot *PHYA*, including that from *Arabidopsis*, is the first plant gene reported with multiple promoters that are differentially regulated by an environmental factor. In this gene the occurrence of three TATA boxes weakly or not regulated by light allows a certain persistent level of *PHYA* expression in light. This could be required to maintain basal levels of phyA protein in the light, where it has an active role in fully de-etiolated plants. Besides, the presence of alternative promoters is postulated to allow more flexibility in the regulation of the gene (Canton and Quail, 1999).
In higher eukaryotes such multiple promoters are frequently associated with genes that are expressed in a tissue-specific and/or developmentally specific manner (Ayoubi and Van De Ven, 1996). PHYA mRNR is the most abundant transcript in etiolated tissue and is the only phytochrome transcript to be regulated by light (Carter et al., 2000). So we can suppose that such variety of PHYA transcripts in our research could be determined because of different PHYA gene promoter regulation depended on light conditions.

The same amount of PHYB gene transcripts, which have been got under different light regime conditions (Fig. 3) in our research, confirms proposition of Kendrick and Kronenberg (1994), Clack et al. (1994), Parks (2003) that the abundance of PHYB transcripts is relatively unaltered by light in Arabidopsis.

GAPDH (glyceraldehyde-3-phosphate dehydrogenase) has been frequently considered as a constitutive housekeeping gene and used to normalize changes in specific gene expression. In some experimental systems its expression is constant at different times and after experimental manipulation (Valenti et al., 2006). Primers, supplied at http://zygem.com/products.php were used to amplify an exon of GAPDH gene of a broad range of common horticultural crops including monocots, stone fruit, citrus and the Solanaceae (apricot, lemon, tobacco, barley, soy, tomato et al.). Our research had shown that this gene could be used as internal control for Arabidopsis PHYA and PHYB gene expression studies also (Fig. 4).

Conclusions. 1. Several transcripts of PHYA gene were got after investigation of Arabidopsis PHYA gene expression. It was estimated, that light quality and time of exposition influences expression of PHYA gene. Supposedly, this might be related to different regulation of PHYA gene promoter dependent on light conditions.

2. Expression studies of PHYB gene have revealed that light quality didn’t have impact for this gene expression and amount of PHYB transcripts under light effect relatively is the same.

Acknowledgement. This research was supported by funds from Lithuanian State Science Studies Foundation. Project ANTOCIANINAI, reg. No. 11/2007.

References


Santrauka

Siekiant ištirti aplinkos signalų priėmime ir perdavime dalyvaujančių baltažiedžio vairenio 
(Arabidopsis thaliana (L.) Heyngh.) PHYA ir PHYB genų raišką, RNR išskirta iš skirtingose 
šviesos režimo sąlygose laikytų augalų. Tyrome naudoti VU Medžiagotyros ir taikomųjų 
mokslų instituto sukonstruoti šviestuvai (kietakūnės šviesos šviestuvai – LED). Palyginimui 
buvo naudota liuminescencinių lempų šviesoje auginto vairenio RNR (šviesos fotonų srautas 
50 µmolm⁻²s⁻¹). Genų raiškos kontrolei naudotas konstitutyvus GAPDH (gliceralaldehid-3-
fosfato dehidrogenazės) genas. Naudojant atvirkštinę transkripciją ir polimerazinę grandinų 
reakciją (PGR), susintetintos PHYA geno fragmento (176–1215 bp regionas), PHYB geno 
fragmento (125–1004 bp regionas) ir GAPDH geno kopijinės DNR (kDNR).

Raiškos tyrimų skirtingomis šviesos režimo sąlygomis metu, po elektroforezės gauti 
skirtingo ilgio PHYA (1 000 bp, 750 bp ir 400 bp) ir PHYB (875 bp ir 560 bp) genų transkriptai. 
Nustatyta, kad PHYA geno raiškos įtakos turėjo šviesos spektro kokybinė sudėtis bei ekspozicijos 
laikas. Manoma, kad tai gali būti susiję su skirtinga PHYA geno promotoriaus reguliacija 
priklausomai nuo šviesos sąlygų. PHYB geno raiškos tyrimas parodė, kad šviesos spektro 
kokybinė sudėtis šio geno raiškai poveikio neturi ir PHYB transkriptų kiekis šviesos poveikyje 
santykinai lieka nepakite.

Reikšminiai žodžiai: Arabidopsis thaliana, fitochromas, genų raiška, kDNR.
ANALYSIS OF DNA POLYMORPHISM OF HERBACEOUS PEONY SPECIES AND CULTIVARS

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DNA polymorphism of *P. officinalis* and *P. lactiflora* species introduced into ornamental plant collection of Lithuanian Institute of Horticulture and polymorphism of *P. lactiflora* cultivars and hybrids introduced or created in Lithuania was investigated; their genetic relationship and RAPD markers were estimated in this research. Ten genotypes of the peony were analyzed. For random polymorphic DNA sequence amplification 18 different primers were used. 14 selected primers produced 147 polymorphic reproducible DNA bands, from 3 to 16 per primer and ranging in size from 200 to 2000 base pairs. It was estimated that Lithuanian cultivars ‘Garbė Motinai’, ‘Virgilijus’, ‘Profesorius K.Grybauskas’ and hybrids ‘Maironis’, ‘Darius Girėnas’ were genetically close to French cultivars ‘Sarah Bernhardt’ and ‘Festiva Maxima’ but genetically distant from wild genotypes of *P. lactiflora*.

**Key words:** DNA polymorphism, *P. lactiflora*, *P. officinalis*, RAPD.

**Introduction.** *P. lactiflora*, *P. officinalis* and *P. tenuifolia* species and several cultivars (‘Edulis Superba’, ‘Festiva Maxima’, ‘Karl Rosenfield’) created in West Europe in XVIII century were more common in Lithuania in the beginning of 20th century. Several viable seedlings of *P. lactiflora* were created using inter-cultivar crossing and selected by O. Skeivienė in 1958; three of them – ‘Garbė Motinai’, ‘Profesorius K. Grybauskas’ and ‘Virgilijus’ were accredited as Lithuanian cultivars (Žliobiene et al., 1985). In order to increase morphological diversity of *Peony* it is important for hybridization to select plants as distant genetically as possible (Hosoki et al., 1997a). It was established that morphologically similar *Peony* cultivars or species could be rather distinct genetically for successful hybridization (Hosoki et al., 1997b). RAPD method was successfully used for genetic relationship analysis and for individual identification of several genotypes of *Paeonia* genus – *P. suffruticosa* subsp. *spontanea* and *P. rockii* populations (Pei et al., 1995), *P. suffruticosa* var. *spontanea* cultivars (Hosoki et al., 1997c), plants of *Paeonia* sect. Mountan DC. (Zouy-Ping et al., 1999). At the Lithuanian Institute of Horticulture investigation of herbaceous peony morphological traits was started in 1996 (Antanaitienė and Stanienė, 2001). The genetic identity of Lithuanian cultivars and hybrids and genetic distance between them and other cultivars or species were not investigated.

The aim of the research is to investigate DNA polymorphism of *P. officinalis* and *P. lactiflora* species introduced into Lithuania and polymorphism of *P. lactiflora* cultivars and hybrids introduced or created in Lithuania and to estimate their genetic
relationship and RAPD markers.

**Materials and methods.** Two morphological types of *P. lactiflora* species with different stigma color of ovary – white (1 genotype) and red (2 genotype), widespread in Lithuania planting genotype of *P. officinalis*, two French cultivars ‘Sarah Bernhardt’ Lemoine (1906) and ‘Festiva Maxima’ Miellez (1851) and five Lithuanian cultivars ‘Garbė Motinai’, ‘Virgilijus’, ‘Profesorius K.Grybauskas’ and hybrids ‘Maironis’ and ‘Darius Girėnas’ created by O. Skeivienė were analyzed by the RAPD method (random amplified polymorphic DNA). Total DNA was extracted from young fresh leaves using CTAB method in the spring of 2006. Eighteen different oligonucleotide primers were used for amplification of random sequences of DNA (Table 1) (‘Fermentas’).

**Table 1.** List of oligonucleotide primers used in RAPD analysis; number and size of amplified DNA bands

<table>
<thead>
<tr>
<th>Primer Pradmenys</th>
<th>Nucleotide sequence</th>
<th>Number of amplified bands</th>
<th>Amplification fragment size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>total bands</td>
<td>monomorphic</td>
</tr>
<tr>
<td>OPA 07</td>
<td>GAAAACGGGTG</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>OPA 08</td>
<td>GTGACGATGG</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>OPA 09</td>
<td>GGTTAACGCC</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>OPA 18</td>
<td>AGGTGACCTG</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>OPA 19</td>
<td>CAAAACGTCC</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>OPA 07</td>
<td>GTGACGCCG</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>OPA 08</td>
<td>GTCCACACGG</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>OPA 17</td>
<td>AGGGACACGG</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>OPA 18</td>
<td>CCACACAGT</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>OPA 19</td>
<td>ACCCGCAGG</td>
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<td>OPA 20</td>
<td>GGAACCTTAC</td>
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<td>MP 01</td>
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</tr>
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<td>MP 02</td>
<td>AGTCGTCCCC</td>
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<td>14</td>
</tr>
<tr>
<td>MP 03</td>
<td>CCATCCCCCA</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>MP 05</td>
<td>GTCATTCCCTGA</td>
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</tr>
<tr>
<td>MP 06</td>
<td>TGAGCTTCAC</td>
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</tr>
<tr>
<td>MP 07</td>
<td>TCGGACCGCA</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>MP 08</td>
<td>GTAACACGACGCCAT</td>
<td>14</td>
<td>12</td>
</tr>
</tbody>
</table>

20 µl mix for amplification reaction was composed of 2 µl Taq DNA polymerase buffer, 4 µl MgCl₂, 2 µl dNTP mix, 2 µl primer, 0.25 µl Taq polymerase and 1 µg of total genomic DNA dissolved in 0.25 µl TE buffer. Amplification reaction was performed in Eppendorf cycler programmed as follows: 45 seconds at 94°C for DNA fragments denaturation, 45 seconds at 40°C (at 45°C for primer MP 8) for primer hybridization and 45 seconds at 72°C for synthesis of DNA fragments. Cycle repeated 45 times with initial DNA denaturation for 3 min at 94°C and final extension 5 min at 72°C. Amplification products were distinguished in 1.5% agarose gel by electrophoresis.
Approximate molecular size of amplification products was estimated using molecular DNA marker a GeneRuler™ 1kb DNA Ladder Mix (‘Fermentas’).

PCR with every primer was repeated three times. Only reproducible bands were used for data analysis. Stable amplification band was documented and analyzed in Herolab camera using EasyWin32 program. Statistic analysis of results was calculated with computer program PopGene 3.2.

**Results.** DNA fragments were amplified with all selected primers (Table 1). They produced 3–16 bands per primer ranging in size from 200 to 2000 base pairs. All fragments amplified with primers MP 1, MP 3, MP 6, MP 7 were (possessed the some size) the same in size and were not used for data analysis.

16 bands of different size were obtained using OPA-19 primer (Fig. 1).


**P. lactiflora** 1 genotype (white ovary) showed a specific band of 1400 bp. *P. officinalis* species showed two specific bands of 730 and 1050 bp. These specific bands were considered as specific marker of genotype. The unique for a cultivar or species composition of polymorphic bands can be used as a representative marker of genotype.

Genetic identity (a font style *Italic*) and genetic distance values calculated using 147 bands obtained from 10 Peony genotypes shown in Table 2. The highest identity value was 0.7703 obtained among genotypes ‘Darius Girėnas’ and ‘Festiva Maxima’, ‘Sarah Bernhardt’ and ‘Festiva Maxima’. *P. officinalis* species was shown to be the
most distant genetically from all investigated genotypes of *P. lactiflora*.

**Table 2.** The genetic identity (*Italic*) and genetic distance of investigated *Paeonia* genus plants (by Nei (1978) (PopGene 3.2 program)

<table>
<thead>
<tr>
<th>Genotype Genotypes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Maironis'</td>
<td>1.0000</td>
<td>0.7432</td>
<td>0.7230</td>
<td>0.7239</td>
<td>0.7027</td>
<td>0.7027</td>
<td>0.6523</td>
<td>0.6456</td>
<td>0.6419</td>
<td>0.5514</td>
</tr>
<tr>
<td>&quot;Virgilijus'</td>
<td>0.2967</td>
<td>1.0000</td>
<td>0.6959</td>
<td>0.6932</td>
<td>0.7162</td>
<td>0.6081</td>
<td>0.6149</td>
<td>0.5135</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Garbe Motilei&quot;</td>
<td>0.3244</td>
<td>0.3625</td>
<td>1.0000</td>
<td>0.7658</td>
<td>0.7320</td>
<td>0.7320</td>
<td>0.6534</td>
<td>0.6014</td>
<td>0.6081</td>
<td>0.5203</td>
</tr>
<tr>
<td>&quot;Profess. K. Grybavaska&quot;</td>
<td>0.3244</td>
<td>0.2098</td>
<td>0.2787</td>
<td>1.0000</td>
<td>0.7500</td>
<td>0.7635</td>
<td>0.7200</td>
<td>0.6691</td>
<td>0.5203</td>
<td></td>
</tr>
<tr>
<td>&quot;Darius Girenas'&quot;</td>
<td>0.3528</td>
<td>0.3244</td>
<td>0.3244</td>
<td>0.2877</td>
<td>1.0000</td>
<td>0.7703</td>
<td>0.7027</td>
<td>0.5135</td>
<td>0.6284</td>
<td>0.4324</td>
</tr>
<tr>
<td>&quot;Sarah Bernhardi&quot;</td>
<td>0.3528</td>
<td>0.3338</td>
<td>0.3244</td>
<td>0.2698</td>
<td>0.2610</td>
<td>1.0000</td>
<td>0.7703</td>
<td>0.5946</td>
<td>0.5878</td>
<td>0.4459</td>
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<tr>
<td>&quot;Festiva Maxima&quot;</td>
<td>0.4754</td>
<td>0.3920</td>
<td>0.4225</td>
<td>0.3244</td>
<td>0.3528</td>
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<td>1.0000</td>
<td>0.5946</td>
<td>0.6419</td>
<td>0.4459</td>
</tr>
<tr>
<td>&quot;P. lactiflora&quot;</td>
<td>0.4329</td>
<td>0.4974</td>
<td>0.5086</td>
<td>0.5784</td>
<td>0.6668</td>
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<td>0.5199</td>
<td>1.0000</td>
<td>0.6524</td>
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</tr>
<tr>
<td>&quot;P. officinalis&quot;</td>
<td>0.4433</td>
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<td>0.5313</td>
<td>0.4433</td>
<td>0.3821</td>
<td>1.0000</td>
<td>0.4932</td>
</tr>
</tbody>
</table>

**Figure 2.** Genetic distance among peony genotypes. Dendrogram was made by Nei’s (1978) on the strength of genetic distance means between *Paeonia* genotypes (PopGene 3.2 program UPGMA PHYLIP version 3.5 modified by NEIGHBOR method)

2 lentelė. *Paeonia* genties augalų genetinis tapatumas (*Italic*) ir genetinis atstumas (pagal Nei (1978) (PopGene 3.2 programa)
The lowest value (0.4324) of genetic identity was estimated between *P. officinalis* species and hybrid ‘Darius Girėnas’. The dendrogram (Fig. 2) clearly shows that both genotypes of *P. lactiflora* with minor morphological distinctions were genetically related closely. Cultivars of *P. lactiflora* and its wild genotypes branched into separate cluster. Cultivar ‘Festiva Maxima’ and hybrid ‘Maironis’ were the most distinct genetically among all investigated *P. lactiflora* cultivars and hybrids. Cultivars ‘Virgilijus’ and ‘Profesorius K. Grybauskas’ as well as hybrid ‘Darius Girėnas’ and cultivar ‘Sarah Bernhardt’ were shown to be genetically closest.

**Discussion.** Heretofore, only morphological and phenological traits of introduced and created by O. Skeivienė peony cultivars were investigated (Varkulevičienė and Stankevičienė, 2006; Antanaitienė and Stanienė, 2001). Comparing such data with *Paeonia* descriptions by other authors, Lithuanian cultivars can be easily grouped into different garden peony groups. Nevertheless such comparison does not show the genetic distance among cultivars regardless their morphological or phenological similarities. RAPD is shown to be the useful method for genetic analysis (genotype identification, investigations of genetic variety after natural and artificial selection) in ornamental plants such as rhododendron (Iqbal et al., 1995), azalea (Kobayashi et al., 1995) and etc. Differently from the other authors who used RAPD method we used not only OPA and OPB primers but also primers of MP group. MP 2, MP 5, and MP 8 primes (10, 12 and 17 oligonucleotides, respectively) were applied successfully for the DNA analysis of investigated species and cultivars.

Wild species of *P. lactiflora* originated from China and were introduced into Japan in 10th century. According to the data of Japanese scientists, the wild species of *P. lactiflora* is genetically closer to the cultivars created in China and Japan than to the European cultivars of peony. We investigated the relationship among Lithuanian and European, Chinese, Japanese herbaceous cultivars of peony (previously genetically described by using RAPD method) (Hosoki et al., 1997a). Analysis proved that Lithuanian cultivars are genetically very distant from wild *P. lactiflora* species that is why it should be included to the schemes of cross-pollination of peony. Our data confirmed Hosoki et al. (1997a) results that *P. officinalis* species are highly genetically distant from *P. lactiflora* cultivars. In our research hybrid ‘Maironis’ with single flowers structured occupied a separate branch in the dendrogram. That is why it should attract the interest of peony breeders.

**Conclusions.** Lithuanian cultivars ‘Garbė Motinai’, ‘Virgilijus’, ‘Profesorius K. Grybauskas’ and hybrids ‘Maironis’ and ‘Darius Girėnas’ are genetically closer to French cultivars ‘Sarah Bernhardt’ and ‘Festiva Maxima’ but are highly genetically distant from both investigated genotypes of wild *P. lactiflora* species.
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SODININKYSTĖ IR DARŽININKYSTĖ. MOKSLO DARBAI. 2007. 26(3).

ŽOLINIŲ BIJŪNŲ RŪŠIŲ IR VEISLIŲ DNR POLIMORFIZMO TYRIMAS

I. Mažeikienė, V. Stanys, G. Stanienė

Santrauka


Reikšminiai žodžiai: DNR polimorfizmas, *P. lactiflora*, *P. officinalis*, RAPD.
INVESTIGATION OF STRAWBERRY HARDENING IN LOW TEMPERATURES IN VITRO

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Cold resistance of different strawberry varieties in vitro and ability to retain hardening after defrosting and repeated hardening. Phytohormons – gibberellin and abscisic acid added in the growing medium were investigated in Horticulture plant genetic and biotechnology department of LIH. We tried to model common conditions in temperate zone when freeze-thaw cycles often occur during wintertime. For investigation in vitro strawberries for the first time hardened in light at the temperature of +4°C for 35 days, kept at the temperature of +22°C for 2, 4, 6 and 14 days and frozen in vitro at the temperature of 9°C for 12 hours. Plant survival rate (%) and cold injury in point (0-not injured plants, 5-plant dead) was evaluated 3 weeks after freezing. It was shown that cold resistant strawberries after hardening remain cold hardy at optimal growing conditions 6–7 days while cold sensitive only 3–4 days. Abscisic acid additions in nutrient medium help to retain cold hardening while gibberellins have opposite effect – decreases level of cold hardening under optimal growing conditions. Repeated freezing and frequent fluctuation of temperature increase plant’s cold injury in vitro. When plants were frozen repeatedly, cold injury depended on genotype: plants of cold tolerant variety ‘Melody’ survived twice more (50–60%) than sensitive plants of variety ‘Holiday’.

Key words: explants, in vitro, Fragaria × ananassa varieties.

Introduction. Cold resistance of plants is not constant feature – it is fluctuating during all season. Before winter, when temperature of weather decrease, cold resistance of plants gradually grows – plants harden off. Cold resistance of plants starts to become weaker in springtime. Strawberries, like most orchard plants, are not enough tolerant to winter under Lithuanian climatic conditions.

Not only in Lithuania, but also in other countries of North Europe, under unfavourable wintering conditions, occurring almost every year, yield decrees of some widely grown strawberry varieties reaches 40% (Nes, 1997). Because of unsatisfactory cold tolerance, most strawberry varieties do not allow to show potential productivity.

It is determined, that cold resistance is partially dominant trait. It is predicted, that this feature is heritable independently from such economically valuable features like plant size, productivity, berry size and quality (Scott et al., 1975).

Essential features characterized winterhardiness – cold resistance at early autumn and beginning of spring, maximum freeze tolerance in the middle of winter, capacity to persist cold resistance during snowbreaks, capacity to harden off repeatedly and resistance of generative parts in spring (Kopan et al., 1997; Stushnoff, 1972). Mentioned
features are very important for strawberries during climate change, when snowbreaks are very frequent and snow cover is very thin in wintertime.

Hardening of plants – very complicated biochemical process, during which plants prepare to winter freeze. Various preservative substances accumulate in plant cells, quantity of water at vacuole decrease, structure and characteristic of protoplasm (semiclear, slimy alive substances of cells) change. Hardened plants became more tolerant not only for major freeze, but also for other negative winter factors.

Strawberry cultivars differ by the level of winterhardiness. Some more tolerant varieties are stable, others, more sensitive varieties are very tolerant during one year, but could be very vulnerable next year, also there are varieties, which freeze every year (Nes, 1997; Zurawicz et al., 1993).

Because of climatic conditions instability evaluation of cold resistance on field takes several years and needs considerable inputs. Screening and evaluation of strawberry cold resistance in vitro allows accelerating process of investigation, reduce its duration and inputs (Palonen et al., 1997; Rugienius et al., 2001). Plant hardening, COR gene expression were investigated at the Lithuanian Institute of Horticulture (Rugienius et al., 2007), but records about plant possibilities for repeated hardening are not enough.

The aim of experiment is to investigate cold resistance of different strawberry varieties in vitro and evaluate ability to retain hardening during fluctuating temperatures, when phytohormons – gibberellin and abscisic acid are added to growing medium.

**Materials and methods.** Investigations were carried out at in Horticulture plant genetic and biotechnology department of the Lithuanian Institute of Horticulture in 2004–2007. Strawberry *Fragaria x ananassa* Duch (Rozier) varieties ‘Melody’ – very cold resistant, ‘Venta’ – cold resistant, ‘Holiday’, ‘Elsanta’ – cold sensitive were used for investigations.

Strawberry plants (proliferous structures) before experiment were grown in flask (in vitro) on agarosed Murashige and Skoog (MS) nutrient medium with 1 mg/l BAP and 3% saccharose addition at the temperature of +22°C. For the experiment they were transferred to test tubes with the same medium. For investigations in vitro strawberries transferred to test tubes, hardened on light at the temperature of +4°C for 35 days, freeze in vitro at the temperature of –9°C for 12 hours, thawed at the temperature of +4°C for 12 hours.

Strawberry cold resistance investigations in vitro were accomplished in different ways: a) plants hardened at the temperature of +4°C 35 days and frozen at the temperature of -9°C in vitro, b) plants hardened for 35 days, then moved to cultivation room (+22°C) for 2, 4, 6 days and after that frozen at the same temperature in vitro, c) plants hardened for 35 days, first variant – plants stayed in cultivation room (+22°C) for 7 days, after that they hardened at the temperature of +4°C and frozen at the temperature of -9°C; second variant – plants stayed at cultivation room (+22°C) for 14 days, after that they hardened at the temperature of +4°C for 1 day and frozen at the temperature of -9°C. After freezing plants were replanted on MS medium in sterile conditions and kept at the temperature of +22°C.

In order to evaluate phytohormon impact on hardening plants of ‘Venta‘ and ‘Holiday’ after hardening for 35 days were replanted to media containing gibberellin
(GA₃) – 2.9 µM (1 mg/l), abscisic acid (ABA) – 100 µM (26.432 mg/l). The plants were kept at the temperature of +22°C for 7 days and frozen, then thawed as described previously.

We used 10–30 plants in test tubes of every variety for each variant. Evaluation of survived plants amount (%) and cold injury (by scale: 0 – not injured plants, 5 – plants dead) was made 30 days after freezing. All plants survived and dead were counted.

Records of both variants were analyzed by statistic program (Tarakanovas, 1996), plants lesion degree and survived plants average established, reliability of difference between varieties, hardening effect persistency dependence on genotype (variety) were evaluated.

**Results.** **Hardening level retain during plants growth in favourable conditions.**

Optimal temperature for strawberries growing *in vitro* is +22°C. The purpose of investigation was to establish, how long strawberry plants manage to keep the hardening, if they are grown after hardening at the temperature of +22°C. This temperature became least advantageous for hardening persisting and could express at the maximum that feature.

Our experiments revealed that strawberry plants are able to persist hardening 2 days after harden off growing them at the temperature of +22°C (Fig. 1.). Plant lesions became major after 4, 6 days at the temperature of +22°C. The lesion degree in comparison with 1 variant is bigger of strawberries, witch were frozen *in vitro* at once after hardening. The biggest point of lesion is of plants grown after hardening 6 days at the temperature of +22°C (4). To compare cold lesion degree between varieties, injury of variety ‘Melody’ plants frozen *in vitro* was the least – 0.2 point.

**Fig. 1.** Strawberry plant lesion by cold freezing *in vitro* at once after hardening (1), growing after hardening for 2 days at the temperature of +22°C (2), 4 days (3), 6 days (4)

**R etaining of hardening level under addition of abscisic acid and gibberellins in the growing medium.** The aim of investigation was to ascertain, what influence have medium additions of different

iš karto po grūdinimo (1), auginant po grūdinimo +22°C temperatūroje

2 paras (2), 4 paras, (3), 6 paras (4)

**Retaining of hardening level under addition of abscisic acid and gibberellins in the growing medium.** The aim of investigation was to ascertain, what influence have medium additions of different
phytohormones for strawberry hardening sustenance and cold resistance in vitro.

Degree of strawberry cold resistance after hardening for 5 days growing at the temperature of +22°C and freezing in vitro was less when strawberries were grown in medium with abscisic acid addition (Fig. 2.). Plants of variety ‘Melody’ grown in medium with abscisic acid addition were injured less (1.5 point) to compare with ‘Holiday’ plants.

**Fig. 2.** Strawberry plants, which survived after freezing in vitro (%).

Frozen after hardening and 5 days grown at the temperature of +22°C in medium with different phytohormone additions.

Strawberry plant injury by cold (points),

Percent of survived after freezing ‘Melody’ plants was higher than ‘Holiday’ plants (Fig. 2.). Percent of survived plants was higher in medium with abscisic acid addition to compare with gibberellins medium.

**Investigation of strawberry reaction to repeated hardening in vitro.**

After repeated hardening 70% of variety ‘Melody’ and 60% of ‘Holiday’ plants survived. 10% of variety ‘Melody’ plants were injured in 1, 2, 3 points, 40% – in 4 points, 30% of plants were dead. 20% of variety ‘Holiday’ plants were injured by 3 points, 40% – by 4 points, 40% of plants was dead (Fig. 3.). 20% of ‘Melody’ variety plants survived, but all ‘Holiday’ plants (100%) were dead, when plants were frozen without repeated hardening.

Part of variety ‘Melody’ plants survived with only small injury (1, 2 points), but when plants were frozen without repeated hardening they all died (‘Holiday’), or get major injury (3-5 points, ‘Melody’) (Fig. 4.).

**Fig. 3.** Strawberry plant freezing in vitro for 3 weeks after their staying in cultivation room at the temperature of +22°C, hardening them for 35 days at the temperature of +4°C before, keeping for 7 days at the temperature of +22°C (variant with repeated hardening) and for 14 days (variant without repeated hardening),
hardening at the temperature of +4°C (variant with repeated hardening), freezing both variant at the temperature of -9°C for 17 hours.

3 pav. Braškių augalų šaldymas in vitro praėjus 3 savaitėms po jų laikymo kultivavimo kambaryje, esant +22°C, prieš tai juos grūdinant +4°C temperatūroje 35 dienas, laikant +22°C temperatūroje 7 dienas (variantas su pakartotino grūdinimu) ir 14 dienų (variantas be pakartotino grūdinimo), grūdinant +4°C temperatūroje (variantas su pakartotinu grūdinimu), šaldant abu variantus -9°C temperatūroje 17 val.

Fig. 4. Strawberry variety injury in points, after plant hardening and without hardening repeatedly

4 pav. Braškių veislių pažeidimas balais, augalus grūdinant ir negrūdinant pakartotinai

Discussion. Strawberries have to survive several periods of freeze-thaw under normal conditions of Lithuanian winter. According to our data, during short-time thaw of 2 days, level of hardening remains unchanged. It was determined previously, that freezing duration at the stable freezing temperature does not have negative effect on strawberry vitality in vitro (Rugienius, Stanys, 2001). Cold sensitive varieties ‘Holiday’
and ‘Elsanta’ plants obtain cold injury after plants are kept for 4 days at the temperature of +22°C, but it does not effect essentially cold resistance of plants. It is also cleared up, that plants of cold sensitive varieties lose hardening, but cold resistance varieties preserve it in part, when plants are frozen for 6 days after hardening.

A lot of biochemical processes – accumulation of carbohydrates, dehidryns and other substances, or composition changes, take place on in plants during hardening (Levitt, 1980). We established in our experiments, that phytohormones abscisic acid addition in nutrient medium supports strawberry plant hardening and vitality after freezing in vitro, while gibberellins have opposite impact on hardening level. Abscisic acid regulates water balance in plants, and additional quantity of it in MS medium may give positive results for plant growing in vitro.

Reaction to phytohormones could be in touch with growing inhibition or induce, connected with phytochrome activity or with specific genes expression (Chinnusamy et al., 2006; Rapacz et al., 2003). Differently from our results, ABA did not have influence on hardening of rape (Rapacz et al., 2003). It shows that phytohormone influence to cold resistance does not have one meaning; it depends on other factors, including genotype.

Our investigations show, that plants can be hardening repeatedly, at least while period between hardening and freezing does not exceed 7 days. Repeated hardening is rather effective, because it increase strawberry plant surviving in 50–60%. Nevertheless cold resistant variety ‘Melody’ does not reach hardening maximum, as it could be decided from percentage of survived plants of variety ‘Melody’. In future investigations we suppose to combine different thaw durations and temperature variants with biochemical factors and genes expressions.

Conclusions. 1. Cold resistant strawberries persist hardening for 6–7 days and cold sensitive – for 3–4 days in optimal growing conditions.

2. Abscisic acid addition in nutrient medium supports and retains strawberry plant cold hardening, while gibberellins have opposite effect.

3. Repeated hardening for 7 days allows surviving freezing in vitro 50–60% more strawberry plants than without repeated hardening. Reaction to fluctuating temperatures after hardening depends on genotype.

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Gauta 2007 06
Parengta spausdinti 2007 06

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BRAŠKIŲ USIGRŪDINIMO ŽEMOMS TEMPERATŪROMS TYRIMAI IN VITRO

V. Lukoševičiūtė, R. Rugienius, D. Kavaliauskaitė

Santrauka

Lietuvos sodininkystės ir daržinininkystės institute Sodo augalų genetikos ir biotechnologijos skyriuje tirtas skirtingų braškių veislių atsparumo šalčiui kūrimas in vitro po grūdinimo ir po atšildymo bei pakartotino grūdinimo. Įvertintas braškių atsparumas šalčiui priklausomai nuo abszizinės rūgšties, giberelino priedų maitinamojoje terpėje. In vitro tyrimams braškės persodintos į mėgintuvėlius, grūdintos šviesoje, esant +4°C temperatūrai 35 dienas, šaldytos in vitro esant -9°C temperatūrai 12 valandų, atšildytos esant +22°C temperatūrai 7 dienas, 14 dienų. Išgyvenusių augalų kiekis (%) ir pažeidimas šalčiui balais (0 – nepažeistas, 5 – augalas žuvęs) vertinti praėjus 3 savaitėms po šaldymo.

Šaldant in vitro atsparų šalčiui braškių užsigrūdinimas optimaliomis augimui sąlygomis išlieka 6-7 paras, o jautrių – 3–4 paras. Braškių augalų užsigrūdinimą šalčiui palaiko abszizinės rūgšties priedai maitinamojoje terpėje, giberelino – ji mažina. Šaldant be pakartotino grūdinimo

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MICROPROPAGATION OF SCHISANDRA CHINENSIS (TURCZ.) BAILL

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The aim was to induce Schisandra chinensis (Turcz.) Baill culture in vitro and investigate the impact of phytohormones BAP and IAA on the growth of microshoots in vitro. The experiment was carried out with plants of five different genotypes obtained from Lithuania and Sweden. Explants taken from donor plants growing in field collection gave high mortality rate (73.9%). While using the donors plants from greenhouse the rate of infection was 11.1%. The impact of phytohormones on the growth of microshoots was shown to be dependent on the composition of microelements in medium. In case DKW medium was used, the best propagation results were obtained if both phytohormones BAP and IAA were added. When MS medium was used the best results were obtained in presence of BAP. The variant of MS medium containing 0.75 mg/l BAP gave the highest multiplication rate of S. chinensis microshoots of all investigated. S. chinensis explant reaction to cultivation in vitro conditions were determined by their genotype.

Key words: indolilacetic acid, in vitro, Schisandra chinensis, 6-benzilaminopurine.

Introduction. Schisandra chinensis (Turcz.) Baill is a ligneous liana from the Schisandraceae family. Plant grows in light wet grounds of mixed forests in Northern China and nearby regions of Russia and Korea (Kamarova, 1984). S. chinensis as a healthful plant has been used in Chinese medicine for ages (Cracer et al., 2002). Nowadays extracts of S. chinensis fruits are widely used by European and American medics because of their clinical and therapeutic applications and low toxicity (Weinberg et al., 1999). Biologically active substances with medical properties are found in all parts of the plant, especially in fruits and seeds. S. chinensis extracts are known as well restorative remedies (Lee et al., 2004), also as useful agents to reduce stress and increase viability of the organism (Chevallier, 1996). S. chinensis is applied in case of liver, kidney, respiratory, heart and vascular diseases and diabetes (Bown, 1995), useful in treatment of vision disorders, gastritis and has a strong adaptogenic effect (Yeung, 1985; Duke, 1985). Unique compounds called lignans have been extracted from S. chinensis (Opletal et al., 2001; Vlasinova, 2004) and some of them are reported as potent anti-human immunodeficiency virus agents (Perez, 2003). Despite wide medical use of S. chinensis, the spread of plantations is restricted by the lack of plant material. Schisandra chinensis (Turcz.) Baill is a primitive dicotyledon with undifferentiated embryos, therefore seeds germinate poorly (Saunders, 2000). S. chinensis is hardly propagated using hardwood cuttings, and only 40–50% of softwood cuttings take roots successfully. Somatic embryogenesis from zygotic embryos of S. chinensis also
gives a poor yield of well developed schizandra plants (Smiskova et al., 2005; Kim et al., 2005). No literature about *S. chinensis* micropropagation *in vitro* has been found.

**The aim** was to induce *Schisandra chinensis* (Turcz.) Baill culture *in vitro* and investigate the impact of phytohormones BAP and IAA on the growth of microshoots *in vitro*.

**Materials and methods.** Investigation was carried out in Lithuanian Institute of Horticulture, Plant Biotechnology Laboratory. Donor plants were raised in Lithuania (Vv, Vm) and Sweden (So, At, Wf, Rf). Explants (segments of shoots with a bud) were taken from plants, which had been grown both in greenhouse and in field collection. Explants were sterilized with sublimate for 10 minutes and three times rinsed with sterile water.

Two types of media were used: Murashige-Skoog (MS) (Murashige, Skoog, 1960) with 50.0 mg/l of inozite, 30.0 g/l of sucrose, and 0.5 mg/l of each pyridoxine, thiamine, ascorbic and nicotinic acids and Driver-Kuniyuki walnut (DKW) (Driver, Kuniyuki, 1984) medium with 100 mg/l of inozite, 30.0 g/l of sucrose and both nicotinic acid and thiamine 0.5 mg/l each. The effect of five different concentrations of phytohormone 6-benzilaminopurine (BAP) 0.125, 0.25, 0.5, 0.75, 1.0, 1.5 mg/l and two different concentrations of indolilacetic acid (IAA) 0.02, 0.04 mg/l were investigated growing 10 explants per variant. The medium pH was 5.8. Explants were grown in growth chamber at 25 ± 1°C temperature, 16 hours-long photoperiod and 50 µmol m⁻² s⁻¹ photosynthetic photon flex density.

The percentage of survived explants and the number of microshoots were registered before every sub-cultivation, which was carried out every 60 days.

Data were processed using statistical analysis for quantitative and qualitative parameters and the set of statistical data analysis software “Selekcija” (Tarakanovas, 1999).

**Results.** *Initial stage.* Donor plants contamination with microorganisms depended on their growing conditions. When explants were taken from donor plants growing in greenhouse (At, Wf, Rf, Vv, Vm) the percentage of infection was significantly lower than those taken from field collection (Table 1).

**Table 1. Effect of donor plant growing conditions on explant contamination frequency *in vitro***

<table>
<thead>
<tr>
<th>Donor plant growing place</th>
<th>Explant number of explants (units)</th>
<th>% Survived</th>
<th>% Dead</th>
<th>% Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field collection</td>
<td>46</td>
<td>21.7 b</td>
<td>0</td>
<td>73.9 a</td>
</tr>
<tr>
<td>Greenhouse</td>
<td>81</td>
<td>74.1 a</td>
<td>12</td>
<td>11.1 b</td>
</tr>
</tbody>
</table>

* = Means in a column marked with the same letter do not differ significantly at P = 0.01 according to Duncan’s multiple range test.

**Table 1. Effect of donor plant growing conditions on explant contamination frequency *in vitro***

Explants taken from So donor plant growing in field gave the highest rate of infection (85.7%). *S. chinensis* explants survived in both DKW and MS media, though intensity
of the growth was strongly dependent on the genotype of the explant. Only explants without infection taken from Vv, So Vm donor plants and grown in DKW medium as well as those taken from Wf, Rf donor plants and grown in MS medium survived 100% during the first sub-cultivation (Table 2).

**Table 2. Effect of plant genotype and medium type on the number of survived S. chinensis explants during the first sub-cultivation**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Medium</th>
<th>Number of explants</th>
<th>Survived</th>
<th>Dead (%)</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vv</td>
<td>DKW</td>
<td>12</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>So</td>
<td>DKW</td>
<td>42</td>
<td>14.3</td>
<td>0</td>
<td>85.7</td>
</tr>
<tr>
<td>Vm</td>
<td>DKW</td>
<td>12</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vm</td>
<td>MS</td>
<td>27</td>
<td>59.3</td>
<td>40.7</td>
<td>0</td>
</tr>
<tr>
<td>At</td>
<td>MS</td>
<td>22</td>
<td>72.7</td>
<td>4.6</td>
<td>22.7</td>
</tr>
<tr>
<td>Wf</td>
<td>MS</td>
<td>12</td>
<td>66.7</td>
<td>0</td>
<td>33.3</td>
</tr>
<tr>
<td>Rf</td>
<td>MS</td>
<td>8</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 3. Effect of plant genotype on the in vitro establishment of S. chinensis sleeping buds**

**3 lentelė. Genotipo įtaka S. chinensis miegančių pumpurų kultūros in vitro stabilizavimui**

**Multiplication stage.** S. chinensis explants of different genotypes began to form proliferating culture in vitro at different time. After four months microshoots
of Vv, At and Wf genotypes were the first which began to multiply. Microshoots of Rf genotype were the last to form proliferating culture (Table 3). No proliferating culture was obtained from So genotype grown in DKW medium and Vm in MS medium during the experiment. All explants of these two genotypes died. The multiplication coefficient of the rest S. chinensis genotypes investigated varied from 1.8 to 3.6.

In order to increase efficiency of S. chinensis successful propagation the effect of various concentrations of phytohormones BAP and IAA were investigated on the growth of Vv microshoots. In DKW medium the biggest number of microshoots was obtained when 1.0–1.5 mg/l of BAP and 0.04 mg/l of IAA had been applied (Table 4).

Table 4. The effect of phytohormones concentrations on the multiplication coefficient of S. chinensis microshoots of Vv genotype

<table>
<thead>
<tr>
<th>concentration of phytohormones</th>
<th>multiplication coefficient after 1 month</th>
<th>multiplication coefficient after 5 months</th>
<th>concentration of phytohormones</th>
<th>multiplication coefficient after 1 month</th>
<th>multiplication coefficient after 5 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/l)</td>
<td>DKW leupe</td>
<td>MS leupe</td>
<td>(mg/l)</td>
<td>DKW leupe</td>
<td>MS leupe</td>
</tr>
<tr>
<td>BAP</td>
<td>IAA</td>
<td></td>
<td>BAP</td>
<td>IAA</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>1.00 ± 0</td>
<td>0</td>
<td>0</td>
<td>1.00 ± 0</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>1.00 ± 0</td>
<td>0.46 ± 0.22</td>
<td>0</td>
<td>1.00 ± 0</td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>1.00 ± 0</td>
<td>1.20 ± 0.12</td>
<td>0.125</td>
<td>1.20 ± 0.12</td>
</tr>
<tr>
<td>1.5</td>
<td>0</td>
<td>1.20 ± 0.12</td>
<td>0.125 ± 0.25</td>
<td>0.125</td>
<td>1.20 ± 0.13</td>
</tr>
<tr>
<td>0</td>
<td>0.04</td>
<td>1.00 ± 0</td>
<td>0.125 ± 0.25</td>
<td>0.02</td>
<td>1.50 ± 0.20</td>
</tr>
<tr>
<td>0.5</td>
<td>0.04</td>
<td>1.00 ± 0</td>
<td>0.125 ± 0.25</td>
<td>0.02</td>
<td>1.20 ± 0.12</td>
</tr>
<tr>
<td>1.0</td>
<td>0.04</td>
<td>1.25 ± 0.16</td>
<td>0.02</td>
<td>1.20 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>0.04</td>
<td>1.20 ± 0.12</td>
<td>0.125 ± 0.25</td>
<td>0.125</td>
<td>1.40 ± 0.16</td>
</tr>
</tbody>
</table>

In case of MS medium, the maximum multiplication coefficient was obtained in variant with 0.75 mg/l of BAP. It was noticed that both DKW and MS media without any phytohormones did not give any proliferation, all explants died if no cytokines were applied.

Discussion. The experiment shows that the impact of phytohormones on the growth of the microshoots is strongly affected by the micro elemental composition of the medium. If DKW medium was used the variant with both phytohormones BAP and IAA was found to be the best for S. chinensis propagation. Using MS medium best results were obtained when 0.75 mg/l BAP was added. The growth of S. chinensis explants in medium of different micro elemental composition was strongly determined by their genotype. Explants of Wf and At genotypes in MS medium survived and began to propagate soon. All explants (100%) of Vv and Vm genotypes in DKW medium survived and started to grow. However, when explants of Vm genotype were planted in MS medium all of them died in a few months. Explants of So and Rf genotypes stayed alive for a few sub-cultivations after beginning of the experiment, and we may suggest that explants of RF genotype adapted to the medium during seven months of the cultivation and began to grow. However, all explants of So genotype have died
during this period. The 100% death rate of So genotype uncontaminated with infection explants is probably associated with inappropriate medium.

Slow growth of *S. chinensis* microshoots *in vitro*, poor germination of seeds and embryos (Smiskova, 2005) are typical for primitive plant families such as the *Paoniaaceae* (Antanaitienė, Stanienė, 2001), the *Orchidaceae* (Islam et al., 2002) and the *Schisandraceae* (Saunders, 2000). Considerable differences among various genotypes of *S. chinensis* were observed in cultures *in vitro* by other authors as well. It was observed that in *S. chinensis* culture *in vitro* one embryogenic line gave 38.9% of normally developing embryos, the rest two lines gave only 19% and 8% (Smiskova, 2005). The amount of biologically active compounds extracted from *S. chinensis* calli cultures *in vitro* also differed significantly depending on genotype (Opletal, 2001). Such significant differences among genotypes of *S. chinensis* reaction to cultivation conditions lead us to an assumption that despite its medical applications known for ages, there was no wide artificial selection carried out with *S. chinensis*. Genotypes of *S. chinensis* are still unique, not equalized that makes experiments and propagation of the plant complicated as well as brings new prospects for selection.

**Conclusions.** 1. It was shown that the impact of phytohormones BAP and IAA on the growth of the microshoots is strongly affected by the microelemental composition of the medium.

2. The highest multiplication coefficient for *S. chinensis* was obtained when MS medium with 0.75 mg/l BAP was used.

3. *S. chinensis* explants reaction to cultivation *in vitro* conditions is determined by their genotype.

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Santrauka

Darbo tikslas – indukti proliferuojančią in vitro kininio citrinyčio - *Schisandra chinensis* (Turcz.) Baill., kultūrą, augantys Lietuvoje ir Švedijoje. Nustatyta, kad po sterilinimo sublimatu, daugiausiai (73,9%) žuvo eksplantų, paimtų nuo lauke augančio augalo. Įvedimui naudojant eksplantus nuo šiltanymoaugusų augalų infectacijos procentas buvo mažesnis (11,1%). Nustatyta, kad fitohormonų įtaka mikroūglių augimui priklausė nuo terpės mineralinės sudėties. Naudojant DKW terpę, geriausi mikroūglių pasidauginimo rezultatai gauti, kai terpėje buvo abu fitohormonai – BAP ir IAR. Naudojant MS terpę geriausi rezultatai gauti, kai terpėje buvo BAP. Didžiausias citrinyčio mikroūglių pasidauginimo procentas gautas auginant eksplantus MS maitinamojoje terpėje su 0,75 mg/l BAP. Citrinyčio genotipas turėjo lemiamą įtaką eksplantų augimui skirtinėse mineralinės sudėties mitybinėse terpėse.

**Reikšminiai žodžiai:** citrinytis, indolilacto rūgštis, *in vitro*, 6-benzilamino purinas.
IDENTIFICATION OF APPLE COLUMNAR HYBRIDS IN JUVENILE PHASE USING MOLECULAR MARKERS

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One of the modern apple breeding program goals is to produce cultivars with compact growing habit, thus minimizing expenses for pruning, tree formation, and allowing to grow more intense orchards. Columnar apple trees are valuable source of this trait. Construction and use of molecular markers, related with desirable physiological traits allows identifying apple trees with columnar habit in juvenile phase. Crosses between columnar and non-columnar apple cultivars and hybrids were performed at the Lithuanian Institute of Horticulture in 2006. Two SCAR and four SSR markers were used in this study to identify hybrids with heterozygous \textit{Coco} gene. Segregation of hybrids with columnar to non-columnar habit fitted 1:1 ratio in the F1 generation, so it was confirmed that parental columnar cultivars has heterozygous \textit{Coco} gene. Hybrids were identified as columnar in juvenile phase, thus breeding process was shortened.

Key words: columnar habit, \textit{Malus × domestica} Borkh., SSR molecular marker.

Introduction. Apple (\textit{Malus × domestica} Borkh.) is one of the most widespread and popular fruit trees in the world. Apple growers demand cultivars with the best fruit quality as cheaply as possible. For obtaining such results one of priorities is control of apple tree growth and structure (Kenis, 2007). Architecture of apple tree canopy is very important for productivity, fruit quality and ripening, grower expenses, so it is one of apple breeding goals (Kenis, 2004). Apple tree architecture is very diverse, but certain tree habits are more desirable than others by growers due to their compact canopy. Apple trees with columnar growth habit perfectly match this requirement.

Columnar growth habit in apple is caused by a mutation at the \textit{co} locus. This mutation was first identified in a spontaneous sport of ‘McIntosh’ with the spur phenotype (Fisher, 1970; Lapins, 1976). The mutation is characterized by a reduced number of lateral shoots, an increased number of spurs and compact internodes (Lapins, 1976; Tobutt, 1984; Kesley, 1992). Columnar growth is controlled by single dominant gene (\textit{Coco}) (Lapins, 1973, 1974, 1976), although Lapins identifies some more modifiers for inheritance of this trait (Lapins, 1976). All available columnar apple varieties are heterozygous (Yi-Ke Tian, 2005).

Construction and use of molecular markers, related to desirable physiological traits, play very important role in modern breeding programs. \textit{Co} gene was located...
on the tenth linkage group of the apple genetic linkage map by Conner et al. His map was mostly constructed from RAPD markers, which are difficult to transfer to other mapping populations (Conner, 1997). The most advanced linkage map of apple genome currently is Liebhard’s et al. saturated map, but Co wasn’t located on it (Liebhard 2002, 2003). The Co gene was finally located between CH03d11 and COL on the tenth linkage group by Yi-Ke Tian et al. (Yi-Ke Tian, 2005). Many different methods are being used to identify Co gene – QTL (quantitative trait loci) analysis (Kenis, 2004; Liebhard, 2003), microsatellite markers – SSR (simple sequence repeat) (Liebhard, 2002; Yi-Ke Tian, 2005; Guarino et al., 2006), RAPD (random amplified polymorphic DNA), (Kim, 2003; Liebhard, 2003) AFLP (amplified fragment length polymorphism) (Kenis, 2005; Yi-Ke Tian, 2005) and SCAR (sequence characterized amplified region) markers (Yi-Ke Tian, 2005). Identification of Co gene in apple during first year growing in nursery enables to select hybrids with columnar growth habit (Kenis, 2004; Kim et al., 2003).

Our goal was to evaluate heterozygosity of Coco gene in parental cultivars, and to identify hybrids during the first year growth using two SCAR and four SSR markers constructed by Yi-Ke Tian (Yi-Ke Tian, 2005).

**Materials and methods.** SSR and SCAR markers were tested on non-columnar cultivars ‘Auksis’, ‘Aldas’, ‘Orlovim’, ‘Priam’, ‘Skaistis’, ‘Katja’; columnar cultivar ‘KB-47’ and selection numbers 17-33-10 (form M38-13), 17-33-15 (form M38-33), 17-33-25 (form M38-35), 17-38-08, 17-38-29, 17-38-50, 17-39-14, 17-39-52, 17-39-56 all of them from ‘KB-43’ × 57-366 crosses, B-0968 - Tuscan x own pollination, (from Sweden), and HCR23T113 (from Germany), which were planted at the Lithuanian Institute of Horticulture (LIH) in Babtai in 2002–2003. Primer pair SSR-COF1 and SSRCOR1 (SSRCOF1 – 5’ATGCCAATGCATGAGACAAA’3, SSRCOR1 – 5’ACACGCAGCTGAAACACTTG’3) was found to be the most efficient in separating Co gene fragments, and it was decided to use it for further hybrid investigation.

For identification of heterozygosity of parental forms, crosses between non-columnar and columnar cultivars – ‘Aldas’ × 17-38-50, form of spreading shoots ‘Podsneznik’ × 17-38-50, B-0968 × weeping shoots ‘Podsnežnik’, CB-0968 × 17-38-50, CB-0968 × ‘Orlovim’, CB-0968 × ‘Rubin’, ‘Katja’ × 17-38-50, ‘Geven Smith Spur’ × 17-38-50, were performed at LIH in 2006. Hybrids were grown in greenhouse; total DNA was extracted when they had 10 leaves. Total DNA was extracted from plant leafs (0.15 g) using CTAB (hexadecyltrimethylammonium bromide) method; according Doyle (Doyle, 1990).

PCR amplification was performed in a 20 µl volume containing 2.5 mM MgCl2, 0.2 mM of each dNTP, 2 µl 10× reaction buffer, 0.2 µM of forward primer, 0.2 µM of reverse primer, 1.25 units ofTaq DNA polymerase (Fermentas) and 350 ng of genomic DNA. The following conditions were used: an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, annealing temperature (see Table 1) for 35 s, 72°C for 1 min 30 s and a final extension at 72°C for 5 min (Mastercycler Gradient S, Eppendorf). The products were detected on a 1.5% agarose gel and photographed under UV light (Herolab).

**Results.** All primers were tested with columnar and non-columnar cultivars.
Primer pair SSRCOF1 and SSRCOR1 was selected for further research. Lithuanian cultivar ‘Auksis’ (Fig. 1) was selected as standard for identification of non-columnar hybrids, and KB-47 (Fig. 1) selected as standard for identification of hybrids with columnar growth habit. Swedish selection number B-0968 and selection number 17-38-50 from LIH (both of them had polymorphic fragments of Co gene) were selected for crosses, in order to evaluate their Coco heterozygosity. These hybrids were crossed with cultivars, which had only recessive (coco) alleles.


The arrow shows the polymorphic fragment.

**Fig. 2.** The molecular markers of specific Co gene fragments in apple hybrids (1–14). 15 – KB-47 (columnar standard); 16 – ‘Auksis’ (non-columnar standard); M1 O’Gene Ruler 100 bb DNA Ladder; M2

55% of all investigated hybrids had polymorphic fragment of approximately 170 bp (Fig. 2), so they are heterozygous (Coco) and have dominant Co allele (Table 1). Segregation of columnar to non-columnar habit fitted 1 : 1 ratio in the F₁ population, so it was shown, that hybrids B-0968 and 17-38-50 has a dominant heterozygous Coco gene.

Only 29 percent of hybrids from ‘Katja’ × 17-38-50 cross had a marker of columnar habit (Table 1), this could be explained by different physiological properties of paternal plants, and reduced viability of hybrid zygotes with Co gene. When both parents had columnar (B-0968 × 17-38-50) habit, similar ratio of heterozygous Coco allele was found, though very few hybrids from this family survived. High ratio of hybrid loosing could have emerged due to reduced viability; probably dominant Co allele reduces viability. That also could be explained by that fact, that we didn’t get any hybrid with two dominant Coco alleles.

**Fig. 2.** The molecular markers of specific Co gene fragments in apple hybrids (1–14). 15 – KB-47 (columnar standard); 16 – ‘Auksis’ (non-columnar standard); M1 O’Gene Ruler 100 bb DNA Ladder; M2
O’Gene Ruler 1 kb DNA Ladder.
The arrow shows the polymorphic fragment.

2 pav. Specifiniai Co genu molekuliniai žymenys obels sėjinukuose
M1 – O’Gene Ruler 100 bp DNR žymuo; M2 – O’Gene Ruler 1 kb DNR žymuo.
Rodykle pažymėtas polimorfinis fragmentas.

Table 1. Segregation of Co gene markers in apple hybrids

<table>
<thead>
<tr>
<th>Crossing combination</th>
<th>Hybrids with marker of dominant Co gene</th>
<th>Hybrids with marker of recessive Co gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>quantity skaičius</td>
<td>percentage procentas</td>
</tr>
<tr>
<td>‘Aldas’ × 17-38-50</td>
<td>4</td>
<td>57</td>
</tr>
<tr>
<td>Spreading shoots ‘Podsneznik’ × 17-38-50</td>
<td>5</td>
<td>62</td>
</tr>
<tr>
<td>Kientačios skais ‘Podsneznik’ × 17-38-50</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>‘Karja’ × 17-38-50</td>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td>‘Geven Smith Spur’ × 17-38-50</td>
<td>5</td>
<td>62</td>
</tr>
<tr>
<td>B-0968 × 17-38-50</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>B-0968 × weeping shoots ‘Podsnežnik’</td>
<td>5</td>
<td>62</td>
</tr>
<tr>
<td>B-0968 × slynačios skais ‘Podsnežnik’</td>
<td>3</td>
<td>43</td>
</tr>
<tr>
<td>Total:</td>
<td>32</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 2. Segregation of Co gene molecular markers in apple hybrids, depending on the direction of the crossing

2 lentelė. Co geno molekulinių žymenų pasiskirstymas sėjinukuose priklausomai nuo kryžminimo krypties
**Discussion.** Yi-Ke Tian found, that the segregation of columnar to non-columnar habit fitted 1 : 1 ratio in the F₁ population, consistent with the hypothesis that Telamon (Coco) carries a single dominant gene conferring columnar habit (Yi-Ke Tian, 2005). In our research we indicated similar ratio in the F₁ population – 55 : 45 percent, so we may state, that our parental columnar selection numbers B-0968 and 17-38-50 also have a single dominant heterozygous Coco gene. When columnar selection number 17-38-50 was crossed with columnar selection number CoB-0968, we indicated 2 : 1 ratio Co and co alleles. Although hybrids with two dominant CoCo alleles were not indicated.

Breeding apple varieties is a time-consuming and challenging task. Long juvenile phase of the plants hamper the efficient crossing and the fast selection of desired genotypes. Between 20 and 25 years elapse between the actual crossing and the release of a new variety (Kellerhals, 1994). Apple breeders in modern breeding programs use marker-assisted selection and breeding, which applies molecular markers, linked to the genes of desired traits, for selection instead of selecting the plants according to their phenotypic behaviour (Kenis, 2007). We used SCAR and SSR markers to identify hybrids with columnar growth habit in juvenile phase, what enables to select valuable genotypes much earlier, thus shortening breeding time. Scientists from Lithuanian Institute of Horticulture use other molecular markers in breeding programs – Gelvonauskienė et al. reported usage of Vf gene specific marker in order to identify scab resistant apple hybrids in juvenile phase (Gelvonauskienė et al., 2005). Our research on the columnar markers enlarges usage of modern methods in apple breeding, because usage of complex molecular identification methods allows shortening breeding time more than usage of a single marker.

**Conclusions.** SSRCOF1 and SSRCOR1 primer pair could be used for identification of heterozygous Coco gene. Segregation of columnar to non-columnar habit fitted 1 : 1 ratio in the F₁ progeny, so Co gene of the selection numbers B-0968 and 17-38-50 is heterozygous.

Cytoplasm inheritance doesn’t influence transferring of dominant Co gene to progeny.

55 percents of F₁ hybrids have dominant Co gene. This was identified in juvenile phase.

**Acknowledgments.** This research was supported by Lithuanian Ministry of Science.
References


Reikšminiai žodžiai: koloninė forma, Malus × domestica Borkh, SSR molekuliniai žymenys.
MODERN TOOLS OF FRUIT PLANT BREEDING – SHORT REVIEW

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Suitability of selected molecular techniques such as qualitative and quantitative PCR-based methods, microarrays and high throughput sequencing for different stages of classical breeding of fruit plants is showed in this review. The following aspects of their role in breeding process are described: elaboration of genetic relationship of putative progenitors on the base of comparable study on DNA polymorphism, early molecular knowledge-based elimination of genotypes with undesired traits and undesired outcrosses in selection process, and molecular identification (DNA fingerprinting) of new cultivars and determination of identity of cultivated plants. Application of these techniques in program of molecular breeding conducted at the Research Institute of Pomology and Floriculture is presented.

Key words: fruit plants, gene expression, genome mapping, identification, markers, molecular breeding.

Introduction. Since prehistoric period many plants have been domesticated by man and then used as a material for the breeding. Consequently, majority of recently cultivated plants are the result of breeders’ activity. However, classical breeding process is characterized by longevity and low percentage of success. Limiting factors are particularly significant in fruit plant breeding where current crossing program comprises of many close related progenitors what increases negative effects of inbred, meanwhile it is difficult to characterize obtained progeny in a short time due to long development of seedlings and long juvenile stages (Janick et al., 1996). The insufficiency of classical methods based on phenotypic observations (Dale, 1996) forced the application of molecular technology, which results are independent on the environmental conditions and stage of plant development (Nybom, 1990; Lavi et al., 1994). Numerous molecular techniques have been available and used for a large scale in many fruit plant-specialized centers since 90s (Nybom, 1990; Higuchi et al., 1993; Ahroni and Vorst, 2001; Gostimsky et al., 2005). In the Research Institute of Pomology and Floriculture the study on molecular aspects of fruit plant breeding were undertaken in 2000. Conducted study covers all species being the objective of breeding program and includes molecular fundamentals of occurrence of economically important plant traits (with elements of functional genomic) as well as practical aspects, such as molecular-based cultivar/genotype identification and selection in progeny populations.

In this paper the role of selected molecular tools in different stages of fruit plant breeding is presented.
Molecular-based crossing program, characterization of parental forms and selected progeny (cultivars). Plant breeding consists of hybridization and selection of desired genotypes. Parental forms chosen for the hybridization ought to be the donors of valuable traits and simultaneously should be genetically different to avoid loss of vigor, yield and fruit quality due to inbreeding (Spangelo et al., 1971). In parallel, the selection is the process, by which a breeder finally chooses the progeny with the best combination of traits from both parents. In both processes full characterization of used and obtained genotypes is very important and should consists of phenotypic study allowing to characterize plant on the base of visible traits as well as basing on molecular techniques making possible to characterize plants also with “invisible traits” coded in plant genetic material and transferred to further generations. Precise phenotypic plus molecular characterization of parental forms from germplasm collection make possible to create proper cross-program and avoid inbreeding effects (Lavi et al., 1994). On the other hand, this kind of characterization of each cultivar allows strongly protect breeder rights according to EU law and to avoid mixing cultivated plant varieties in the nursery or in regular orchards.

In general, molecular characterization of genotype is based on analysis of genetic material (DNA) polymorphism. Several techniques being able to cut DNA according to this molecule structure and obtain fragments (markers) creating specific DNA pattern called “DNA fingerprinting” were described as a perfect tool for polymorphism study. The Restricted Fragment Length Polymorphism (RFLP) basing on the digesting properties of restriction enzymes (Botstein et al., 1980) was the first technique used for study on genotype polymorphism. More recently, molecular techniques based on the enzyme polymerase abilities to amplify digested DNA (Polymerase Chain Reaction, PCR) (Mulis and Faloona, 1987; Powell et al., 1996) have become increasingly popular for fingerprinting and cultivar identification. Among others, the following techniques have been described most frequently: Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), Inter-Simple Sequence Repeats (ISSR), Amplified Fragment Length Polymorphism (AFLP) (Williams et al., 1990; Zietkiewicz et al, 1994; Vos et al., 1995). RAPD, utilizing PCR amplification from single arbitrary and generating dominant differentiating markers have been used for the genotype identification in many plant species, as well as for assessing plant genetic diversity (Koller et al., 1993; Graham et al., 1994; Lanham et al., 1995; Moreno et al., 1995, Landry et al., 1997; Degani et al., 1998). However, RAPD-identifying markers do not properly contribute to understand the allelic diversity in the population. Additionally, lack of sequence specificity and in consequence unrepeatability of RAPD limit markers cross-lab application (Meunier et al., 1993). SSR and ISSR (anchored microsatellites) using simple sequence repeats or inter-sequences anchored at the 5' or 3' are ideal markers for genetic mapping and population study because of their abundance, and the high degree of polymorphism between individuals within a population of closely related genotypes (Hokanson et al., 1981; Cregan et al., 1994; Graham et al., 1996; Levi and Rowland, 1997; Lanham and Brennan, 1998; Joshi et al., 2000; Arnau et al., 2003). AFLP generating the third type of markers used for identification combines
RFLP with PCR techniques by amplification fragments of restriction digests. AFLP analysis is a powerful tool for breeding purpose due to high reproducibility and the large number of detectable loci (Vos et al., 1995; Degani et al., 2001; Patzak et al., 2001).

Markers in RIPF investigations were generated using different PCR-based techniques. Totally, over four hundred cultivars (genotypes) of *Fragaria x ananassa*, *Ribes*, *Rubus*, *Malus domestica*, *Prunus domestica*, *Prunus cerasus* and *Prunus persica* used in the breeding programs were screened for RAPD, SSR, ISSR and AFLP. Most of the reactions produced DNA patterns that were easily scored. Some of them created putative markers and allowed to differentiate all investigated genotypes with specific DNA patterns (Fig.1) (Korbin et al., 2002a; Kuras et al., 2002; Lisek et al., 2006; Korbin et al., 2007a).

Reproducible, informative DNA bands were chosen also for dendrograms generation (SEQ 8000, Dnastar, MegAlign) (Fig. 2). The presence/absence of the markers was the basis for the creation of computer databases required for the estimation of genetic distance between cultivars and then their actual relatedness. The parallel study with data sets derived from different type of PCR-based reactions allows reducing the number of potential mistakes connected with each method and their technical limitations, and ensuring determination of the actual relationship (Korbin et al., 2002a; Kuras et al., 2004; Korbin et al., 2007a, b).

**Fig. 1.** The example of cultivars differentiation with ISSR markers – DNA fingerprints of selected strawberry cultivars: ‘Aga’ (a), ‘Ananasova’ (b), ‘Astra’ (c).

**Fig. 2.** Dendrograms generated on the base of DNA polymorphism data obtained in RAPD (a), ISSR (b) and AFLP reactions (c) for apple germplasm collection (Korbin et al., 2007b)

**F i g. 1.** Veislų nustatymas naudojant ISSR žymenis – braškių veislių veislių ‘Aga’ (a), ‘Ananasova’ (b), ‘Astra’ (c) DNR pasai.

**F i g. 2.** Obelų genetinių išteklių giminingumo nustatymas, panaudojant RAPD (a), ISSR (b) ir AFLP (c) žymenis
Molecular tools for the progeny selection. During the breeding process breeders are obligated to select the progeny with the most valuable traits. In the first stage comparison of DNA patterns characterizing each parental forms and population of progeny allows to select outcrosses being a result of open pollination. Open pollination is well known phenomenon sometimes very useful in searching for new gene sources (Rejman, 1994). However, precise pedigree determination is indispensable in modern breeding, especially that knowledge concerning real parental forms eliminates the problems with identity determination in license study, and simultaneously gives breeder opportunity for optimization of crossing program (Antonius-Klemola, 1999).

Study on progeny purity conducted in RIPF apple population (over 1000 seedlings) with SSR technique showed that it is impossible to avoid the open pollination at all, but using good protection system is possible to limit the percentage of outcrosses to the level lower than 10% (Fig. 3) (Keller-Przybylkowicz et al., 2005).

Analysis of DNA patterns is very convenient not only for plant identification and determination of seedling genetic purity. Generation of specific DNA-markers connected with valuable traits is also needed for elimination of undesirable genotypes. Molecular-based selection of desired progeny can be quite easy in case of monogenic traits. Comparable study on genotypes being a donor of the trait and genotypes that do not express it allows identifying DNA fragment diversifying all plants from the both populations (BSA), and generate markers for early selection of valuable seedlings. Study on black current producing black and green fruits carried out in our laboratory (Fig. 4) showed polymorphism of genetic material and allowed generating marker for plant selection at early stage of their development without necessity to wait 2–3 years for the phenotypic observations (Keller-Przybylkowicz et al., 2006).

Fig. 3. Electrophorograms showing the segregation of genes for progeny derived from controlled pollination and for outcross

Fig. 4. Marker of fruit colour in black currant. In lines 1, 2, 4 and 5:
genotypes BB or Bb with black fruits,
in 3 – DNA of plant with green fruits (Keller et al., 2006)

Generation of polygenic trait markers needs very precise tools. Majority of polygenic traits are regulated by activating or deactivating the expression of the genes and the markers for them can be found just after study on gene expression (Korbin et al., 2002b). The gene expression corresponds to the number of copies of RNA (messenger type) produced in investigated phase of the life processes. Traditionally the amount of particular mRNA produced, and thus the activation status of the gene has been measured by northern-blotting. The limitation of this method was the amount of RNA, thus in order to detect gene expression even from single cell techniques allowing amplification of genetic material are suitable.

Microarrays also called DNA chips are new analytical devices that allow the parallel detection of thousands biological compounds (Ahroni and Vorst, 2001). In the technique introduced by Fodor and co-workers (1991) microscopic arrays (microarrays) immobilize on solid surface, meanwhile tested biological samples (DNA or RNA) are labeled enzymatically by incorporating nucleotides bearing reporter tags and hybridize to microarrays. Hybridization reactions yield heteroduplexes between individual components of the fluorescent sample (probe) and a complementary sequence (target) on the chip surface. The identity and quantity of fluorescent mixture can be determined by measuring the fluorescence intensity at each position on the microarrays with
newest electronic technology including confocal laser scanning and charge-coupled
device. At the beginning of XXI century microarray technology was identified as a key
element for study on functional genomics, large-scale sequencing and EST (expressed
sequence tag) analysis (Lemieux et al., 1998, Ahroni and Vorst, 2001; Wan et al., 2002).
Simultaneously some limitations of the technique such as very high cost of tests, lack
of available chips for majority of organisms, lack of possibilities to monitor genes
expressing transiently and to measure correlation between mRNA and protein level,
and finally lack of standards for data analysis (Jansen et al., 2002; Wan et al., 2002)
were found together with necessity to verify microarray results with other methods,
e.g. northern-blot and new PCR-based quantitative techniques (Maleck et al., 2000;
Kawasaki et al., 2001, Carbone et al., 2006).

Quantitative-competitive QC-PCR (Giacca et al., 1994) and real-time RT-PCR
(Higuchi et al., 1993) became important tools not only for verification of microarrays
but also for individual study on gene expression (Gibson et al., 1996; Heid et al., 1996;
Elsas et al., 1998; Freeman et al., 1999). The technology may be in study on genetic
expression of a particular gene in aspect of response of tissue and cell cultures to
changes in biotic and abiotic conditions (Orlando et al., 1998). The QC-PCR is semi-
quantitative techniques needing standard sample for comparable analysis in final point
of the reaction. RT-PCR allows monitoring reaction in time of its development. PCR is
correlated with fluorescent signal emission proportional to the amount of the recently
generated product. For signal detection different staining systems (SYBR Green,
ethidium bromide) and probes (TaqMan, Fluorescence Resonance Energy Transfer,
Molecular Beacons, Scorpions, TaqMan Mini Groove Binder) are used. Generally,
quantitative PCR is significantly cheaper than microarrays. This technique offers
also very high sensitivity, reproducibility for quantification range on gene expression
levels (Ullmannova et al., 2003), and high correlation between gene expression data
and biochemical analyses (Llyop-Tours et al., 1999; Carbone et al., 2006).

The third method useful for analysis of gene expression is throughput sequencing
called also 454 sequencing. The breakthrough technology by 454 companies has
revolutionized the field of sequencing (Margulies, 2005) technology is based on large
scale pyrosequencing reactions and allows reads of around 100MB with read lengths of
200-300bp at a reasonable price. The reaction is carried out directly on cDNA without
any cloning stages as the primers are physically added to every molecule as part of the
sequencing process (Cheung et al., 2006; Emrich et al., 2007). Clones that are expressed
differentially in the different experiments are identified by bioinformatics analysis.

In our study on generation of molecular markers of polygenic traits in fruit
plants we started from analysis of genes involved in processes correlated with
desired traits. Some polymorphic markers/genes responsible for fruit quality such as
AAZA – M9 Xylem, 1-peroxidase–Glicine Max, MALDO2-MT2_MALDO
Metallothionein like protein type 2, MALDO3–MT2_MALDO Metallothionein
like protein type 3, GluSra-Glutathione S-Tranferase class PHI, Defensine –
1-defensin protein were identified using microarrays (cooperation with UNIMI) and
introduced into the map of apple genome (Keller-Przybylkowicz and Korbin, HiDRAS,
5 FP). Since 2005 we have conducted also the study on candidate genes involved in
strawberry tolerance to soil-borne pathogen. Among 56 analyzed LRR genes (Leucine
Reach Repeats), 29 RGAs and DGAs (Resistance Genes Analogs and Defense Genes
Analogs) originating from cotton and tomato respectively twenty and four fragments
(three from cotton and one from wild tomato) showed high level of homology with strawberry sequence (Korbin and Keller-Przybylkowicz, in press) meanwhile some of CGs from polyphenolic pathway showed high level of expression measured by RT-PCR (Fig. 5) after infection with *Verticillium dahliae*. Study on expression of genes in strawberry affected with soil-borne disease was continued with 454 sequencing in cooperation with Volcani Center.

**Fig. 5.** Curve of RT-PCR amplification with 18S RNA reference gene

5 pav. Kontrolinio RNR 18S geno amplifikavimo RT-PCR metodu kriove
Keller-Przybylkowicz 2007. RIPF, Skierniewice.

**Conclusions.** Successive creation of new plant variety is hindered by difficulties in precise identification and selection of plants used and obtained in breeding program. These obstacles are reduced by application of molecular techniques. They are useful in a) identification and selection of parental forms and determination of their genetic relationship, b) determination of plant genetic purity and selection of outcrosses, c) mapping and mass selection of progenies with markers of selected traits.

*Gauta 2007 06*

*Parensta spausdinti 2007 06*

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Šioje apžvalgoje parodytas šiuolaikinių molekulinės biologijos metodų – kaip kokybinė ir kiekybinė PGR, mikrogardelių metodas, greitas sekvenavimas – tinkamumas įvairiems klasiškiems selekcijos etapams. Šių svarbių metodų pritaikymo sritys yra tinkamų tėvinių formų bei jų genetinių ryšių nustatymas tiriant DNR polimorfizmą, molekuliniais tyrimais paremtas ankstvęs genotipų su nenorimais požymiais brokavimas, DNR pasų sudarymas veislėms ir jų identifikavimas. Visi šie metodai naudojami Gėlininkystės ir pomologijos mokslinių tyrimų instituto molekulinės selekcijos programose.

Reikšminiai žodžiai: genomo žemėlapis, genų raiška, identifikavimas, molekulinė selekcija vaisinių augalai, žymenys.
PRODUCTION AND PROCESSING VALUE OF NEW BLACKCURRANT CULTIVARS AND BREEDING CLONES IN CENTRAL POLAND

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Yield, fruit size, field resistance of plants to fungal diseases and chemical content of the fruit of 19 blackcurrant cultivars and breeding clones are presented in this paper. Studies were carried out in the field of the Experimental Orchard of the Research Institute of Pomology and Floriculture at Dabrowice, near Skierniewice, Central Poland in 2004–2006. The results showed that the tested genotypes differed in evaluated traits. New Polish cultivars (‘Tisel’ ‘Tiben’ and ‘Ruben’) and Lithuanian cultivars (‘Gagatai’, ‘Kupoliniai’ and ‘Laimiai’) as well as Scottish clone (14-1-9) produced considerably higher yields than the standard cultivars (‘Ojebyn’, and ‘Ben Lomond’). ‘Gagatai’ and ‘Vyčiai’ (Lithuania) as well as ‘Tisel’ and ‘Tines’ (Poland) produced the largest fruits. The most resistant to fungal diseases (powdery mildew, leaf spot and white pine blister rust) were cultivars ‘Tisel’, ‘Ores’ and ‘Ruben’. The highest content of bioactive chemical components of fruits were found in Polish cultivars ‘Tiben’, ‘Tisel’, ‘Ruben’ and ‘Ores’ and breeding clones No 1/4 and No 7/13 as well as Scottish cultivars ‘Ben Lomond’ and ‘Ben Hope’.

Key words: blackcurrant, chemical content of fruit, cultivar trial, fruit size, fungal diseases, yield, Ribes nigrum L.

Introduction. Valuable cultivars of all species of crop plants, including blackcurrants, are the most important carriers of biological progress and the focus of technological progress of fruit production. Good yield, good fruit quality and reduced pesticide applications as well as the incorporation of modern cultivation technologies is the goal (Żurawicz, 2006). New cultivars with these improved traits will better serve the needs and desires of the processing industry and consumers and the reduced application of pesticides is better for the environment. In addition, the cost of fruit production is significantly lowered, which results in greater economic competitiveness. Growers are always seeking new and better cultivars. These cultivars should be
characterized by a higher yields, better fruit quality and their conduciveness for the processing and freezing as well as the fresh market (Pluta et al., 1998; Markowski and Pluta, 2002). The resistance of plants to serious pests and diseases is also important (Bielenin, 2001; Broniarek et al., 1999, 2000; Broniarek and Pluta, 2003). Therefore, the Research Institute of Pomology and Floriculture in Skierniewice, evaluates foreign cultivars and is engaged in an applied breeding program of native cultivars. Careful comparisons of these studied and newly developed cultivars to those presently grown in our country determine the overall economic value.

New blackcurrant cultivars released at the Fruit Plant Breeding Department (Pluta and Żurawicz, 1998; Pluta 2001, 2003) are evaluated for cultivar traits with regards to economic value to determine their usefulness for cultivation in Poland’s climate and soil conditions. Analyses of the chemical content of fruit are also studied to evaluate both health and food-processing value.

The aim of this paper is to evaluate the yield and fruit qualities of 19 blackcurrant cultivars and breeding clones in order to determine their usefulness for cultivation in Poland.

**Materials and methods.** Studies and observation were carried out in three consecutive years, 2004–2006. Bushes of new blackcurrant cultivars and clones bred at the Research Institute of Pomology and Floriculture in Skierniewice and a few foreign cultivars were the material used in the study. A total of 19 genotypes were included in evaluation. The standard cultivars were ‘Ojebyn’ and ‘Ben Lomond’ (Table 1). One-year-old bushes, first class were planted in the field experiment at the Experimental Orchard (SD) Dabrowice near Skierniewice (Central Poland) in the autumn of 2002.

The experiment was conducted on the mineral soil (fawn floor, poorly humus content, the mechanical composition of loams, laid on a matrix of the medium clay, pH 6.0–6.5). It was set in the random complete blocks design, in 3 replications and 3 plants on the plot. Planting density was 3.5 × 0.75 m with the break of 1.25 m between plots.

No chemical protection of plants was applied against the main fungal diseases of blackcurrants. Fertilization of the plants was in accordance with recommendations for commercial plantations. Weeds were controlled with soil and contact herbicides in accordance with actual Programs of Fruit Plant Protection. If necessary, weeds were destroyed mechanically and manually.

The following features of the plants were noted: date of the fruit harvest (ripening date), fruit yield in kg/plot or kg/bush and the size of fruits defined as weight of 100 berries randomly chosen from every plot in grams, susceptibility to American powdery mildew (*Sphaerotheca mors-uvae* Berk.), leaf spot (*Drepanopeziza ribis* Kelb.) and white pine blister rust (*Cronartium ribicola* Fish). The assessment of the level of plant infection by these pathogens was done at the beginning of July and in the second-half of the August each individual year of investigation using 5 grade ranking scale (1 – no symptoms, 3 – medium level of symptoms, 5 – very strong symptoms of diseases) according to the method by Karolczak et al. (1973).

Fruits were picked at commercial maturity. Study of the fruits’ chemical composition was conducted in at least two analytical replications. Content of soluble
solids was determined by means of RE 50 refractometer (Mettler Toledo) according to Polish Standard (PN-90/A-75101/04). The results were expressed in % and calculated as anhydrous citric acid. Titratable acidity was determined according to PN-90/A-75101/04. Anthocyanin content was determined by pH differential method (Wrolstad, 1976) and expressed in mg/100 g fresh weight of berries. Ascorbic acid content was determined by HPLC using HP 1100 (Agilent) equipped with DAD detector. Supelco LC-18 columns connected in series were used. The mobile phase was 1% KH$_2$PO$_4$ buffer pH 2.5 with flow 0.8 ml/min for 30 min. at 30°C. Ascorbic acid was detected at 244 nm, bandwidth 4 nm with reference at 360 nm, bandwidth 60 nm. Retention time of ascorbic acid was 11.2 min. The results were expressed as mg/100 g.

All results were elaborated statistically using the method of a one-factor analysis of variance, according to the model of randomly block design. Significance of the differences between means was evaluated using the Duncan’s test at P = 0.05.

**Results and discussion.** Collected results from measurements and observation as well as chemical analyses of fruits are presented in Tables 1–4. As demonstrated, tested blackcurrant genotypes differed considerably at the date of ripening and the harvest of fruits. Under conditions of Central Poland, the harvest of fruits started usually at the beginning of July and finished after 20 July, depending on the year of investigation. However, the earliest fruits from the standard cultivar (‘Ojebyn’), Lithuanian cultivars (‘Kupoliniai’, ‘Laimiai’, ‘Vyčiai’ and ‘Gagatai’), Scottish (‘Ben Gairn’ and clone 14-1-9) and Polish cultivars (‘Tisel’, ‘Tines’) ripened independently of the year of examinations. The majority of the tested cultivars and clones ripened later (3–6 days) than those mentioned above. Fruits of the Scottish cultivar ‘Ben Hope’ and Lithuanian ‘Vakariai’ ripened latest. The difference in the time of fruit ripening of the earliest and latest genotypes was from 11 to 13 days (Table 1).

The yield of examined genotypes was diversified and mainly depended on the genotype and weather conditions occurring in years studied. As it was expected, the lowest fruit yield was in 2004 i.e. in the first year of cropping. From 2005 bushes entered into full yield. On the average in 2004–2006 some of cultivars equaled or even exceeded the yields of the standard cultivars ‘Ojebyn’ and ‘Ben Lomond’. New Polish cultivars (‘Tisel’, ‘Tiben’ and ‘Ruben’) produced 2.27–2.42 kg of fruits from the bush. These yields were 184–203% higher than this of the standard cultivar ‘Ojebyn’ and 32–41% higher than this of ‘Ben Lomond’. The cultivars from Lithuania ‘Gagatai’, ‘Kupoliniai’ and ‘Laimiai’ produced the best yields; on the average 1.89–2.39 kg/bush. The new Scottish cultivars (‘Ben Gairn’ and ‘Ben Hope’) and the clone (14-1-9) yielded 1.36–2.35 kg/bush. The yields of these cultivars exceeded those of the standard cultivar ‘Ojebyn’, and the breeding clone produced higher yields than the standard cultivar ‘Ben Lomond’. In this experiment, the British cultivar (‘Farliegh’) and the Polish clones produced the lowest yields. On the average they were 0.73–1.14 kg/bush (Table 1).

**Table 1.** Yield, fruit size of blackcurrant cultivars and clones and susceptibility of plants to main fungal diseases (experimental orchard at Dabrowice, 2004–2006)
Juodųjų serbentų veislų ir selekcinių numerių derlius, vaisių dydis ir jautrumas grybinėms ligoms (Dambrovicos eksperimentinis sodas, 2004–2006 m.)

**Table 1 continued**

<table>
<thead>
<tr>
<th>Cultivar, clone</th>
<th>Country of origin</th>
<th>Average harvest date, 2004–2006</th>
<th>Yield (kg/ha)</th>
<th>Average weight of 100 fruits</th>
<th>Susceptibility of plants to fungal diseases, average of 2004–2006 (ranking scale 1–5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Oxblayn’</td>
<td>Scotland</td>
<td>15-07</td>
<td>0.32</td>
<td>1.29</td>
<td>0.78</td>
</tr>
<tr>
<td>‘Ben’</td>
<td>Scotland</td>
<td>21-07</td>
<td>0.34</td>
<td>2.60</td>
<td>1.72</td>
</tr>
<tr>
<td>Lorandiff</td>
<td>Lithuania</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘Ben Gaim’</td>
<td>Scotland</td>
<td>12-07</td>
<td>0.67</td>
<td>1.96</td>
<td>1.45</td>
</tr>
<tr>
<td>‘Ben Hope’</td>
<td>Scotland</td>
<td>24-07</td>
<td>0.46</td>
<td>2.93</td>
<td>1.47</td>
</tr>
<tr>
<td>‘Foxwoodstan’</td>
<td>England</td>
<td>16-07</td>
<td>0.39</td>
<td>1.67</td>
<td>1.37</td>
</tr>
<tr>
<td>‘Farleigh’</td>
<td>England</td>
<td>19-07</td>
<td>0.39</td>
<td>1.45</td>
<td>1.07</td>
</tr>
<tr>
<td>‘Gogatan’</td>
<td>Lithuania</td>
<td>13-07</td>
<td>0.70</td>
<td>1.17</td>
<td>1.31</td>
</tr>
<tr>
<td>‘Kopolinia’</td>
<td>Lithuania</td>
<td>11-07</td>
<td>0.81</td>
<td>1.56</td>
<td>2.18</td>
</tr>
<tr>
<td>‘Laimia’</td>
<td>Lithuania</td>
<td>11-07</td>
<td>0.64</td>
<td>2.37</td>
<td>2.48</td>
</tr>
<tr>
<td>‘Vakaria’</td>
<td>Lithuania</td>
<td>24-07</td>
<td>0.42</td>
<td>1.60</td>
<td>1.76</td>
</tr>
<tr>
<td>‘Vyšlia’</td>
<td>Lithuania</td>
<td>11-07</td>
<td>1.33</td>
<td>2.00</td>
<td>1.39</td>
</tr>
<tr>
<td>‘Tisel’</td>
<td>Poland</td>
<td>11-07</td>
<td>1.10</td>
<td>3.61</td>
<td>2.55</td>
</tr>
<tr>
<td>‘Tiben’</td>
<td>Poland</td>
<td>21-07</td>
<td>0.98</td>
<td>3.83</td>
<td>2.46</td>
</tr>
<tr>
<td>‘Ores’</td>
<td>Poland</td>
<td>18-07</td>
<td>0.82</td>
<td>2.54</td>
<td>1.85</td>
</tr>
<tr>
<td>‘Ruben’</td>
<td>Poland</td>
<td>18-07</td>
<td>1.08</td>
<td>3.15</td>
<td>2.58</td>
</tr>
<tr>
<td>‘Times’</td>
<td>Poland</td>
<td>18-07</td>
<td>0.96</td>
<td>2.87</td>
<td>1.92</td>
</tr>
<tr>
<td>14-1-9</td>
<td>Scotland</td>
<td>14-07</td>
<td>1.33</td>
<td>2.94</td>
<td>2.73</td>
</tr>
</tbody>
</table>

(SCR1)
The fruit size of the tested genotypes also differed dependently both on the genotype and the year of examinations (Table 1). On the average, during the years of the study, the largest fruits were produced by Lithuanian cultivars (‘Gagatai’ and ‘Vyčiai’); a weight of 100 berries totaled 128.7 g and 135.8 g, respectively. Similar fruit size (weight) results were obtained from Lithuanian cultivar ‘Vyčiai’ by Kawecki and co-authors (Kawecki et al., 2000a and 2000b). Similarly the Polish cultivars produced large fruits; ‘Tisel’ (124.8 g) and ‘Tines’ (134.9 g). The smallest fruits were found on the cultivars ‘Ojebyn’, ‘Foxendown’ and ‘Vakariai’ as well as the Polish and Scottish breeding clones (70.4–84.5 g). Remaining tested genotypes were characterized by medium sized fruit, averaging weight ranges from 96.1 g to 113.5 g per 100 berries.

The tested cultivars and breeding clones differed in the field resistance to the main fungal diseases (American powdery mildew, leaf spot and white pine blister rust). The results of evaluations done in 2004–2006 showed that the most of the genotypes were resistant to powdery mildew (Table 1). Plants of the standard cultivar ‘Ben Lomond’ were susceptible (3.6 in 5 grade ranking scale). These results are in concordance with those obtained by other authors (Pedersen, 1998; Broniarek et al., 1999, 2000; Gwozdecki et al., 2002; Broniarek and Pluta, 2003; Pluta, 2003). In addition, on the plants of two Lithuanian cultivars (‘Kupoliniai’ and ‘Vakariai’) exhibited small symptoms (1.3–1.4) of these diseases were observed, probably resulting from the effect of breaking down of resistance.

None of tested genotypes was fully resistant to leaf spot. The average level of infection by this pathogen of all the genotypes ranged from 2.3 to 3.6 in 5 grade of the ranking scale (Table 1). However, the plants of the cultivars: ‘Ben Hope’, ‘Gagatai’, ‘Laimiai’, ‘Vakariai’, ‘Ores’, ‘Tisel’ and ‘Tines’ appeared to be the least susceptible to this disease. The level of the disease symptoms ranged from 2.3 to 2.6.

Only small degrees of infections of the tested genotypes by C. ribicola, causing the white pine blister rust were observed. During 3 years of observations, only Lithuanian cultivar ‘Kupoliniai’ and three Polish cultivars ‘Tisel’, ‘Ores’ and ‘Ruben’ demonstrated full field resistance to this disease.

All tested genotypes differed significantly in chemical fruit content (Table 2). The average content of soluble solids in 21 blackcurrant cultivars and clones over three years of tests was 15.0%. The average content of soluble solids was calculated to be

<table>
<thead>
<tr>
<th>No</th>
<th>Cultivar</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/4</td>
<td>Poland</td>
<td>17-07</td>
<td>0.35</td>
<td>1.03</td>
<td>1.37</td>
<td>2.75</td>
<td>0.92</td>
<td>70.4</td>
<td>1.0</td>
<td>2.8</td>
<td>1.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/9</td>
<td>Poland</td>
<td>20-07</td>
<td>0.27</td>
<td>0.94</td>
<td>1.33</td>
<td>2.54</td>
<td>0.85</td>
<td>77.7</td>
<td>1.0</td>
<td>3.3</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/13</td>
<td>Poland</td>
<td>22-07</td>
<td>0.41</td>
<td>1.55</td>
<td>1.46</td>
<td>3.41</td>
<td>1.14</td>
<td>72.9</td>
<td>1.0</td>
<td>3.1</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>138×76/69</td>
<td>Poland</td>
<td>17-07</td>
<td>0.27</td>
<td>1.27</td>
<td>0.66</td>
<td>2.20</td>
<td>0.73</td>
<td>84.5</td>
<td>1.0</td>
<td>2.6</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* - means in the columns marked in the same letter do not differ significantly according to Duncan’s t-test (5%)
* - reikšmes, pažymėtos ta pačia raide, nesiskiria pagal Dunčano kriterijų (%)
1 ranking scale 1–5: 1 – no infection, 5 – very severe infection of plants
2 skalė 1–5: 1 – pažeidimo nėra, 5 – labai stiprus augalų pažeidimas
14.4% in 2004 and 2005 and 16.3% in 2006. This result implies the strong effect the weather conditions had during the spring and summer of 2006. Very high soluble solids content was found in the fruit from Polish cultivars ‘Tisel’, ‘Tiben’, ‘Tines’ and standard cultivars ‘Ojebyn’ and ‘Ben Lomond’. However, only cultivars ‘Vyčiai’, ‘Gagatai’, ‘Laimiai’, ‘Ores’ and clone No 7/13 were characterized by significantly lower soluble solids content comparing to cultivar ‘Tisel’. It is suggested that these genotypes are less suitable for processing into blackcurrant juice and concentrate (Table 2).

Table 2. Chemical contents of fruits of blackcurrant cultivars and breeding clones (experimental orchard at Dabrowice, 2004–2006)

<table>
<thead>
<tr>
<th>Cultivar, clone</th>
<th>Soluble solids</th>
<th>Titratable acidity</th>
<th>Anthocyanins</th>
<th>Ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Ojebyn'</td>
<td>14.0</td>
<td>15.6</td>
<td>18.2</td>
<td>15.9</td>
</tr>
<tr>
<td>'Ben Lomond'</td>
<td>15.9</td>
<td>15.9</td>
<td>16.6</td>
<td>16.1</td>
</tr>
<tr>
<td>'Ben Hope'</td>
<td>15.0</td>
<td>14.7</td>
<td>15.2</td>
<td>15.0</td>
</tr>
<tr>
<td>'Froxenilman'</td>
<td>13.3</td>
<td>13.3</td>
<td>17.4</td>
<td>14.7</td>
</tr>
<tr>
<td>'Farleigh'</td>
<td>13.4</td>
<td>15.3</td>
<td>17.1</td>
<td>15.3</td>
</tr>
<tr>
<td>'Gagatai'</td>
<td>13.9</td>
<td>12.5</td>
<td>14.7</td>
<td>13.5</td>
</tr>
<tr>
<td>'Kupoliniai'</td>
<td>15.3</td>
<td>13.0</td>
<td>14.9</td>
<td>14.4</td>
</tr>
<tr>
<td>'Laimiai'</td>
<td>14.2</td>
<td>12.8</td>
<td>14.4</td>
<td>13.2</td>
</tr>
<tr>
<td>'Vakariai'</td>
<td>13.7</td>
<td>13.8</td>
<td>17.8</td>
<td>15.1</td>
</tr>
<tr>
<td>'Vyčiai'</td>
<td>12.0</td>
<td>12.0</td>
<td>13.3</td>
<td>12.4</td>
</tr>
<tr>
<td>'Tisel'</td>
<td>15.6</td>
<td>16.3</td>
<td>19.3</td>
<td>16.9</td>
</tr>
<tr>
<td>'Ores'</td>
<td>13.1</td>
<td>14.0</td>
<td>15.2</td>
<td>14.8</td>
</tr>
<tr>
<td>(PC-36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Ruben'</td>
<td>14.6</td>
<td>14.3</td>
<td>17.4</td>
<td>13.4</td>
</tr>
<tr>
<td>'Tines' (PC-106)</td>
<td>16.0</td>
<td>16.1</td>
<td>17.1</td>
<td>16.4</td>
</tr>
<tr>
<td>'No 14-1-9 (SCR1)</td>
<td>14.6</td>
<td>16.0</td>
<td>16.4</td>
<td>15.3</td>
</tr>
<tr>
<td>'No 14'</td>
<td>14.2</td>
<td>14.8</td>
<td>18</td>
<td>15.7</td>
</tr>
<tr>
<td>'No 7/9'</td>
<td>14.0</td>
<td>14.2</td>
<td>15.4</td>
<td>14.5</td>
</tr>
<tr>
<td>'No 7/13'</td>
<td>13.3</td>
<td>13.9</td>
<td>15.6</td>
<td>13.9</td>
</tr>
</tbody>
</table>

The average content of titratable acidity in tested blackcurrant genotypes reached 3.0% with relatively low variation over three years of studies (Table 2). The highest content of acids in the fruit during each year of experiments and comparisons of the averages was found in cultivars ‘Tiben’, ‘Ores’, ‘Ruben’, ‘Vakariai’, ‘Ben Lomond’
The anthocyanins’ content in blackcurrant fruit of these genotypes differed significantly within each year and on the average for the three years of investigation. The average content for over three years of analyses was 291 mg/100 g berries and depended strongly on weather conditions and genotype. Individual year analyses revealed the following results: 294 mg/100 g (2004), 261 mg/100 g (2005) and 318 mg/100 g (2006). Generally, the highest content of this pigment was analyzed in the fruit of Polish cultivars (‘Tiben’, ‘Ores’, ‘Ruben’) and clones (No 1/4, No 7/9, No 7/13) as well as Scottish cultivars ‘Ben Lomond’ and ‘Ben Hope’ (Table 2).

The tested blackcurrant genotypes differed significantly in contents of ascorbic acid (vitamin C) in the fruit. The average content of ascorbic acid of all genotypes over the years of investigation (2004–2006) was 171 mg/100 g berries, ranging from 153 mg/100 g (2005) to 196 mg/100 g (2006) (Table 2).

Based on the conclusions of the presented results, we can state that the majority of the Polish bred cultivars considerably exceed the standard cultivars ‘Ojebyn’ and ‘Ben Lomond’ with regards to the evaluated traits. This means that the breeding program of blackcurrants conducted at RIPF in Skierniewice has produced good results. The ongoing breeding program of this crop will produce new cultivars, which are characterized by higher productivity, better quality of fruits for processing and plant resistance to fungal diseases compared to the standard cultivars, like ‘Ojebyn’, ‘Titania’ and ‘Ben Lomond’ presently cultivated.

Conclusions. 1. New Polish cultivars (‘Tisel’, ‘Tiben’ and ‘Ruben’) and Lithuanian cultivars (‘Gagatai’ and ‘Kupoliniai’) are considerably more productive than the standard cultivars (‘Ojebyn’ and ‘Ben Lomond’) under growing conditions of Central Poland.

2. Among the studied genotypes, Lithuanian cultivars (‘Gagatai’ and ‘Vyčiai’) and Polish cultivars (‘Tisel’ and ‘Tines’) produced the largest fruits.

3. The highest content of bioactive chemical components of fruits were found in Polish cultivars ‘Tiben’, ‘Tisel’, ‘Ruben’ and ‘Ores’ and breeding clones No 1/4 and No 7/13 as well as Scottish cultivars ‘Ben Lomond’ and ‘Ben Hope’.

4. Cultivars ‘Tisel’, ‘Ores’ and ‘Ruben’ were the most resistant to American powdery mildew, leaf spot and white pine blister rust evaluated in the field conditions.

5. Lithuanian cultivars (‘Gagatai’ and ‘Vyčiai’), producing large fruits, will be included in the crossing program aimed to obtain new “dessert type” cultivars with attractive and tasty fruit.


**Reikšmingiai žodžiai:** grybinės ligos, juodieji serbentai, Ribes nigrum L., vaisiaus dydis, vaisių cheminė sudėtis, veislų tyrimas.
GUIDELINES FOR THE PREPARATION AND SUBMISSION OF ARTICLES TO THE VOLUMES OF SCIENTIFIC WORKS “SODININKYSTĖ IR DARŽININKYSTĖ“

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  – Abstract (should not exceed 1400 characters or 250 words);
    Should contain the statement of the aims, methods and main results in short.
  – Key words (should not exceed 10 words in alphabetical order);
– Introduction
  Should present the investigated subject, results of earlier related research, reasons of the study, innovation.
– Materials and methods;
– Results
  Should present concisely the collected data during investigation, documentation (tables, figures).
– Discussion
  Should not repeat results presented in “Results” but should interpret them with reference to the results obtained by other authors, explain the reasons of the investigated phenomena, and raise new ideas, hypotheses.
– Conclusions;
– References
Should be kept to a minimum of 10 latest references on this theme.

– **Summary in English** (up to 2000 characters or 350 words);
– **Acknowledgements** (not compulsory);

The paper should be ended by a **signature** of the author(s) or other persons responsible for the article and the **date**.

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The article **should not exceed 10–15 pages**, tables and figures included (longer articles are agreed with the chairman of the Editorial Board).

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The manuscripts should be submitted in Lithuanian, English or Russian, typed on a PC, used Microsoft **WORD for Windows 2000, 2003 or XP** word-processor format, the font to be typed – **TIMES NEW ROMAN size 12**, on A4 paper (210 / 297 mm) one side, **for a manuscript – single spaced**, for the text **after reviews – 1,5 lines**, justified. Margins: top – 2 cm, bottom – 2 cm, right – 1.5 cm, left – 3 cm.

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If results are already given in figures, tables should not be used. Double documentation is not acceptable.

Text in tables is written in Lithuanian and English languages. If the text is written in Russian – in Russian and English. If Lithuanian and English texts are in one line they are separated by /. Do not use vertical and horizontal lines to separate parts of the text. A horizontal line separates only headings of columns and the end of the table. Orientation in a page only vertical (**Portrait**).

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**Statistical data, figures, numerals**

It is desirable to describe in detail the applied research methods and indicate references. The information on the scheme (design) of field, vegetative and other trials and motivation of their choice are very important. Data presented in tables and figures **must be statistically processed**: means, standard errors, correlation coefficients, significance of differences, (most acceptable at 95% or and – 90.99% level), etc. calculated. Abbreviations of parameters should be explained if they are not international standard abbreviations (ISO) [13].

Value figures should not be more than the trial method allows. Means of values should be rounded off to 1/10, their standard errors calculated.
For figures indicating quantity should be used Arabic numerals, e.g.: 15 tons, and for consecutive numbering can be used both Roman and Arabic numerals, e.g.: XX century, 2nd sample.

Numbers from one to nine are written in words, except when they mean measurement size (e.g.: 5 km, but “three variants”) or numbered consecutively (e.g.: phase 6, 9, 12). In many-figured numbers between classes an interval is made, e.g., 42 351. Percent is noted as %, when a specific number is implied, though “percent unit“ is written in words. In decimals use the decimal point.

Figures

Figures must be drafted in black colour in Microsoft Office 2000, 2003 or XP package EXCEL or other programs of this packet and included into the text and submitted as a separate EXCEL file in a diskette. Drawings must be professionally drafted or they can be redrafted by a professional and the work will be paid by the author according to the price list. Notice that the Editorial Board has the right to change their format according to the design of the article or the whole publication.

Letters and symbols in figures are recommended not smaller than size 10. Block parts of figures should be numbered consecutively by letters a, b, c, etc.

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Thesis abstract

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– Didžiosiomis raidėmis
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  Trumpai išdėstomu tyrimų metu surinkti duomenys, dokumentai (lentelės, grafikai).
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Straipsnis turi būti ne daugiau kaip 10–15 puslapių apimties, įskaitant lenteles ir paveikslus (didžesnės apimties straipsniai derinami su redkoligijos pirmininku).

Teksto parengimas

Straipsnis rašomas lietuvių, anglų ar rusų kalba ir spausdinamas Microsoft WORD teksto redaktoriu į Windows 2000, XP ar Vistą operacinėse sistemose. Tekstas rašomas TIMES NEW ROMAN 12 dydžio šriftu, vienoje A4 formato (210 × 297 mm) lapo pusėje, atstumas tarp rankraščio eilučių – 1 (single), tekstui po recenzijų – 1,5 (1,5 lines), išlyginamas iš abiejų pusiių. Paraščių plotis viršuje – 2 cm, apačioje – 3 cm, dešinėje – 1,5 cm, kairėje – 3 cm. Straipsnis pateikiamas elektroninėje laikmenėje – 1,44 MB diskelyje arba kompaktiniame diske.

Paryškintai (Bold) rašoma: straipsnio pavadinimas visomis kalbomis, antraštės bei svarbiausieji struktūriniai elementai (santrauka, įvadas, tyrimo objektas ir metodai, sąlygos, rezultatai, aptarimas arba diskusija, išvados, padėka, literatūra, santrauka anglių kalba). Kursyvu (Italic) rašomi lotyniški augalų rūšių, genčių, ligų, kenkėjų ir mikroorganizmų pavadinimai. Augalų veislių pavadinimai rašomi viengubose kabutėse (pvz.: ‘Auksis’).

Cituojamas šaltinis tekste nurodomas lenktiniuose skliaustuose (autoriaus pavardė, metai).

Lentelės

Lentelėse neturi būti kartojama paveiksluose ar kitose iliustracijose pateikta informacija.


Bandymų variantai lentelėse neturi būti žymimi skaičiais, sudėtingomis santrumpomis, o pateikiamai visa arba suprantamai sutrupinta aprašo forma.

Statistiniai duomenys, skaičiai ir skaitmenys

Pageidautina detalaviai aprašyti taikytus tyrimų metodus ir nurodyti jų originalius šaltinius. Labai svarbūs informacijos apie lauko, vegetaciniių ir kt. bandymų išdėstymo schemų ir jos pasirinkimo motyvus. Lentelėse ir paveiksluose pateikiamai duomenys privalo būti statistiškai apdoroti: apskaičiuoti vidurkius, jų kitimo paklaidos, ryšio ir
jo tikslumo koeficientai, patikimo skirtumo ribos (priimtiniausiaapskaičiuoti 95% arba ir 90,99% tikimybės lygį) ir pan. Rodiklių žymėjimo santrumpos turi būti paaškintos, jeigu jos neatitinka tarptautinių ISO standartų [13].

Reikšminių skaičių turi būti ne daugiau negu leidžia bandymo metodas. Variantų vidurkiai turi būti suapvalinti iki 1/10, apskaičiuotos jų standartinės paklaidos.

Kiekį žymintys skaičiai rašomi arabiškais skaitmenimis, pvz.: 15 tonų, o eilę žymintys gali būti rašomi ir romėniskai, pvz.: XX amžius, 2 pavyzdys.

Skaičiai nuo vieneto iki devynių rašomi žodžiu, išskyrus tuos atvejus, kai jie reiškia matavimo vienetų dydį (pvz.: 5 km, bet „trys variantai“) arba yra prasminė skaičių seka (pvz.: 6, 9, 12 tarpsnis). Tarp daugiauenklių skaičių klasių paliekanamas tarpelis, pvz., 42 351. Procentai žymimi %, kai reiškia konkrečų skaičių, tačiau „procentiniai vienetai” rašomi tik žodžiu. Dešimtainės trupmenos dalys nuo sveikųjų skaičių atskiriamos kableliu.

**Paveikslai**

Visa iliustracinė medžiaga – brėžiniai, grafikai, diagramos, fotografijos, piešiniai ir kt. – vadinami bendru paveikslų vardu. Tekstas juose rašomas lietuvių ir anglų kalbomis.


Irašai ir simboliai paveiksluose turi būti parašyti ne mažesni kaip 10 dydžio šriftu. Paveikslų blokų dalys turi būti sužymėtos raidėmis a, b, c ir t.t.

**Literatūros sąrašas**

Į literatūros sąrašą gali būti įtraukiami:

– straipsniai, atspausdinti moksliuose periodiniuose žurnaluose, moksliuose recenzuotuose leidiniuose (knygose, monografiuose), mokslių konferencijų, simpoziumų, kurių medžiaga buvo recenzuota arba struktūros ir apimties požiūriu atitinka mokslių periodinių leidinių straipsnių reikalavimus, straipsnių rinkiniuose;

– moksliškos knygos, monografiuose, mažesnės apimties recenzuoti ir tik išimta moksliškos paskirties leidiniai (t.y. disertacijų mokslo laipsniui įgyti santraukos) arba jų dalys;


Moksliškas ataskaitos, rankraštinė medžiaga, vadovėliai, žinynai, konferencijų medžiaga (tezės ar trumpai paranešimai), rekomendacijos, reklaminiai bukletai bei laikraščiai literatūros moksliuose nelaikomi ir į sąrašą neįtraukiami. Nuorodos į standartus, žinynus ir kitus normatyvinius teisinius dokumentus pateikiamos puslapių išnašose.

Užsienioje leistų žurnalų, konferencijų rinkinių ir kt. pavadinimai netrumpinami.

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**Knygos**


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